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Design, Synthesis and Biological Activity of α-Acylaminoamide Derivatives as Influenza Nucleoprotein Inhibitors

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By

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Table of Contents

LIST OF ABBREVIATIONS

δ	Chemical shift
ṽ	Wavenumber
®	Registered trademark
Ø	Diameter of the column
µM	Micromolar
Acetone-d_6	Deuterated acetone
Boc	*tert*-Butyloxycarbonyl
CC_{50}	50% Cytotoxic concentration
CDCl3	Deuterated chloroform
CDI	1,1'-Carbonyldiimidazole
COMU	1-[(1-(Cyano-2-ethoxy-2-oxoethylideneaminooxy) dimethylaminomorpholino)] uronium hexafluorophosphate
COSY	Correlated spectroscopy
COVID-19	Coronavirus disease 2019
CPA	Chiral phosphoric acid
CPE	Cytopathic effect
DCC	N,N'-Dicyclohexylcarbodiimide
DCM	Dichloromethane
DIC	N,N'-Diisopropylcarbodiimide
DIEA	N,N'-Diisopropylethylamine
DMAP	4-Dimethylaminopyridine
DMF	Dimethylformamide
DMSO	Dimethylsulfoxide
DMSO-d_6	Deuterated dimethylsulfoxide
EC_{50}	50% Effective concentration
EDCI	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
ee	Enantiomeric excess
EMEM	Eagle’s minimal essential medium
ESI	Electrospray ionization
Et_3N	Triethylamin
EtOAc	Ethyl acetate
GCI	Grating-Coupled Interferometry
HA	Hemagglutinin
HATU	Hexafluorophosphate Azabenzotriazole Tetramethyl Uronium
HBTU	Hexafluorophosphate Benzotriazole Tetramethyl Uronium
HEPES	4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid
His	Histidine
HMBC	Heteronuclear Multiple Bond Correlation
HOBt	Hydroxybenzotriazole
HPLC	High performance liquid chromatography
HRMS	High-resolution mass spectrometry
HSQC	Heteronuclear single quantum Correlation

HTS	High-throughput screening
Hz	Hertz
IAV	Influenza A virus
IC_{50}	50% Inhibitory concentration
IR	Infrared
K_D	Dissociation constant
M	Molar
m.p.	Melting point
m/z	Mass-to-charge ratio
MDCK	Madin Darby canine kidney
MeCN	Acetonitrile
MeOH	Methanol
MOI	Multiplicity of infection
NA	Neuraminidase
NES	Nuclear Export Signal
NHS	*N*-Hydroxysuccinimide
NLS	Nuclear localization signals
NMR	Nuclear magnetic resonance
No.	Number
NP	Nucleoprotein
Oxyma	Ethyl cyanohydroxyiminoacetate
PA	Polymerase acidic protein
PB1	Polymerase basic protein 1
PB2	Polymerase basic protein 2
PDB	Protein data bank
ppm	Parts per million
R_f	Retention factor
RNP	Ribonucleoprotein
rt	Room temperature
SAR	Structure-activity relationship
SI	Selectivity index
SPR	Surface plasmon resonance
TCEP	Tris(2-carboxyethyl)phosphine
$TCID_{50}$	Median Tissue Culture Infectious Dose
THF	Tetrahydrofuran
TLC	Thin layer chromatography
TMS	Tetramethylsilane
t_R	Retention time
Ugi-4CR	Ugi four-component reaction
V/V	Volume/volume
vRNP	Viral ribonucleoprotein
WHO	World Health Organization

ZUSAMMENFASSUNG

Influenza ist eine Atemwegsinfektion, die erhebliche Auswirkungen auf die weltweite Gesundheit hat. Historische Pandemien haben ihre tiefgreifenden Auswirkungen auf das gesellschaftliche Wohlergehen und die wirtschaftliche Stabilität unterstrichen. Die sich wandelnde Natur des Influenza-A-Virus, kombiniert mit seinem wachsenden Wirtsspektrum, verstärkt diese Bedrohung. Daher hat die Bekämpfung der Influenza weltweit höchste Priorität im öffentlichen Gesundheitswesen erhalten.

Das Nukleoprotein des Influenzavirus, ein durchgehend konserviertes Strukturprotein, ist zentral für seinen Replikationsprozess und fungiert als wesentlicher Vermittler zwischen dem Virus und den Wirtszellen. Angesichts dieser Funktionen stellt das Nukleoprotein ein attraktives Ziel für therapeutische Eingriffe gegen Influenza dar. Empirische Forschung unterstützt die Machbarkeit der Entwicklung von Medikamenten, die auf dieses Nukleoprotein abzielen, was es zu einem Schwerpunkt in der Anti-Influenza-Wirkstoffforschung macht.

Prof. Dr. Johannes Kirchmair und Prof. Dr. Michaela Schmidtke identifizierten α-Acylaminoamid-Derivate als potenzielle Inhibitoren des Influenza-Nukleoproteins durch virtuelles Screening. Nachfolgende *in vitro* Tests zeigten die Wirksamkeit dieser Verbindungen, wobei **JK9** (**1**) eine besonders vielversprechende inhibitorische Aktivität gegen verschiedene Influenzastämme zeigte, insbesondere gegen die Nucleozizn-resistente A/pdm09(H1N1)-Variante. Angesichts der geringen Toxizität und des großen Potenzials für strukturelle Modifikationen von **JK9** (**1**), zusammen mit seiner Aktivität gegen das Influenza-A-Virus, wurde es als optimale Hit-Verbindung identifiziert.

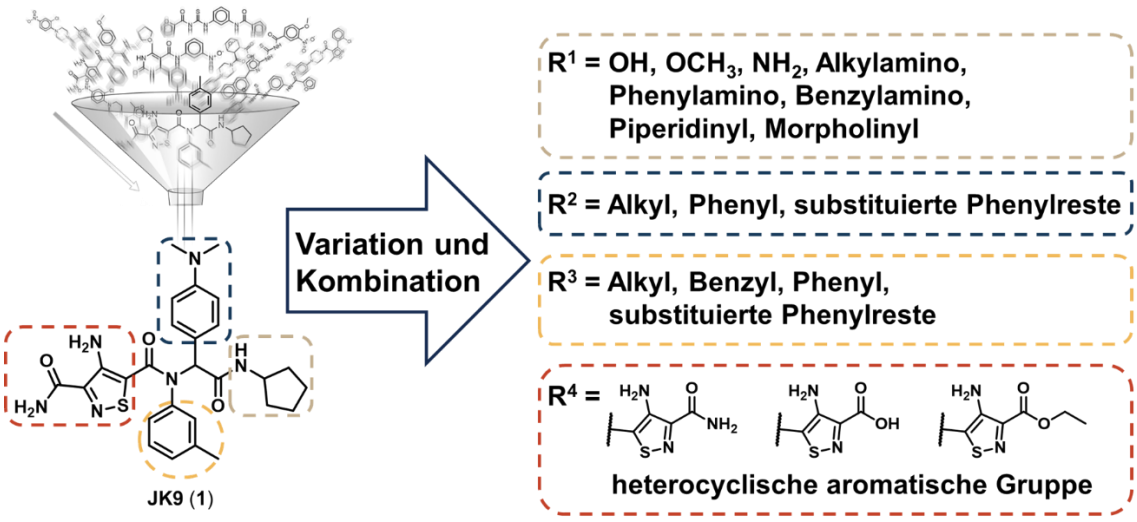


Abbildung I. Entdeckung von **JK9** (**1**) und Strategien zur Modifikation der Verbindung.

In dieser Studie wurden ausgehend von der Hit-Verbindung **JK9** (**1**) systematische strukturelle Modifikationen an R^1, R^2, R^3 und R^4 vorgenommen mit dem Fokus auf der Hemmung der Aktivität des Influenza A-Virus. Die Struktur-Wirkungs-Beziehung für die Hemmung der Aktivität des A/(H1N1)pdm09-Stamms kann wie folgt systematisch zusammengefasst werden:

1. Das Vorhandensein eines sekundären Amids an der R^1-Position ist für die Aktivität entscheidend. Substituenten am Amidstickstoff beeinflussen ebenfalls die Aktivität, wobei Cyclopentyl- und Cyclohexyl-Reste am günstigsten sind.
2. Die Einführung eines Phenylrings an der R^2-Position ist förderlich für die Aktivität. *Ortho*-Substitution am Phenylring ist ungünstig. Elektronenentziehende Gruppen, insbesondere Halogene und Cyanogruppen, führten im Vergleich zu elektronenschiebenden Gruppen zu einer höheren Aktivität. Verbindungen mit Chlor- oder Cyanosubstituenten am Phenylring zeigten die höchste Aktivität.
3. Die Einführung von Methyl- und Benzhydryl-Gruppen an der R^3-Position führte zu einer reduzierten Aktivität, während Verbindungen, die mit einem Phenylring substituiert waren, erhöhte Aktivität zeigten. Bei den Phenyl-Derivaten erwiesen sich *ortho*-Substituenten als nachteilig für die Aktivität. Die Aktivität nahm im Allgemeinen mit elektronenschiebenden Gruppen ab, während elektronenentziehende Gruppen, insbesondere Fluor, Trifluormethyl-, und Cyano-Gruppen, außergewöhnliche Aktivität zeigten.
4. Ein fünfgliedriger aromatischer Heterozyklus an der R^4-Position ist für die Aktivität unerlässlich. Phenyl- und Chinolinderivate zeigten keine Aktivität, wobei die Phenylgruppe zu Zytotoxizität führte. Die Modifikation des Isothiazolrings, insbesondere die Einführung einer Carboxylgruppe, erhöhte sowohl die Aktivität als auch die Löslichkeit.
5. Weder die (*S*)- noch die (*R*)-Enantiomere zeigten einzeln eine signifikante Wirksamkeit, was im starken Kontrast zur vielversprechenden Aktivität ihrer racemischen Mischungen steht.

Darüber hinaus wurde die Bindungsaffinität von sieben charakteristischen Verbindungen zum Influenza-Nukleoprotein bestimmt. **88** zeigte die stärkste Bindungsaffinität zum Nukleoprotein, wobei ihr K_D-Wert den der Positivkontrolle Nukleozin übertraf. **49c** zeigte ebenfalls eine bemerkenswerte Bindungsaffinität für das Nukleoprotein, die besser als die von Nukleozin, aber schlechter als die von **88** war. Obwohl sowohl (*R*)-**58a** als auch (*S*)-**58a** an das Nukleoprotein banden, waren ihre Affinitäten niedriger, wobei (*S*)-**58a** günstiger war. Dies

deutet darauf hin, dass diese Verbindungen tatsächlich die Vermehrung des Influenza-A-Virus hemmen, indem sie das Nukleoprotein als Zielstruktur haben.

In dieser Studie wurden 84 neue Zielverbindungen entworfen und synthetisiert, die alle mittels ^{1}H-NMR-, ^{13}C-NMR-, und IR-Spektroskopie sowie HRMS identifiziert wurden. Es wurde eine umfassende Zusammenfassung der Struktur-Wirkungs-Beziehung der Zielverbindungen bezüglich der Hemmung des Influenza A-Virus erstellt. Besonders hervorzuheben ist die repräsentative Verbindung **88**, die eine einzigartige Wechselwirkung mit dem Nukleoprotein zeigte und sowohl vielversprechende *in vitro* Aktivität als auch eine günstige Wasserlöslichkeit aufwies. Dies zeichnet **88** als führenden Wirkstoffkandidaten aus, wobei derzeit umfangreiche Studien laufen. Darüber hinaus stellt dieses Isothiazol-haltige α-Acylaminoamid eine vielversprechende Leitstruktur für die weitere Entwicklung von Influenza A Nukleoprotein-Inhibitoren dar.

ABSTRACT

Influenza is a respiratory infection that significantly impacts global health. Historical pandemics have underscored its profound effects on both societal well-being and economic stability. The evolving nature of the influenza A virus, coupled with its expanding host range, amplifies this threat. Consequently, addressing influenza has emerged as a paramount public health priority globally.

The influenza virus nucleoprotein (NP), a conserved structural protein, is central to virus replication process and acts as an essential mediator between the virus and host cells. Given these functions, the NP represents an attractive target for therapeutic intervention against influenza. Empirical research supports the feasibility of devising drugs aimed at this NP, making it a focal point in anti-influenza drug discovery.

Prof. Dr. Johannes Kirchmair and Prof. Dr. Michaela Schmidtke identified α-acylaminoamide derivatives as potential inhibitors of the influenza NP through virtual screening. Subsequent *in vitro* tests have demonstrated the efficacy of these compounds, with **JK9** (**1**) displaying particularly promising inhibitory activity across various influenza strains, notably the nucleozin-resistant A/pdm09(H1N1) variant. Given the low toxicity and extensive structural modification potential of **JK9** (**1**), along with its activity against the influenza A virus (IAV), it has been identified as an optimal hit compound.

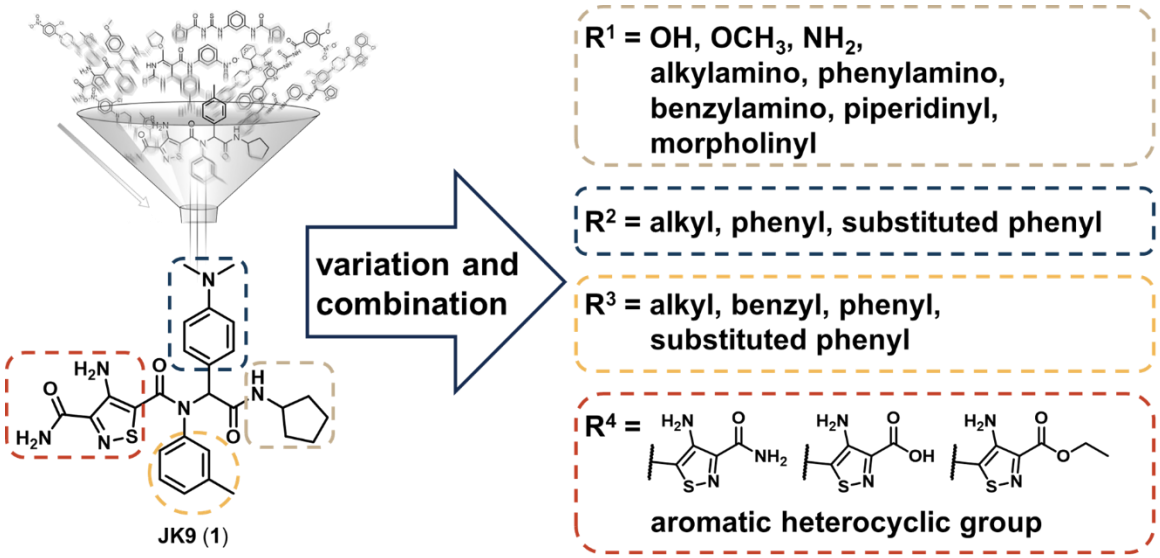


Figure 2. Discovery and modification strategies of **JK9** (**1**).

In this study, with **JK9** (**1**) as the hit compound and inhibition of the IAV as the focus, systematic structural modifications were carried out at R^1, R^2, R^3, and R^4. The structure-activity relationship (SAR) for the inhibition of the A/(H1N1)pdm09 strain activity was systematically summarized:

1. The presence of a secondary amide at the R^1 position is crucial for activity. Substituents on the amide nitrogen also influence the activity, with cyclopentyl and cyclohexyl being the most optimal.
2. Introducing a phenyl ring at the R^2 position is beneficial for activity. *Ortho*-substitution on the phenyl ring is detrimental. Electron-withdrawing groups, especially halogens and cyano groups on phenyl, demonstrated higher potency compared to electron-donating groups. Compounds with chlorine or cyano substituents on phenyl ring exhibited the highest activity.
3. The introduction of methyl and benzhydryl at the R^3 position led to reduced activity, while compounds substituted with a phenyl ring enhanced activity. Among the phenyl derivatives, *ortho*-substituents are disadvantageous for activity. Activity generally decreased with electron-donating groups, while electron-withdrawing groups, especially trifluoromethyl, fluorine, and cyano groups, caused exceptional activity.
4. A five-membered aromatic heterocycle at the R^4 position is essential for activity. Phenyl and quinoline derivatives lacked activity, with the phenyl derivative showing cytotoxicity. Modifying the isothiazole ring, especially by introducing a carboxylic acid group, increased both activity and solubility.
5. Neither the (*S*)- nor the (*R*)-enantiomers demonstrated significant efficacy individually, contrasting sharply with the promising activity of their racemic mixtures.

Additionally, the binding affinity of seven representative compounds to the influenza NP was determined. **88** exhibited the strongest NP binding affinity, with its K_D value surpassing that of the positive control, nucleozin. **49c** also showed a notable binding affinity for the NP, better than nucleozin but inferior to **88**. Although both (*R*)**-58a** and (*S*)**-58a** bound to the NP, their affinities were lower, with (*S*)**-58a** being more favorable. This indicates that these compounds indeed inhibit the proliferation of the IAV by targeting the NP.

In this study, 84 novel target compounds were designed and synthesized, all of which were authenticated by ^{1}H NMR, ^{13}C NMR, and IR spectroscopy as well as HRMS. A comprehensive summary of the SAR of the target compounds in inhibiting the IAV was provided. Significantly, the representative compound **88** showcased a unique interaction with the NP and displayed both,

promising *in vitro* activity and favorable water solubility. This positions **88** as a leading candidate compound, with comprehensive studies currently underway. Moreover, this isothiazole-containing α-acylaminoamide represents a promising lead structure for the further development of influenza A NP inhibitors.

1.0 INTRODUCTION

1.1 Influenza and Influenza Virus

Influenza is a highly contagious viral infection that typically manifests in seasonal epidemics, often peaking during the winter months[1-3]. It has continuously posed a significant impact on human life and health, further introducing substantial economic and social burdens[4, 5]. The influenza A virus (IAV) is recognized as a prominent respiratory pathogen, capable of infecting a wide variety of species[6, 7]. As a highly transmissible respiratory virus, it exhibits a plethora of symptoms commonly including, but not limited to, fever, chills, cough, sore throat, nasal congestion, musculoskeletal pain, headaches, and fatigue[8,9]. Additionally, certain cases, particularly among children, might present gastrointestinal symptoms such as vomiting and diarrhea[10, 11]. Although frequently self-limiting, IAV can cause severe, and at times, fatal complications, especially within susceptible populations like the elderly, young children, and individuals with pre-existing health afflictions[12]. Such complications might span pneumonia, bronchitis, sinus and ear infections, myocarditis, encephalitis, and secondary bacterial infections, in addition to the exacerbation of pre-existing chronic medical conditions[13, 14].

Epidemiological data highlight the significant global impact of IAV, with approximately one billion cases of seasonal influenza reported annually, including 3-5 million cases of severe illness[15]. Additionally, IAV is responsible for 290,000 to 650,000 respiratory deaths each year[16]. Beyond the regular annual epidemics, IAV has historically been the culprit behind several catastrophic pandemics[17].

Three major influenza pandemics occurred worldwide in the 20th century. The outbreak of the 'Spanish Flu' in 1918 consisted of three waves: the first was relatively mild, while the second and third were extremely severe, with a mortality rate exceeding 2.5%, far higher than the usual mortality rate of less than 0.1% during typical influenza outbreaks. Caused by the H1N1 influenza virus, the 'Spanish Flu' was the deadliest influenza pandemic recorded in history, infecting about one-third of the world's population and resulting in approximately 50 million deaths. Unusually, this pandemic primarily caused deaths among young adults aged 20-40, a non-typical mortality pattern that remains unexplained to this day[18-21]. The 'Asian Flu' of 1957-1958, originating in southern China and caused by the H2N2 influenza virus, resulted in high morbidity and mortality rates worldwide, causing about 4 million deaths. The 'Hong Kong Flu' of 1968, caused by the H3N2 influenza virus, had both, lower morbidity and mortality rates than the 1957 'Asian Flu,' resulting in approximately 1 million deaths[22, 23].

In 2009, a novel H1N1 swine flu broke out in Mexico and rapidly spread worldwide[24]. As person-to-person transmission continued to occur globally within a short period of time, the World Health Organization (WHO) declared in June 2009 that the pandemic alert level would be raised from phase 5 to 6, marking the arrival of the first influenza pandemic of the new century. It was not until August 2010 that the WHO announced the end of this influenza pandemic. This pandemic affected 214 countries and regions worldwide, causing at least 18,000 deaths[25, 26].

During the COVID-19 pandemic, the implementation of widespread protective measures led to a significant decline in influenza incidences. However, a resurgence was observed in the winter of 2022[27]. The morbidity, mortality, and heightened susceptibility to infections associated with IAV continue to be a focal point of concern and research.

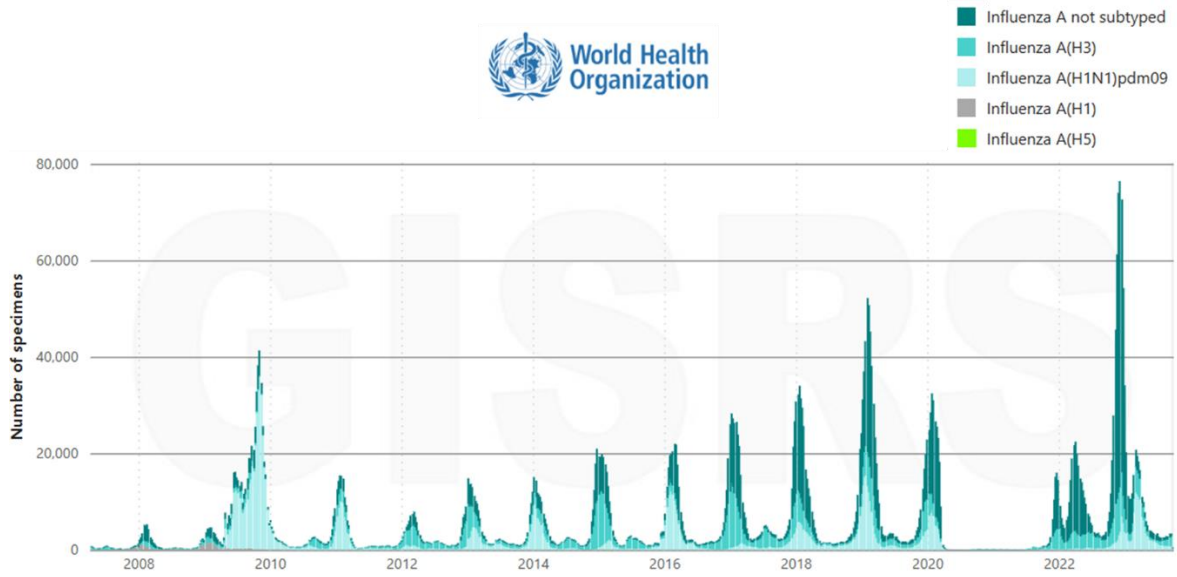


Figure 3. Virus detections by subtype reported to FluNet (WHO)[27].

As a member of the Orthomyxoviridae family, the IAV is categorized into 16 hemagglutinin (HA) and 9 neuraminidase (NA) subtypes, denoted H1-H16 and N1-N9, respectively, based on the antigenic properties of the two surface glycoproteins[28-30].

IAV virions, though exhibiting various shapes, are primarily spherical and enveloped[31]. Their lipid envelope is obtained from the host cell membrane during the viral budding process and is punctuated with an abundance of the glycoproteins HA and NA, critical for cellular entry and exit, respectively[32-35]. The lipid envelope also harbors the ion channel protein M2, albeit in smaller quantities. As a membrane homotetramer, M2 plays an essential role in uncoating and

HA maturation[36-38]. Underneath the lipid envelope, the peripheral membrane protein M1 forms a layer, providing structural integrity to the virion and safeguarding the internal ribonucleoprotein (RNP) particles while maintaining the virion's overall morphology[39, 40].

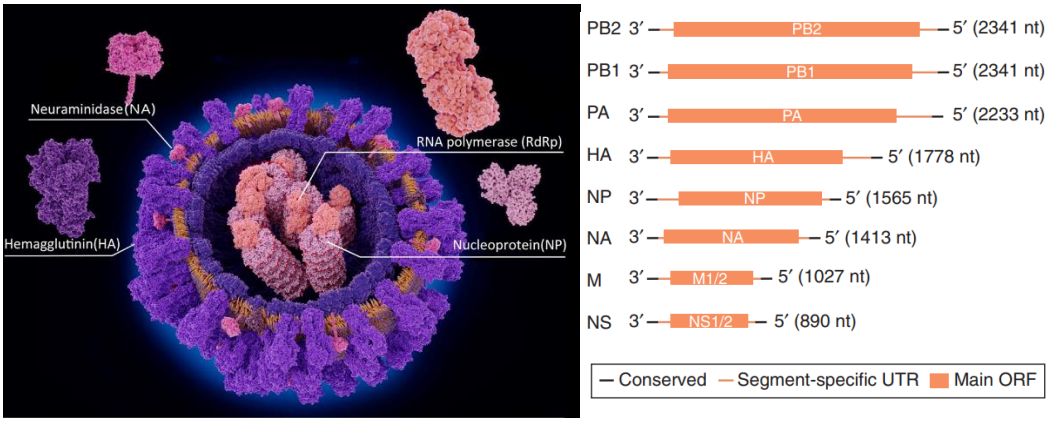


Figure 4. Influenza virus structure (left) and RNA segments with their corresponding encoding proteins (right)[41].

The lipid envelope and the M1 protein layer constitute a protective shell, encasing the viral RNA, a fundamental element responsible for encoding various viral proteins. The RNA is composed of eight negative-sense viral gene segments, each encoding one or two proteins essential for the viral life cycle and infectivity. The three largest segments encode the three subunits of an RNA-dependent RNA polymerase: polymerase basic protein 1 (PB1), polymerase basic protein 2 (PB2), and polymerase acidic protein (PA). The segment encoding the PB1 subunit also encodes a small non-structural protein, PB1-F2. Following these, the 4th, 5th, and 6th segments encode HA, NP, and NA, respectively[42]. The 7th segment is responsible for encoding the M1 matrix protein and M2 ion channel protein. The smallest segment also encodes two proteins: non-structural protein NS1 and nuclear export protein NS2.

The intricacy of the viral structure is further exhibited in the formation of the viral ribonucleoprotein (vRNP) complex. Viral RNA binds meticulously with various proteins including PB1, PB2, PA, and NP, to coil into the vRNP[43, 44]. This coiling results in the vRNP existing in an exceptionally dense form[32, 36, 45]. The NP not only serves as a principal component of the vRNP but also plays a pivotal role in the transcription and replication of vRNP within cells, thereby facilitating the proliferation of the virus within the host organism.

1.2 Influenza A virus life cycle

The replication cycle of the influenza virus can be categorized into the following stages: binding, endocytosis, membrane fusion and viral uncoating, synthesis of viral RNA, synthesis of viral proteins, assembly, and release of progeny viral particles.

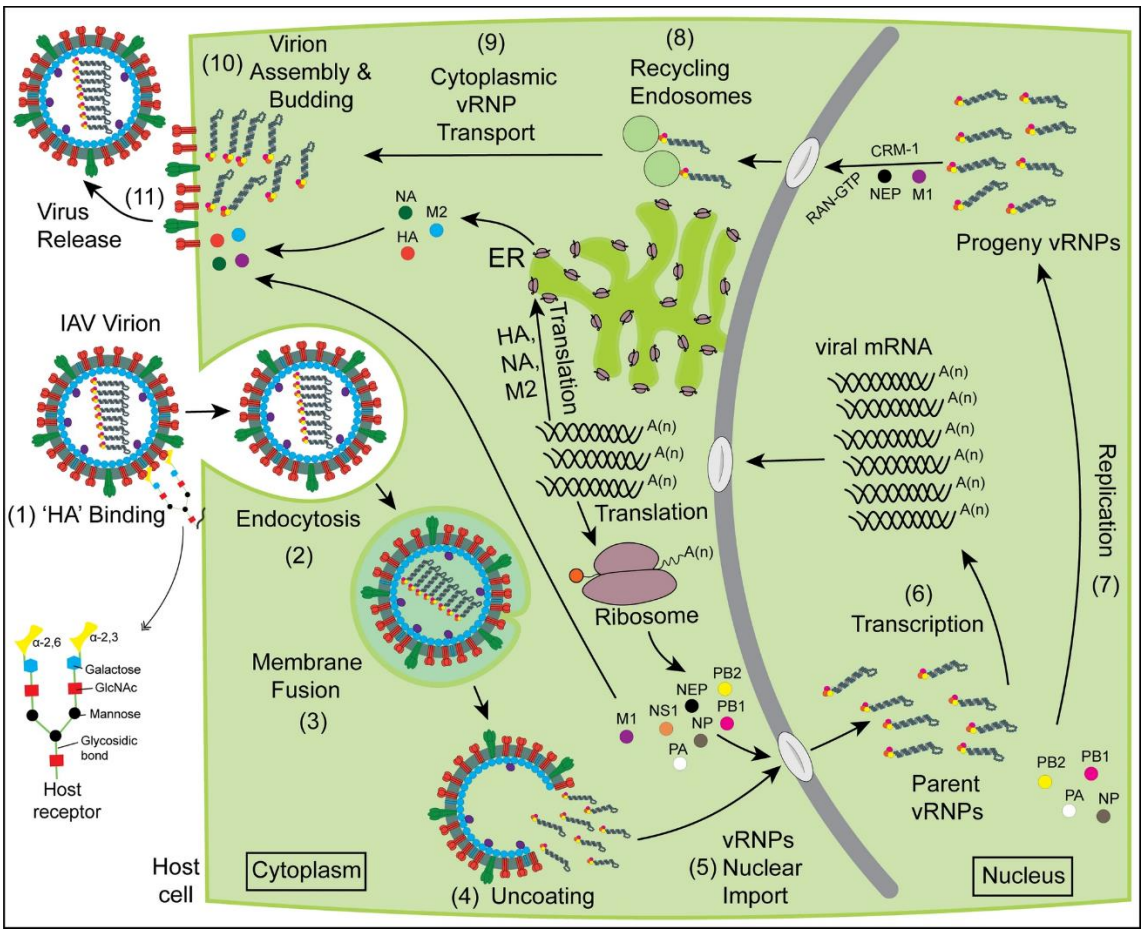


Figure 5. IAV replication cycle[46].

Binding: The influenza virus initiates its replication cycle *via* adsorption, where HA recognizes and engages with sialic acid (*N*-acetylneuraminic acid) receptors on host cells, establishing a preliminary interaction that facilitates the subsequent viral entry[47, 48].

Endocytosis: Subsequent to adherence, the virus is internalized into the host cell through receptor-mediated endocytosis, wherein the viral particles are enclosed within endosomes, navigating through the intracellular environment.

Membrane fusion and uncoating: Within the endosome, a mildly acidic pH provokes an irreversible conformational change in HA, exposing the fusion peptide of its HA2 subunit. The fusion peptide facilitates the fusion of viral and endosomal membranes, constructing a conduit for the vRNP complex to enter the cytoplasm[49, 50]. Additionally, M2 ion channels are activated,

permitting proton influx, internally acidifying the viral particle and thereby facilitating vRNP release into the host cytoplasm through the pore formed by membrane fusion[51-53].

Synthesis of viral RNA: Upon cytoplasmic entry, the vRNP complex is transported to the nucleus, guided by nuclear localization signals (NLS)[54-56]. Within the nucleus, viral RNA synthesis occurs, involving a cap-snatching mechanism for mRNA synthesis and subsequent replication of the viral genome *via* complementary RNA intermediates[57-59].

Synthesis of viral proteins: The newly synthesized mRNA is shuttled to the cytoplasm where viral protein synthesis ensues. HA, NA, and M2 proteins are subjected to folding and post-translational modifications within the endoplasmic reticulum and Golgi apparatus, culminating in their transport to the host cell membrane, while NPs and the RNA polymerase associate with nascent vRNA within the nucleus[46, 60].

Assembly: Progeny vRNP complexes interact with M1 matrix proteins and are exported from the nucleus to the cytoplasm *via* an M1-NS2 complex, which cooperates with the human CRM1 protein. Subsequently, the vRNP complex congregates with viral proteins at the host cell membrane, leading to the assembly of progeny viral particles[61, 62].

Release of viral particles: The progeny viral particles bud from the host cell membrane, facilitated by host factors. HA and sialic acid residues facilitate the temporary adherence of the nascent virions to the cell surface. NA activity cleaves sialic acid moieties, enabling the release and dissemination of progeny viruses to infect neighboring cells[63-65].

1.3 Influenza nucleoprotein

vRNP assumes multifarious roles throughout the lifecycle of the influenza virus, engaging not only in its gene expression and replication but also playing a pivotal role in its packaging, release, penetration into new host cells, and immune evasion, among other processes. Consequently, comprehending the structure and function of vRNP is vitally important for devising new therapeutic strategies targeting the influenza virus.

A vRNP complex is composed of eight single-stranded, negative-sense RNA segments. Each segment comprises one or more open reading frames, segment-specific untranslated regions, and partially complementary, conserved 3’ and 5’ termini. The assembly of vRNP involves the binding of the conserved 3’ and 5’ termini to a viral RNA-dependent RNA polymerase, while the remaining RNA binds to oligomerized viral NP.

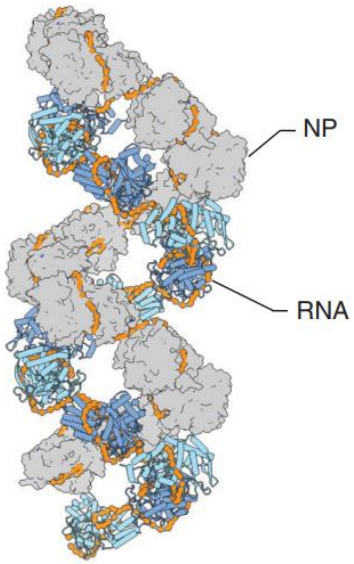


Figure 6. Crystal Structure of vRNP[41].

The elucidation of its crystal structure revealed that vRNP adopts a double-helical, rod-like complex, comprising single-stranded vRNA segments, RNA polymerase, and oligomeric NP (Figure 6). Despite embodying a double-helical conformation, the establishment of a universal and clear structural model for vRNPs is hindered due to their diverse helical properties and handedness. Such heterogeneity challenges the mapping of RNA and NP-NP junctions within vRNPs[59, 66].

As a crucial component of vRNP, the NP plays a pivotal role in the viral replication process and is encoded by the 5th segment of influenza virus RNA. It is composed of 498 amino acids, rich in arginine, glycine, and serine residues, carrying a net positive charge under neutral pH conditions and primarily exists in a trimeric form *in vitro*. The crystal structures of H1N1[67] and H5N1[68] NPs were first delineated. Subsequently, the monomeric structure of H1N1 NP, with an R416A mutation, was elucidated[69].

The crystal structures revealed that the NP monomer is predominantly composed of α-helical structures, presenting a crescent-like shape. Each molecule encompasses two structural domains: head and body, with the head domain being structurally more conservative than the body domain. They are connected by three loop structures to maintain the protein conformation. Between the head and body domains on the external surface, a viral RNA-binding groove is situated, within which some highly conserved basic amino acid residues are located[70]. On the back of each crescent-shaped NP molecule a tail loop structure is located, composed of amino acid residues 402–428[67]. This tail loop can insert into the body domain of adjacent NP

molecules, forming intermolecular β-sheet structures and binding through hydrophobic interactions and salt bridges, leading to the oligomerization of NP molecules. Appropriate oligomerization between NP molecules plays a crucial role in maintaining the biological activity of the NPs. Mutations of single amino acid residues in the tail loop can completely abolish NP oligomerization.

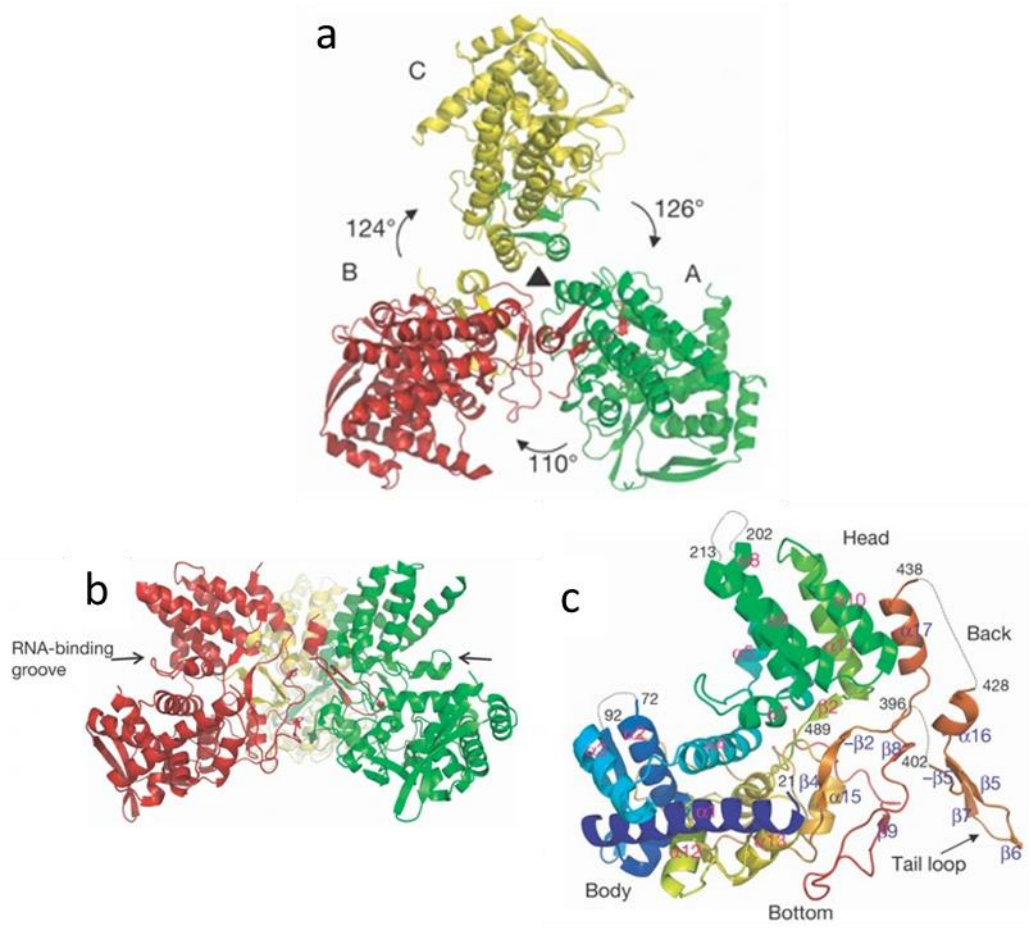


Figure 7. The crystal structure of H1N1 NP. **a)** the NP trimer with three subunits shown in different colors. **b)** side view of a NP trimer, mainly representing the RNA-binding groove. **c)** structure of NP subunit B[67].

As a significant structural protein of the influenza virus, the primary function of the NP is to form the scaffold of the helical genomic RNP complexes and to maintain RNA templates in an optimal orientation for transcription, replication, and assembly into virions[71]. NP demonstrates a high affinity for RNA binding, without exhibiting notable sequence specificity, and the RNA bound to NP remains sensitive to ribonucleases[72]. The stoichiometry of the chemical binding between NP and RNA is approximately one NP molecule per 24 nucleotides of RNA[73]. A

plausible mechanism for NP-RNA binding could involve an alkaline loop structure near the NP RNA-binding groove, which detects and captures RNA, subsequently transporting the RNA molecule to the arginine-rich binding groove. Within the groove, the side chains of the arginine residues are pointing towards each other, effectively clamping the RNA within the groove, with additional interactions facilitating the binding between the two entities[68]. Additionally, NP can interact directly with the PB1 and PB2 subunits of the RNA polymerase but does not bind with the PA subunit[74, 75].

NP also plays a pivotal role in the transportation of vRNP complexes into the cell nucleus. There are at least two NLS in NP molecules: the *N*-terminus NLS1, composed of amino acid residues 1-13, and the mid-protein NLS2, comprised of amino acid residues 198-216. NLS1 mediates the transportation of vRNP and newly synthesized NP into the cell nucleus after decapsidation and release into the cytoplasm. Given that vRNPs, ranging from 100 to 200 Å, cannot traverse the 9 Å nuclear pore, the NLS sequences are crucial for facilitating its transportation between cytoplasm and nucleus. This is achieved by binding of the NLS sequence in NP to a cellular protein, importin-α, which mediates the importation of vRNPs[61, 76-78]. When newly formed NP assembles with progeny RNA to form progeny vRNP in the nucleus, which is then transported to the cytoplasm, the NLS1 of NP becomes concealed. This prevents the vRNP complexes from being transported back into the nucleus. The selective exposure and concealment of NLS1 constitutes a key mechanism regulating the directional transport of vRNP complexes[79].

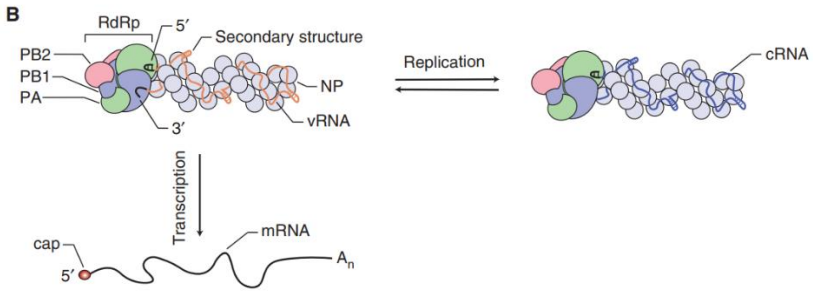


Figure 8. Influenza virus vRNP with its potential structural changes in the process of transcription and replication[41].

In addition, NP is vital for the transcription and replication of the viral genome. The influenza virus synthesizes three forms of viral RNAs: messenger RNA, viral genome RNA, and complementary positive-sense RNA, with vRNA replication utilizing cRNA as an intermediary. The binding of NP with one or more subunits of the RNA polymerase may induce conformational changes in the polymerase, thereby activating the synthesis of complementary

RNA and progeny RNA[80]. The effective synthesis of each RNA type, and the transition from mRNA transcription to viral RNA replication, requires a functional NP[81, 82]. Consequently, NP serves as a crucial functional link between the virus and host cells.

1.4 Influenza nucleoprotein inhibitors

Although vaccines are a directly effective method for preventing influenza, their efficacy against sudden outbreaks may not be guaranteed due to the antigenic shift and drift characteristics of the influenza virus[83, 84]. Furthermore, given the lengthy production cycle of influenza vaccines, antiviral drugs play a crucial role in the prevention and treatment of influenza.

Due to NP's conserved protein sequence and crucial roles throughout the influenza virus replication cycle, it has emerged as a promising target in the field of anti-influenza virus drug development. However, research into inhibitors targeting NP experienced a prolonged period of relative stagnation. It was not until 2010 that researchers consecutively discovered a series of small-molecule compounds targeting NP, further enhancing the allure of NP as antiviral drug target. Generally, NP inhibitors interfere with NP oligomerization, inhibit viral replication, and *via* additional mechanisms that warrant further exploration[85].

1.4.1 Nucleozin and its analogs

Nucleozin (**2**, Figure 10), one of the initial inhibitors targeting NP, was identified through cell-based antiviral screening[86-88]. It was effective in inhibiting the infection of influenza virus strains such as WSN/33 (H1N1), H3N2, and Vietnam/1194/04 (H5N1), with EC_{50} values of 0.07 μM, 0.16 μM, and 0.33 μM, respectively. In mice infected with the highly pathogenic Vietnam/1194/04 virus strain, treatment with nucleozin significantly reduced viral load in the lungs and improved survival rates. However, it has some shortcomings related to suboptimal solubility and metabolic stability in mouse liver microsomes[89]. After conducting successive passage experiments, it was observed that the H1N1 virus develops resistance under conditions of increasing concentrations of nucleozin or its analogs[86]. Sequencing of the viral genome enabled the mapping of resistance mutations to the corresponding viral proteins, and multiple research groups independently identified NP mutations that diminish the efficacy of nucleozin[86-88].

Three notable NP mutations are Y289H, N309K/N309T, and Y52H. The emergence of these mutations in the IAV, whether singularly or in combination, can confer resistance to nucleozin

and its analogs, suggesting that NP is the pharmacological target of this substance family. Furthermore, these mutations provide insight into potential binding sites (Figure 9a). Molecular docking models suggest that a small groove on the backbone region of the nucleoprotein may be the binding site for nucleozin, where N309 and Y289 in this region can form hydrogen bonds and hydrophobic interactions with nucleozin, respectively.

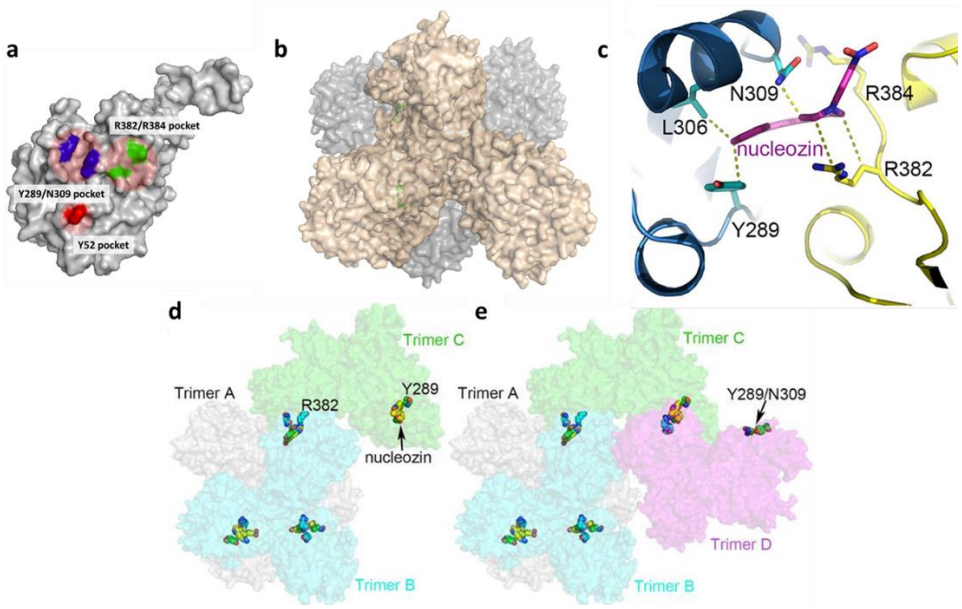
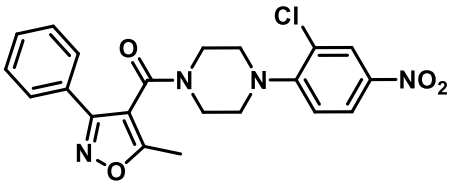


Figure 9. Mechanism of nucleozin binding **a**) Surface representation of three drug-binding pockets in the body domain of NP. **b**) Top view of dimer of trimers. **c**) Key NP-nucleozin interactions in one NP trimer. **d**) Docking of trimer C onto the hexamer of NP. **e**) Docking of trimer D onto trimer C. (PDB: 5B7B)[90].

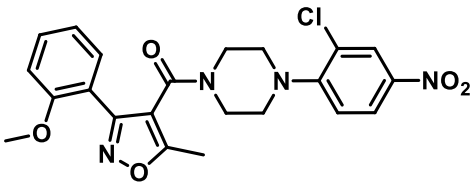
Subsequent investigations into the crystal structure of the NP-nucleozin complex have revealed that nucleozin act as bridging ligands between two NP molecules belonging to adjacent trimers.[90] The binding site for nucleozin consists between two distinct pockets from separate NP trimers: a Y289/N309 pocket from one trimer and a R382/R384 pocket from the other. In this configuration, a π-stacking interaction between the aromatic ring of residue Y289 and the unsubstituted phenyl ring of nucleozin, while N309 engages both hydrophobic and weak hydrogen bond interactions with the middle part of nucleozin. Additionally, the study identified the R382/R384 as a secondary binding pocket which supports the nitro-phenyl moiety of nucleozin like a horse saddle. These two residues interact with nucleozin through weak polar interactions. Despite offering lower binding affinity, this pocket is significant for nucleozin attachment, as the Y289/N309 or Y52/Y313 pocket alone is insufficient for nucleozin binding.

The intermolecular bridging facilitated by nucleozin facilitates the formation of an NP 'dimer of trimers,' effectively crosslinking two trimers. With these insights, a model of nucleozin-induced NP aggregation was constructed using computational tools.

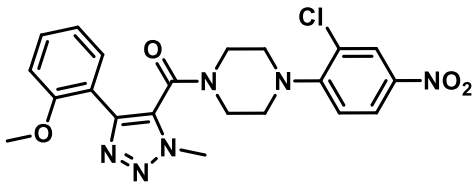
According to the crystal structure of NP-nucleozin complex, there are six NP molecules (chains **a**–**f**) in the asymmetric unit. These six molecules formed dimer of trimers. Trimer A was composed of chains **a**, **c** and **f** while trimer B was composed of chains **b**, **d** and **e**. Two nucleozin molecules were found between chains **a** and **b**, **c** and **d**, respectively. In the model of nucleozin-induced NP aggregation, nucleozin binds to chain **e** in trimer B *via* the Y289/N309 pocket and another NP molecule of a third trimer (Trimer C) through the R382 pocket (Figure 9d). And two NP molecules from trimer C still present unoccupied Y289/N309 and R382 pockets. This arrangement allows a fourth trimer (Trimer D) to attach to Trimer C through nucleozin, maintaining consistent binding properties (Figure 9e). Notably, several binding pockets in Trimer D remain exposed, capable of accommodating an additional NP trimer. Thus, in this model, NP molecules can continue to bind to each other until all binding sites are engaged.



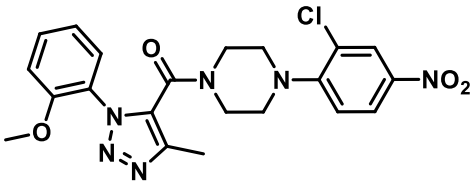
Nucleozin (2)
EC_{50} (H1N1) = 0.07 μM
EC_{50} (H3N2) = 0.16 μM
EC_{50} (H5N1) = 0.33 μM



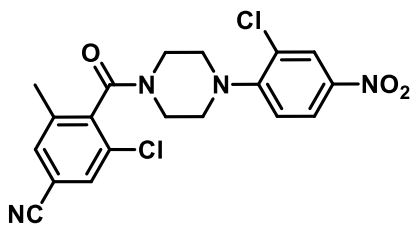
3
EC_{50} (H1N1) = 0.04 μM
EC_{50} (H5N1) = 0.07 μM



4
EC_{50} (H1N1) = 0.07 μM
EC_{50} (H5N1) = 0.04 μM



5



6
EC_{50} (H1N1) = 0.24 μM
EC_{50} (H3N2) = 0.46 μM

Figure 10. Structures and antiviral activities of nucleozin and its analogs.

Moreover, the exploration and study of nucleozin analogs burgeoned, revealing increasingly effective compounds. Gerritz *et al.* undertook structural modifications of the lead compound nucleozin, identifying two compounds, **3** and **4**, exhibiting relatively potent activity[86]. These compounds demonstrated 2-4 times higher antiviral activity than nucleozin and showed enhanced solubility and stability. Notably, at doses exceeding 10 mg/kg, compound **3** fully protected mice from influenza virus-induced mortality.

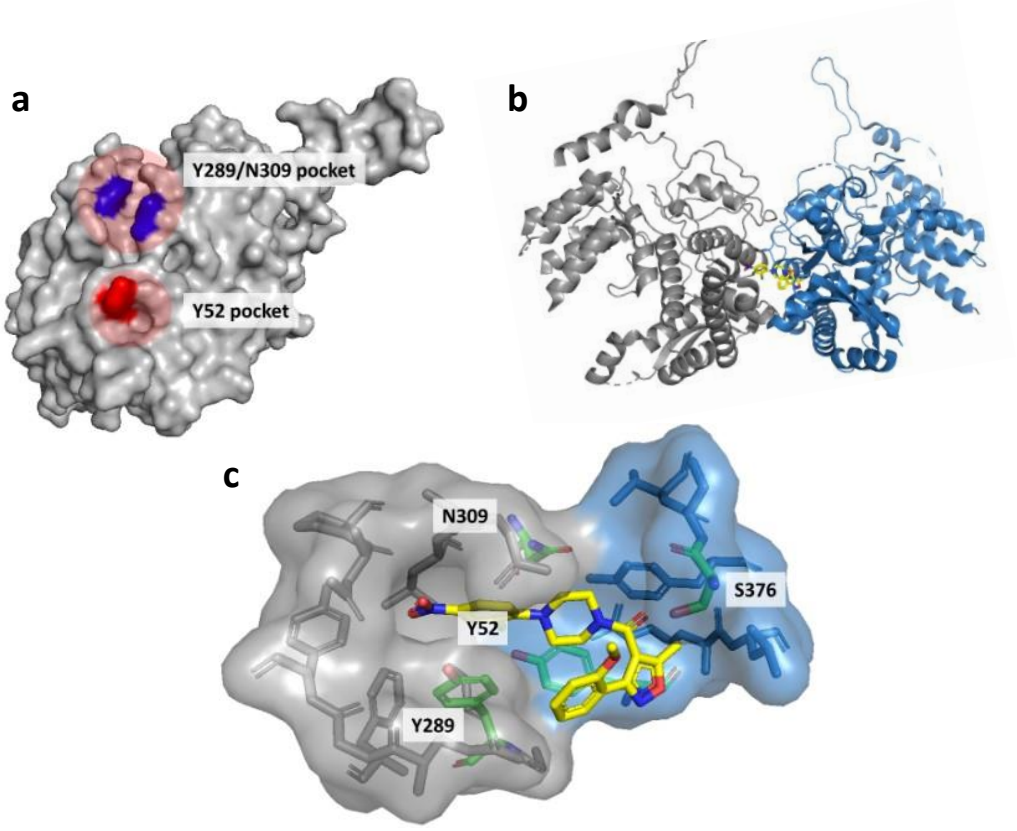


Figure 11. Binding of nucleozin analog **3** to NP **a)** Surface representation of two drug-binding pockets in the body domain of NP. **b)** Dimer structure of H1N1 NP in complex with **3**. **c)** Surface representation of the drug-binding pocket located at the interface of two NP monomers and interactions between **3** with key residue (PDB: 3RO5)[86].

The interaction of Compound **3**, a nucleozin analog, with NP is mediated through two key binding sites similar to those of nucleozin: the Y289/N309 pocket and the Y52 pocket (Figure 11a). While both compounds engage the Y289/N309 pocket during binding, only Compound **3** utilizes the Y52 pocket, which is not involved in nucleozin's interaction with NP. Notably, the binding conformation of Compound **3** within the Y289/N309 pocket is inversely oriented compared to that of nucleozin. The nitro-phenyl part of Compound **3** forms hydrophobic

interactions with the Y289 pocket. Furthermore, the methoxy phenyl ring is repositioned to form hydrophobic interactions with the side chains of both Y289 and R99 residues. Additionally, the Y52 residue is implicated in hydrophobic interactions with the central structure of Compound **3**.

The distinct conformational arrangements of Compound **3** and nucleozin in relation to NP suggest that structural variations underpin their differential effects on NP aggregation. Specifically, nucleozin is capable of inducing the formation of extensive NP aggregates, whereas Compound **3** predominantly promotes the assembly of NP hexamers, illustrating the influence of molecular architecture on the nucleation and polymerization of NP aggregates.

In 2012, Cheng *et al.* designed a series of nucleozin analogs utilizing scaffold hopping and bioisosteric replacement strategies[91]. Notably, compound **5** emerged as the most potent, with IC_{50} values against different IAV strains ranging from 0.5 to 4.6 μM. Thus, the compound showed antiviral activity being comparable to the one of nucleozin and exhibited sub-micromolar IC_{50} values against amantadine- and oseltamivir-resistant strains.

In 2016, Liao *et al.* discovered the NP inhibitor **6**, which has a mechanism of action similar to nucleozin, with IC_{50} values of 0.27 and 0.46 μM against H1N1 and H3N2 strains, respectively[92]. Pharmacokinetic studies in mice showed that this inhibitor has a half-life of more than 4 h and a bioavailability of more than 20%, demonstrating certain drug-like properties.

1.4.2 Targeting tail-loop binding pocket

Crystallographic data elucidated that while NP exists as monomers and oligomers, trimers are its most prevalent forms *in vitro*[67]. Interactions among the three subunits, mediated by the tail-loop, spotlighted the disruption of the salt bridge between the tail-loop and its binding pocket as a viable anti-influenza strategy. In contrast to nucleozin and its analogs, which induce NP aggregation to inhibit the formation of functional vRNP, certain small-molecule compounds spoil the aforementioned salt bridge, disabling NP and RNP functions.

Following Ye's publication in 2006[67], research by Yu-Fang Shen *et al.* identified that E339A, R416A, and the tail-loop deletion (Δ402–428) mutants perturb NP-NP interactions and inhibit interactions between NP and the RNA-dependent RNA polymerase[93, 94]. The researchers initially designed and expressed peptides that bind to the tail-loop, inhibiting NP trimer formation. Three peptides were developed, a linear short peptide, comprising tail-loop residue 411-417, and two circularized forms, one incorporating two cysteine residues at the ends of the

7-residue peptide and one spanning residues 409-418. The latter exhibited the highest inhibitory activity on viral replication (IC_{50} = 904 μM).

Furthermore, a virtual screening of small molecules was conducted, identifying several compounds that inhibit NP trimerization and promote the formation of NP monomers. Among these compounds, thiazole derivative **7** demonstrated notable potency against the H1N1 virus, exhibiting an EC_{50} value of 2.7 μM [94]. As shown in the modeled structure of the **7**-NP complex, the dichloro-anilino group of this inhibitor can bind into a hydrophobic site, which is formed by Phe304, Trp330, Ala336, Ile347, and Ala387, and is naturally occupied by Phe412 from the tail-loop. The aromatic ring of the inhibitor has the capacity to form aromatic-π interactions with Phe304 and Trp330, and a cation-π interaction with Arg389. Moreover, the nitrogen atoms of the anilino-thiazole-carboxamide groups of **7** can mimic Arg416 of the tail-loop and interact with the carboxylate moiety of Glu339. Additionally, the morpholino and propyl moieties of **7** are capable of docking into the pocket initially occupied by Ile408 and Pro419 of the tail-loop.

Although compound **7** demonstrated lower efficacy than nucleozin and its analogs in inhibiting viral replication, it was effective against viral variants that exhibit resistance to nucleozin. Additionally, due to the high conservation of the E339···R416 salt bridge, the probability of the virus developing resistance against drugs targeting this specific site is diminished.

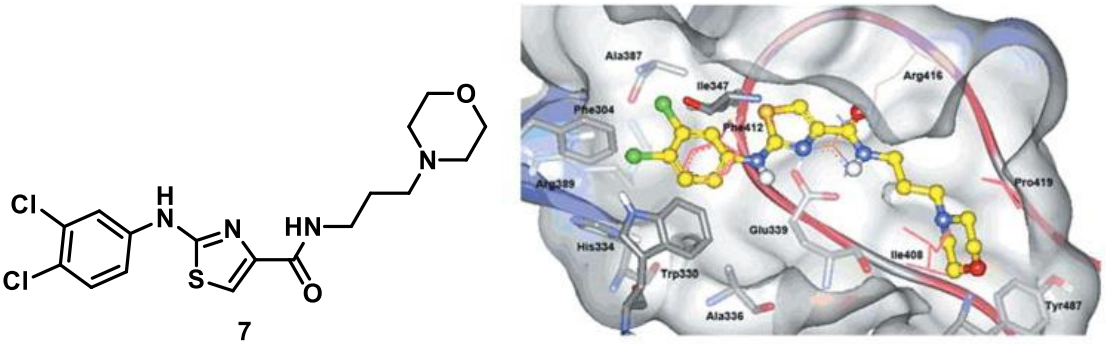


Figure 12. Structure of **7** and modeled structure of the **7**-NP complex[94].

1.4.3 Naproxen and its analogs

Naproxen (**8**), a marketed non-steroidal anti-inflammatory drug, has been verified to combat IAV by inhibiting the binding of NP to RNA. *In vitro* and *in vivo* tests against H1N1 and H3N2 strains have demonstrated good antiviral effects of the compound, and no resistant strains were found after 8 cell passages[95]. Surface plasmon resonance experiments and single-point mutation experiments indicated that naproxen targets the RNA binding groove of NP, inhibiting virus

replication by preventing the interaction between NP and viral RNA. Naproxen and its further modified derivatives have become dual-target inhibitors for anti-inflammatory and antiviral effects[96, 97].

Based on this, Tarus *et al.* designed a series of naproxen analogs through fragment extension strategies and molecular dynamics simulations, among which compounds **9** and **10** have prominent affinity toward NP, with IC_{50} values of 1.1 and 0.32 μM, respectively[98]. Studies on their binding sites found that **9** and **10** form two salt bridges with amino acid residues R152 and R355, while naproxen forms only one salt bridge with R361, which may be the reason for the increased affinity of **9** and **10** compared to naproxen.

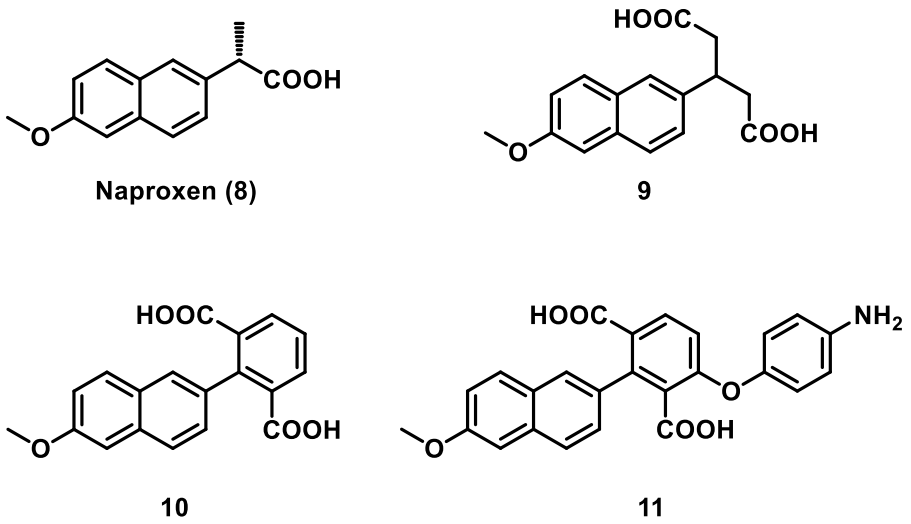


Figure 13. Structures of naproxen and its analogs.

The team further modified compound **10** and conducted antiviral experiments at the cellular level. The results showed that derivative **11** exhibits significantly enhanced activity compared to compound **10** with an IC_{50} value of 1.3 μM against A/WSN/33 (H1N1). Mechanistic studies speculate that these compounds not only hinder NP-RNA interaction but also block the binding of NP to the RNA polymerase.

1.4.4 Quinoline and quinolinone derivative

Quinoline is a potent pharmacophore in antiviral compounds against influenza. The following compounds are either derived from quinoline or have a similar chemical structure.

Aida's team undertook a comprehensive examination of 50,000 chemicals utilizing a cell-based high-throughput screening (HTS) assay targeting the A/WSN/33 (H1N1) virus[99]. Quinoline derivative **12** emerged as a standout, displaying broad-spectrum antiviral activity against a variety of influenza A strains, thereby securing its position as the most potent compound among the identified hits. Investigations of its mechanism of action revealed that **12** inhibits viral replication during the initial stages post-viral entry, potentially impacting viral genome replication. Subsequent investigations using a mini-genome assay and western blotting techniques revealed a notable reduction in viral protein (HA, NP, NA, M1, and M2) expression in the **12** treatment group, implicating that **12** hinders vRNP activity. Further exploration of its influence on NP localization in HeLa cells six hours post-infection was conducted *via* fluorescence microscopy. The compound was observed to constrain NP export from the nucleus, proposing NP as a promising therapeutic target for **12**. Further analysis through molecular docking revealed that **12** binds to a pocket formed by R162, S165, L264, and Y487, according to the degree of binding free energy.

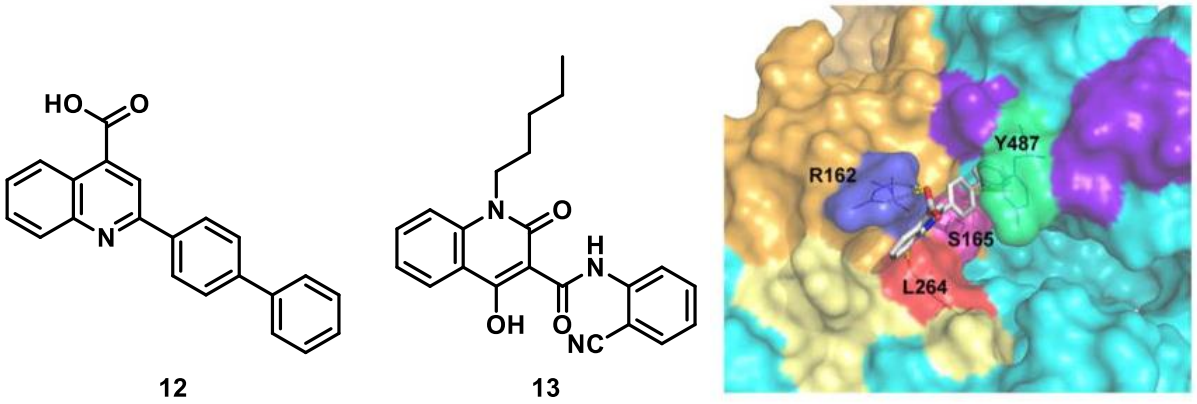


Figure 14. Structure of **12**, **13** and NP binding site of **12**.

Specifically, the carboxyl group, the biphenyl moiety, and the phenyl ring of the quinoline moiety bind to R162, Y487, and L264, respectively. Given that the binding site is situated adjacent to the RNA binding groove and the NP dimer interface, this interaction inhibits the typical functioning of NP.

In the research conducted by Nishida *et al.*, the impact of **13**, an inhibitor that prevents the oligomerization of NP, was meticulously investigated[100]. As NP oligomerization is pivotal for the formation of vRNPs, **13** exerts an inhibitory effect on viral transcription, protein synthesis, and the nuclear export associated with NP. The quinolinone derivative was effective against

various types of influenza A viruses including a clinical isolate of A(H1N1)pdm09 influenza with an IC_{50} value range of 1.8 – 2.1 μM.

Through the application of BN-PAGE, the formation of high-molecular-weight structures, indicative of the binding between yeast DNA and NP, was quantitatively analyzed. Remarkably, the presence of **13** as well as naproxen, caused a substantial decrease in the band intensity of the high-molecular-weight oligomer, while oseltamivir did not induce a comparable phenomenon. These findings underscore the targeted action of **13** in perturbing NP oligomerization, thereby obstructing vRNA formation and hindering the viral replication process.

1.4.5 Other nucleoprotein inhibitors

In 2018, Huang *et al.*[101] conducted a HTS of a library containing 20,000 compounds through a cell-based infection assay and identified compound **14** as having significant antiviral effects against H1N1 and H3N2 viral strains,, with IC_{50} values ranging from 0.41 to 1.41 μM. Further research confirmed that the binding site of this compound is similar to that of **12**, being the NES3 region and the NP dimer interface. *In vivo* test results indicated that **14** could effectively protect mice from influenza virus infection.

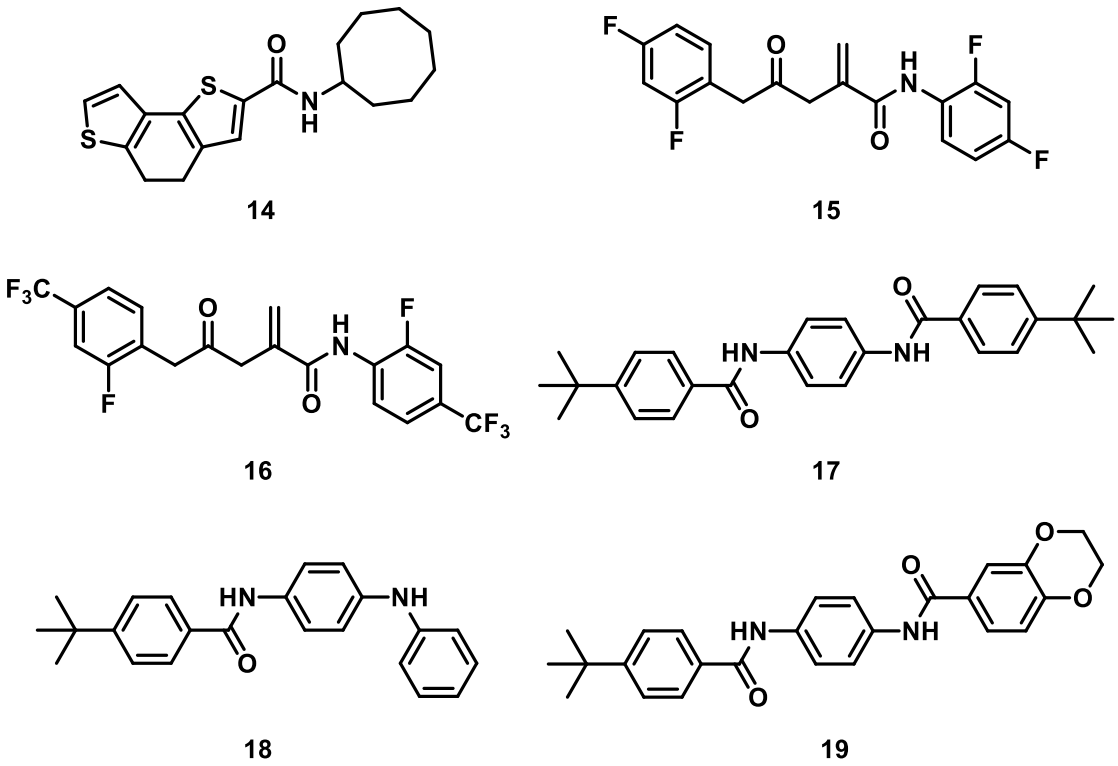


Figure 15. Structures of some NP inhibitors from the literature[82, 101-103].

Recently, Sethy *et al.*[102] identified lead compound **15** in a cell-based screening assay, which has an EC_{50} value of 2.84 μM against the IAV H1N1 and a selectivity index (SI) of 30. Structural modifications yielded compound **16**, with an EC_{50} value of 0.14 μM and an SI > 785.

White *et al.*[103] utilized an ultra-high throughput screening method to discover compound **17**, which exhibited a promising IC_{50} value of 0.02 μM against H1N1 A/WSN/1933 and an IC_{50} value of 27.43 μM against H1N1 A/California/04/2009. Mechanistic studies indicated that **17** can induce NP aggregation and that it has a higher affinity towards monomeric NP compared to NP in an oligomeric state in vRNP structures. Optimization based on SAR yielded **18**, which demonstrated good inhibitory effects against both type A and B influenza viruses, with IC_{50} values ranging from 1.43 to 15.15 μM and a CC_{50} value greater than 40 μM. When used in combination with oseltamivir, it exhibited synergistic antiviral effects.

Fang Y *et al.*[82] employed a HTS approach to identify compound **19**, which exhibits potent anti-influenza virus properties by inhibiting the NP across various IAV strains, with IC_{50} values ranging from 1.61 to 6.22 μM. It was shown to prevent body weight loss, enhance survival rates, and reduce viral titers in infected mice.

Insights from X-ray crystallography and molecular docking models reveal that **19** engages with several amino acid residues surrounding the I41-binding pocket in NP. Since I41 is highly conserved, **19** may possess broad applicability. Furthermore, **19** disrupts multiple stages of the influenza virus life cycle, exerting pleiotropic inhibitory effects on viral replication, transcription, and export processes. Given the rise of resistance to existing antivirals, **19** represents a promising candidate for inclusion in antiviral cocktails, potentially working synergistically with existing therapeutics like zanamivir or baloxavir, to treat and prevent influenza infections effectively[82].

2.0 AIM OF THE PROJECT

The objective of this project was the development of novel, potent inhibitors targeting the IAV-NP. To achieve this aim, the following goals have been set:

1. Development of efficient synthesis strategies for the systematic exploration and optimization of α-acylaminoamide inhibitors of IAV-NP.
2. Synthesis of a large set of α-acylaminoamide inhibitors for biological evaluation. The synthesis effort should be guided by continuous SAR analysis.

2.1 Discovery and modification of hit compound JK9

Several small-molecule inhibitors of IAV-NP have been identified and crystal structures of some inhibitor-NP complexes are available (see Section 1.4). Consequently, the structure-based identification and optimization of novel inhibitors can be pursued. However, clinically isolated influenza A viruses resistant to nucleozin, one of the most crucial NP inhibitors, have been identified. One example is the pandemic H1N1 IAV (A(H1N1)pdm09), which emerged in 2009 and carries a Y289H mutation, conferring resistance to nucleozin. Therefore, further efforts are needed to discover resistance-breaking NP inhibitors that exhibit broad-spectrum activity against influenza A viruses.

The research teams of Prof. Dr. Johannes Kirchmair at the University of Vienna (formerly at the University of Innsbruck and the University of Hamburg) and Prof. Dr. Michaela Schmidtke at the University of Jena have been committed to discovering and designing new anti-influenza drugs with the help of computational methods. In Prof. Kirchmair's laboratory, approximately 400,000 commercially available compounds from the Asinex Gold and Platinum screening libraries were virtually screened through docking to an X-ray structure of influenza NP[104]. Fifteen hit compounds were manually selected for purchasing and tested in the lab of Prof. Schmidtke against a nucleozin-sensitive as well as a nucleozin-resistant H1N1 IAV using a cytopathic effect-inhibition assay. Among the tested compounds, an α-acylaminoamide derivative, **JK9** (**1**), exhibited promising activity against the assayed IAV strains and demonstrated low cytotoxicity. Importantly, the identified compound also effectively inhibited the influenza A/pdm09(H1N1) virus, which exhibits resistance to nucleozin, warranting further investigation.

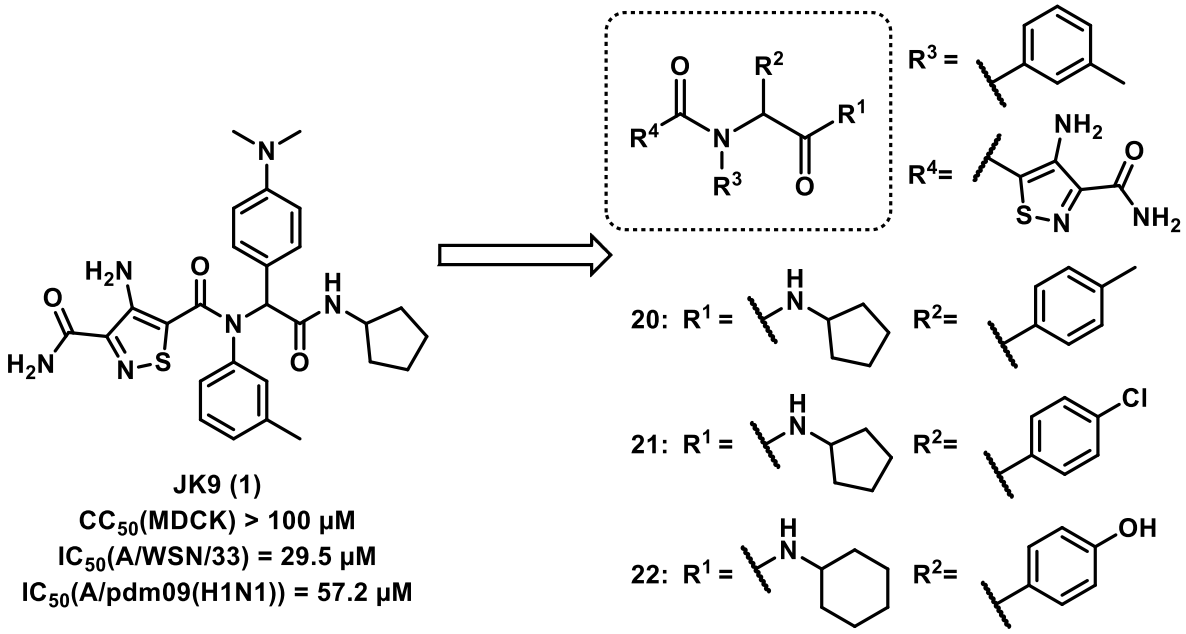


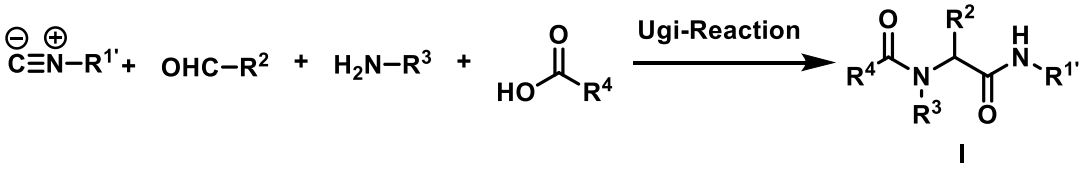
Figure 16. Structures of hit compound **JK9** (**1**) and most promising compounds **20**-**22**.

During follow-up studies on compounds that are structurally closely related to **JK9**, three additional α-acylaminoamide derivatives **20**, **21** and **22** were identified as promising compounds. Together with **JK9**, these compounds form the basis of the synthesis-driven, systematic compound optimization work presented in this PhD thesis. The optimization included the exploration of different substituents at positions R^1, R^2, R^3, and R^4 (Figure 16). The synthesis efforts were guided by continuous evaluation of the SAR. Structure-based approaches were not relied on because the binding poses obtained from protein-ligand docking did not seem plausible or consistent with the observed SAR. Complications are likely related to the conformational flexibility and the mainly hydrophobic nature of the ligand binding site.

Overall, a series of novel α-acylaminoamide derivatives should be designed and synthesized, their SAR should be systematically explored, and detailed studies should be conducted on key compounds.

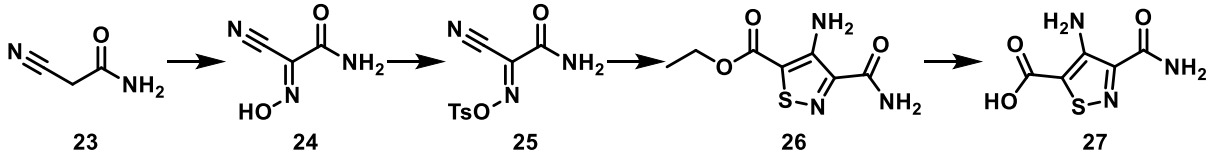
2.2 Synthesis plan

Generally, α-acylaminoamide derivatives **I** should be obtained through the Ugi reaction. To introduce different groups at positions $R^{1'}$, R^2, R^3, and R^4, the desired target compounds should be produced by using different isocyanides, aldehydes, amines, and carboxylic acids as starting materials (Scheme 1).



Scheme 1. Synthetic strategy for α-acylaminoamide derivatives by Ugi reaction.

The synthesis of 4-amino-3-carbamoylisothiazole-5-carboxylic acid (**27**) should be conducted following the methods described in the patent literature and the master thesis of Mr. Max Schwenk from our research team (Scheme 2). Cyanoacetamide served as the starting material and was subjected to nitrosation followed by sulfonylation. This intermediate was then reacted with ethyl 2-sulfanylacetate to yield the isothiazole derivative (**26**). Subsequently, **27** was obtained through the hydrolysis and it should be used as the carboxylic acid building block in the Ugi reaction.

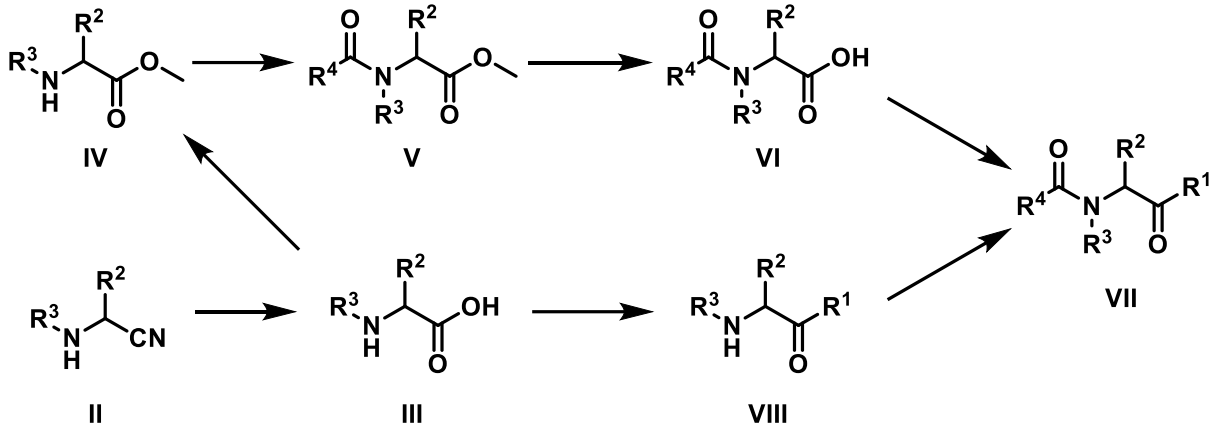


Scheme 2. Syntheses of 4-amino-3-carbamoylisothiazole-5-carboxylic acid **27**.

For isocyanides that are not directly available for purchase, a linear synthesis should be performed (Scheme 3). Initially the corresponding amino acid **III** should be produced *via* the Strecker synthesis, followed by esterification to produce *N*-substituted amino acid methyl esters **IV**. After an amide condensation to introduce the R^4 group, subsequent hydrolysis and amide condensation should yield the target compounds **VII**.

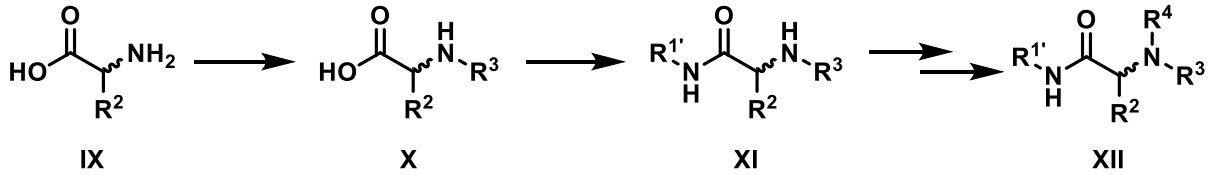
Alternatively, upon obtaining the *N*-substituted amino acid, an initial amide condensation could be conducted to produce *N*-substituted amino amides with different R^1 substituents **VIII**. This

should be followed by another amide condensation to introduce the R^4 group to yield the target compound **VII**.



Scheme 3. Synthetic strategy for α-acylaminoamide derivatives by liner synthesis.

For optically pure target compounds, a stereoselective synthesis of amino acids **IX** should be employed, followed by the introduction of the appropriate R^3 group on the nitrogen atom **X** (Scheme 4). The subsequent amide condensation and introduce the R^4 group should lead to the optically pure target compounds **XII**.



Scheme 4. Synthetic strategy for optically pure α-acylaminoamide derivatives

3.0 SYNTHESES AND BIOLOGICAL EVALUATION RESULTS

In this chapter, the synthesis, anti-influenza A activity, influenza A NP inhibitory potency, and SAR of *N*-acyl amino acid-derived influenza A NP inhibitors, possessing the general formula depicted in Figure 17, are described.

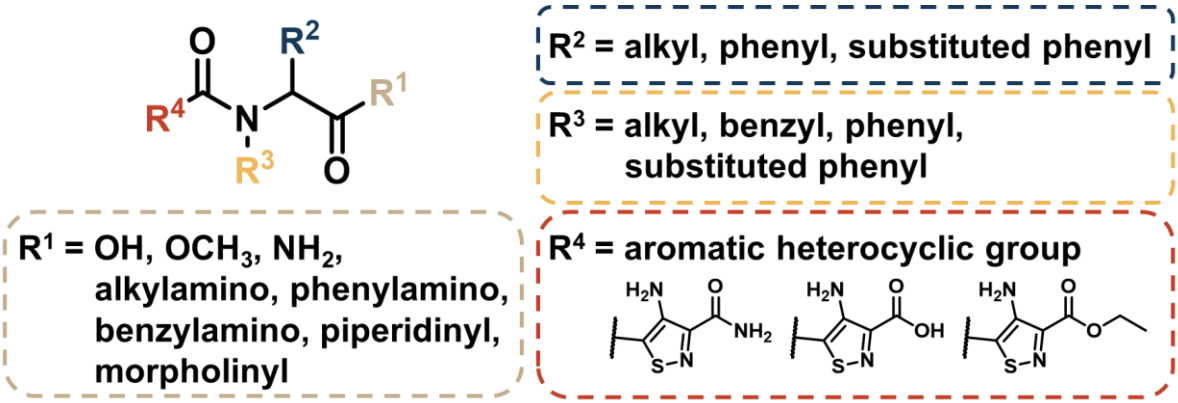


Figure 17. General structure of target compounds and modification strategies.

3.1 α-Acylaminoamides 20-22

3.1.1 Synthesis of α-acylaminoamides 20-22

In order to ascertain the *in vitro* activity of the α-acylaminoamides and to investigate the SAR of this series of compounds, synthetic routes were established. At first, α-acylaminoamides **20-22** (Figure 18) being the most promising compounds identified were synthesized.

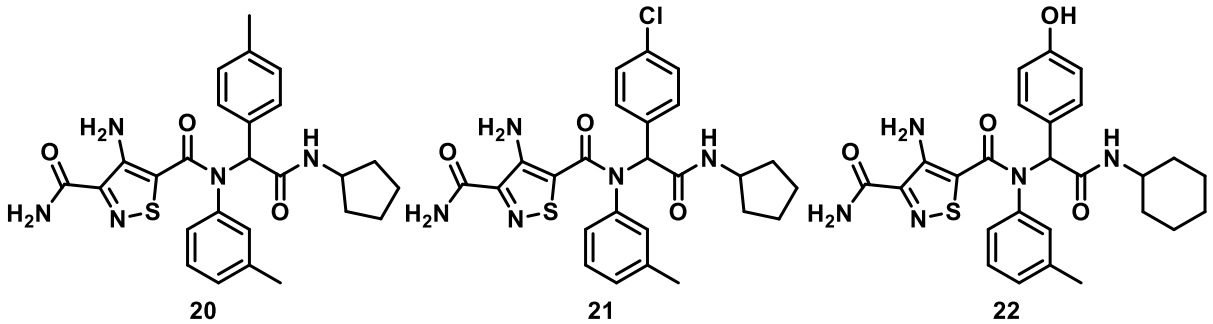


Figure 18. Structures of compounds **20-22**.

The Ugi four-component reaction (Ugi-4CR), renowned for its extensive application and adaptability in organic synthesis and medicinal chemistry, enables the expeditious assembly of

an aldehyde or a ketone, an amine, an isocyanide, and a carboxylic acid featuring α-acylaminoamides[105, 106] (Figure 19).

Figure 19 shows the mechanism of the Ugi reaction. The primary amine reacts with the carbonyl compound, yielding an imine. This imine is subsequently protonated, usually by the carboxylic acid present in the reaction mixture, leading to the formation of an iminium ion. The isocyanide then undergoes a nucleophilic attack on the iminium ion, producing a nitrilium intermediate. Following this, the carboxylic acid attacks the nitrilium intermediate, a step facilitated by the positive charge on the nitrilium ion, making it electrophilic. Then, a rearrangement occurs *via* the formation of a cyclic intermediate. A proton transfer, typically facilitated by the carboxylic acid or another proton source in the reaction mixture, finally leads to the formation of the α-acylaminoamide product[107, 108].

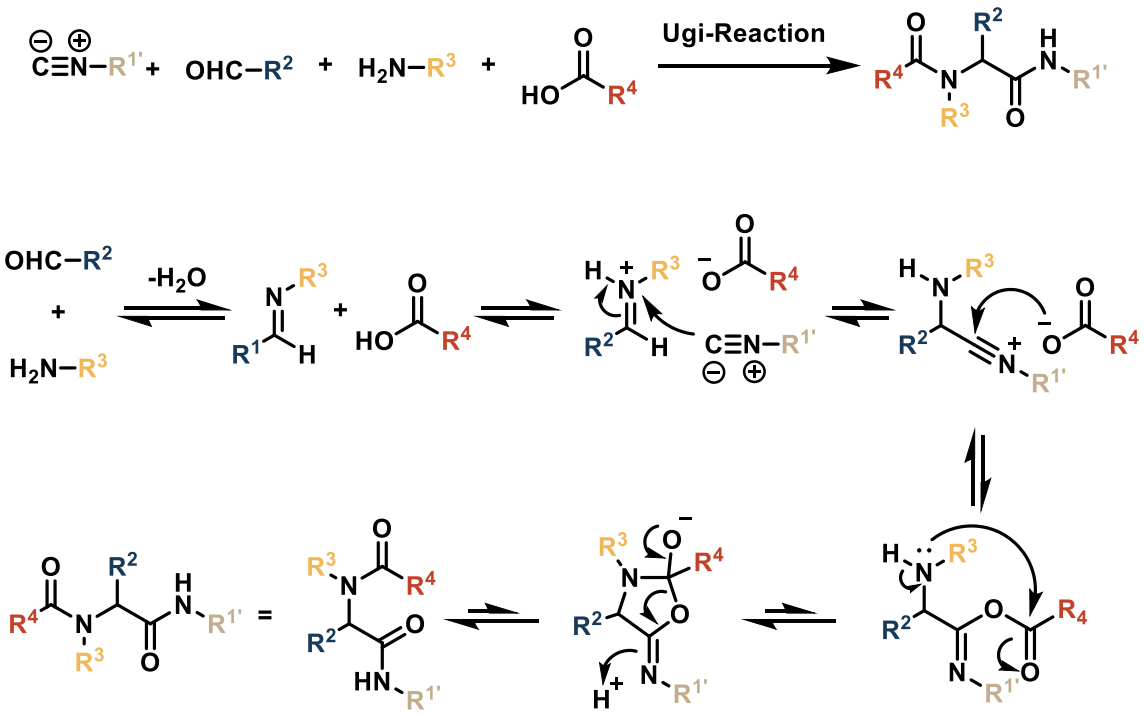
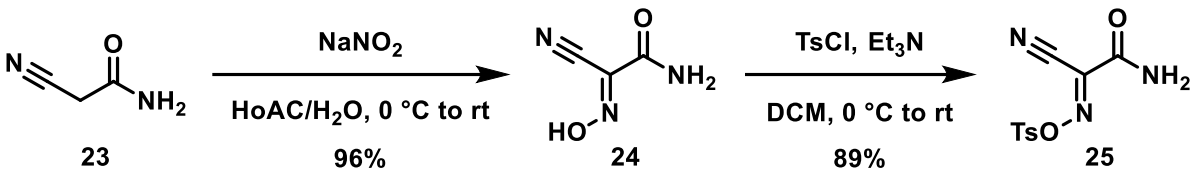


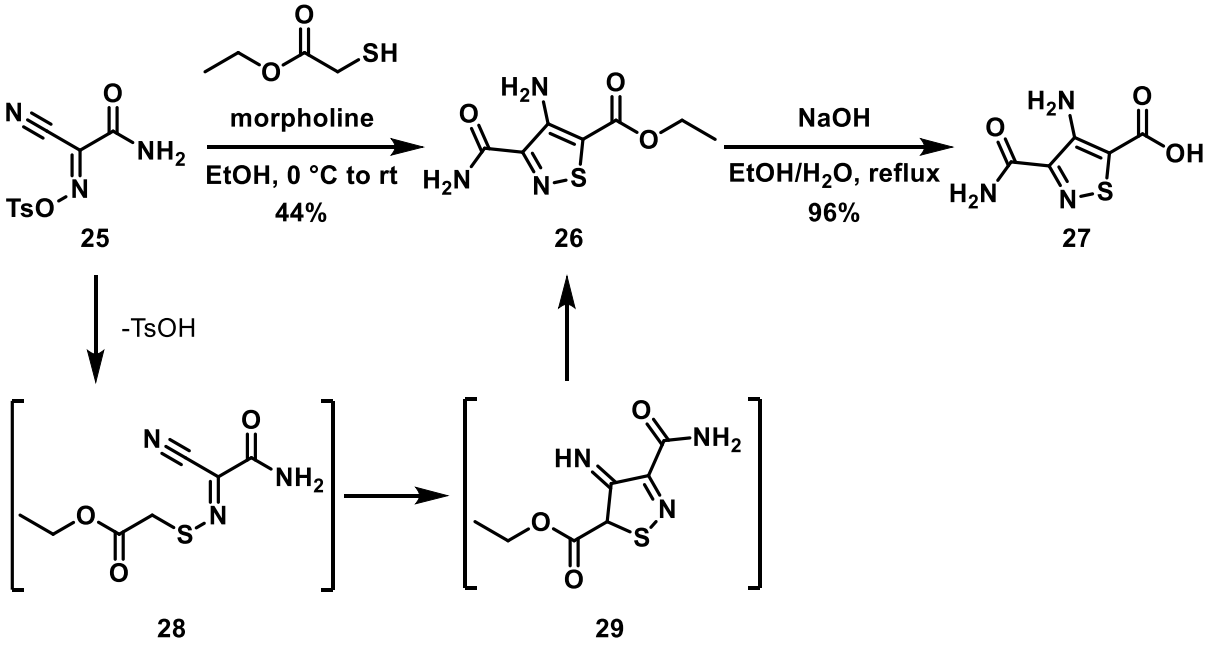
Figure 19. Mechanism of the Ugi reaction.

For the synthesis of compounds **20**-**22**, the first step involved the synthesis of an isothiazole derivative, which would later serve as the carboxylic acid building block in the Ugi reaction.



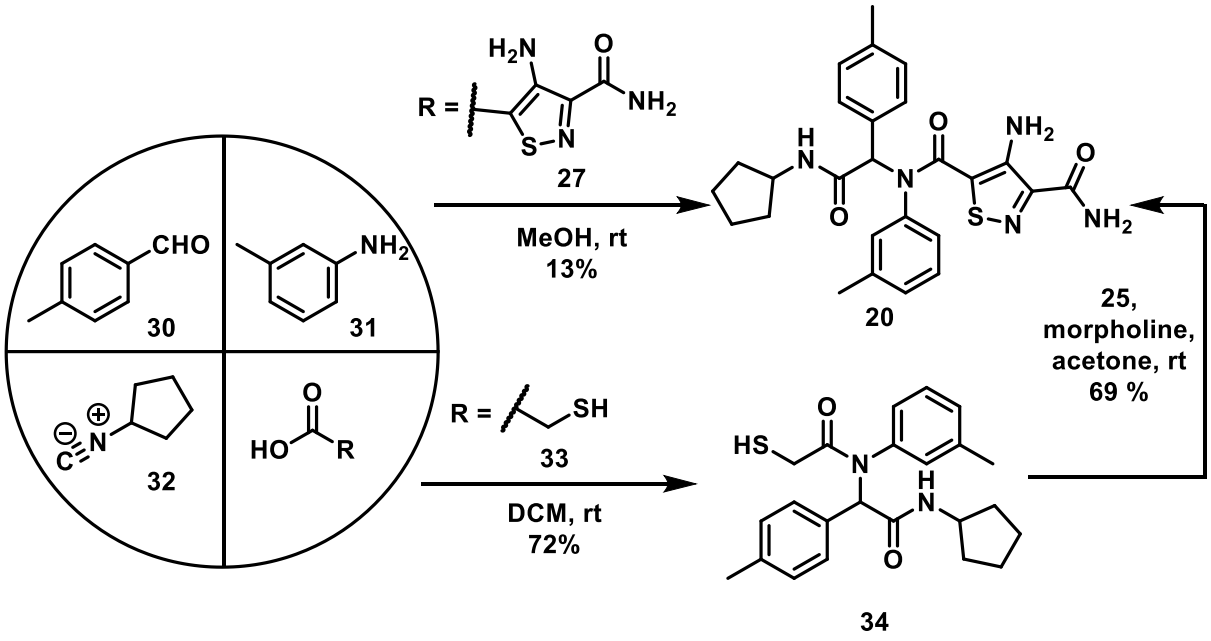
Scheme 5. Syntheses of key intermediate **25**.

The synthetic methodology applied in this study was adopted from procedures described in the patent literature[109, 110] and the master thesis of Mr. Max Schwenk from our research team. As shown in Scheme 5, cyanoacetamide **23** was chosen as the starting material. Key intermediate **25** was synthesized through a nitrosylation reaction of **23** in 2-position, followed by the reaction of oxime **24** with *p*-toluenesulfonyl chloride using triethylamine as base. Historically, column chromatography was employed for the separation and purification of intermediate **25**. However, this method was time-consuming and gave limited results. Considering the large amount of this intermediate necessary for subsequent syntheses, the column chromatography step was replaced by recrystallization using a petroleum ether/ethyl acetate (2/1) mixture. This change increased synthesis capacity from 1 g/batch to 20 g/batch and improved yields from 60% to 89%.



Scheme 6. Syntheses of carboxylic acid **27**.

Subsequently, a ring-closing reaction should establish the desired isothiazole ring. Thus, sulfonate **25** was reacted with ethyl 2-sulfanylacetate in ethanol in the presence of morpholine. Following the coupling reaction, the resulting intermediate **28** underwent a Thorpe-like cyclization reaction. After the deprotonation of the active methylene group, the generated enolate ion underwent a nucleophilic attack on the cyano group, yielding imine **29**. Through a final tautomerization, isothiazole derivative **26**, which aromatic isothiazole ring system effectively stabilizes the structure, was obtained in a yield of 44%. This ester was then hydrolyzed using sodium hydroxide to give isothiazole carboxylic acid **27** in 92% yield.



Scheme 7. Syntheses of α-acylaminoamides **20**.

Finally, carboxylic acid **27** was subjected to an Ugi reaction with cyclopentylisocyanide, *m*-toluidine, and *p*-tolualdehyde in methanol at room temperature for 24 h, ultimately yielding the desired α-acylaminoamide **20** in 13% yield. The low yield might be attributed to the limited reactivity of the primary aromatic amine **31**. Additionally, it might be related to the unsatisfactory solubility of **27** and subsequent products in methanol.

Efforts were made to enhance the solubility of isothiazole-based carboxylic acid **27** by elevating the reaction temperature to facilitate the reaction; however, the yield remained largely unchanged. Similarly, extending the reaction duration proved unsuccessful in obtaining a greater quantity of the product. Given the presence of an amino and a carboxyl group, compound **27** can be classified as an amphoteric compound. Typically, enhancing the polarity

of the solvent can enhance solubility; however, when a mixture of methanol and water was employed as solvent, the yield diminished, and the formation of the product was not discernible when water was the sole solvent.

Table 1. Reaction conditions for the synthesis of α-acylaminoamide **20**.

No.	Reaction conditions			Yield of **20**
	Solvent	Temperature	Time [h]	
1	MeOH	rt	24	13%
2	MeOH	reflux	24	15%
3	MeOH	rt	48	12%
4	MeOH	rt	72	13%
5	MeOH/H_2O (1/1)	rt	24	9%
6	H_2O	rt	24	-

The structure of α-acylaminoamide **20** was confirmed by ^{1}H NMR and ^{13}C NMR spectroscopy as well as high-resolution mass spectrometry. As shown in Figure 20, the eight protons of the methylene groups of the cyclopentyl moiety gave a multiplet pattern at high field between 1.18 ppm and 1.86 ppm. The signal for the hydrogen atom bound to the secondary amide nitrogen atom appeared at 8.08 ppm as a doublet. The signal for the proton of methine moiety of the cyclopentyl group was observed between 4.00 ppm and 4.10 ppm. The latter two nuclei were coupling with each other as corroborated by the COSY spectrum (Figure 21). The signals for the two methyl groups attached to the benzene rings were found at 2.16 ppm and 2.20 ppm, respectively. Notably, the latter signal appeared as a broad peak, indicating that it likely corresponds to the methyl group positioned at the 3' location. This broadening can be attributed to hindered rotation due to the direct connection between the *meta*-substituted benzene ring and the amide nitrogen. The restriction causes a decrease in the spin-spin relaxation time (T_2) for substituents and hydrogens on this ring, leading to an increase in peak width. Similarly, at low field, significantly broadened peaks can be observed at 6.55 ppm, 7.02 ppm - 7.35 ppm, and 7.65 ppm, which are presumed to represent the signals for the protons on the *meta*-substituted benzene ring. Conversely, the rotational motion of the other benzene ring was unaffected,

allowing the clear observation of the two signals for the four protons of the *para*-substituted benzene ring at 6.92 ppm-6.98 ppm.

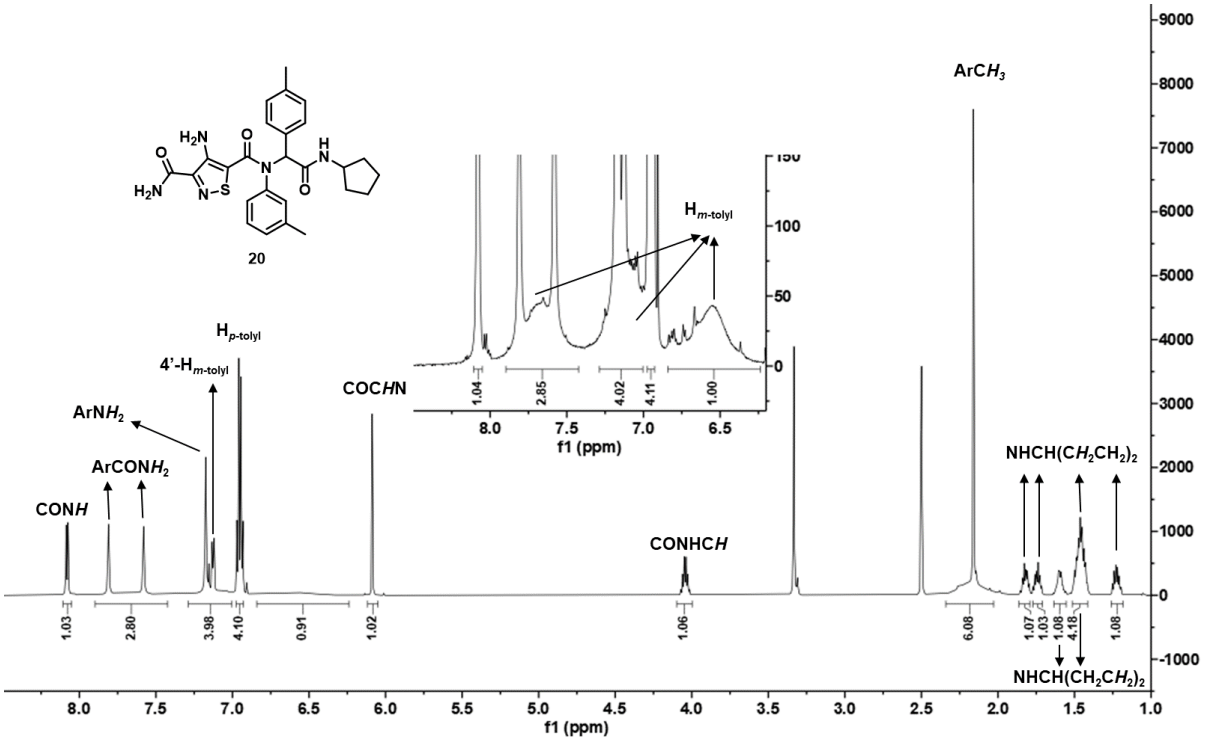


Figure 20. ^{1}H NMR (DMSO-d_6) spectrum of compound **20**.

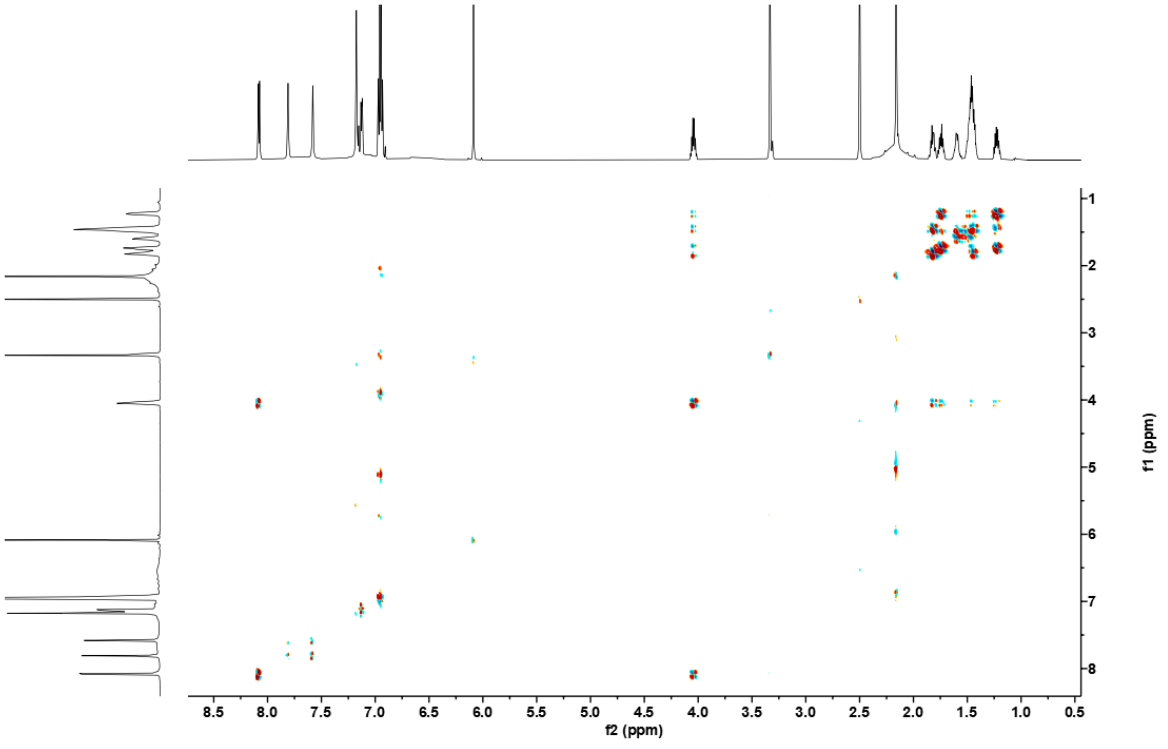


Figure 21. COSY (DMSO-d_6) spectrum of compound **20**.

The protons belonging to the primary amide moiety on the isothiazole ring give two signals at 7.58 ppm and 7.81 ppm, respectively, with the COSY spectrum suggesting that these nuclei couple with each other. This signal pattern arises due to the partial double-bond nature of the amide bond, resulting in the two hydrogens being in distinct chemical environments and exhibiting different chemical shifts.

Upon analyzing the ^{1}H NMR spectrum of compound **20** obtained at a temperature of 363 K (Figure 22), it was observed that the broad peak at low field transformed into a relatively narrow peak. Additionally, the broad signal for the methyl group of the *meta*-substituted benzene ring at high field became sharp, supporting the aforementioned hypothesis. As the temperature increased, the rotational barrier of the amide N-C bonds diminished. Consequently, the proton signals associated with the primary amide moiety on the isothiazole ring coalesced, resulting in a broad peak at 7.37 ppm instead of two distinct peaks.

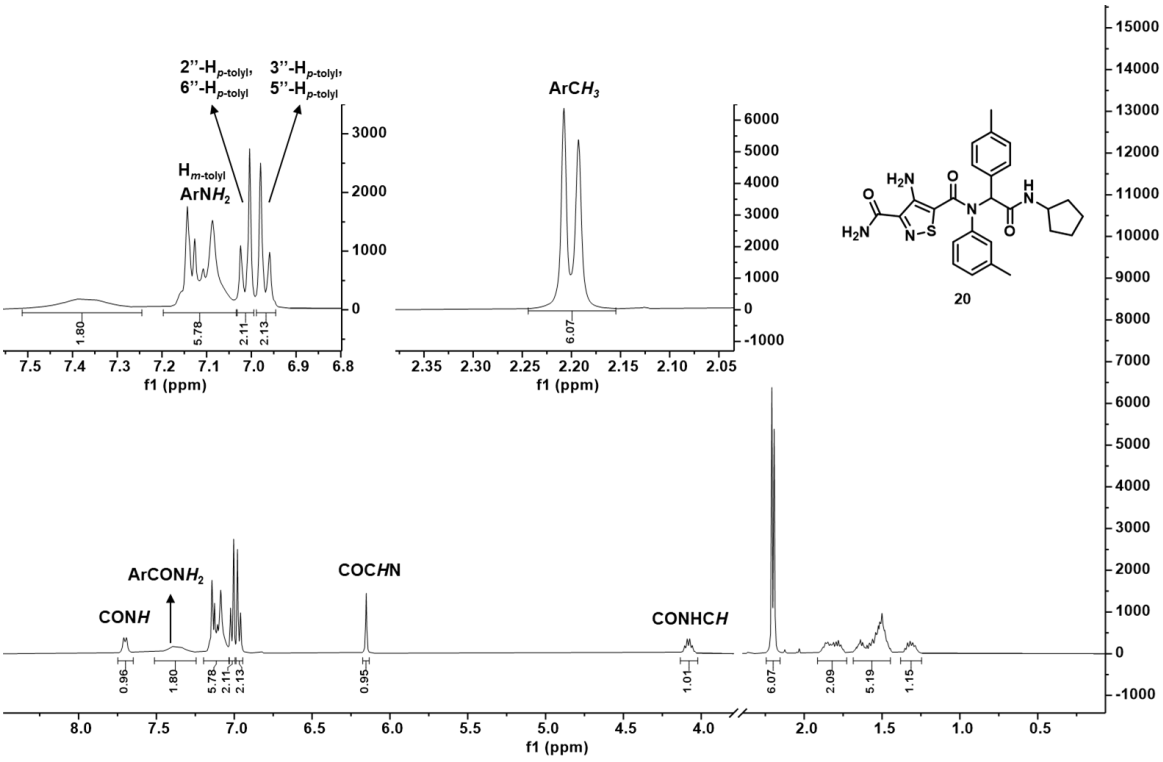


Figure 22. ^{1}H NMR (DMSO-d_6) spectra of **20** at 363 K.

Given the limitations of NMR spectroscopy in elucidating the structure of compound **20**, X-ray crystallography was employed for definitive structural confirmation.

At room temperature, an excess of pure compound **20** was dissolved in 20 mL of methanol. After thorough stirring, the mixture was filtered to obtain a saturated methanol solution of **20**.

This solution was then divided into ten small glass vials. To ensure a controlled evaporation rate, the vials were left open to the atmosphere and partially covered with aluminum foil punctured with small holes. After standing for a week, crystals precipitated as the methanol evaporated. The crystals were carefully removed from the solution, dried, and inspected for quality. Subsequent X-ray crystal structure analysis confirmed that the synthesized racemic compound **20** conformed to the expected structure.

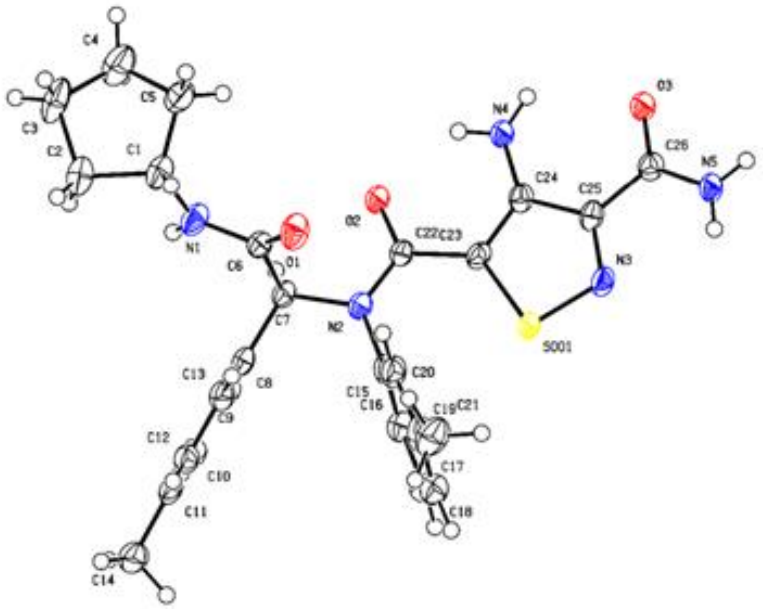
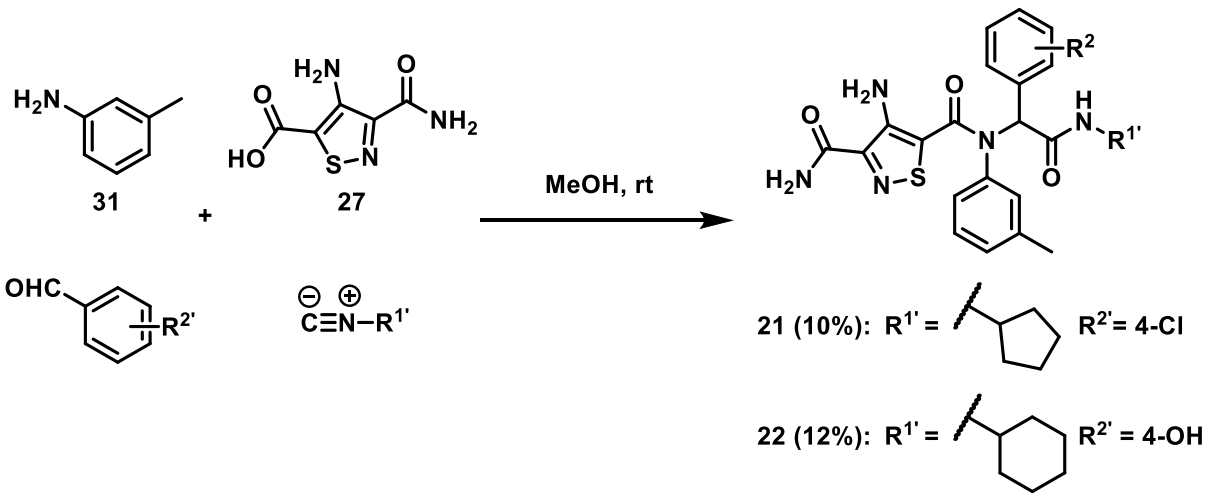


Figure 23. Crystal structure of **20** (only the (*S*)-configured enantiomer is shown).



Scheme 8. Syntheses of α-acylaminoamides **21** and **22**.

α-Acylaminoamides **21** and **22**, the other most promising compounds, were synthesized in an analogous way *via* Ugi reactions and were obtained in similarly low yields as compound **20** (Scheme 8).

Thus, for the synthesis of further α-acylaminoamides, refining the synthetic methodology was essential. In addition to the previously mentioned factors, the mechanism demonstrates that the protonation of the intermediately formed imines, yielding iminium ions, promotes the nucleophilic attack of the isocyanides (Figure 19). However, it is crucial to recognize that 4-amino-3-carbamoylisothiazole-5-carboxylic acid (**27**) contains an amino group. This group might exhibit a higher basicity compared to the intermediate imine, potentially hindering subsequent reactions. Therefore, in an alternative two-step synthetic route the α-acylaminoamide scaffold should be established at first *via* an Ugi reaction, using 2-sulfanylacetic acid (**33**) as carboxylic acid (Scheme 7). The 2-sulfanylacetyl moiety of the thereby obtained α-acylaminoamide should subsequently be employed to build up the isothiazole ring. This strategy to establish the α-acylaminoamide scaffold before the cyclization not only addresses the solubility challenges of the starting materials but also ensures the efficient generation of iminium ions.

As depicted in Scheme 7, utilizing the refined method, *p*-tolualdehyde and *m*-toluidine were initially combined in dichloromethane. This mixture was allowed to react for 10 min, facilitating the formation of the imine. Subsequently, cyclopentylisocyanide (**32**) and 2-sulfanylacetic acid (**33**) were added. After stirring the mixture at ambient temperature for 48 h, the Ugi reaction product **34** was isolated in a yield of 72%.

The structure of bisamide **34** was confirmed by ^{1}H NMR and ^{13}C NMR spectroscopy as well as high-resolution mass spectrometry.

It is noteworthy that the ^{1}H NMR spectrum of **34** (Figure 24) is similar to the previously shown spectrum of compound **20** (Figure 20). The signals for the protons of the phenyl ring directly connected to the amide nitrogen atom as well as the signal for the protons of the respective methyl group appear as broad peaks. The reason might be consistent with the previous speculation, according to which the formation of the amide bond inhibits the rotation of this phenyl ring. Also of note are the signals for the diastereotopic protons of the methylene group of the 2-sulfanylacetyl moiety, which appear as two doublet of doublets (dd) and are located at 2.97 ppm and 3.02 ppm, respectively. The proton of the thiol is an active hydrogen, but it can be well observed in the NMR spectrum with DMSO-d_6 as the solvent. It couples with the adjacent methylene group, splitting into a triplet being located at 2.57 ppm, even if the peak area is somewhat smaller than expected.

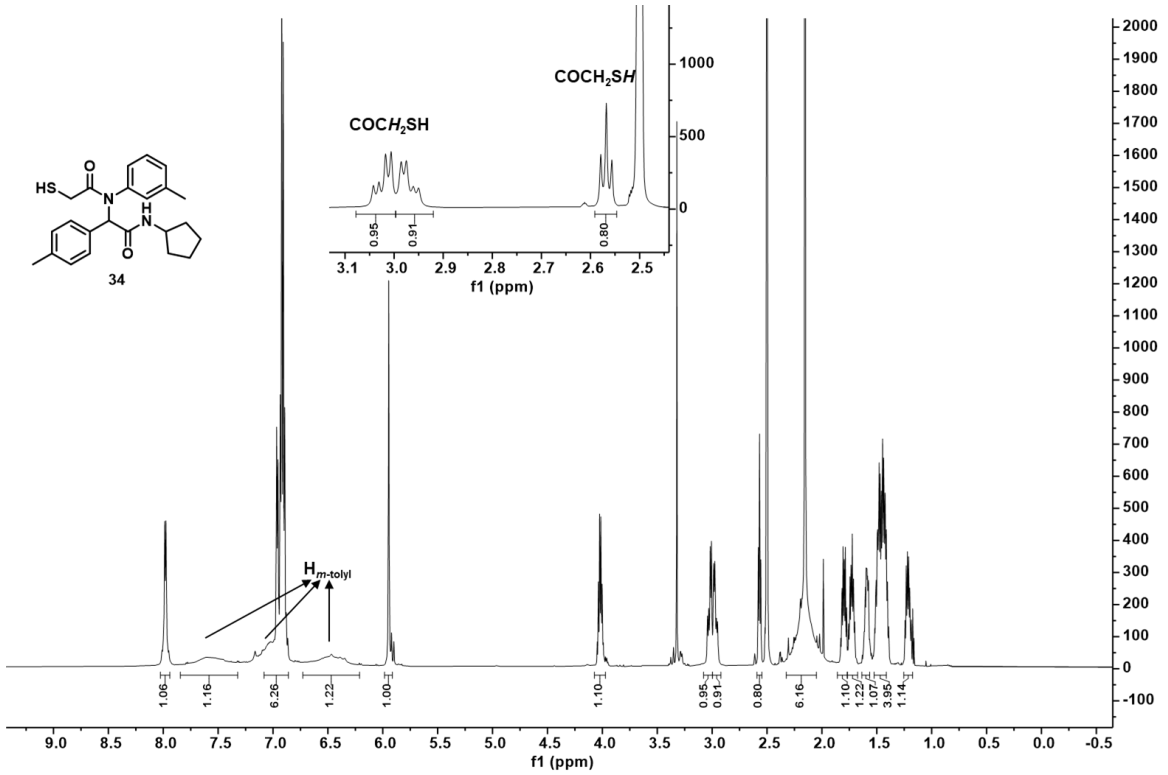


Figure 24. ^{1}H NMR (DMSO-d_6) spectruma of bismaide **34**.

Intermediate **34** was then subjected to a cyclization reaction using sulfonate **25** in acetone in the presence of morpholine. This step afforded the desired isothiazole derivative **20** in 69% yield. Cumulatively, the overall yield of the compound was 50%, marking an improvement of nearly fivefold compared to the previous methodology.

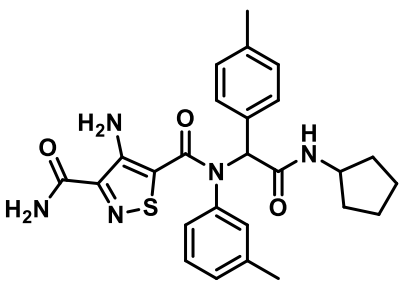
3.1.2 Biological evaluation of α-acylaminoamides 20-22

In this section, the antiviral efficacy of the three most promising compounds is described.

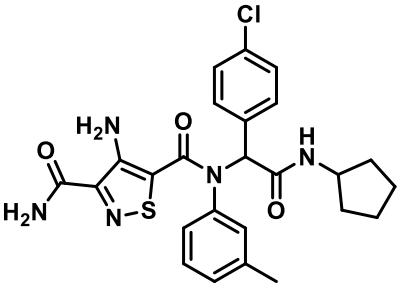
The evaluation of the antiviral efficacy of the compounds was performed by Prof. Dr. Michaela Schmidtke at the University of Jena, whose contribution is gratefully acknowledged.

Cytotoxicity and the inhibition of the influenza virus-induced cytopathic effect (CPE) of the synthesized compounds were analyzed in confluent Madin Darby canine kidney (MDCK) cell monolayers as published with some modifications. Cell-culture assays were performed with MDCK cells, with Eagle's minimal essential medium (EMEM) supplemented with 2 µg/mL trypsin, 2 mM L-glutamine, 1% nonessential amino acids, 100 U/mL penicillin, and 100 U/mL streptomycin.

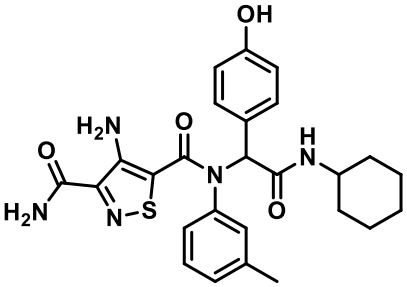
Table 2. Activity of the α-acylaminoamides **20**-**22** against different influenza A strains.



20



21



22

Compound	**IC_{50} [µM]**				
	A/1519/19 (H1N1)*	A/2588/19 (H3N2)*	A/WSN/33 (H1N1)**	A/pdm09 (H1N1)**	A/HK/1/68 (H3N2)**
20	10.22 ± 3.35	9.95 ± 2.89	10.86 ± 1.94	12.95 ± 2.52	6.67
21	6.18 ± 0.50	3.70 ± 2.11	11.41 ± 3.37	11.58 ± 7.50	9.20
22	8.20 ± 2.18	10.34 ± 3.78	11.04 ± 4.03	14.51 ± 1.61	7.35
nucleozin	-	-	0.48	>100	0.87

* 50% inhibition of plaque production of influenza virus

** 50% cytopathic effect inhibitory concentration against influenza virus

Briefly, cytotoxicity was assessed in MDCK cells using serial half-log dilutions of test compounds (up to 100 μM), analyzed 72 h post-addition. For CPE inhibition, similar compound dilutions were used alongside varying MOIs of three viruses (MOI: 0.004, 0.005, 0.006, 0.01, and 0.001 for A(H1N1)pdm09[111], WSN/33[112], and HK/68, respectively). After a 48-hour incubation at 37 °C in 5% CO_2, virus proliferation and CPE were evaluated. Methods included crystal violet staining, optical density reading, and determining the 50% cytotoxic concentration (CC_{50}) and 50% inhibitory concentration (IC_{50}), with a minimum of three independent tests performed[113].

Plaque reduction assays were conducted on A/1519/19(H1N1) and A/2588/19(H3N2)[114] strains in MDCK cells. Confluent monolayers in 12-well plates were exposed to virus suspensions with/without test compounds (0.316, 1, 3.16, 10 μg/mL). Post-inoculation, wells received 1 mL of 0.4% agar-infused medium, with or without the compounds, followed by a 48-hour incubation at 37 °C. The process was replicated three times[113].

As shown in Table 2, all three compounds exhibited significant anti-influenza activity in both, the plaque assay as well as the cytopathic effect assay. Furthermore, the CC_{50} of the three compounds in MDCK cells exceeded 100 μM, indicating a favorable safety profile.

Against the influenza strains A/1519/19(H1N1) and A/2588/19(H3N2) isolated from clinical samples, the compounds showed IC_{50} values between 6.18-10.22 μM and 3.70-10.34 μM, respectively, in the plaque assay. In the cytopathic effect-inhibition assay, the compounds displayed IC_{50} values of 10.86-11.41 μM and 6.67-9.20 μM against the nucleozin-sensitive strains A/WSN/33(H1N1) and A/HK/1/68(H3N2). Thus, the compounds were less potent than nucleozin, a recognized NP inhibitor, serving as positive control in the assay. The 2009-emerged pandemic influenza A(H1N1) virus, termed A/pdm09(H1N1), exhibits resistance to nucleozin and its analogues, attributed to the Y289H amino acid substitution of NP. Yet, the three compounds retained inhibitory activity, with IC_{50} values between 11.58-14.51 μM, suggesting a potentially different mechanism of action from nucleozin that merits further exploration.

Among the tested compounds, **20** consistently displayed moderate to strong inhibitory activity across all strains. Its IC_{50} values were comparable to, if not better than, the other two compounds, particularly against nucleozin-resistant strains. While **21** demonstrated the lowest IC_{50} for the A/2588/19 (H3N2) strain, its efficacy varied considerably across the other investigated strains. Consequently, **20** emerged as lead compound for subsequent studies.

3.2 *N*-acyl amino acid derivatives with varied R^1 group

3.2.1 Synthesis of α-acylamino ester 35 and acid 36

To verify the necessity of an amide moiety at the R^1 position, isothiazole-derived α-acylamino ester **35** and acid **36** were synthesized (Figure 25, Scheme 9).

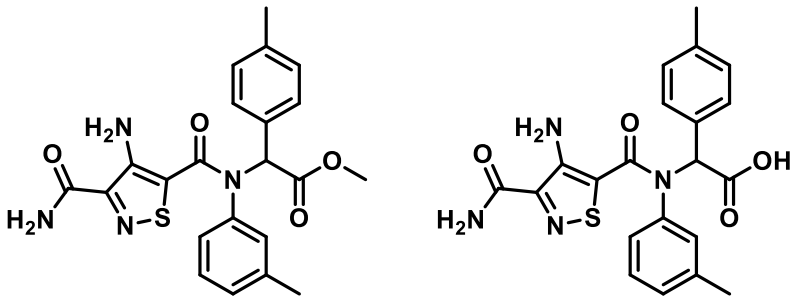
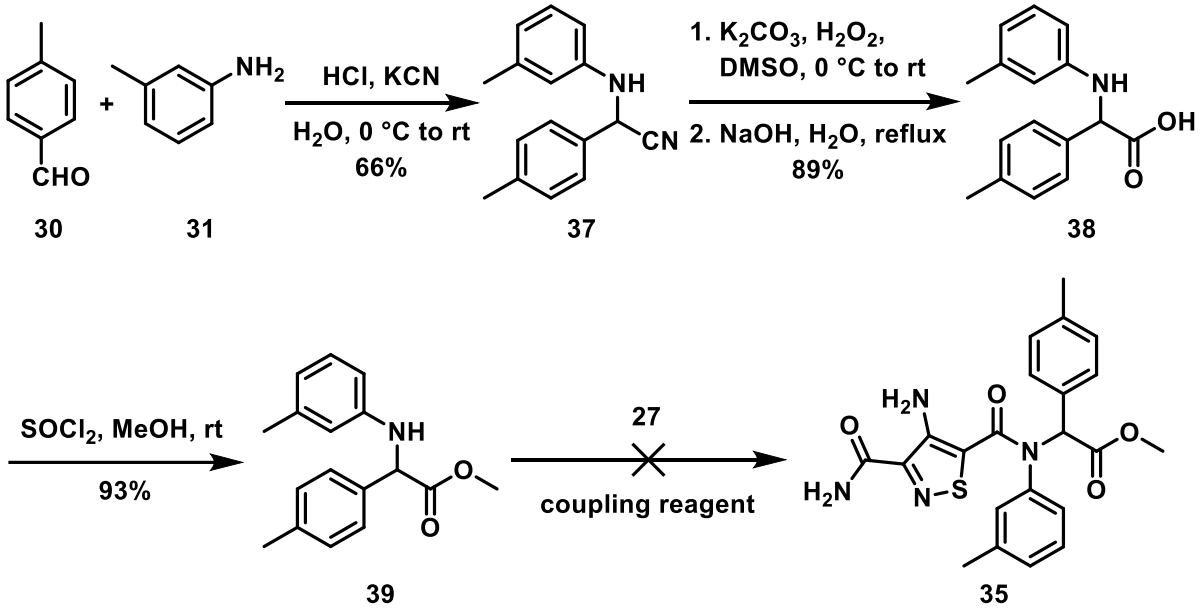


Figure 25. Structures of α-acylamino ester **35** and acid **36**.



Scheme 9. Syntheses of **35**.

Initially, racemic aminonitrile **37** was obtained *via* a Strecker synthesis[115]. An aqueous solution of potassium cyanide was added dropwise to a mixture of *m*-toluidine, *p*-tolualdehyde, and hydrochloric acid. This reaction mixture was stirred at 0 °C for 1 h. After extraction with ethyl acetate and recrystallization from petroleum ether/ethyl acetate (3/1), 25 g of the desired aminonitrile **37** were obtained in a single batch, in a yield of 66%. The ^{13}C NMR spectrum of this compound prominently displayed a characteristic signal for the carbon atom of the cyano group at 119.7 ppm.

In subsequent steps, nitrile **37** was treated with hydrogen peroxide and DMSO in the presence of an excess of potassium carbonate. After filtration, the respective primary amide was efficiently obtained, which was then hydrolyzed by refluxing the compound in a 0.5 M aqueous solution of NaOH for 9 h[116]. After cooling to ambient temperature, impurities were removed by extraction with ethyl acetate. The pH of the resulting aqueous phase was adjusted to 4 and the product was extracted with dichloromethane. Thus, carboxylic acid **38** was obtained over two steps, achieving an overall yield of 89%. Subsequently, carboxylic acid **38** was dissolved in methanol and treated with 5% (V/V) thionyl chloride in methanol, yielding methyl ester **39** in 93% yield (Scheme 9).

Subsequently, secondary amine **39** should be linked with carboxylic acid **27** *via* amide coupling reactions. Initial attempts to obtain the desired amide **35** were unsuccessful. As shown in Table 3, despite exploring a variety of common coupling agents[117, 118], the reaction did not proceed as intended. This might be attributed to the relatively low reactivity and steric hindrance associated with aromatic amines.

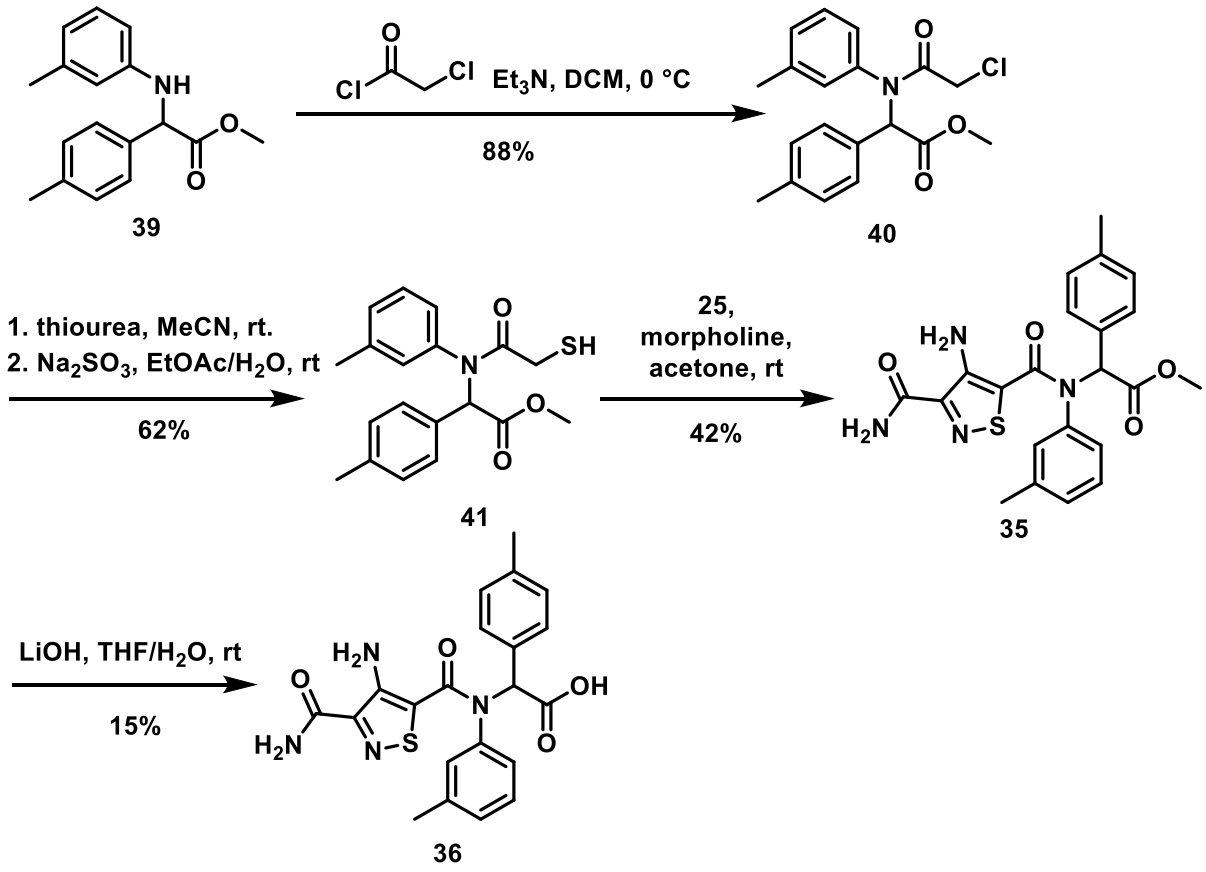
Table 3. Reaction conditions for the synthesis of amide **35**.

No.	Reaction conditions	Yield of **35**
1	DCC, DMAP, DMF, rt, 6 h	-
2	DIC, HOBt, Et_3N, DMF, rt, 6 h	-
3	EDCI, HOBt, Et_3N, DMF, rt, 6 h	-
4	EDCI, Oxyma, DIEA, DMF, rt, 6 h	-
5	CDI, DCM, rt, 6 h	-
6	COMU, DIEA, MeCN, rt, 6 h	-

Given this, an attempt was made to perform a coupling using a highly reactive acyl chloride. However, the amino group in isothiazole derivative **27** theoretically prevents its conversion into an acyl chloride. Considering the strategy of introducing a 2-sulfanylacetyl group followed by cyclization to construct the isothiazole ring, the acquisition of 2-sulfanylacetyl chloride appeared to be challenging. Thus, ester **39** was initially reacted with chloroacetyl chloride in

the presence of triethylamine in dichloromethane to yield amide **40** (Scheme 10). Subsequently, **40** was treated with thiourea then an aqueous solution of sodium sulfite to obtain thiol **41**[119]. Subsequently, isothiazole derivative **35** was obtained under the aforementioned conditions.

Ester **35** was then subjected to hydrolysis using lithium hydroxide, resulting in the formation of carboxylic acid **36**. However, the yield was disappointingly low at only 15%. A comparative analysis *via* TLC indicated a significant formation of 4-amino-3-carbamoylisothiazole-5-carboxylic acid (**27**). Attempts to enhance the yield by reducing the quantity of lithium hydroxide or substituting it with other inorganic bases such as NaOH or KOH or changing solvents were unsuccessful.



Scheme 10. Syntheses of α-acylamino ester **35** and acid **36**.

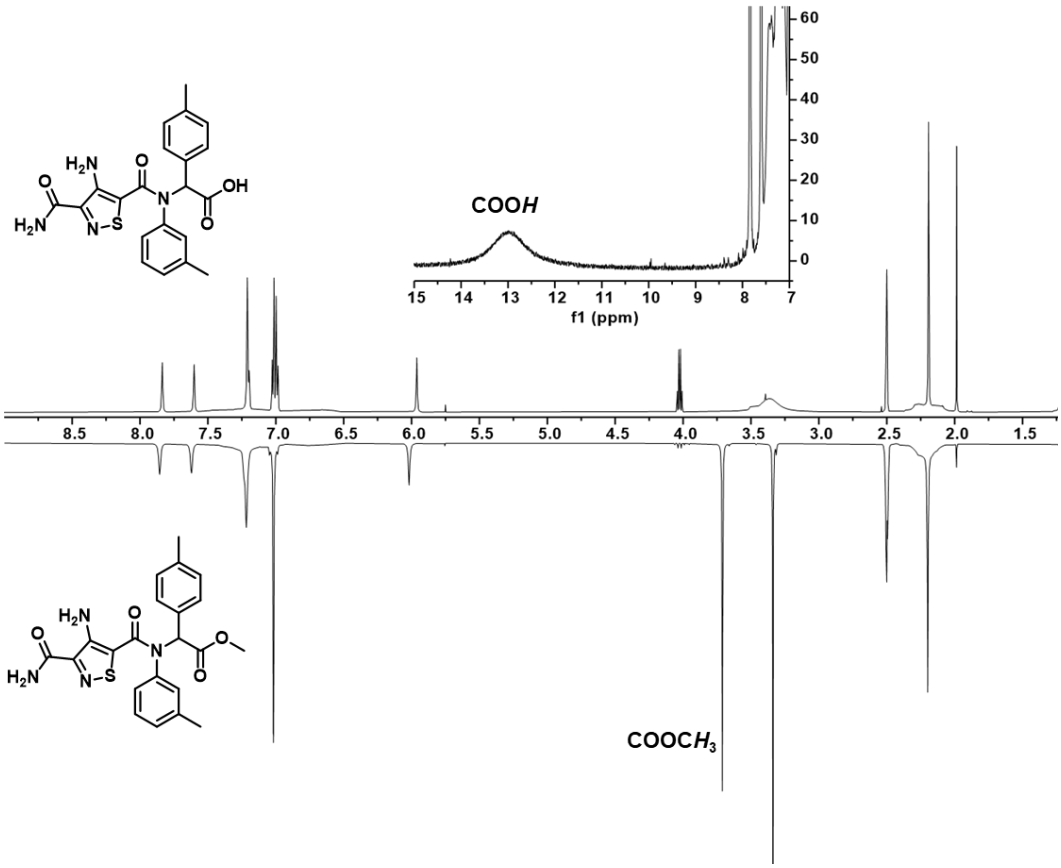


Figure 26. ^{1}H NMR (DMSO-d_6) spectra of α-acylamino acid **36** (up) and ester **35** (down).

In the ^{1}H NMR spectrum of ester **35**, a singlet signal for the methyl ester group can be observed at 3.71 ppm. This signal disappears in the spectrum of carboxylic acid **36**, being replaced by a broad peak at 13.00 ppm corresponding to the proton of the carboxylic acid.

3.2.2 Linear synthesis of α-acylaminoamide derivatives 42, 43, 47a-e

To investigate the influence of different amide substituents at the R^1 position on the biological activity of the compounds, α-acylaminoamides containing primary (**42**) or tertiary amides (**43**) were synthesized (Figure 27). Concurrently, secondary amides with cycloalkyl rings of varying sizes (**47a**, **47b**, **49a**), alkyl chains of differing lengths (**47c**, **49b**, **49c**), as well as phenyl (**49d**) and benzyl (**49e**) groups at the amide nitrogen were introduced at this position. Additionally, to enhance the water solubility of the compounds, oxygen atoms were attempted to be incorporated into the chain or ring (**47d**, **47e**).

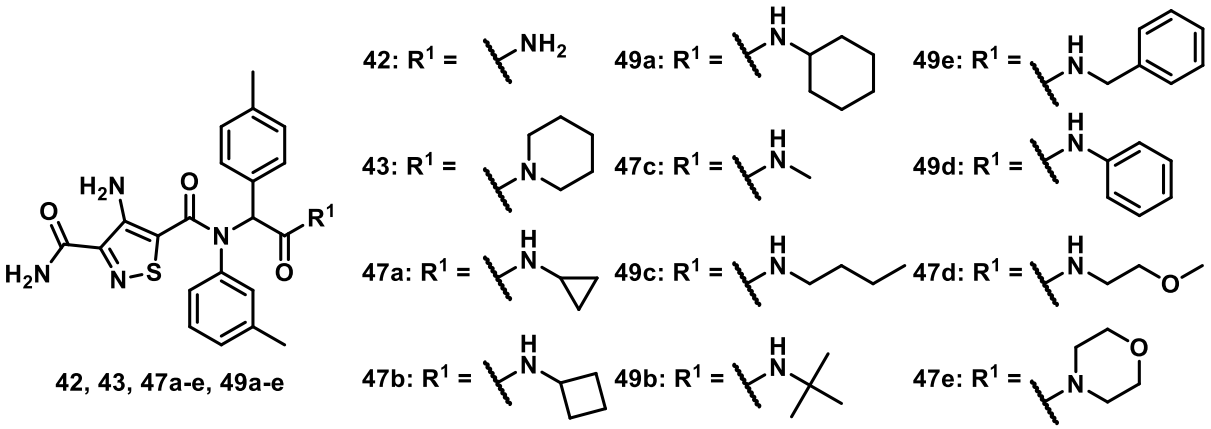
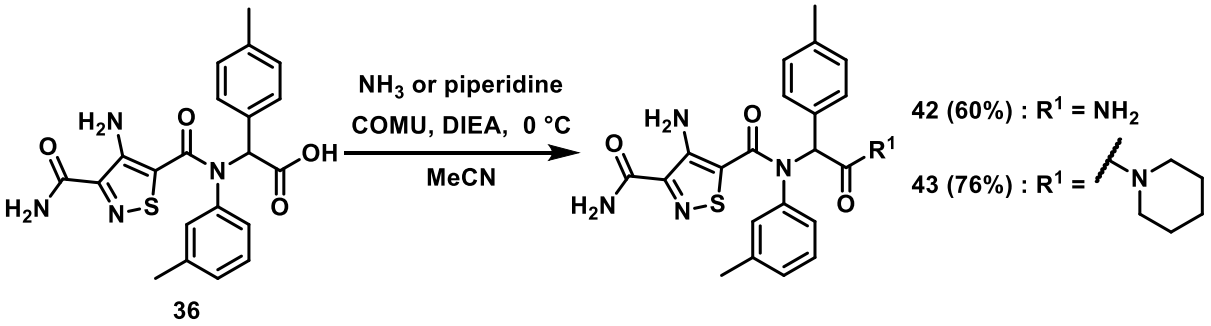


Figure 27. Structures of isothiazole derivatives **42, 43, 47a-e, 49a-e**.

In addition to the Ugi reaction, α-acylaminoamide derivatives can be synthesized *via* the sequential assembly of amino acids. For isocyanides that are not commercially available, a flexible linear synthesis strategy was adopted. This approach involved the initial preparation of a carboxylic acid **36** as described in the previous section. Subsequently, **36** underwent a one-step coupling with the appropriate amines to yield the desired products (Scheme 11).

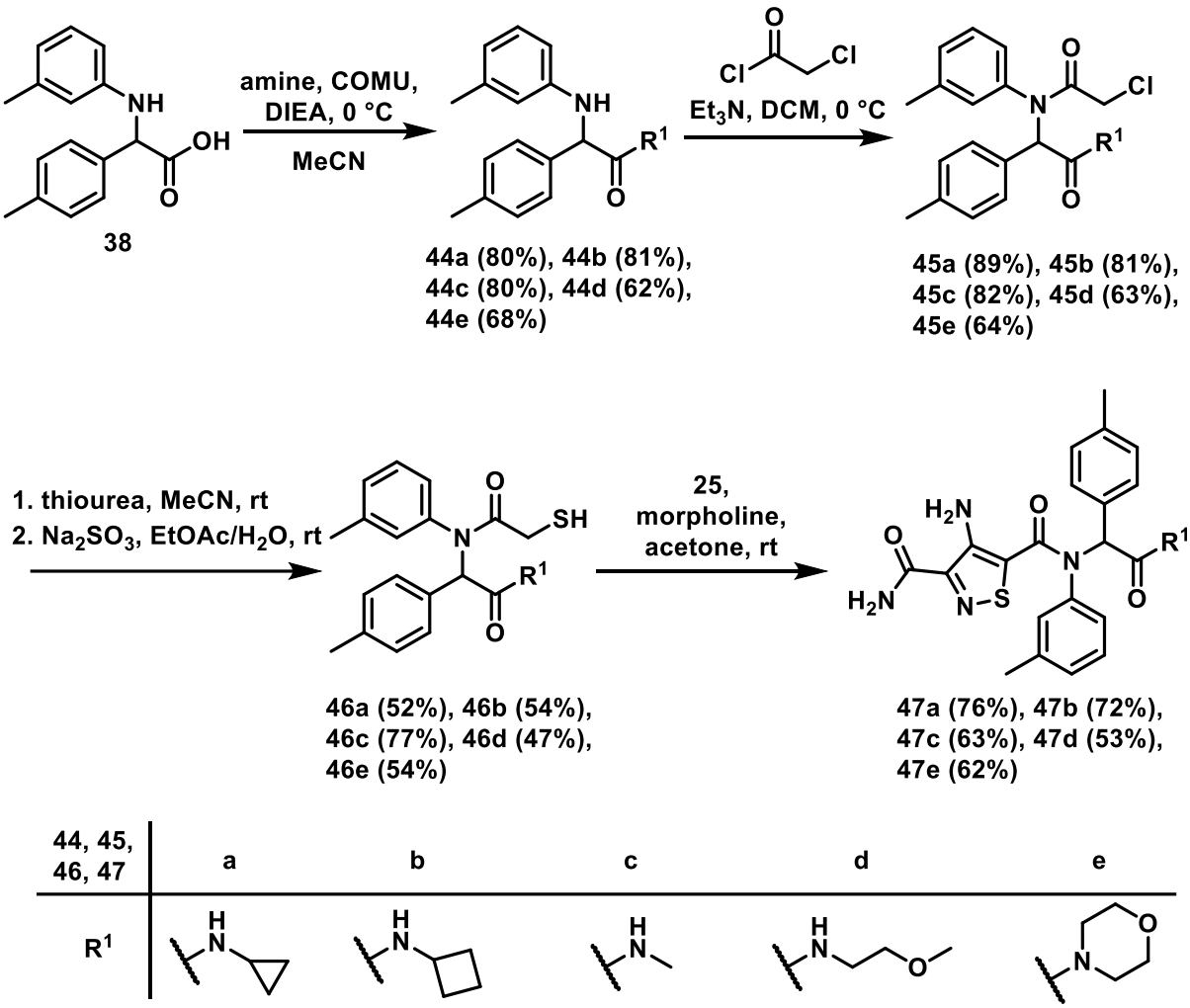


Scheme 11. Syntheses of α-acylaminoamides **42** and **43**.

Given the subsequent need to prepare optically pure α-acylaminoamides, the choice of the coupling reagent is crucial to minimize racemization. The contemporary peptide coupling reagent COMU tends to exhibit lower racemization compared to its counterparts[120]. Furthermore, COMU is renowned for its high coupling efficiency and is viewed as a safer and more efficient alternative to benzotriazole-based uronium reagents[121]. Unlike HATU and HBTU, which release the hazardous byproduct HOBt upon activation, COMU does not release the harmful 1,2,3-triazole, rendering it a more environmentally friendly option for synthesis.

Following unsuccessful efforts to synthesize the target compounds using EDCI/HOBt and HBTU, COMU was eventually chosen as the coupling reagent for the reaction. Initially,

carboxylic acid **36** was dissolved in acetonitrile in the presence of DIEA. With the subsequent introduction of COMU at 0 °C to activate the carboxylic acid, the reaction proceeded at the same temperature upon adding ammonia or piperidine. This led to the successful synthesis of the target compounds **42** and **43** in yields of 60% and 76%, respectively (Scheme 11).



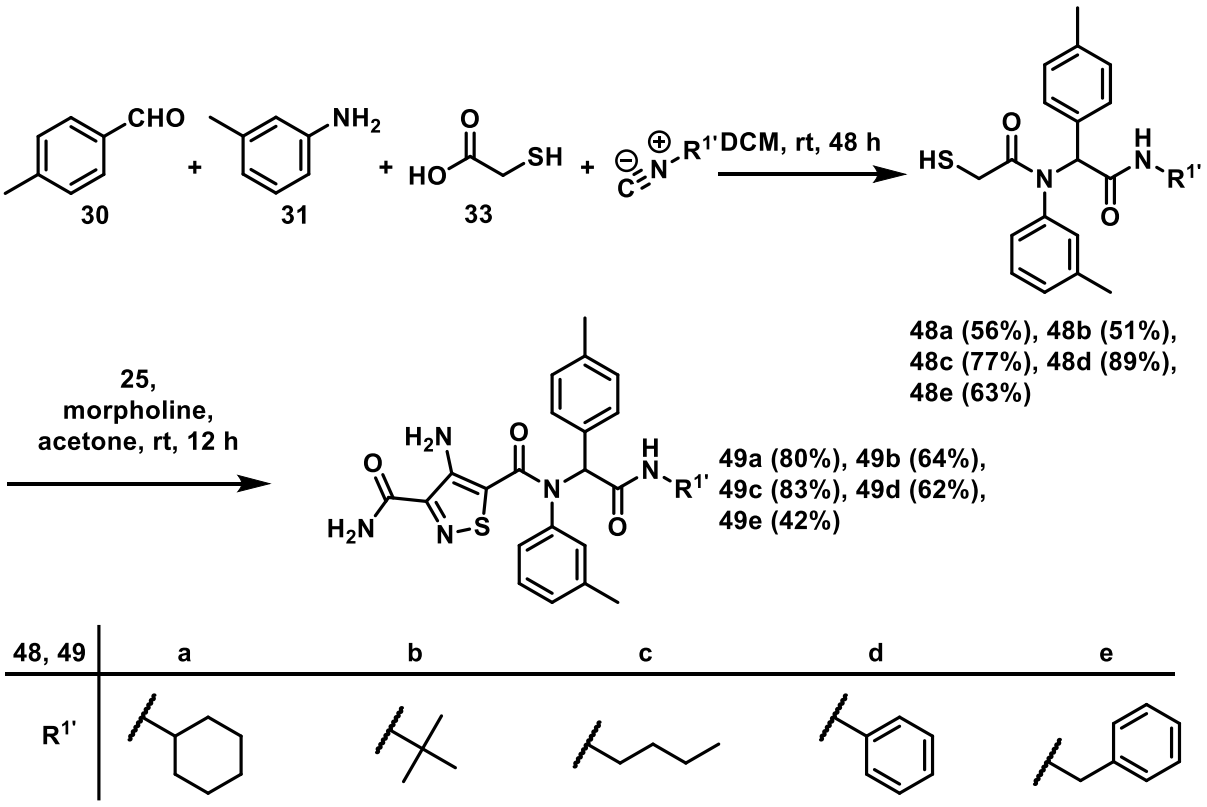
Scheme 12. Syntheses of α-acylaminoamides **47a-e**.

Although amide coupling reactions of carboxylic acid **36** could produce the envisaged products, the extremely low overall yields of amides **42** and **43** (2.1 % and 2.7 %) resulted in significant wastage, leading to the decision to abandon this method. As an alternative strategy, the *N*-aryl substituted carboxylic acid **38** was first condensed with appropriate amines, achieving yields ranging from 62% to 81% (Scheme 12). The thereby obtained amides **44a-e** were reacted with chloroacetyl chloride and subsequently treated with thiourea then sodium sulfite to obtain thiols

46a-e. The final cyclization step yielded the target compounds **47a-e**, achieving marginally improved overall yields ranging from 5.7 % to 18.7 %.

3.2.3 Synthesis of α-acylaminoamide derivatives 49a-e *via* the Ugi reaction

For commercially available isocyanides, the method comprising the Ugi reaction, which was described in section 3.1.1, was followed (Scheme 13). By utilizing *p*-tolualdehyde, *m*-methylaniline, 2-sulfanylacetic acid, and the corresponding isocyanides as starting materials, the reactions were conducted in dichloromethane for a duration of 48 h, resulting in the production of intermediates **48a-e** in yields from 51% to 89%. Subsequently, the obtained intermediates were reacted with sulfonate **25** in the presence of morpholine in acetone, leading to the formation of the desired compounds **49a-e** in yields from 42% to 83%.

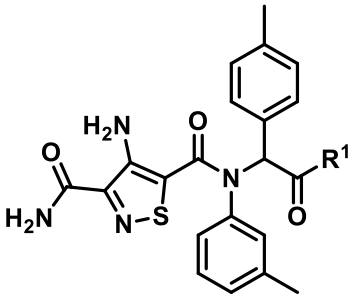


Scheme 13. Syntheses of α-acylaminoamides **49a-e**.

3.2.4 Biological evaluation of α-acylamino acid derivatives 35, 36, 42, 43, 47a-e, 49a-e

To investigate the impact of different structural variations of lead compound **20** on antiviral activity, the synthesized series of *N*-acyl amino acid derivatives featuring variations specifically at the R^1 position were tested for antiviral activity against influenza virus A/(H1N1)pdm09 (Table 4).

Table 4. Cytotoxicity and antiviral activity of *N*-acyl amino acid derivatives **35, 36, 42, 43, 47a-e, 49a-e** against influenza virus A/(H1N1)pdm09.



35, 36, 42, 43, 47a-e, 49a-e

Compound	R^1	CC_{50} [μM]	IC_{50} [μM]
36	OH	>100	n.a.*
35	OCH_3	>100	>100
42	NH_2	63.82 ± 3.92	n.a.*
47c	$NHCH_3$	>100	n.a.*
49c	$NHCH_2CH_2CH_2CH_3$	>100	25.74 ± 4.95
49b	$NHC(CH_3)_3$	>100	29.42 ± 14.71
47a	NHcyclopropyl	>100	n.a.*
47b	NHcyclobutyl	>100	48.96 ± 12.80
20	NHcyclopentyl	>100	12.95 ± 2.52
49a	NHcyclohexyl	>100	16.92 ± 5.37
49d	NHPh	>100	59.00 ± 22.95
49e	NHBn	>100	40.14 ± 10.57
47d	$NHCH_2CH_2OCH_3$	>100	87.12 ± 12.21
43	$N(CH_2CH_2)_2CH_2$	50.14 ± 20.75	n.a.*
47e	$N(CH_2CH_2)_2O$	>100	n.a.*
nucleozin	-	-	>100

* n.a. - not active

Carboxylic acid **36** was found to exhibit no antiviral activity in the performed influenza virus-induced cytopathic effect assay. Similarly, methyl ester **35** exhibited an IC_{50} value exceeding 100 μM. Considering the potential metabolic instability of esters and the inactivity of **36**, more

ester-containing derivatives were not synthesized. Both, the primary amide **42** and the *N*-methyl substituted amide **47c,** were devoid of antiviral activity. When compared to lead compound **20** exhibiting a *N*-cyclopentyl substituted amide moiety, these findings underscore the importance of a substituent at the amide nitrogen of a specific volume for optimal activity.

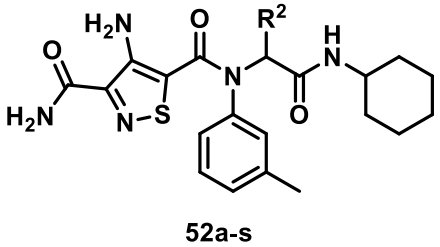
The introduction of a *n*-butyl chain (**49c**) or a bulky *tert*-butyl substituent (**49b**) at the amide nitrogen resulted in IC_{50} values of 25.74 μM and 29.42 μM, respectively. While these values represent an improvement over **47c**, they remain inferior to lead compound **20**. When rings of varying sizes were introduced to the amide nitrogen, most derivatives exhibited activity. The cyclohexyl derivative **49a** exhibited an IC_{50} value of 16.92 μM, comparable to the lead compound. However, *N*-cyclopropyl derivative **47a**, *N*-cyclobutyl derivative **47b**, and *N*-phenyl derivative **49d** had significantly diminished activities, suggesting that small saturated rings and phenyl groups might not be favorable for activity. The *N*-benzyl-substituted derivative **49e** had an IC_{50} of 40.14 μM, thus being slightly better than *N*-phenyl derivative **49d** but still lagging behind the lead compound. Derivatives modified with oxygen atoms within the chain, intended to exhibit enhanced solubility, unfortunately experienced reduced activity. For instance, the substitution of the γ-carbon atom of the *n*-butyl chain of secondary amide **49c** with an oxygen atom led to compound **47d**, which showed a significant drop-in activity, exhibiting an IC_{50} value of 87.12 μM.

Additionally, studies into tertiary amides with six-membered rings indicated that both the piperidine-based tertiary amide **43** and the morpholine derivative **47e** lacked activity, with **43** also showing heightened toxicity. These results suggest that introducing an oxygen atom into the substituent at the amide nitrogen may be counterproductive for maintaining activity, thereby underscoring the critical role of the secondary amide moiety.

3.3 α-Acylaminoamides with varied R^2 and R^3 groups

3.3.1 Synthesis of α-acylaminoamide derivatives 52a-s, 54a-u *via* Ugi reactions

Considering the comparable biological activity of cyclohexyl derivative **49a** and cyclopentyl derivative **20** (section 3.2.4) differing only in the substituent at the $R^{1'}$ position as well as the easier accessibility of cyclohexylisocyanide, the *N*-cyclohexyl-substituted amide moiety was fixed at this position. To systematically study the influence of substituents on the phenyl ring at the R^2 position, substituents of varying size, electronic properties, and polarity were introduced at different positions. To enhance solubility, hydroxyl and carboxyl groups were incorporated. Additionally, the synthesis of α-acylaminoamide derivatives with cyclohexyl and methyl groups at the R^2 position was undertaken to assess the essential role of the phenyl ring.

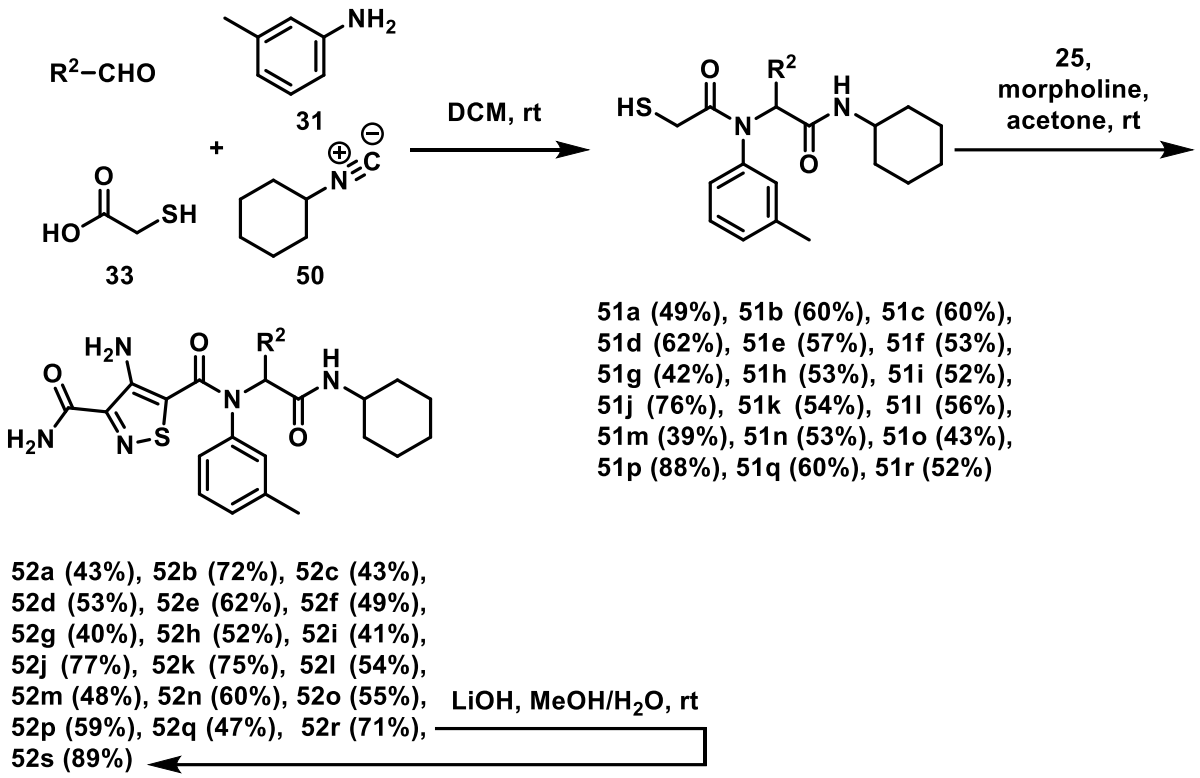


52a-s

52a: R^2 = Ph
52b: R^2 = 3-$CH_3C_6H_4$
52c: R^2 = 2-$CH_3C_6H_4$
52d: R^2 = 4-$CH_2CH_3C_6H_4$
52e: R^2 = 4-$(CH_3)_2CHC_6H_4$
52f: R^2 = 3,5-$(CH_3)_2C_6H_3$
52g: R^2 = 4-FC_6H_4
52h: R^2 = 4-ClC_6H_4
52i: R^2 = 3-ClC_6H_4
52j: R^2 = 2-ClC_6H_4
52k: R^2 = 4-BrC_6H_4
52l: R^2 = 4-CNC_6H_4
52m: R^2 = 4-$CF_3C_6H_4$
52n: R^2 = 4-$OCH_3C_6H_4$
52o: R^2 = 4-$OCF_3C_6H_4$
52p: R^2 = cyclohexyl
52q: R^2 = 4-$COOCH_3C_6H_4$
52r: R^2 = CH_3
52s: R^2 = 4-$COOHC_6H_4$

Figure 28. Structures of α-acylaminoamide derivatives **52a**-**s** with varied R^2 groups.

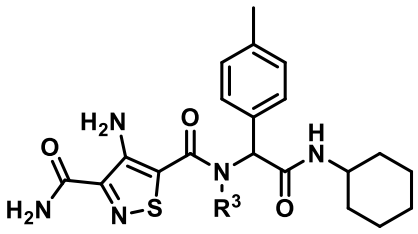
As depicted in Scheme 14 and following the methodology described in the previous section, intermediates **51a**-**r** were synthesized *via* Ugi reactions in yields ranging from 39% to 88%. This was achieved by reacting benzaldehydes with diverse substituents, cyclohexanecarbaldehyde, or acetaldehyde with *m*-toluidine, 2-sulfanylacetic acid, and cyclohexylisocyanide. The obtained intermediates were then cyclized to produce target compounds **52a**-**r**, achieving yields between 41% and 80%. Additionally, ester **52q** was subjected to a basic hydrolysis using lithium hydroxide, leading to the formation of benzoic acid derivative **52s** in 89% yield.



51, 52	a	b	c	d	e	f
R^2	Ph	3-$CH_3C_6H_4$	2-$CH_3C_6H_4$	4-$CH_2CH_3C_6H_4$	4-$(CH_3)_2CHC_6H_4$	3,5-$(CH_3)_2C_6H_3$
51, 52	g	h	i	j	k	l
R^2	4-FC_6H_4	4-ClC_6H_4	3-ClC_6H_4	2-ClC_6H_4	4-BrC_6H_4	4-CNC_6H_4
51, 52	m	n	o	p	q	r
R^2	4-$CF_3C_6H_4$	4-$OCH_3C_6H_4$	4-$OCF_3C_6H_4$	cyclohexyl	CH_3	4-$COOCH_3C_6H_4$
51, 52	s					
R^2	4-$COOHC_6H_4$					

Scheme 14. Syntheses of α-acylaminoamides **52a-s** with varied R^2 groups.

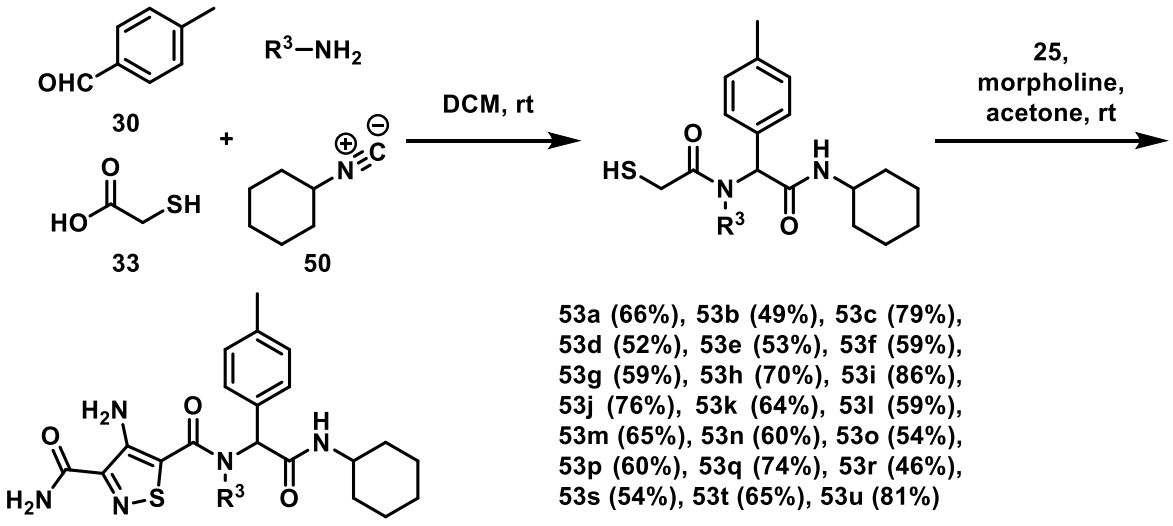
To systematically understand the effects of substituents on the phenyl ring at the R^3 position, substituents of diverse sizes and electronic characteristics were strategically introduced at various sites. To assess the essential role of the phenyl ring and its distance from the core structure, α-acylaminoamide derivatives with a methyl group on the nitrogen atom and compounds substituted with benzyl and benzhydryl groups were synthesized (Figure 29, Scheme 15).



54a-u

54a: R^3 = Ph
54b: R^3 = 4-$CH_3C_6H_4$
54c: R^3 = 2-$CH_3C_6H_4$
54d: R^3 = 3-$CH_2CH_3C_6H_4$
54e: R^3 = 3,4-$(CH_3)_2C_6H_3$
54f: R^3 = 3,5-$(CH_3)_2C_6H_3$
54g: R^3 = 4-$OCH_3C_6H_4$
54h: R^3 = 3-$OCH_3C_6H_4$
54i: R^3 = 4-FC_6H_4
54j: R^3 = 3-FC_6H_4
54k: R^3 = 4-ClC_6H_4
54l: R^3 = 3-ClC_6H_4
54m: R^3 = 3,4-$Cl_2C_6H_3$
54n: R^3 = 3,5-$Cl_2C_6H_3$
54o: R^3 = 3-CNC_6H_4
54p: R^3 = 3-$CF_3C_6H_4$
54q: R^3 = 2-$CF_3C_6H_4$
54r: R^3 = 4-$NO_2C_6H_4$
54s: R^3 = CH_3
54t: R^3 = $PhCH_2$
54u: R^3 = Ph_2CH

Figure 29. Structures of α-acylaminoamides derivatives **54a-u** with varied R^3 groups.



53a (66%), 53b (49%), 53c (79%),
53d (52%), 53e (53%), 53f (59%),
53g (59%), 53h (70%), 53i (86%),
53j (76%), 53k (64%), 53l (59%),
53m (65%), 53n (60%), 53o (54%),
53p (60%), 53q (74%), 53r (46%),
53s (54%), 53t (65%), 53u (81%)

54a (66%), 54b (59%), 54c (79%),
54d (76%), 54e (65%), 54f (56%),
54g (87%), 54h (89%), 54i (63%),
54j (59%), 54k (72%), 54l (75%),
54m (71%), 54n (51%), 54o (74%),
54p (85%), 54q (55%), 54r (73%),
54s (43%), 54t (79%), 54u (70%)

53, 54	a	b	c	d	e	f
R^3	Ph	4-$CH_3C_6H_4$	2-$CH_3C_6H_4$	3-$CH_2CH_3C_6H_4$	3,4-$(CH_3)_2C_6H_3$	3,5-$(CH_3)_2C_6H_3$
53, 54	g	h	i	j	k	l
R^3	4-$OCH_3C_6H_4$	3-$OCH_3C_6H_4$	4-FC_6H_4	3-FC_6H_4	4-ClC_6H_4	3-ClC_6H_4
53, 54	m	n	o	p	q	r
R^3	3,4-$Cl_2C_6H_3$	3,5-$Cl_2C_6H_3$	3-CNC_6H_4	3-$CF_3C_6H_4$	2-$CF_3C_6H_4$	4-$NO_2C_6H_4$
53, 54	s	t	u			
R^3	CH_3	$PhCH_2$	$(Ph)_2CH$			

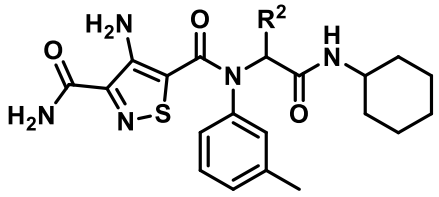
Scheme 15. Syntheses of α-acylaminoamides **54a-u**.

As depicted in Scheme 15, following the methodology developed in section 3.1.1, intermediates **53a**-**u** were synthesized be reacting anilines with different substituents, benzylamine, benzhydrylamine, or methylamine with *p*-tolualdehyde, 2-sulfanylacetic acid, and cyclohexylisocyanide *via* the Ugi reaction. These thiols were obtained in yields ranging from 46% to 86%. Subsequent cyclization produced target compounds **54a**-**u** in yields between 43% and 89%.

3.3.2 Biological evaluation of α-acylaminoamides 52a-s, 54a-u with varied R^2 and R^3 groups

In this section, the substituents at the α-acylaminoamide scaffold were consistently modified, while retaining a cyclohexyl group at the $R^{1'}$ position and the isothiazole ring at the R^4 position. Diverse substituents were introduced at the R^2 and R^3 positions, with a particular focus on phenyl rings bearing various substituents to systematically assess their effects. To further probe the significance of the phenyl ring, α-acylaminoamide derivatives were synthesized with alternative groups, including methyl, cyclohexyl, benzyl, and benzhydryl residues. The structural details and anti-influenza A activities for these 41 novel compounds can be found in Table 5, Table 6, and Table 7.

Table 5. Cytotoxicity and antiviral activity of α-acylaminoamides derivatives **52a-s** against influenza virus A/(H1N1)pdm09.



52a-s

Compound	R^2	CC_{50} [μM]	IC_{50} [μM]
52s	CH_3	>100	21.05 ± 5.94
52q	cyclohexyl	>100	34.77 ± 5.83
52a	Ph	>100	10.10 ± 3.23
52c	2-$CH_3C_6H_4$	>100	44.44 ± 11.69
52b	3-$CH_3C_6H_4$	>100	13.94 ± 6.74
49a	4-$CH_3C_6H_4$	>100	16.92 ± 5.32
52d	4-$CH_3CH_2C_6H_4$	>100	20.06 ± 4.82
52e	4-$(CH_3)_2CHC_6H_4$	>100	30.04 ± 12.93
52f	3,5-$(CH_3)_2C_6H_3$	>100	27.57 ± 6.14
52n	4-$OCH_3C_6H_4$	>100	17.36 ± 3.99
22	4-OHC_6H_4	>100	14.51 ± 1.61
52g	4-FC_6H_4	>100	21.29 ± 7.78
52h	4-ClC_6H_4	>100	8.97 ± 1.91
52i	3-ClC_6H_4	>100	12.65 ± 3.62
52j	2-ClC_6H_4	>100	18.27 ± 0.99
52k	4-BrC_6H_4	>100	16.16 ± 6.92
52l	4-CNC_6H_4	>100	7.35 ± 1.21
52m	4-$CF_3C_6H_4$	>100	14.73 ± 5.12
52o	4-$OCF_3C_6H_4$	>100	36.27 ± 17.47
52q	4-$COOCH_3C_6H_4$	>100	12.21 ± 2.98
52s	4-$COOHC_6H_4$	>100	n.a.*
nucleozin	-	-	>100

* n.a. - not active

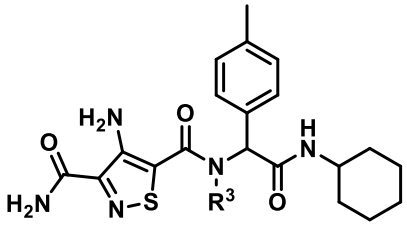
The introduction of a phenyl group at the R^2 position, as seen in compound **52a**, caused enhanced activity, with an IC_{50} value of 10.10 μM. In contrast, the methyl (**52s**) and cyclohexyl (**52q**) substitutions at the same position were less effective. For compounds with electron-donating groups at the phenyl ring, the activity varied based on the position of the substituent. Specifically, the *ortho*-substituted methyl compound (**52c**) exhibited significantly reduced activity compared to its *meta*- (**52b**) and *para*-substituted (**49a**) counterparts. The 3,5-dimethylphenyl derivative (**52f**) displayed an intermediate activity with an IC_{50} value ranging between the ones of *ortho*-monosubstituted compound **52c** and *meta*-monosubstituted compound **52b**. Furthermore, increasing the length of the alkyl chain at the *para*-position as well as introducing a bulkier group at this position resulted in diminished activity. Thus, among the *para*-substituted compounds, ethyl (**52d**) and isopropyl (**52e**) derivatives exhibited higher IC_{50} values compared to the methyl-substituted compound **49a**. In case of substituents containing heteroatoms, compound **52n** with a methoxy substituent exhibited an IC_{50} value of 17.36 μM, thus being less active compared to the hydroxyl-substituted compound **22**. Altogether, all compounds with electron-donating groups at the phenyl ring were less active than the unsubstituted compound **52a**.

When a halogen atom was introduced at the *para*-position of the phenyl ring, the observed activity followed the order: chlorine (**52h**) > bromine (**52k**) > fluorine (**52g**). Remarkably, 4-chlorophenyl derivative **52h** showed the highest efficacy, displaying an IC_{50} value of 8.97 μM, which surpasses that of the unsubstituted compound **52a**. In case of the chlorine-substituted compounds, the *meta*- (**52i**) and particularly the *ortho*-substituted (**52j**) regioisomers exhibited a significant decline in activity compared to 4-chlorophenyl derivative **52h**. This indicates that the introduction of a substituent at the *ortho*-position of the phenyl ring is unfavorable for activity. Among the other electron-withdrawing groups introduced at the *para*-position, the cyano group stood out, achieving an IC_{50} of 7.35 μM. The activities of the trifluoromethyl- (**52m**) and methoxycarbonyl-substituted (**52q**) compounds were only slightly lower than the one of the unsubstituted compound **52a**, while trifluoromethoxy derivative (**52o**) showed a considerable reduction in activity. Efforts to enhance solubility by incorporating a carboxyl group at the *para*-position were disappointing, as this modification completely abolished the anti-influenza activity. All synthesized compounds demonstrated favorable safety profiles, with CC_{50} values consistently exceeding 100 μM.

These findings suggest that a substituent on the phenyl ring at the R^2 position can strongly influence activity against the A/(H1N1)pdm09 strain. Specifically, *ortho*-substitution appears

to be detrimental, whereas electron-withdrawing groups are generally superior to electron-donating groups.

Table 6. Cytotoxicity and antiviral activity of α-acylaminoamides derivatives **54a-u** against influenza virus A/(H1N1)pdm09.



54a-u

Compound	R^3	CC_{50} [μM]	IC_{50} [μM]
54s	CH_3	>100	n.a.*
54a	Ph	>100	7.96 ± 1.22
54t	Bn	>100	26.31 ± 7.94
54u	Ph_2CH	33.27 ± 4.33	n.a.*
49a	3-$CH_3C_6H_4$	>100	16.92 ± 5.32
54c	2-$CH_3C_6H_4$	60.06 ± 4.03	15.26 ± 2.03
54b	4-$CH_3C_6H_4$	>100	13.39 ± 5.45
54e	3,4-$(CH_3)_2C_6H_3$	>100	21.69 ± 6.16
54f	3,5-$(CH_3)_2C_6H_3$	>100	10.75 ± 1.14
54d	3-$CH_3CH_2C_6H_4$	>100	35.69 ± 6.69
54h	3-$OCH_3C_6H_4$	>100	19.86 ± 6.78
54g	4-$OCH_3C_6H_4$	>100	19.06 ± 8.04
54i	4-FC_6H_4	>100	10.72 ± 3.35
54j	3-FC_6H_4	>100	10.76 ± 0.62
54k	4-ClC_6H_4	>100	16.07 ± 5.56
54l	3-ClC_6H_4	>100	12.86 ± 4.29
54m	3,4-$Cl_2C_6H_3$	>100	20.20 ± 6.00
54n	3,5-$Cl_2C_6H_3$	>100	9.46 ± 5.75
54q	2-$CF_3C_6H_4$	>100	42.29 ± 4.97
54p	3-$CF_3C_6H_4$	>100	11.13 ± 1.54
54o	3-CNC_6H_4	>100	7.56 ± 0.30
54r	4-$NO_2C_6H_4$	>100	18.96 ± 5.14
nucleozin	-	-	>100

* n.a. - not active

Modifications at the R^3 position revealed that the introduction of both methyl (**54s**) and benzhydryl (**54u**) groups, negatively impacted activity, as neither compound exhibited anti-

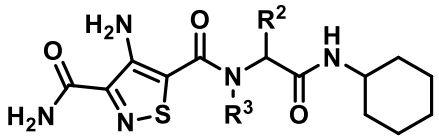
influenza A activity. Notably, benzhydryl-substituted compound **54u** had a CC_{50} value of 33.27 µM, indicating cytotoxicity. Also benzyl-substituted compound **54t** showed only weak anti-influenza A activity. In contrast, the phenyl-substituted compound **54a** displayed enhanced activity with an IC_{50} value of 7.96 µM.

For derivatives with electron-donating groups on the benzene ring, the introduction of either a methyl (**49a**, **54b**, **54c**) or methoxy group (**54h**, **54g**) led to activity below the one of the unsubstituted compounds, with the position of these substituents having minimal influence on the outcome. However, compound **54c** with an *ortho*-methyl substituted benzene ring demonstrated cytotoxicity, evidenced by a CC_{50} value of 66.06 µM. When compared to *meta*-methyl derivative **49a**, the introduction of a *meta*-ethyl group (**54d**) resulted in reduced activity. Among the derivatives with two methyl groups on the benzene ring, the 3,5-disubstituted variant **54f** outperformed the 3,4-disubstituted regioisomer **54e**. Notably, compound **54f** showed an IC_{50} value of 10.75 µM, thus being the most active compound with electron-donating groups.

Among the halogenated phenyl derivatives, the fluorine-substituted compounds proved superior to the chlorine-substituted derivatives. 3-Fluorophenyl derivative **54j** and its 4-fluorophenyl substituted regioisomer **54i** exhibited IC_{50} values of 10.76 µM and 10.72 µM, respectively. The *meta*-chlorine substituted derivative **54l** was slightly more effective than its *para*-substituted counterpart **54k**, and both were superior to the 3,4-dichloro substituted variant. However, the 3,5-dichloro substituted derivative **54n** showed improved activity with an IC_{50} of 9.46 µM. In case of the trifluoromethyl-substituted compounds **54p** and **54q**, the *ortho*-substituted compound **54q** exhibited considerably reduced antiviral activity compared to its methyl-substituted counterpart **54c**, whereas the *meta*-substituted regioisomer outperformed the 3-methylphenyl derivative **49a**, displaying an IC_{50} value of 11.13 µM. Considering the other investigated electron-withdrawing groups, a *para*-nitro substituent (**54r**) caused reduced activity compared to the 4-fluorophenyl (**54i**) and 4-chlorophenyl (**54k**) derivatives, whereas a *meta*-cyano group (**54o**) was highly beneficial. With an IC_{50} value of 7.56 µM, compound **54o** exhibited superior activity compared to the unsubstituted compound **54a**.

These findings indicated that the benzene ring at the R^3 position was also crucial for activity against the A/(H1N1)pdm09 strain. Whereas *ortho*-substitution was shown to cause unfavorable effects with respect to activity and cytotoxicity, an unsubstituted phenyl ring or one with an electron-withdrawing cyano group in *meta*-position were found to be more favorable for antiviral activity.

Table 7. Cytotoxicity and antiviral activity of α-acylaminoamides derivatives **22**, **52a**, **52h**, **52i**, **52n**, **54a**, **54f**, **54i**, **54j**, **54l**. **54n**, **54o**, **54p** against influenza virus A/HK/1/68 (H3N2).



22, 52a, 52h, 52i, 52n, 54a, 54f, 54i, 54j, 54l. 54n, 54o, 54p

Compound	R^2	R^3	CC_{50} [μM]	IC_{50} [μM]
52a	Ph	3-$CH_3C_6H_4$	>100	11.8
52h	4-ClC_6H_4	3-$CH_3C_6H_4$	>100	12.6
52i	3-ClC_6H_4	3-$CH_3C_6H_4$	>100	18.2
52n	4-$OCH_3C_6H_4$	3-$CH_3C_6H_4$	>100	5.6
22	4-OHC_6H_4	3-$CH_3C_6H_4$	>100	7.4
54a	4-$CH_3C_6H_4$	Ph	>100	21.7
54i	4-$CH_3C_6H_4$	4-FC_6H_4	>100	19.6
54j	4-$CH_3C_6H_4$	3-FC_6H_4	>100	28.6
54l	4-$CH_3C_6H_4$	3-ClC_6H_4	>100	7.9
54p	4-$CH_3C_6H_4$	3-$CF_3C_6H_4$	>100	34.5
54n	4-$CH_3C_6H_4$	3,5-$Cl_2C_6H_3$	>100	35.0
54o	4-$CH_3C_6H_4$	3-CNC_6H_4	>100	8.9
54f	4-$CH_3C_6H_4$	3,5-$(CH_3)_2C_6H_3$	>100	28.4
nucleozin	-	-	-	0.87

Compounds demonstrating promising inhibitory effects against the A/(H1N1)pdm09 strain were further assessed for their efficacy against the A/HK/1/68 (H3N2) strain. The SAR against the A/HK/1/68 (H3N2) strain exhibited subtle differences when compared to their effects on H1N1.

Regarding the R^2 position, the derivative featuring an unsubstituted phenyl moiety (**52a**) consistently displayed inhibitory against the A/HK/1/68 (H3N2) strain, with IC_{50} values around 10 μM against both strains. Remarkably, the introduction of electron-donating groups at the *para*-position improved activity against the A/HK/1/68 (H3N2) strain. Compound **52n**, which bears a methoxy group, showed an IC_{50} of 5.6 μM, surpassing the efficacy of the unsubstituted compound against the H3N2 strain. In contrast, the monochloro-substituted derivatives **52h** and **52i** saw a slight decrease in activity, diverging from their behavior against the H1N1 strain.

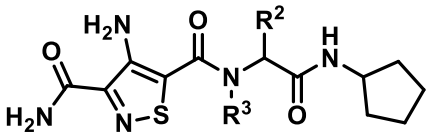
With respect to the phenyl ring at the R^3 position, particularly compounds **54l** and **54o**, exhibiting an electron-withdrawing chloro or cyano group, stood out for their pronounced effects. The strategic placement of these groups on the benzene ring markedly influenced

efficacy of the compounds against both virus strains. Detailed studies on these substituents could provide insights into optimizing the compounds for enhanced antiviral efficacy.

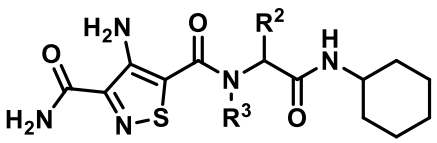
3.4 α-Acylaminoamides with variations at several positions

This chapter describes the synthesis and biological activity of compounds resulting from combinations of substituents at R^1, R^2, and R^3 that individually caused favorable activity and solubility characteristics. The R^4 position was consistently fixed as 4-amino-3-carbamoylisothiazol-5-yl residue. At the R^1 position, cyclopentylamino, cyclohexylamino, and morpholino residues were selected. For the R^2 position, choices included phenyl, methoxyphenyl, and hydroxyphenyl residues, while options like phenyl, methylphenyl, cyanophenyl, and chlorophenyl moieties should be introduced at the R^3 position. These components were systematically combined and integrated into the α-acylaminoamide scaffold.

3.4.1 Synthesis of α-acylaminoamide derivatives 57a-k, 58a-c, 66



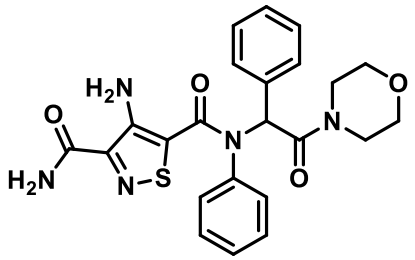
57a-n



58a-c

57a: R^2 = Ph, R^3 = Ph
57b: R^2 = 4-OHC$_6$H$_4$, R^3 = 3-CH$_3$C$_6$H$_4$
57c: R^2 = 4-OHC$_6$H$_4$, R^3 = 3-CNC$_6$H$_4$
57d: R^2 = 4-OHC$_6$H$_4$, R^3 = 3-ClC$_6$H$_4$
57e: R^2 = 4-OHC$_6$H$_4$, R^3 = Ph
57f: R^2 = 4-OCH$_3$C$_6$H$_4$, R^3 = 3-CH$_3$C$_6$H$_4$
57g: R^2 = 4-OCH$_3$C$_6$H$_4$, R^3 = 3-CNC$_6$H$_4$
57h: R^2 = 4-OCH$_3$C$_6$H$_4$, R^3 = 3-ClC$_6$H$_4$
57i: R^2 = 2-OCH$_3$C$_6$H$_4$, R^3 = 3-ClC$_6$H$_4$
57j: R^2 = 3-OCH$_3$C$_6$H$_4$, R^3 = 3-ClC$_6$H$_4$
57k: R^2 = 4-N(CH$_3$)$_2$C$_6$H$_4$, R^3 = 3-CH$_3$C$_6$H$_4$
57l: R^2 = 4-N(CH$_3$)$_2$C$_6$H$_4$, R^3 = Ph
57m: R^2 = 4-N(CH$_3$)$_2$C$_6$H$_4$, R^3 = 3,5-(CH$_3$)$_2$C$_6$H$_3$
57n: R^2 = 4-N(CH$_3$)$_2$C$_6$H$_4$, R^3 = 2,5-(CH$_3$)$_2$C$_6$H$_3$

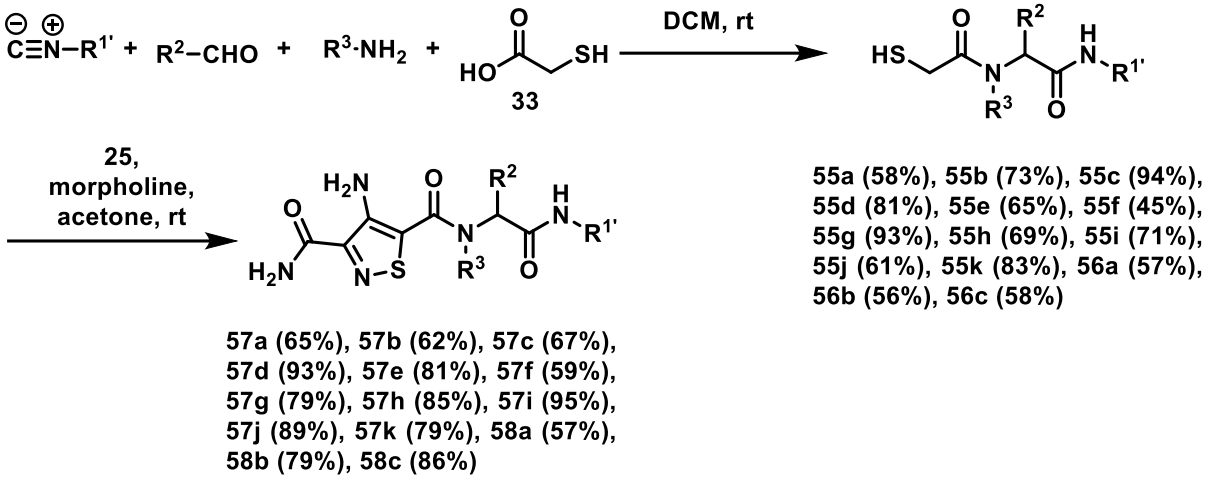
58a: R^2 = Ph, R^3 = Ph
58b: R^2 = 4-OCH$_3$C$_6$H$_4$, R^3 = Ph
58c: R^2 = 4-OCH$_3$C$_6$H$_4$, R^3 = 3-ClC$_6$H$_4$



66

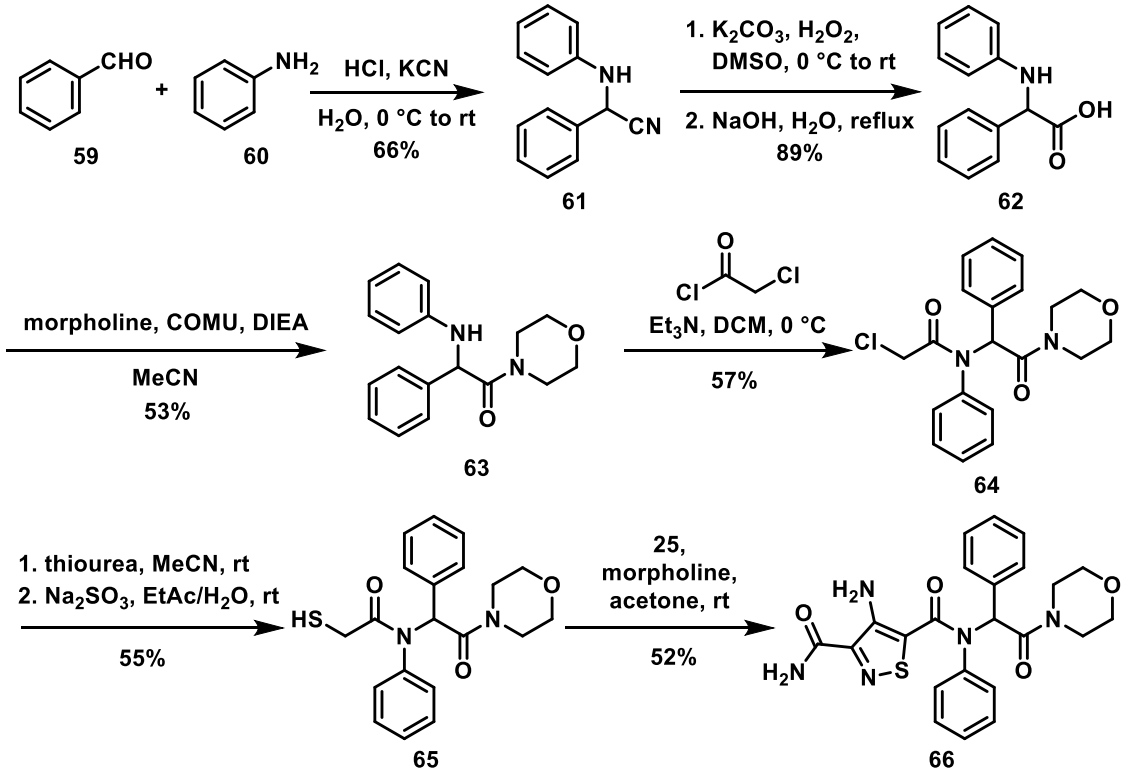
Figure 30. Structures of α-acylaminoamide derivatives **57a-n, 58a-c, 66**.

As illustrated in Scheme 16 and following the methodology previously developed (section 3.1.1), intermediates **55a-k** and **56a-c** were synthesized in yields ranging from 45% to 94% by reacting various aldehydes, amines, isocyanides, and 2-sulfanylacetic acid *via* the Ugi reaction. These intermediates were then cyclized with sulfonate **25** to produce the target compounds **57a-k** and **58a-c** in yields between 57% and 95%.



Scheme 16. Syntheses of α-acylaminoamides **57a-k, 58a-c**.

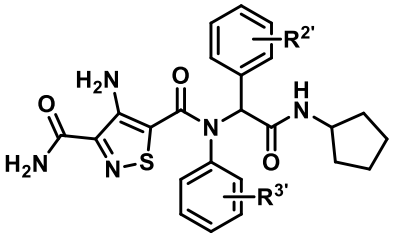
The morpholine-based amide **66** was synthesized as shown in Scheme 17. Starting from benzaldehyde and aniline, a Strecker synthesis was employed to produce nitrile **61**, which was then hydrolyzed to yield carboxylic acid **62**. Subsequently, an amide coupling with morpholine was performed to obtain morpholine-based amide **63**, followed by an acylation with chloroacetyl chloride to give bisamide **64**. After treating with thiourea then sodium sulfite produced thiol **65**, which was finally cyclized with sulfonate **25** to yield the targeted compound **66**.



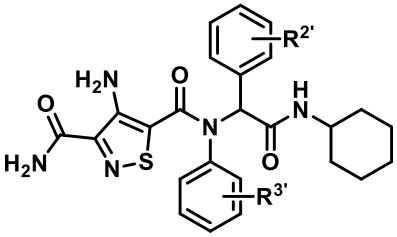
Scheme 17. Syntheses of morpholine derivative **66**.

3.4.2 Biological evaluation of 57a-n, 58a-c, 66

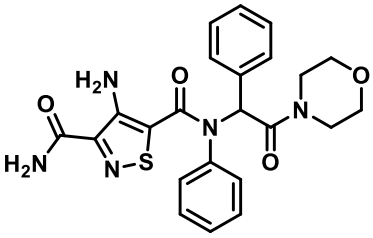
Table 8. Cytotoxicity and antiviral activity of α-acylaminoamides derivatives **57a-n, 58a-c, 66** against influenza viruses A/pdm09 (H1N1) and A/HK/1/68 (H3N2).



57a-n, JK9



22, 49a, 58a-c



66

Compound	$R^{2'}$	$R^{3'}$	CC_{50} [μM]	IC_{50} [μM]	
				A/pdm09 (H1N1)	A/HK/1/68 (H3N2)
58a	H	H	>100	17.33 ± 5.38	2.71
57a	H	H	>100	10.45 ± 3.84	24.5
66	-	-	>100	68.02 ± 28.58	not tested
58b	4-OCH_3	H	>100	12.84 ± 2.27	18.4
58c	4-OCH_3	3-Cl	>100	13.76 ± 4.96	19.3
57i	2-OCH_3	3-Cl	>100	33.78 ± 5.86	not tested
57j	3-OCH_3	3-Cl	>100	23.56 ± 7.10	43.0
57h	4-OCH_3	3-Cl	>100	17.60 ± 3.07	not tested
57g	4-OCH_3	3-CN	>100	17.67 ± 1.50	not tested
57f	4-OCH_3	3-CH_3	>100	11.58 ± 7.50	not tested
57d	4-OH	3-Cl	>100	48.17 ± 7.88	not tested
57c	4-OH	3-CN	44.62 ± 22.52	n.a.**	not tested
57b	4-OH	3-CH_3	>100	n.a.**	n.a.**
57e	4-OH	H	>100	>100	not tested
22	4-OH	3-CH_3	>100	14.51 ± 1.61	7.35
57k	4-$N(CH_3)_2$	3-CH_3	>100	23.06 ± 14.31	not tested
57l*	4-$N(CH_3)_2$	H	>100	55.06 ± 10.29	not tested
57m*	4-$N(CH_3)_2$	3,5-$(CH_3)_2$	>100	29.25 ± 13.90	not tested
57n*	4-$N(CH_3)_2$	2,5-$(CH_3)_2$	>100	46.14 ± 14.52	not tested
JK9*	4-$N(CH_3)_2$	3-CH_3	>100	57.2	not tested
49a	4-CH_3	3-CH_3	>100	16.92 ± 5.32	35.27
nucleozin	-	-	-	>100	0.87

* purchased compound

**n.a.- not active

The attempts to combine beneficial groups for activity and solubility on the α-acylaminoamide scaffold did not always produce the anticipated ideal compounds. Specifically, compounds **57a** and **58a** with both phenyl rings unsubstituted showed commendable activity. The derivative containing an *N*-cyclohexylamide moiety (**58a**) is particularly noteworthy, exhibiting an 50% inhibitory concentration of 2.71 μM against the A/HK/1/68 (H3N2) strain. This performance significantly surpassed the one of compound **20** and closely approached the efficacy of nucleozin. However, **57a**, differing from **58a** only in the presence of a cyclohexyl instead of cyclopentyl group, exhibited a surprisingly reduced efficacy against the H3N2 strain, being 9 times less potent than **58a**. Tertiary amide **66** was inactive, further confirming the importance of a secondary amide moiety.

In case of the *N*-cyclohexylamide derivatives, where R^2 is a *para*-methoxyphenyl residue, a slight increase in the antiviral activity against H1N1 was observed. However, these compounds were less active against H3N2 than the unsubstituted compound **58a**. In contrast, in case of the investigated *N*-cyclopentylamide derivatives, all tested compounds with a methoxyphenyl moiety at the R^2 position were less active against the H1N1 strain than the unsubstituted compound **57a**. The introduction of a hydroxyl group at the *para*-position of the phenyl ring at the R^2 position led to diminished activity (**57b, 57c, 57d** and **57e**). Even though groups were introduced at the phenyl ring at the R^3 position, which had been shown to enhance activity, with R^2 fixed as para-hydroxyphenyl, activity was not regained. Surprisingly, also 4-hydroxyphenyl derivative **57b**, which only differs from the most potent compound **22** in having a cyclopentyl group at the amide nitrogen instead of a cyclohexyl group, did not show any inhibitory activity.

Similarly, attempts to enhance the water solubility by introducing a para-dimethylamino group to the phenyl ring at the R^2 position resulted in compounds with reduced antiviral efficacy. Typically, having a phenyl group or a methyl-substituted phenyl at the R^3 position is advantageous for activity. However, neither the initially discovered hit compound **JK9** nor the subsequent compounds **57k**, **57l**, **57m**, **57n** achieved the anticipated level of activity.

Among the tested compounds, the outstanding performance of compound **58a**, with its impressive antiviral activity against the A/HK/1/68 (H3N2) strain, makes it a prime candidate for further investigation. Therefore, based on its superior activity profile, compound **58a** was selected as the representative compound for subsequent research endeavors.

3.5 Enantiomerically pure α-acylaminoamides

3.6.1 Synthesis of α-acylaminoamide derivatives (*S*)/(*R*)-20 and (*S*)/(*R*)-58a

This chapter describes the synthesis of both enantiomers of α-acylaminoamide derivatives **20** and **58a** along with the analysis of their respective biological activity.

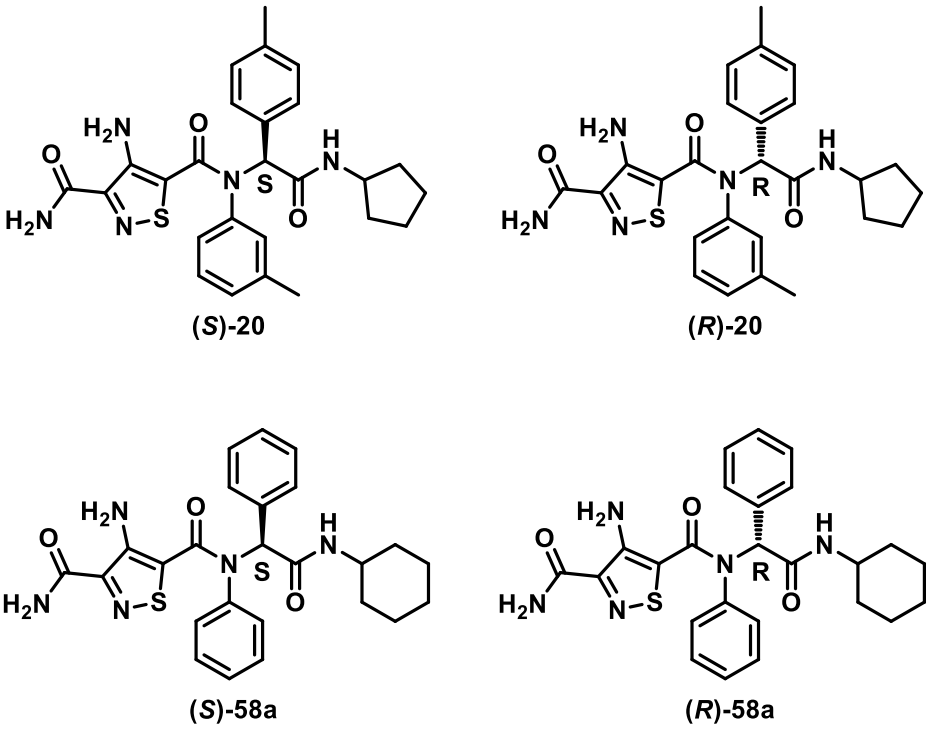
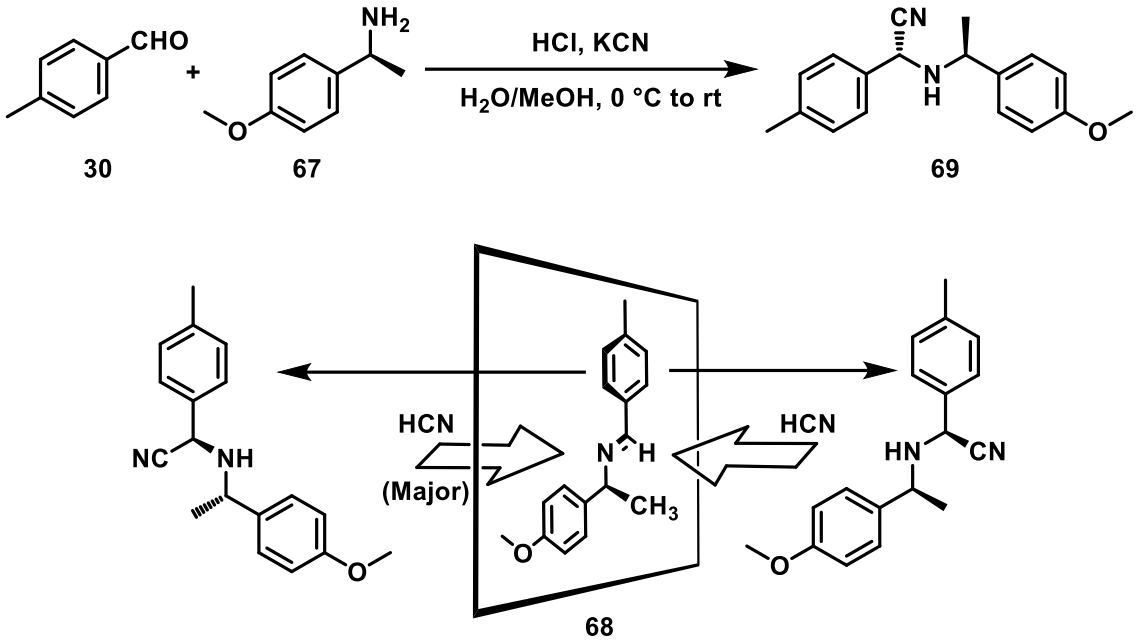


Figure 31. Structures of enantiopure α-acylaminoamides **(*S*)/(*R*)-20 and (*S*)/(*R*)-58a**.

Considering the activity against various IAV strains of all previously described α-acylaminoamides, which were synthesized and tested as racemates, compounds **20** and **58a** were selected as representative molecules. To ascertain which enantiomer exhibits enhanced activity, both stereoisomers should be synthesized in enantiomerically pure form, each.

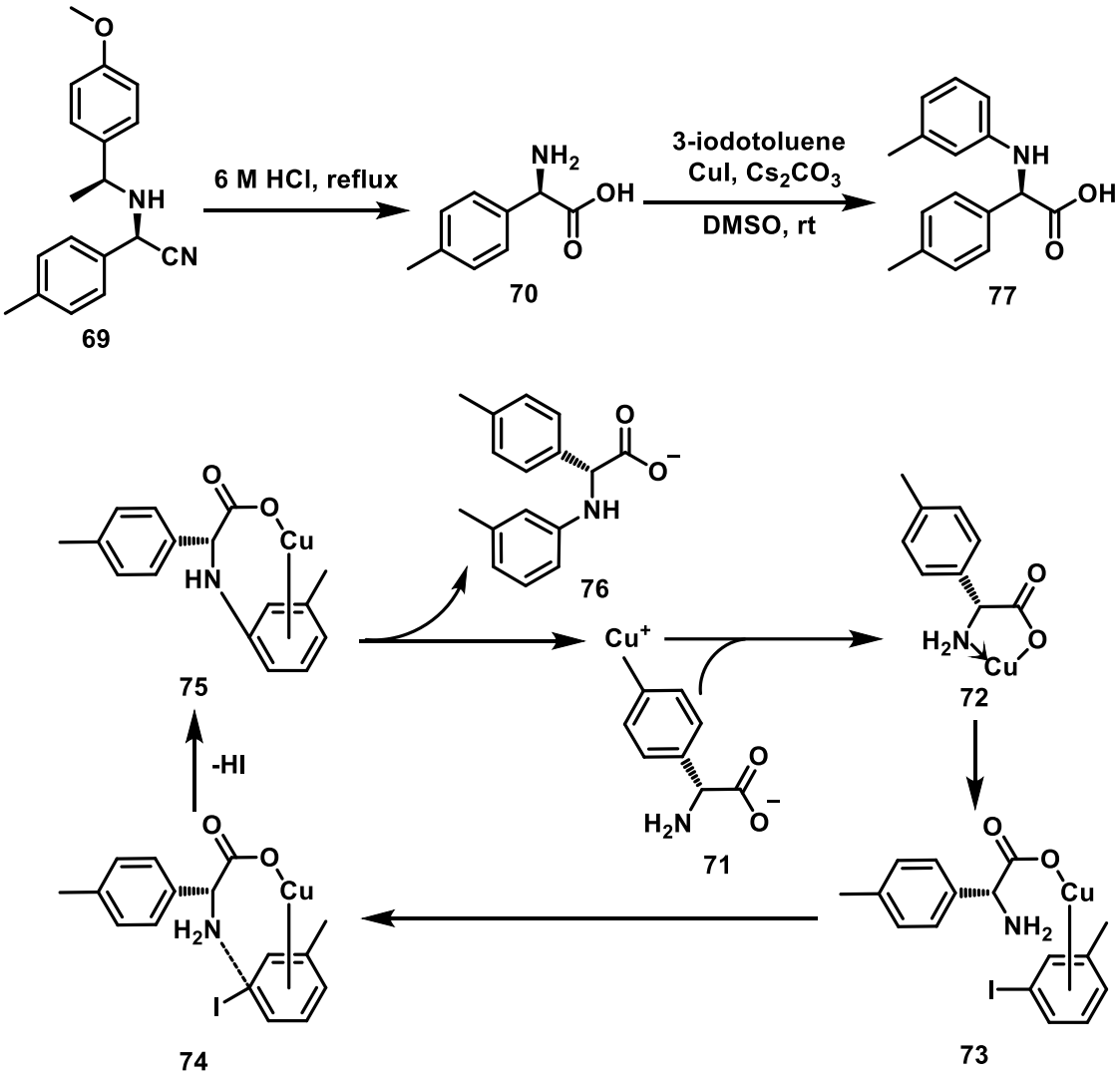
While the Ugi reaction offers a convenient approach to construct α-acylaminoamide derivatives, its lack of stereoselectivity remains an inherent limitation[122]. Although there have been reports of using chiral phosphoric acid (CPA) derivatives as catalysts in the Ugi reaction to prepare chiral α-acylaminoamide derivatives with high stereoselectivity, those approaches are not applicable when both, aromatic aldehydes and aromatic amines, are used as substrates[122, 123]. Therefore, this study opted for the previously described linear synthesis method (section 3.2.2), utilizing enantiopure amino acids as starting materials to obtain the desired optically pure compounds.



Scheme 18. Stereoselective synthesis of α-aminonitrile **69**.

Given that the required enantiopure amino acid is not a natural amino acid and is not commercially available, it had to be synthesized first. As reported in the literature, optically pure (1*S*)-1-(4-methoxyphenyl)ethanamine can be used as chiral auxiliary to obtain a chiral α-aminonitrile *via* the Strecker synthesis[124, 125]. Since this α-aminonitrile comprises two stereocenters, one being newly formed and one originating from the chiral auxiliary, it is formed as a mixture of two diastereomers, which can be separated either by chromatography or recrystallization.

As illustrated in Scheme 18, following the formation of intermediate imine **68**, hydrocyanic acid undergoes a preferential nucleophilic attack from the sterically less hindered side, resulting in (*R*)-2-{[(*S*)-1-(4-methoxyphenyl)ethyl]amino}-2-(*p*-tolyl)acetonitrile **69** as the primary product. The initial attempts to purify **69** through recrystallization from methanol involved heating to 40 °C and subsequent cooling to 0 °C. Based on TLC monitoring, it was observed that the ratio of an impurity in the precipitate and the mother liquor significantly increased, which was supposed to be another diastereomer. It was hypothesized that in a neutral protic solvent, increasing the temperature promotes isomer interconversion. However, when petroleum ether was employed as solvent for recrystallization, **69** was successfully isolated in high purity after heating to 40 °C and subsequent cooling to -10 °C. Subsequently, refluxing α-aminonitrile **69** in 6 M hydrochloric acid led to the formation of (*R*)-2-amino-2-(*p*-tolyl)acetic acid (**70**).

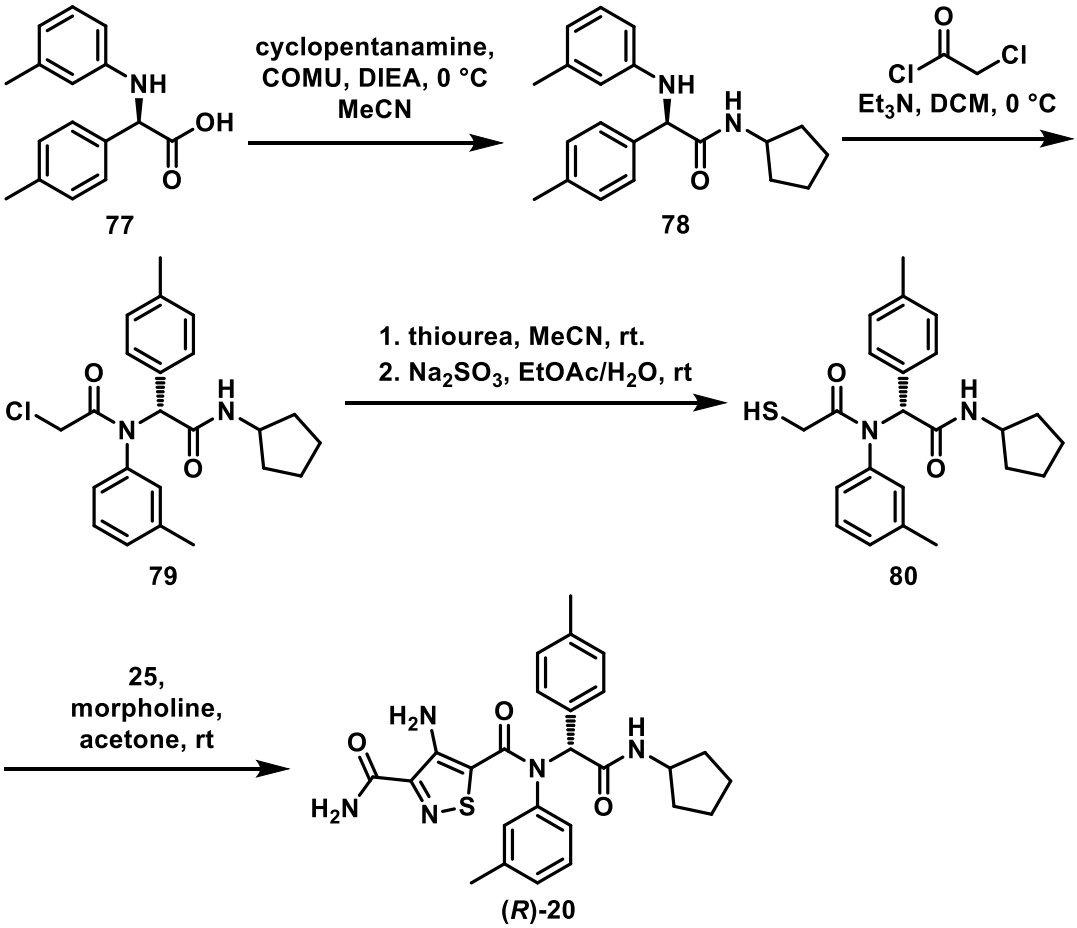


Scheme 19. Syntheses of (*R*)-configured amino acid **77**.

The subsequent step required an *N*-arylation while maintaining optical purity. Literature reports indicate that optically pure *N*-aryl amino acids can be synthesized by reacting aryl iodides with amino acids under alkaline conditions using copper(I) iodide as catalyst[126-128]. The mechanism of the reaction is illustrated in Scheme 19. Initially, the copper(I) ion reacts with α-amino acid salt **71** to form chelate **71**, which then coordinates with an aryl iodide to yield π-complex **72**. This is followed by an intramolecular nucleophilic substitution on the aromatic ring *via* transition state **74**. Ultimately, HI is eliminated with the aid of caesium carbonate, resulting in another π-complex **75**. This complex can decompose to produce coupling product **76** and regenerate the copper(I) ion.

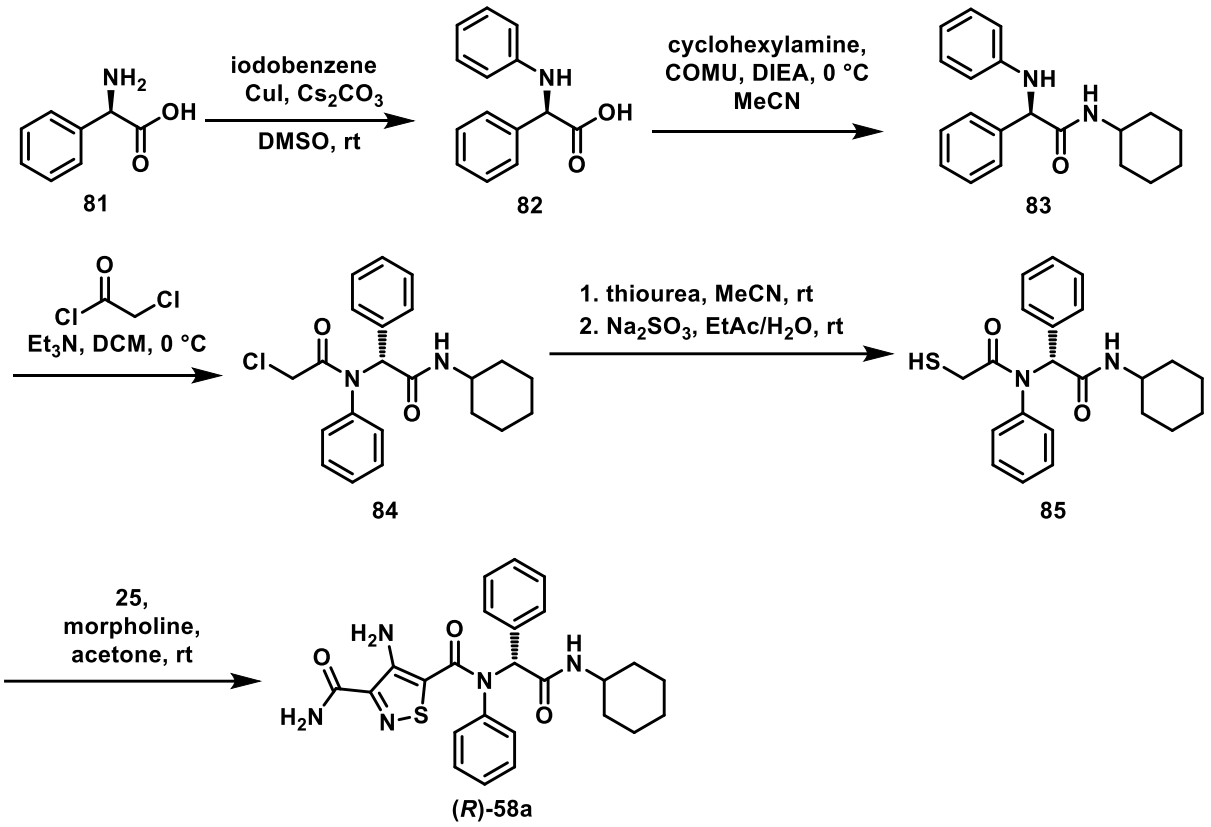
As copper(I) ions are prone to oxidation, in initial experiments, where dissolved oxygen was not rigorously removed from the solvents and reactants, the reaction mixture rapidly turned

blue, and no product formation was observed. Based on the reaction mechanism, the copper(I) ions need to coordinate with the α-amino acid salt to act as a catalyst, whereas copper(II) ions cannot. Thus, by rigorously treating all liquid reagents involved in the reaction *via* the freeze-pump-thaw method, (*R*)-2-(*p*-tolyl)-2-(*m*-tolylamino)acetic acid (**77**) was successfully synthesized with an ee value of 95.6%.



Scheme 20. Syntheses of α-acylaminoamide (*R*)-**20**.

Lastly, following the previously described linear synthesis method (section 3.2.2), *N*-aryl amino acid **77** was coupled with cyclopentanamine using COMU as the coupling agent (Scheme 20). Acylation with chloroacetyl chloride was meticulously temperature-controlled to suppress racemization. The thereby obtained alkyl chloride **79** was treated with thiourea then sodium sulfite to produce thiol **80**, culminating in the cyclization with sulfonate **25** to yield the (*R*)-**20** with an ee value of 97.2%. Similarly, using (1*R*)-1-(4-methoxyphenyl)ethanamine as the chiral auxiliary for the initial preparation of the respective α-aminonitrile, the (*S*)-**20** could be obtained with an ee of 97.0%.

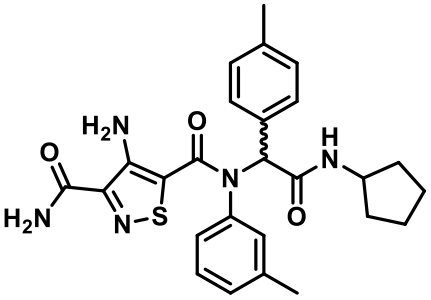


Scheme 21. Syntheses of α-acylaminoamide (*R*)-**58a**.

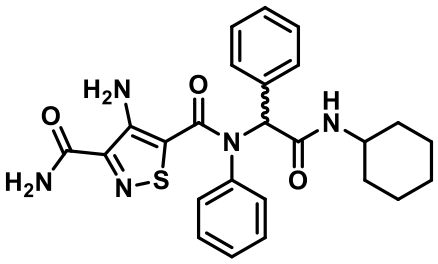
For the synthesis of compounds (*R*)-**58a** and (*S*)-**58a**, (*R*)- and (*S*)-configured 2-amino-2-phenylacetic acid, which were commercially available in optically pure form, were used as starting material (Scheme 21). Each of the two enantiomers was *N*-arylated, coupled with cyclohexylamine, acylated with chloroacetyl chloride, treated with thiourea and sodium sulfite to produce the respective thiols, and finally cyclized to yield the enantiopure target compounds (*R*)-**58a** and (*S*)-**58a** with ee values of 96.6% and 99.8%, respectively.

3.5.2 Biological evaluation of (*S*)/(*R*)-20 and (*S*)/(*R*)-58a

Table 9. Antiviral activity of racemic and enantiomerically pure α-acylaminoamide derivatives (*S*)/(*R*)-**20** and (*S*)/(*R*)-**58a** against influenza viruses A/pdm09 (H1N1) and A/HK/1/68 (H3N2).



R: (*R*)-20
S: (*S*)-20
Rac: 20



R: (*R*)-58a
S: (*S*)-58a
Rac: 58a

Compound	IC_{50} [μM]		CPE inhibition [%]*	
	A/pdm09(H1N1)	A/HK/1/68(H3N2)	@100 μM	@31.6 μM
20	12.95 ± 2.52	6.67	102.64 ± 10.48	87.37 ± 9.30
(*R*)-**20**	n.a.*	~100	45.39 ± 18.36	13.02 ± 3.02
(*S*)-**20**	n.a.*	~100	52.19 ± 12.72	13.63 ± 7.07
58a	17.33 ± 5.38	2.71	101.20 ± 12.23	85.62 ±10.25
(*R*)-**58a**	n.a.*	not tested	52.46 ± 6.15	-2.45 ± 15.60
(*S*)-**58a**	n.a.*	not tested	4.04 ± 18.35	-5.39 ±11.63
nucleozin	> 100	0.87	-	-

* n.a.- not active

** Inhibition of influenza virus A/pdm09(H1N1)-induced CPE at different concentrations.

The initial objective behind synthesizing the enantiomers of the representative compound **58a** and lead compound **20** was to discern which stereoconfiguration would be more favorable for activity. Intriguingly, the data presented in Table 9 and Figure 32 reveal a notable discrepancy in the antiviral activity between the racemic mixtures and their respective enantiomers. While the racemic mixtures of both compounds exhibited appreciable activity, neither the (*S*)- nor the (*R*)-enantiomers individually manifested significant efficacy, with IC_{50} values exceeding 100 μM for all four compounds.

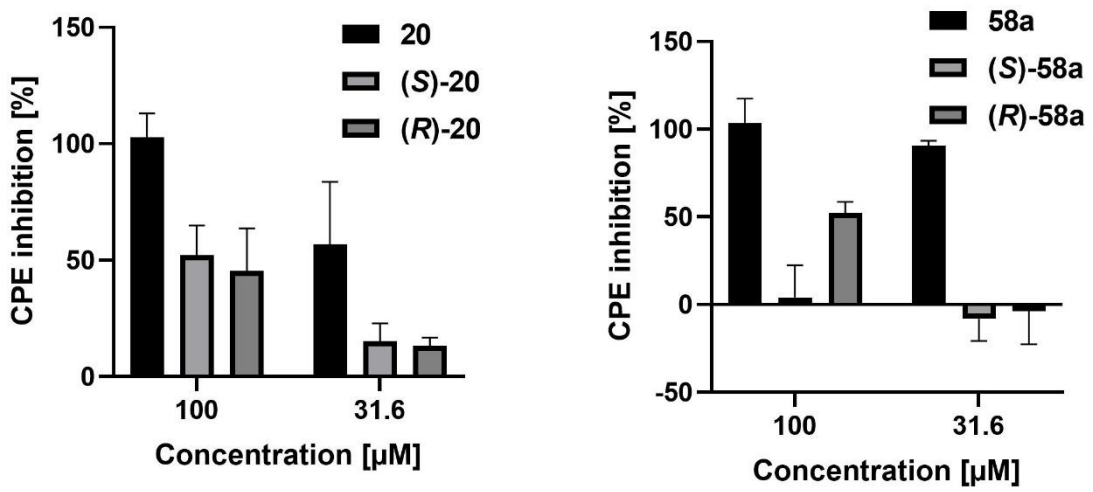


Figure 32. Inhibition of the influenza virus A/pdm09(H1N1)-induced CPE by **20** and **58a** as well as the enantiomers of these compounds in MDCK cells. Means and standard deviations of 4 independent assays each with 2 parallels per concentration are shown.

Specifically, (*S*)-**20** and (*R*)-**20**, even at a dosing concentration of 100 µM, only achieved approximately 50% inhibition of influenza virus A/pdm09(H1N1)-induced CPE, without a discernible difference between them. Similarly, at a dosing concentration of 31.6 µM, both enantiomers exhibited a comparable activity level of around 13%. In contrast, (*R*)-**58a** demonstrated a superior inhibition rate of 52% at 100 µM compared to (*S*)-**58a**. However, at a concentration of 31.6 µM, neither (*S*)-**58a** nor (*R*)-**58a** exhibited discernible inhibitory effects against the virus.

The occurrence where a racemic mixture displays biological activity, but its individual enantiomers remain inactive, is a rarity in literature. Several hypotheses can be postulated to explain this phenomenon:

1. **Heterochiral Interactions**: The racemic mixture might form dimers or aggregates wherein one enantiomer interacts with its counterpart. These heterochiral species could be the biologically active entities. When the enantiomers are isolated, these interactions are disrupted, leading to a loss in activity.
2. **Multi-site Binding**: The target protein or receptor might possess multiple binding sites. The racemic mixture, due to the presence of both enantiomers, might be capable of engaging with these sites simultaneously. In contrast, individual enantiomers might not facilitate this multi-site interaction.

3. **Cooperative Binding**: One enantiomer might bind to the target, inducing a structural alteration that facilitates the binding of the other enantiomer at a distinct site. The racemic mixture would enable this synergistic binding, whereas the individual enantiomers would be unable to replicate this effect.

While the racemic mixtures of compounds **58a** and **20** showed significant activity, their individual enantiomers did not, suggesting unique interactions or binding mechanisms exclusive to the combined form. Further studies are warranted to pinpoint the exact mechanism behind this observation.

3.6 α-Acylaminoamides with varied R^4 groups

3.6.1 Synthesis of α-acylaminoamide derivatives 87, 88, 89a-f

While prior studies had not considered modifying the isothiazole ring, the efforts to introduce solubility-enhancing groups at the three alternative modification sites had led to diminished or even complete loss of activity. To enhance either activity or solubility and to particularly investigate the role of the isothiazole ring system, a series of α-acylaminoamide derivatives featuring various aromatic and heteroaromatic rings at the R^4 position were synthesized. Their respective structures are depicted in Figure 33.

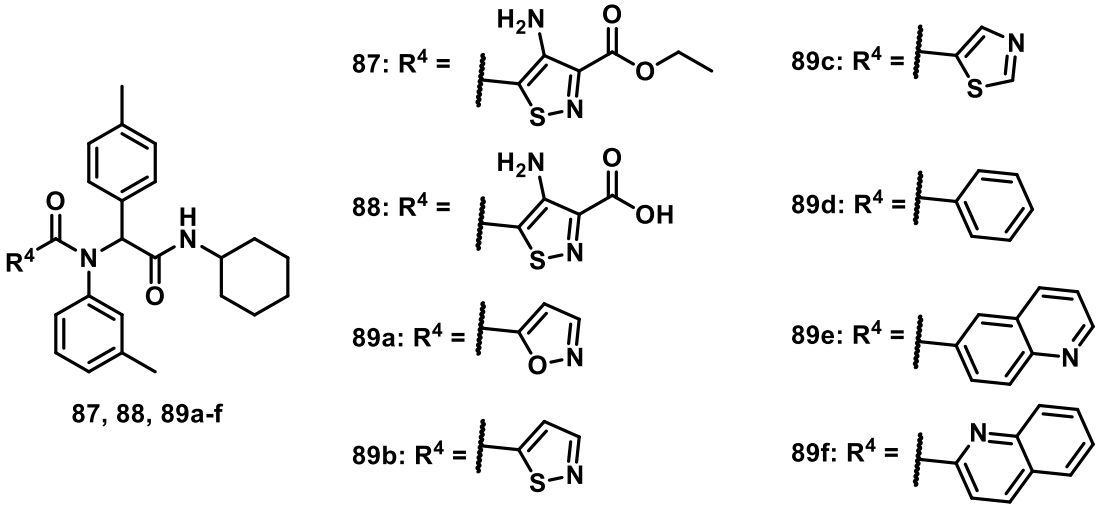
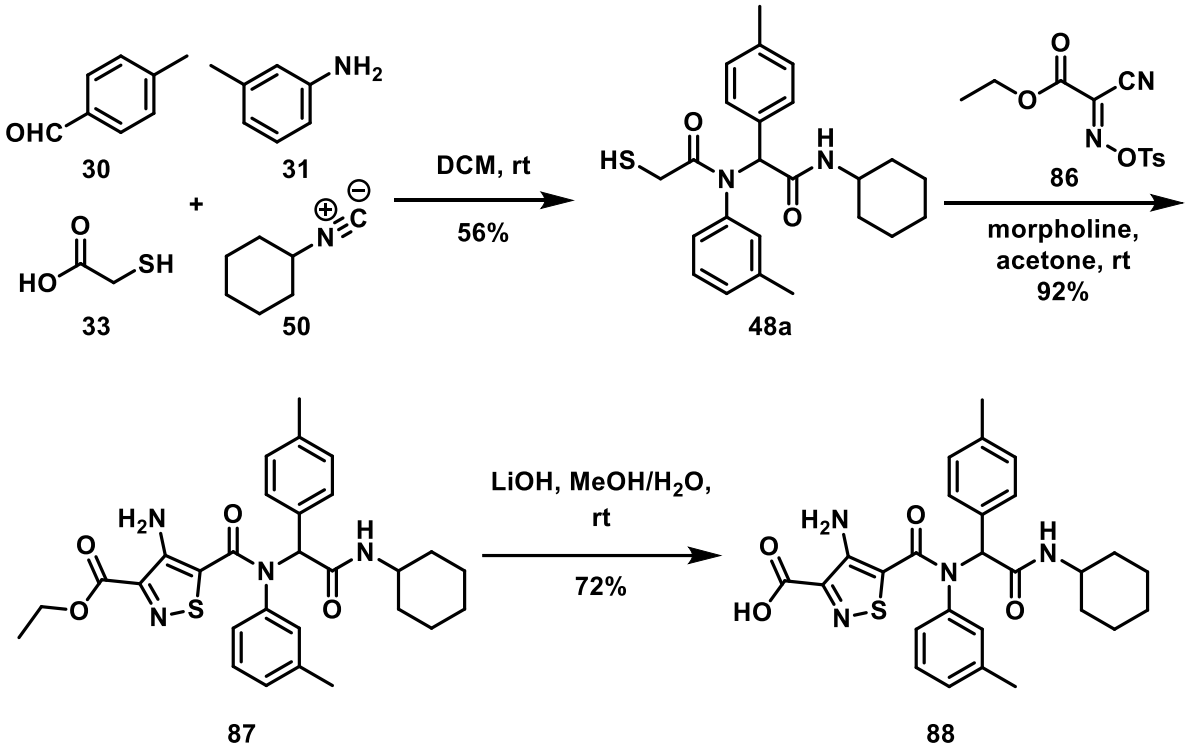


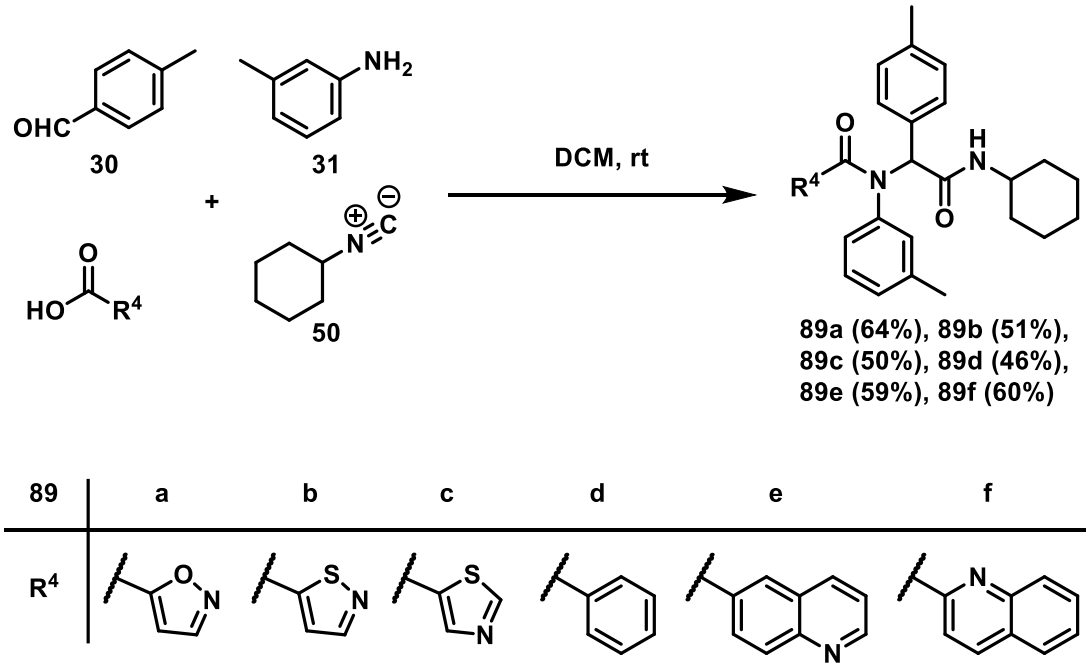
Figure 33. Structures of compounds **87, 88, 89a-f**.

The synthetic methodology outlined in section 3.1.1 remained applicable for the preparation of isothiazole derivatives **87** and **88**. However, the cyclization step was modified, employing ester **86** as substrate, which led to the formation of isothiazole derivative **87** in 92% yield. Subsequently, this compound was subjected to hydrolysis utilizing lithium hydroxide, resulting in the formation of carboxylic acid **88** in a yield of 72%.

The substituent at the R^4 position is derived from the carboxylic acid component in the Ugi reaction. For commercially available aromatic carboxylic acids, the procedure outlined in section 3.1.1 was followed. Using *p*-tolualdehyde, *m*-toluidine, cyclohexylisocyanide, and the respective aromatic carboxylic acid as reactants, α-acylaminoamide derivatives containing various aromatic rings at the R^4 position were synthesized *via* Ugi reactions, achieving yields ranging from 46% to 64%.



Scheme 22. Syntheses of α-acylaminoamides **87** and **88**.

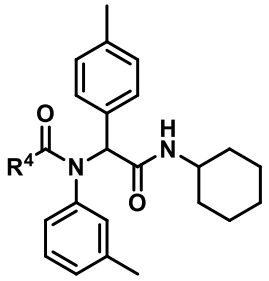


Scheme 23. Syntheses of α-acylaminoamides **89a-f** with varied R^4 groups.

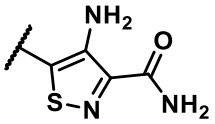
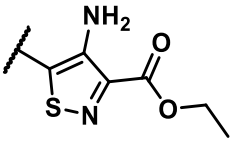
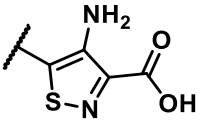
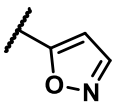
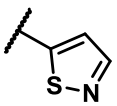
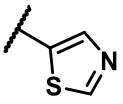
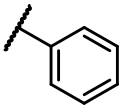
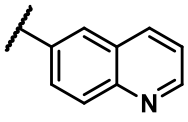
3.6.2 Biological evaluation of 87, 88, 89a-f

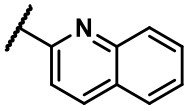
The synthesized series of α-acylaminoamide derivatives featuring various aromatic and heteroaromatic rings at the R^4 position were tested for antiviral activity against the influenza A/(H1N1)pdm09 strain. The results of the biological evaluation are summarized in Table 10.

Table 10. Cytotoxicity and activity of α-acylaminoamide derivatives **87, 88, 89a-f** against influenza virus A/pdm09 (H1N1).



87, 88, 89a-f

Compound	R^4	CC_{50} [μM]	IC_{50} [μM]
49a		>100	16.92 ± 5.32
87		>100	10.53 ± 3.86
88		>100	7.14 ± 0.42
89a		>100	17.86 ± 4.73
89b		>100	14.55 ± 4.87
89c		>100	13.97 ± 2.92
89d		30.34 ± 10.26	n.a.*
89e		>100	n.a.*

Compound	R^4	CC_{50} [μM]	IC_{50} [μM]
89f		>100	~100
nucleozin			

*n.a.- not active

Results demonstrated the importance of the five-membered aromatic heterocycle for activity. The phenyl (**89d**) and quinoline derivatives (**89e** and **89f**) showed no activity, with **89d** additionally exhibiting notable cytotoxicity. Both, the thiazole (**89c**) and isothiazole (**89b**) derivatives, displayed comparable activities, marginally surpassing isoxazole derivative **89a**. Maintaining an amino group at the 4-position of the isothiazole ring but altering the 3-position amide to either an ethyl ester moiety (**87**) or a carboxylic acid (**88**) enhanced activity. Notably, carboxylic acid **88**, with an IC_{50} of 7.14 μM, not only showed promising activity but also displayed enhanced water solubility due to its carboxyl group, marking it a good candidate for further investigation.

3.7 Evaluation of binding affinity between influenza virus nucleoprotein and α-acylaminoamides

Grating-Coupled Interferometry (GCI) is a highly sensitive optical detection technique used to study molecular interactions on surfaces. This technique hinges on the principle of detecting minute changes in the refractive index on a sensor surface where biomolecules bind. In GCI, light is directed into a waveguide using a grating, creating an interference pattern sensitive to surface refractive index changes. When molecules interact on the sensor surface, alterations in this pattern occur, allowing for precise, real-time analysis of binding events without the need for labeling.

This study utilized GCI to investigate the affinity of α-acylaminoamide derivatives toward influenza virus NP, aiming to confirm the NP as the target of the compounds. Nucleozin was employed as positive control. The influenza A/HK/1/68(H3N2) NP, equipped with a His-Tag, was chosen for the experiments, and a PCH-NTA chip was utilized due to its proficiency in capturing and coupling His-tagged ligands to the surface.

The GCI experiment was carried out under the expertise of Mr. Sebastian Bayer at the University of Vienna, and we express our sincere appreciation for his contribution.

Table 11. Binding affinity of α-acylaminoamide derivatives for influenza virus NP.

Compound	IC_{50} [μM]		Calculated K_D [μM]
	A/pdm09(H1N1)	A/HK/1/68(H3N2)	
49c	25.74 ± 4.95	18.12	8.0
52n	17.36 ± 3.99	5.55	-
54l	12.86 ± 4.29	7.94	-
57h	17.60 ± 3.07	not tested	-
(*R*)-**58a**	n.a.*	not tested	432.0
(*S*)-**58a**	n.a.*	not tested	265.5
88	7.14 ± 0.42	not tested	3.0
nucleozin	> 100	0.87	21.4

*n.a.- not active

The procedure was executed at 25 °C using a Creoptix® WAVEsystem. The chip surface was initially activated with EDCI/NHS, followed by NP immobilization, achieving a level of 7,000 Units over 7 minutes. Subsequently, any remaining active sites were neutralized with 1 M ethanolamine.

Compounds were prepared through serial dilution in a 20 mM HEPES buffer (pH 7.0), supplemented with 150 mM NaCl, 2 mM TCEP, 0.2% DMSO, and 0.005% Tween. The analysis protocol included 10 startup measurements, a blank measurement between each run, and three DMSO corrections (at the beginning, middle, and end), utilizing samples at five two-fold dilutions (10 μM, 5 μM, 2.5 μM, 1.25 μM, 0.625 μM). Each sample was analyzed in ascending order of concentration and then in the reverse order. The K_D values were determined using a steady-state affinity model provided by the WAVEsystem analysis software.

The results indicated that **88**, which has the best activity against the A/pdm09(H1N1) strain, demonstrates strong affinity for the NP with a calculated K_D of 3 μM, which is lower than the one for the positive control nucleozin. *N*-butylamide **49c**, which exhibited moderate antiviral activity *in vitro*, showed a substantial affinity for the NP with a calculated K_D value of 8.0 μM, thus exhibiting higher affinity than nucleozin but weaker than **88**. Despite demonstrating no significant antiviral activity, the optically pure compounds (*R*)-**58a** and (*S*)-**58a** were found to be able to bind to the NP, however with low affinity. Specifically, (*S*)-**58a** exhibited higher affinity compared to (*R*)-**58a**, with respective K_D values of 265.5 μM and 432.0 μM.

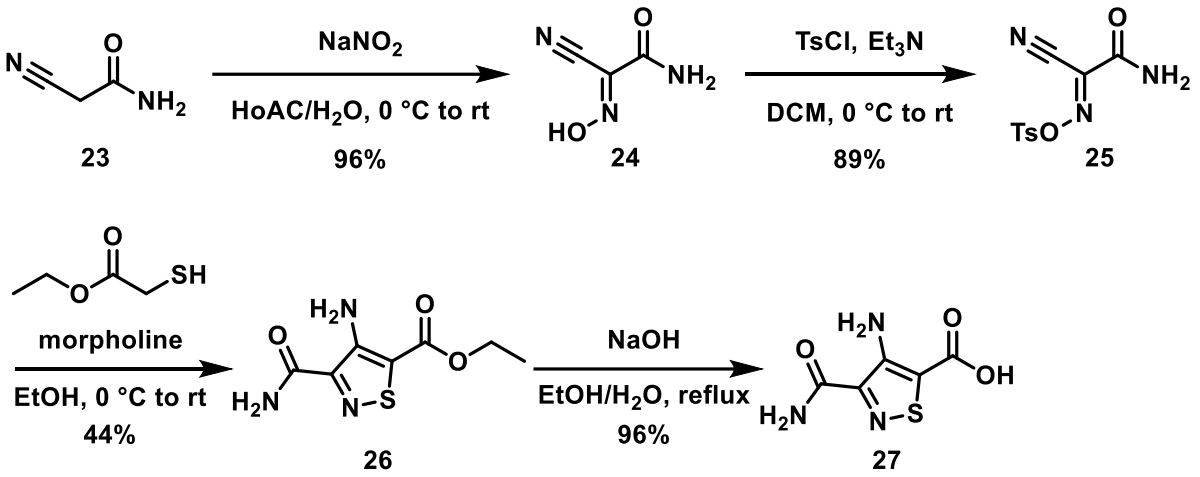
Typically, the trend of GCI response alterations positively correlates with the concentration of the analyte, wherein an elevated analyte concentration generally causes a larger GCI response. Nonetheless, for **52n, 54l**, and **57h**, which demonstrated commendable activity against various strains, an inverse relationship was observed, with their GCI response values diminishing with escalating concentration. Thus, potential experimental inaccuracies or technical complications require exclusion.

4.0 CONCLUSION AND SUMMARY

In this study, a total of 84 novel target compounds were designed and synthesized, with their structures being confirmed through ^{1}H NMR, ^{13}C NMR, and IR spectroscopy as well as HRMS. The SAR of these target compounds in inhibiting the IAV was systematically elaborated.

Lead compound **20** was identified through virtual screening, and its structure exhibits an α-acylaminoamide scaffold containing an isothiazole moiety.

As the isothiazole moiety represents a crucial component of the target compounds, 4-amino-3-carbamoylisothiazole-5-carboxylic acid (**27**) and the essential intermediate **25** required for its synthesis were produced (Scheme 39). The synthetic methods were adapted from literature procedures.



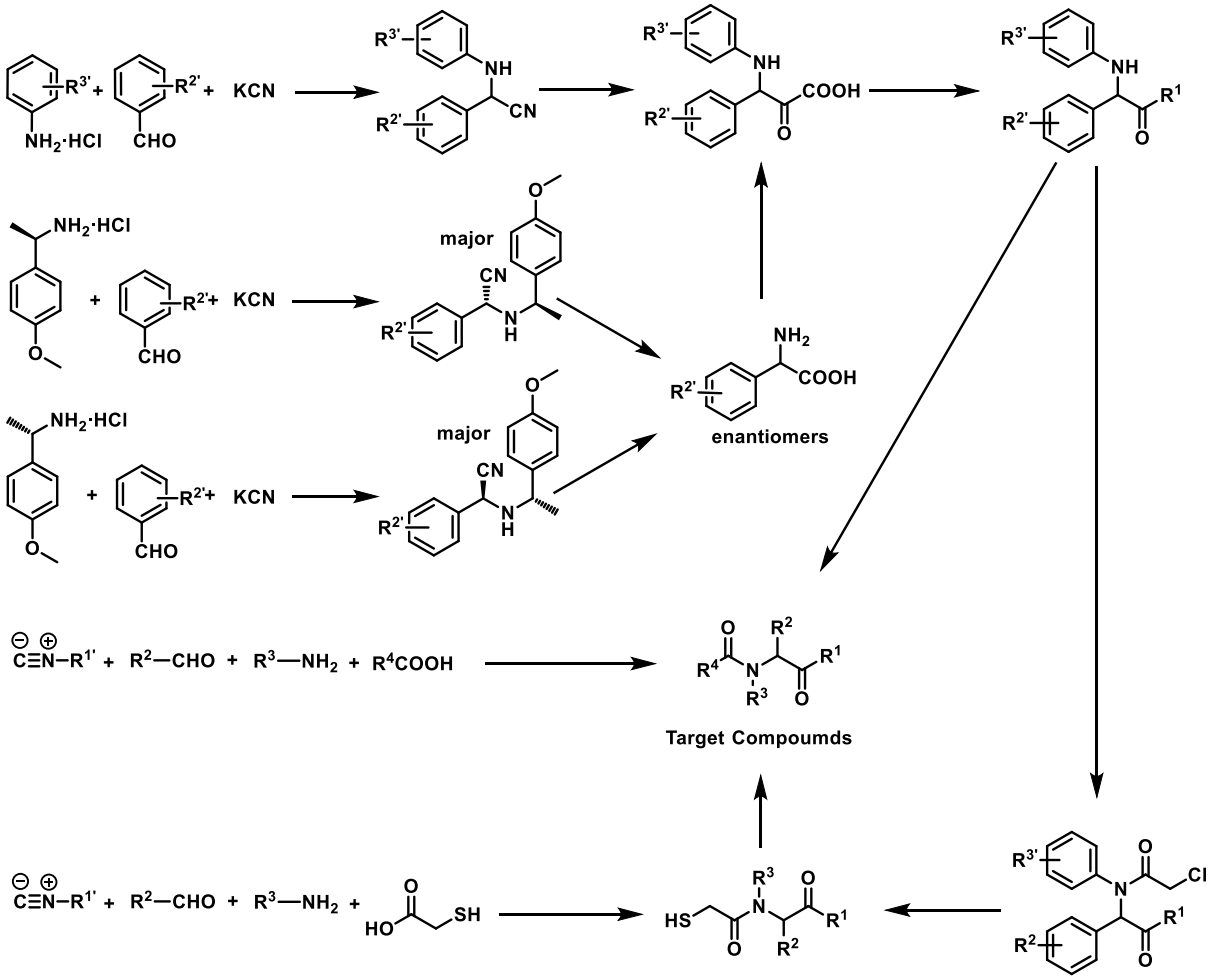
Scheme 24. Syntheses of key intermediate **25** and 4-amino-3-carbamoylisothiazole-5-carboxylic acid **27**.

Cyanoacetamide (**23**) was nitrated using sodium nitrite in acetic acid, resulting in oxime (**24**). This oxime was then reacted with *p*-toluenesulfonyl chloride. After recrystallization from a petroleum ether/ethyl acetate (2:1) mixture, the key intermediate **25** was obtained in 89% yield in batches of 20 g.

Following the coupling reaction of sulfonate **25** with ethyl 2-sulfanylacetate, the resulting intermediate underwent a Thorpe-like cyclization reaction. Subsequent hydrolysis with sodium hydroxide yielded 4-amino-3-carbamoylisothiazole-5-carboxylic acid **27**.

The target compounds, representing α-acylaminoamide derivatives, were typically obtained *via* Ugi reactions or through linear syntheses (Scheme 25).

The racemic α-acylaminoamide derivatives were synthesized using various isocyanides, aldehydes, amines, and carboxylic acids, serving as sources for residues R^1, R^2, R^3, and R^4 in the target molecules, respectively. The target compounds could be achieved in a single step *via* the Ugi reaction, either in methanol or dichloromethane. However, in case of the isothiazole derivatives, due to the poor solubility of both, the starting materials and the final products, in common solvents, their yields were significantly low.



Scheme 25. Summary of the performed syntheses to access the envisaged α-acylaminoamide derivatives.

Therefore, an alternative two-step synthetic route was adopted. The α-acylaminoamide scaffold was initially established *via* an Ugi reaction, utilizing 2-sulfanylacetic acid as the carboxylic acid. The 2-sulfanylacetyl group of the resulting α-acylaminoamide was then used to construct the isothiazole ring. This was achieved by condensing it with **25** in the presence of morpholine,

leading to the isothiazole-containing α-acylaminoamide derivatives. Overall, this new approach has significantly improved the total yield compared to previous methods.

The racemic forms of α-acylaminoamide derivatives could also be synthesized through the sequential assembly of amino acids. For commercially unavailable isocyanides, a flexible linear synthesis strategy was employed. Initially, aldehydes and amines were used as the sources for the R^2 and R^3 portions of the target compounds, respectively. Through the Strecker synthesis, racemic aminonitriles was obtained. In subsequent steps, the nitriles were treated with hydrogen peroxide and DMSO in the presence of an excess of potassium carbonate. The resulting aminocarbamides were then hydrolyzed in a sodium hydroxide solution, yielding *N*-substituted amino acids. Considering the subsequent need to prepare optically pure α-acylaminoamides, COMU was chosen as the coupling agent. In the presence of DIEA, the *N*-substituted amino acids, once activated with COMU, reacted with the corresponding amines, producing intermediates with the respective R^1 substitution. After subsequent reactions with chloroacetyl chloride and thiourea, the thiol-containing intermediates were obtained. The isothiazole-containing α-acylaminoamide derivatives were then synthesized using the aforementioned method.

For the synthesis of optically pure α-acylaminoamide derivatives, enantiomerically pure 1-(4-methoxyphenyl)ethan-1-amine was used as chiral auxiliary. Utilizing aromatic aldehydes as the source for the R^2 portion of the target compounds, chiral α-aminonitriles could be acquired through the Strecker synthesis. These α-aminonitriles contain two stereocenters, and their diastereomers could be separated either chromatographically or by recrystallization. Subsequent reflux in 6 M hydrochloric acid yielded the corresponding amino acids. Following this, using copper(I) iodide as catalyst under basic conditions, the amino acids were reacted with an aryl iodide, which served as the source for the R^3 portion of the target compounds, to produce the enantiomerically pure *N*-arylamino acids. Finally, following the aforementioned linear synthesis strategy, the envisaged optically pure α-acylaminoamide derivatives were obtained, with ee values consistently exceeding 95%.

In conclusion, based on the methods described above, it was possible to introduce the envisaged groups at various positions on the target molecule or to synthesize the required enantiomers. For racemic forms, starting materials are not restricted to aromatic amines and aldehydes; aliphatic amines and aldehydes can also be employed in this synthetic route. However, for the stereoisomers of the representative compounds, due to the limitations of the reaction mechanism, the aforementioned methods are only applicable to targets where the R^3 position is an aromatic ring.

Based on the biological evaluation of the synthesized target compounds as well as the performed GCI studies, the following conclusions were drawn:

1. The majority of synthesized α-acylaminoamide derivatives effectively inhibited the proliferation of the IAV with relatively low toxicity. Furthermore, these compounds also demonstrated a clear inhibitory effect on strain resistant to nucleozin.
2. When modifying the R^1 position, carboxylic acid derivative **35**, carboxylic acid derivative **36**, primary amide **42**, and *N*-methyl substituted amide **47c** did not exhibit significant antiviral activity. However, the introduction of an n-butyl chain (**49c**) or a bulky tert-butyl substituent (**49b**) on the amide nitrogen enhanced the activity. When introducing rings of different sizes onto the amide nitrogen, most derivatives displayed activity. Yet, the antiviral properties of *N*-cyclopropyl derivative **47a**, *N*-cyclobutyl derivative **47b**, and *N*-phenyl derivative **49d** were significantly reduced, with the *N*-benzyl substituted derivative **49e** slightly outperforming **49d**. Substituting the γ-carbon of the n-butyl chain in secondary amide **49c** with an oxygen atom yielded compound **47d**, which manifested a notable reduction in activity. Furthermore, studies on tertiary amides bearing a six-membered ring revealed that both pyridine derivative **43** and morpholine derivative **47e** lacked activity, with **43** also showing enhanced toxicity.
   These findings suggest that substituents of a specific volume on the secondary amide nitrogen play a crucial role in modulating activity. Smaller saturated rings and phenyl groups might be detrimental to the activity, and introducing an oxygen atom into the substituent on the amide nitrogen may be unfavorable for maintaining activity.
3. At the R^2 position, methyl (**52s**) and cyclohexyl (**52q**) substituents caused weaker activity, while the introduction of a phenyl ring enhanced the activity. For compounds bearing electron-donating groups on the phenyl ring, the activity varied depending on the position of the substituent. Notably, the *ortho*-substituted methyl compound (**52c**) demonstrated significantly reduced activity compared to its *meta*- (**52b**) and *para*-substituted (**49a**) counterparts. The 3,5-dimethylphenyl derivative (**52f**) displayed intermediate activity. Moreover, increasing the alkyl chain length at the *para* position or introducing larger groups at this position led to decreased activity. Among the *para*-substituted compounds, the ethyl (**52d**) and isopropyl (**52e**) derivatives showed superior activity compared to methyl-substituted compound **49a**. Among the compounds with heteroatom-containing substituents, methoxy-substituted compound **52n** was less active than hydroxy-substituted compound **22**. Overall, all compounds with electron-donating groups on the R2 phenyl ring showed lower activity than the unsubstituted compounds.

When introducing a halogen atom at the *para* position of the phenyl ring, the observed activity followed the order: chlorine (**52h**) > bromine (**52k**) > fluorine (**52g**). Notably, *para*-chlorophenyl derivative **52h** exhibited the highest potency with an IC_{50} value of 8.97 μM, surpassing the unsubstituted compound **52a**. Among the chlorine-substituted compounds, both *meta*- (**52i**) and *ortho*-substitution (**52j**) showed significantly decreased activity compared to the *para*-substituted version. This suggests that introducing substituents at the *ortho* position on the phenyl ring might be detrimental to activity. Among the other electron-withdrawing groups introduced at the *para*-position, the nitrile stood out with an IC_{50} value of 7.35 μM. The trifluoromethyl (**52m**) and methoxycarbonyl substituted compounds (**52q**) exhibited activities only slightly lower than the unsubstituted compound **52a**, while the activity of the trifluoromethoxy derivative (**52o**) was substantially reduced. Efforts to enhance solubility by introducing a carboxylic group at the *para*-position were unsuccessful, as this modification completely abrogated the anti-influenza activity.

These findings highlight the significant influence of substituents on the phenyl ring at the R^2 position on activity. Specifically, *ortho*-substitution appears to be detrimental, while electron-withdrawing groups generally outperform electron-donating groups.

4. Modifications at the R^3 position indicated that the introduction of a methyl (**54s**) and a benzhydryl (**54u**) group negatively impacts activity. Specifically, compound **54u** displayed cytotoxicity with a CC_{50} value of 33.27 μM. The benzyl-substituted compound **54t** showed only weak activity. In contrast, the phenyl-substituted compound **54a** demonstrated enhanced activity, with an IC_{50} value of 7.96 μM.

   For derivatives with electron-donating groups on the phenyl ring, the introduction of either a methyl (e.g., **49a**, **54b**, **54c**) or a methoxy group (e.g., **54g**, **54h**) resulted in activities lower than the one of unsubstituted compound, with the position of these substituents having minimal impact on the outcomes. However, compound **54c** with *ortho*-methyl substitution exhibited cytotoxicity. Compared to the *meta*-methyl derivative **49a**, the introduction of a *meta*-ethyl group (**54d**) resulted in decreased activity. Among the derivatives with two methyl groups on the phenyl ring, the 3,5-disubstituted **54f** outperformed the 3,4-disubstituted compound **54e**. Notably, **54f** had an IC_{50} value of 10.75 μM, making it the most active among the compounds with electron-donating groups.

   Among the halogenated phenyl derivatives, the fluorine-substituted compounds outperformed those substituted with chlorine. However, the 3,5-dichloro derivative **54n** displayed improved activity, with an IC_{50} value of 9.46 μM. For the trifluoromethyl-

substituted derivatives, the *meta*-substituted regioisomer **54p**, with an IC_{50} value of 11.13 μM, was superior to the *ortho*-substituted compound **54q**. For other electron-withdrawing groups, the *para*-nitro substituted compound (**54r**) had decreased activity, whereas the *meta*-cyanide (**54o**) exhibited better activity with an IC_{50} value of 7.56 μM, surpassing the activity of the unsubstituted compound **54a**.

These findings suggest that the phenyl ring at the R^3 position is also pivotal for activity. *Ortho* substitution on the phenyl ring adversely affects activity and induces cytotoxicity, whereas an unsubstituted phenyl ring or one with a *meta*-electron-withdrawing cyanide group is more beneficial for antiviral activity.

5. Modifications at the R^4 position revealed that the five-membered heteroaromatic ring is crucial for activity. Both phenyl (**89d**) and quinoline derivatives (**89e** and **89f**) displayed no activity, with **89d** also demonstrating significant cytotoxicity. The activities of the investigated isothiazole (**89b**) and thiazole (**89c**) derivatives were comparable, both slightly surpassing that of isothiazole derivative, **89a**. Retaining the amino group at position 4 of the isothiazole ring while substituting the amide at position 3 with an ethyl ester group (**87**) or a carboxylic acid (**88**) led to enhanced activity. Notably, **88**, with an IC_{50} value of 7.14 μM, not only displayed superior activity but also exhibited better water solubility due to its carboxyl group, making it a promising candidate for further research.
6. Neither the (S)- nor the (R)-enantiomers demonstrated significant efficacy individually. Both (*S*)-**20** and (*R*)-**20** only achieved about 50% inhibition of influenza virus A/pdm09(H1N1)-induced CPE at 100 μM, and roughly 13% at 31.6 μM. (*R*)-**58a** had a slightly higher inhibition rate than (*S*)-**58a** at 100 μM, but both were ineffective at 31.6 μM, contrasting sharply with the promising antiviral activity of their racemic mixtures.
7. Binding affinity results of α-acylaminoamide derivatives to the influenza virus NP revealed that both **88** and **49c** exhibit strong binding affinities, with their K_D values surpassing that of the positive control nucleozin. Interestingly, (*R*)-**58a** and (*S*)-**58a**, which did not display significant antiviral activity *in vitro*, were still able to bind to the NP, albeit with lower affinities. Among them, (*S*)-**58a** demonstrated a slightly higher affinity. This suggests that these compounds indeed inhibit the proliferation of the IAV by targeting NP. Therefore, the isothiazole-containing α-acylaminoamide represent promising lead structures for the further development of influenza A NP inhibitors.

5.0 EXPERIMENTAL SECTION

5.1 General procedures and materials

5.1.1 General remarks

Moisture and oxygen sensitive reactions were carried out under nitrogen, dried with molecular sieves (3 Å, 8 to 12 mesh, Acros Organics), in dry glassware (Schlenk flasks, sealed with rubbersepta). Reaction mixtures were stirred with magnetic stirrer RCT basic (IKA).

Reaction temperatures were controlled with:

- Cryostat (Julabo FT902),
- Ice/water (0 °C),
- Magnetic stirrer MR 3001 K (Heidolph), together with temperature controller EKT HeiCON (Heidolph) and paraffin oil bath.

5.1.2 Solvents

Anhydrous solvents were purchased from Acros Organics (extra dried over molecular sieves). Solvents for flash column chromatography were of technical grade and distilled prior to use. Ultrapure water for HPLC was obtained by using a Sartorius arium® pro system (Sartopore 0.2 µm, UV). Acetonitrile, ethanol and n-Hexane for HPLC was purchased from VWR (HPLC grade).

5.1.3 Thin layer chromatography (TLC)

Thin-layer chromatography was performed on Macherey Nagel precoated TLC sheets (ALUGRAM® Xtra SIL G/UV254) and conducted in a saturated chamber at ambient temperature. Visualization was achieved by UV light (254 nm) and by heat-staining using a cerium molybdate dipping bath [$Ce(SO_4)_2$ (1.8 g), $(NH_4)_6Mo_7O_{24} \times 4\ H_2O$ (45 g), conc. H_2SO_4 (45 g), H_2O (900 mL)]. Compositions of the mobile phase and retention factors (R_f) of the compounds are given in the descriptions of the synthetic procedures. As the Rf-values strongly depend on the exact ratio of the components of the mobile phase and some of these are highly volatile, the given R_f-values are just approximate values.

5.1.4 Flash column chromatography

For flash column chromatography, Macherey Nagel silica gel 60 M (40-63 µm) was used as stationary phase. Pressure was applied with compressed air. In the descriptions of the synthetic procedures, the diameter of the column (Ø), height of the stationary phase (h), fraction size (V), the eluent, and R_f-values are given in brackets.

5.1.5 Melting points

Melting points were determined with a Büchi Melting Point M-565. The values are uncorrected.

5.1.6 HPLC

All HPLC methods were carried out at ambient temperature.

HPLC method 1

- VWR Hitachi equipment:
  - pump: 5160
  - autosampler: 5260
  - column oven: 5310
  - UV/VIS detector: 5420
  - interface: organizer
  - data acquisition and evaluation: Chromaster software
- Column:
  - LiChrospher® 60 RP-select B (5 µm)
  - LiCroCART® 250-4 mm cartridge
- Guard Column:
  - LiChrospher® 60 RP-select B (5 µm)
  - LiCroCART® 4-4 mm cartridge
- Solvents:
  - A: water with 0.05 % (V/V) trifluoroacetic acid
  - B: acetonitrile with 0.05 % (V/V) trifluoroacetic acid
- Gradient:
-

Time [min]	Solvent A [%]	Solvent B [%]
0.0	90.0	10.0
4.0	90.0	10.0
29.0	0.0	100.0
31.0	0.0	100.0
31.5	90.0	10.0
40.0	90.0	10.0

- Flow rate: 1.0 mL/min
- Injection volume: 5.0 µL, method: cut
- Detection wavelength: 210 nm
- Stop time: 30.0 min

- Calculation:
  - integration: manual
  - calculation method: area %
  - use of blank substraction from the same series

Chiral HPLC method 2

- KNAUER Isocratic HPLC system:
  - Pump: AZURA P 4.1S
  - UV/VIS detector: AZURA UVD 2.1S
  - data acquisition and evaluation: Mobile Control, ClarityChrom®
- Column:
  - CHIRALPAK® IB (5 µm)
  - Sized 250 mm × 4.6 mm
- Guard Column:
  - GUARD CARTRIDGES 100 mm × 4.6 mm
- Solvents:
  - A: n-Hexane
  - B: Ethanol
- Mobile Phase: A/B = 80/20
- Flow rate: 1.0 mL/min
- Injection volume: 5.0 µL, method: cut
- Detection wavelength: 220 nm
- Stop time: 12.0 min
- Calculation:
  - integration: manual
  - calculation method: area %

Chiral HPLC method 3

- KNAUER Isocratic HPLC system:
  - Pump: AZURA P 4.1S
  - UV/VIS detector: AZURA UVD 2.1S
  - data acquisition and evaluation: Mobile Control, ClarityChrom®
- Column:
  - CHIRALPAK® IB (5 µm)
  - Sized 250 mm × 4.6 mm
- Guard Column:

    - GUARD CARTRIDGES 100 mm × 4.6 mm
- Solvents:
    - A: n-Hexane
    - B: Ethanol with 0.01 % (V/V) trifluoroacetic acid
- Mobile Phase: A/B = 98/2
- Flow rate: 1.0 mL/min
- Injection volume: 5.0 µL, method: cut
- Detection wavelength: 220 nm
- Stop time: 30.0 min
- Calculation:
    - integration: manual
    - calculation method: area %

5.1.7 High-resolution mass spectrometry (HRMS)-Electrospray ionization (ESI)

The electrospray ionization (ESI) mass spectra were recorded in both positive and negative ion mode with a 6224 ESI-TOF spectrometer (Agilent). Data were analyzed with OpenChrom 1.1.0 (Lablicate). The mass-to-charge ratios [*m/z*] and the relative intensity of the signals [%] are given.

5.1.8 NMR spectroscopy

NMR spectra were recorded at room temperature on Bruker Avance I 400 MHz, DRX 500 MHz and Avance III 600 MHz. The software MestReNova (version 14.1.0-24037, © 2019 by Mestrelab Research S.L.) was used for analyzation of the NMR spectra. NMR solvents were purchased from Eurisotop (Acetone-d_6 and CDCl3) and Deutero (DMSO-d_6).

^{1}H NMR spectroscopy

- NMR frequency: 400, 500 or 600 MHz

Chemical shifts (δ) are reported in parts per million [ppm] and are referenced to the solvent signal[129].

- Abbreviations for the multiplicities of the signals: br = broad, s = singlet, d = doublet, dd = doublet of doublets, dd = doublet of a doublet of doublets, td = triplet of doublets, t = triplet, q = quartet, m = multiplet. The multiplicities are given as they were seen in the spectra.

^{13}C NMR spectroscopy

- NMR frequency: 101, 126 or 151 MHz

Chemical shifts (δ) are reported in parts per million [ppm] and are referenced to the solvent signal[129].

Two-dimensional (2D) NMR spectroscopy

Where necessary, 1H and 13C NMR assignments were supported by the following two-dimensional (2D) NMR spectroscopy techniques:

- COSY (^{1}H, ^{1}H-correlation spectroscopy)
- HSQC (heteronuclear single-quantum coherence)
- HMBC (heteronuclear multiple-bond correlation)

5.1.9 IR spectroscopy

IR spectra were recorded on a Bruker Alpha FT-IR Platinum ATR Spectro-photometer. All samples were applied to the device without solvent and were directly measured. Absorption bands are characterized by their wavenumbers ($\tilde{\nu}$).

5.1.10 X-ray diffractometry

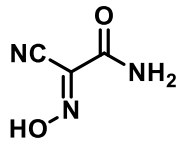
For the compounds **20**:

The single crystal X-ray experiment was performed using a SuperNova four-circle diffractometer in Kappa geometry with 50 W Cu microfocus tubes, an Atlas CCD detector (Rigaku Oxford Diffraction), and a Cryostream 700 Plus cooler (100 K, Oxford Cryosystems Ltd). Data collection, cell refinement, data reduction, and absorption correction were done using CrysAlisPro (Rigaku Oxford Diffraction).

Absorption correction was done with *multi-scan*[130] or *gaussian*[131] methods. Determination and refinement of space group: GRAL (Rigaku Oxford Diffraction) or XPREP (Bruker AXS Inc.) and OLEX2[132]; structure solution: SHELXT[133] (dual-space algorithm) or SHELXS (structure invariant direct method); full-matrix least-squares refinement done on F^2: SHELXL. Missing secondary atom sites were located from the difference Fourier map. Non-hydrogen atoms were refined using individual, anisotropic temperature factors. Heteroatom-bound hydrogen atoms were freely refined in their positions. Carbon atom-bound hydrogen atoms were positioned geometrically and refined riding on their respective parent atoms. U_{iso}(H) was fixed at 1.5 (OH, CH_3) or 1.2 (all other H atoms) of the parent atom's U_{eq} isotropic displacement parameter. The fully refined data were reviewed using PLATON[134].

5.2 Synthetic procedures and analytical data

2-Amino-*N*-hydroxy-2-oxoacetimidoyl cyanide (24)



$C_3H_3N_3O_2$

M_r = 113.1

2-Cyanoacetamid (10 g, 120 mmol) was suspended in 30 mL acetic acid. Under stirring a solution of sodium nitrite (21 g, 310 mmol) in water (26 mL) was added dropwise. The resulting mixture was stirred at 0 °C for 12 h. Then the formed precipitate was filtered off and washed with ethanol. The filtrate was evaporated and the residue was washed with cold ethanol. The collected solids were combined and dried to give **24** as yellow solid (11 g, 96 mmol, 80 % yield).

m.p. = 181 °C;

^{1}H NMR (400 MHz, DMSO-d_6): δ [ppm] = 7.84 (d, J = 29.7 Hz, 2H, CONH_2), 14.45 (s, 1H, CNO*H*);

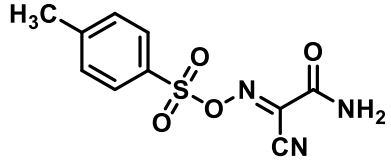
^{13}C NMR (101 MHz, DMSO-d_6): δ [ppm] = 109.1 (1C, C*C*N), 128.3 (1C, *C*NOH), 159.9 (1C, *C*ONH$_2$);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3453, 3369, 3332, 3166, 2725, 2634, 1664, 1597, 1580, 1443, 1056, 701, 678, 568, 525, 509, 425, 412;

HRMS (*m*/*z*): [M+H]$^+$ calcd for $C_3H_4N_3O_2$: 114.0298, found: 114.0299;

HPLC (method 1): t_R = 4.7 min, purity 96.2%.

2-Amino-2-oxo-*N*-(tosyloxy)acetimidoyl cyanide (25)



$C_{10}H_9N_3O_2$

M_r = 267.3

Under nitrogen atmosphere, **24** (11.0 g, 96 mmol) was suspended in dichloromethane (250 mL) and triethylamine (11.9 mL, 8.3 g, 99 mmol) was added at 0 °C. 4-Toluenesulfonyl chloride (18.8 g, 99 mmol) was added dropwise and the mixture was stirred for 2 h at 0 °C. After warming to ambient temperature, the organic suspension was subsequently washed with saturated aqueous solution of ammonium chloride (2× 100 mL) and brine (100 mL). The organic layer was dried with sodium sulfate and concentrated *in vacuo*. The residue was recrystallized from petroleum ether/ethyl acetate = 2/1 (150 mL) to give **25** as colorless solid (22.8 g, 85 mmol, 89 % yield).

m.p. = 80 °C;

TLC: R_f = 0.44 (petroleum ether/ethyl acetate = 1/1);

^{1}H NMR (400 MHz, DMSO-d_6): δ [ppm] = 2.45 (s, 3H, ArCH_3), 7.54 (d, J = 8.5 Hz, 2H, 2-H_{tolyl}, 6-H_{tolyl}), 8.03 (d, J = 8.4 Hz, 2H, 3-H_{tolyl}, 5-H_{tolyl}), 8.22 (d, J = 23.0 Hz, 2H, CONH_2);

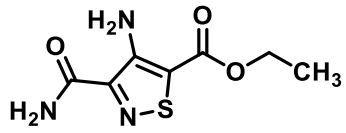
^{13}C NMR (101 MHz, DMSO-d_6): δ [ppm] = 21.2 (1C, CH$_3$), 107.0 (1C, CN), 129.3 (2C, C-2_{tolyl}, C-6_{tolyl}), 129.6 (1C, C-4_{tolyl}), 130.4 (2C, C-3_{tolyl}, C-5_{tolyl}), 135.5 (1C, CNO), 147.2 (1C, C-1_{tolyl}), 156.7 (1C, CONH$_2$);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3433, 3273, 3191, 2984, 1731, 1700, 1594, 1396, 1373, 1234, 1197, 1182, 1167, 1088, 1039, 933, 836, 812, 704, 693, 664, 645, 607, 540, 475, 382;

HRMS (m/z): [M+H]$^+$ calcd for $C_{10}H_{10}N_3O_4S$: 290.0206, found: 290.0207;

HPLC (method 1): t_R = 20.2 min, purity 96.7%.

Ethyl 4-amino-3-carbamoylisothiazole-5-carboxylate (26)



$C_7H_9N_3O_3S$

M_r = 212.5

Ethyl 2-sulfanylacetate (0.90 mL, 0.98 g, 8.2 mmol) was added in one portion to a solution of **25** (1.5 g, 5.4 mmol) in ethanol (150 mL). The solution was cooled to 0 °C and morpholine (0.9 mL, 0.89 g, 10 mmol) was added dropwise. After stirring the mixture for 12 h at ambient temperature, the solvent was removed *in vacuo*. The residue was dissolved in dichloromethane

(100 mL), washed with saturated aqueous solution of ammonium chloride (2×50 mL) and brine (50 mL), dried with sodium sulfate, filtered, and the solvent was removed in *vacuo*. The residue was purified by flash column chromatography (Ø = 6 cm, h = 15 cm, V = 15 mL, petroleum ether/ethyl acetate = 2/1 to 1/1) to give **26** as yellowish solid (0.52 g, 2.4 mmol, 44 % yield).

m.p.: 198 °C;

TLC: R_f = 0.77 (petroleum ether/ethyl acetate = 1/1);

^{1}H NMR (400 MHz, DMSO-d_6): δ [ppm] = 1.29 (t, J = 7.1 Hz, 3H, $COCH_2CH_3$), 4.30 (q, J = 7.1 Hz, 2H, $COCH_2CH_3$), 6.83 (s, 2H, $ArNH_2$), 7.76 (s br, 1H, $ArCONH_2$), 8.12 (s br, 1H, $ArCONH_2$);

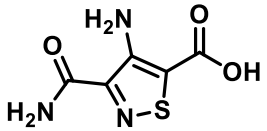
^{13}C NMR (101 MHz, DMSO-d_6): δ [ppm] = 14.1 (1C, $COCH_2CH_3$), 60.7 (1C, $COCH_2CH_3$), 120.8 (1C, C-5'$_{isothiazole}$), 148.8 (1C, C-3'$_{isothiazole}$), 149.3 (1C, C-4'$_{isothiazole}$), 161.0 (1C, $COCH_2CH_3$), 163.5 (1C, $ArCONH_2$);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3469, 3418, 3359, 3217, 3170, 2995, 2972, 2928, 1678, 1574, 1511, 1460, 1375, 1364, 1289, 1165, 1130, 1018, 857, 797, 760, 682, 605, 529, 500, 461, 430, 380;

HRMS (m/z): $[M+H]^+$ calcd for $C_7H_{10}N_3O_3S$: 216.0437, found: 216.0416;

HPLC (method 1): t_R = 19.9 min, purity 91.3%.

4-Amino-3-carbamoylisothiazole-5-carboxylic acid (27)



$C_5H_5N_3O_3S$

M_r = 187.5

Sodium hydroxide (3.5 g, 88 mmol) and **26** (9.4 g, 43.9 mmol) were dissolved in a mixture of water (80 mL) and ethanol (40 mL) and the mixture was stirred at ambient temperature overnight. Then water was added until a clear solution was obtained, and the mixture was washed with ethyl acetate (100 mL). Upon addition of hydrochloric acid (12 M), a precipitate formed which was filtered off and dried to give **27** as yellow solid (7.6 g, 42.1 mmol, 96 % yield).

m.p. = 217 °C;

^{1}H NMR (400 MHz, DMSO-d_6): δ [ppm] = 7.70 (s br, 1H, CONH_2), 8.07 (s br, 1H, CONH_2), the signals for $ArNH_2$, COOH cannot be observed in the spectrum;

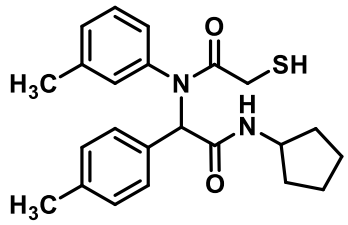
^{13}C NMR (101 MHz, DMSO-d_6): δ [ppm] = 123.6 (1C, C-$5_{isothiazole}$), 146.8 (1C, C-$3_{isothiazole}$), 149.9 (1C, C-$4_{isothiazole}$), 162.7 (1C, *C*OOH), 163.7 (1C, Ar*C*$CONH_2$);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3486, 3447, 3367, 3179, 2825, 2494, 1690, 1637, 1508, 1461, 1386, 1277, 1119, 731, 713, 670, 533, 436;

HRMS (*m*/*z*): $[M\text{-}CONH_2]^+$ calcd for $C_4H_3N_2O_2S$: 142.9915, found: 142.9920;

HPLC (method 1): t_R = 11.2 min, purity 78.5%;

***N*-Cyclopentyl-2-(2-mercapto-*N*-(*m*-tolyl)acetamido)-2-(*p*-tolyl)acetamide (34)**



$C_{23}H_{28}N_2O_2S$

M_r = 396.5

3-Methylaniline (0.22 mL, 214 mg, 2.0 mmol) and 4-methylbenzaldehyde (0.24 mL, 240 mg, 2.0 mmol) were dissolved in dichloromethane (15 mL) and the solution was stirred at ambient temperature for 10 min. Then, 2-sulfanylacetic acid (0.14 mL, 180 mg, 2.0 mmol) and isocyanocyclopentane (0.21 mL, 190 mg, 2.0 mmol) were added. After stirring the reaction mixture for 48 h at ambient temperature, the solvent was removed *in vacuo* and the residue was purified by flash column chromatography (Ø = 2 cm, h = 15 cm, V = 5 mL, petroleum ether/ethyl acetate = 4/1) to give **34** (570 mg, 1.44 mmol, 72% yield) as colorless solid.

m.p. = 158 °C;

TLC: R_f = 0.27 (petroleum ether/ethyl acetate = 2/1);

^{1}H NMR (600 MHz, $CDCl_3$): δ [ppm] = 1.15 – 1.27 (m, 1H, NHCH(C$H_2$$CH_2$)$_2$), 1.38 – 1.55 (m, 4H, NHCH(C$H_2$$CH_2$)$_2$ (1H), NHCH(CH_2CH_2)$_2$ (2H)), 1.56 – 1.64 (m, 1H,

NHCH(CH_2CH_2)$_2$), 1.69 – 1.86 (m, 2H, NHCH(CH_2CH_2)$_2$), 2.15 (s, 3H, ArCH_3), 2.16 (s br, 3H, ArCH_3), 2.57 (t, J = 6.7 Hz, 1H, HSCH_2CO), 2.97 (dd, J = 14.9, 6.7 Hz, 1H, HSCH_2CO), 3.02 (dd, J = 14.9, 6.7 Hz, 1H, HSCH_2CO), 3.98 – 4.06 (m, 1H, NHCH(CH_2CH_2)$_2$), 5.95 (s, 1H, NCHCO), 6.91 (q, J = 8.1 Hz, 4H, 2"-H$_{p\text{-tolyl}}$, 3"-H$_{p\text{-tolyl}}$, 5"-H$_{p\text{-tolyl}}$, 6"-H$_{p\text{-tolyl}}$), 6.94 – 6.98 (m, 1H, H$_{m\text{-tolyl}}$), 7.98 (d, J = 7.1 Hz, 1H, NHCH(CH_2CH_2)$_2$), the signals for 3 H$_{m\text{-tolyl}}$ cannot be observed in the spectrum;

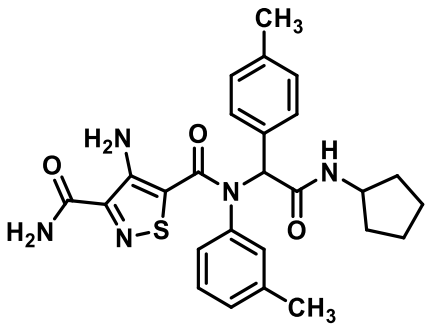
^{13}C NMR (151 MHz, DMSO-d_6): δ [ppm] = 20.5 (1C, ArCH_3), 20.6 (1C, ArCH_3), 23.4 (1C, NHCH(CH_2CH_2)$_2$), 23.5 (1C, NHCH(CH_2CH_2)$_2$), 27.3 (1C, HSCH_2CO), 31.8 (1C, NHCH(CH_2CH_2)$_2$), 32.1 (1C, NHCH(CH_2CH_2)$_2$), 50.5 (1C, NHCH(CH_2CH_2)$_2$), 63.5 (1C, COCHN), 127.7 (1C, C$_{m\text{-tolyl}}$), 128.1 (1C, C$_{m\text{-tolyl}}$), 128.3 (2C, C-3'''$_{p\text{-tolyl}}$, C-5'''$_{p\text{-tolyl}}$), 128.3 (1C, C$_{m\text{-tolyl}}$), 129.9 (2C, C-4'''$_{p\text{-tolyl}}$, C-6'''$_{p\text{-tolyl}}$), 131.3 (1C, C$_{m\text{-tolyl}}$), 132.1 (1C, C-1'''$_{p\text{-tolyl}}$), 136.7 (1C, C-4'''$_{p\text{-tolyl}}$), 137.6 (1C, C-3"$_{m\text{-tolyl}}$), 139.1 (1C, C-1"$_{m\text{-tolyl}}$), 168.8 (1C, NCOCH_2), 168.9 (1C, CONHCH);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3271, 2953, 2865, 1649, 1555, 1487, 1365, 1246, 1228, 1190, 977, 860, 818, 792, 733, 703, 675, 631, 573, 548, 504 449;

HRMS (m/z): [M+H]$^+$ calcd for $C_{23}H_{29}N_2O_2S$: 397.1944, found: 397.1942;

HPLC (method 1): t_R = 24.3 min, purity 81.2%.

4-Amino-N^5-(2-(cyclopentylamino)-2-oxo-1-(p-tolyl)ethyl)-N^5-(m-tolyl)isothiazole-3,5-dicarboxamide (20)



$C_{26}H_{29}N_5O_3S$

M_r = 491.6

Method 1: 3-Methylaniline (0.22 mL, 214 mg, 2.0 mmol) and 4-methylbenzaldehyde (0.24 mL, 240 mg, 2.0 mmol) were added to methanol (40 mL) and the solution was stirred at ambient temperature for 30 min. Afterwards, **27** (377 mg, 2.0 mmol) and isocyanocyclopentane (0.21 mL, 190 mg, 2.0 mmol) were added. After stirring for 24 h the mixture was filtered and

the precipitate was washed with cold methanol, dried to give **20** as colorless solid (128 mg, 0.26 mmol, 13 % yield).

Method 2: 34 (396 mg, 1.0 mmol) was added in one portion to a solution of **25** (320 mg, 1.2 mmol) in acetone (100 mL). The mixture was cooled to 0 °C and morpholine (0.17 mL, 170 mg, 1.9 mmol) was added dropwise. After stirring the mixture for 12 h at ambient temperature, the solvent was removed *in vacuo*. The residue was purified by flash column chromatography (Ø = 6 cm, h = 20 cm, V = 15 mL, dichloromethane/acetone = 10/1) and recrystallized from acetonitrile to give **20** (338 mg, 0.69 mmol, 69% yield) as colorless solid.

m.p. = 264 °C;

TLC: R_f = 0.26 (dichloromethane/acetone = 10/1);

^{1}H NMR (600 MHz, DMSO-d_6): δ [ppm] = 1.18 – 1.28 (m, 1H, NHCH(CH_2CH$_2$)$_2$), 1.39 – 1.53 (m, 4H, NHCH(CH_2CH$_2$)$_2$ (1H), NHCH(CH$_2$CH_2)$_2$ (3H)), 1.55 – 1.65 (m, 1H, NHCH(CH$_2$CH_2)$_2$), 1.69 – 1.87 (m, 2H, NHCH(CH_2CH$_2$)$_2$), 2.16 (s, 3H, ArCH_3), 2.17 (s br, 3H, ArCH_3), 4.04 (h, J = 7.0 Hz, 1H, NHCH(CH$_2$CH$_2$)$_2$), 6.09 (s, 1H, NCHCO), 6.55 (br, 1H, H$_{m\text{-tolyl}}$), 6.95 (q, J = 8.2 Hz, 4H, 2'''-H$_{p\text{-tolyl}}$, 3'''-H$_{p\text{-tolyl}}$, 5'''-H$_{p\text{-tolyl}}$, 6'''-H$_{p\text{-tolyl}}$), 7.11 (br, 1H, H$_{m\text{-tolyl}}$), 7.13 (d, J = 7.1 Hz, 1H, H$_{m\text{-tolyl}}$), 7.15 – 7.20 (br, 2H, ArNH_2), 7.58 (s br, 1H, ArCONH_2), 7.69 (br, 1H, H$_{m\text{-tolyl}}$), 7.81 (s br, 1H, ArCONH_2), 8.08 (d, J = 7.0 Hz, 1H, NHCH(CH$_2$CH$_2$)$_2$;

^{13}C NMR (151 MHz, DMSO-d_6): δ [ppm] = 20.6 (1C, ArCH$_3$), 20.7 (1C, ArCH$_3$), 23.4 (1C, NHCH(CH$_2$$CH_2$)$_2$), 23.5 (1C, NHCH(CH$_2$$CH_2$)$_2$), 31.7 (1C, NHCH($CH_2CH_2$)$_2$), 32.2 (1C, NHCH($CH_2CH_2$)$_2$), 50.5 (1C, NH$C$H(CH$_2CH_2$)$_2$), 63.7 (1C, CO$C$HN), 123.1 (1C, C-5'$_{isothiazole}$), 128.3 (2C, C-3'''$_{p\text{-tolyl}}$, C-5'''$_{p\text{-tolyl}}$), 128.6 (1C, C$_{m\text{-tolyl}}$), 130.0 (1C, C$_{m\text{-tolyl}}$), 130.1 (1C, C$_{m\text{-tolyl}}$), 130.2 (2C, C-2'''$_{p\text{-tolyl}}$, C-6'''$_{p\text{-tolyl}}$), 131.8 (1C, C-1'''$_{p\text{-tolyl}}$), 133.7 (1C, C$_{m\text{-tolyl}}$), 136.9 (1C, C-4'''$_{p\text{-tolyl}}$), 137.1 (1C, C-1''$_{m\text{-tolyl}}$), 138.3 (1C, C$_{m\text{-tolyl}}$), 146.4 (1C, C-3'$_{isothiazole}$), 151.2 (1C, C-4'$_{isothiazole}$), 162.2 (1C, Ar$C$ONCH), 163.9 (1C, Ar$CONH_2$), 169.1 (1C, CONHCH);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3468, 3427, 3395, 3351, 3183, 2959, 2866, 1689, 1670, 1619, 1557, 1513, 1486, 1447, 1409, 1376, 1354, 1334, 1226, 1070, 801, 757, 744, 728, 707, 684, 614, 503, 440, 411;

HRMS (m/z): [M+H]$^+$ calcd for $C_{26}H_{30}N_5O_3S$: 492.2064, found: 492.2081;

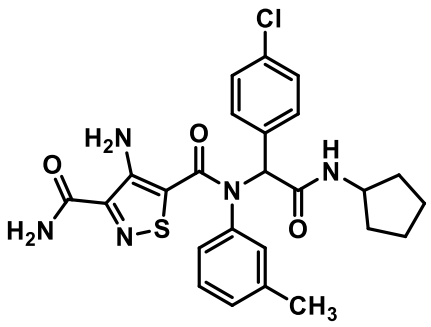
HPLC (method 1): t_R = 24.0 min, purity 96.0%;

X-ray crystal structure analysis:

Table 12. Crystal data, data collection, and refinement of **20**.

Bond precision	C-C = 0.0068 Å
Cell	a = 8.7582(6) Å α = 79.823(9)º b = 10.6466(12) Å β = 77.012(7) º c = 14.2908(13) Å γ = 70.831(8) º V = 1237.061(17) Å^3
Chemical formula	$C_{26}H_{29}N_5O_3S$
Z	2
M_r	491.6
Crystal system, space group	Triclinic, P -1
Crystal colour, morphology	colourless, plate
F(000)	520.0
$\boldsymbol{M_u}$	1.494 mm^{-1}
$\boldsymbol{D_x}$	1.340 g/cm³
$\boldsymbol{\theta_{min}}$, $\boldsymbol{\theta_{max}}$	4.43 º, 75.7º
Radiation type	Cu K_α (λ = 1.54184 Å)
Temperature	100 K
Diffractometer	SuperNova, Dual, Cu at home/near, Atlas
T_{min}, T_{max}	0.348, 1.000

4-Amino-N^5-[1-(4-chlorophenyl)-2-(cyclopentylamino)-2-oxoethyl]-N^5-(*m*-tolyl)isothiazole-3,5-dicarboxamide (21)



$C_{25}H_{26}ClN_5O_3S$

M_r = 512.0

3-Methylaniline (0.22 mL, 210 mg, 2.0 mmol) and 4-chlorobenzaldehyde (280 mg, 2.0 mmol) were dissolved in methanol (50 mL) and the solution was stirred at ambient temperature for 10 min. Then, **27** (380 mg, 2.0 mmol) and isocyanocyclopentane (0.22 mL, 190 mg, 2.0 mmol) were added. After stirring the reaction mixture for 48 h at ambient temperature, the solvent was removed *in vacuo* and the residue was purified by flash column chromatography (Ø = 4 cm, h = 15 cm, V = 15 mL, dichloromethane/methanol = 100/1) to give **21** (100 mg, 0.20 mmol, 10% yield) as colorless solid.

m.p. = 240 °C;

TLC: R_f = 0.43 (petroleum ether/ethyl acetate = 2/1);

^{1}H NMR (400 MHz, DMSO-*d*$_6$): δ [ppm] = 1.17 – 1.29 (m, 1H, NHCH(C*H*$_2$CH$_2$)$_2$), 1.37 – 1.67 (m, 5H NHCH(CH$_2$C*H*$_2$)$_2$, NHCH(C*H*$_2$CH$_2$)$_2$ (1H)), 1.69 – 1.89 (m, 2H NHCH(C*H*$_2$CH$_2$)$_2$), 2.19 (s br, 3H, ArC*H*$_3$), 3.98 – 4.10 (m, 1H, NHC*H*(CH$_2$CH$_2$)$_2$), 6.11 (s, 1H, NC*H*CO), 7.04 – 7.37 (m, 8H, 2'''-H$_{4\text{-chlorophenyl}}$, 3'''-H$_{4\text{-chlorophenyl}}$, 5'''-H$_{4\text{-chlorophenyl}}$, 6'''-H$_{4\text{-chlorophenyl}}$, ArN*H*$_2$, H$_{m\text{-tolyl}}$ (2H)), 7.58 (s br, 1H, CON*H*$_2$), 7.81 (s br, 1H, CON*H*$_2$), 8.17 (d, *J* = 7.0 Hz, 1H, N*H*CH(CH$_2$CH$_2$)$_2$), the signals for 2 H$_{m\text{-tolyl}}$ cannot be observed in the spectrum;

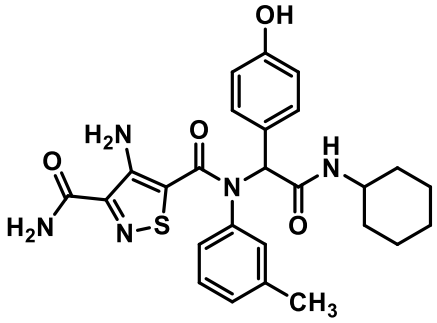
^{13}C NMR (101 MHz, DMSO-*d*$_6$): δ [ppm] = 20.7 (1C, Ar*C*H$_3$), 23.4 (1C, NHCH(CH$_2$*C*H$_2$)$_2$), 23.5 (1C, NHCH(CH$_2$*C*H$_2$)$_2$), 31.8 (1C, NHCH(*C*H$_2$CH$_2$)$_2$), 32.2 (1C, NHCH(*C*H$_2$CH$_2$)$_2$), 50.6 (1C, NH*C*H(CH$_2$CH$_2$)$_2$), 63.2 (1C, N*C*HCO), 122.9 (1C, C-5'$_{\text{isothiazole}}$), 127.8 (2C, C-3'''$_{4\text{-chlorophenyl}}$, C-5'''$_{4\text{-chlorophenyl}}$), 128.8 (1C, C$_{m\text{-tolyl}}$), 130.1 (1C, C$_{m\text{-tolyl}}$), 130.2 (1C, C$_{m\text{-tolyl}}$), 132.2 (2C, C-2'''$_{4\text{-chlorophenyl}}$, C-6'''$_{4\text{-chlorophenyl}}$), 132.5 (1C, C-4'''$_{4\text{-chlorophenyl}}$), 133.7 (1C, C$_{m\text{-tolyl}}$), 133.9 (1C, C-1'''$_{4\text{-chlorophenyl}}$), 136.9 (1C, C-1''$_{m\text{-tolyl}}$), 138.6 (1C, C$_{m\text{-tolyl}}$), 146.4 (1C, C-3'$_{\text{isothiazole}}$), 151.3 (1C, C-4'$_{\text{isothiazole}}$), 162.2 (1C, Ar*C*ONCH), 163.9 (1C, Ar*C*ONH$_2$), 168.6 (1C, CH*C*ONHCH);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3446, 3293, 2959, 1651, 1613, 1598, 1557, 1492, 1446, 1409, 1370, 1340, 1322, 1277, 1247, 1192, 1154, 1092, 1074, 1018, 975, 933, 877, 853, 824, 801, 771, 740, 727, 705, 688, 672, 595, 538, 523;

HRMS (*m*/*z*): [M+H]$^+$ calcd for C$_{25}$H$_{27}$^{35}ClN$_5$O$_3$S: 512.1518, found: 512.1526;

HPLC (method 1): t_R = 21.2 min, purity 94.1%.

4-Amino-N^5-[2-(cyclohexylamino)-1-(4-hydroxyphenyl)-2-oxoethyl]-N^5-(*m*-tolyl)isothiazole-3,5-dicarboxamide (22)



$C_{26}H_{29}N_5O_4S$

M_r = 507.6

3-Methylaniline (0.22 mL, 210 mg, 2.0 mmol) and 4-hydroxybenzaldehyde (240 mg, 2.0 mmol) were dissolved in methanol (50 mL) and the solution was stirred at ambient temperature for 10 min. Then, **27** (380 mg, 2.0 mmol) and isocyanocyclohexane (0.25 mL, 220 mg, 2.0 mmol) were added. After stirring the reaction mixture for 48 h at ambient temperature, the solvent was removed *in vacuo* and the residue was purified by flash column chromatography (Ø = 4 cm, h = 15 cm, V = 15 mL, dichloromethane/methanol = 50/1) to give **22** (120 mg, 0.24 mmol, 12% yield) as colorless solid.

m.p. = 271 °C;

TLC: R_f = 0.23 (petroleum ether/ethyl acetate = 2/1);

^{1}H NMR (600 MHz, DMSO-d_6): δ [ppm] = 0.94 – 1.02 (m, 1H, NHCH(CH_2CH$_2$)$_2$CH$_2$), 1.03 – 1.12 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$CH_2), 1.16 – 1.31 (m, 3H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (1H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H)), 1.50 – 1.56 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$CH_2), 1.56 – 1.62 (m, 1H, NHCH(CH$_2$CH_2)$_2$CH$_2$), 1.62 – 1.73 (m, 2H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (1H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (1H)), 1.74 – 1.80 (m, 1H, NHCH(CH_2CH$_2$)$_2$CH$_2$), 2.21 (s br, 3H, ArCH_3), 3.53 – 3.63 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), 6.02 (s, 1H, NCHCO), 6.46 – 6.50 (m, 2H, 3'''-H$_{4\text{-hydroxyphenyl}}$, 5'''-H$_{4\text{-hydroxyphenyl}}$), 6.82 – 6.88 (m, 2H, 2'''-H$_{4\text{-hydroxyphenyl}}$, 6'''-H$_{4\text{-hydroxyphenyl}}$), 7.11 – 7.14 (m, 1H, H$_{m\text{-tolyl}}$), 7.15 (s br, 2H, ArN$H_2$), 7.57 (s br, 1H, CON$H_2$), 7.80 (s br, 1H, CON$H_2$), 7.93 (d, J = 7.7 Hz, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), 9.31 (s, 1H, ArOH), the signals for 3 H$_{m\text{-tolyl}}$ cannot be observed in the spectrum;

^{13}C NMR (151 MHz, DMSO-d_6): δ [ppm] = 24.5 (1C, NHCH(CH$_2$$CH_2$)$_2CH_2$), 24.7 (1C, NHCH(CH$_2$$CH_2$)$_2CH_2$), 25.2 (1C, NHCH(CH$_2CH_2$)$_2$$CH_2$), 32.2 (1C, NHCH($CH_2CH_2$)$_2CH_2$),

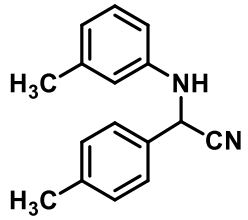
32.3 (1C, NHCH(*C*H$_2$CH$_2$)$_2$CH$_2$), 47.9 (1C, NH*C*H(CH$_2$CH$_2$)$_2$CH$_2$), 63.6 (1C, N*C*HCO), 114.6 (2C, C-3'''$_{4\text{-hydroxyphenyl}}$, C-5'''$_{4\text{-hydroxyphenyl}}$), 123.3 (1C, C-5'$_{isothiazole}$), 124.9 (1C, C-1'''$_{4\text{-hydroxyphenyl}}$), 128.6 (1C, C$_{m\text{-tolyl}}$), 130.0 (1C, C$_{m\text{-tolyl}}$), 130.2 (1C, C$_{m\text{-tolyl}}$), 131.6 (2C, C-2'''$_{4\text{-hydroxyphenyl}}$, C-6'''$_{4\text{-hydroxyphenyl}}$), 133.8 (1C, C$_{m\text{-tolyl}}$), 137.2 (1C, C-1''$_{m\text{-tolyl}}$), 146.4 (1C, C-3'$_{isothiazole}$), 151.2 (1C, C-4'$_{isothiazole}$), 156.8 (1C, C-4'''$_{4\text{-hydroxyphenyl}}$), 162.2 (1C, Ar*C*ONCH), 163.9 (1C, Ar*C*ONH$_2$), 168.9 (1C, CH*C*ONHCH), the signals for Ar*C*H$_3$ and 1 C$_{m\text{-tolyl}}$ cannot be observed in the spectrum;

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3468, 3402, 3355, 3174, 2952, 2923, 2848, 1673, 1662, 1624, 1611, 1579, 1556, 1515, 1487, 1445, 1406, 1362, 1331, 1276, 1252, 1231, 1212, 1188, 1171, 1148, 1101, 1091, 1076, 871, 853, 831, 802, 792, 762, 735, 711, 684, 668, 655, 639, 532, 523, 512, 493;

HRMS (*m*/*z*): [M+H]$^+$ calcd for $C_{26}H_{30}N_5O_4S$: 508.2013, found: 508.2010;

HPLC (method 1): t_R = 24.3 min, purity 97.5%.

2-(*p*-Tolyl)-2-(*m*-tolylamino)acetonitrile (37)



$C_{16}H_{16}N_2$

Mr = 236.3

3-Methylaniline hydrochloride (5.5 g, 38 mmol) was dissolved in water (38 mL) and the mixture was cooled to 0 °C. A solution of potassium cyanide (4.0 g, 61 mmol) in water (19 mL) was added dropwise. Under vigorous stirring, 4-methylbenzaldehyde (4.6 mL, 4.7 g, 39 mmol) was added and the mixture was stirred for 1 h at 0 °C. Then petroleum ether (50 mL) was added and the mixture was stirred for 12 h at ambient temperature. After phase separation, the aqueous phase was extracted with ethyl acetate (3×100 mL). The combined organic phases were washed with water (200 mL) and brine (200 mL), dried with sodium sulfate, filtered, and the solvent was removed *in vacuo*. The residue was purified by recrystallization with petroleum ether/ethyl acetate = 3/1 (50 mL) to give **37** as colorless solid (5.9 g, 25 mmol, 66 % yield).

m.p. = 110 °C;

TLC: R_f = 0.51 (petroleum ether/ethyl acetate = 9/1);

^{1}H NMR (500 MHz, DMSO-d_6): δ [ppm] = 2.22 (s, 3H, C$H_{3,m\text{-tolyl}}$), 2.33 (s, 3H, C$H_{3,p\text{-tolyl}}$), 5.89 (d, J = 9.5 Hz, 1H, C*H*CN), 6.52 (d, J = 9.5 Hz, 1H, CHN*H*), 6.54 (d, J = 7.6 Hz, 1H, 4'-H$_{m\text{-tolyl}}$), 6.62 (dd, J = 8.0, 2.4 Hz, 1H, 6'-H$_{m\text{-tolyl}}$), 6.66 (t, J = 2.1 Hz, 1H, 2'-H$_{m\text{-tolyl}}$), 7.04 (t, J = 7.8 Hz, 1H, 5'-H$_{m\text{-tolyl}}$), 7.27 (d, J = 8.0 Hz, 2H, 3"-H$_{p\text{-tolyl}}$, 5"-H$_{p\text{-tolyl}}$), 7.46 (d, J = 8.0 Hz, 2H, 2"-H$_{p\text{-tolyl}}$, 6"-H$_{p\text{-tolyl}}$);

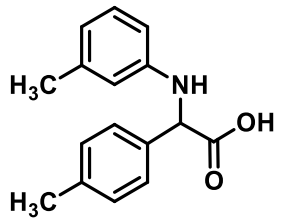
^{13}C NMR (101 MHz, DMSO-d_6): δ [ppm] = 20.6 (1C, *C*H$_{3,p\text{-tolyl}}$), 21.3 (1C, *C*H$_{3,m\text{-tolyl}}$), 47.9 (1C, *C*HCN), 111.0 (1C, C-6'$_{m\text{-tolyl}}$), 114.4 (1C, C-2'$_{m\text{-tolyl}}$), 119.1 (1C, C-4'$_{m\text{-tolyl}}$), 119.7 (1C, CH*C*N), 127.1 (1C, C-2"$_{p\text{-tolyl}}$, C-6"$_{p\text{-tolyl}}$), 128.8 (1C, C-5'$_{m\text{-tolyl}}$), 129.3 (1C, C-3"$_{p\text{-tolyl}}$, C-5"$_{p\text{-tolyl}}$), 132.1 (1C, C-1"$_{p\text{-tolyl}}$), 138.0 (1C, C-3'$_{m\text{-tolyl}}$), 138.1 (1C, C-4"$_{p\text{-tolyl}}$), 146.0 (1C, C-1'$_{m\text{-tolyl}}$);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3324, 3021, 2915, 2858, 2235, 1915, 1605, 1589, 1516, 1481, 1426, 1294, 1167, 1104, 1072, 853, 817, 773, 718, 688, 614, 601, 545, 499, 438, 416;

HRMS (m/z): [M+H]$^+$ calcd for $C_{16}H_{17}N_2$: 237.1386, found: 237.1384;

HPLC (method 1): t_R = 24.5 min, purity 92.6%;

2-(*p*-Tolyl)-2-(*m*-tolylamino)acetic acid (38)



$C_{16}H_{17}NO_2$

Mr = 255.3

Hydrogen peroxide (30 %, 2 mL) was added dropwise to the mixture of **37** (460 mg, 2 mmol) and potassium carbonate (140 mg, 1 mmol) in dimethyl sulfoxide (2.5 mL) at 0 °C. After stirring for 2 h, the resulting precipitate was filtered to give colorless solid.

The obtained crude product and sodium hydroxide (480 mg, 12 mmol) were dissolved in a mixture of methanol and water (4:1) (20 mL) and the mixture was stirred at 90 °C for 6 h. After cooling, the mixture was concentrated and diluted with water (30 mL) and the resulting mixture was extracted with ethyl acetate (30 mL). The aqueous phase was acidified to pH 4 with an aqueous solution of hydrogen chloride (6 M) and extracted with dichloromethane (3× 20 mL). The combined organic layers were washed with brine (50 mL), dried with sodium sulfate, filtered, and the solvent was removed *in vacuo* to give **38** as colorless solid (460 mg, 1.8 mmol, 92 % yield).

m.p. = 190 °C (decomposition);

TLC: R_f = 0.71 (dichloromethane/methanol = 10/1);

^{1}H NMR (400 MHz, DMSO-d_6): δ [ppm] = 2.15 (s, 3H, C$H_{3,m\text{-tolyl}}$), 2.28 (s, 3H, C$H_{3,p\text{-tolyl}}$), 3.41 (br, 2H, COO*H*, COCHN*H*), 5.02 (s, 1H, NHC*H*CO), 6.34 – 6.48 (m, 2H, 4'-H$_{m\text{-tolyl}}$, 6'-H$_{m\text{-tolyl}}$), 6.50 (t, J = 1.9 Hz, 1H, 2'-H$_{m\text{-tolyl}}$), 6.92 (t, J = 7.7 Hz, 1H, 5'-H$_{m\text{-tolyl}}$), 7.16 (d, J = 7.9 Hz, 2H, 3''-H$_{p\text{-tolyl}}$, 5''-H$_{p\text{-tolyl}}$), 7.39 (d, J = 7.9 Hz, 2H, 2''-H$_{p\text{-tolyl}}$, 6''-H$_{p\text{-tolyl}}$);

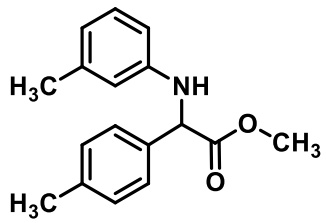
^{13}C NMR (101 MHz, DMSO-d_6): δ [ppm] = 20.6 (1C, *C*H$_{3,m\text{-tolyl}}$), 21.3 (1C, *C*H$_{3,p\text{-tolyl}}$), 59.4 (1C, CO*C*HNH), 110.2 (1C, C-4'$_{m\text{-tolyl}}$), 113.7 (1C, C-2'$_{m\text{-tolyl}}$), 117.4 (1C, C-6'$_{m\text{-tolyl}}$), 127.3 (2C, C-2''$_{p\text{-tolyl}}$, C-6''$_{p\text{-tolyl}}$), 128.5 (1C, C-5'$_{m\text{-tolyl}}$), 128.9 (2C, C-3''$_{p\text{-tolyl}}$, C-5''$_{p\text{-tolyl}}$), 135.5 (1C, C-1''$_{p\text{-tolyl}}$), 136.8 (1C, C-4''$_{p\text{-tolyl}}$), 137.6 (1C, C-3'$_{m\text{-tolyl}}$), 146.9 (1C, C-1'$_{m\text{-tolyl}}$), 173.1 (1C, *C*OOH);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3021, 2917, 2865, 2539, 1719, 1605, 1589, 1509, 1489, 1436, 1377, 1324, 1307, 1256, 1181, 1127, 769, 725, 691, 503, 440, 404;

HRMS (*m*/*z*): [M+H]$^+$ calcd for $C_{16}H_{18}NO_2$: 256.1332, found: 256.1354;

HPLC (method 1): t_R = 15.3 min, purity 90.5%.

Methyl 2-(*p*-tolyl)-2-(*m*-tolylamino)acetate (39)



$C_{17}H_{19}NO_2$

M_r = 269.3

Thionyl chloride (5 mL) was added dropwise to the solution of **38** (2.4 g, 10 mmol) in methanol (50 mL) at 0 °C. Then the solution was allowed to warm to room temperature and stirred for 12 h. Afterwards, the mixture was concentrated *in vacuo* and a saturated aqueous solution of sodium bicarbonate (30 mL) was added. The aqueous phase was extracted with dichloromethane (2×50 mL). The combined organic phases were dried with sodium sulfate, filtered, and the solvent was removed *in vacuo*. The residue was purified by flash column chromatography (Ø = 4 cm, h = 15 cm, V = 15 mL petroleum ether/dichloromethane = 1/1) to give **39** as colorless solid (2.5 g, 9.3 mmol, 93 % yield).

m.p. = 71 °C;

TLC: R_f = 0.88 (petroleum ether/ethyl acetate = 1/1);

^{1}H NMR (400 MHz, DMSO-d_6): δ [ppm] = 2.15 (s, 3H, C$H_{3,m\text{-tolyl}}$), 2.28 (s, 3H, C$H_{3,p\text{-tolyl}}$), 3.61 (s, 3H, COCH_3), 5.16 (d, J = 8.2 Hz, 1H, NHC*H*CO), 6.15 (d, J = 8.2 Hz, 1H, N*H*CHCO), 6.35 – 6.42 (m, 1H, 4'-H$_{m\text{-tolyl}}$), 6.44 (dd, J = 7.9, 2.3 Hz, 1H, 6'-H$_{m\text{-tolyl}}$), 6.47 – 6.53 (m, 1H, 2'-H$_{m\text{-tolyl}}$), 6.92 (t, J = 7.9 Hz, 1H, 5'-H$_{m\text{-tolyl}}$), 7.13 – 7.20 (m, 2H, 3''-H$_{p\text{-tolyl}}$, 5''-H$_{p\text{-tolyl}}$), 7.37 (d, J = 8.1 Hz, 2H, 2''-H$_{p\text{-tolyl}}$, 6''-H$_{p\text{-tolyl}}$);

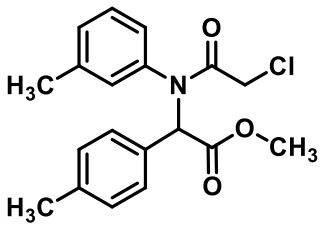
^{13}C NMR (101 MHz, DMSO-d_6): δ [ppm] = 20.7 (1C, *C*H$_{3,p\text{-tolyl}}$), 21.3 (1C, *C*H$_{3,m\text{-tolyl}}$), 52.1 (1C, COO*C*H$_3$), 59.3 (1C, CO*C*HNH), 110.2 (1C, C-6'$_{m\text{-tolyl}}$), 113.7 (1C, C-2'$_{m\text{-tolyl}}$), 117.7 (1C, C-4'$_{m\text{-tolyl}}$), 127.4 (2C, C-2''$_{p\text{-tolyl}}$, C-6''$_{p\text{-tolyl}}$), 128.6 (1C, C-5'$_{m\text{-tolyl}}$), 129.1 (2C, C-3''$_{p\text{-tolyl}}$, C-5''$_{p\text{-tolyl}}$), 134.8 (1C, C-1''$_{p\text{-tolyl}}$), 137.2 (1C, C-4''$_{p\text{-tolyl}}$), 137.7 (1C, C-3'$_{m\text{-tolyl}}$), 146.9 (1C, C-1'$_{m\text{-tolyl}}$), 172.4 (1C, *C*OOCH$_3$);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3404, 1729, 1605, 1588, 1509, 1488, 1437, 1314, 1295, 1253, 1209, 1193, 1180, 1166, 1141, 1106, 991, 867, 818, 790, 764, 739, 690, 499, 445, 302;

HRMS (*m*/*z*): [M+H]$^+$ calcd for $C_{17}H_{20}NO_2$: 270.1489, found: 270.142;

HPLC (method 1): t_R = 24.9 min, purity 99.5%.

Methyl 2-(2-chloro-*N*-(*m*-tolyl)acetamido)-2-(*p*-tolyl)acetate (40)



$C_{19}H_{20}ClNO_3$

M_r = 345.8

Triethylamine (242 mg, 0.34 mL, 2.4 mmol) was added to an ice-cooled solution of **39** (538 mg, 2 mmol) and chloroacetyl chloride (258 mg, 0.19 mL, 2.4 mmol) in dichloromethane (40 mL). After stirring at ambient temperature for 12 h, the reaction mixture was washed with saturated ammonium chloride solution (2× 40 mL) and brine (40 mL), dried over sodium sulfate, filtered, and the solvent was removed *in vacuo*. The resulting residue was purified by flash

column chromatography (Ø = 4 cm, h = 10 cm, V = 15 mL petroleum ether/ethyl acetate = 10/1) to give **40** as colorless oil (604 mg, 1.76 mmol, 88 % yield).

TLC: R_f = 0.40 (petroleum ether/ethyl acetate = 6/1);

^{1}H NMR (600 MHz, DMSO-d_6): δ [ppm] = 2.19 (s br, 6H, ArCH_3), 3.69 (s, 3H, COOCH_3), 3.93 – 4.05 (m, 2H, ClCH_2CO), 5.89 (s, 1H, NC*H*CO), 6.94 (br, 2H, H$_{m\text{-tolyl}}$), 6.98 (q, J = 8.2 Hz, 4H, 2"-H$_{p\text{-tolyl}}$, 3"-H$_{p\text{-tolyl}}$, 5"-H$_{p\text{-tolyl}}$, 6"-H$_{p\text{-tolyl}}$), 7.07 (d, J = 7.5 Hz, 1H, H$_{m\text{-tolyl}}$), 7.11 – 7.15 (m, 1H, H$_{m\text{-tolyl}}$);

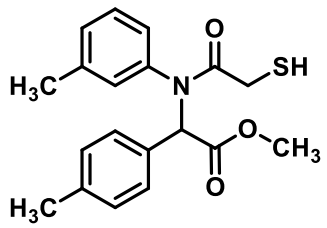
^{13}C NMR (101 MHz, DMSO-d_6): δ [ppm] = 20.5 (1C, Ar*C*H$_3$), 20.6 (1C, Ar*C*H$_3$), 42.6 (1C, Cl*C*H$_2$CO), 52.2 (1C, COO*C*H$_3$), 64.1 (1C, CO*C*HN), 126.7 (1C, C$_{m\text{-tolyl}}$), 128.6 (2C, C-3"$_{p\text{-tolyl}}$, C-5"$_{p\text{-tolyl}}$), 128.7 (1C, C$_{m\text{-tolyl}}$), 129.2 (1C, C$_{m\text{-tolyl}}$), 129.7 (2C, C-2"$_{p\text{-tolyl}}$, C-6"$_{p\text{-tolyl}}$), 130.1 (1C, C$_{m\text{-tolyl}}$), 130.2 (1C, C$_{arom.}$), 137.5 (1C, C-4"$_{p\text{-tolyl}}$), 138.2 (1C, C$_{m\text{-tolyl}}$), 138.5 (1C, C$_{arom.}$), 165.7 (1C, N*C*OCH$_2$), 170.2 (1C, *C*OOCH$_3$);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 2951, 2923, 1745, 1667, 1604, 1587, 1515, 1488, 1433, 1376, 1344, 1307, 1238, 1216, 1208, 1174, 1091, 1068, 1025, 1003, 988, 818, 793, 761, 738, 705, 636, 499;

HRMS (*m*/*z*): [M+Na]$^+$ calcd for C$_{19}$H$_{20}$^{35}ClNNaO$_3$: 368.1024, found: 368.0676;

HPLC (method 1): t_R = 24.5 min, purity 94.1%.

Methyl 2-(2-mercapto-*N*-(*m*-tolyl)acetamido)-2-(*p*-tolyl)acetate (41)



C$_{19}$H$_{21}$NO$_3$S

M$_r$ = 343.4

Finely ground thiourea (91.2 mg, 1.2 mmol) was added to a solution of **40** (345 mg, 1.0 mmol) in acetone (20 mL) and the resulting mixture stirred for 16 h at ambient temperature. Removal of the solvent left a residue, which was treated with ethyl acetic (25 mL). Under vigorous stirring, a solution of sodium sulfite (252 mg, 2.0 mmol) in water (25 mL) was added, and the mixture was stirred at ambient temperature for 3 h. The organic phase was washed with water (20 mL) and brine (20 mL), dried with sodium sulfate, filtered, and the solvent was removed *in*

vacuo. The residue was purified by flash column chromatography (Ø = 4 cm, h = 15 cm, V = 15 mL petroleum ether/ethyl acetate = 8/1) to give **41** as colorless oil (212 mg, 0.62 mmol, 62 % yield).

TLC: R_f = 0.38 (petroleum ether/ethyl acetate = 6/1);

^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 2.07 (dd, *J* = 8.1, 7.3 Hz, 1H, *H*SCH$_2$CO), 2.25 (s br, 6H, ArC*H*$_3$), 3.03 (dd, *J* = 14.8, 8.1 Hz, 1H, HSC*H*$_2$CO), 3.10 (dd, *J* = 14.8, 7.3 Hz, 1H, HSC*H*$_2$CO), 3.76 (s, 3H, COOC*H*$_3$), 6.10 (s, 1H, NC*H*CO), 6.33 (br, 1H, H$_{m\text{-tolyl}}$), 6.89 (d, *J* = 8.1 Hz, 2H, 3"-H$_{p\text{-tolyl}}$, 5"-H$_{p\text{-tolyl}}$), 6.97 (d, *J* = 7.9 Hz, 2H, 2"-H$_{p\text{-tolyl}}$, 6"-H$_{p\text{-tolyl}}$), 6.99 – 7.05 (m, 1H, H$_{m\text{-tolyl}}$), 7.12 (br, 1H, H$_{m\text{-tolyl}}$), 7.44 (br, 1H, H$_{m\text{-tolyl}}$);

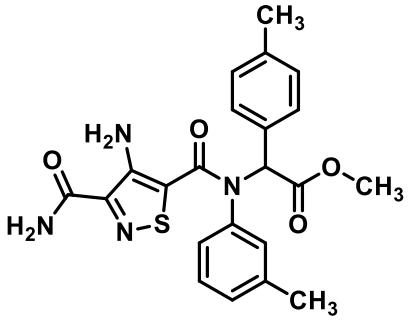
^{13}C NMR (126 MHz, $CDCl_3$): δ [ppm] = 21.1 (1C, Ar*C*H$_3$), 21.2 (1C, Ar*C*H$_3$), 27.6 (1C, HS*C*H$_2$CO), 52.6 (1C, COO*C*H$_3$), 64.1 (1C, CO*C*HN), 127.5 (1C, C$_{arom.}$), 128.9 (1C, C$_{arom.}$), 129.1 (2C, C-3"$_{p\text{-tolyl}}$, C-5"$_{p\text{-tolyl}}$), 129.3 (1C, C$_{arom.}$), 130.1 (2C, C-2"$_{p\text{-tolyl}}$, C-6"$_{p\text{-tolyl}}$), 130.5 (1C, C$_{arom.}$)131.1 (1C, C$_{arom.}$), 138.4 (2C, C$_{arom.}$), 138.9 (1C, C$_{arom.}$), 170.5 (1C, *C*OOCH$_3$), 171.1 (1C, Ar*C*ONCH);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3026, 2950, 2922, 1744, 1655, 1603, 1515, 1487, 1433, 1372, 1339, 1207, 1173, 1090, 1069, 1002, 872, 816, 793, 705, 635, 549, 523, 499, 446;

HRMS (*m*/*z*): [M+Na]$^+$ calcd for $C_{19}H_{21}NNaO_3S$: 366.1134, found: 366.1119;

HPLC (method 1): t_R = 24.2 min, purity 85.3%.

Methyl 2-(4-amino-3-carbamoyl-*N*-(*m*-tolyl)isothiazole-5-carboxamido)-2-(*p*-tolyl)acetate (35)



$C_{22}H_{22}N_4O_4S$

M_r = 438.5

41 (343 mg, 1.0 mmol) was added in one portion to a solution of **25** (320 mg, 1.2 mmol) in acetone (100 mL). The solution was cooled to 0 °C and morpholine (0.17 mL, 170 mg, 1.9 mmol) was added dropwise. After stirring the mixture for 12 h at ambient temperature, the solvent was removed *in vacuo*. The residue was purified by flash column chromatography (Ø = 6 cm, h = 20 cm, V = 15 mL, petroleum ether/ethyl acetate = 2/1) and recrystallized from acetonitrile to give **35** as colorless solid (184 mg, 0.42 mmol, 42 % yield).

m.p. = 192 °C;

TLC: R_f = 0.19 (petroleum ether/ethyl acetate = 4/1);

^{1}H NMR (300 MHz, DMSO-d_6): δ [ppm] = 2.20 (s, 3H, ArCH_3), 2.21 (s br, 3H, ArCH_3), 3.71 (s, 3H, COOCH_3), 6.02 (s, 1H, NCHCO), 6.72 (br, 1H, H$_{m\text{-tolyl}}$), 7.02 (s, 4H, 2'''-H$_{p\text{-tolyl}}$, 3'''-H$_{p\text{-tolyl}}$, 5'''-H$_{p\text{-tolyl}}$, 6'''-H$_{p\text{-tolyl}}$), 7.12 – 7.47 (m, 5H, H$_{m\text{-tolyl}}$ (2H), ArNH_2), 7.59 – 7.66 (m, 1H, ArCONH_2), 7.81 – 7.89 (m, 1H, ArCONH_2);

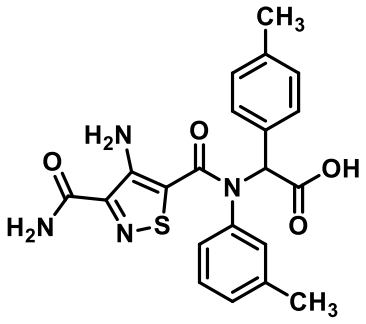
^{13}C NMR (151 MHz, DMSO-d_6): δ [ppm] = 20.6 (1C, ArCH$_3$), 20.7 (1C, ArCH$_3$), 52.3 (1C, COOCH$_3$), 64.0 (1C, COCHN), 122.0 (1C, C-5'$_{\text{isothiazole}}$), 128.7 (2C, C-3'''$_{p\text{-tolyl}}$, C-5'''$_{p\text{-tolyl}}$), 129.2 (1C, C$_{m\text{-tolyl}}$), 130.0 (2C, C-2'''$_{p\text{-tolyl}}$, C-6'''$_{p\text{-tolyl}}$), 130.6 (1C, C$_{m\text{-tolyl}}$), 132.7 (1C, C$_{m\text{-tolyl}}$), 137.0 (1C, C$_{m\text{-tolyl}}$), 137.6 (1C, C-4'''$_{p\text{-tolyl}}$), 139.1 (1C, C$_{\text{arom.}}$), 146.5 (1C, C-3'$_{\text{isothiazole}}$), 151.5 (1C, C-4'$_{\text{isothiazole}}$), 162.5 (1C, ArCONCH), 163.8 (1C, ArCONH$_2$), 170.6 (1C, COOCH$_3$), the signals for 2 C$_{\text{arom.}}$ cannot be observed in the spectrum;

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3462, 3422, 3345, 3188, 2951, 1742, 1670, 1617, 1572, 1556, 1486, 1443, 1365, 1319, 1208, 1175, 1069, 874, 848, 812, 798, 729, 706, 679, 615, 525, 440;

HRMS (m/z): [M+Na]$^+$ calcd for $C_{22}H_{22}N_4NaO_4S$: 461.1254, found: 461.1247;

HPLC (method 1): t_R = 24.3 min, purity 96.6%.

2-(4-Amino-3-carbamoyl-*N*-(*m*-tolyl)isothiazole-5-carboxamido)-2-(*p*-tolyl)acetic acid (36)



$C_{21}H_{20}N_4O_4S$

$M_r = 424.5$

35 (438 mg, 1 mmol) and lithium hydroxide (36 mg, 1.5 mmol) were dissolved in a mixture of tetrahydrofuran and water (1:1) (20 mL) and the mixture was stirred at ambient temperature for 12 h. The mixture was concentrated and diluted with water (30 mL). The aqueous phase was acidified to pH 4 with an aqueous solution of hydrogen chloride (6 M) and extracted with dichloromethane (3×20 mL). The combined organic layers were washed with brine (50 mL), dried with sodium sulfate, filtered, and the solvent was removed *in vacuo*. The residue was purified by flash column chromatography (Ø = 4 cm, h = 15 cm, V = 15 mL, dichloromethane/methanol/acetic acid = 10/1/0.1) to give **36** as colorless solid (63 mg, 0.15 mmol, 15 % yield).

m.p. = 194 °C;

TLC: R_f = 0.51 (dichloromethane/methanol/acetic acid = 10/1/0.1);

^{1}H NMR (600 MHz, DMSO-d_6): δ [ppm] = 2.19 (s, 3H, ArCH_3), 2.20 (s br, 3H, ArCH_3), 5.96 (s, 1H, NC*H*CO), 6.63 (br, 1H, H$_{m\text{-tolyl}}$), 7.01 (q, J = 8.1 Hz, 4H, 2'''-H$_{p\text{-tolyl}}$, 3'''-H$_{p\text{-tolyl}}$, 5'''-H$_{p\text{-tolyl}}$, 6'''-H$_{p\text{-tolyl}}$), 7.11 (br, 1H, H$_{m\text{-tolyl}}$), 7.17 – 7.25 (m, 3H, H$_{m\text{-tolyl}}$ (1H), ArNH_2), 7.44 (br, 1H, H$_{m\text{-tolyl}}$), 7.60 (s br, 1H, ArCONH_2), 7.84 (s br, 1H, ArCONH_2), 12.99 (br, 1H, COO*H*);

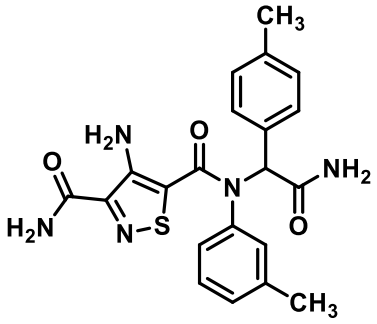
^{13}C NMR (151 MHz, DMSO-d_6): δ [ppm] = 20.6 (1C, Ar*C*H$_3$), 20.7 (1C, Ar*C*H$_3$), 64.2 (1C, CO*C*HN), 122.5 (1C, C-5'$_{isothiazole}$), 128.5 (2C, C-3'''$_{p\text{-tolyl}}$, C-5'''$_{p\text{-tolyl}}$), 129.1 (1C, C$_{m\text{-tolyl}}$), 129.4 (1C, C$_{m\text{-tolyl}}$), 130.2 (2C, C-2'''$_{p\text{-tolyl}}$, C-6'''$_{p\text{-tolyl}}$), 130.4 (1C, C-1'''$_{p\text{-tolyl}}$), 130.7 (1C, C$_{m\text{-tolyl}}$), 132.8 (1C, C$_{m\text{-tolyl}}$), 137.2 (1C, C$_{m\text{-tolyl}}$), 137.3 (1C, C-4'''$_{p\text{-tolyl}}$), 138.9 (1C, C$_{m\text{-tolyl}}$), 146.5 (1C, C-3'$_{isothiazole}$), 151.4 (1C, C-4'$_{isothiazole}$), 162.3 (1C, Ar*C*ONCH), 163.9 (1C, Ar*C*ONH$_2$), 171.5 (1C, *C*OOH);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3481,3398, 3365, 3196, 2920, 2364, 1706, 1611, 1599, 1496, 1374, 1362, 1230, 1184, 1087, 754, 726, 703, 688, 648, 631, 525, 508, 497, 424;

HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{21}H_{21}N_4O_4S$: 425.1278, found: 425.1174;

HPLC (method 1): t_R = 21.8 min, purity 85.6%.

4-Amino-N^5-[2-amino-2-oxo-1-(*p*-tolyl)ethyl]-N^5-(*m*-tolyl)isothiazole-3,5-dicarboxamide (42)



$C_{21}H_{21}N_5O_3S$

M_r = 423.5

DIPEA (13 mg, 0.10 mmol) was added to an ice-cooled solution of **36** (42 mg, 0.099 mmol) and COMU (43 mg, 0.10 mmol) in acetonitrile (5 mL) and the mixture was stirred for 10 min at 0 °C. After the addition of aqueous ammonia (1 M, 0.2 mL) at 0 °C, the reaction mixture was stirred for 4 h at ambient temperature. Subsequently, the mixture was concentrated *in vacuo* and the residue was dissolved in dichloromethane (30 mL). The organic phase was washed with water (2× 20 mL) and brine (30 mL), dried over sodium sulfate, filtered, and the solvent was removed *in vacuo*. The residue was purified by flash column chromatography (Ø = 4 cm, h = 15 cm, V = 15 mL, dichloromethane/ethyl acetate = 2/1) to give **42X** (25 mg, 0.059 mmol, 60% yield) as colorless solid.

m.p. = 232 °C;

TLC: R_f = 0.32 (dichloromethane/ethyl acetate = 2/1);

^{1}H NMR (600 MHz, DMSO-d_6): δ [ppm] = 1.95 – 2.38 (m, 6H, ArCH_3), 6.04 (s, 1H, NC*H*CO), 6.93 – 6.96 (m, 2H, 3'''-$H_{p\text{-tolyl}}$, 5'''-$H_{p\text{-tolyl}}$), 6.97 – 7.01 (m, 2H, 2'''-$H_{p\text{-tolyl}}$, 6'''-$H_{p\text{-tolyl}}$), 7.12 – 7.16 (m, 2H, $H_{m\text{-tolyl}}$ (1H), CHCONH_2 (1H)), 7.18 (s br, 2H, ArNH_2), 7.52 (s br, 1H, CHCONH_2),

7.58 (s br, 1H, ArCONH_2), 7.81 (s br, 1H, ArCONH_2), the signals for 3 $H_{m\text{-tolyl}}$ cannot be observed in the spectrum;

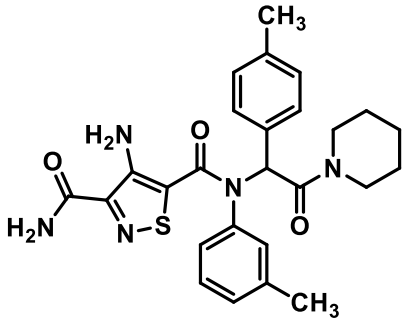
^{13}C NMR (151 MHz, DMSO-d_6): δ [ppm] = 20.6 (2C, ArCH$_3$), 64.0 (1C, NCHCO), 123.1 (1C, C-5'$_{isothiazole}$), 128.4 (2C, C-3'''$_{p\text{-tolyl}}$, C-5'''$_{p\text{-tolyl}}$), 128.8 (1C, $C_{arom.}$), 130.09 (1C, $C_{arom.}$), 130.13 (1C, $C_{arom.}$), 130.5 (2C, C-2'''$_{p\text{-tolyl}}$, C-6'''$_{p\text{-tolyl}}$), 131.6 (1C, C-1'''$_{p\text{-tolyl}}$), 133.6 (1C, $C_{arom.}$), 137.0 (1C, $C_{arom.}$), 137.2 (1C, $C_{arom.}$), 138.5 (1C, $C_{arom.}$), 146.4 (1C, C-3'$_{isothiazole}$), 151.3 (1C, C-4'$_{isothiazole}$), 162.3 (1C, ArCONCH), 163.9 (1C, ArCONH$_2$), 171.5 (CHCONH$_2$);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3487, 3448, 3381, 3331, 2987, 2919, 1702, 1665, 1596, 1569, 1489, 1447, 1400, 1355, 1313, 1070, 814, 794, 734, 713, 696, 659, 569, 545;

HRMS (*m*/*z*): [M+H]$^+$ calcd for $C_{21}H_{22}N_5O_3S$: 424.1438, found: 424.1449;

HPLC (method 1): t_R = 20.1 min, purity 97.9%.

4-Amino-N^5-[2-oxo-2-(piperidin-1-yl)-1-(*p*-tolyl)ethyl]-N^5-(*m*-tolyl)isothiazole-3,5-dicarboxamide (43)



$C_{26}H_{29}N_5O_3S$

M_r = 491.6

DIPEA (13 mg, 0.10 mmol) was added to an ice-cooled solution of **36** (42 mg, 0.099 mmol) and COMU (43 mg, 0.10 mmol) in acetonitrile (5 mL) and the mixture was stirred for 10 min at 0 °C. After the addition of piperidine (8.5 mg, 0.10 mmol) at 0 °C, the reaction mixture was stirred for 4 h at ambient temperature. Subsequently, the mixture was concentrated *in vacuo* and the residue was dissolved in dichloromethane (30 mL). The organic phase was washed with water (2× 20 mL) and brine (30 mL), dried over sodium sulfate, filtered, and the solvent was removed *in vacuo*. The residue was purified by flash column chromatography (Ø = 2 cm, h = 15 cm, V = 5 mL, dichloromethane/acetone = 50/1 → 10/1) to give **43** (37 mg, 0.075 mmol, 76% yield) as colorless solid.

m.p. = 279 °C;

TLC: R_f = 0.26 (dichloromethane/acetone = 10/1);

^{1}H NMR (5 00 MHz, DMSO-*d*$_6$): δ [ppm] = 0.61 – 0.70 (m, 1H, N(CH$_2$C*H*$_2$)$_2$CH$_2$), 1.21 – 1.38 (m, 2H, N(CH$_2$C*H*$_2$)$_2$CH$_2$), 1.39 – 1.54 (m, 3H, N(CH$_2$CH$_2$)$_2$C*H*$_2$ (2H), N(CH$_2$C*H*$_2$)$_2$CH$_2$ (1H)), 2.02 (s br, 1.5H, ArC*H*$_3$), 2.16 (s, 3H, ArC*H*$_3$), 2.28 (s br, 1.5H, ArC*H*$_3$), 3.19 – 3.36 (m, 3H, N(C*H*$_2$CH$_2$)$_2$CH$_2$), 3.70 – 3.82 (m, 1H, N(C*H*$_2$CH$_2$)$_2$CH$_2$), 6.46 (s br, 1H, H$_{m\text{-tolyl}}$), 6.51 (s, 1H, NC*H*CO), 6.91 – 7.04 (m, 4.5H, 2'''-H$_{p\text{-tolyl}}$, 3'''-H$_{p\text{-tolyl}}$, 5'''-H$_{p\text{-tolyl}}$, 6'''-H$_{p\text{-tolyl}}$, H$_{m\text{-tolyl}}$ (0.5H)), 7.10 – 7.15 (m, 1H, 4''-H$_{m\text{-tolyl}}$), 7.18 (s br, 2H, ArN*H*$_2$), 7.25 (s br, 0.5H, H$_{m\text{-tolyl}}$), 7.59 (s br, 1H, CON*H*$_2$), 7.67 – 7.81 (m, 1H, H$_{m\text{-tolyl}}$), 7 82 (s br, 1H, CON*H*$_2$),

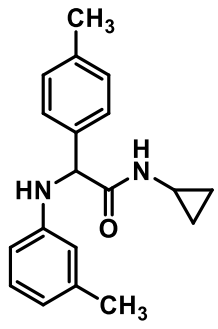
^{13}C NMR (151 MHz, DMSO-*d*$_6$): δ [ppm] = 20.6 (2C, Ar*C*H$_3$), 23.8 (1C, N(CH$_2$CH$_2$)$_2$*C*H$_2$), 25.1 (1C, N(CH$_2$*C*H$_2$)$_2$CH$_2$), 25.2 (1C, N(CH$_2$*C*H$_2$)$_2$CH$_2$), 42.8 (1C, N(*C*H$_2$CH$_2$)$_2$CH$_2$), 45.9 (1C, N(*C*H$_2$CH$_2$)$_2$CH$_2$), 61.8 (1C, N*C*HCO), 123.0 (1C, C-5'$_{isothiazole}$), 128.8 (3C, C-3'''$_{p\text{-tolyl}}$, C-5'''$_{p\text{-tolyl}}$, 1 C$_{m\text{-tolyl}}$), 130.1 (1C, C$_{arom.}$), 130.27 (1C, C$_{arom.}$), 130.30 (1C, C$_{arom.}$), 130.4 (2C, C-2'''$_{p\text{-tolyl}}$, C-6'''$_{p\text{-tolyl}}$), 133.8 (1C, C$_{m\text{-tolyl}}$), 137.1 (1C, C-1''$_{m\text{-tolyl}}$), 137.3 (1C, C-4'''$_{p\text{-tolyl}}$), 146.4 (1C, C-3'$_{isothiazole}$), 151.3 (1C, C-4'$_{isothiazole}$), 162.1 (1C, Ar*C*ONCH), 163.9 (1C, Ar*C*ONH$_2$), 167.4 (1C, *C*ON(CH$_2$CH$_2$)$_2$CH$_2$), the signal for 1 C$_{arom.}$ cannot be observed in the spectrum;

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3463, 3342, 3280, 3237, 2935, 2859, 1681, 1630, 1580, 1568, 1515, 1490, 1444, 1402, 1374, 1342, 1299, 1253, 1226, 1190, 1156, 1139, 1121, 1073, 1024, 1011, 849, 818, 794, 734, 707, 693, 663, 654, 642, 621, 555, 529, 500, 464, 440, 417;

HRMS (*m*/*z*): [M+H]$^+$ calcd for C$_{26}$H$_{30}$N$_5$O$_3$S: 492.2064, found: 492.2076;

HPLC (method 1): t$_R$ = 24.4 min, purity 95.1%.

***N*-Cyclopropyl-2-(*p*-tolyl)-2-(*m*-tolylamino)acetamide (44a)**



C$_{19}$H$_{22}$N$_2$O

M$_r$ = 294.4

DIPEA (0.52 mL, 390 mg, 3.0 mmol) and COMU (1.3 g, 3.0 mmol) were added to an ice-cooled solution of **38** (770 mg, 3.0 mmol) in acetonitrile (40 mL) and the mixture was stirred for 10 min at 0 °C. Then, cyclopropylamine (0.21 mL, 170 mg, 3.0 mmol) and DIPEA (0.52 mL, 390 mg, 3.0 mmol) were added. After stirring the reaction mixture at ambient temperature for 8 h, the volatiles were removed *in vacuo*. The residue was dissolved in dichloromethane (100 mL) and the organic solvent was washed with a saturated aqueous solution of ammonium chloride (2× 50 mL), water (100 mL), and brine (100 mL), dried with sodium sulfate, filtered, and removed *in vacuo*. The residue was purified by flash column chromatography (Ø = 6 cm, h = 20 cm, V = 30 mL, petroleum ether/ethyl acetate = 8/1) to give **44a** (710 mg, 2.4 mmol, 80% yield) as pink oil.

TLC: R_f = 0.42 (petroleum ether/ethyl acetate = 4/1);

^{1}H NMR (400 MHz, $CDCl_3$): δ [ppm] = 0.39 – 0.51 (m, 2H, NHCH(CH_2)$_2$), 0.67 – 0.81 (m, 2H, NHCH(CH_2)$_2$), 2.27 (s, 3H, ArCH_3), 2.34 (s, 3H, ArCH_3), 2.69 – 2.77 (m, 1H, NHC*H*(CH$_2$)$_2$), 4.66 (s, 1H, NHC*H*CO), 6.38 – 6.43 (m, 1H, 6'-H$_{m\text{-tolyl}}$), 6.43 – 6.45 (m, 1H, 2'-H$_{m\text{-tolyl}}$), 6.60 – 6.66 (m, 1H, 4'-H$_{m\text{-tolyl}}$), 6.82 – 6.88 (m, 1H, CON*H*CH), 7.07 (t, *J* = 7.7 Hz, 1H, 5'-H$_{m\text{-tolyl}}$), 7.14 – 7.20 20 (m, 2H, 3"-H$_{p\text{-tolyl}}$, 5"-H$_{p\text{-tolyl}}$), 7.24 – 7.30 (m, 2H, 2"-H$_{p\text{-tolyl}}$, 6"-H$_{p\text{-tolyl}}$), the signal for ArN*H*CH cannot be observed in the spectrum;

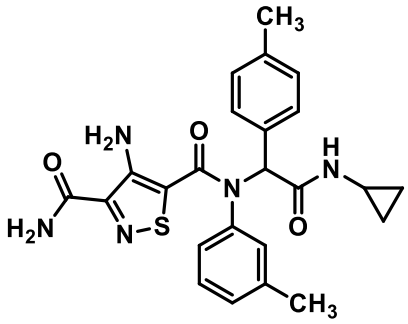
^{13}C NMR (101 MHz, $CDCl_3$): δ [ppm] = 6.5 (1C, NHCH(*C*H$_2$)$_2$), 6.8 (1C, NHCH(*C*H$_2$)$_2$), 21.3 (1C, Ar*C*H$_3$), 21.7 (1C, Ar*C*H$_3$), 22.7 (1C, NH*C*H(CH$_2$)$_2$), 64.1 (1C, NHCHCO), 111.1 (1C, C-6'$_{m\text{-tolyl}}$), 114.7 (1C, C-2'$_{m\text{-tolyl}}$), 120.3 (1C, C-4'$_{m\text{-tolyl}}$), 127.3 (2C, C-2"$_{p\text{-tolyl}}$, C-6"$_{p\text{-tolyl}}$), 129.3 (1C, C-5'$_{m\text{-tolyl}}$), 130.0 (2C, C-3"$_{p\text{-tolyl}}$, C-5"$_{p\text{-tolyl}}$), 135.9 (1C, C-1"$_{p\text{-tolyl}}$), 138.5 (1C, C-4"$_{p\text{-tolyl}}$), 139.4 (1C, C-3'$_{m\text{-tolyl}}$), 146.8 (1C, C-1'$_{m\text{-tolyl}}$), 173.2 (1C, *C*ONH);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3453, 3071, 3049, 2929, 2856, 1721, 1589, 1472, 1462, 1427, 1379, 1370, 1244, 1214, 1187, 1166, 1137, 1111, 1077, 998, 939, 909, 859, 822, 792, 740, 701, 613, 503, 489, 435;

HRMS (*m*/*z*): [M+H]$^+$ calcd for $C_{19}H_{23}N_2O$: 295.1805, found: 295.1796;

HPLC (method 1): t_R = 22.3 min, purity 81.1%.

4-Amino-N^5-[2-(cyclopropylamino)-2-oxo-1-(*p*-tolyl)ethyl]-N^5-(*m*-tolyl)isothiazole-3,5-dicarboxamide (47a)



$C_{24}H_{25}N_5O_3S$

M_r = 463.6

Triethylamine (0.34 mL, 240 mg, 2.4 mmol) and chloroacetyl chloride (0.19 mL, 270 mg, 2.4 mmol) were added to an ice-cooled solution of **44a** (590 mg, 2.0 mmol) in dichloromethane (40 mL) and the reaction mixture was stirred at ambient temperature for 12 h. Then, the organic solvent was washed with a saturated aqueous solution of ammonium chloride (2× 40 mL), a saturated aqueous solution of sodium bicarbonate (2× 40 mL), and brine (40 mL), dried over sodium sulfate, filtered, and removed *in vacuo*.

A share (370 mg, 56%) of the obtained crude product (660 mg) was dissolved in acetone (20 mL). Finely ground thiourea (91 mg, 1.2 mmol) was added and the resulting mixture was stirred for 16 h at ambient temperature. After removal of the solvent, the residue was treated with ethyl acetate (50 mL). Under vigorous stirring, a solution of sodium sulfite (250 mg, 2.0 mmol) in water (50 mL) was added and the mixture was stirred for 3 h at ambient temperature. The organic phase was washed with water (50 mL) and brine (50 mL), dried over sodium sulfate, filtered, and the solvent was removed *in vacuo*. The residue was washed with acetonitrile.

A share (180 mg, 96%) of the obtained crude product (190 mg) was added in one portion to a solution of **25** (160 mg, 0.60 mmol) in acetone (50 mL). The solution was cooled to 0 °C and morpholine (0.09 mL, 87 mg, 1.0 mmol) was added. After stirring the mixture for 12 h at ambient temperature, the solvent was removed *in vacuo*. The residue was purified by flash column chromatography (Ø = 6 cm, h = 20 cm, V = 15 mL, dichloromethane/acetone = 15/1) to give **47a** (180 mg, 0.38 mmol, 35% yield) as colorless solid.

m.p. = 221 °C;

TLC: R_f = 0.19 (dichloromethane/acetone = 15/1);

^{1}H NMR (400 MHz, DMSO-d_6): δ [ppm] = 0.23 – 0.31 (m, 1H, NHCH(CH_2)$_2$), 0.34 – 0.42 (m, 1H, NHCH(CH_2)$_2$), 0.54 – 0.66 (m, 2H, NHCH(CH_2)$_2$), 1.95 – 2.37 (m, 6H, ArCH_3), 2.63 – 2.71 (m, 1H, NHCH(CH$_2$)$_2$), 6.01 (s, 1H, NCHCO), 6.90 – 6.99 (m, 4H, 2'''-H$_{p\text{-tolyl}}$, 3'''-H$_{p\text{-tolyl}}$, 5'''-H$_{p\text{-tolyl}}$, 6'''-H$_{p\text{-tolyl}}$), 6.99 – 7.25 (m, 4H, ArNH_2, 4''-H$_{m\text{-tolyl}}$, H$_{m\text{-tolyl}}$ (1H)), 7.57 (s br, 1H, CONH_2), 7.80 (s br, 1H, CONH_2), 8.19 (d, J = 4.1 Hz, 1H, CONHCH), the signals for 2 H$_{m\text{-tolyl}}$ cannot be observed in the spectrum;

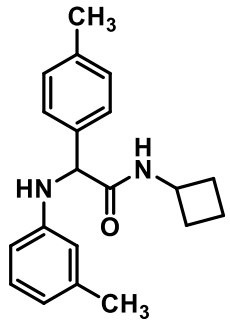
^{13}C NMR (151 MHz, DMSO-d_6): δ [ppm] = 5.5 (1C, NHCH(CH_2)$_2$), 5.6 (1C, NHCH(CH_2)$_2$), 20.2 (2C, ArCH_3), 22.5 (1C, NHCH(CH$_2$)$_2$), 63.7 (1C, NCHCO), 123.1 (1C, C-5'$_{isothiazole}$), 128.4 (2C, C-3'''$_{p\text{-tolyl}}$, C-5'''$_{p\text{-tolyl}}$), 128.7 (1C, C$_{m\text{-tolyl}}$), 130.1 (2C, C$_{m\text{-tolyl}}$), 130.3 (2C, C-2'''$_{p\text{-tolyl}}$, C-6'''$_{p\text{-tolyl}}$), 131.5 (1C, C-1'''$_{p\text{-tolyl}}$), 133.7 (1C, C$_{m\text{-tolyl}}$), 137.01 (1C, C$_{arom.}$), 137.04 (1C, C$_{arom.}$), 146.4 (1C, C-3'$_{isothiazole}$), 151.3 (1C, C-4'$_{isothiazole}$), 162.3 (1C, ArCONCH), 163.9 (1C, ArCONH$_2$), 170.9 (1C, CHCONH), the signal for 1 C$_{m\text{-tolyl}}$ cannot be observed in the spectrum;

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3477, 3446, 3280, 1657, 1612, 1597, 1559, 1517, 1488, 1450, 1395, 1369, 1340, 1325, 1308, 1256, 1191, 1157, 1091, 1073, 1041, 1025, 1001, 916, 815, 792, 755, 731, 707, 688, 666, 631, 600, 554, 527, 503, 483;

HRMS (m/z): [M+H]$^+$ calcd for $C_{24}H_{26}N_5O_3S$: 464.1751, found: 464.1759;

HPLC (method 1): t_R = 22.1 min, purity 96.0%.

N-Cyclobutyl-2-(*p*-tolyl)-2-(*m*-tolylamino)acetamide (44b)



$C_{20}H_{24}N_2O$

M_r = 308.4

DIPEA (0.52 mL, 390 mg, 3.0 mmol) and COMU (1.3 g, 3.0 mmol) were added to an ice-cooled solution of **38** (770 mg, 3.0 mmol) in acetonitrile (40 mL) and the mixture was stirred for 10 min at 0 °C. Then, cyclobutylamine (0.26 mL, 210 mg, 3.0 mmol) and DIPEA (0.52 mL,

390 mg, 3.0 mmol) were added. After stirring the reaction mixture at ambient temperature for 8 h, the volatiles were removed *in vacuo*. The residue was dissolved in dichloromethane (100 mL) and the organic solvent was washed with a saturated aqueous solution of ammonium chloride (2× 50 mL), water (100 mL), and brine (100 mL), dried with sodium sulfate, filtered, and removed *in vacuo*. The residue was purified by flash column chromatography (Ø = 6 cm, h = 20 cm, V = 30 mL, petroleum ether/ethyl acetate = 8/1) to give **44b** (750 mg, 2.4 mmol, 81% yield) as pink oil.

TLC: R_f = 0.46 (petroleum ether/ethyl acetate = 4/1);

^{1}H NMR (400 MHz, $CDCl_3$): δ [ppm] = 1.63 – 1.92 (m, 4H, NHCH(C*H*$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$)$_2$C*H*$_2$), 2.24 – 2.37 (m, 8H, ArC*H*$_3$, NHCH(C*H*$_2$)$_2$CH$_2$ (2H)), 4.34 – 4.47 (m, 1H, NHC*H*(CH$_2$)$_2$CH$_2$), 4.64 (s, 1H, NHC*H*CO), 6.40 – 6.45 (m, 1H, 6'-H$_{m\text{-tolyl}}$), 6.45 – 6.48 (m, 1H, 2'-H$_{m\text{-tolyl}}$), 6.61 – 6.66 (m, 1H, 4'-H$_{m\text{-tolyl}}$), 6.86 (d, J = 7.9 Hz, 1H, CON*H*CH), 7.07 (t, J = 7.7 Hz, 1H, 5'-H$_{m\text{-tolyl}}$), 7.15 – 7.20 (m, 2H, 3"-H$_{p\text{-tolyl}}$, 5"-H$_{p\text{-tolyl}}$), 7.27 – 7.31 (m, 2H, 2"-H$_{p\text{-tolyl}}$, 6"-H$_{p\text{-tolyl}}$), the signal for ArN*H*CH cannot be observed in the spectrum;

^{13}C NMR (101 MHz, $CDCl_3$): δ [ppm] = 15.2 (1C, NHCH(CH$_2$)$_2$*C*H$_2$), 21.3 (1C, Ar*C*H$_3$), 21.7 (1C, Ar*C*H$_3$), 31.0 (1C, NHCH(*C*H$_2$)$_2$CH$_2$), 31.1 (1C, NHCH(*C*H$_2$)$_2$CH$_2$),

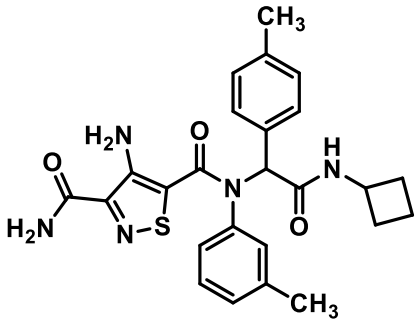
44.8 (1C, NH*C*H(CH$_2$)$_2$CH$_2$), 64.2 (1C, NH*C*HCO), 111.1 (1C, C-6'$_{m\text{-tolyl}}$), 114.8 (1C, C-2'$_{m\text{-tolyl}}$), 120.2 (1C, C-4'$_{m\text{-tolyl}}$), 127.4 (2C, C-2"$_{p\text{-tolyl}}$, C-6"$_{p\text{-tolyl}}$), 129.3 (1C, C-5'$_{m\text{-tolyl}}$), 130.0 (2C, C-3"$_{p\text{-tolyl}}$, C-5"$_{p\text{-tolyl}}$), 136.1 (1C, C-1"$_{p\text{-tolyl}}$), 138.4 (1C, C-4"$_{p\text{-tolyl}}$), 139.3 (1C, C-3'$_{m\text{-tolyl}}$), 147.0 (1C, C-1'$_{m\text{-tolyl}}$), 170.6 (1C, *C*ONH);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3305, 2979, 2941, 2866, 1737, 1646, 1605, 1590, 1508, 1489, 1444, 1372, 1321, 1300, 1241, 1194, 1180, 1153, 1112, 1044, 1022, 993, 843, 830, 795, 767, 734, 691, 637, 606, 581, 502, 441;

HRMS (*m*/*z*): [M+H]$^+$ calcd for $C_{20}H_{25}N_2O$: 309.1961, found: 309.1963;

HPLC (method 1): t_R = 23.6 min, purity 98.0%.

4-Amino-N^5-[2-(cyclobutylamino)-2-oxo-1-(*p*-tolyl)ethyl]-N^5-(*m*-tolyl)isothiazole-3,5-dicarboxamide (47b)



$C_{25}H_{27}N_5O_3S$

M_r = 477.6

Triethylamine (0.34 mL, 240 mg, 2.4 mmol) and chloroacetyl chloride (0.19 mL, 270 mg, 2.4 mmol) were added to an ice-cooled solution of **44b** (620 mg, 2.0 mmol) in dichloromethane (40 mL) and the reaction mixture was stirred at ambient temperature for 12 h. Then, the organic solvent was washed with a saturated aqueous solution of ammonium chloride (2× 40 mL), a saturated aqueous solution of sodium bicarbonate (2× 40 mL), and brine (40 mL), dried over sodium sulfate, filtered, and removed *in vacuo*.

A share (380 mg, 62%) of the obtained crude product (620 mg) was dissolved in acetone (20 mL). Finely ground thiourea (91 mg, 1.2 mmol) was added and the resulting mixture was stirred for 16 h at ambient temperature. After removal of the solvent, the residue was treated with ethyl acetate (25 mL). Under vigorous stirring, a solution of sodium sulfite (250 mg, 2.0 mmol) in water (50 mL) was added and the mixture was stirred for 3 h at ambient temperature. The organic phase was washed with water (50 mL) and brine (50 mL), dried with sodium sulfate, filtered, and the solvent was removed *in vacuo*. The residue was washed with acetonitrile.

A share (190 mg, 88%) of the obtained crude product (210 mg) was added in one portion to a solution of **25** (160 mg, 0.60 mmol) in acetone (50 mL). The solution was cooled to 0 °C and morpholine (0.09 mL, 87 mg, 1.0 mmol) was added. After stirring the mixture for 12 h at ambient temperature, the solvent was removed *in vacuo*. The residue was purified by flash column chromatography (Ø = 4 cm, h = 15 cm, V = 15 mL, dichloromethane/acetone = 15/1) to give **47b** (170 mg, 0.36 mmol, 33% yield) as colorless solid.

m.p. = 226 °C;

TLC: R_f = 0.20 (dichloromethane/acetone = 15/1);

^{1}H NMR (400 MHz, DMSO-d_6): δ [ppm] = 1.54 – 1.66 (m, 2H, NHCH(CH_2)$_2$CH_2), 1.66 – 1.78 (m, 1H, NHCH(CH_2)$_2$CH_2), 1.87 – 2.00 (m, 1H, NHCH(CH_2)$_2$CH_2), 2.02 – 2.33 (m, 8H, ArCH_3, NHCH(CH_2)$_2$CH_2 (2H)), 4.15 – 4.27 (m, 1H, NHCH(CH_2)$_2$CH_2), 6.05 (s, 1H, NCHCO), 6.54 (s br, 1H, H$_{m\text{-tolyl}}$), 6.78 – 7.34 (m, 8H, 2'''-H$_{p\text{-tolyl}}$, 3'''-H$_{p\text{-tolyl}}$, 5'''-H$_{p\text{-tolyl}}$, 6'''-H$_{p\text{-tolyl}}$, ArNH_2, 4''-H$_{m\text{-tolyl}}$, H$_{m\text{-tolyl}}$ (1H)), 7.42 – 7.78 (m, 2H, CONH_2 (1H), H$_{m\text{-tolyl}}$ (1H)), 7.83 (s br, 1H, CONH_2), 8.37 (d, J = 7.3 Hz, 1H, CONHCH);

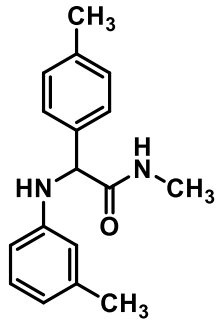
^{13}C NMR (101 MHz, DMSO-d_6): δ [ppm] = 14.8 (1C, NHCH(CH_2)$_2$CH_2), 20.6 (2C, ArCH$_3$), 29.9 (1C, NHCH(CH$_2$)$_2$CH_2), 30.0 (1C, NHCH(CH$_2$)$_2$CH_2), 44.3 (1C, NHCH(CH_2)$_2$CH_2), 63.7 (1C, NCHCO), 123.1 (1C, C-5'$_{isothiazole}$), 128.5 (2C, C-3'''$_{p\text{-tolyl}}$, C-5'''$_{p\text{-tolyl}}$), 128.7 (1C, C$_{m\text{-tolyl}}$), 130.1 (2C, C$_{m\text{-tolyl}}$), 130.3 (2C, C-2'''$_{p\text{-tolyl}}$, C-6'''$_{p\text{-tolyl}}$), 131.6 (1C, C-1'''$_{p\text{-tolyl}}$), 133.7 (1C, C$_{m\text{-tolyl}}$), 137.0 (2C, C-4'''$_{p\text{-tolyl}}$, C-1''$_{m\text{-tolyl}}$), 138.4 (1C, C$_{m\text{-tolyl}}$), 146.4 (1C, C-3'$_{isothiazole}$), 151.3 (1C, C-4'$_{isothiazole}$), 162.3 (1C, ArCONCH), 164.0 (1C, ArCONH$_2$), 168.6 (1C, CHCONH);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3471, 3393, 3355, 3183, 2983, 2925, 2855, 1690, 1672, 1621, 1575, 1558, 1511, 1486, 1447, 1406, 1378, 1337, 1327, 1304, 1289, 1246, 1228, 1206, 1187, 1144, 1113, 1072, 1027, 866, 850, 817, 801, 755, 744, 731, 707, 684, 663, 622, 551, 530, 505, 488, 461, 439, 420;

HRMS (m/z): [M+H]$^+$ calcd for $C_{25}H_{28}N_5O_3S$: 478.1907, found: 478.1896;

HPLC (method 1): t_R = 23.5 min, purity 95.3%.

***N*-Methyl-2-(*p*-tolyl)-2-(*m*-tolylamino)acetamide (44c)**



$C_{17}H_{20}N_2O$

M_r = 268.4

DIPEA (0.70 mL, 516 mg, 4.0 mmol) and COMU (1.7 g, 4.0 mmol) were added to an ice-cooled solution of **38** (1.02 g, 4.0 mmol) in acetonitrile (50 mL) and the mixture was stirred for

10 min at 0 °C. Then, a solution of methylamine in tetrahydrofuran (2M, 2.0 mL) and DIPEA (0.70 mL, 516 mg, 4.0 mmol) were added. After stirring the reaction mixture at ambient temperature for 8 h, the volatiles were removed *in vacuo*. The residue was dissolved in dichloromethane (100 mL) and the organic solvent was washed with a saturated aqueous solution of ammonium chloride (2× 50 mL), water (100 mL), and brine (100 mL), dried with sodium sulfate, filtered, and removed *in vacuo*. The residue was purified by flash column chromatography (Ø = 6 cm, h = 20 cm, V = 30 mL, petroleum ether/ethyl acetate = 8/1) to give **44c** (951 mg, 3.6 mmol, 89% yield) as colorless oil.

TLC: R_f = 0.40 (petroleum ether/ethyl acetate = 4/1);

^{1}H NMR (400 MHz, DMSO-d_6): δ [ppm] = 2.15 (s, 3H, ArCH_3), 2.27 (s, 3H, ArCH_3), 2.59 (d, J = 4.6 Hz, 3H, NHCH_3), 4.87 (d, J = 7.2 Hz, 1H, NHCHCO), 5.94 (d, J = 7.2 Hz, 1H, NHCHCO), 6.36 – 6.43 (m, 2H, 4'-H$_{m\text{-tolyl}}$, 6'-H$_{m\text{-tolyl}}$), 6.45 – 6.48 (m, 1H, 2'-H$_{m\text{-tolyl}}$), 6.89 – 6.95 (m, 1H, 5'-H$_{m\text{-tolyl}}$), 7.10 – 7.16 (m, 2H, 3"-H$_{p\text{-tolyl}}$, 5"-H$_{p\text{-tolyl}}$), 7.34 – 7.40 (m, 2H, 2"-H$_{p\text{-tolyl}}$, 6"-H$_{p\text{-tolyl}}$), 8.10 – 8.18 (m, 1H, NHCH$_3$);

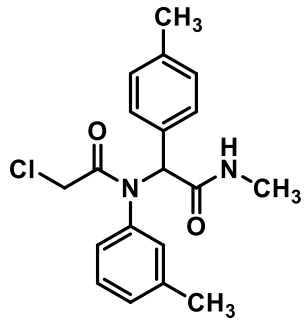
^{13}C NMR (126 MHz, DMSO-d_6): δ [ppm] = 20.6 (1C, ArCH$_3$), 21.3 (1C, ArCH$_3$), 25.6 (1C, NHCH$_3$), 60.6 (1C, NHCHCO), 110.3 (1C, C-6'$_{m\text{-tolyl}}$), 113.8 (1C, C-2'$_{m\text{-tolyl}}$), 117.5 (1C, C-4'$_{m\text{-tolyl}}$), 127.1 (2C, C-2"$_{p\text{-tolyl}}$, C-6"$_{p\text{-tolyl}}$), 128.6 (1C, C-5'$_{m\text{-tolyl}}$), 128.8 (2C, C-3"$_{p\text{-tolyl}}$, C-5"$_{p\text{-tolyl}}$), 136.6 (1C, C-4"$_{p\text{-tolyl}}$), 136.7 (1C, C-1"$_{p\text{-tolyl}}$), 137.6 (1C, C-3'$_{m\text{-tolyl}}$), 147.2 (1C, C-1'$_{m\text{-tolyl}}$), 171.4 (1C, CONHCH$_3$);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3383, 3303, 3040, 2919, 2875, 1665, 1604, 1591, 1513, 1478, 1449, 1422, 1407, 1380, 1331, 1305, 1270, 1258, 1231, 1206, 1191, 1178, 1162, 1133, 1114, 1023, 993, 841, 821, 793, 771, 754, 742, 699, 680, 644, 596, 560, 501, 446, 400;

HRMS (m/z): [M+H]$^+$ calcd for $C_{17}H_{21}N_2O$: 269.1648, found: 269.1638;

HPLC (method 1): t_R = 21.2 min, purity 99.1%.

2-Chloro-*N*-[2-(methylamino)-2-oxo-1-(*p*-tolyl)ethyl]-*N*-(*m*-tolyl)acetamide (45c)



$C_{19}H_{21}ClN_2O_2$

$M_r = 344.8$

Triethylamine (0.34 mL, 240 mg, 2.4 mmol) and chloroacetyl chloride (0.19 mL, 270 mg, 2.4 mmol) were added to an ice-cooled solution of **44c** (540 mg, 2.0 mmol) in dichloromethane (40 mL) and the reaction mixture was stirred at ambient temperature for 12 h. Then, the organic solvent was washed with a saturated aqueous solution of ammonium chloride (2× 40 mL), a saturated aqueous solution of sodium bicarbonate (2× 40 mL), and brine (40 mL), dried over sodium sulfate, filtered, and removed *in vacuo*. The residue was purified by flash column chromatography (Ø = 4 cm, h = 15 cm, V = 15 mL, dichloromethane/ethyl acetate = 4/1) to give **45c** (570 mg, 1.6 mmol, 82% yield) as colorless solid.

m.p. = 150 °C;

TLC: R_f = 0.51 (dichloromethane/ethyl acetate = 1/1);

^{1}H NMR (400 MHz, DMSO-*d*$_6$): δ [ppm] = 2.00 – 2.31 (m, 6H, ArC*H*$_3$), 2.61 (d, *J* = 4.5 Hz, 3H, NHC*H*$_3$), 3.88 (d, *J* = 14.0 Hz, 1H, ClC*H*$_2$CO), 3.95 (d, *J* = 14.0 Hz, 1H, ClC*H*$_2$CO), 5.92 (s, 1H, NC*H*CO), 6.86 – 6.97 (m, 4H, 2"-H$_{p\text{-tolyl}}$, 3"-H$_{p\text{-tolyl}}$, 5"-H$_{p\text{-tolyl}}$, 6"-H$_{p\text{-tolyl}}$), 6.97 – 7.02 (m, 1H, 4'-H$_{m\text{-tolyl}}$), 7.05 (s br, 1H, H$_{m\text{-tolyl}}$), 7.93 – 8.00 (m, 1H, N*H*CH$_3$), the signals for 2 H$_{m\text{-tolyl}}$ cannot be observed in the spectrum;

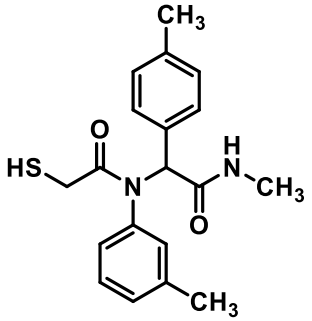
^{13}C NMR (151 MHz, DMSO-*d*$_6$): δ [ppm] = 20.6 (1C, Ar*C*H$_3$), 20.7 (1C, Ar*C*H$_3$), 25.7 (1C, NH*C*H$_3$), 43.0 (1C, Cl*C*H$_2$CO), 64.1 (1C, N*C*HCO), 127.6 (1C, C$_{m\text{-tolyl}}$), 128.3 (1C, C$_{m\text{-tolyl}}$), 128.4 (2C, C-3"$_{p\text{-tolyl}}$, C-5"$_{p\text{-tolyl}}$), 128.8 (1C, C-4'$_{m\text{-tolyl}}$), 130.1 (2C, C-2"$_{p\text{-tolyl}}$, C-6"$_{p\text{-tolyl}}$), 131.2 (1C, C$_{m\text{-tolyl}}$), 131.6 (1C, C-1"$_{p\text{-tolyl}}$), 137.0 (1C, C-4"$_{p\text{-tolyl}}$), 137.9 (1C, C-3'$_{m\text{-tolyl}}$), 138.2 (1C, C-1'$_{m\text{-tolyl}}$), 165.3 (1C, ClCH$_2$*C*O), 169.7 (1C, *C*ONHCH$_3$);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3267, 3095, 2950, 1673, 1651, 1604, 1573, 1516, 1488, 1450, 1409, 1364, 1312, 1249, 1189, 1161, 1039, 929, 823, 795, 763, 741, 705, 688, 652, 632, 552, 501, 485;

HRMS (*m*/*z*): $[M+Na]^+$ calcd for $C_{19}H_{21}{}^{35}ClN_2NaO_2$: 367.1184, found: 367.1183;

HPLC (method 1): t_R = 21.5 min, purity 95.8%.

2-Mercapto-*N*-[2-(methylamino)-2-oxo-1-(*p*-tolyl)ethyl]-*N*-(*m*-tolyl)acetamide (46c)



$C_{19}H_{22}N_2O_2S$

M_r = 342.5

Finely ground thiourea (91 mg, 1.2 mmol) was added to a solution of **45c** (340 mg, 1.0 mmol) in acetone (20 mL) and the resulting mixture stirred for 16 h at ambient temperature. After removal of the solvent, the residue was treated with ethyl acetic (25 mL). Under vigorous stirring, a solution of sodium sulfite (250 mg, 2.0 mmol) in water (25 mL) was added and the mixture was stirred for 3 h at ambient temperature. The organic phase was washed with water (20 mL) and brine (20 mL), dried with sodium sulfate, filtered, and the solvent was removed *in vacuo*. The residue was purified by flash column chromatography (Ø = 4 cm, h = 15 cm, V = 15 mL, dichloromethane/ethyl acetate = 2/1) to give **46c** (260 mg, 0.77 mmol, 77% yield) as colorless solid.

m.p. = 122 °C;

TLC: R_f = 0.40 (dichloromethane/ethyl acetate = 1/1);

^{1}H NMR (500 MHz, DMSO-d_6): δ [ppm] = 2.03 – 2.26 (m, 6H, ArCH_3), 2.57 (t, *J* = 6.9 Hz, 1H, *H*SCH_2CO), 2.60 (d, *J* = 4.6 Hz, 3H, NHCH_3), 2.98 (dd, *J* = 14.9/6.7 Hz, 1H, HSCH_2CO), 3.03 (dd, *J* = 14.9/7.0 Hz, 1H, HSCH_2CO), 5.91 (s, 1H, NC*H*CO), 6.86 – 6.91 (m, 2H, 2''-$H_{p\text{-tolyl}}$, 6''-$H_{p\text{-tolyl}}$), 6.91 – 6.95 (m, 2H, 3''-$H_{p\text{-tolyl}}$, 5''-$H_{p\text{-tolyl}}$), 6.96 – 7.00 (m, 1H, 4'-$H_{m\text{-tolyl}}$), 7.04

(s br, 1H, $H_{m\text{-tolyl}}$), 7.89 – 7.96 (m, 1H, N*H*CH_3), the signals for 2 $H_{m\text{-tolyl}}$ cannot be observed in the spectrum;

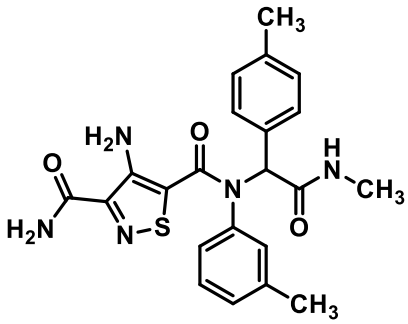
^{13}C NMR (101 MHz, DMSO-d_6): δ [ppm] = 20.6 (1C, Ar*C*H_3), 20.7 (1C, Ar*C*H_3), 25.7 (1C, NH*C*H_3), 27.3 (1C, HS*C*H_2CO), 63.9 (1C, N*C*HCO), 127.7 (1C, $C_{m\text{-tolyl}}$), 128.2 (1C, $C_{m\text{-tolyl}}$), 128.4 (2C, C-3"$_{p\text{-tolyl}}$, C-5"$_{p\text{-tolyl}}$), 128.5 (1C, $C_{m\text{-tolyl}}$), 130.1 (2C, C-2"$_{p\text{-tolyl}}$, C-6"$_{p\text{-tolyl}}$), 131.2 (1C, $C_{m\text{-tolyl}}$), 132.0 (1C, C-1"$_{p\text{-tolyl}}$), 136.9 (1C, C-4"$_{p\text{-tolyl}}$), 137.8 (1C, C-3'$_{m\text{-tolyl}}$), 139.1 (1C, C-1'$_{m\text{-tolyl}}$), 168.9 (1C, HSCH_2*C*O), 170.0 (1C, *C*ONHCH_3);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3232, 3063, 2932, 2560, 1672, 1651, 1603, 1575, 1515, 1487, 1448, 1407, 1365, 1336, 1313, 1260, 1245, 1221, 1189, 1151, 1123, 1095, 1083, 1039, 1023, 999, 926, 899, 869, 825, 796, 771, 742, 705, 636, 571, 552, 505, 485, 461, 445;

HRMS (*m*/*z*): $[M+Na]^+$ calcd for $C_{19}H_{22}N_2NaO_2S$: 365.1294, found: 365.1293;

HPLC (method 1): t_R = 21.2 min, purity 95.3%.

4-Amino-N^5-[2-(methylamino)-2-oxo-1-(*p*-tolyl)ethyl]-N^5-(*m*-tolyl)isothiazole-3,5-dicarboxamide (47c)



$C_{22}H_{23}N_5O_3S$

M_r = 437.5

46c (340 mg, 1.0 mmol) was added in one portion to a solution of **6** (320 mg, 1.2 mmol) in acetone (100 mL). The solution was cooled to 0 °C and morpholine (0.17 mL, 170 mg, 1.9 mmol) was added dropwise. After stirring the mixture for 12 h at ambient temperature, the solvent was removed *in vacuo*. The residue was purified by flash column chromatography (Ø = 6 cm, h = 20 cm, V = 15 mL, dichloromethane/acetone = 10/1) and recrystallized from acetonitrile to give **47c** (270 mg, 0.63 mmol, 63% yield) as colorless solid.

m.p. = 231 °C;

TLC: R_f = 0.31 (dichloromethane/acetone = 4/1);

^{1}H NMR (500 MHz, DMSO-d_6): δ [ppm] = 1.97 – 2.36 (m, 6H, ArCH_3), 2.62 (d, J = 4.6 Hz, 3H, NHCH_3), 6.05 (s, 1H, NCHCO), 6.91 – 6.99 (m, 4H, 2'''-H$_{p\text{-tolyl}}$, 3'''-H$_{p\text{-tolyl}}$, 5'''-H$_{p\text{-tolyl}}$, 6'''-H$_{p\text{-tolyl}}$), 7.13 – 7.16 (m, 1H, H$_{m\text{-tolyl}}$), 7.19 (s br, 2H, ArNH_2), 7.59 (s br, 1H, CONH_2), 7.82 (s br, 1H, CONH_2), 8.02 (q, J = 4.6 Hz, 1H, NHCH$_3$), the signals for 3 H$_{m\text{-tolyl}}$ cannot be observed in the spectrum;

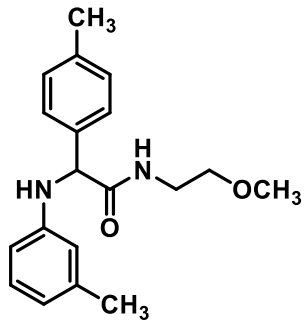
^{13}C NMR (101 MHz, DMSO-d_6): δ [ppm] = 20.6 (1C, ArCH$_3$), 20.7 (1C, ArCH$_3$), 25.7 (1C, NHCH$_3$), 64.1 (1C, NCHCO), 123.0 (1C, C-5'$_{isothiazole}$), 128.4 (2C, C-3'''$_{p\text{-tolyl}}$, C-5'''$_{p\text{-tolyl}}$), 128.7 (1C, C$_{arom.}$), 130.09 (1C, C$_{arom.}$), 130.15 (1C, C$_{arom.}$), 130.4 (2C, C-2'''$_{p\text{-tolyl}}$, C-6'''$_{p\text{-tolyl}}$), 131.6 (1C, C-1'''$_{p\text{-tolyl}}$), 133.6 (1C, C$_{arom.}$), 137.08 (1C, C$_{arom.}$), 137.10 (1C, C$_{arom.}$), 138.5 (1C, C$_{arom.}$), 146.4 (1C, C-3'$_{isothiazole}$), 151.3 (1C, C-4'$_{isothiazole}$), 162.3 (1C, ArCONCH), 163.9 (1C, ArCONH$_2$), 170.1 (1C, CONHCH$_3$);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3451, 3419, 3333, 3308, 2921, 1682, 1663, 1610, 1566, 1522, 1489, 1444, 1406, 1371, 1320, 1262, 1242, 1195, 1182, 1152, 1115, 1089, 1073, 1031, 846, 819, 795, 762, 731, 704, 685, 669, 650, 599, 580, 544, 526, 509, 485, 456, 440;

HRMS (m/z): [M+H]$^+$ calcd for $C_{22}H_{24}N_5O_3S$: 438.1594, found: 438.1594;

HPLC (method 1): t_R = 21.0 min, purity 97.9%.

***N*-(2-Methoxyethyl)-2-(*p*-tolyl)-2-(*m*-tolylamino)acetamide (44d)**



$C_{19}H_{24}N_2O_2$

M_r = 312.4

DIPEA (0.52 mL, 390 mg, 3.0 mmol) and COMU (1.3 g, 3.0 mmol) were added to an ice-cooled solution of **38** (770 mg, 3.0 mmol) in acetonitrile (40 mL) and the mixture was stirred for 10 min at 0 °C. Then, 2-methoxyethanamine (0.26 mL, 230 mg, 3.0 mmol) and DIPEA

(0.52 mL, 390 mg, 3.0 mmol) were added. After stirring the reaction mixture at ambient temperature for 8 h, the volatiles were removed *in vacuo*. The residue was dissolved in dichloromethane (100 mL) and the organic solvent was washed with a saturated aqueous solution of ammonium chloride (2× 30 mL), water (50 mL), and brine (50 mL), dried over sodium sulfate, filtered, and removed *in vacuo*. The residue was purified by flash column chromatography (Ø = 6 cm, h = 20 cm, V = 30 mL, dichloromethane/ethyl acetate = 20/1) to give **44d** (580 mg, 1.9 mmol, 62% yield) as colorless oil.

TLC: R_f = 0.22 (dichloromethane/ethyl acetate = 20/1);

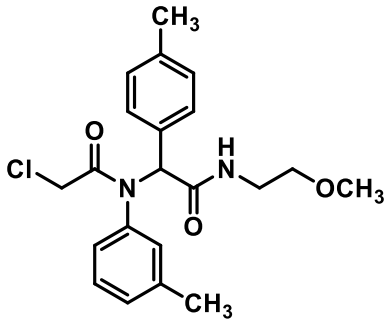
^{1}H NMR (400 MHz, DMSO-d_6): δ [ppm] = 2.14 (s, 3H, C$H_{3,\ m\text{-tolyl}}$), 2.26 (s, 3H, C$H_{3,\ p\text{-tolyl}}$), 3.10 – 3.18 (m, 1H, NHCH_2CH$_2$O), 3.19 (s, 3H, OCH_3), 3.22 – 3.34 (m, 3H, NHCH_2CH$_2$O (1H), NHCH$_2$CH_2O), 4.93 (d, J = 6.4 Hz, 1H, NHCHCO), 5.95 (d, J = 7.0 Hz, 1H, ArNHCHCO), 6.34 – 6.39 (m, 1H, 4'-H$_{m\text{-tolyl}}$), 6.39 – 6.44 (m, 1H, 6'-H$_{m\text{-tolyl}}$), 6.44 – 6.48 (m, 1H, 2'-H$_{m\text{-tolyl}}$), 6.91 (t, J = 7.8 Hz, 1H, 5'-H$_{m\text{-tolyl}}$), 7.09 – 7.16 (m, 2H, 3''-H$_{p\text{-tolyl}}$, 5''-H$_{p\text{-tolyl}}$), 7.33 – 7.40 (m, 2H, 2''-H$_{p\text{-tolyl}}$, 6''-H$_{p\text{-tolyl}}$), 8.26 – 8.35 (m, 1H, CONHCH$_2$);

^{13}C NMR (101 MHz, DMSO-d_6): δ [ppm] = 20.6 (1C, ArCH$_{3,\ p\text{-tolyl}}$), 21.3 (1C, ArCH$_{3,\ m\text{-tolyl}}$), 38.5 (1C, NHCH$_2$CH$_2$O), 57.9 (1C, OCH$_3$), 60.3 (1C, NHCHCO), 70.5 (1C, NHCH$_2$$CH_2$O), 110.4 (1C, C-6'$_{m\text{-tolyl}}$), 113.7 (1C, C-2'$_{m\text{-tolyl}}$), 117.4 (1C, C-4'$_{m\text{-tolyl}}$), 127.1 (2C, C-2''$_{p\text{-tolyl}}$, C-6''$_{p\text{-tolyl}}$), 128.6 (1C, C-5'$_{m\text{-tolyl}}$), 128.8 (2C, C-3''$_{p\text{-tolyl}}$, C-5''$_{p\text{-tolyl}}$), 136.5 (1C, C$_{p\text{-tolyl}}$), 136.6 (1C, C$_{p\text{-tolyl}}$), 137.6 (1C, C-3'$_{m\text{-tolyl}}$), 147.2 (1C, C-1'$_{m\text{-tolyl}}$), 171.2 (1C, CH$C$ONH);

HRMS (m/z): $[M+H]^+$ calcd for $C_{19}H_{25}N_2O_2$: 313.1911, found: 313.1912;

HPLC (method 1): t_R = 21.6 min, purity 93.5%.

2-Chloro-*N*-{2-[(2-methoxyethyl)amino]-2-oxo-1-(*p*-tolyl)ethyl}-*N*-(*m*-tolyl)acetamide (45d)



$C_{21}H_{25}ClN_2O_3$

M_r = 388.9

Triethylamine (0.34 mL, 240 mg, 2.4 mmol) and chloroacetyl chloride (0.19 mL, 270 mg, 2.4 mmol) were added to an ice-cooled solution of **44d** (620 mg, 2.0 mmol) in dichloromethane (40 mL) and the reaction mixture was stirred at ambient temperature for 12 h. Then, the organic solvent was washed with a saturated aqueous solution of ammonium chloride (2× 40 mL), a saturated aqueous solution of sodium bicarbonate (2× 40 mL), and brine (40 mL), dried over sodium sulfate, filtered, and removed *in vacuo*. The residue was purified by flash column chromatography (Ø = 4 cm, h = 15 cm, V = 15 mL, dichloromethane/ethyl acetate = 4/1) to give **45d** (490 mg, 1.3 mmol, 63% yield) as colorless solid.

m.p. = 179 °C;

TLC: R_f = 0.33 (dichloromethane/ethyl acetate = 10/1);

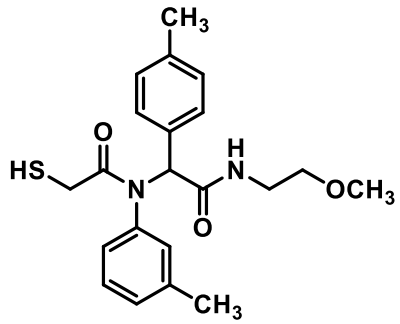
^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 2.09 – 2.34 (m, 6H, ArC*H*$_3$), 3.27 (s, 3H, OC*H*$_3$), 3.37 – 3.53 (m, 4H, NHC*H*$_2$CH$_2$O, NHCH$_2$C*H*$_2$O), 3.82 (d, *J* = 13.8 Hz, 1H, ClC*H*$_2$CO), 3.86 (d, *J* = 13.8 Hz, 1H, ClC*H*$_2$CO), 5.90 (s br, 1H, NC*H*CO), 5.97 – 6.06 (m, 1H, CON*H*CH$_2$), 6.97 – 7.02 (m, 4H, 2"-H$_{p\text{-tolyl}}$, 3"-H$_{p\text{-tolyl}}$, 5"-H$_{p\text{-tolyl}}$, 6"-H$_{p\text{-tolyl}}$), 7.03 – 7.06 (m, 1H, 4'-H$_{m\text{-tolyl}}$), the signals for 3 H$_{m\text{-tolyl}}$ cannot be observed in the spectrum;

^{13}C NMR (101 MHz, $CDCl_3$): δ [ppm] = 21.2 (2C, Ar*C*H$_3$), 39.7 (1C, NH*C*H$_2$CH$_2$O), 42.8 (1C, ClCH$_2$CO), 58.8 (1C, OCH$_3$), 66.1 (1C, NCHCO), 71.7 (1C, NHCH$_2$*C*H$_2$O), 127.3 (1C, C$_{m\text{-tolyl}}$), 129.0 (1C, C$_{m\text{-tolyl}}$), 129.3 (2C, C-3"$_{p\text{-tolyl}}$, C-5"$_{p\text{-tolyl}}$), 129.6 (1C, C-4'$_{m\text{-tolyl}}$), 130.4 (2C, C-2"$_{p\text{-tolyl}}$, C-6"$_{p\text{-tolyl}}$), 130.8 (1C, C$_{m\text{-tolyl}}$), 130.9 (1C, C-1"$_{p\text{-tolyl}}$), 138.7 (1C, C-4"$_{p\text{-tolyl}}$), 138.9 (1C, C$_{m\text{-tolyl}}$), 139.4 (1C, C$_{m\text{-tolyl}}$), 166.8 1C, ClCH$_2$*C*O), 169.4 (1C, CH*C*ONH);

HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{21}H_{26}{}^{35}ClN_2O_3$: 389.1626, found: 389.1627;

HPLC (method 1): t_R = 22.0 min, purity 97.3%.

2-Mercapto-*N*-{2-[(2-methoxyethyl)amino]-2-oxo-1-(*p*-tolyl)ethyl}-*N*-(*m*-tolyl)acetamide (46d)



$C_{21}H_{26}N_2O_3S$

M_r = 386.5

Finely ground thiourea (91 mg, 1.2 mmol) was added to a solution of **45d** (390 mg, 1.0 mmol) in acetone (20 mL) and the resulting mixture stirred for 16 h at ambient temperature. After removal of the solvent, the residue was treated with ethyl acetate (25 mL). Under vigorous stirring, a solution of sodium sulfite (250 mg, 2.0 mmol) in water (25 mL) was added and the mixture was stirred for 3 h at ambient temperature. The organic phase was washed with water (20 mL) and brine (20 mL), dried over sodium sulfate, filtered, and the solvent was removed *in vacuo*. The residue was purified by flash column chromatography (Ø = 4 cm, h = 15 cm, V = 15 mL, dichloromethane/ethyl acetate = 10/1) to give **46d** (180 mg, 0.47 mmol, 47% yield) as colorless solid.

m.p. = 164 °C;

TLC: R_f = 0.24 (dichloromethane/ethyl acetate = 10/1);

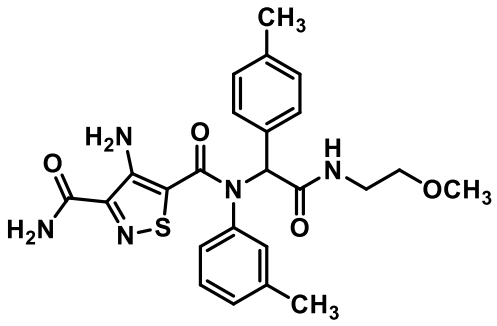
^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 2.07 (t, *J* = 7.7 Hz, 1H, *H*SCH$_2$CO), 2.10 – 2.37 (m, 6H, ArC*H*$_3$), 3.03 (dd, *J* = 14.7/7.9 Hz, 1H, HSC*H*$_2$CO), 3.09 (dd, *J* = 14.7/7.5 Hz, 1H, HSC*H*$_2$CO), 3.27 (s, 3H, OC*H*$_3$), 3.37 – 3.54 (m, 4H, NHC*H*$_2$CH$_2$O, NHCH$_2$C*H*$_2$O), 5.90 (s br, 1H, NC*H*CO), 6.01 – 6.11 (m, 1H, CON*H*CH$_2$), 6.96 – 7.05 (m, 5H, 2"-H$_{p\text{-tolyl}}$, 3"-H$_{p\text{-tolyl}}$, 5"-H$_{p\text{-tolyl}}$, 6"-H$_{p\text{-tolyl}}$, 4'-H$_{m\text{-tolyl}}$), the signals for 3 H$_{m\text{-tolyl}}$ cannot be observed in the spectrum;

^{13}C NMR (101 MHz, $CDCl_3$): δ [ppm] = 21.2 (2C, Ar*C*H$_3$), 27.7 (1C, HS*C*H$_2$CO), 39.6 (1C, NH*C*H$_2$CH$_2$O), 58.8 (1C, O*C*H$_3$), 65.5 (1C, N*C*HCO), 71.1 (1C, NHCH$_2$*C*H$_2$O), 127.2 (1C, C$_{m\text{-tolyl}}$), 128.9 (1C, C$_{m\text{-tolyl}}$), 129.2 (2C, C-3"$_{p\text{-tolyl}}$, C-5"$_{p\text{-tolyl}}$), 129.3 (1C, C-4'$_{m\text{-tolyl}}$), 130.3 (2C, C-2"$_{p\text{-tolyl}}$, C-6"$_{p\text{-tolyl}}$), 130.8 (1C, C$_{m\text{-tolyl}}$), 131.3 (1C, C-1"$_{p\text{-tolyl}}$), 138.5 (1C, C-4"$_{p\text{-tolyl}}$), 139.3 (1C, C-3'$_{m\text{-tolyl}}$), 139.8 (1C, C-1'$_{m\text{-tolyl}}$), 169.7 (1C, CH*C*ONH), 170.6 (1C, HSCH$_2$*C*O);

HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{21}H_{27}N_2O_3S$: 387.1737, found: 387.1743;

HPLC (method 1): t_R = 21.7 min, purity 99.0%.

4-Amino-*N*^{5}-{2-[(2-methoxyethyl)amino]-2-oxo-1-(*p*-tolyl)ethyl}-*N*^{5}-(*m*-tolyl)isothiazole-3,5-dicarboxamide (47d)



$C_{24}H_{27}N_5O_4S$

M_r = 481.6

46d (390 mg, 1.0 mmol) was added in one portion to a solution of **25** (320 mg, 1.2 mmol) in acetone (100 mL). The solution was cooled to 0 °C and morpholine (0.17 mL, 170 mg, 1.9 mmol) was added dropwise. After stirring the reaction mixture for 12 h at ambient temperature, the solvent was removed *in vacuo*. The residue was purified by flash column chromatography (Ø = 6 cm, h = 20 cm, V = 15 mL, dichloromethane/acetone = 15/1) and recrystallized from acetonitrile to give **47d** (260 mg, 0.53 mmol, 53% yield) as colorless solid.

m.p. = 232 °C;

TLC: R_f = 0.24(dichloromethane/acetone = 10/1);

^{1}H NMR (400 MHz, DMSO-d_6): δ [ppm] = 2.00 – 2.32 (m, 6H, ArC*H*$_3$), 3.12 – 3.25 (m, 4H, OC*H*$_3$, NHC*H*$_2$CH$_2$O (1H)), 3.25 – 3.41 (m, 3H, NHC*H*$_2$CH$_2$O (1H), NHCH$_2$C*H*$_2$O), 6.11 (s, 1H, NC*H*CO), 6.90 – 7.00 (m, 4H, 2'''-H$_{p\text{-tolyl}}$, 3'''-H$_{p\text{-tolyl}}$, 5'''-H$_{p\text{-tolyl}}$, 6'''-H$_{p\text{-tolyl}}$), 7.00 – 7.26 (m,

4H, ArN*H*$_2$, 4''-H$_{m\text{-tolyl}}$, H$_{m\text{-tolyl}}$ (1H)), 7.57 (s br, 1H, CON*H*$_2$), 7.80 (s br, 1H, CON*H*$_2$), 8.16 – 8.21 (m, 1H, CON*H*CH$_2$), the signals for 2 H$_{m\text{-tolyl}}$ cannot be observed in the spectrum;

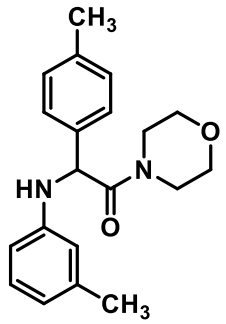
^{13}C NMR (101 MHz, DMSO-*d*$_6$): δ [ppm] = 20.6 (2C, Ar*C*H$_3$), 38.7 (1C, NH*C*H$_2$CH$_2$O), 57.9 (1C, O*C*H$_3$), 63.9 (1C, N*C*HCO), 70.4 (1C, NHCH$_2$*C*H$_2$O), 123.1 (1C, C-5'$_{isothiazole}$), 128.4 (2C, C-3'''$_{p\text{-tolyl}}$, C-5'''$_{p\text{-tolyl}}$), 128.8 (1C, C$_{m\text{-tolyl}}$), 130.2 (1C, C-4''$_{m\text{-tolyl}}$), 130.4 (2C, C-2'''$_{p\text{-tolyl}}$, C-6'''$_{p\text{-tolyl}}$), 131.6 (1C, C-1'''$_{p\text{-tolyl}}$), 137.0 (1C, C$_{arom.}$), 137.1 (1C, C$_{arom.}$), 146.4 (1C, C-3'$_{isothiazole}$), 151.3 (1C, C-4'$_{isothiazole}$), 162.3 (1C, Ar*C*ONCH), 163.9 (1C, Ar*C*ONH$_2$), 169.8 (1C, *C*ONHCH$_2$), the signals for 3 C$_{m\text{-tolyl}}$ cannot be observed in the spectrum;

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3448, 3416, 3331, 3308, 3178, 2954, 2919, 2885, 2831, 1684, 1666, 1613, 1568, 1516, 1489, 1445, 1404, 1370, 1322, 1239, 1212, 1190, 1124, 1088, 1069, 1022, 1004, 850, 812, 796, 758, 727, 706, 683, 671, 648, 602, 582, 565, 544, 523, 487, 456, 440;

HRMS (*m*/*z*): [M+H]$^+$ calcd for C$_{24}$H$_{28}$N$_5$O$_4$S: 482.1857, found: 482.1860;

HPLC (method 1): t$_R$ = 21.4 min, purity 99.4%.

1-Morpholino-2-(*p*-tolyl)-2-(*m*-tolylamino)ethan-1-one (44e)



C$_{20}$H$_{24}$N$_2$O$_2$

M$_r$ = 324.4

DIPEA (0.52 mL, 390 mg, 3.0 mmol) and COMU (1.3 g, 3.0 mmol) were added to an ice-cooled solution of **38** (770 mg, 3.0 mmol) in acetonitrile (40 mL) and the mixture was stirred for 10 min at 0 °C. Then, morpholine (0.26 mL, 260 mg, 3.0 mmol) and DIPEA (0.52 mL, 390 mg, 3.0 mmol) were added. After stirring the reaction mixture at ambient temperature for 8 h, the volatiles were removed *in vacuo*. The residue was dissolved in dichloromethane (100 mL) and the organic solvent was washed with a saturated aqueous solution of ammonium chloride (2× 30 mL), water (50 mL), and brine (50 mL), dried over sodium sulfate, filtered, and

removed *in vacuo*. The residue was purified by flash column chromatography (Ø = 6 cm, h = 20 cm, V = 30 mL, dichloromethane/ethyl acetate = 20/1) to give **44e** (660 mg, 2.0 mmol, 68% yield) as colorless solid.

m.p. = 107 °C;

TLC: R_f = 0.28 (dichloromethane/ethyl acetate = 20/1);

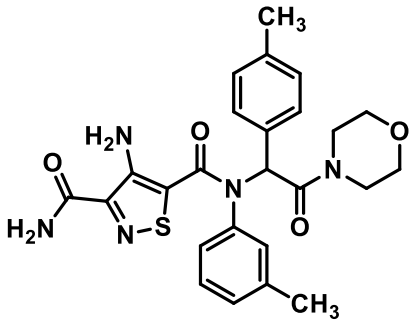
^{1}H NMR (400 MHz, DMSO-d_6): δ [ppm] = 2.14 (s, 3H, C$H_{3,\ m\text{-tolyl}}$), 2.26 (s, 3H, C$H_{3,\ p\text{-tolyl}}$), 3.22 – 3.63 (m, 7H, N(CH_2CH$_2$)$_2$O (3H), N(CH$_2$CH_2)$_2$O), 3.72 – 3.84 (m, 1H, N(CH_2CH$_2$)$_2$O), 5.55 (d, J = 8.6 Hz, 1H, NHCHCO), 5.94 (d, J = 8.6 Hz, 1H, NHCHCO), 6.31 – 6.38 (m, 1H, 4'-H$_{m\text{-tolyl}}$), 6.48 – 6.57 (m, 2H, 2'-H$_{m\text{-tolyl}}$, 6'-H$_{m\text{-tolyl}}$), 6.90 (t, J = 7.7 Hz, 1H, 5'-H$_{m\text{-tolyl}}$), 7.11 – 7.16 (m, 2H, 3"-H$_{p\text{-tolyl}}$, 5"-H$_{p\text{-tolyl}}$), 7.34 – 7.38 (m, 2H, 2"-H$_{p\text{-tolyl}}$, 6"-H$_{p\text{-tolyl}}$);

^{13}C NMR (101 MHz, DMSO-d_6): δ [ppm] = 20.7 (1C, CH$_{3,\ p\text{-tolyl}}$), 21.3 (1C, CH$_{3,\ m\text{-tolyl}}$), 42.2 (1C, N(CH$_2$CH$_2$)$_2$O), 45.5 (1C, N(CH$_2$CH$_2$)$_2$O), 55.4 (1C, NHCHCO), 66.0 (1C, N(CH$_2C$H$_2$)$_2$O), 66.1 (1C, N(CH$_2C$H$_2$)$_2$O), 110.5 (1C, C-6'$_{m\text{-tolyl}}$), 114.0 (1C, C-2'$_{m\text{-tolyl}}$), 117.3 (1C, C-4'$_{m\text{-tolyl}}$), 127.7 (2C, C-2"$_{p\text{-tolyl}}$, C-6"$_{p\text{-tolyl}}$), 128.5 (1C, C-5'$_{m\text{-tolyl}}$), 129.0 (2C, C-3"$_{p\text{-tolyl}}$, C-5"$_{p\text{-tolyl}}$), 135.8 (1C, C-1"$_{p\text{-tolyl}}$), 136.6 (1C, C-4"$_{p\text{-tolyl}}$), 137.6 (1C, C-3'$_{m\text{-tolyl}}$), 146.9 (1C, C-1'$_{m\text{-tolyl}}$), 169.3 (1C, CHCON);

HRMS (m/z): [M+H]$^+$ calcd for $C_{20}H_{25}N_2O_2$: 325.1911, found: 325.1915;

HPLC (method 1): t_R = 21.2 min, purity 93.8%.

4-Amino-N^5-[2-morpholino-2-oxo-1-(*p*-tolyl)ethyl]-N^5-(*m*-tolyl)isothiazole-3,5-dicarboxamide (47e)



$C_{25}H_{27}N_5O_4S$

M_r = 493.6

Triethylamine (0.34 mL, 240 mg, 2.4 mmol) and chloroacetyl chloride (0.19 mL, 270 mg, 2.4 mmol) were added to an ice-cooled solution of **44e** (650 mg, 2.0 mmol) in dichloromethane (40 mL) and the reaction mixture was stirred at ambient temperature for 12 h. Then, the organic solvent was washed with a saturated aqueous solution of ammonium chloride (2× 40 mL), a saturated aqueous solution of sodium bicarbonate (2× 40 mL), and brine (40 mL), dried over sodium sulfate, filtered, and removed *in vacuo*.

A share (400 mg, 78%) of the obtained crude product (510 mg) was dissolved in acetone (20 mL). Finely ground thiourea (91 mg, 1.2 mmol) was added and the resulting mixture was stirred for 16 h at ambient temperature. After removal of the solvent, the residue was treated with ethyl acetate (50 mL). Under vigorous stirring, a solution of sodium sulfite (250 mg, 2.0 mmol) in water (50 mL) was added and the mixture was stirred for 3 h at ambient temperature. The organic phase was washed with water (50 mL) and brine (50 mL), dried over sodium sulfate, filtered, and the solvent was removed *in vacuo*. The residue was washed with acetonitrile.

A share (200 mg, 92%) of the obtained crude product (220 mg) was added in one portion to a solution of **25** (160 mg, 0.60 mmol) in acetone (50 mL). The solution was cooled to 0 °C and morpholine (0.09 mL, 87 mg, 1.0 mmol) was added. After stirring the mixture for 12 h at ambient temperature, the solvent was removed *in vacuo*. The residue was purified by flash column chromatography (Ø = 4 cm, h = 20 cm, V = 15 mL, dichloromethane/acetone = 10/1) and recrystallized from acetonitrile to give **47e** (150 mg, 0.31 mmol, 22% yield) as yellowish solid.

m.p. = 299 °C;

TLC: R_f = 0.22 (dichloromethane/acetone = 10/1);

^{1}H NMR (400 MHz, DMSO-d_6): δ [ppm] = 1.95 – 2.34 (m, 6H, ArCH_3), 2.82 – 2.99 (m, 1H, N(CH_2CH_2)$_2$O), 3.11 – 3.24 (m, 1H, N(CH_2CH_2)$_2$O), 3.36 – 3.54 (m, 4H, N(CH_2CH_2)$_2$O (2H), N(CH_2CH_2)$_2$O (2H)), 3.54 – 3.70 (m, 2H, N(CH_2CH_2)$_2$O (1H), N(CH_2CH_2)$_2$O (1H)), 6.40 – 6.59 (m, 2H, NC*H*CO, H$_{m\text{-tolyl}}$ (1H)), 6.90 – 7.07 (m, 4H, 2'''-H$_{p\text{-tolyl}}$, 3'''-H$_{p\text{-tolyl}}$, 5'''-H$_{p\text{-tolyl}}$, 6'''-H$_{p\text{-tolyl}}$), 7.07 – 7.32 (m, 4H, ArNH_2, 4''-H$_{m\text{-tolyl}}$, H$_{m\text{-tolyl}}$ (1H)), 7.59 (s br, 1H, CONH_2), 7.62 – 7.86 (m, 2H, CONH_2 (1H), H$_{m\text{-tolyl}}$ (1H));

^{13}C NMR (101 MHz, DMSO-d_6): δ [ppm] = 20.7 (1C, Ar*C*H$_3$), 42.4 (1C, N(*C*H$_2$CH$_2$)$_2$O), 45.7 (1C, N(*C*H$_2$CH$_2$)$_2$O), 61.7 (1C, N*C*HCO), 65.5 (1C, N(CH_2*C*H$_2$)$_2$O), 66.0 (1C, N(CH_2*C*H$_2$)$_2$O), 122.9 (1C, C-5'$_{isothiazole}$), 128.9 (3C, C-3'''$_{p\text{-tolyl}}$, C-5'''$_{p\text{-tolyl}}$, 1 C$_{m\text{-tolyl}}$), 129.9 (1C, C-1'''$_{p\text{-tolyl}}$),

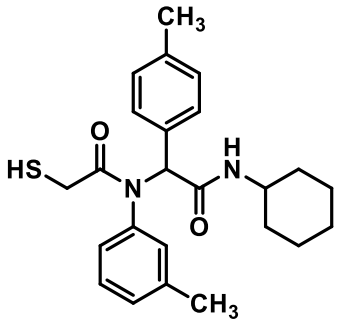
130.2 (2C, C$_{m\text{-tolyl}}$), 130.5 (2C, C-2'''$_{p\text{-tolyl}}$, C-6'''$_{p\text{-tolyl}}$), 133.8 (1C, C$_{m\text{-tolyl}}$), 137.0 (1C, C-1''$_{m\text{-tolyl}}$), 137.5 (1C, C-4'''$_{p\text{-tolyl}}$), 146.5 (1C, C-3'$_{\text{isothiazole}}$), 151.3 (1C, C-4'$_{\text{isothiazole}}$), 162.1 (1C, Ar*C*ONCH), 164.0 (1C, Ar*C*ONH$_2$), 168.0 (1C, NCH*C*ON), the signals for 1 Ar*C*H$_3$ and 1 C$_{m\text{-tolyl}}$ cannot be observed in the spectrum;

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3471, 3430, 3352, 3287, 3242, 2955, 2918, 1679, 1631, 1593, 1572, 1514, 1491, 1447, 1427, 1398, 1366, 1339, 1299, 1289, 1261, 1234, 1218, 1186, 1174, 1146, 1123, 1103, 1064, 1030, 1003, 948, 865, 848, 818, 791, 736, 706, 655, 644, 631, 586, 552, 507, 492;

HRMS (*m*/*z*): [M+H]$^+$ calcd for $C_{25}H_{28}N_5O_4S$: 494.1857, found: 494.1855;

HPLC (method 1): t_R = 22.0 min, purity 99.1%.

N-Cyclohexyl-2-[2-mercapto-*N*-(*m*-tolyl)acetamido]-2-(*p*-tolyl)acetamide (48a)



$C_{24}H_{30}N_2O_2S$

M_r = 410.6

3-Methylaniline (0.22 mL, 210 mg, 2.0 mmol) and 4-methylbenzaldehyde (0.24 mL, 240 mg, 2.0 mmol) were dissolved in dichloromethane (15 mL) and the solution was stirred at ambient temperature for 10 min. Then, sulfanylacetic acid (0.14 mL, 180 mg, 2.0 mmol) and isocyanocyclohexane (0.25 mL, 220 mg, 2.0 mmol) were added. After stirring the reaction mixture for 48 h at ambient temperature, the solvent was removed *in vacuo* and the residue was purified by flash column chromatography (Ø = 2 cm, h = 15 cm, V = 5 mL, petroleum ether/ethyl acetate = 4/1) to give **48a** (460 mg, 1.1 mmol, 56% yield) as colorless solid.

m.p. = 184 °C;

TLC: R_f = 0.39 (petroleum ether/ethyl acetate = 2/1);

^{1}H NMR (400 MHz, DMSO-d_6): δ [ppm] = 0.89 – 1.32 (m, 5H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 1.47 – 1.79 (m, 5H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 2.03 – 2.25 (m, 6H, ArCH_3), 2.57 (t, J = 6.8 Hz, 1H, HSCH$_2$CO), 2.91 – 3.07 (m, 2H, HSCH_2CO), 3.51 – 3.63 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), 5.96 (s, 1H, NCHCO), 6.50 (s br, 1H, H$_{m\text{-tolyl}}$), 6.83 – 7.18 (m, 6H, 2''-H$_{p\text{-tolyl}}$, 3''-H$_{p\text{-tolyl}}$, 5''-H$_{p\text{-tolyl}}$, 6''-H$_{p\text{-tolyl}}$, 4'-H$_{m\text{-tolyl}}$, H$_{m\text{-tolyl}}$), 7.57 (s br, 1H, H$_{m\text{-tolyl}}$), 7.89 (d, J = 7.7 Hz, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$);

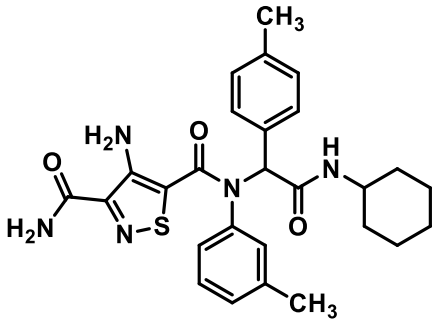
^{13}C NMR (101 MHz, DMSO-d_6): δ [ppm] = 20.6 (1C, ArCH$_3$), 20.7 (1C, ArCH$_3$), 24.5 (1C, NHCH(CH$_2$$CH_2$)$_2CH_2$), 24.6 (1C, NHCH(CH$_2$$CH_2$)$_2CH_2$), 25.2 (1C, NHCH(CH$_2CH_2$)$_2$$CH_2$), 27.3 (1C, HS$CH_2$CO), 32.15 (1C, NHCH($CH_2CH_2$)$_2CH_2$), 32.19 (1C, NHCH($CH_2CH_2$)$_2CH_2$), 47.9 (1C, NH$C$H(CH$_2CH_2$)$_2CH_2$), 63.6 (1C, N$C$HCO), 127.7 (1C, C$_{m\text{-tolyl}}$), 128.1 (1C, C$_{m\text{-tolyl}}$), 128.3 (2C, C-3''$_{p\text{-tolyl}}$, C-5''$_{p\text{-tolyl}}$), 128.4 (1C, C$_{m\text{-tolyl}}$), 129.9 (2C, C-2''$_{p\text{-tolyl}}$, C-6''$_{p\text{-tolyl}}$), 131.3 (1C, C$_{m\text{-tolyl}}$), 132.2 (1C, C-1''$_{p\text{-tolyl}}$), 136.7 (1C, C-4''$_{p\text{-tolyl}}$), 137.6 (1C, C-3'$_{m\text{-tolyl}}$), 139.1 (1C, C-1'$_{m\text{-tolyl}}$), 168.5 (1C, CH$C$ONHCH), 168.8 (1C, HSCH$_2$$C$O);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3275, 3082, 2927, 2850, 1649, 1603, 1586, 1557, 1518, 1487, 1452, 1371, 1357, 1252, 1231, 1189, 1104, 817, 802, 754, 731, 708, 662, 644, 631, 548, 504;

HRMS (m/z): [M+H]$^+$ calcd for C$_{24}$H$_{31}$N$_2$O$_2$S: 411.2101, found: 411.2107;

HPLC (method 1): t_R = 25.2 min, purity 92.0%.

4-Amino-N^5-[2-(cyclohexylamino)-2-oxo-1-(*p*-tolyl)ethyl]-N^5-(*m*-tolyl)isothiazole-3,5-dicarboxamide (49a)



C$_{27}$H$_{31}$N$_5$O$_3$S

M$_r$ = 505.6

48a (410 mg, 1.0 mmol) was added in one portion to a solution of **25** (320 mg, 1.2 mmol) in acetone (100 mL). The mixture was cooled to 0 °C and morpholine (0.17 mL, 170 mg, 1.9 mmol) was added dropwise. After stirring the reaction mixture for 12 h at ambient temperature, the solvent was removed *in vacuo*. The residue was purified by flash column chromatography (Ø = 6 cm, h = 20 cm, V = 15 mL, dichloromethane/methanol = 100/1) and recrystallized from acetonitrile to give **49a** (400 mg, 0.80 mmol, 80% yield) as colorless solid.

m.p. = 243 °C;

TLC: R_f = 0.41 (dichloromethane/methanol = 50/1);

^{1}H NMR (6500 MHz, DMSO-d_6): δ [ppm] = 0.92 – 1.02 (m, 1H, NHCH(CH_2CH$_2$)$_2$CH$_2$), 1.02 – 1.12 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$CH_2), 1.14 – 1.31 (m, 3H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (1H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H)), 1.48 – 1.61 (m, 2H, NHCH(CH$_2$CH_2)$_2$CH$_2$ (1H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 1.61 – 1.73 (m, 2H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (1H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (1H)), 1.73 – 1.81 (m, 1H, NHCH(CH_2CH$_2$)$_2$CH$_2$), 1.95 – 2.37 (m, 6H, ArCH_3), 3.55 – 3.64 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), 6.09 (s, 1H, NCHCO), 6.54 (s br, 1H, H$_{m\text{-tolyl}}$), 6.88 – 7.00 (m, 4H, 2'''-H$_{p\text{-tolyl}}$, 3'''-H$_{p\text{-tolyl}}$, 5'''-H$_{p\text{-tolyl}}$, 6'''-H$_{p\text{-tolyl}}$), 7.08 (s br, 1H, H$_{m\text{-tolyl}}$), 7.11 – 7.14 (m, 1H, 4''-H$_{m\text{-tolyl}}$), 7.17 (s br, 2H, ArNH_2), 7.58 (s br, 1H, CONH_2), 7.67 (s br, 1H, H$_{m\text{-tolyl}}$), 7.81 (s br, 1H, CONH_2), 7.98 (d, J = 7.7 Hz, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$);

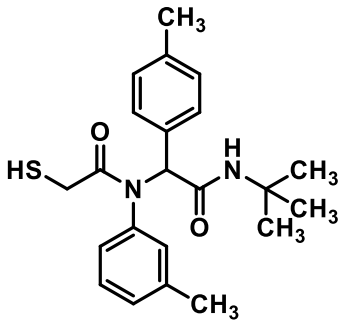
^{13}C NMR (151 MHz, DMSO-d_6): δ [ppm] = 20.6 (2C, ArCH$_3$), 24.5 (1C, NHCH(CH$_2$$CH_2$)$_2CH_2$), 24.6 (1C, NHCH(CH$_2$$CH_2$)$_2CH_2$), 25.2 (1C, NHCH(CH$_2CH_2$)$_2$$CH_2$), 32.15 (1C, NHCH($CH_2CH_2$)$_2CH_2$), 32.22 (1C, NHCH($CH_2CH_2$)$_2CH_2$), 48.0 (1C, NH$C$H(CH$_2CH_2$)$_2CH_2$), 63.7 (1C, N$C$HCO), 123.2 (1C, C-5'$_{\text{isothiazole}}$), 128.4 (3C, C-3'''$_{p\text{-tolyl}}$, C-5'''$_{p\text{-tolyl}}$, 1 C$_{m\text{-tolyl}}$), 128.7 (1C, C$_{m\text{-tolyl}}$), 130.1 (1C, C$_{m\text{-tolyl}}$), 130.2 (1C, C$_{m\text{-tolyl}}$), 130.3 (2C, C-2'''$_{p\text{-tolyl}}$, C-6'''$_{p\text{-tolyl}}$), 131.9 (1C, C-1'''$_{p\text{-tolyl}}$), 133.7 (1C, C$_{m\text{-tolyl}}$), 136.9 (1C, C-4'''$_{p\text{-tolyl}}$), 137.1 (1C, C-1''$_{m\text{-tolyl}}$), 146.4 (1C, C-3'$_{\text{isothiazole}}$), 151.2 (1C, C-4'$_{\text{isothiazole}}$), 162.2 (1C, Ar$C$ONCH), 163.9 (1C, Ar$CONH_2$), 168.6 (1C, CH$C$ONHCH);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3479, 3449, 3293, 2929, 2850, 1664, 1649, 1612, 1599, 1557, 1518, 1489, 1449, 1369, 1345, 1325, 1252, 1238, 1192, 1152, 1106, 1073, 815, 794, 760, 734, 708, 668, 631, 548, 528, 504;

HRMS (m/z): [M+H]$^+$ calcd for $C_{27}H_{32}N_5O_3S$: 506.2220, found: 506.2232;

HPLC (method 1): t_R = 24.9 min, purity 95.0%.

***N*-(*tert*-Butyl)-2-[2-mercapto-*N*-(*m*-tolyl)acetamido]-2-(*p*-tolyl)acetamide (48b)**



$C_{22}H_{28}N_2O_2S$

$M_r = 384.5$

3-Methylaniline (0.22 mL, 210 mg, 2.0 mmol) and 4-methylbenzaldehyde (0.24 mL, 240 mg, 2.0 mmol) were dissolved in dichloromethane (15 mL) and the solution was stirred at ambient temperature for 10 min. Then, sulfanylacetic acid (0.14 mL, 180 mg, 2.0 mmol) and 2-isocyano-2-methylpropane (0.22 mL, 170 mg, 2.0 mmol) were added. After stirring the reaction mixture for 48 h at ambient temperature, the solvent was removed *in vacuo* and the residue was purified by flash column chromatography (Ø = 4 cm, h = 15 cm, V = 15 mL, petroleum ether/ethyl acetate = 10/1) to give **48b** (400 mg, 1.0 mmol, 52% yield) as colorless solid.

m.p. = 157 °C;

TLC: R_f = 0.43 (petroleum ether/ethyl acetate = 4/1);

^{1}H NMR (300 MHz, DMSO-*d*$_6$): δ [ppm] = 1.22 (s, 9H, NHC(C*H*$_3$)$_3$), 2.03 – 2.27 (m, 6H, ArC*H*$_3$), 2.56 (t, *J* = 6.8 Hz, 1H, *H*SCH$_2$CO), 2.91 – 3.08 (m, 2H, HSC*H*$_2$CO), 5.92 (s, 1H, NC*H*CO), 6.85 – 7.13 (m, 6H, 2"-H$_{p\text{-tolyl}}$, 3"-H$_{p\text{-tolyl}}$, 5"-H$_{p\text{-tolyl}}$, 6"-H$_{p\text{-tolyl}}$, 4'-H$_{m\text{-tolyl}}$, H$_{m\text{-tolyl}}$ (1H)), 7.63 (s, 1H, CON*H*C(CH$_3$)$_3$), the signals for 2 H$_{m\text{-tolyl}}$ cannot be observed in the spectrum;

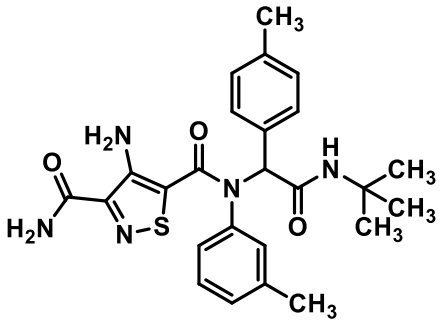
^{13}C NMR (101 MHz, DMSO-*d*$_6$): δ [ppm] = 20.6 (1C, Ar*C*H$_3$), 20.7 (1C, Ar*C*H$_3$), 27.4 (1C, HS*C*H$_2$CO), 28.4 (3C, NHC(*C*H$_3$)$_3$), 50.2 (1C, NH*C*(CH$_3$)$_3$), 63.9 (1C, N*C*HCO), 127.8 (1C, C$_{m\text{-tolyl}}$), 128.1 (1C, C$_{m\text{-tolyl}}$), 128.3 (2C, C-3"$_{p\text{-tolyl}}$, C-5"$_{p\text{-tolyl}}$), 128.4 (1C, C$_{m\text{-tolyl}}$), 129.9 (2C, C-2"$_{p\text{-tolyl}}$, C-6"$_{p\text{-tolyl}}$), 131.3 (1C, C$_{m\text{-tolyl}}$), 132.5 (1C, C-1"$_{p\text{-tolyl}}$), 136.6 (1C, C-4"$_{p\text{-tolyl}}$), 137.6 (1C, C-3'$_{m\text{-tolyl}}$), 139.2 (1C, C-1'$_{m\text{-tolyl}}$), 168.8 (1C, HSCH$_2$*C*O), 169.0 (1C, *C*ONHC(CH$_3$)$_3$);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3290, 3204, 3151, 3108, 3052, 2916, 2566, 1676, 1652, 1614, 1556, 1487, 1417, 1378, 1330, 1276, 1256, 1222, 1201, 1174, 1145, 1089, 1037, 969, 909, 892, 871, 777, 738, 686, 613, 562, 534, 513, 443;

HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{22}H_{29}N_2O_2S$: 385.1944, found: 385.1941;

HPLC (method 1): t_R = 24.5 min, purity 78.1%.

4-Amino-N^5-[2-(*tert*-butylamino)-2-oxo-1-(*p*-tolyl)ethyl]-N^5-(*m*-tolyl)isothiazole-3,5-dicarboxamide (49b)



$C_{25}H_{29}N_5O_3S$

M_r = 479.6

48b (380 mg, 1.0 mmol) was added in one portion to a solution of **25** (320 mg, 1.2 mmol) in acetone (100 mL). The mixture was cooled to 0 °C and morpholine (0.17 mL, 170 mg, 1.9 mmol) was added dropwise. After stirring the reaction mixture for 12 h at ambient temperature, the solvent was removed *in vacuo*. The residue was purified by flash column chromatography (Ø = 4 cm, h = 20 cm, V = 15 mL, dichloromethane/ethyl acetate = 4/1) and recrystallized from acetonitrile to give **49b** (310 mg, 0.64 mmol, 64% yield) as colorless solid.

m.p. = 261 °C;

TLC: R_f = 0.48 (dichloromethane/ethyl acetate = 3/2);

^{1}H NMR (500 MHz, DMSO-d_6): δ [ppm] = 1.24 (s, 9H, NHC(CH_3)$_3$), 2.00 – 2.33 (m, 6H, ArCH_3), 6.07 (s, 1H, NC*H*CO), 6.91 – 6.96 (m, 2H, 3'''-$H_{p\text{-tolyl}}$, 5'''-$H_{p\text{-tolyl}}$), 6.96 – 7.00 (m, 2H, 2'''-$H_{p\text{-tolyl}}$, 6'''-$H_{p\text{-tolyl}}$), 7.10 – 7.14 (m, 1H, 4''-$H_{m\text{-tolyl}}$), 7.16 (s br, 2H, ArNH_2), 7.55 (s br, 1H, CONH_2), 7.75 (s, 1H, CO*N*HC(CH_3)$_3$), 7.80 (s br, 1H, CONH_2), the signals for 3 $H_{m\text{-tolyl}}$ cannot be observed in the spectrum;

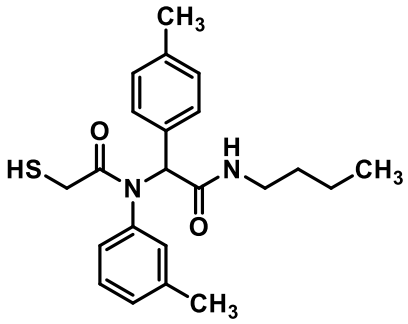
^{13}C NMR (151 MHz, DMSO-*d*$_6$): δ [ppm] = 20.6 (2C, Ar*C*H$_3$), 28.4 (3C, NHC(*C*H$_3$)$_3$), 50.3 (1C, NH*C*(CH$_3$)$_3$), 64.0 (1C, N*C*HCO), 123.3 (1C, C-5'$_{isothiazole}$), 128.3 (2C, C-3'''$_{p\text{-tolyl}}$, C-5'''$_{p\text{-tolyl}}$), 128.7 (1C, C$_{m\text{-tolyl}}$), 130.0 (1C, C$_{m\text{-tolyl}}$), 130.2 (1C, C-4''$_{m\text{-tolyl}}$), 130.3 (2C, C-2'''$_{p\text{-tolyl}}$, C-6'''$_{p\text{-tolyl}}$), 132.0 (1C, C-1'''$_{p\text{-tolyl}}$), 133.8 (1C, C$_{m\text{-tolyl}}$), 136.8 (1C, C-4'''$_{p\text{-tolyl}}$), 137.2 (1C, C-1''$_{m\text{-tolyl}}$), 138.3 (1C, C$_{m\text{-tolyl}}$), 146.4 (1C, C-3'$_{isothiazole}$), 151.2 (1C, C-4'$_{isothiazole}$), 162.2 (1C, Ar*C*ONCH), 163.9 (1C, Ar*C*ONH$_2$), 169.1 (1C, *C*ONHC(CH$_3$)$_3$);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3463, 3339, 3297, 3245, 2972, 2921, 1688, 1664, 1591, 1570, 1542, 1516, 1491, 1444, 1406, 1393, 1377, 1363, 1341, 1307, 1258, 1220, 1187, 1145, 1120, 1091, 1073, 851, 809, 792, 760, 747, 728, 700, 657, 641, 595, 549, 533, 509, 497, 462, 407;

HRMS (*m*/*z*): [M+H]$^+$ calcd for C$_{25}$H$_{30}$N$_5$O$_3$S: 480.2064, found: 480.2067;

HPLC (method 1): t$_R$ = 24.3 min, purity 98.5%.

N-Butyl-2-[2-mercapto-*N*-(*m*-tolyl)acetamido]-2-(*p*-tolyl)acetamide (48c)



C$_{22}$H$_{28}$N$_2$O$_2$S

M$_r$ = 384.5

3-Methylaniline (0.22 mL, 210 mg, 2.0 mmol) and 4-methylbenzaldehyde (0.24 mL, 240 mg, 2.0 mmol) were dissolved in dichloromethane (15 mL) and the solution was stirred at ambient temperature for 10 min. Then, sulfanylacetic acid (0.14 mL, 180 mg, 2.0 mmol) and 1-isocyanobutane (0.21 mL, 170 mg, 2.0 mmol) were added. After stirring the reaction mixture for 48 h at ambient temperature, the solvent was removed *in vacuo* and the residue was purified by flash column chromatography (Ø = 4 cm, h = 15 cm, V = 15 mL, dichloromethane/ethyl acetate = 8/1) to give **48c** (590 mg, 1.5 mmol, 77% yield) as colorless solid.

m.p. = 121 °C;

TLC: R_f = 0.32 (dichloromethane/ethyl acetate = 7/1);

^{1}H NMR (400 MHz, DMSO-d_6): δ [ppm] = 0.83 (t, J = 7.3 Hz, 3H, NHCH$_2$CH$_2$CH$_2$CH_3), 1.15 – 1.26 (m, 2H, NHCH$_2$CH$_2$CH_2CH$_3$), 1.29 – 1.39 (m, 2H, NHCH$_2$CH_2CH$_2$CH$_3$), 2.00 – 2.31 (m, 6H, ArCH_3), 2.56 (t, J = 6.8 Hz, 1H, HSCH$_2$CO), 2.92 – 3.17 (m, 4H, NHCH_2CH$_2$CH$_2$CH$_3$, HSCH_2CO), 5.94 (s, 1H, NCHCO), 6.87 – 6.95 (m, 4H, 2"-H$_{p\text{-tolyl}}$, 3"-H$_{p\text{-tolyl}}$, 5"-H$_{p\text{-tolyl}}$, 6"-H$_{p\text{-tolyl}}$), 6.95 – 7.00 (m, 1H, 4'-H$_{m\text{-tolyl}}$), 7.03 (s br, 1H, H$_{m\text{-tolyl}}$), 7.97 (t, J = 5.5 Hz, 1H, NHCH$_2$CH$_2$CH$_2$CH$_3$), the signals for 2 H$_{m\text{-tolyl}}$ cannot be observed in the spectrum;

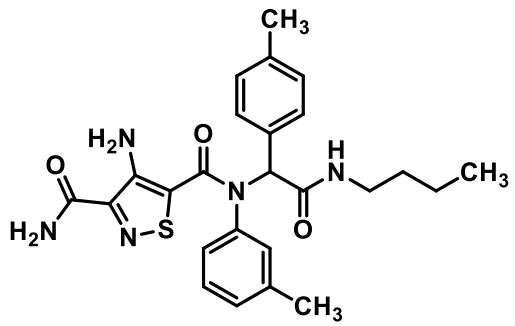
^{13}C NMR (101 MHz, DMSO-d_6): δ [ppm] = 13.6 (1C, NHCH$_2$CH$_2$CH$_2$$CH_3$), 19.5 (1C, NHCH$_2CH_2$$CH_2CH_3$), 20.6 (1C, Ar$CH_3$), 20.7 (1C, Ar$CH_3$), 27.4 (1C, HS$CH_2$CO), 31.1 (1C, NHCH$_2$$CH_2CH_2CH_3$), 38.4 (1C, NH$CH_2CH_2CH_2CH_3$), 63.8 (1C, N$C$HCO), 127.7 (1C, C$_{m\text{-tolyl}}$), 128.2 (1C, C$_{m\text{-tolyl}}$), 128.3 (2C, C-3"$_{p\text{-tolyl}}$, C-5"$_{p\text{-tolyl}}$), 128.5 (1C, C$_{m\text{-tolyl}}$), 130.0 (2C, C-2"$_{p\text{-tolyl}}$, C-6"$_{p\text{-tolyl}}$), 131.3 (1C, C$_{m\text{-tolyl}}$), 132.1 (1C, C-1"$_{p\text{-tolyl}}$), 136.8 (1C, C-4"$_{p\text{-tolyl}}$), 137.7 (1C, C-3'$_{m\text{-tolyl}}$), 139.1 (1C, C-1'$_{m\text{-tolyl}}$), 168.9 (1C, HSCH$_2$$C$O), 169.3 (1C, CH$C$ONH);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3260, 3063, 2956, 2927, 2870, 1647, 1602, 1558, 1516, 1487, 1465, 1370, 1308, 1245, 1225, 1190, 1154, 1133, 1092, 1040, 989, 963, 820, 793, 736, 707, 676, 631, 553, 496, 448;

HRMS (m/z): [M+H]$^+$ calcd for $C_{22}H_{29}N_2O_2S$: 385.1944, found: 385.1948;

HPLC (method 1): t_R = 24.1 min, purity 92.3%.

4-Amino-N^5-[2-(butylamino)-2-oxo-1-(p-tolyl)ethyl]-N^5-(m-tolyl)isothiazole-3,5-dicarboxamide (49c)



$C_{25}H_{29}N_5O_3S$

M_r = 479.6

48c (380 mg, 1.0 mmol) was added in one portion to a solution of **25** (320 mg, 1.2 mmol) in acetone (100 mL). The mixture was cooled to 0 °C and morpholine (0.17 mL, 170 mg, 1.9 mmol) was added dropwise. After stirring the reaction mixture for 12 h at ambient temperature, the solvent was removed *in vacuo*. The residue was purified by flash column chromatography (Ø = 6 cm, h = 20 cm, V = 15 mL, dichloromethane/acetone = 20/1) and recrystallized from acetonitrile to give **49c** (400 mg, 0.83 mmol, 83% yield) as colorless solid.

m.p. = 227 °C;

TLC: R_f = 0.22 (dichloromethane/acetone = 15/1);

^{1}H NMR (400 MHz, DMSO-d_6): δ [ppm] = 0.83 (t, J = 7.3 Hz, 3H, NHCH$_2$CH$_2$CH$_2$CH_3), 1.16 – 1.26 (m, 2H, NHCH$_2$CH$_2$CH_2CH$_3$), 1.30 – 1.40 (m, 2H, NHCH$_2$CH_2CH$_2$CH$_3$), 2.00 – 2.34 (m, 6H, ArCH_3), 2.98 – 3.09 (m, 1H, NHCH_2CH$_2$CH$_2$CH$_3$), 3.10 – 3.21 (m, 1H, NHCH_2CH$_2$CH$_2$CH$_3$), 6.07 (s, 1H, NCHCO), 6.91 – 7.00 (m, 4H, 2'''-H$_{p\text{-tolyl}}$, 3'''-H$_{p\text{-tolyl}}$, 5'''-H$_{p\text{-tolyl}}$, 6'''-H$_{p\text{-tolyl}}$), 7.01 – 7.24 (m, 4H, ArNH_2, 4''-H$_{m\text{-tolyl}}$, H$_{m\text{-tolyl}}$ (1H)), 7.57 (s br, 1H, CONH_2), 7.80 (s br, 1H, CONH_2), 8.07 (t, J = 5.7 Hz, 1H, NHCH$_2$CH$_2$CH$_2$CH$_3$), the signals for 2 H$_{m\text{-tolyl}}$ cannot be observed in the spectrum;

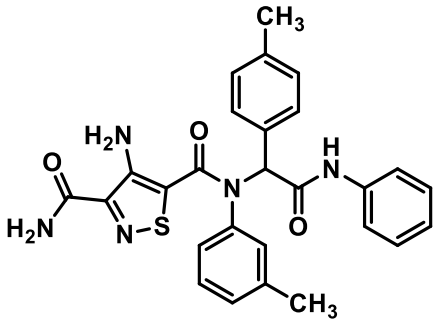
^{13}C NMR (101 MHz, DMSO-d_6): δ [ppm] = 13.6 (1C, NHCH$_2$CH$_2$CH$_2$$CH_3$), 19.5 (1C, NHCH$_2CH_2$$CH_2CH_3$), 20.6 (2C, Ar$CH_3$), 31.1 (1C, NHCH$_2$$CH_2CH_2CH_3$), 38.4 (1C, NH$CH_2CH_2CH_2CH_3$), 63.9 (1C, N$C$HCO), 123.1 (1C, C-5'$_{\text{isothiazole}}$), 128.4 (2C, C-3'''$_{p\text{-tolyl}}$, C-5'''$_{p\text{-tolyl}}$), 128.7 (1C, C$_{m\text{-tolyl}}$), 130.1 (1C, C$_{m\text{-tolyl}}$), 130.2 (1C, C$_{m\text{-tolyl}}$), 130.3 (2C, C-2'''$_{p\text{-tolyl}}$, C-6'''$_{p\text{-tolyl}}$), 131.7 (1C, C-1'''$_{p\text{-tolyl}}$), 133.7 (1C, C$_{m\text{-tolyl}}$), 137.0 (1C, C$_{\text{arom.}}$), 137.1 (1C, C$_{\text{arom.}}$), 138.4 (1C, C$_{m\text{-tolyl}}$), 146.4 (1C, C-3'$_{\text{isothiazole}}$), 151.3 (1C, C-4'$_{\text{isothiazole}}$), 162.3 (1C, Ar$C$ONCH), 163.9 (1C, Ar$CONH_2$), 169.5 (1C, CH$C$ONH);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3464, 3405, 3350, 3184, 2956, 2926, 2871, 1692, 1671, 1620, 1559, 1514, 1488, 1448, 1406, 1374, 1326, 1225, 1187, 1146, 1118, 1069, 800, 730, 706, 682, 606, 504;

HRMS (m/z): [M+H]$^+$ calcd for $C_{25}H_{30}N_5O_3S$: 480.2064, found: 480.2107;

HPLC (method 1): t_R = 23.9 min, purity 98.2%.

4-Amino-N^5-[2-oxo-2-(phenylamino)-1-(*p*-tolyl)ethyl]-N^5-(*m*-tolyl)isothiazole-3,5-dicarboxamide (49d)



$C_{27}H_{25}N_5O_3S$

M_r = 499.6

3-Methylaniline (0.22 mL, 210 mg, 2.0 mmol) and 4-methylbenzaldehyde (0.24 mL, 240 mg, 2.0 mmol) were dissolved in acetonitrile (50 mL) and the solution was stirred at ambient temperature for 10 min. Then, sulfanylacetic acid (0.14 mL, 180 mg, 2.0 mmol) and isocyanobenzene (0.21 mL, 210 mg, 2.0 mmol) were added. After stirring the reaction mixture for 48 h at ambient temperature, the formed precipitate was filtered off and used for the next step without further purification.

A share (400 mg, 56%) of the obtained crude product (720 mg) was added in one portion to a solution of **25** (320 mg, 1.2 mmol) in acetone (100 mL). The mixture was cooled to 0 °C and morpholine (0.17 mL, 170 mg, 1.9 mmol) was added dropwise. After stirring the reaction mixture for 12 h at ambient temperature, the solvent was removed *in vacuo*. The residue was purified by flash column chromatography (Ø = 6 cm, h = 20 cm, V = 15 mL, dichloromethane/acetone = 15/1) and recrystallized from acetonitrile to give **49d** (310 mg, 0.62 mmol, 55% yield) as colorless solid.

m.p. = 297 °C;

TLC: R_f = 0.24 (dichloromethane/acetone = 15/1);

^{1}H NMR (500 MHz, DMSO-d_6): δ [ppm] = 2.06 – 2.36 (m, 6H, ArCH_3), 6.29 (s, 1H, NC*H*CO), 6.95 – 7.00 (m, 2H, 3'''-H$_{p\text{-tolyl}}$, 5'''-H$_{p\text{-tolyl}}$), 7.00 – 7.05 (m, 1H, 4''''-H$_{phenyl}$), 7.06 – 7.11 (m, 2H, 2'''-H$_{p\text{-tolyl}}$, 6'''-H$_{p\text{-tolyl}}$), 7.14 – 7.18 (m, 1H, 4''-H$_{m\text{-tolyl}}$), 7.24 (s br, 2H, ArNH_2), 7.26 – 7.32 (m, 2H, 3''''-H$_{phenyl}$, 5''''-H$_{phenyl}$), 7.55 (s br, 1H, CONH_2), 7.64 – 7.68 (m, 2H, 2''''-H$_{phenyl}$, 6''''-H$_{phenyl}$),

7.72 (s br, 1H, CON*H*$_2$), 10.26 (s, 1H, CON*H*Ph), the signals for 3 H$_{m\text{-tolyl}}$ cannot be observed in the spectrum;

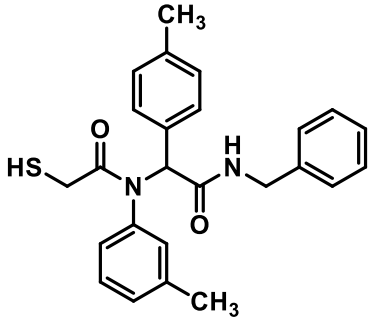
^{13}C NMR (151 MHz, DMSO-*d*$_6$): δ [ppm] = 20.5 (2C, Ar*C*H$_3$), 64.8 (1C, N*C*HCO), 119.0 (2C, C-2''''$_{phenyl}$, C-6''''$_{phenyl}$), 123.0 (1C, C-5'$_{isothiazole}$), 123.2 (1C, C-4''''$_{phenyl}$), 128.6 (2C, C-3''''$_{phenyl}$, C-5''''$_{phenyl}$), 128.7 (2C, C-3'''$_{p\text{-tolyl}}$, C-5'''$_{p\text{-tolyl}}$), 128.8 (1C, C$_{m\text{-tolyl}}$), 130.2 (1C, C$_{m\text{-tolyl}}$), 130.3 (1C, C$_{m\text{-tolyl}}$), 130.5 (2C, C-2'''$_{p\text{-tolyl}}$, C-6'''$_{p\text{-tolyl}}$), 130.8 (1C, C-1'''$_{p\text{-tolyl}}$), 133.8 (1C, C$_{m\text{-tolyl}}$), 137.0 (1C, C-1''$_{m\text{-tolyl}}$), 137.5 (1C, C-4'''$_{p\text{-tolyl}}$), 138.6 (1C, C$_{m\text{-tolyl}}$), 139.3 (1C, C-1''''$_{phenyl}$), 146.5 (1C, C-3'$_{isothiazole}$), 151.5 (1C, C-4'$_{isothiazole}$), 162.6 (1C, Ar*C*ONCH), 164.0 (1C, Ar*C*ONH$_2$), 168.9 (1C, CH*C*ONHPh);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3476, 3447, 3301, 3273, 3140, 1674, 1663, 1599, 1561, 1546, 1518, 1490, 1443, 1397, 1369, 1344, 1315, 1291, 1251, 1207, 1188, 1157, 1115, 1074, 1027, 970, 817, 793, 748, 732, 708, 691, 670, 634, 544, 528, 490, 470, 440, 416;

HRMS (*m*/*z*): [M+H]$^+$ calcd for $C_{27}H_{26}N_5O_3S$: 500.1751, found: 500.1736;

HPLC (method 1): t_R = 24.7 min, purity 95.2%.

N-Benzyl-2-[2-mercapto-*N*-(*m*-tolyl)acetamido]-2-(*p*-tolyl)acetamide (48e)



$C_{25}H_{26}N_2O_2S$

M_r = 418.6

3-Methylaniline (0.22 mL, 210 mg, 2.0 mmol) and 4-methylbenzaldehyde (0.24 mL, 240 mg, 2.0 mmol) were dissolved in dichloromethane (15 mL) and the solution was stirred at ambient temperature for 10 min. Then, sulfanylacetic acid (0.14 mL, 180 mg, 2.0 mmol) and (isocyanomethyl)benzene (0.24 mL, 230 mg, 2.0 mmol) were added. After stirring the reaction mixture for 48 h at ambient temperature, the solvent was removed *in vacuo* and the residue was purified by flash column chromatography (Ø = 4 cm, h = 15 cm, V = 15 mL,

dichloromethane/ethyl acetate = 10/1) to give **48e** (530 mg, 1.3 mmol, 63% yield) as colorless solid.

m.p. = 119 °C;

TLC: R_f = 0.40 (dichloromethane/ethyl acetate = 7/1);

^{1}H NMR (400 MHz, DMSO-d_6): δ [ppm] = 2.03 – 2.27 (m, 6H, ArCH_3), 2.59 (t, J = 6.9 Hz, 1H, HSCH_2CO), 2.95 – 3.09 (m, 2H, HSCH_2CO), 4.32 (d, J = 5.9 Hz, 2H, NHCH_2Ph), 6.02 (s, 1H, NCHCO), 6.90 – 6.95 (m, 4H, 2"-H$_{p\text{-tolyl}}$, 3"-H$_{p\text{-tolyl}}$, 5"-H$_{p\text{-tolyl}}$, 6"-H$_{p\text{-tolyl}}$), 6.96 – 7.01 (m, 1H, 4'-H$_{m\text{-tolyl}}$), 7.04 (s br, 1H, H$_{m\text{-tolyl}}$), 7.17 – 7.24 (m, 3H, 2'"-H$_{phenyl}$, 4'"-H$_{phenyl}$, 6'"-H$_{phenyl}$), 7.24 – 7.31 (m, 2H, 3'"-H$_{phenyl}$, 5'"-H$_{phenyl}$), 8.56 (t, J = 5.9 Hz, 1H, NHCH$_2$Ph), the signals for 2 H$_{m\text{-tolyl}}$ cannot be observed in the spectrum;

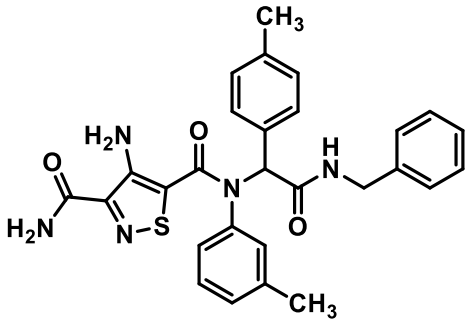
^{13}C NMR (101 MHz, DMSO-d_6): δ [ppm] = 20.6 (1C, ArCH$_3$), 20.7 (1C, ArCH$_3$), 27.3 (1C, HSCH$_2$CO), 42.2 (1C, NHCH$_2$Ph), 64.0 (1C, NCHCO), 126.7 (1C, C-4'"$_{phenyl}$), 127.1 (2C, C-2'"$_{phenyl}$, C-6'"$_{phenyl}$), 127.7 (1C, C$_{m\text{-tolyl}}$), 128.1 (2C, C-3'"$_{phenyl}$, C-5'"$_{phenyl}$), 128.2 (1C, C$_{m\text{-tolyl}}$), 128.3 (2C, C-3"$_{p\text{-tolyl}}$, C-5"$_{p\text{-tolyl}}$), 128.5 (1C, C$_{m\text{-tolyl}}$), 130.2 (2C, C-2"$_{p\text{-tolyl}}$, C-6"$_{p\text{-tolyl}}$), 131.3 (1C, C$_{m\text{-tolyl}}$), 131.8 (1C, C-1"$_{p\text{-tolyl}}$), 137.0 (1C, C-4"$_{p\text{-tolyl}}$), 137.8 (1C, C-3'$_{m\text{-tolyl}}$), 139.1 (1C, C-1'$_{m\text{-tolyl}}$), 139.4 (1C, C-1'"$_{phenyl}$), 169.0 (1C, HSCH$_2$$C$O), 169.7 (1C, CH$C$ONHCH$_2$Ph);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3247, 3058, 3030, 2922, 2866, 1648, 1604, 1586, 1548, 1515, 1499, 1488, 1465, 1455, 1406, 1370, 1308, 1227, 1189, 1158, 1113, 1092, 1075, 1031, 988, 904, 817, 791, 748, 733, 706, 694, 632, 575, 552, 511, 496, 473, 444;

HRMS (m/z): [M+H]$^+$ calcd for $C_{25}H_{27}N_2O_2S$: 419.1788, found: 419.1795;

HPLC (method 1): t_R = 24.3 min, purity 93.8%.

4-Amino-N^5-[2-(benzylamino)-2-oxo-1-(*p*-tolyl)ethyl]-N^5-(*m*-tolyl)isothiazole-3,5-dicarboxamide (49e)



$C_{28}H_{27}N_5O_3S$

M_r = 513.6

48e (420 mg, 1.0 mmol) was added in one portion to a solution of **25** (320 mg, 1.2 mmol) in acetone (100 mL). The mixture was cooled to 0 °C and morpholine (0.17 mL, 170 mg, 1.9 mmol) was added dropwise. After stirring the reaction mixture for 12 h at ambient temperature, the solvent was removed *in vacuo*. The residue was purified by flash column chromatography (Ø = 6 cm, h = 20 cm, V = 15 mL, dichloromethane/ethyl acetate = 5/1) and recrystallized from acetonitrile to give **49e** (220 mg, 0.42 mmol, 42% yield) as colorless solid.

m.p. = 228 °C;

TLC: R_f = 0.36 (dichloromethane/ethyl acetate = 5/2);

^{1}H NMR (500 MHz, DMSO-d_6): δ [ppm] = 1.99 – 2.35 (m, 6H, ArCH_3), 4.31 (dd, J = 15.4/5.9 Hz, 1H, NHCH_2Ph), 4.37 (dd, J = 15.4/6.1 Hz, 1H, NHCH_2Ph), 6.15 (s, 1H, NCHCO), 6.93 – 6.97 (m, 2H, 3'''-$H_{p\text{-tolyl}}$, 5'''-$H_{p\text{-tolyl}}$), 6.97 – 7.01 (m, 2H, 2'''-$H_{p\text{-tolyl}}$, 6'''-$H_{p\text{-tolyl}}$), 7.13 – 7.17 (m, 1H, 4''-$H_{m\text{-tolyl}}$), 7.18 – 7.25 (m, 5H, 2''''-H_{phenyl}, 4''''-H_{phenyl}, 6''''-H_{phenyl}, ArNH_2), 7.25 – 7.30 (m, 2H, 3''''-H_{phenyl}, 5''''-H_{phenyl}), 7.59 (s br, 1H, CONH_2), 7.82 (s br, 1H, CONH_2), 8.67 (t, J = 6.0 Hz, 1H, NHCH$_2$Ph), the signals for 3 $H_{m\text{-tolyl}}$ cannot be observed in the spectrum;

^{13}C NMR (101 MHz, DMSO-d_6): δ [ppm] = 20.6 (2C, ArCH$_3$), 42.3 (1C, NHCH$_2$Ph), 64.1 (1C, NCHCO), 123.0 (1C, C-5'$_{isothiazole}$), 126.7 (1C, C-4''''$_{phenyl}$), 127.1 (2C, C-2''''$_{phenyl}$, C-6''''$_{phenyl}$), 128.1 (2C, C-3''''$_{phenyl}$, C-5''''$_{phenyl}$), 128.4 (2C, C-3'''$_{p\text{-tolyl}}$, C-5'''$_{p\text{-tolyl}}$), 128.8 (1C, $C_{m\text{-tolyl}}$), 130.1 (1C, $C_{m\text{-tolyl}}$), 130.2 (1C, $C_{m\text{-tolyl}}$), 130.5 (2C, C-2'''$_{p\text{-tolyl}}$, C-6'''$_{p\text{-tolyl}}$), 131.4 (1C, C-1'''$_{p\text{-tolyl}}$), 133.7 (1C, $C_{m\text{-tolyl}}$), 137.1 (1C, $C_{arom.}$), 137.2 (1C, $C_{arom.}$), 138.4 (1C, $C_{m\text{-tolyl}}$), 139.4 (1C, C-1''''$_{phenyl}$),

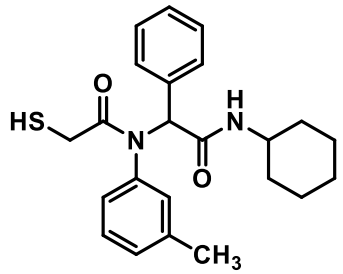
146.4 (1C, C-3'$_{isothiazole}$), 151.3 (1C, C-4'$_{isothiazole}$), 162.4 (1C, Ar*C*ONCH), 163.9 (1C, Ar*C*ONH$_2$), 169.9 (1C, CH*C*ONHCH$_2$Ph);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3447, 3284, 3171, 3061, 3031, 2922, 1674, 1653, 1613, 1562, 1515, 1488, 1452, 1401, 1369, 1327, 1306, 1248, 1224, 1191, 1157, 1114, 1073, 1029, 1002, 873, 816, 797, 752, 735, 706, 697, 682, 529, 503, 477, 440, 423;

HRMS (*m*/*z*): [M+H]$^+$ calcd for $C_{28}H_{28}N_5O_3S$: 514.1907, found: 514.1935;

HPLC (method 1): t_R = 24.1 min, purity 99.0%.

_N_-Cyclohexyl-2-[2-mercapto-_N_-(_m_-tolyl)acetamido]-2-phenylacetamide (51a)



$C_{23}H_{28}N_2O_2S$

M_r = 396.5

3-Methylaniline (0.22 mL, 210 mg, 2.0 mmol) and benzaldehyde (0.20 mL, 210 mg, 2.0 mmol) were dissolved in dichloromethane (15 mL) and the solution was stirred at ambient temperature for 10 min. Then, sulfanylacetic acid (0.14 mL, 180 mg, 2.0 mmol) and isocyanocyclohexane (0.25 mL, 220 mg, 2.0 mmol) were added. After stirring the reaction mixture for 48 h at ambient temperature, the solvent was removed *in vacuo* and the residue was purified by flash column chromatography (Ø = 4 cm, h = 15 cm, V = 15 mL, dichloromethane/ethyl acetate = 20/1) to give **51a** (390 mg, 0.99 mmol, 49% yield) as colorless solid.

m.p. = 219 °C;

TLC: R_f = 0.39 (dichloromethane/ethyl acetate = 15/1);

^{1}H NMR (400 MHz, DMSO-d_6): δ [ppm] = 0.91 – 1.33 (m, 5H, NHCH(C*H*$_2$CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$C*H*$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH$_2$)$_2$C*H*$_2$ (1H)), 1.47 – 1.81 (m, 5H, NHCH(C*H*$_2$CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$C*H*$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH$_2$)$_2$C*H*$_2$ (1H)), 2.13 (s br, 3H, ArC*H*$_3$), 2.57 (t, *J* = 6.9 Hz, 1H, *H*SCH$_2$CO), 2.98 (dd, *J* = 14.9/6.8 Hz, 1H, HSC*H*$_2$CO),

3.04 (dd, J = 14.9/7.0 Hz, 1H, HSCH_2CO), 3.52 – 3.64 (m, 1H, NHCH(CH_2CH_2)$_2CH_2$), 6.01 (s, 1H, NCHCO), 6.91 – 6.98 (m, 1H, 4'-H$_{m\text{-tolyl}}$), 6.98 – 7.07 (m, 2H, 2"-H$_{phenyl}$, 6"-H$_{phenyl}$), 7.07 – 7.17 (m, 3H, 3"-H$_{phenyl}$, 4"-H$_{phenyl}$, 5"-H$_{phenyl}$), 7.94 (d, J = 7.7 Hz, 1H, NHCH(CH_2CH_2)$_2CH_2$), the signals for 3 H$_{m\text{-tolyl}}$ cannot be observed in the spectrum;

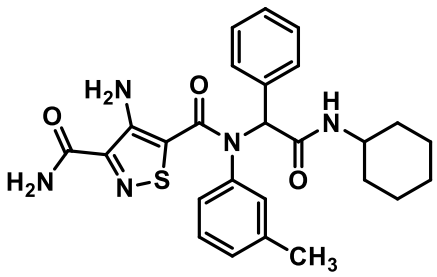
^{13}C NMR (101 MHz, DMSO-d_6): δ [ppm] = 20.7 (1C, ArCH$_3$), 24.4 (1C, NHCH(CH_2CH$_2$)$_2CH_2$), 24.6 (1C, NHCH(CH_2CH$_2$)$_2CH_2$), 25.2 (1C, NHCH(CH_2CH_2)$_2C$H$_2$), 27.3 (1C, HSCH$_2$CO), 32.1 (1C, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), 32.2 (1C, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), 47.9 (1C, NHCH(CH_2CH_2)$_2CH_2$), 63.9 (1C, NCHCO), 127.6 (1C, C-4"$_{phenyl}$), 127.7 (3C, C-3"$_{phenyl}$, C-5"$_{phenyl}$, 1 C$_{m\text{-tolyl}}$), 128.1 (1C, C$_{m\text{-tolyl}}$), 128.4 (1C, C$_{m\text{-tolyl}}$), 130.0 (2C, C-2"$_{phenyl}$, C-6"$_{phenyl}$), 131.3 (1C, C$_{m\text{-tolyl}}$), 135.3 (1C, C-1"$_{phenyl}$), 137.6 (1C, C-3'$_{m\text{-tolyl}}$), 139.1 (1C, C-1'$_{m\text{-tolyl}}$), 168.3 (1C, CHCONHCH), 168.9 (1C, HSCH$_2C$O);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3282, 3061, 2923, 2851, 1645, 1603, 1586, 1553, 1487, 1451, 1407, 1367, 1316, 1251, 1234, 1192, 1151, 1106, 1090, 1065, 1036, 978, 964, 891, 802, 748, 730, 698, 670, 646, 581, 564, 521;

HRMS (m/z): [M+H]$^+$ calcd for $C_{23}H_{29}N_2O_2S$: 397.1944, found: 397.1953;

HPLC (method 1): t_R = 24.5 min, purity 93.4%.

4-Amino-N^5-[2-(cyclohexylamino)-2-oxo-1-phenylethyl]-N^5-(m-tolyl)isothiazole-3,5-dicarboxamide (52a)



$C_{26}H_{29}N_5O_3S$

M_r = 491.6

51a (400 mg, 1.0 mmol) was added in one portion to a solution of **25** (320 mg, 1.2 mmol) in acetone (100 mL). The mixture was cooled to 0 °C and morpholine (0.17 mL, 170 mg, 1.9 mmol) was added dropwise. After stirring the reaction mixture for 12 h at ambient temperature, the solvent was removed *in vacuo*. The residue was purified by flash column

chromatography (Ø = 6 cm, h = 20 cm, V = 15 mL, dichloromethane/ethyl acetate = 4/1) to give **52a** (210 mg, 0.43 mmol, 43% yield) as colorless solid.

m.p. = 259 °C;

TLC: R_f = 0.26 (dichloromethane/ethyl acetate = 4/1);

^{1}H NMR (500 MHz, DMSO-d_6): δ [ppm] = 0.97 – 1.15 (m, 2H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (1H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 1.18 – 1.35 (m, 3H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (1H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H)), 1.50 – 1.65 (m, 2H, NHCH(CH$_2$CH_2)$_2$CH$_2$ (1H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 1.65 – 1.75 (m, 2H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (1H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (1H)), 1.79 – 1.87 (m, 1H, NHCH(CH_2CH$_2$)$_2$CH$_2$), 2.17 (s br, 3H, ArCH_3), 3.60 – 3.70 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), 6.21 (s, 1H, NCHCO), 7.00 – 7.19 (m, 7H, 2'''-H$_{phenyl}$, 3'''-H$_{phenyl}$, 4'''-H$_{phenyl}$, 5'''-H$_{phenyl}$, 6'''-H$_{phenyl}$, H$_{m\text{-}tolyl}$ (2H)), 7.25 (s br, 2H, ArNH_2), 7.52 (s br, 1H, CONH_2), 7.67 (s br, 1H, CONH_2), 7.95 (d, J = 7.7 Hz, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), the signals for 2 H$_{m\text{-}tolyl}$ cannot be observed in the spectrum;

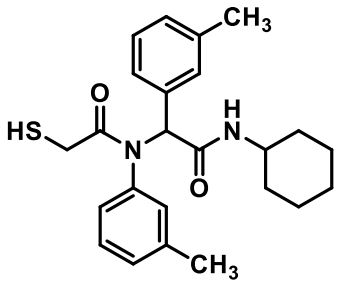
^{13}C NMR (151 MHz, DMSO-d_6): δ [ppm] = 24.6 (1C, NHCH(CH$_2$$CH_2$)$_2CH_2$), 24.7 (1C, NHCH(CH$_2$$CH_2$)$_2CH_2$), 25.3 (1C, NHCH(CH$_2CH_2$)$_2$$CH_2$), 32.3 (2C, NHCH($CH_2CH_2$)$_2CH_2$), 48.1 (1C, NH$C$H(CH$_2CH_2$)$_2CH_2$), 64.1 (1C, N$C$HCO), 123.4 (1C, C-5'$_{isothiazole}$), 127.7 (3C, C-3'''$_{phenyl}$, C-4'''$_{phenyl}$, C-5'''$_{phenyl}$), 128.6 (1C, C$_{m\text{-}tolyl}$), 130.0 (1C, C$_{m\text{-}tolyl}$), 130.3 (1C, C$_{m\text{-}tolyl}$), 130.5 (2C, C-2'''$_{phenyl}$, C-6'''$_{phenyl}$), 134.0 (1C, C$_{m\text{-}tolyl}$), 135.1 (1C, C-1'''$_{phenyl}$), 137.3 (1C, C-1''$_{m\text{-}tolyl}$), 146.5 (1C, C-3'$_{isothiazole}$), 151.5 (1C, C-4'$_{isothiazole}$), 162.5 (1C, Ar$C$ONCH), 164.1 (1C, Ar$CONH_2$), 168.5 (1C, CH$C$ONHCH), the signals for ArCH$_3$ and 1 C$_{m\text{-}tolyl}$ cannot be observed in the spectrum;

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3481, 3451, 3335, 3274, 3085, 3060, 2925, 2851, 1665, 1646, 1600, 1560, 1490, 1450, 1396, 1369, 1337, 1253, 1239, 1193, 1152, 1107, 1074, 1003, 979, 891, 868, 804, 793, 762, 748, 726, 710, 698, 667, 583, 568, 532, 517;

HRMS (m/z): [M+H]$^+$ calcd for $C_{26}H_{30}N_5O_3S$: 492.2064, found: 492.2057;

HPLC (method 1): t_R = 24.2 min, purity 96.1%.

_N_-Cyclohexyl-2-[2-mercapto-_N_-(_m_-tolyl)acetamido]-2-(_m_-tolyl)acetamide (51b)



$C_{24}H_{30}N_2O_2S$

$M_r = 410.6$

3-Methylaniline (0.22 mL, 210 mg, 2.0 mmol) and 3-methylbenzaldehyde (0.24 mL, 240 mg, 2.0 mmol) were dissolved in dichloromethane (15 mL) and the solution was stirred at ambient temperature for 10 min. Then, sulfanylacetic acid (0.14 mL, 180 mg, 2.0 mmol) and isocyanocyclohexane (0.25 mL, 220 mg, 2.0 mmol) were added. After stirring the reaction mixture for 48 h at ambient temperature, the solvent was removed *in vacuo* and the residue was purified by flash column chromatography (Ø = 4 cm, h = 15 cm, V = 15 mL, dichloromethane/ethyl acetate = 18/1) to give **51b** (490 mg, 1.2 mmol, 59% yield) as colorless solid.

m.p. = 154 °C;

TLC: R_f = 0.35 (dichloromethane/ethyl acetate = 15/1);

^{1}H NMR (600 MHz, DMSO-d_6): δ [ppm] = 0.94 – 1.03 (m, 1H, NHCH(CH_2CH$_2$)$_2$CH$_2$), 1.03 – 1.12 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$CH_2), 1.14 – 1.30 (m, 3H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (1H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H)), 1.48 – 1.78 (m, 5H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 1.94 – 2.32 (m, 6H, ArCH_3), 2.57 (t, J = 6.8 Hz, 1H, HSCH$_2$CO), 2.98 (dd, J = 14.9/6.6 Hz, 1H, HSCH_2CO), 3.04 (dd, J = 14.9/7.0 Hz, 1H, HSCH_2CO), 3.50 – 3.62 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), 5.96 (s, 1H, NCHCO), 6.46 (s br, 1H, H$_{m\text{-tolyl}}$), 6.74 – 6.81 (m, 1H, 6"-H$_{m\text{-tolyl}}$), 6.81 – 7.15 (m, 5H, 2"-H$_{m\text{-tolyl}}$, 4"-H$_{m\text{-tolyl}}$, 5"-H$_{m\text{-tolyl}}$, H$_{m\text{-tolyl}}$ (2H)), 7.56 (s br, 1H, H$_{m\text{-tolyl}}$), 7.92 (d, J = 7.6 Hz, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$);

^{13}C NMR (151 MHz, DMSO-d_6): δ [ppm] = 20.7 (1C, ArCH$_3$), 20.8 (1C, ArCH$_3$), 24.5 (1C, NHCH(CH$_2$$CH_2$)$_2CH_2$), 24.6 (1C, NHCH(CH$_2$$CH_2$)$_2CH_2$), 25.2 (1C, NHCH(CH$_2CH_2$)$_2$$CH_2$), 27.3 (1C, HS$CH_2$CO), 32.15 (1C, NHCH($CH_2CH_2$)$_2CH_2$), 32.18 (1C, NHCH($CH_2CH_2$)$_2CH_2$), 47.9 (1C, NH$C$H(CH$_2CH_2$)$_2CH_2$), 63.8 (1C, N$C$HCO), 127.0 (1C, C-6"$_{m\text{-tolyl}}$), 127.6 (1C, C-

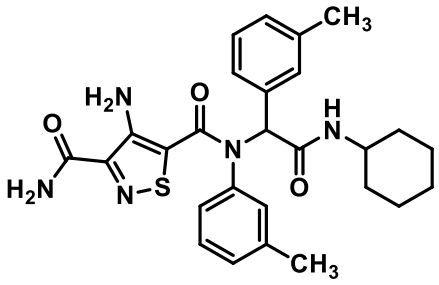
5"$_{m\text{-tolyl}}$), 127.7 (1C, C$_{m\text{-tolyl}}$), 128.0 (1C, C$_{m\text{-tolyl}}$), 128.1 (1C, C-4"$_{m\text{-tolyl}}$), 128.3 (1C, C$_{m\text{-tolyl}}$), 130.9 (1C, C-2"$_{m\text{-tolyl}}$), 131.3 (1C, C$_{m\text{-tolyl}}$), 135.1 (1C, C-1"$_{m\text{-tolyl}}$), 136.7 (1C, C-3"$_{m\text{-tolyl}}$), 137.6 (1C, C-3'$_{m\text{-tolyl}}$), 139.1 (1C, C-1'$_{m\text{-tolyl}}$), 168.3 (1C, CH*C*ONHCH), 168.8 (1C, HSCH$_2$*C*O);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3285, 2926, 2849, 1649, 1606, 1589, 1551, 1488, 1450, 1411, 1370, 1328, 1266, 1250, 1236, 1213, 1186, 1154, 1106, 1042, 1003, 979, 965, 891, 862, 802, 787, 748, 729, 707, 671, 650, 576, 545, 522, 497, 465;

HRMS (*m*/*z*): [M+H]$^+$ calcd for $C_{24}H_{31}N_2O_2S$: 411.2101, found: 411.2093;

HPLC (method 1): t_R = 25.1 min, purity 98.2%.

4-Amino-*N*^{5}-[2-(cyclohexylamino)-2-oxo-1-(*m*-tolyl)ethyl]-*N*^{5}-(*m*-tolyl)isothiazole-3,5-dicarboxamide (52b)



$C_{27}H_{31}N_5O_3S$

M_r = 505.6

51b (410 mg, 1.0 mmol) was added in one portion to a solution of **25** (320 mg, 1.2 mmol) in acetone (100 mL). The mixture was cooled to 0 °C and morpholine (0.17 mL, 170 mg, 1.9 mmol) was added dropwise. After stirring the reaction mixture for 12 h at ambient temperature, the solvent was removed *in vacuo*. The residue was purified by flash column chromatography (Ø = 6 cm, h = 20 cm, V = 15 mL, dichloromethane/ethyl acetate = 5/1) and recrystallized from acetonitrile to give **52b** (360 mg, 0.72 mmol, 72% yield) as colorless solid.

m.p. = 216 °C;

TLC: R_f = 0.27 (dichloromethane/ethyl acetate = 5/1);

^{1}H NMR (600 MHz, DMSO-d_6): δ [ppm] = 0.95 – 1.03 (m, 1H, NHCH(C*H*$_2$CH$_2$)$_2$CH$_2$), 1.03 – 1.12 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$C*H*$_2$), 1.17 – 1.31 (m, 3H, NHCH(C*H*$_2$CH$_2$)$_2$CH$_2$ (1H),

NHCH(CH_2CH_2)$_2CH_2$ (2H)), 1.50 – 1.56 (m, 1H, NHCH(CH_2CH_2)$_2CH_2$), 1.56 – 1.62 (m, 1H, NHCH(CH_2CH_2)$_2CH_2$), 1.63 – 1.72 (m, 2H, NHCH(CH_2CH_2)$_2CH_2$ (1H), NHCH(CH_2CH_2)$_2CH_2$ (1H)), 1.74 – 1.80 (m, 1H, NHCH(CH_2CH_2)$_2CH_2$), 1.95 – 2.39 (m, 6H, ArCH_3), 3.55 – 3.64 (m, 1H, NHCH(CH_2CH_2)$_2CH_2$), 6.10 (s, 1H, NCHCO), 6.55 (s br, 1H, $H_{m\text{-tolyl}}$), 6.81 – 6.86 (m, 1H, 6'''-$H_{m\text{-tolyl}}$), 6.91 – 6.95 (m, 2H, 2'''-$H_{m\text{-tolyl}}$, 4'''-$H_{m\text{-tolyl}}$), 6.97 – 7.02 (m 1H, 5'''-$H_{m\text{-tolyl}}$), 7.10 – 7.14 (m, 1H, 4''-$H_{m\text{-tolyl}}$), 7.17 (s br, 2H, ArNH_2), 7.58 (s br, 1H, CONH_2), 7.66 (s br, 1H, $H_{m\text{-tolyl}}$), 7.81 (s br, 1H, CONH_2), 8.02 (d, J = 7.7 Hz, 1H, NHCH(CH_2CH_2)$_2CH_2$), the signal for 1 $H_{m\text{-tolyl}}$ cannot be observed in the spectrum;

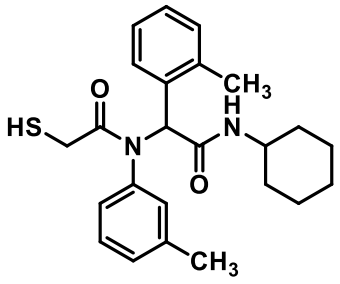
^{13}C NMR (151 MHz, DMSO-d_6): δ [ppm] = 20.8 (1C, ArCH_3), 24.5 (1C, NHCH(CH_2CH_2)$_2CH_2$), 24.6 (1C, NHCH(CH_2CH_2)$_2CH_2$), 25.2 (1C, NHCH(CH_2CH_2)$_2CH_2$), 32.1 (1C, NHCH(CH_2CH_2)$_2CH_2$), 32.2 (1C, NHCH(CH_2CH_2)$_2CH_2$), 48.0 (1C, NHCH(CH_2CH_2)$_2CH_2$), 64.0 (1C, NCHCO), 123.1 (1C, C-5'$_{isothiazole}$), 127.3 (1C, C-6'''$_{m\text{-tolyl}}$), 127.6 (1C, C-5'''$_{m\text{-tolyl}}$), 128.3 (1C, C-4'''$_{m\text{-tolyl}}$), 128.5 (1C, $C_{m\text{-tolyl}}$), 130.0 (1C, $C_{m\text{-tolyl}}$), 130.2 (1C, $C_{m\text{-tolyl}}$), 131.2 (1C, C-2'''$_{m\text{-tolyl}}$), 133.8 (1C, $C_{m\text{-tolyl}}$), 134.8 (1C, C-1'''$_{m\text{-tolyl}}$), 136.8 (1C, C-3'''$_{m\text{-tolyl}}$), 137.0 (1C, C-1''$_{m\text{-tolyl}}$), 146.4 (1C, C-3'$_{isothiazole}$), 151.2 (1C, C-4'$_{isothiazole}$), 162.2 (1C, ArCONCH), 163.9 (1C, ArCONH_2), 168.4 (1C, CHCONHCH), the signals for ArCH_3 and 1 $C_{m\text{-tolyl}}$ cannot be observed in the spectrum;

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3451, 3335, 3272, 3163, 2926, 2851, 1674, 1651, 1611, 1561, 1488, 1448, 1400, 1367, 1343, 1324, 1266, 1248, 1213, 1188, 1158, 1103, 1073, 1039, 979, 890, 799, 783, 757, 727, 703, 678, 579, 543, 528, 502;

HRMS (m/z): [M+H]$^+$ calcd for $C_{27}H_{32}N_5O_3S$: 506.2220, found: 506.2203;

HPLC (method 1): t_R = 25.0 min, purity 99.5%.

N-Cyclohexyl-2-[2-mercapto-N-(m-tolyl)acetamido]-2-(o-tolyl)acetamide (51c)



$C_{24}H_{30}N_2O_2S$

M_r = 410.6

3-Methylaniline (0.22 mL, 210 mg, 2.0 mmol) and 2-methylbenzaldehyde (0.23 mL, 240 mg, 2.0 mmol) were dissolved in dichloromethane (15 mL) and the solution was stirred at ambient temperature for 10 min. Then, sulfanylacetic acid (0.14 mL, 180 mg, 2.0 mmol) and isocyanocyclohexane (0.25 mL, 220 mg, 2.0 mmol) were added. After stirring the reaction mixture for 48 h at ambient temperature, the solvent was removed *in vacuo* and the residue was purified by flash column chromatography (Ø = 4 cm, h = 15 cm, V = 15 mL, dichloromethane/ethyl acetate = 15/1) to give **51c** (490 mg, 1.2 mmol, 60% yield) as colorless solid.

m.p. = 176 °C;

TLC: R_f = 0.43 (dichloromethane/ethyl acetate = 10/1);

^{1}H NMR (400 MHz, DMSO-d_6): δ [ppm] = 0.90 – 1.35 (m, 5H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 1.48 – 1.64 (m, 2H, NHCH(CH$_2$CH_2)$_2$CH$_2$ (1H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 1.64 – 1.81 (m, 3H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (1H)), 1.88 – 2.31 (m, 3H, ArCH_3), 2.36 (s, 3H, ArCH_3), 2.60 (t, J = 6.9 Hz, 1H, HSCH$_2$CO), 2.96 (dd, J = 14.7/6.7 Hz, 1H, HSCH_2CO), 3.08 (dd, J = 14.7/7.1 Hz, 1H, HSCH_2CO), 3.54 – 3.68 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), 6.20 (s, 1H, NCHCO), 6.41 (s br, 1H, H$_{m\text{-tolyl}}$), 6.62 – 6.75 (m, 1H, 6"-H$_{o\text{-tolyl}}$,), 6.75 – 6.85 (m, 1H, 5"-H$_{o\text{-tolyl}}$), 6.85 – 6.96 (m, 1H, 4'-H$_{m\text{-tolyl}}$), 6.96 – 7.04 (m, 1H, 4"-H$_{o\text{-tolyl}}$), 7.04 – 7.15 (m, 1H, 3"-H$_{o\text{-tolyl}}$), 7.71 (s br, 1H, H$_{m\text{-tolyl}}$), 7.92 (d, J = 7.6 Hz, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), the signal for 1 H$_{m\text{-tolyl}}$ cannot be observed in the spectrum, the signals of the major rotamer are given;

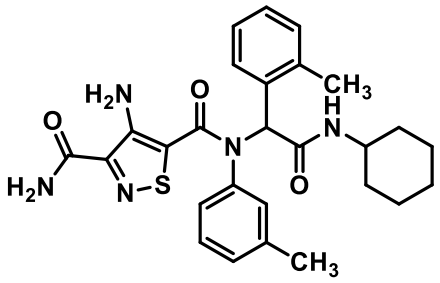
^{13}C NMR (101 MHz, DMSO-d_6): δ [ppm] = 19.0 (1C, ArCH$_3$), 24.5 (1C, NHCH(CH$_2$$CH_2$)$_2CH_2$), 24.7 (1C, NHCH(CH$_2$$CH_2$)$_2CH_2$), 25.2 (1C, NHCH(CH$_2CH_2$)$_2$$CH_2$), 27.1 (1C, HS$CH_2$CO), 32.2 (1C, NHCH($CH_2CH_2$)$_2CH_2$), 32.3 (1C, NHCH($CH_2CH_2$)$_2CH_2$), 48.0 (1C, NH$C$H(CH$_2CH_2$)$_2CH_2$), 60.4 (1C, N$C$HCO), 125.3 (1C, C-5"$_{o\text{-tolyl}}$), 127.8 (1C, C-4"$_{o\text{-tolyl}}$), 128.4 (1C, C-4'$_{m\text{-tolyl}}$), 129.2 (1C, C-6"$_{o\text{-tolyl}}$), 129.7 (1C, C-3"$_{o\text{-tolyl}}$), 133.9 (1C, C-1"$_{o\text{-tolyl}}$), 137.6 (1C, C-2"$_{o\text{-tolyl}}$), 138.9 (1C, C-1'$_{m\text{-tolyl}}$), 168.9 (1C, CH$C$ONHCH), 169.2 (1C, HSCH$_2$$C$O), the signals for 1 Ar$CH_3$ and 4 C$_{m\text{-tolyl}}$ cannot be observed in the spectrum;

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3274 3071, 2925, 2851, 1644, 1604, 1587, 1551, 1488, 1448, 1354, 1323, 1252, 1228, 1186, 1151, 1103, 1090, 1066, 1052, 978, 890, 873, 862, 843, 816, 802, 751, 729, 710, 662, 646, 590, 547, 514, 499, 451;

HRMS (m/z): [M+H]$^+$ calcd for C$_{24}$H$_{31}$N$_2$O$_2$S: 411.2101, found: 411.2110;

HPLC (method 1): t$_R$ = 25.1 min, purity 82.2%.

4-Amino-N^5-[2-(cyclohexylamino)-2-oxo-1-(*o*-tolyl)ethyl]-N^5-(*m*-tolyl)isothiazole-3,5-dicarboxamide (52c)



$C_{27}H_{31}N_5O_3S$

M_r = 505.6

51c (410 mg, 1.0 mmol) was added in one portion to a solution of **25** (320 mg, 1.2 mmol) in acetone (100 mL). The mixture was cooled to 0 °C and morpholine (0.17 mL, 170 mg, 1.9 mmol) was added dropwise. After stirring the reaction mixture for 12 h at ambient temperature, the solvent was removed *in vacuo*. The residue was purified by flash column chromatography (Ø = 6 cm, h = 20 cm, V = 15 mL, dichloromethane/ethyl acetate = 5/1) to give **52c** (220 mg, 0.43 mmol, 43% yield) as colorless solid.

m.p. = 203 °C;

TLC: R_f = 0.19 (dichloromethane/ethyl acetate = 5/1);

^{1}H NMR (500 MHz, DMSO-d_6): δ [ppm] = 0.92 – 1.02 (m, 1H, NHCH(CH_2CH$_2$)$_2$CH$_2$), 1.02 – 1.13 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$CH_2), 1.14 – 1.33 (m, 3H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (1H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H)), 1.50 – 1.64 (m, 2H, NHCH(CH$_2$CH_2)$_2$CH$_2$ (1H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 1.65 – 1.75 (m, 2H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (1H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (1H)), 1.75 – 1.82 (m, 1H, NHCH(CH_2CH$_2$)$_2$CH$_2$), 1.92 – 2.34 (m, 3H, ArCH_3), 2.40 (s, 3H, ArCH_3), 3.57 – 3.67 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), 6.36 (s, 1H, NCHCO), 6.43 (s br, 1H, H$_{m\text{-tolyl}}$), 6.67 – 6.75 (m, 1H, 6'''-H$_{o\text{-tolyl}}$,), 6.79 – 6.87 (m, 1H, 5'''-H$_{o\text{-tolyl}}$), 6.87 – 6.99 (m, 0.5H, H$_{m\text{-tolyl}}$), 6.99 – 7.06 (m, 1H, 4'''-H$_{o\text{-tolyl}}$), 7.06 – 7.15 (m, 2H, 3'''-H$_{o\text{-tolyl}}$, H$_{m\text{-tolyl}}$ (1H)), 7.15 – 7.31 (m, 2.5H, ArNH_2, H$_{m\text{-tolyl}}$ (0.5H)), 7.57 (s br, 1H, CONH_2), 7.73 – 7.89 (m, 2H, CONH_2 (1H), H$_{m\text{-tolyl}}$ (1H)), 8.02 (d, J = 7.7 Hz, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$);

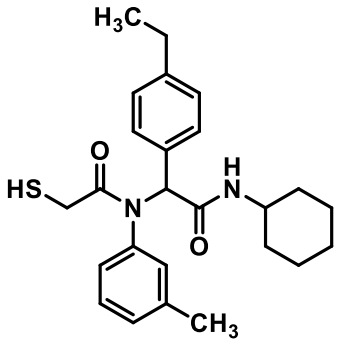
^{13}C NMR (126 MHz, DMSO-d_6): δ [ppm] = 19.0 (1C, Ar*C*H$_3$), 24.5 (1C, NHCH(CH$_2$*C*H$_2$)$_2$CH$_2$), 24.7 (1C, NHCH(CH$_2$*C*H$_2$)$_2$CH$_2$), 25.2 (1C, NHCH(CH$_2$CH$_2$)$_2$*C*H$_2$), 32.2 (1C, NHCH(*C*H$_2$CH$_2$)$_2$CH$_2$), 32.3 (1C, NHCH(*C*H$_2$CH$_2$)$_2$CH$_2$), 48.1 (1C, NH*C*H(CH$_2$CH$_2$)$_2$CH$_2$), 60.4 (1C, N*C*HCO), 123.0 (1C, C-5'$_{isothiazole}$), 125.3 (1C, C-5'''$_{o\text{-tolyl}}$), 127.9 (1C, C-4'''$_{o\text{-tolyl}}$), 129.4 (1C, C-6'''$_{o\text{-tolyl}}$), 129.8 (1C, C-3'''$_{o\text{-tolyl}}$), 130.1 (1C, C$_{m\text{-tolyl}}$), 133.6 (1C, C-1'''$_{o\text{-tolyl}}$), 136.9 (1C, C-1''$_{m\text{-tolyl}}$), 137.7 (1C, C-2'''$_{o\text{-tolyl}}$), 146.4 (1C, C-3'$_{isothiazole}$), 151.3 (1C, C-4'$_{isothiazole}$), 162.4 (1C, Ar*C*ONCH), 163.9 (1C, Ar*C*ONH$_2$), 169.0 (1C, CH*C*ONHCH), the signals for 1 Ar*C*H$_3$ and 4 C$_{m\text{-tolyl}}$ cannot be observed in the spectrum;

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3457, 3339, 3281, 3067, 2924, 2850, 1674, 1646, 1615, 1600, 1561, 1488, 1449, 1382, 1344, 1318, 1252, 1237, 1189, 1151, 1104, 1071, 1057, 979, 890, 873, 863, 803, 792, 767, 731, 712, 686, 654, 588, 528, 506;

HRMS (*m*/*z*): [M+H]$^+$ calcd for C$_{27}$H$_{32}$N$_5$O$_3$S: 506.2220, found: 506.2224;

HPLC (method 1): t_R = 24.8 min, purity 95.3%.

N-Cyclohexyl-2-(4-ethylphenyl)-2-[2-mercapto-*N*-(*m*-tolyl)acetamido]acetamide (51d)



C$_{25}$H$_{32}$N$_2$O$_2$S

M$_r$ = 424.6

3-Methylaniline (0.22 mL, 210 mg, 2.0 mmol) and 4-ethylbenzaldehyde (0.27 mL, 270 mg, 2.0 mmol) were dissolved in dichloromethane (15 mL) and the solution was stirred at ambient temperature for 10 min. Then, sulfanylacetic acid (0.14 mL, 180 mg, 2.0 mmol) and isocyanocyclohexane (0.25 mL, 220 mg, 2.0 mmol) were added. After stirring the reaction mixture for 48 h at ambient temperature, the solvent was removed *in vacuo* and the residue was purified by flash column chromatography (Ø = 4 cm, h = 15 cm, V = 15 mL,

dichloromethane/ethyl acetate = 15/1) to give **51d** (530 mg, 1.2 mmol, 62% yield) as colorless solid.

m.p. = 198 °C;

TLC: R_f = 0.36 (dichloromethane/ethyl acetate = 15/1);

^{1}H NMR (400 MHz, DMSO-*d*$_6$): δ [ppm] = 0.90 – 1.32 (m, 8H, NHCH(C*H*$_2$CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$C*H*$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH$_2$)$_2$C*H*$_2$ (1H), ArCH$_2$C*H*$_3$), 1.48 – 1.79 (m, 5H, NHCH(C*H*$_2$CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$C*H*$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH$_2$)$_2$C*H*$_2$ (1H)), 2.12 (s br, 3H, ArC*H*$_3$), 2.45 (q, *J* = 7.6 Hz, 2H, ArC*H*$_2$CH$_3$), 2.57 (t, *J* = 6.8 Hz, 1H, *H*SCH$_2$CO), 2.92 – 3.07 (m, 2H, HSC*H*$_2$CO), 3.50 – 3.63 (m, 1H, NHC*H*(CH$_2$CH$_2$)$_2$CH$_2$), 5.97 (s, 1H, NC*H*CO), 6.46 (s br, 1H, H$_{m\text{-tolyl}}$), 6.83 – 7.18 (m, 6H, 2"-H$_{4\text{-ethylphenyl}}$, 3"-H$_{4\text{-ethylphenyl}}$, 5"-H$_{4\text{-ethylphenyl}}$, 6"-H$_{4\text{-ethylphenyl}}$, H$_{m\text{-tolyl}}$ (2H)), 7.56 (s br, 1H, H$_{m\text{-tolyl}}$), 7.92 (d, *J* = 7.7 Hz, 1H, N*H*CH(CH$_2$CH$_2$)$_2$CH$_2$);

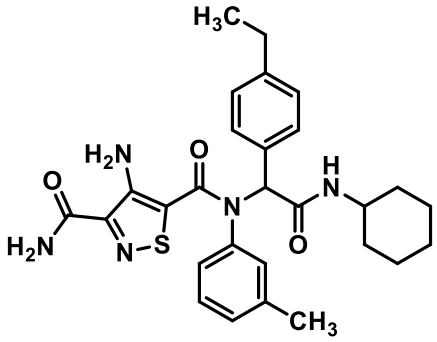
^{13}C NMR (151 MHz, DMSO-*d*$_6$): δ [ppm] = 15.5 (1C, ArCH$_2$*C*H$_3$), 24.5 (1C, NHCH(CH$_2$*C*H$_2$)$_2$CH$_2$), 24.6 (1C, NHCH(CH$_2$*C*H$_2$)$_2$CH$_2$), 25.2 (1C, NHCH(CH$_2$CH$_2$)$_2$*C*H$_2$), 27.3 (1C, HS*C*H$_2$CO), 27.7 (1C, Ar*C*H$_2$CH$_3$), 32.17 (1C, NHCH(*C*H$_2$CH$_2$)$_2$CH$_2$), 32.24 (1C, NHCH(*C*H$_2$CH$_2$)$_2$CH$_2$), 47.9 (1C, NH*C*H(CH$_2$CH$_2$)$_2$CH$_2$), 63.6 (1C, N*C*HCO), 127.1 (2C, C-3"$_{4\text{-ethylphenyl}}$, C-5"$_{4\text{-ethylphenyl}}$), 127.8 (1C, C$_{m\text{-tolyl}}$), 128.0 (1C, C$_{m\text{-tolyl}}$), 128.3 (1C, C$_{m\text{-tolyl}}$), 130.0 (2C, C-2"$_{4\text{-ethylphenyl}}$, C-6"$_{4\text{-ethylphenyl}}$), 131.3 (1C, C$_{m\text{-tolyl}}$), 132.6 (1C, C-1"$_{4\text{-ethylphenyl}}$), 139.1 (1C, C-1'$_{m\text{-tolyl}}$), 143.1 (1C, C-4"$_{4\text{-ethylphenyl}}$), 168.4 (1C, CH*C*ONHCH), 168.8 (1C, HSCH$_2$*C*O), the signals for Ar*C*H$_3$ and 1 C$_{m\text{-tolyl}}$ cannot be observed in the spectrum;

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3270, 3078, 2926, 2852, 1650, 1604, 1587, 1556, 1513, 1488, 1449, 1411, 1373, 1251, 1234, 1187, 1162, 1106, 1041, 979, 889, 850, 829, 801, 727, 709, 664, 642, 594, 570, 496;

HRMS (*m*/*z*): [M+H]$^+$ calcd for C$_{25}$H$_{33}$N$_2$O$_2$S: 425.2257, found: 425.2262;

HPLC (method 1): t_R = 26.1 min, purity 99.2%.

4-Amino-N^5-[2-(cyclohexylamino)-1-(4-ethylphenyl)-2-oxoethyl]-N^5-(*m*-tolyl)isothiazole-3,5-dicarboxamide (52d)



$C_{28}H_{33}N_5O_3S$

M_r = 519.7

51d (420 mg, 1.0 mmol) was added in one portion to a solution of **25** (320 mg, 1.2 mmol) in acetone (100 mL). The mixture was cooled to 0 °C and morpholine (0.17 mL, 170 mg, 1.9 mmol) was added dropwise. After stirring the reaction mixture for 12 h at ambient temperature, the solvent was removed *in vacuo*. The residue was purified by flash column chromatography (Ø = 6 cm, h = 20 cm, V = 15 mL, dichloromethane/ethyl acetate = 5/1) to give **52d** (270 mg, 0.53 mmol, 53% yield) as colorless solid.

m.p. = 232 °C;

TLC: R_f = 0.29 (dichloromethane/ethyl acetate = 5/1);

^{1}H NMR (600 MHz, DMSO-d_6): δ [ppm] = 0.91 – 1.02 (m, 1H, NHCH(CH_2CH$_2$)$_2$CH$_2$), 1.02 – 1.12 (m, 4H, NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H), ArCH$_2$CH_3), 1.13 – 1.31 (m, 3H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (1H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H)), 1.48 – 1.62 (m, 2H, NHCH(CH$_2$CH_2)$_2$CH$_2$ (1H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 1.62 – 1.74 (m, 2H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (1H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (1H)), 1.74 – 1.81 (m, 1H, NHCH(CH_2CH$_2$)$_2$CH$_2$), 2.16 (s br, 3H, ArCH_3), 2.46 (q, *J* = 7.6 Hz, 2H, ArCH_2CH$_3$), 3.55 – 3.64 (m, 1H, NHC*H*(CH$_2$CH$_2$)$_2$CH$_2$), 6.11 (s, 1H, NC*H*CO), 6.50 (s br, 1H, H$_{m\text{-tolyl}}$), 6.81 – 7.29 (m, 8H, 2'''-H$_{4\text{-ethylphenyl}}$, 3'''-H$_{4\text{-ethylphenyl}}$, 5'''-H$_{4\text{-ethylphenyl}}$, 6'''-H$_{4\text{-ethylphenyl}}$, H$_{m\text{-tolyl}}$ (2H), ArNH_2), 7.58 (s br, 1H, CONH_2), 7.65 (s br, 1H, H$_{m\text{-tolyl}}$), 7.89 (s br, 1H, CONH_2), 8.00 (d, *J* = 7.7 Hz, 1H, N*H*CH(CH$_2$CH$_2$)$_2$CH$_2$), the signals of the major rotamer are given;

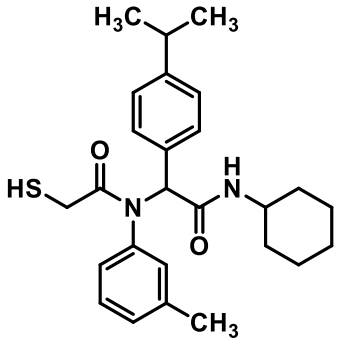
^{13}C NMR (151 MHz, DMSO-*d*$_6$): δ [ppm] = 15.6 (1C, ArCH$_2$*C*H$_3$), 24.5 (1C, NHCH(CH$_2$*C*H$_2$)$_2$CH$_2$), 24.7 (1C, NHCH(CH$_2$*C*H$_2$)$_2$CH$_2$), 25.2 (1C, NHCH(CH$_2$CH$_2$)$_2$*C*H$_2$), 27.7 (1C, Ar*C*H$_2$CH$_3$), 32.2 (1C, NHCH(*C*H$_2$CH$_2$)$_2$CH$_2$), 32.3 (1C, NHCH(*C*H$_2$CH$_2$)$_2$CH$_2$), 48.0 (1C, NH*C*H(CH$_2$CH$_2$)$_2$CH$_2$), 63.7 (1C, N*C*HCO), 123.2 (1C, C-5'$_{isothiazole}$), 127.2 (2C, C-3'''$_{4\text{-ethylphenyl}}$, C-5'''$_{4\text{-ethylphenyl}}$), 130.0 (1C, C$_{m\text{-tolyl}}$), 130.2 (1C, C$_{m\text{-tolyl}}$), 130.3 (2C, C-2'''$_{4\text{-ethylphenyl}}$, C-6'''$_{4\text{-ethylphenyl}}$), 132.2 (1C, C-1'''$_{4\text{-ethylphenyl}}$), 133.7 (1C, C$_{m\text{-tolyl}}$), 137.9 (1C, C-1''$_{m\text{-tolyl}}$), 143.3 (1C, C-4'''$_{4\text{-ethylphenyl}}$), 146.4 (1C, C-3'$_{isothiazole}$), 151.2 (1C, C-4'$_{isothiazole}$), 162.2 (1C, Ar*C*ONCH), 163.9 (1C, Ar*C*ONH$_2$), 168.5 (1C, CH*C*ONHCH), the signals for Ar*C*H$_3$ and 2 C$_{m\text{-tolyl}}$ cannot be observed in the spectrum;

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3454, 3283, 3081, 2968, 2926, 2851, 1673, 1648, 1600, 1560, 1516, 1489, 1449, 1369, 1347, 1322, 1251, 1238, 1191, 1152, 1104, 1073, 979, 890, 873, 829, 799, 756, 732, 708, 678, 531, 501;

HRMS (*m*/*z*): [M+H]$^+$ calcd for C$_{28}$H$_{34}$N$_5$O$_3$S: 520.2377, found: 520.2377;

HPLC (method 1): t$_R$ = 25.7 min, purity 97.7%.

N-Cyclohexyl-2-(4-isopropylphenyl)-2-[2-mercapto-*N*-(*m*-tolyl)acetamido]acetamide (51e)



C$_{26}$H$_{34}$N$_2$O$_2$S

M$_r$ = 438.6

3-Methylaniline (0.22 mL, 210 mg, 2.0 mmol) and 4-isopropylbenzaldehyde (0.31 mL, 300 mg, 2.0 mmol) were dissolved in dichloromethane (15 mL) and the solution was stirred at ambient temperature for 10 min. Then, sulfanylacetic acid (0.14 mL, 180 mg, 2.0 mmol) and isocyanocyclohexane (0.25 mL, 220 mg, 2.0 mmol) were added. After stirring the reaction mixture for 48 h at ambient temperature, the solvent was removed *in vacuo* and the residue was purified by flash column chromatography (Ø = 4 cm, h = 15 cm, V = 15 mL,

dichloromethane/methanol = 100/1) to give **51e** (500 mg, 1.1 mmol, 57% yield) as colorless solid.

m.p. = 199 °C;

TLC: R_f = 0.49 (dichloromethane/methanol = 40/1);

^{1}H NMR (500 MHz, DMSO-d_6): δ [ppm] = 0.93 – 1.13 (m, 8H, NHCH(C*H*$_2$CH$_2$)$_2$CH$_2$ (1H), NHCH(CH$_2$CH$_2$)$_2$C*H*$_2$ (1H), ArCH(C*H*$_3$)$_2$), 1.14 – 1.31 (m, 3H, NHCH(C*H*$_2$CH$_2$)$_2$CH$_2$ (1H), NHCH(CH$_2$C*H*$_2$)$_2$CH$_2$ (2H)), 1.49 – 1.78 (m, 5H, NHCH(C*H*$_2$CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$C*H*$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH$_2$)$_2$C*H*$_2$ (1H)), 2.09 (s br, 3H, ArC*H*$_3$), 2.56 (t, *J* = 6.9 Hz, 1H, *H*SCH$_2$CO), 2.75 (sep, *J* = 6.9 Hz, 1H, ArC*H*(CH$_3$)$_2$), 2.94 – 3.05 (m, 2H, HSC*H*$_2$CO), 3.51 – 3.61 (m, 1H, NHC*H*(CH$_2$CH$_2$)$_2$CH$_2$), 5.98 (s, 1H, NC*H*CO), 6.38 (s br, 1H, H$_{m\text{-tolyl}}$), 6.83 – 7.17 (m, 6H, 2"-H$_{4\text{-isopropylphenyl}}$, 3"-H$_{4\text{-isopropylphenyl}}$, 5"-H$_{4\text{-isopropylphenyl}}$, 6"-H$_{4\text{-isopropylphenyl}}$, 4'-H$_{m\text{-tolyl}}$, H$_{m\text{-tolyl}}$ (1H)), 7.53 (s br, 1H, H$_{m\text{-tolyl}}$), 7.90 (d, *J* = 7.6 Hz, 1H, N*H*CH(CH$_2$CH$_2$)$_2$CH$_2$);

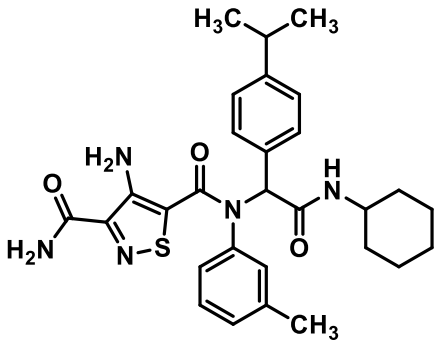
^{13}C NMR (101 MHz, DMSO-d_6): δ [ppm] = 20.6 (1C, ArCH$_3$), 23.7 (2C, ArCH(*C*H$_3$)$_2$), 24.5 (1C, NHCH(CH$_2$*C*H$_2$)$_2$CH$_2$), 24.7 (1C, NHCH(CH$_2$*C*H$_2$)$_2$CH$_2$), 25.2 (1C, NHCH(CH$_2$CH$_2$)$_2$*C*H$_2$), 27.3 (1C, HS*C*H$_2$CO), 32.2 (1C, NHCH(*C*H$_2$CH$_2$)$_2$CH$_2$), 32.3 (1C, NHCH(*C*H$_2$CH$_2$)$_2$CH$_2$), 33.0 (1C, Ar*C*H(CH$_3$)$_2$), 47.9 (1C, NH*C*H(CH$_2$CH$_2$)$_2$CH$_2$), 63.5 (1C, N*C*HCO), 125.6 (2C, C-3"$_{4\text{-isopropylphenyl}}$, C-5"$_{4\text{-isopropylphenyl}}$), 127.8 (1C, C$_{m\text{-tolyl}}$), 128.0 (1C, C$_{m\text{-tolyl}}$), 128.3 (1C, C$_{m\text{-tolyl}}$), 130.0 (2C, C-2"$_{4\text{-isopropylphenyl}}$, C-6"$_{4\text{-isopropylphenyl}}$), 131.3 (1C, C$_{m\text{-tolyl}}$), 132.8 (1C, C-1"$_{4\text{-isopropylphenyl}}$), 137.5 (1C, C-3'$_{m\text{-tolyl}}$), 139.1 (1C, C-1'$_{m\text{-tolyl}}$), 147.8 (1C, C-4"$_{4\text{-isopropylphenyl}}$), 168.4 (1C, CH*C*ONHCH), 168.8 (1C, HSCH$_2$*C*O);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3675, 3276, 3076, 2965, 2929, 2853, 1649, 1604, 1587, 1554, 1510, 1488, 1452, 1407, 1378, 1359, 1322, 1304, 1265, 1251, 1233, 1188, 1152, 1103, 1076, 1053, 979, 889, 829, 805, 768, 748, 709, 659, 631, 569, 544;

HRMS (*m*/*z*): [M+H]$^+$ calcd for C$_{26}$H$_{35}$N$_2$O$_2$S: 439.2414, found: 439.2411;

HPLC (method 1): t_R = 26.9 min, purity 98.0%.

4-Amino-N^5-[2-(cyclohexylamino)-1-(4-isopropylphenyl)-2-oxoethyl]-N^5-(*m*-tolyl)isothiazole-3,5-dicarboxamide (52e)



$C_{29}H_{35}N_5O_3S$

M_r = 533.7

51e (440 mg, 1.0 mmol) was added in one portion to a solution of **25** (320 mg, 1.2 mmol) in acetone (100 mL). The mixture was cooled to 0 °C and morpholine (0.17 mL, 170 mg, 1.9 mmol) was added dropwise. After stirring the reaction mixture for 12 h at ambient temperature, the solvent was removed *in vacuo*. The residue was purified by flash column chromatography (Ø = 6 cm, h = 20 cm, V = 15 mL, dichloromethane/ethyl acetate = 5/1) to give **52e** (330 mg, 0.62 mmol, 62% yield) as colorless solid.

m.p. = 231 °C;

TLC: R_f = 0.23 (dichloromethane/ethyl acetate = 5/1);

^{1}H NMR (400 MHz, DMSO-d_6): δ [ppm] = 0.90 – 1.14 (m, 8H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (1H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H), ArCH(CH_3)$_2$), 1.14 – 1.33 (m, 3H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (1H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H)), 1.48 – 1.81 (m, 5H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 2.10 (s br, 3H, ArCH_3), 2.75 (sep, J = 6.9 Hz, 1H, ArCH(CH$_3$)$_2$), 3.50 – 3.65 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), 6.11 (s, 1H, NCHCO), 6.43 (s br, 1H, H$_{m\text{-tolyl}}$), 6.75 – 7.30 (m, 8H, 2'''-H$_{4\text{-isopropylphenyl}}$, 3'''-H$_{4\text{-isopropylphenyl}}$, 5'''-H$_{4\text{-isopropylphenyl}}$, 6'''-H$_{4\text{-isopropylphenyl}}$, H$_{m\text{-tolyl}}$ (2H), ArNH_2), 7.41 – 7.78 (m, 2H, H$_{m\text{-tolyl}}$ (1H), CONH_2 (1H)), 7.82 (s br, 1H, CONH_2), 8.01 (d, J = 7.7 Hz, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$);

^{13}C NMR (151 MHz, DMSO-d_6): δ [ppm] = 23.72 (1C, ArCH(*C*H$_3$)$_2$), 23.75 (1C, ArCH(*C*H$_3$)$_2$), 24.5 (1C, NHCH(CH$_2$*C*H$_2$)$_2$CH$_2$), 24.7 (1C, NHCH(CH$_2$*C*H$_2$)$_2$CH$_2$), 25.2 (1C, NHCH(CH$_2$CH$_2$)$_2$*C*H$_2$), 32.2 (1C, NHCH(*C*H$_2$CH$_2$)$_2$CH$_2$), 32.3 (1C, NHCH(*C*H$_2$CH$_2$)$_2$CH$_2$),

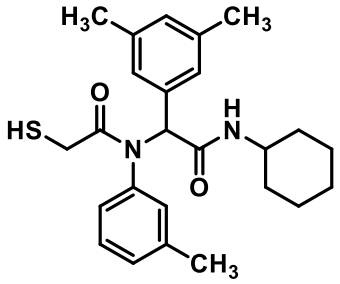
33.0 (1C, Ar*C*H(CH_3)$_2$), 48.0 (1C, NH*C*H(CH_2CH_2)$_2CH_2$), 63.7 (1C, N*C*HCO), 123.1 (1C, C-5'$_{isothiazole}$), 125.6 (2C, C-3'''$_{4\text{-isopropylphenyl}}$, C-5'''$_{4\text{-isopropylphenyl}}$), 129.9 (1C, C$_{m\text{-tolyl}}$), 130.3 (3C, C-2'''$_{4\text{-isopropylphenyl}}$, C-6'''$_{4\text{-isopropylphenyl}}$, 1 C$_{m\text{-tolyl}}$), 132.4 (1C, C-1'''$_{4\text{-isopropylphenyl}}$), 133.7 (1C, C$_{m\text{-tolyl}}$), 137.0 (1C, C$_{m\text{-tolyl}}$), 146.4 (1C, C-3'$_{isothiazole}$), 148.0 (1C, C-4'''$_{4\text{-isopropylphenyl}}$), 151.2 (1C, C-4'$_{isothiazole}$), 162.2 (1C, Ar*C*ONCH), 163.9 (1C, Ar*C*ONH_2), 168.5 (1C, CH*C*ONHCH), the signals for Ar*C*H_3 and 2 C$_{m\text{-tolyl}}$ cannot be observed in the spectrum;

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3275, 2927, 2851, 1649, 1602, 1585, 1558, 1487, 1448, 1370, 1352, 1252, 1232, 1189, 1104, 801, 753, 731, 709, 665, 644, 631, 548, 534, 503;

HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{29}H_{36}N_5O_3S$: 534.2533, found: 534.2539;

HPLC (method 1): t_R = 26.6 min, purity 98.6%.

N-Cyclohexyl-2-(3,5-dimethylphenyl)-2-[2-mercapto-*N*-(*m*-tolyl)acetamido]acetamide (51f)



$C_{25}H_{32}N_2O_2S$

M_r = 424.6

3-Methylaniline (0.22 mL, 210 mg, 2.0 mmol) and 3,5-dimethylbenzaldehyde (0.27 mL, 270 mg, 2.0 mmol) were dissolved in dichloromethane (15 mL) and the solution was stirred at ambient temperature for 10 min. Then, sulfanylacetic acid (0.14 mL, 180 mg, 2.0 mmol) and isocyanocyclohexane (0.25 mL, 220 mg, 2.0 mmol) were added. After stirring the reaction mixture for 48 h at ambient temperature, the solvent was removed *in vacuo* and the residue was purified by flash column chromatography (Ø = 4 cm, h = 15 cm, V = 15 mL, dichloromethane/ethyl acetate = 18/1) to give **51f** (450 mg, 1.1 mmol, 53% yield) as colorless solid.

m.p. = 156 °C;

TLC: R_f = 0.36 (dichloromethane/ethyl acetate = 15/1);

^{1}H NMR (600 MHz, DMSO-d_6): δ [ppm] = 0.95 – 1.13 (m, 2H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (1H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 1.14 – 1.30 (m, 3H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (1H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H)), 1.49 – 1.56 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$CH_2), 1.56 – 1.77 (m, 4H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H)), 2.08 (s, 6H, Ar(CH_3)$_2$), 2.15 (s br, 3H, ArCH_3), 2.57 (t, J = 6.9 Hz, 1H, HSCH$_2$CO), 2.58 (dd, J = 14.9/6.8 Hz, 1H, HSCH_2CO), 3.04 (dd, J = 14.9/7.0 Hz, 1H, HSCH_2CO), 3.51 – 3.62 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), 5.92 (s, 1H, NCHCO), 6.47 (s br, 1H, H$_{m\text{-tolyl}}$), 6.59 – 6.65 (m, 2H, 2"-H$_{3,5\text{-dimethylphenyl}}$, 6"-H$_{3,5\text{-dimethylphenyl}}$), 6.71 – 6.75 (m, 1H, 4"-H$_{3,5\text{-dimethylphenyl}}$), 6.94 – 6.98 (m, 1H, 4'-H$_{m\text{-tolyl}}$), 7.02 (s br, 1H, H$_{m\text{-tolyl}}$), 7.55 (s br, 1H, H$_{m\text{-tolyl}}$), 7.89 (d, J = 7.4 Hz, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$);

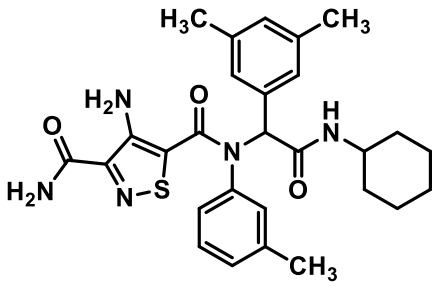
^{13}C NMR (151 MHz, DMSO-d_6): δ [ppm] = 20.6 (1C, ArCH$_3$), 20.7 (2C, Ar(CH$_3$)$_2$), 24.5 (1C, NHCH(CH$_2C$H$_2$)$_2$CH$_2$), 24.6 (1C, NHCH(CH$_2C$H$_2$)$_2$CH$_2$), 25.2 (1C, NHCH(CH$_2$CH$_2$)$_2C$H$_2$), 27.3 (1C, HSCH$_2$CO), 32.2 (2C, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), 47.8 (1C, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), 63.8 (1C, NCHCO), 127.7 (1C, C$_{m\text{-tolyl}}$), 127.89 (2C, C-2"$_{3,5\text{-dimethylphenyl}}$, C-6"$_{3,5\text{-dimethylphenyl}}$), 127.94 (1C, C$_{m\text{-tolyl}}$), 128.3 (1C, C-4'$_{m\text{-tolyl}}$), 128.8 (1C, C-4"$_{3,5\text{-dimethylphenyl}}$), 131.4 (1C, C$_{m\text{-tolyl}}$), 135.0 (1C, C-1"$_{3,5\text{-dimethylphenyl}}$), 136.5 (2C, C-3"$_{3,5\text{-dimethylphenyl}}$, C-5"$_{3,5\text{-dimethylphenyl}}$), 137.5 (1C, C-3'$_{m\text{-tolyl}}$), 139.1 (1C, C-1'$_{m\text{-tolyl}}$), 168.3 (1C, CH$C$ONHCH), 168.8 (1C, HSCH$_2C$O);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3268, 3090, 2925, 2852, 1651, 1604, 1564, 1488, 1449, 1410, 1369, 1295, 1266, 1251, 1232, 1221, 1186, 1163, 1151, 1106, 1064, 1039, 981, 912, 889, 867, 852, 800, 727, 710, 669, 594, 572, 558, 532, 494, 469;

HRMS (m/z): [M+H]$^+$ calcd for C$_{25}$H$_{33}$N$_2$O$_2$S: 425.2257, found: 425.2259;

HPLC (method 1): t_R = 25.9 min, purity 98.4%.

4-Amino-N^5-[2-(cyclohexylamino)-1-(3,5-dimethylphenyl)-2-oxoethyl]-N^5-(*m*-tolyl)isothiazole-3,5-dicarboxamide (52f)



C$_{28}$H$_{33}$N$_5$O$_3$S

M$_r$ = 519.7

51f (420 mg, 1.0 mmol) was added in one portion to a solution of **25** (320 mg, 1.2 mmol) in acetone (100 mL). The mixture was cooled to 0 °C and morpholine (0.17 mL, 170 mg, 1.9 mmol) was added dropwise. After stirring the reaction mixture for 12 h at ambient temperature, the solvent was removed *in vacuo*. The residue was purified by flash column chromatography (Ø = 6 cm, h = 20 cm, V = 15 mL, dichloromethane/ethyl acetate = 7/1) to give **52f** (250 mg, 0.49 mmol, 49% yield) as colorless solid.

m.p. = 204 °C;

TLC: R_f = 0.29 (dichloromethane/ethyl acetate = 5/1);

^{1}H NMR (500 MHz, DMSO-d_6): δ [ppm] = 0.94 – 1.14 (m, 2H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (1H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 1.15 – 1.32 (m, 3H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (1H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H)), 1.48 – 1.80 (m, 5H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 2.08 (s, 6H, Ar(CH_3)$_2$), 2.18 (s br, 3H, ArCH_3), 3.54 – 3.65 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), 6.05 (s, 1H, NCHCO), 6.54 (s br, 1H, H$_{m\text{-tolyl}}$), 6.67 – 6.70 (m, 2H, 2'''-H$_{3,5\text{-dimethylphenyl}}$, 6'''-H$_{3,5\text{-dimethylphenyl}}$), 6.73 – 6.76 (m, 1H, 4'''-H$_{3,5\text{-dimethylphenyl}}$), 6.93 – 7.28 (m, 4H, 4''-H$_{m\text{-tolyl}}$, H$_{m\text{-tolyl}}$ (1H), ArNH_2), 7.59 (s br, 1H, CONH_2), 7.66 (s br, 1H, H$_{m\text{-tolyl}}$), 7.82 (s br, 1H, CONH_2), 8.00 (d, J = 7.7 Hz, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$);

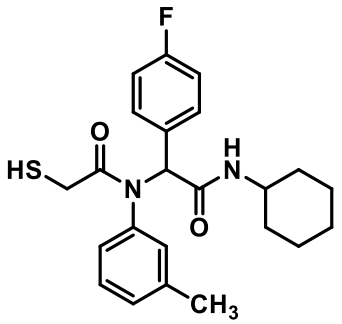
^{13}C NMR (151 MHz, DMSO-d_6): δ [ppm] = 20.7 (2C, Ar(CH_3)$_2$), 24.5 (1C, NHCH(CH$_2$CH_2)$_2$CH$_2$), 24.6 (1C, NHCH(CH$_2$CH_2)$_2$CH$_2$), 25.2 (1C, NHCH(CH$_2$CH$_2$)$_2$CH_2), 32.2 (2C, NHCH(CH_2CH$_2$)$_2$CH$_2$), 47.9 (1C, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), 63.9 (1C, NCHCO), 123.2 (1C, C-5'$_{isothiazole}$), 128.2 (2C, C-2'''$_{3,5\text{-dimethylphenyl}}$, C-6'''$_{3,5\text{-dimethylphenyl}}$), 128.5 (1C, C$_{m\text{-tolyl}}$), 129.0 (1C, C-4'''$_{3,5\text{-dimethylphenyl}}$), 129.9 (1C, C$_{m\text{-tolyl}}$), 130.1 (1C, C$_{m\text{-tolyl}}$), 133.8 (1C, C$_{m\text{-tolyl}}$), 134.7 (1C, C-1'''$_{3,5\text{-dimethylphenyl}}$), 136.5 (2C, C-3'''$_{3,5\text{-dimethylphenyl}}$, C-5'''$_{3,5\text{-dimethylphenyl}}$), 137.0 (1C, C-1''$_{m\text{-tolyl}}$), 146.4 (1C, C-3'$_{isothiazole}$), 151.2 (1C, C-4'$_{isothiazole}$), 162.2 (1C, Ar$C$ONCH), 163.9 (1C, Ar$CONH_2$), 168.4 (1C, CH$C$ONHCH), the signals for ArCH$_3$ and 1 C$_{m\text{-tolyl}}$ cannot be observed in the spectrum;

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3450, 3284, 3078, 2926, 2852, 1672, 1650, 1599, 1558, 1488, 1448, 1367, 1345, 1320, 1291, 1266, 1249, 1237, 1223, 1188, 1149, 1104, 1072, 1037, 1002, 979, 888, 863, 846, 798, 753, 740, 728, 704, 676, 658, 588, 532;

HRMS (m/z): [M+H]$^+$ calcd for $C_{28}H_{34}N_5O_3S$: 520.2377, found: 520.2369;

HPLC (method 1): t_R = 25.6 min, purity 99.5%.

_N_-Cyclohexyl-2-(4-fluorophenyl)-2-[2-mercapto-_N_-(_m_-tolyl)acetamido]acetamide (51g)



$C_{23}H_{27}FN_2O_2S$

$M_r = 414.5$

3-Methylaniline (0.22 mL, 210 mg, 2.0 mmol) and 4-fluorobenzaldehyde (0.22 mL, 250 mg, 2.0 mmol) were dissolved in dichloromethane (15 mL) and the solution was stirred at ambient temperature for 10 min. Then, sulfanylacetic acid (0.14 mL, 180 mg, 2.0 mmol) and isocyanocyclohexane (0.25 mL, 220 mg, 2.0 mmol) were added. After stirring the reaction mixture for 48 h at ambient temperature, the solvent was removed *in vacuo* and the residue was purified by flash column chromatography (Ø = 4 cm, h = 15 cm, V = 15 mL, dichloromethane/ethyl acetate = 18/1) to give **51g** (350 mg, 0.83 mmol, 42% yield) as colorless solid.

m.p. = 219 °C;

TLC: R_f = 0.31 (dichloromethane/ethyl acetate = 15/1);

^{1}H NMR (400 MHz, DMSO-d_6): δ [ppm] = 0.90 – 1.33 (m, 5H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 1.45 – 1.80 (m, 5H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 2.16 (s br, 3H, ArCH_3), 2.57 (t, J = 6.8 Hz, 1H, HSCH$_2$CO), 2.94 – 3.08 (m, 2H, HSCH_2CO), 3.49 – 3.63 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), 5.99 (s, 1H, NCHCO), 6.89 – 7.12 (m, 6H, 2"-H$_{4\text{-fluorophenyl}}$, 3"-H$_{4\text{-fluorophenyl}}$, 5"-H$_{4\text{-fluorophenyl}}$, 6"-H$_{4\text{-fluorophenyl}}$, H$_{m\text{-tolyl}}$ (2H)), 7.96 (d, J = 7.7 Hz, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), the signals for 2 H$_{m\text{-tolyl}}$ cannot be observed in the spectrum;

^{13}C NMR (101 MHz, DMSO-d_6): δ [ppm] = 20.7 (1C, ArCH$_3$), 24.4 (1C, NHCH(CH$_2$$CH_2$)$_2CH_2$), 24.6 (1C, NHCH(CH$_2$$CH_2$)$_2CH_2$), 25.1 (1C, NHCH(CH$_2CH_2$)$_2$$CH_2$), 27.3 (1C, HS$CH_2$CO), 32.1 (2C, NHCH($CH_2CH_2$)$_2CH_2$), 47.9 (1C, NH$C$H(CH$_2CH_2$)$_2CH_2$), 63.0 (1C, N$C$HCO), 114.6 (d, J = 21.3 Hz, 2C, C-3"$_{4\text{-fluorophenyl}}$, C-5"$_{4\text{-fluorophenyl}}$), 127.7 (1C, C$_{m\text{-}}$

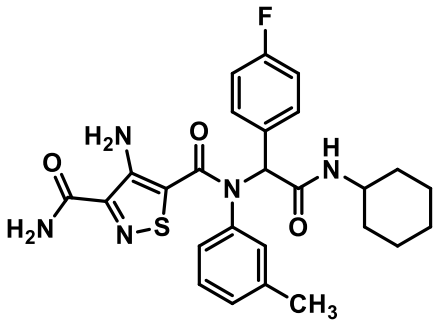
$_{tolyl}$), 128.2 (1C, C$_{m\text{-tolyl}}$), 128.5 (1C, C$_{m\text{-tolyl}}$), 131.3 (1C, C$_{m\text{-tolyl}}$), 131.5 (d, J = 3.3 Hz, 1C, C-1"$_{4\text{-fluorophenyl}}$), 132.1 (d, J = 8.4 Hz, 2C, C-2"$_{4\text{-fluorophenyl}}$, C-6"$_{4\text{-fluorophenyl}}$), 137.8 (1C, C$_{m\text{-tolyl}}$), 139.0 (1C, C-1'$_{m\text{-tolyl}}$), 161.4 (d, J = 244.0 Hz, 1C, C-4"$_{4\text{-fluorophenyl}}$), 168.3 (1C, CH*C*ONHCH), 168.8 (1C, HSCH$_2$*C*O);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3286, 3080, 2937, 2922, 2852, 2556, 1645, 1603, 1588, 1556, 1510, 1488, 1452, 1407, 1373, 1252, 1236, 1223, 1193, 1159, 1104, 1065, 979, 964, 892, 863, 835, 803, 786, 740, 707, 653, 639, 628, 580, 548, 507, 494;

HRMS (*m*/*z*): [M+H]$^+$ calcd for $C_{23}H_{28}FN_2O_2S$: 415.1850, found: 415.1843;

HPLC (method 1): t_R = 24.7 min, purity 98.9%.

4-Amino-N^5-[2-(cyclohexylamino)-1-(4-fluorophenyl)-2-oxoethyl]-N^5-(*m*-tolyl)isothiazole-3,5-dicarboxamide (52g)



$C_{26}H_{28}FN_5O_3S$

M_r = 509.6

51g (410 mg, 1.0 mmol) was added in one portion to a solution of **25** (320 mg, 1.2 mmol) in acetone (100 mL). The mixture was cooled to 0 °C and morpholine (0.17 mL, 170 mg, 1.9 mmol) was added dropwise. After stirring the reaction mixture for 12 h at ambient temperature, the solvent was removed *in vacuo*. The residue was purified by flash column chromatography (Ø = 6 cm, h = 20 cm, V = 15 mL, dichloromethane/ethyl acetate = 5/1) to give **52g** (200 mg, 0.40 mmol, 40% yield) as colorless solid.

m.p. = 257 °C;

TLC: R_f = 0.27 (dichloromethane/ethyl acetate = 5/1);

^{1}H NMR (400 MHz, DMSO-d_6): δ [ppm] = 0.88 – 1.35 (m, 5H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 1.44 – 1.83 (m, 5H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 2.17 (s br, 3H, ArCH_3), 3.52 – 3.65 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), 6.13 (s, 1H, NCHCO), 6.88 – 7.23 (m, 8H, 2'''-H$_{4\text{-fluorophenyl}}$, 3'''-H$_{4\text{-fluorophenyl}}$, 5'''-H$_{4\text{-fluorophenyl}}$, 6'''-H$_{4\text{-fluorophenyl}}$, ArNH_2, H$_{m\text{-tolyl}}$ (2H)), 7.57 (s br, 1H, CONH_2), 7.80 (s br, 1H, CONH_2), 8.06 (d, J = 7.7 Hz, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), the signals for 2 H$_{m\text{-tolyl}}$ cannot be observed in the spectrum;

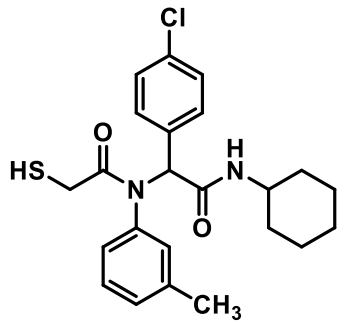
^{13}C NMR (151 MHz, DMSO-d_6): δ [ppm] = 24.5 (1C, NHCH(CH$_2$$CH_2$)$_2CH_2$), 24.7 (1C, NHCH(CH$_2$$CH_2$)$_2CH_2$), 25.2 (1C, NHCH(CH$_2CH_2$)$_2$$CH_2$), 32.3 (2C, NHCH($CH_2CH_2$)$_2CH_2$), 48.1 (1C, NH$C$H(CH$_2CH_2$)$_2CH_2$), 63.3 (1C, N$C$HCO), 114.5 (d, J = 21.8 Hz, 2C, C-3'''$_{4\text{-fluorophenyl}}$, C-5'''$_{4\text{-fluorophenyl}}$), 123.2 (1C, C-5'$_{isothiazole}$), 130.1 (1C, C$_{m\text{-tolyl}}$), 130.3 (1C, C$_{m\text{-tolyl}}$), 131.4 (d, J = 3.1 Hz, 1C, C-1'''$_{4\text{-fluorophenyl}}$), 132.6 (d, J = 8.3 Hz, 2C, C-2'''$_{4\text{-fluorophenyl}}$, C-6'''$_{4\text{-fluorophenyl}}$), 133.9 (1C, C$_{m\text{-tolyl}}$), 137.2 (1C, C-1''$_{m\text{-tolyl}}$), 146.5 (1C, C-3'$_{isothiazole}$), 151.5 (1C, C-4'$_{isothiazole}$), 161.8 (d, J = 244.9 Hz, 1C, C-4'''$_{4\text{-fluorophenyl}}$), 162.4 (1C, ArCONCH), 164.0 (1C, ArCONH$_2$), 168.5 (1C, CHCONHCH), the signals for ArCH$_3$ and 2 C$_{m\text{-tolyl}}$ cannot be observed in the spectrum;

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3450, 3279, 3080, 2936, 2852, 1675, 1647, 1604, 1561, 1510, 1489, 1449, 1404, 1369, 1348, 1252, 1236, 1224, 1193, 1158, 1103, 1073, 979, 891, 858, 835, 803, 744, 707, 665, 548, 529, 507, 464;

HRMS (m/z): [M+H]$^+$ calcd for C$_{26}$H$_{29}$FN$_5$O$_3$S: 510.1970, found: 510.1978;

HPLC (method 1): t$_R$ = 24.4 min, purity 95.1%.

2-(4-Chlorophenyl)-*N*-cyclohexyl-2-[2-mercapto-*N*-(*m*-tolyl)acetamido]acetamide (51h)



C$_{23}$H$_{27}$ClN$_2$O$_2$S

M$_r$ = 431.0

3-Methylaniline (0.22 mL, 210 mg, 2.0 mmol) and 4-chlorobenzaldehyde (280 mg, 2.0 mmol) were dissolved in dichloromethane (15 mL) and the solution was stirred at ambient temperature for 10 min. Then, sulfanylacetic acid (0.14 mL, 180 mg, 2.0 mmol) and isocyanocyclohexane (0.25 mL, 220 mg, 2.0 mmol) were added. After stirring the reaction mixture for 48 h at ambient temperature, the solvent was removed *in vacuo* and the residue was purified by flash column chromatography (Ø = 4 cm, h = 15 cm, V = 15 mL, dichloromethane/ethyl acetate = 20/1) to give **51h** (460 mg, 1.1 mmol, 53% yield) as colorless solid.

m.p. = 208 °C;

TLC: R_f = 0.37 (dichloromethane/ethyl acetate = 15/1);

^{1}H NMR (500 MHz, DMSO-d_6): δ [ppm] = 0.90 – 1.31 (m, 5H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 1.46 – 1.79 (m, 5H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 2.16 (s, 3H, ArCH_3), 2.59 (t, J = 6.8 Hz, 1H, HSCH$_2$CO), 2.98 (dd, J = 15.0/6.7 Hz, 1H, HSCH_2CO), 3.04 (dd, J = 15.0/6.9 Hz, 1H, HSCH_2CO), 3.51 – 3.61 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), 5.98 (s, 1H, NCHCO), 6. 56 (s br, 1H, H$_{m\text{-tolyl}}$), 6.93 – 7.16 (m, 4H, 2"-H$_{4\text{-chlorophenyl}}$, 6"-H$_{4\text{-chlorophenyl}}$, H$_{m\text{-tolyl}}$ (2H)), 7.16 – 7.24 (m, 2H, 3"-H$_{4\text{-chlorophenyl}}$, 5"-H$_{4\text{-chlorophenyl}}$), 7.53 (s br, 1H, H$_{m\text{-tolyl}}$), 8.01 (d, J = 7.7 Hz, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$);

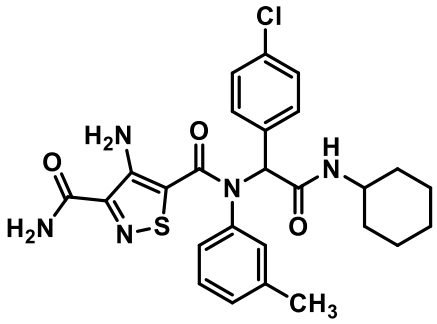
^{13}C NMR (126 MHz, DMSO-d_6): δ [ppm] = 20.7 (1C, ArCH$_3$), 24.4 (1C, NHCH(CH$_2$$CH_2$)$_2CH_2$), 24.6 (1C, NHCH(CH$_2$$CH_2$)$_2CH_2$), 25.1 (1C, NHCH(CH$_2CH_2$)$_2$$CH_2$), 27.3 (1C, HS$CH_2$CO), 32.1 (2C, NHCH($CH_2CH_2$)$_2CH_2$), 47.9 (1C, NH$C$H(CH$_2CH_2$)$_2CH_2$), 63.1 (1C, N$C$HCO), 127.70 (1C, C$_{m\text{-tolyl}}$), 127.74 (2C, C-3"$_{4\text{-chlorophenyl}}$, C-5"$_{4\text{-chlorophenyl}}$), 128.3 (1C, C$_{m\text{-tolyl}}$), 128.6 (1C, C-4'$_{m\text{-tolyl}}$), 131.3 (1C, C$_{m\text{-tolyl}}$), 131.8 (2C, C-2"$_{4\text{-chlorophenyl}}$, C-6"$_{4\text{-chlorophenyl}}$), 132.3 (1C, C-4"$_{4\text{-chlorophenyl}}$), 134.3 (1C, C-1"$_{4\text{-chlorophenyl}}$), 137.9 (1C, C-3'$_{m\text{-tolyl}}$), 138.9 (1C, C-1'$_{m\text{-tolyl}}$), 168.0 (1C, CH$C$ONHCH), 168.9 (1C, HSCH$_2$$C$O);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3292, 3058, 2933, 2850, 2552, 1645, 1602, 1589, 1550, 1495, 1487, 1449, 1408, 1380, 1328, 1283, 1251, 1233, 1192, 1151, 1104, 1093, 1019, 978, 963, 920, 892, 873, 857, 828, 802, 784, 761, 739, 725, 707, 683, 656, 632, 609, 578, 536;

HRMS (m/z): [M+H]$^+$ calcd for $C_{23}H_{28}{}^{35}ClN_2O_2S$: 431.1555, found: 431.1556;

HPLC (method 1): t_R = 22.9 min, purity 69.5%.

4-Amino-N^5-[1-(4-chlorophenyl)-2-(cyclohexylamino)-2-oxoethyl]-N^5-(*m*-tolyl)isothiazole-3,5-dicarboxamide (52h)



$C_{26}H_{28}ClN_5O_3S$

M_r = 526.1

51h (430 mg, 1.0 mmol) was added in one portion to a solution of **25** (320 mg, 1.2 mmol) in acetone (100 mL). The mixture was cooled to 0 °C and morpholine (0.17 mL, 170 mg, 1.9 mmol) was added dropwise. After stirring the reaction mixture for 12 h at ambient temperature, the solvent was removed *in vacuo*. The residue was purified by flash column chromatography (Ø = 6 cm, h = 20 cm, V = 15 mL, dichloromethane/ethyl acetate = 5/1) to give **52h** (270 mg, 0.52 mmol, 52% yield) as colorless solid.

m.p. = 269 °C;

TLC: R_f = 0.16 (dichloromethane/ethyl acetate = 5/1);

^{1}H NMR (400 MHz, DMSO-d_6): δ [ppm] = 0.91 – 1.33 (m, 5H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 1.47 – 1.82 (m, 5H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 2.19 (s br, 3H, ArCH_3), 3.53 – 3.65 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), 6.12 (s, 1H, NCHCO), 7.06 – 7.26 (m, 8H, 2'''-H$_{4\text{-chlorophenyl}}$, 3'''-H$_{4\text{-chlorophenyl}}$, 5'''-H$_{4\text{-chlorophenyl}}$, 6'''-H$_{4\text{-chlorophenyl}}$, ArNH_2, H$_{m\text{-tolyl}}$ (2H)), 7.60 (s br, 1H, CONH_2), 7.83 (s br, 1H, CONH_2), 8.09 (d, J = 7.7 Hz, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), the signals for 2 H$_{m\text{-tolyl}}$ cannot be observed in the spectrum;

^{13}C NMR (126 MHz, DMSO-d_6): δ [ppm] = 20.7 (1C, ArCH$_3$), 24.4 (1C, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), 24.6 (1C, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), 25.1 (1C, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), 32.11 (1C, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), 32.14 (1C, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), 48.0 (1C, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), 63.2 (1C, NCHCO), 122.9 (1C, C-5'$_{\text{isothiazole}}$), 127.8 (2C, C-3'''$_{4\text{-chlorophenyl}}$, C-5'''$_{4\text{-chlorophenyl}}$), 128.8 (1C, C$_{m\text{-tolyl}}$), 130.1 (1C, C$_{m\text{-tolyl}}$), 130.2 (1C, C$_{m\text{-tolyl}}$), 132.2

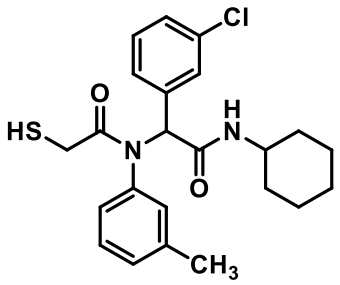
(2C, C-2'''$_{4\text{-chlorophenyl}}$, C-6'''$_{4\text{-chlorophenyl}}$), 132.5 (1C, C-4'''$_{4\text{-chlorophenyl}}$), 133.7 (1C, C$_{m\text{-tolyl}}$), 134.0 (1C, C-1'''$_{4\text{-chlorophenyl}}$), 136.9 (1C, C-1''$_{m\text{-tolyl}}$), 138.6 (1C, C$_{m\text{-tolyl}}$), 146.4 (1C, C-3'$_{\text{isothiazole}}$), 151.3 (1C, C-4'$_{\text{isothiazole}}$), 162.2 (1C, Ar*C*ONCH), 163.9 (1C, Ar*C*ONH$_2$), 168.1 (1C, CH*C*ONHCH);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3447, 3292, 2933, 2850, 1664, 1648, 1596, 1556, 1492, 1449, 1410, 1368, 1342, 1322, 1251, 1236, 1192, 1152, 1094, 1073, 1018, 977, 891, 872, 852, 824, 794, 769, 740, 727, 706, 689, 666, 592, 536, 506;

HRMS (*m*/*z*): [M+H]$^+$ calcd for $C_{26}H_{29}{}^{35}ClN_5O_3S$: 526.1674, found: 526.1690;

HPLC (method 1): t_R = 25.2 min, purity 97.9%.

2-(3-Chlorophenyl)-*N*-cyclohexyl-2-[2-mercapto-*N*-(*m*-tolyl)acetamido]acetamide (51i)



$C_{23}H_{27}ClN_2O_2S$

M_r = 431.0

3-Methylaniline (0.22 mL, 210 mg, 2.0 mmol) and 3-chlorobenzaldehyde (0.23 mL, 280 mg, 2.0 mmol) were dissolved in dichloromethane (15 mL) and the solution was stirred at ambient temperature for 10 min. Then, sulfanylacetic acid (0.14 mL, 180 mg, 2.0 mmol) and isocyanocyclohexane (0.25 mL, 220 mg, 2.0 mmol) were added. After stirring the reaction mixture for 48 h at ambient temperature, the solvent was removed *in vacuo* and the residue was purified by flash column chromatography (Ø = 4 cm, h = 15 cm, V = 15 mL, dichloromethane/ethyl acetate = 20/1) to give **51i** (450 mg, 1.0 mmol, 52% yield) as colorless solid.

m.p. = 184 °C;

TLC: R_f = 0.39 (dichloromethane/ethyl acetate = 15/1);

^{1}H NMR (500 MHz, DMSO-*d*$_6$): δ [ppm] = 0.93 – 1.31 (m, 5H, NHCH(C*H*$_2$CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$C*H*$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH$_2$)$_2$C*H*$_2$ (1H)), 1.48 – 1.78 (m, 5H, NHCH(C*H*$_2$CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$C*H*$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH$_2$)$_2$C*H*$_2$ (1H)), 2.16 (s br, 3H, ArC*H*$_3$), 2.60 (t, *J* = 6.8 Hz, 1H, *H*SCH$_2$CO), 2.99 (dd, *J* = 14.9/6.7 Hz, 1H, HSC*H*$_2$CO), 3.06 (dd, *J* = 14.9/6.9 Hz, 1H, HSC*H*$_2$CO), 3.52 – 3.61 (m, 1H, NHC*H*(CH$_2$CH$_2$)$_2$CH$_2$), 5.97 (s, 1H, NC*H*CO), 6.94 – 7.22 (m, 6H, 2''-H$_{3\text{-chlorophenyl}}$, 4''-H$_{3\text{-chlorophenyl}}$, 5''-H$_{3\text{-chlorophenyl}}$, 6''-H$_{3\text{-chlorophenyl}}$, H$_{m\text{-tolyl}}$ (2H)), 8.05 (d, *J* = 7.7 Hz, 1H, N*H*CH(CH$_2$CH$_2$)$_2$CH$_2$), the signals for 2 H$_{m\text{-tolyl}}$ cannot be observed in the spectrum;

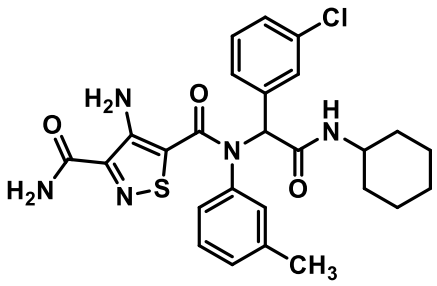
^{13}C NMR (101 MHz, DMSO-*d*$_6$): δ [ppm] = 20.7 (1C, Ar*C*H$_3$), 24.4 (1C, NHCH(CH$_2$*C*H$_2$)$_2$CH$_2$), 24.5 (1C, NHCH(CH$_2$*C*H$_2$)$_2$CH$_2$), 25.1 (1C, NHCH(CH$_2$CH$_2$)$_2$*C*H$_2$), 27.3 (1C, HS*C*H$_2$CO), 32.1 (2C, NHCH(*C*H$_2$CH$_2$)$_2$CH$_2$), 47.9 (1C, NH*C*H(CH$_2$CH$_2$)$_2$CH$_2$), 63.3 (1C, N*C*HCO), 127.6 (1C, C-4'$_{3\text{-chlorophenyl}}$), 127.7 (1C, C$_{m\text{-tolyl}}$), 128.3 (1C, C$_{m\text{-tolyl}}$), 128.6 (1C, C$_{m\text{-tolyl}}$), 128.7 (1C, C-6'$_{3\text{-chlorophenyl}}$), 129.6 (1C, C-5'$_{3\text{-chlorophenyl}}$), 129.9 (1C, C-2'$_{3\text{-chlorophenyl}}$), 131.3 (1C, C$_{m\text{-tolyl}}$), 132.3 (1C, C-3'$_{3\text{-chlorophenyl}}$), 137.7 (1C, C-1'$_{3\text{-chlorophenyl}}$), 137.9 (1C, C$_{m\text{-tolyl}}$), 138.9 (1C, C$_{m\text{-tolyl}}$), 167.8 (1C, CH*C*ONHCH), 168.9 (1C, HSCH$_2$*C*O);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3290, 3089, 2926, 2852, 2553, 1650, 1601, 1588, 1559, 1487, 1448, 1433, 1408, 1380, 1322, 1308, 1263, 1248, 1237, 1193, 1151, 1102, 1090, 1000, 978, 961, 919, 891, 860, 805, 785, 768, 741, 720, 697, 669, 576, 546;

HRMS (*m*/*z*): [M+H]$^+$ calcd for C$_{23}$H$_{28}$^{35}ClN$_2$O$_2$S: 431.1555, found: 431.1556;

HPLC (method 1): t$_R$ = 25.5 min, purity 94.0%.

4-Amino-*N*^{5}-[1-(3-chlorophenyl)-2-(cyclohexylamino)-2-oxoethyl]-*N*^{5}-(*m*-tolyl)isothiazole-3,5-dicarboxamide (52i)



C$_{26}$H$_{28}$ClN$_5$O$_3$S

M$_r$ = 526.1

51i (430 mg, 1.0 mmol) was added in one portion to a solution of **25** (320 mg, 1.2 mmol) in acetone (100 mL). The mixture was cooled to 0 °C and morpholine (0.17 mL, 170 mg, 1.9 mmol) was added dropwise. After stirring the reaction mixture for 12 h at ambient temperature, the solvent was removed *in vacuo*. The residue was purified by flash column chromatography (Ø = 6 cm, h = 20 cm, V = 15 mL, dichloromethane/ethyl acetate = 5/1) to give **52i** (220 mg, 0.42 mmol, 42% yield) as colorless solid.

m.p. = 232 °C;

TLC: R_f = 0.29 (dichloromethane/ethyl acetate = 5/1);

^{1}H NMR (600 MHz, DMSO-d_6): δ [ppm] = 0.94 – 1.04 (m, 1H, NHCH(CH_2CH$_2$)$_2$CH$_2$), 1.04 – 1.14 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$CH_2), 1.14 – 1.31 (m, 3H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (1H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H)), 1.48 – 1.80 (m, 5H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 2.15 (s br, 3H, ArCH_3), 3.50 – 3.64 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), 6.11 (s, 1H, NCHCO), 6.94 – 7.25 (m, 8H, 2'''-H$_{3\text{-chlorophenyl}}$, 4'''-H$_{3\text{-chlorophenyl}}$, 5'''-H$_{3\text{-chlorophenyl}}$, 6'''-H$_{3\text{-chlorophenyl}}$, H$_{m\text{-tolyl}}$ (2H), ArNH_2), 7.59 (s br, 1H, CONH_2), 7.82 (s br, 1H, CONH_2), 8.11 (d, J = 7.7 Hz, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), the signals for 2 H$_{m\text{-tolyl}}$ cannot be observed in the spectrum, the signals of the major rotamer are given;

^{13}C NMR (151 MHz, DMSO-d_6): δ [ppm] = 20.7 (1C, ArCH$_3$), 24.4 (1C, NHCH(CH$_2$$CH_2$)$_2CH_2$), 24.5 (1C, NHCH(CH$_2$$CH_2$)$_2CH_2$), 25.1 (1C, NHCH(CH$_2CH_2$)$_2$$CH_2$), 32.08 (1C, NHCH($CH_2CH_2$)$_2CH_2$), 32.12 (1C, NHCH($CH_2CH_2$)$_2CH_2$), 48.0 (1C, NH$C$H(CH$_2CH_2$)$_2CH_2$), 63.4 (1C, N$C$HCO), 122.9 (1C, C-5'$_{isothiazole}$), 127.6 (1C, C$_{arom.}$), 127.7 (1C, C$_{arom.}$), 128.6 (1C, C$_{arom.}$), 129.0 (1C, C$_{arom.}$), 129.6 (1C, C$_{arom.}$), 130.1 (1C, C$_{arom.}$), 130.2 (1C, C$_{arom.}$), 132.3 (1C, C$_{arom.}$), 133.7 (1C, C$_{arom.}$), 136.9 (1C, C$_{arom.}$), 137.3 (1C, C$_{arom.}$), 138.6 (1C, C$_{arom.}$), 146.4 (1C, C-3'$_{isothiazole}$), 151.3 (1C, C-4'$_{isothiazole}$), 162.2 (1C, Ar$C$ONCH), 163.9 (1C, Ar$CONH_2$),

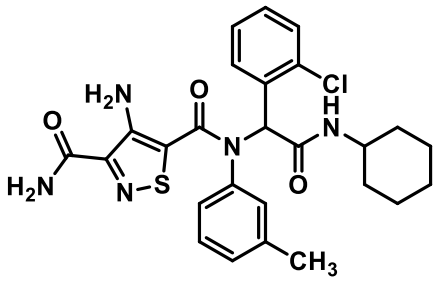
167.9 (1C, CHCONHCH);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3453, 3276, 3094, 2927, 2852, 1675, 1651, 1599, 1563, 1489, 1449, 1433, 1405, 1368, 1347, 1321, 1266, 1249, 1239, 1210, 1193, 1152, 1099, 1081, 1000, 978, 891, 788, 774, 743, 723, 679, 666, 578, 544, 497;

HRMS (m/z): [M+H]$^+$ calcd for C$_{26}$H$_{29}$^{35}ClN$_5$O$_3$S: 526.1674, found: 526.1690;

HPLC (method 1): t$_R$ = 25.2 min, purity 95.3%.

4-Amino-N^5-[1-(2-chlorophenyl)-2-(cyclohexylamino)-2-oxoethyl]-N^5-(*m*-tolyl)isothiazole-3,5-dicarboxamide (52j)



$C_{26}H_{28}ClN_5O_3S$

M_r = 526.1

3-Methylaniline (0.22 mL, 220 mg, 2.0 mmol) and 2-chlorobenzaldehyde (0.22 mL, 280 mg, 2.0 mmol) were dissolved in dichloromethane (15 mL) and the solution was stirred at ambient temperature for 10 min. Then, sulfanylacetic acid (0.14 mL, 180 mg, 2.0 mmol) and isocyanocyclohexane (0.25 mL, 220 mg, 2.0 mmol) were added. After stirring the reaction mixture for 48 h at ambient temperature, the solvent was removed *in vacuo* and the residue was washed with acetonitrile.

A share (430 mg, 66%) of the obtained crude product (650 mg) was added in one portion to a solution of **25** (320 mg, 1.2 mmol) in acetone (100 mL). The mixture was cooled to 0 °C and morpholine (0.17 mL, 170 mg, 1.9 mmol) was added dropwise. After stirring the reaction mixture for 12 h at ambient temperature, the solvent was removed *in vacuo*. The residue was purified by flash column chromatography (Ø = 6 cm, h = 20 cm, V = 15 mL, dichloromethane/ethyl acetate = 4/1) to give **52j** (410 mg, 0.77 mmol, 59% yield) as colorless solid.

m.p. = 216 °C;

TLC: R_f = 0.22 (dichloromethane/ethyl acetate = 4/1);

^{1}H NMR (600 MHz, DMSO-d_6): δ [ppm] = 0.94 – 1.13 (m, 2H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (1H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 1.15 – 1.32 (m, 3H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (1H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H)), 1.50 – 1.57 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$CH_2), 1.57 – 1.64 (m, 1H, NHCH(CH$_2$CH_2)$_2$CH$_2$), 1.66 – 1.81 (m, 3H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (1H)), 2.05 (s br, 1.5H, ArCH_3), 2.26 (s br, 1.5H, ArCH_3), 3.59 – 3.67 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), 6.47 (s, 1H, NCHCO), 6.59 – 6.73 (s br, 1H, H$_{m\text{-tolyl}}$), 6.84 –

6.95 (m, 1H, 6'''-$H_{2\text{-chlorophenyl}}$), 6.95 – 7.08 (m, 1.5H, 5'''-$H_{2\text{-chlorophenyl}}$, $H_{m\text{-tolyl}}$ (0.5H)), 7.11 – 7.15 (m, 1H, 4''-$H_{m\text{-tolyl}}$), 7.15 – 7.22 (m, 3H, ArNH_2, 4'''-$H_{2\text{-chlorophenyl}}$), 7.22 – 7.29 (m, 0.5H, $H_{m\text{-tolyl}}$), 7.37 – 7.42 (m, 1H, 3'''-$H_{2\text{-chlorophenyl}}$), 7.59 (s br, 1H, CON$H_2$), 7.70 – 7.81 (m, 1H, $H_{m\text{-tolyl}}$), 7.82 (s br, 1H, CONH_2), 8.26 (d, J = 7.7 Hz, 1H, NHCH$(CH_2CH_2)_2CH_2$);

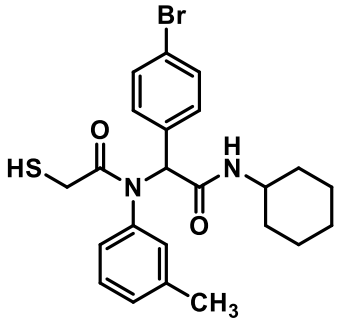
^{13}C NMR (126 MHz, DMSO-d_6): δ [ppm] = 24.4 (1C, NHCH$(CH_2CH_2)_2CH_2$), 24.6 (1C, NHCH$(CH_2CH_2)_2CH_2$), 25.1 (1C, NHCH$(CH_2CH_2)_2CH_2$), 32.08 (1C, NHCH$(CH_2CH_2)_2CH_2$), 32.15 (1C, NHCH$(CH_2CH_2)_2CH_2$), 48.1 (1C, NHCH$(CH_2CH_2)_2CH_2$), 61.0 (1C, NCHCO), 122.6 (1C, C-5'$_{\text{isothiazole}}$), 126.8 (1C, C-5'''$_{\text{2-chlorophenyl}}$), 129.0 (1C, C-3'''$_{\text{2-chlorophenyl}}$), 129.8 (1C, C-4'''$_{\text{2-chlorophenyl}}$), 130.4 (1C, C-4''$_{m\text{-tolyl}}$), 131.2 (1C, C-6'''$_{\text{2-chlorophenyl}}$), 133.2 (1C, C-1'''$_{\text{2-chlorophenyl}}$), 134.8 (1C, C-2'''$_{\text{2-chlorophenyl}}$), 136.8 (1C, C-1''$_{m\text{-tolyl}}$), 146.5 (1C, C-3'$_{\text{isothiazole}}$), 151.3 (1C, C-4'$_{\text{isothiazole}}$), 162.2 (1C, ArCONCH), 163.9 (1C, ArCONH$_2$), 167.8 (1C, CHCONHCH), the signals for ArCH$_3$ and 4 $C_{m\text{-tolyl}}$ cannot be observed in the spectrum;

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3473, 3399, 3359, 3179, 2928, 2853, 1678, 1622, 1580, 1561, 1511, 1488, 1448, 1401, 1375, 1339, 1330, 1317, 1275, 1248, 1219, 1188, 1131, 1070, 1052, 1036, 802, 768, 740, 708, 682, 638, 529, 504;

HRMS (m/z): [M+H]$^+$ calcd for $C_{26}H_{29}{}^{35}ClN_5O_3S$: 526.1674, found: 526.1690;

HPLC (method 1): t_R = 24.9 min, purity 98.5%.

2-(4-Bromophenyl)-*N*-cyclohexyl-2-[2-mercapto-*N*-(*m*-tolyl)acetamido]acetamide (51k)



$C_{23}H_{27}BrN_2O_2S$

M_r = 475.4

3-Methylaniline (0.22 mL, 210 mg, 2.0 mmol) and 4-bromobenzaldehyde (370 mg, 2.0 mmol) were dissolved in dichloromethane (15 mL) and the solution was stirred at ambient temperature for 10 min. Then, sulfanylacetic acid (0.14 mL, 180 mg, 2.0 mmol) and isocyanocyclohexane

(0.25 mL, 220 mg, 2.0 mmol) were added. After stirring the reaction mixture for 48 h at ambient temperature, the solvent was removed *in vacuo* and the residue was purified by flash column chromatography (Ø = 4 cm, h = 15 cm, V = 15 mL, dichloromethane/ethyl acetate = 18/1) to give **51k** (510 mg, 1.1 mmol, 54% yield) as colorless solid.

m.p. = 196 °C;

TLC: R_f = 0.33 (dichloromethane/ethyl acetate = 15/1);

^{1}H NMR (400 MHz, DMSO-d_6): δ [ppm] = 0.91 – 1.32 (m, 5H, NHCH(C*H*$_2$CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$C*H*$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH$_2$)$_2$C*H*$_2$ (1H)), 1.48 – 1.78 (m, 5H, NHCH(C*H*$_2$CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$C*H*$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH$_2$)$_2$C*H*$_2$ (1H)), 2.17 (s br, 3H, ArC*H*$_3$), 2.58 (t, *J* = 6.9 Hz, 1H, *H*SCH$_2$CO), 2.98 (dd, *J* = 14.9/6.8 Hz, 1H, HSC*H*$_2$CO), 3.04 (dd, *J* = 14.9/7.0 Hz, 1H, HSC*H*$_2$CO), 3.51 – 3.62 (m, 1H, NHC*H*(CH$_2$CH$_2$)$_2$CH$_2$), 5.96 (s, 1H, NC*H*CO), 6.95 – 7.03 (m, 3H, 2"-H$_{4\text{-bromophenyl}}$, 6"-H$_{4\text{-bromophenyl}}$, H$_{m\text{-tolyl}}$ (1H)), 7.07 (s br, 1H, H$_{m\text{-tolyl}}$), 7.30 – 7.37 (m, 2H, 3"-H$_{4\text{-bromophenyl}}$, 5"-H$_{4\text{-bromophenyl}}$), 7.98 (d, *J* = 7.7 Hz, 1H, N*H*CH(CH$_2$CH$_2$)$_2$CH$_2$), the signals for 2 H$_{m\text{-tolyl}}$ cannot be observed in the spectrum;

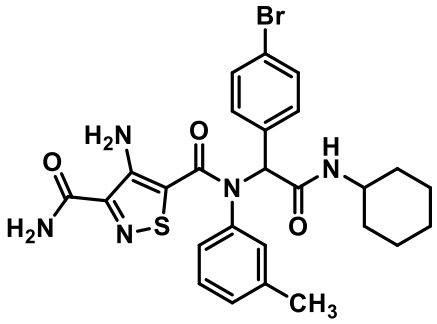
^{13}C NMR (151 MHz, DMSO-d_6): δ [ppm] = 24.4 (1C, NHCH(CH$_2$*C*H$_2$)$_2$CH$_2$), 24.6 (1C, NHCH(CH$_2$*C*H$_2$)$_2$CH$_2$), 25.1 (1C, NHCH(CH$_2$CH$_2$)$_2$*C*H$_2$), 27.3 (1C, HS*C*H$_2$CO), 32.1 (2C, NHCH(*C*H$_2$CH$_2$)$_2$CH$_2$), 47.9 (1C, NH*C*H(CH$_2$CH$_2$)$_2$CH$_2$), 63.2 (1C, N*C*HCO), 121.0 (1C, C-4"$_{4\text{-bromophenyl}}$), 127.7 (1C, C$_{m\text{-tolyl}}$), 128.3 (1C, C$_{m\text{-tolyl}}$), 128.6 (1C, C$_{m\text{-tolyl}}$), 130.7 (2C, C-3"$_{4\text{-bromophenyl}}$, C-5"$_{4\text{-bromophenyl}}$), 131.3 (1C, C$_{m\text{-tolyl}}$), 132.2 (2C, C-2"$_{4\text{-bromophenyl}}$, C-6"$_{4\text{-bromophenyl}}$), 134.8 (1C, C-1"$_{4\text{-bromophenyl}}$), 167.9 (1C, CH*C*ONHCH), 168.9 (1C, HSCH$_2$*C*O), the signals for Ar*C*H$_3$ and 2 C$_{m\text{-tolyl}}$ cannot be observed in the spectrum;

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3297, 3058, 2931, 2849, 2549, 1646, 1603, 1589, 1548, 1491, 1449, 1407, 1380, 1325, 1282, 1252, 1233, 1191, 1151, 1104, 1091, 1078, 1014, 978, 963, 891, 873, 856, 823, 801, 783, 757, 736, 718, 707, 672, 649, 595, 577, 531;

HRMS (*m*/*z*): [M+H]$^+$ calcd for C$_{23}$H$_{28}$^{79}BrN$_2$O$_2$S: 475.1049, found: 475.1057;

HPLC (method 1): t_R = 25.8 min, purity 99.4%.

4-Amino-N^5-[1-(4-bromophenyl)-2-(cyclohexylamino)-2-oxoethyl]-N^5-(*m*-tolyl)isothiazole-3,5-dicarboxamide (52k)



$C_{26}H_{28}BrN_5O_3S$

$M_r = 570.5$

51k (480 mg, 1.0 mmol) was added in one portion to a solution of **25** (320 mg, 1.2 mmol) in acetone (100 mL). The mixture was cooled to 0 °C and morpholine (0.17 mL, 170 mg, 1.9 mmol) was added dropwise. After stirring the reaction mixture for 12 h at ambient temperature, the solvent was removed *in vacuo*. The residue was purified by flash column chromatography (Ø = 6 cm, h = 20 cm, V = 15 mL, dichloromethane/ethyl acetate = 7/1) to give **52k** (430 mg, 0.75 mmol, 75% yield) as colorless solid.

m.p. = 253 °C;

TLC: R_f = 0.30 (dichloromethane/ethyl acetate = 5/1);

^{1}H NMR (400 MHz, DMSO-d_6): δ [ppm] = 0.91 – 1.33 (m, 5H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 1.46 – 1.81 (m, 5H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 2.19 (s br, 3H, ArCH_3), 3.52 – 3.66 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), 6.10 (s, 1H, NCHCO), 6.63 (s br, 1H, H$_{m\text{-tolyl}}$), 7.02 – 7.08 (m, 2H, 2'''-H$_{4\text{-bromophenyl}}$, 6'''-H$_{4\text{-bromophenyl}}$), 7.08 – 7.30 (m, 4H, ArNH_2, H$_{m\text{-tolyl}}$ (2H)), 7.32 – 7.37 (m, 2H, 3'''-H$_{4\text{-bromophenyl}}$, 5'''-H$_{4\text{-bromophenyl}}$), 7.42 – 7.78 (m, 2H, H$_{m\text{-tolyl}}$ (1H), CONH_2 (1H)), 7.83 (s br, 1H, CONH_2), 8.09 (d, J = 7.7 Hz, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$);

^{13}C NMR (101 MHz, DMSO-d_6): δ [ppm] = 20.7 (1C, ArCH$_3$), 24.4 (1C, NHCH(CH$_2$$CH_2$)$_2CH_2$), 24.6 (1C, NHCH(CH$_2$$CH_2$)$_2CH_2$), 25.2 (1C, NHCH(CH$_2CH_2$)$_2$$CH_2$), 32.1 (1C, NHCH($CH_2CH_2$)$_2CH_2$), 32.2 (1C, NHCH($CH_2CH_2$)$_2CH_2$), 48.0 (1C, NH$C$H(CH$_2CH_2$)$_2CH_2$), 63.3 (1C, N$C$HCO), 121.2 (1C, C-4'''$_{4\text{-bromophenyl}}$), 122.9 (1C, C-5'$_{isothiazole}$), 128.9 (1C, C$_{m\text{-tolyl}}$), 130.2 (1C, C$_{m\text{-tolyl}}$), 130.3 (1C, C$_{m\text{-tolyl}}$), 130.8 (2C, C-3'''$_{4\text{-}}$

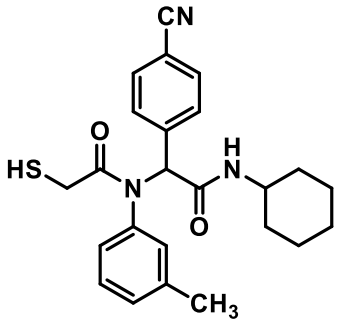
bromophenyl, C-5'''4-bromophenyl), 132.5 (2C, C-2'''4-bromophenyl, C-6'''4-bromophenyl), 133.7 (1C, C*m*-tolyl), 134.4 (1C, C-1'''4-bromophenyl), 136.9 (1C, C*m*-tolyl), 138.6 (1C, C*m*-tolyl), 146.4 (1C, C-3'isothiazole), 151.3 (1C, C-4'isothiazole), 162.3 (1C, Ar*C*ONCH), 163.9 (1C, Ar*C*ONH$_2$), 168.1 (1C, CH*C*ONHCH);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3449, 3291, 2931, 2849, 1675, 1666, 1649, 1611, 1557, 1491, 1448, 1406, 1369, 1345, 1322, 1252, 1237, 1192, 1152, 1103, 1074, 1014, 978, 820, 799, 768, 737, 720, 705, 688, 671, 589, 534;

HRMS (*m*/*z*): [M+H]$^+$ calcd for $C_{26}H_{29}{}^{79}BrN_5O_3S$: 570.1169, found: 570.1159;

HPLC (method 1): t_R = 25.5 min, purity 96.3%.

2-(4-Cyanophenyl)-*N*-cyclohexyl-2-[2-mercapto-*N*-(*m*-tolyl)acetamido]acetamide (51l)



$C_{24}H_{27}N_3O_2S$

M_r = 421.6

3-Methylaniline (0.22 mL, 210 mg, 2.0 mmol) and 4-formylbenzonitrile (260 mg, 2.0 mmol) were dissolved in dichloromethane (15 mL) and the solution was stirred at ambient temperature for 10 min. Then, sulfanylacetic acid (0.14 mL, 180 mg, 2.0 mmol) and isocyanocyclohexane (0.25 mL, 220 mg, 2.0 mmol) were added. After stirring the reaction mixture for 48 h at ambient temperature, the solvent was removed *in vacuo* and the residue was purified by flash column chromatography (Ø = 4 cm, h = 15 cm, V = 15 mL, dichloromethane/ethyl acetate = 18/1) to give **51l** (470 mg, 1.1 mmol, 56% yield) as colorless solid.

m.p. = 211 °C;

TLC: R_f = 0.31 (dichloromethane/ethyl acetate = 15/1);

^{1}H NMR (400 MHz, DMSO-*d*$_6$): δ [ppm] = 0.93 – 1.32 (m, 5H, NHCH(C*H*$_2$CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$C*H*$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH$_2$)$_2$C*H*$_2$ (1H)), 1.47 – 1.77 (m, 5H, NHCH(C*H*$_2$CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$C*H*$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH$_2$)$_2$C*H*$_2$ (1H)), 2.16 (s br, 3H, ArC*H*$_3$), 2.59 (t, *J* = 6.9 Hz, 1H, *H*SCH$_2$CO), 3.01 (dd, *J* = 14.9/6.8 Hz, 1H, HSC*H*$_2$CO), 3.07 (dd, *J* = 14.9/7.0 Hz, 1H, HSC*H*$_2$CO), 3.49 – 3.62 (m, 1H, NHC*H*(CH$_2$CH$_2$)$_2$CH$_2$), 6.05 (s, 1H, NC*H*CO), 6.97 – 7.15 (m, 2H, H$_{m\text{-tolyl}}$), 7.23 – 7.30 (m, 2H, 2"-H$_{4\text{-cyanophenyl}}$, 6"-H$_{4\text{-cyanophenyl}}$), 7.60 – 7.66 (m, 2H, 3"-H$_{4\text{-cyanophenyl}}$, 5"-H$_{4\text{-cyanophenyl}}$), 8.07 (d, *J* = 7.7 Hz, 1H, N*H*CH(CH$_2$CH$_2$)$_2$CH$_2$), the signals for 2 H$_{m\text{-tolyl}}$ cannot be observed in the spectrum;

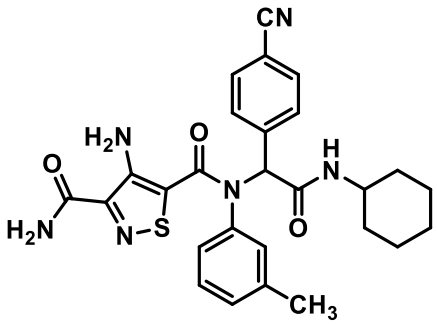
^{13}C NMR (126 MHz, DMSO-*d*$_6$): δ [ppm] = 20.6 (1C, Ar*C*H$_3$), 24.4 (1C, NHCH(CH$_2$*C*H$_2$)$_2$CH$_2$), 24.5 (1C, NHCH(CH$_2$*C*H$_2$)$_2$CH$_2$), 25.1 (1C, NHCH(CH$_2$CH$_2$)$_2$*C*H$_2$), 27.2 (1C, HS*C*H$_2$CO), 32.1 (2C, NHCH(*C*H$_2$CH$_2$)$_2$CH$_2$), 47.9 (1C, NH*C*H(CH$_2$CH$_2$)$_2$CH$_2$), 63.6 (1C, N*C*HCO), 110.3 (1C, C-4"$_{4\text{-cyanophenyl}}$), 118.4 (1C, Ar*C*≡N), 127.5 (1C, C$_{m\text{-tolyl}}$), 128.4 (1C, C$_{m\text{-tolyl}}$), 128.7 (1C, C$_{m\text{-tolyl}}$), 130.9 (2C, C-2"$_{4\text{-cyanophenyl}}$, C-6"$_{4\text{-cyanophenyl}}$), 131.1 (1C, C$_{m\text{-tolyl}}$), 131.6 (2C, C-3"$_{4\text{-cyanophenyl}}$, C-5"$_{4\text{-cyanophenyl}}$), 138.1 (1C, C$_{m\text{-tolyl}}$), 138.8 (1C, C$_{m\text{-tolyl}}$), 141.1 (1C, C-1"$_{4\text{-cyanophenyl}}$), 167.3 (1C, CH*C*ONHCH), 169.0 (1C, HSCH$_2$*C*O);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3294, 3081, 2927, 2852, 2549, 2232, 1647, 1603, 1589, 1553, 1506, 1487, 1450, 1408,1381, 1330, 1311, 1250, 1232, 1192, 1152, 1100, 1065, 1025, 977, 960, 892, 874, 860, 836, 803, 779, 740, 710, 666, 645, 624, 577, 563;

HRMS (*m*/*z*): [M+H]$^+$ calcd for C$_{24}$H$_{28}$N$_3$O$_2$S: 422.1897, found: 422.1889;

HPLC (method 1): t$_R$ = 23.8 min, purity 94.4%.

4-Amino-*N*^{5}-[1-(4-cyanophenyl)-2-(cyclohexylamino)-2-oxoethyl]-*N*^{5}-(*m*-tolyl)isothiazole-3,5-dicarboxamide (52l)



C$_{27}$H$_{28}$N$_6$O$_3$S

M$_r$ = 516.6

51l (420 mg, 1.0 mmol) was added in one portion to a solution of **25** (320 mg, 1.2 mmol) in acetone (100 mL). The mixture was cooled to 0 °C and morpholine (0.17 mL, 170 mg, 1.9 mmol) was added dropwise. After stirring the reaction mixture for 12 h at ambient temperature, the solvent was removed *in vacuo*. The residue was purified by flash column chromatography (Ø = 6 cm, h = 20 cm, V = 15 mL, dichloromethane/acetone = 10/1) to give **52l** (280 mg, 0.54 mmol, 54% yield) as colorless solid.

m.p. = 257 °C;

TLC: R_f = 0.24 (dichloromethane/acetone = 10/1);

^{1}H NMR (500 MHz, DMSO-d_6): δ [ppm] = 0.92 – 1.31 (m, 5H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 1.48 – 1.80 (m, 5H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 2.19 (s br, 3H, ArCH_3), 3.53 – 3.64 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), 6.19 (s, 1H, NCHCO), 6.69 (s br, 1H, H$_{m\text{-tolyl}}$), 6.96 – 7.30 (m, 4H, ArNH_2, H$_{m\text{-tolyl}}$ (2H)), 7.30 – 7.35 (m, 2H, 2'''-H$_{4\text{-cyanophenyl}}$, 6'''-H$_{4\text{-cyanophenyl}}$), 7.60 (s br, 1H, CONH_2), 7.62 – 7.66 (m, 2H, 3'''-H$_{4\text{-cyanophenyl}}$, 5'''-H$_{4\text{-cyanophenyl}}$), 7.83 (s br, 1H, CONH_2), 8.17 (d, J = 7.7 Hz, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), the signal for 1 H$_{m\text{-tolyl}}$ cannot be observed in the spectrum;

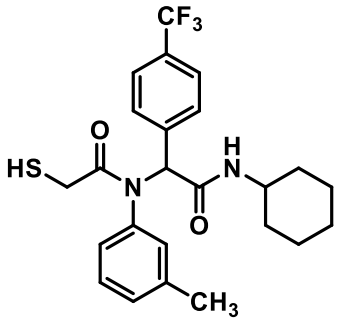
^{13}C NMR (126 MHz, DMSO-d_6): δ [ppm] = 20.6 (1C, ArCH$_3$), 24.4 (1C, NHCH(CH$_2$$CH_2$)$_2CH_2$), 24.5 (1C, NHCH(CH$_2$$CH_2$)$_2CH_2$), 25.1 (1C, NHCH(CH$_2CH_2$)$_2$$CH_2$), 32.1 (2C, NHCH($CH_2CH_2$)$_2CH_2$), 48.0 (1C, NH$C$H(CH$_2CH_2$)$_2CH_2$), 63.7 (1C, N$C$HCO), 110.5 (1C, C-4'''$_{4\text{-cyanophenyl}}$), 118.4 (1C, Ar$C$≡N), 122.6 (1C, C-5'$_{isothiazole}$), 130.0 (1C, C$_{m\text{-tolyl}}$), 130.4 (1C, C$_{m\text{-tolyl}}$), 131.2 (2C, C-2"$_{4\text{-cyanophenyl}}$, C-6"$_{4\text{-cyanophenyl}}$), 131.7 (2C, C-3"$_{4\text{-cyanophenyl}}$, C-5"$_{4\text{-cyanophenyl}}$), 133.6 (1C, C$_{m\text{-tolyl}}$), 136.8 (1C, C-1"$_{m\text{-tolyl}}$), 140.7 (1C, C-1"$_{4\text{-cyanophenyl}}$), 146.4 (1C, C-3'$_{isothiazole}$), 151.3 (1C, C-4'$_{isothiazole}$), 162.3 (1C, Ar$C$ONCH), 163.8 (1C, Ar$CONH_2$), 167.5 (1C, CH$C$ONHCH), the signals for 2 C$_{m\text{-tolyl}}$ cannot be observed in the spectrum;

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3476, 34478 3296, 2926, 2852, 2232, 1651, 1611, 1558, 1490, 1448, 1398, 1368, 1343, 1326, 1251, 1236, 1192, 1152, 1099, 1073, 976, 892, 845, 831, 793, 744, 708, 667, 563, 530;

HRMS (m/z): [M+H]$^+$ calcd for $C_{27}H_{29}N_6O_3S$: 517.2016, found: 517.2027;

HPLC (method 1): t_R = 23.5 min, purity 95.9%.

_N_-Cyclohexyl-2-[2-mercapto-_N_-(_m_-tolyl)acetamido]-2-[4-(trifluoromethyl)phenyl]acetamide (51m)



$C_{24}H_{27}F_3N_2O_2S$

$M_r = 464.5$

3-Methylaniline (0.22 mL, 210 mg, 2.0 mmol) and 4-(trifluoromethyl)benzaldehyde (0.27 mL, 350 mg, 2.0 mmol) were dissolved in dichloromethane (15 mL) and the solution was stirred at ambient temperature for 10 min. Then, sulfanylacetic acid (0.14 mL, 180 mg, 2.0 mmol) and isocyanocyclohexane (0.25 mL, 220 mg, 2.0 mmol) were added. After stirring the reaction mixture for 48 h at ambient temperature, the solvent was removed *in vacuo* and the residue was purified by flash column chromatography (Ø = 2 cm, h = 15 cm, V = 5 mL, petroleum ether/ethyl acetate = 3/1) to give **51m** (360 mg, 0.77 mmol, 39% yield) as colorless solid.

m.p. = 209 °C;

TLC: R_f = 0.24 (petroleum ether/ethyl acetate = 3/1);

^{1}H NMR (400 MHz, DMSO-d_6): δ [ppm] = 0.92 – 1.32 (m, 5H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 1.47 – 1.78 (m, 5H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 2.13 (s br, 3H, ArCH_3), 2.61 (t, J = 6.8 Hz, 1H, HSCH$_2$CO), 2.97 – 3.10 (m, 2H, HSCH_2CO), 3.49 – 3.63 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), 6.08 (s, 1H, NCHCO), 6.95 – 7.02 (m, 1H, 4'-H$_{m\text{-tolyl}}$), 7.06 (s br, 1H, H$_{m\text{-tolyl}}$), 7.24 – 7.30 (m, 2H, 2"-H$_{4\text{-(trifluoromethyl)phenyl}}$, 6"-H$_{4\text{-(trifluoromethyl)phenyl}}$), 7.48 – 7.55 (m, 2H, 3"-H$_{4\text{-(trifluoromethyl)phenyl}}$, 5"-H$_{4\text{-(trifluoromethyl)phenyl}}$), 8.07 (d, J = 7.7 Hz, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), the signals for 2 H$_{m\text{-tolyl}}$ cannot be observed in the spectrum;

^{13}C NMR (126 MHz, DMSO-d_6): δ [ppm] = 20.6 (1C, ArCH$_3$), 24.4 (1C, NHCH(CH$_2$$CH_2$)$_2CH_2$), 24.6 (1C, NHCH(CH$_2$$CH_2$)$_2CH_2$), 25.1 (1C, NHCH(CH$_2CH_2$)$_2$$CH_2$), 27.2 (1C, HS$CH_2$CO), 32.1 (2C, NHCH($CH_2CH_2$)$_2CH_2$), 48.0 (1C, NH$C$H(CH$_2CH_2$)$_2CH_2$),

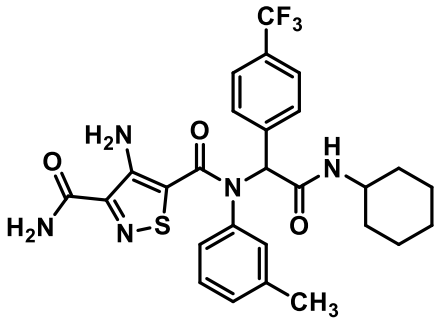
63.4 (1C, N*C*HCO), 124.0 (q, *J* = 272.5 Hz, 1C, Ar*C*F_3), 124.6 (2C, C-3"$_{4\text{-(trifluoromethyl)phenyl}}$, C-5"$_{4\text{-(trifluoromethyl)phenyl}}$), 127.7 (1C, C$_{m\text{-tolyl}}$), 128.1 (q, *J* = 31.1 Hz, 1C, C-4"$_{4\text{-(trifluoromethyl)phenyl}}$), 128.3 (1C, C$_{m\text{-tolyl}}$), 128.6 (1C, C-4'$_{m\text{-tolyl}}$), 130.8 (2C, C-2"$_{4\text{-(trifluoromethyl)phenyl}}$, C-6"$_{4\text{-(trifluoromethyl)phenyl}}$), 131.2 (1C, C$_{m\text{-tolyl}}$), 137.9 (1C, C-3'$_{m\text{-tolyl}}$), 138.8 (1C, C-1'$_{m\text{-tolyl}}$), 140.2 (1C, C-1"$_{4\text{-(trifluoromethyl)phenyl}}$), 167.6 (1C, CH*C*ONHCH), 169.1 (1C, HSCH$_2$*C*O);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3277, 3080, 2931, 2856, 1650, 1619, 1605, 1587, 1556, 1488, 1453, 1419, 1378, 1357, 1322, 1251, 1236, 1191, 1164, 1125, 1112, 1068, 1020, 978, 891, 876, 862, 844, 835, 805, 779, 756, 707, 676, 602, 578, 530;

HRMS (*m*/*z*): [M+H]$^+$ calcd for $C_{24}H_{28}F_3N_2O_2S$: 465.1818, found: 465.1822;

HPLC (method 1): t_R = 26.0 min, purity 93.2%.

4-Amino-*N*^{5}-{2-(cyclohexylamino)-2-oxo-1-[4-(trifluoromethyl)phenyl]ethyl}-*N*^{5}-(*m*-tolyl)isothiazole-3,5-dicarboxamide (52m)



$C_{27}H_{28}F_3N_5O_3S$

M_r = 559.6

51m (470 mg, 1.0 mmol) was added in one portion to a solution of **25** (320 mg, 1.2 mmol) in acetone (100 mL). The mixture was cooled to 0 °C and morpholine (0.17 mL, 170 mg, 1.9 mmol) was added dropwise. After stirring the reaction mixture for 12 h at ambient temperature, the solvent was removed *in vacuo*. The residue was purified by flash column chromatography (Ø = 6 cm, h = 20 cm, V = 15 mL, dichloromethane/methanol = 50/1) to give **52m** (270 mg, 0.48 mmol, 48% yield) as colorless solid.

m.p. = 241 °C;

TLC: R_f = 0.27 (dichloromethane/methanol = 40/1);

^{1}H NMR (500 MHz, DMSO-*d*$_6$): δ [ppm] = 0.92 – 1.13 (m, 2H, NHCH(C*H*$_2$CH$_2$)$_2$CH$_2$ (1H), NHCH(CH$_2$CH$_2$)$_2$C*H*$_2$ (1H)), 1.13 – 1.32 (m, 3H, NHCH(C*H*$_2$CH$_2$)$_2$CH$_2$ (1H), NHCH(CH$_2$C*H*$_2$)$_2$CH$_2$ (2H)), 1.46 – 1.63 (m, 2H, NHCH(CH$_2$C*H*$_2$)$_2$CH$_2$ (1H), NHCH(CH$_2$CH$_2$)$_2$C*H*$_2$ (1H)), 1.63 – 1.73 (m, 2H, NHCH(C*H*$_2$CH$_2$)$_2$CH$_2$ (1H), NHCH(CH$_2$C*H*$_2$)$_2$CH$_2$ (1H)), 1.73 – 1.81 (m, 1H, NHCH(C*H*$_2$CH$_2$)$_2$CH$_2$), 2.16 (s br, 3H, ArC*H*$_3$), 3.54 – 3.65 (m, 1H, NHC*H*(CH$_2$CH$_2$)$_2$CH$_2$), 6.22 (s, 1H, NC*H*CO), 6.64 (s br, 1H, H$_{m\text{-tolyl}}$), 7.13 – 7.18 (m, 1H, 4''-H$_{m\text{-tolyl}}$), 7.19 (s br, 2H, ArN*H*$_2$), 7.31 – 7.36 (m, 2H, 2'''-H$_{4\text{-(trifluoromethyl)phenyl}}$, 6'''-H$_{4\text{-(trifluoromethyl)phenyl}}$), 7.50 – 7.55 (m, 2H, 3'''-H$_{4\text{-(trifluoromethyl)phenyl}}$, 5'''-H$_{4\text{-(trifluoromethyl)phenyl}}$), 7.59 (s br, 1H, CON*H*$_2$), 7.82 (s br, 1H, CON*H*$_2$), 8.15 (d, *J* = 7.7 Hz, 1H, N*H*CH(CH$_2$CH$_2$)$_2$CH$_2$), the signals for 2 H$_{m\text{-tolyl}}$ cannot be observed in the spectrum;

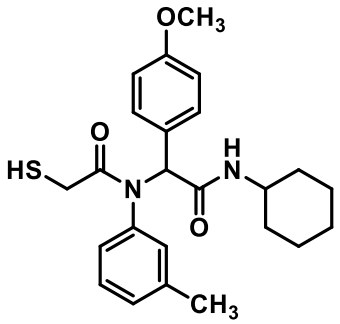
^{13}C NMR (101 MHz, DMSO-*d*$_6$): δ [ppm] = 20.5 (1C, Ar*C*H$_3$), 24.4 (1C, NHCH(CH$_2$*C*H$_2$)$_2$CH$_2$), 24.6 (1C, NHCH(CH$_2$*C*H$_2$)$_2$CH$_2$), 25.1 (1C, NHCH(CH$_2$CH$_2$)$_2$*C*H$_2$), 32.09 (1C, NHCH(*C*H$_2$CH$_2$)$_2$CH$_2$), 32.13 (1C, NHCH(*C*H$_2$CH$_2$)$_2$CH$_2$), 48.0 (1C, NH*C*H(CH$_2$CH$_2$)$_2$CH$_2$), 63.5 (1C, N*C*HCO), 122.7 (1C, C-5'$_{\text{isothiazole}}$), 124.0 (q, *J* = 272.4 Hz, 1C, Ar*C*F$_3$), 124.6 (2C, C-3'''$_{\text{4-(trifluoromethyl)phenyl}}$, C-5'''$_{\text{4-(trifluoromethyl)phenyl}}$), 128.2 (q, *J* = 31.7 Hz, 1C, C-4'''$_{\text{4-(trifluoromethyl)phenyl}}$), 128.8 (1C, C$_{m\text{-tolyl}}$), 130.1 (1C, C$_{m\text{-tolyl}}$), 130.3 (1C, C$_{m\text{-tolyl}}$), 131.1 (2C, C-2'''$_{\text{4-(trifluoromethyl)phenyl}}$, C-6'''$_{\text{4-(trifluoromethyl)phenyl}}$), 133.6 (1C, C$_{m\text{-tolyl}}$), 136.8 (1C, C-1''$_{m\text{-tolyl}}$), 139.8 (1C, C-1'''$_{\text{4-(trifluoromethyl)phenyl}}$), 146.5 (1C, C-3'$_{\text{isothiazole}}$), 151.3 (1C, C-4'$_{\text{isothiazole}}$), 162.3 (1C, Ar*C*ONCH), 163.9 (1C, Ar*C*ONH$_2$), 167.7 (1C, CH*C*ONHCH), the signal for 1 C$_{m\text{-tolyl}}$ cannot be observed in the spectrum;

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3448, 3290, 2928, 2853, 1667, 1651, 1600, 1560, 1489, 1451, 1421, 1369, 1347, 1325, 1253, 1238, 1193, 1163, 1118, 1069, 1021, 978, 891, 874, 835, 793, 755, 709, 691, 667, 585, 533, 504;

HRMS (*m*/*z*): [M+H]$^+$ calcd for C$_{27}$H$_{29}$F$_3$N$_5$O$_3$S: 560.1938, found: 560.1935;

HPLC (method 1): t$_R$ = 25.7 min, purity 96.5%.

_N_-Cyclohexyl-2-[2-mercapto-_N_-(_m_-tolyl)acetamido]-2-(4-methoxyphenyl)acetamide (51n)



$C_{24}H_{30}N_2O_3S$

$M_r = 426.6$

3-Methylaniline (0.22 mL, 210 mg, 2.0 mmol) and 4-methoxybenzaldehyde (0.24 mL, 270 mg, 2.0 mmol) were dissolved in dichloromethane (15 mL) and the solution was stirred at ambient temperature for 10 min. Then, sulfanylacetic acid (0.14 mL, 180 mg, 2.0 mmol) and isocyanocyclohexane (0.25 mL, 220 mg, 2.0 mmol) were added. After stirring the reaction mixture for 48 h at ambient temperature, the solvent was removed *in vacuo* and the residue was purified by flash column chromatography (Ø = 4 cm, h = 15 cm, V = 15 mL, dichloromethane/ethyl acetate = 25/1) to give **51n** (450 mg, 1.1 mmol, 53% yield) as colorless solid.

m.p. = 164 °C;

TLC: R_f = 0.41 (dichloromethane/ethyl acetate = 15/1);

^{1}H NMR (500 MHz, DMSO-*d*$_6$): δ [ppm] = 0.91 – 1.02 (m, 1H, NHCH(C*H*$_2$CH$_2$)$_2$CH$_2$), 1.02 – 1.13 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$C*H*$_2$), 1.13 – 1.31 (m, 3H, NHCH(C*H*$_2$CH$_2$)$_2$CH$_2$ (1H), NHCH(CH$_2$C*H*$_2$)$_2$CH$_2$ (2H)), 1.48 – 1.79 (m, 5H, NHCH(C*H*$_2$CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$C*H*$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH$_2$)$_2$C*H*$_2$ (1H)), 2.16 (s br, 3H, ArC*H*$_3$), 2.56 (t, *J* = 6.8 Hz, 1H, *H*SCH$_2$CO), 2.96 (dd, *J* = 14.9/6.7 Hz, 1H, HSC*H*$_2$CO), 3.02 (dd, *J* = 14.9/6.9 Hz, 1H, HSC*H*$_2$CO), 3.52 – 3.61 (m, 1H, NHC*H*(CH$_2$CH$_2$)$_2$CH$_2$), 3.63 (s, 3H, ArOC*H*$_3$), 5.94 (s, 1H, NC*H*CO), 6.47 (s br, 1H, H$_{m\text{-tolyl}}$), 6.63 – 6.71 (m, 2H, 3"-H$_{4\text{-methoxyphenyl}}$, 5"-H$_{4\text{-methoxyphenyl}}$), 6.89 – 6.95 (m, 2H, 2"-H$_{4\text{-methoxyphenyl}}$, 6"-H$_{4\text{-methoxyphenyl}}$), 6.95 – 6.99 (m, 1H, 4'-H$_{m\text{-tolyl}}$), 7.02 (s br, 1H, H$_{m\text{-tolyl}}$), 7.58 (s br, 1H, H$_{m\text{-tolyl}}$), 7.88 (d, *J* = 7.7 Hz, 1H, N*H*CH(CH$_2$CH$_2$)$_2$CH$_2$);

^{13}C NMR (101 MHz, DMSO-*d*$_6$): δ [ppm] = 20.7 (1C, Ar*C*H$_3$), 24.5 (1C, NHCH(CH$_2$*C*H$_2$)$_2$CH$_2$), 24.7 (1C, NHCH(CH$_2$*C*H$_2$)$_2$CH$_2$), 25.2 (1C, NHCH(CH$_2$CH$_2$)$_2$*C*H$_2$),

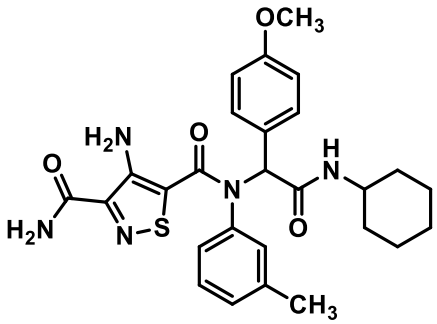
27.4 (1C, HS*C*H$_2$CO), 32.2 (1C, NHCH(*C*H$_2$CH$_2$)$_2$CH$_2$), 32.3 (1C, NHCH(*C*H$_2$CH$_2$)$_2$CH$_2$), 47.9 (1C, NH*C*H(CH$_2$CH$_2$)$_2$CH$_2$), 55.0 (1C, ArO*C*H$_3$), 63.3 (1C, N*C*HCO), 113.1 (2C, C-3"$_{4\text{-methoxyphenyl}}$, C-5"$_{4\text{-methoxyphenyl}}$), 127.1 (1C, C-1"$_{4\text{-methoxyphenyl}}$), 127.8 (1C, C$_{m\text{-tolyl}}$), 128.1 (1C, C$_{m\text{-tolyl}}$), 128.4 (1C, C$_{m\text{-tolyl}}$), 131.3 (2C, C-2"$_{4\text{-methoxyphenyl}}$, C-6"$_{4\text{-methoxyphenyl}}$), 131.4 (1C, C$_{m\text{-tolyl}}$), 137.6 (1C, C-3'$_{m\text{-tolyl}}$), 139.2 (1C, C-1'$_{m\text{-tolyl}}$), 158.5 (1C, C-4"$_{4\text{-methoxyphenyl}}$), 168.7 (1C, *C*ON), 168.8 (1C, *C*ON);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3264, 3079, 2924, 2851, 1644, 1605, 1586, 1558, 1515, 1488, 1452, 1442, 1409, 1373, 1335, 1309, 1249, 1239, 1181, 1105, 1038, 979, 890, 833, 809, 791, 744, 709, 678, 642, 627, 556, 521;

HRMS (*m*/*z*): [M+H]$^+$ calcd for $C_{24}H_{31}N_2O_3S$: 427.2050, found: 427.2064;

HPLC (method 1): t_R = 24.1 min, purity 93.6%.

4-Amino-*N*^{5}-[2-(cyclohexylamino)-1-(4-methoxyphenyl)-2-oxoethyl]-*N*^{5}-(*m*-tolyl)isothiazole-3,5-dicarboxamide (52n)



$C_{27}H_{31}N_5O_4S$

M_r = 521.6

51n (430 mg, 1.0 mmol) was added in one portion to a solution of **25** (320 mg, 1.2 mmol) in acetone (100 mL). The mixture was cooled to 0 °C and morpholine (0.17 mL, 170 mg, 1.9 mmol) was added dropwise. After stirring the reaction mixture for 12 h at ambient temperature, the solvent was removed *in vacuo*. The residue was purified by flash column chromatography (Ø = 6 cm, h = 20 cm, V = 15 mL, dichloromethane/ethyl acetate = 5/1) to give **52n** (310 mg, 0.60 mmol, 60% yield) as colorless solid.

m.p. = 251 °C;

TLC: R_f = 0.26 (dichloromethane/ethyl acetate = 5/1);

^{1}H NMR (500 MHz, DMSO-*d*$_6$): δ [ppm] = 0.92 – 1.02 (m, 1H, NHCH(C*H*$_2$CH$_2$)$_2$CH$_2$), 1.02 – 1.13 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$C*H*$_2$), 1.14 – 1.31 (m, 3H, NHCH(C*H*$_2$CH$_2$)$_2$CH$_2$ (1H), NHCH(CH$_2$C*H*$_2$)$_2$CH$_2$ (2H)), 1.48 – 1.62 (m, 2H, NHCH(CH$_2$C*H*$_2$)$_2$CH$_2$ (1H), NHCH(CH$_2$CH$_2$)$_2$C*H*$_2$ (1H)), 1.62 – 1.74 (m, 2H, NHCH(C*H*$_2$CH$_2$)$_2$CH$_2$ (1H), NHCH(CH$_2$C*H*$_2$)$_2$CH$_2$ (1H)), 1.74 – 1.81 (m, 1H, NHCH(C*H*$_2$CH$_2$)$_2$CH$_2$), 2.20 (s br, 3H, ArC*H*$_3$), 3.54 – 3.63 (m, 1H, NHC*H*(CH$_2$CH$_2$)$_2$CH$_2$), 3.64 (s, 3H, ArOC*H*$_3$), 6.07 (s, 1H, NC*H*CO), 6.65 – 6.72 (m, 2H, 3'''-H$_{4\text{-methoxyphenyl}}$, 5'''-H$_{4\text{-methoxyphenyl}}$), 6.95 – 7.03 (m, 2H, 2'''-H$_{4\text{-methoxyphenyl}}$, 6'''-H$_{4\text{-methoxyphenyl}}$), 7.11 – 7.16 (m, 1H, 4''-H$_{m\text{-tolyl}}$), 7.17 (s br, 2H, ArN*H*$_2$), 7.58 (s br, 1H, CON*H*$_2$), 7.81 (s br, 1H, CON*H*$_2$), 7.97 (d, *J* = 7.7 Hz, 1H, N*H*CH(CH$_2$CH$_2$)$_2$CH$_2$), the signals for 3 H$_{m\text{-tolyl}}$ cannot be observed in the spectrum;

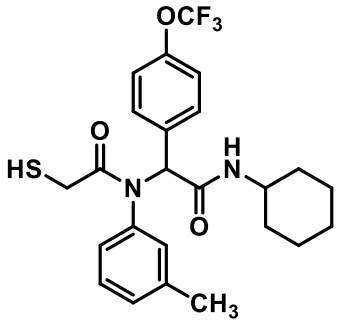
^{13}C NMR (151 MHz, DMSO-*d*$_6$): δ [ppm] = 20.6 (1C, Ar*C*H$_3$), 24.5 (1C, NHCH(CH$_2$*C*H$_2$)$_2$CH$_2$), 24.6 (1C, NHCH(CH$_2$*C*H$_2$)$_2$CH$_2$), 25.2 (1C, NHCH(CH$_2$CH$_2$)$_2$*C*H$_2$), 32.17 (1C, NHCH(*C*H$_2$CH$_2$)$_2$CH$_2$), 32.25 (1C, NHCH(*C*H$_2$CH$_2$)$_2$CH$_2$), 48.0 (1C, NH*C*H(CH$_2$CH$_2$)$_2$CH$_2$), 55.0 (1C, ArO*C*H$_3$), 63.4 (1C, N*C*HCO), 113.2 (2C, C-3'''$_{4\text{-methoxyphenyl}}$, C-5'''$_{4\text{-methoxyphenyl}}$), 123.2 (1C, C-5'$_{isothiazole}$), 126.7 (1C, C-1'''$_{4\text{-methoxyphenyl}}$), 128.7 (1C, C$_{m\text{-tolyl}}$), 130.0 (1C, C$_{m\text{-tolyl}}$), 130.2 (1C, C$_{m\text{-tolyl}}$), 131.6 (2C, C-2'''$_{4\text{-methoxyphenyl}}$, C-6'''$_{4\text{-methoxyphenyl}}$), 133.8 (1C, C$_{m\text{-tolyl}}$), 137.2 (1C, C-1''$_{m\text{-tolyl}}$), 146.4 (1C, C-3'$_{isothiazole}$), 151.2 (1C, C-4'$_{isothiazole}$), 158.7 (1C, C-4'''$_{4\text{-methoxyphenyl}}$), 162.2 (1C, Ar*C*ONCH), 163.9 (1C, Ar*C*ONH$_2$), 168.8 (1C, CH*C*ONHCH), the signal for 1 C$_{m\text{-tolyl}}$ cannot be observed in the spectrum;

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3459, 3340, 3266, 3173, 3081, 2923, 2850 ,1674, 1645, 1610, 1560, 1514, 1489, 1444, 1403, 1370, 1349, 1330, 1310, 1249, 1181, 1152, 1105, 1072, 1041, 979, 890, 830, 797, 745, 707, 678, 555, 532, 502;

HRMS (*m*/*z*): [M+H]$^+$ calcd for C$_{27}$H$_{32}$N$_5$O$_4$S: 522.2170, found: 522.2139;

HPLC (method 1): t$_R$ = 23.9 min, purity 97.8%.

_N_-Cyclohexyl-2-[2-mercapto-_N_-(_m_-tolyl)acetamido]-2-[4-(trifluoromethoxy)phenyl]acetamide (51o)



$C_{24}H_{27}F_3N_2O_3S$

$M_r = 480.5$

3-Methylaniline (0.22 mL, 210 mg, 2.0 mmol) and 4-(trifluoromethoxy)benzaldehyde (0.29 mL, 380 mg, 2.0 mmol) were dissolved in dichloromethane (15 mL) and the solution was stirred at ambient temperature for 10 min. Then, sulfanylacetic acid (0.14 mL, 180 mg, 2.0 mmol) and isocyanocyclohexane (0.25 mL, 220 mg, 2.0 mmol) were added. After stirring the reaction mixture for 48 h at ambient temperature, the solvent was removed *in vacuo* and the residue was purified by flash column chromatography (Ø = 4 cm, h = 15 cm, V = 15 mL, dichloromethane/ethyl acetate = 18/1) to give **51o** (410 mg, 0.85 mmol, 43% yield) as colorless solid.

m.p. = 166 °C;

TLC: R_f = 0.26 (dichloromethane/ethyl acetate = 15/1);

^{1}H NMR (500 MHz, DMSO-d_6): δ [ppm] = 0.94 – 1.13 (m, 2H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (1H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 1.14 – 1.31 (m, 3H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (1H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H)), 1.48 – 1.79 (m, 5H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 2.12 (s br, 3H, ArCH_3), 2.59 (t, J = 6.8 Hz, 1H, HSCH$_2$CO), 3.00 (dd, J = 15.0/6.7 Hz, 1H, HSCH_2CO), 3.05 (dd, J = 15.0/6.9 Hz, 1H, HSCH_2CO), 3.52 – 3.62 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), 6.03 (s, 1H, NCHCO), 6.95 – 7.00 (m, 1H, 4'-H$_{m\text{-tolyl}}$), 7.06 (s br, 1H, H$_{m\text{-tolyl}}$), 7.10 – 7.18 (m, 4H, 2"-H$_{4\text{-(trifluoromethoxy)phenyl}}$, 3"-H$_{4\text{-(trifluoromethoxy)phenyl}}$, 5"-H$_{4\text{-(trifluoromethoxy)phenyl}}$, 6"-H$_{4\text{-(trifluoromethoxy)phenyl}}$), 8.03 (d, J = 7.7 Hz, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), the signals for 2 H$_{m\text{-tolyl}}$ cannot be observed in the spectrum;

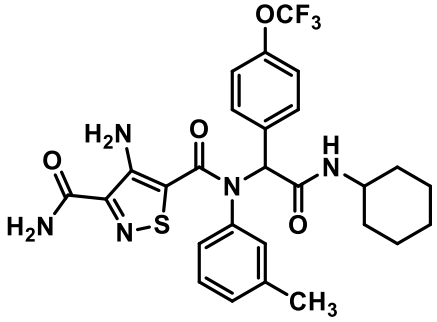
^{13}C NMR (126 MHz, DMSO-*d*$_6$): δ [ppm] = 20.5 (1C, Ar*C*H$_3$), 24.4 (1C, NHCH(CH$_2$*C*H$_2$)$_2$CH$_2$), 24.6 (1C, NHCH(CH$_2$*C*H$_2$)$_2$CH$_2$), 25.1 (1C, NHCH(CH$_2$CH$_2$)$_2$*C*H$_2$), 27.3 (1C, HS*C*H$_2$CO), 32.11 (1C, NHCH(*C*H$_2$CH$_2$)$_2$CH$_2$), 32.14 (1C, NHCH(*C*H$_2$CH$_2$)$_2$CH$_2$), 47.9 (1C, NH*C*H(CH$_2$CH$_2$)$_2$CH$_2$), 63.0 (1C, N*C*HCO), 119.9 (q, *J* = 256.4 Hz, 1C, ArO*C*F$_3$), 120.3 (2C, C-3"$_{4\text{-(trifluoromethoxy)phenyl}}$, C-5"$_{4\text{-(trifluoromethoxy)phenyl}}$), 127.7 (1C, C$_{m\text{-tolyl}}$), 128.2 (1C, C$_{m\text{-tolyl}}$), 128.5 (1C, C$_{m\text{-tolyl}}$), 131.2 (1C, C$_{m\text{-tolyl}}$), 131.9 (2C, C-2"$_{4\text{-(trifluoromethoxy)phenyl}}$, C-6"$_{4\text{-(trifluoromethoxy)phenyl}}$), 134.9 (1C, C-1"$_{4\text{-(trifluoromethoxy)phenyl}}$), 137.8 (1C, C-3'$_{m\text{-tolyl}}$), 138.9 (1C, C-1'$_{m\text{-tolyl}}$), 147.6 (1C, C-4"$_{4\text{-(trifluoromethoxy)phenyl}}$), 167.9 (1C, CH*C*ONHCH), 168.9 (1C, HSCH$_2$*C*O);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3274, 3080, 2931, 2856, 1652, 1605, 1587, 1557, 1513, 1489, 1448, 1405, 1378, 1355, 1325, 1303, 1251, 1218, 1208, 1189, 1167, 1101, 1064, 1020, 978, 921, 890, 849, 808, 762, 740, 707, 670, 649, 588, 579, 554, 508;

HRMS (*m*/*z*): [M+H]$^+$ calcd for C$_{24}$H$_{28}$F$_3$N$_2$O$_3$S: 481.1767, found: 481.1770;

HPLC (method 1): t$_R$ = 26.3 min, purity 95.5%.

4-Amino-*N*^{5}-{2-(cyclohexylamino)-2-oxo-1-[4-(trifluoromethoxy)phenyl]ethyl}-*N*^{5}-(*m*-tolyl)isothiazole-3,5-dicarboxamide (52o)



C$_{27}$H$_{28}$F$_3$N$_5$O$_4$S

M$_r$ = 575.6

51o (480 mg, 1.0 mmol) was added in one portion to a solution of **25** (320 mg, 1.2 mmol) in acetone (100 mL). The mixture was cooled to 0 °C and morpholine (0.17 mL, 170 mg, 1.9 mmol) was added dropwise. After stirring the reaction mixture for 12 h at ambient temperature, the solvent was removed *in vacuo*. The residue was purified by flash column chromatography (Ø = 6 cm, h = 20 cm, V = 15 mL, dichloromethane/acetone = 12/1) to give **52o** (320 mg, 0.55 mmol, 55% yield) as colorless solid.

m.p. = 271 °C;

TLC: R_f = 0.30 (dichloromethane/acetone = 10/1);

^{1}H NMR (500 MHz, DMSO-d_6): δ [ppm] = 0.93 – 1.14 (m, 2H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (1H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 1.14 – 1.32 (m, 3H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (1H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H)), 1.48 – 1.63 (m, 2H, NHCH(CH$_2$CH_2)$_2$CH$_2$ (1H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 1.63 – 1.74 (m, 2H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (1H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (1H)), 1.74 – 1.82 (m, 1H, NHCH(CH_2CH$_2$)$_2$CH$_2$), 2.14 (s br, 3H, ArCH_3), 3.55 – 3.65 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), 6.17 (s, 1H, NCHCO), 6.56 (s br, 1H, H$_{m\text{-tolyl}}$), 6.90 – 7.38 (m, 8H, 2'''-H$_{4\text{-(trifluoromethoxy)phenyl}}$, 3'''-H$_{4\text{-(trifluoromethoxy)phenyl}}$, 5'''-H$_{4\text{-(trifluoromethoxy)phenyl}}$, 6'''-H$_{4\text{-(trifluoromethoxy)phenyl}}$, ArN$H_2$, H$_{m\text{-tolyl}}$ (2H)), 7.41 – 7.79 (m, 2H, H$_{m\text{-tolyl}}$ (1H), CONH_2 (1H)), 7.82 (s br, 1H, CONH_2), 8.12 (d, J = 7.7 Hz, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$);

^{13}C NMR (101 MHz, DMSO-d_6): δ [ppm] = 20.5 (1C, ArCH$_3$), 24.4 (1C, NHCH(CH$_2$$CH_2$)$_2CH_2$), 24.6 (1C, NHCH(CH$_2$$CH_2$)$_2CH_2$), 25.1 (1C, NHCH(CH$_2CH_2$)$_2$$CH_2$), 32.1 (1C, NHCH($CH_2CH_2$)$_2CH_2$), 32.2 (1C, NHCH($CH_2CH_2$)$_2CH_2$), 48.0 (1C, NH$C$H(CH$_2CH_2$)$_2CH_2$), 63.1 (1C, N$C$HCO), 119.9 (q, J = 256.0 Hz, 1C, ArOCF$_3$), 120.3 (2C, C-3'''$_{4\text{-(trifluoromethoxy)phenyl}}$, C-5'''$_{4\text{-(trifluoromethoxy)phenyl}}$), 122.9 (1C, C-5'$_{\text{isothiazole}}$), 128.8 (1C, C$_{m\text{-tolyl}}$), 130.1 (1C, C$_{m\text{-tolyl}}$), 130.2 (1C, C$_{m\text{-tolyl}}$), 132.3 (2C, C-2'''$_{4\text{-(trifluoromethoxy)phenyl}}$, C-6'''$_{4\text{-(trifluoromethoxy)phenyl}}$), 133.7 (1C, C$_{m\text{-tolyl}}$), 134.5 (1C, C-1'''$_{4\text{-(trifluoromethoxy)phenyl}}$), 136.9 (1C, C-1''$_{m\text{-tolyl}}$), 138.4 (1C, C$_{m\text{-tolyl}}$), 146.4 (1C, C-3'$_{\text{isothiazole}}$), 147.8 (1C, C-4'''$_{4\text{-(trifluoromethoxy)phenyl}}$), 151.3 (1C, C-4'$_{\text{isothiazole}}$), 162.2 (1C, Ar$C$ONCH), 163.9 (1C, Ar$CONH_2$), 168.0 (1C, CH$C$ONHCH);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3446, 3278, 2929, 2854, 1650, 1603, 1562, 1509, 1490, 1450, 1348, 1328, 1251, 1210, 1158, 1104, 1072, 1022, 978, 849, 804, 747, 710, 690, 669, 545, 527;

HRMS (m/z): [M+H]$^+$ calcd for C$_{27}$H$_{29}$F$_3$N$_5$O$_4$S: 576.1887, found: 576.1875;

HPLC (method 1): t_R = 26.0 min, purity 99.0%.

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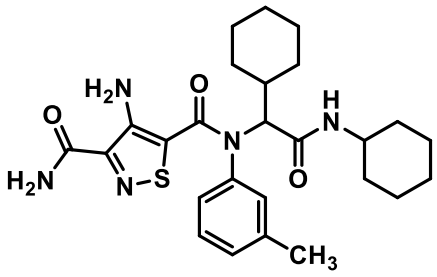
4-Amino-N^5-[1-cyclohexyl-2-(cyclohexylamino)-2-oxoethyl]-N^5-(*m*-tolyl)isothiazole-3,5-dicarboxamide (52p)



$C_{26}H_{35}N_5O_3S$

M_r = 497.7

3-Methylaniline (0.22 mL, 220 mg, 2.0 mmol) and cyclohexanecarboxaldehyde (0.24 mL, 220 mg, 2.0 mmol) were dissolved in dichloromethane (15 mL) and the solution was stirred at ambient temperature for 10 min. Then, sulfanylacetic acid (0.14 mL, 180 mg, 2.0 mmol) and isocyanocyclohexane (0.25 mL, 220 mg, 2.0 mmol) were added. After stirring the reaction mixture for 48 h at ambient temperature, the solvent was removed *in vacuo* and the residue was washed with acetonitrile.

A share (400 mg, 96%) of the obtained crude product (420 mg) was added in one portion to a solution of **25** (320 mg, 1.2 mmol) in acetone (100 mL). The mixture was cooled to 0 °C and morpholine (0.17 mL, 170 mg, 1.9 mmol) was added dropwise. After stirring the reaction mixture for 12 h at ambient temperature, the solvent was removed *in vacuo*. The residue was purified by flash column chromatography (Ø = 6 cm, h = 20 cm, V = 15 mL, dichloromethane/acetone = 12/1) and recrystallized from acetonitrile to give **52p** (290 mg, 0.59 mmol, 31% yield) as colorless solid.

m.p. = 184 °C;

TLC: R_f = 0.34 (dichloromethane/acetone = 10/1);

^{1}H NMR (500 MHz, DMSO-d_6): δ [ppm] = 0.91 – 1.28 (m, 10H, CH_2), 1.45 – 1.92 (m, 11H, CH_2 (10H), CHCH(CH$_2$CH$_2$)$_2$CH$_2$), 2.32 (s br, 3H, ArCH_3), 3.38 – 3.50 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), 4.92 (d, J = 10.4 Hz, 1H, CHCH(CH$_2$CH$_2$)$_2$CH$_2$), 7.03 – 7.32 (m, 4H,

ArN*H*$_2$, H$_{m\text{-tolyl}}$ (2H)), 7.33 – 7.43 (m, 2H, H$_{m\text{-tolyl}}$), 7.57 (s br, 1H, CON*H*$_2$), 7.82 (s br, 1H, CON*H*$_2$), 8.03 (d, *J* = 7.8 Hz, 1H, N*H*CH(CH$_2$CH$_2$)$_2$CH$_2$);

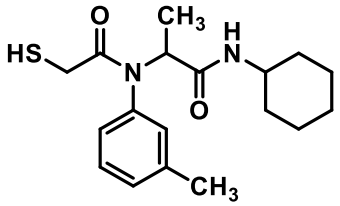
^{13}C NMR (151 MHz, DMSO-*d*$_6$): δ [ppm] = 20.8 (1C, Ar*C*H$_3$), 24.62 (1C, NHCH(CH$_2$*C*H$_2$)$_2$CH$_2$), 24.65 (1C, NHCH(CH$_2$*C*H$_2$)$_2$CH$_2$), 25.2 (1C, NHCH(CH$_2$CH$_2$)$_2$*C*H$_2$), 25.4 (2C, CHCH(CH$_2$*C*H$_2$)$_2$CH$_2$), 26.0 (1C, CHCH(CH$_2$CH$_2$)$_2$*C*H$_2$), 29.6 (1C, CHCH(*C*H$_2$CH$_2$)$_2$CH$_2$), 29.8 (1C, CHCH(*C*H$_2$CH$_2$)$_2$CH$_2$), 32.2 (2C, NHCH(*C*H$_2$CH$_2$)$_2$CH$_2$), 36.6 (1C, CH*C*H(CH$_2$CH$_2$)$_2$CH$_2$), 47.7 (1C, NH*C*H(CH$_2$CH$_2$)$_2$CH$_2$), 64.1 (1C, *C*HCH(CH$_2$CH$_2$)$_2$CH$_2$), 123.1 (1C, C-5'$_{isothiazole}$), 129.4 (1C, C$_{m\text{-tolyl}}$), 130.6 (1C, C$_{m\text{-tolyl}}$), 139.1 (1C, C$_{m\text{-tolyl}}$), 146.4 (1C, C-3'$_{isothiazole}$), 151.3 (1C, C-4'$_{isothiazole}$), 162.8 (1C, Ar*C*ONCH), 163.9 (1C, Ar*C*ONH$_2$), 167.1 (1C, CH*C*ONHCH), the signals for 3 C$_{m\text{-tolyl}}$ cannot be observed in the spectrum;

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3481, 3446, 3331, 3285, 2927, 2849, 1664, 1650, 1614, 1597, 1560, 1541, 1487, 1447, 1369, 1344, 1322, 1309, 1269, 1247, 1227, 1196, 1150, 1105, 1071, 983, 958, 892, 868, 851, 803, 792, 755, 741, 710, 666, 578, 562, 529, 516, 506;

HRMS (*m*/*z*): [M+H]$^+$ calcd for C$_{26}$H$_{36}$N$_5$O$_3$S: 498.2533, found: 498.2511;

HPLC (method 1): t$_R$ = 26.0 min, purity 97.4%.

N-Cyclohexyl-2-[2-mercapto-*N*-(*m*-tolyl)acetamido]propanamide (51q)



C$_{18}$H$_{26}$N$_2$O$_2$S

M$_r$ = 334.5

3-Methylaniline (0.22 mL, 210 mg, 2.0 mmol) and a 5 M solution of acetaldehyde in tetrahydrofuran (0.40 mL, 2.0 mmol) were dissolved in dichloromethane (15 mL) and the solution was stirred at ambient temperature for 10 min. Then, sulfanylacetic acid (0.14 mL, 180 mg, 2.0 mmol) and isocyanocyclohexane (0.25 mL, 220 mg, 2.0 mmol) were added. After stirring the reaction mixture for 48 h at ambient temperature, the solvent was removed *in vacuo* and the residue was purified by flash column chromatography (Ø = 4 cm, h = 15 cm, V = 15 mL,

dichloromethane/ethyl acetate = 15/1) to give **51q** (400 mg, 1.2 mmol, 60% yield) as colorless oil.

TLC: R_f = 0.19 (dichloromethane/ethyl acetate = 15/1);

^{1}H NMR (500 MHz, DMSO-d_6): δ [ppm] = 0.98 (d, J = 7.3 Hz, 3H, NCHCH_3), 1.05 – 1.31 (m, 5H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 1.50 – 1.58 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$CH_2), 1.61 – 1.75 (m, 4H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H)), 2.31 (s, 3H, ArCH_3), 2.55 (t, J = 6.8 Hz, 1H, HSCH$_2$CO), 2.94 (dd, J = 14.8/6.7 Hz, 1H, HSCH_2CO), 3.01 (dd, J = 14.8/6.9 Hz, 1H, HSCH_2CO), 3.45 – 3.55 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), 4.87 (q, J = 7.3 Hz, 1H, NCHCH$_3$), 7.15 – 7.37 (m, 4H, 2'-H$_{m\text{-tolyl}}$, 4'-H$_{m\text{-tolyl}}$, 5'-H$_{m\text{-tolyl}}$, 6'-H$_{m\text{-tolyl}}$), 7.73 (d, J = 7.8 Hz, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$);

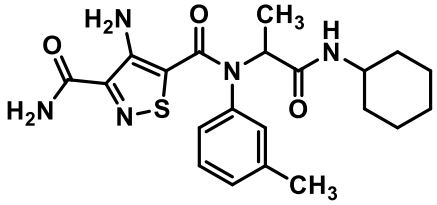
^{13}C NMR (101 MHz, DMSO-d_6): δ [ppm] = 16.2 (1C, NCHCH$_3$), 20.8 (1C, ArCH$_3$), 24.59 (1C, NHCH(CH$_2$$CH_2$)$_2CH_2$), 24.62 (1C, NHCH(CH$_2$$CH_2$)$_2CH_2$), 25.2 (1C, NHCH(CH$_2CH_2$)$_2$$CH_2$), 27.2 (1C, HS$CH_2$CO), 32.33 (1C, NHCH($CH_2CH_2$)$_2CH_2$), 32.35 (1C, NHCH($CH_2CH_2$)$_2CH_2$), 47.7 (1C, NH$C$H(CH$_2CH_2$)$_2CH_2$), 54.3 (1C, N$CHCH_3$), 127.2 (1C, C-6'$_{m\text{-tolyl}}$), 128.8 (1C, C-5'$_{m\text{-tolyl}}$), 129.1 (1C, C-4'$_{m\text{-tolyl}}$), 130.7 (1C, C-2'$_{m\text{-tolyl}}$), 138.57 (1C, C$_{m\text{-tolyl}}$), 138.65 (1C, C$_{m\text{-tolyl}}$), 168.9 (1C, HSCH$_2$$C$O), 170.0 (1C, CH$C$ONHCH);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3287, 2923, 2852, 2525, 1643, 1601, 1585, 1537, 1487, 1449, 1420, 1390, 1376, 1357, 1319, 1296, 1269, 1251, 1232, 1194, 1165, 1150, 1122, 1088, 1047, 1003, 970, 891, 869, 847, 788, 702, 636, 620, 591, 507;

HRMS (m/z): [M+H]$^+$ calcd for $C_{18}H_{27}N_2O_2S$: 335.1788, found: 335.1780;

HPLC (method 1): t_R = 22.4 min, purity 87.3%.

4-Amino-N^5-[1-(cyclohexylamino)-1-oxopropan-2-yl]-N^5-(m-tolyl)isothiazole-3,5-dicarboxamide (52q)



$C_{21}H_{27}N_5O_3S$

M_r = 429.5

51q (330 mg, 1.0 mmol) was added in one portion to a solution of **25** (320 mg, 1.2 mmol) in acetone (100 mL). The mixture was cooled to 0 °C and morpholine (0.17 mL, 170 mg, 1.9 mmol) was added dropwise. After stirring the reaction mixture for 12 h at ambient temperature, the solvent was removed *in vacuo*. The residue was purified by flash column chromatography (Ø = 6 cm, h = 20 cm, V = 15 mL, dichloromethane/acetone = 20/1) and recrystallized from acetonitrile to give **52q** (200 mg, 0.47 mmol, 47% yield) as colorless solid.

m.p. = 221 °C;

TLC: R_f = 0.47 (dichloromethane/acetone = 10/1);

^{1}H NMR (400 MHz, DMSO-d_6): δ [ppm] = 1.06 (d, J = 7.3 Hz, 3H, NCHCH_3), 1.08 – 1.31 (m, 5H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 1.50 – 1.59 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$CH_2), 1.61 – 1.79 (m, 4H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H)), 2.34 (s, 3H, ArCH_3), 3.45 – 3.58 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), 5.02 (q, J = 7.3 Hz, 1H, NCHCH$_3$), 7.14 (s br, 2H, ArNH_2), 7.16 – 7.64 (m, 5H, 2"-H$_{m\text{-tolyl}}$, 4"-H$_{m\text{-tolyl}}$, 5"-H$_{m\text{-tolyl}}$, 6"-H$_{m\text{-tolyl}}$, CONH_2 (1H)), 7.81 (s br, 1H, CONH_2), 7.88 (d, J = 7.8 Hz, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$);

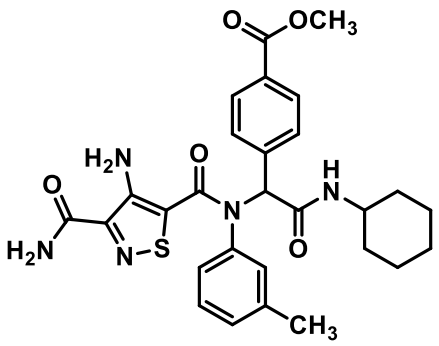
^{13}C NMR (101 MHz, DMSO-d_6): δ [ppm] = 16.3 (1C, NCHCH$_3$), 20.8 (1C, ArCH$_3$), 24.57 (1C, NHCH(CH$_2$$CH_2$)$_2CH_2$), 24.61 (1C, NHCH(CH$_2$$CH_2$)$_2CH_2$), 25.2 (1C, NHCH(CH$_2CH_2$)$_2$$CH_2$), 32.3 (2C, NHCH($CH_2CH_2$)$_2CH_2$), 47.8 (1C, NH$C$H(CH$_2CH_2$)$_2CH_2$), 54.4 (1C, N$CHCH_3$), 123.2 (1C, C-5'$_{isothiazole}$), 129.3 (1C, C$_{m\text{-tolyl}}$), 129.7 (1C, C$_{m\text{-tolyl}}$), 130.7 (1C, C-4"$_{m\text{-tolyl}}$), 133.1 (1C, C-2"$_{m\text{-tolyl}}$), 136.7 (1C, C-1"$_{m\text{-tolyl}}$), 139.2 (1C, C-3"$_{m\text{-tolyl}}$), 146.4 (1C, C-3'$_{isothiazole}$), 151.1 (1C, C-4'$_{isothiazole}$), 162.3 (1C, Ar$C$ONCH), 163.9 (1C, Ar$CONH_2$), 170.1 (CH$C$ONHCH);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3464, 3352, 3256, 2936, 2854, 1672, 1648, 1614, 1600, 1564, 1489, 1450, 1398, 1373, 1320, 1278,1188, 1153, 1125, 1101, 796, 780, 770, 745, 705, 682, 596, 504;

HRMS (m/z): [M+H]$^+$ calcd for $C_{21}H_{28}N_5O_3S$: 430.1907, found: 430.1897;

HPLC (method 1): t_R = 22.3 min, purity 98.5%.

Methyl 4-{1-[4-amino-3-carbamoyl-*N*-(*m*-tolyl)isothiazole-5-carboxamido]-2-(cyclohexylamino)-2-oxoethyl}benzoate (52r)



$C_{28}H_{31}N_5O_5S$

M_r = 549.6

3-Methylaniline (0.22 mL, 220 mg, 2.0 mmol) and methyl 4-formylbenzoate (330 mg, 2.0 mmol) were dissolved in dichloromethane (15 mL) and the solution was stirred at ambient temperature for 10 min. Then, sulfanylacetic acid (0.14 mL, 180 mg, 2.0 mmol) and isocyanocyclohexane (0.25 mL, 220 mg, 2.0 mmol) were added. After stirring the reaction mixture for 48 h at ambient temperature, the solvent was removed *in vacuo* and the residue was washed with acetonitrile.

A share (450 mg, 57%) of the obtained crude product (800 mg) was added in one portion to a solution of **25** (320 mg, 1.2 mmol) in acetone (100 mL). The mixture was cooled to 0 °C and morpholine (0.17 mL, 170 mg, 1.9 mmol) was added dropwise. After stirring the reaction mixture for 12 h at ambient temperature, the solvent was removed *in vacuo*. The residue was purified by flash column chromatography (Ø = 6 cm, h = 20 cm, V = 15 mL, dichloromethane/acetone = 12/1) and recrystallized from acetonitrile to give **52r** (390 mg, 0.71 mmol, 62% yield) as colorless solid.

m.p. = 244 °C;

TLC: R_f = 0.20 (dichloromethane/acetone = 12/1);

^{1}H NMR (500 MHz, DMSO-d_6): δ [ppm] = 0.92 – 1.13 (m, 2H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (1H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 1.13 – 1.33 (m, 3H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (1H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H)), 1.47 – 1.62 (m, 2H, (m, 2H, NHCH(CH$_2$CH_2)$_2$CH$_2$ (1H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 1.62 – 1.73 (m, 2H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (1H),

NHCH(CH_2CH_2)$_2CH_2$ (1H)), 1.73 – 1.81 (m, 1H, NHCH(CH_2CH_2)$_2CH_2$), 2.17 (s br, 3H, ArCH_3), 3.55 – 3.66 (m, 1H, NHCH(CH_2CH_2)$_2CH_2$), 3.79 (s, 3H, CO_2CH_3), 6.21 (s, 1H, NCHCO), 7.11 – 7.15 (m, 1H, 4''-H$_{m\text{-tolyl}}$), 7.19 (s br, 2H, ArNH_2), 7.24 – 7.29 (m, 2H, 3'''-H$_{benzoate}$, 5'''-H$_{benzoate}$), 7.59 (s br, 1H, CONH_2), 7.70 – 7.75 (m, 2H, 2'''-H$_{benzoate}$, 6'''-H$_{benzoate}$), 7.82 (s br, 1H, CONH_2), 8.14 (d, J = 7.7 Hz, 1H, NHCH(CH_2CH_2)$_2CH_2$), the signals for 3 H$_{m\text{-tolyl}}$ cannot be observed in the spectrum;

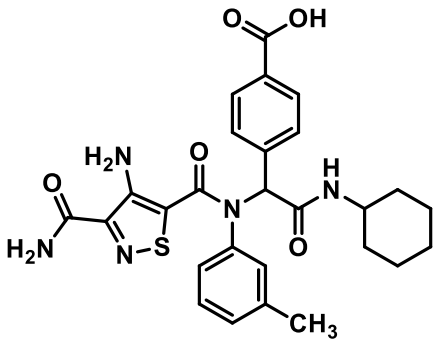
^{13}C NMR (101 MHz, DMSO-d_6): δ [ppm] = 20.6 (1C, ArCH_3), 24.4 (1C, NHCH(CH_2CH_2)$_2CH_2$), 24.5 (1C, NHCH(CH_2CH_2)$_2CH_2$), 25.1 (1C, NHCH(CH_2CH_2)$_2CH_2$), 32.1 (2C, NHCH(CH_2CH_2)$_2CH_2$), 48.0 (1C, NHCH(CH_2CH_2)$_2CH_2$), 52.1 (1C, CO_2CH_3), 63.6 (1C, NCHCO), 122.8 (1C, C-5'$_{isothiazole}$), 128.6 (2C, C-2'''$_{benzoate}$, C-6'''$_{benzoate}$), 128.8 (1C, C-1'''$_{benzoate}$), 130.3 (1C, C-4''$_{m\text{-tolyl}}$), 130.7 (2C, C-3'''$_{benzoate}$, C-5'''$_{benzoate}$), 133.6 (1C, C$_{m\text{-tolyl}}$), 136.9 (1C, C-1''$_{m\text{-tolyl}}$), 138.6 (1C, C-3''$_{m\text{-tolyl}}$), 140.4 (1C, C-4'''$_{benzoate}$), 146.4 (1C, C-3'$_{isothiazole}$), 151.3 (1C, C-4'$_{isothiazole}$), 162.3 (1C, ArCONCH), 163.9 (1C, ArCONH_2), 165.8 (1C, CO_2CH_3), 167.9 (1C, CHCONHCH), the signals for 2 C$_{m\text{-tolyl}}$ cannot be observed in the spectrum;

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3454, 3285, 2933, 2854, 1724, 1664, 1650, 1611, 1565, 1489, 1437, 1405, 1367, 1348, 1324, 1274, 1247, 1188, 1161, 1150, 1104, 1074, 1024, 976, 855, 762, 750, 713, 687, 670, 642, 588, 515;

HRMS (m/z): [M+H]$^+$ calcd for $C_{28}H_{32}N_5O_5S$: 550.2119, found: 550.2140;

HPLC (method 1): t_R = 23.8 min, purity 98.2%.

4-{1-[4-Amino-3-carbamoyl-*N*-(*m*-tolyl)isothiazole-5-carboxamido]-2-(cyclohexylamino)-2-oxoethyl}benzoic acid (52s)



$C_{27}H_{29}N_5O_5S$

M_r = 535.6

Lithium hydroxide (48 mg, 2.0 mmol) and **52r** (280 mg, 0.50 mmol) were dissolved in a mixture of water (20 mL) and methanol (20 mL). After stirring the reaction mixture at ambient temperature overnight, the mixture was concentrated *in vacuo*, diluted with water (40 mL), and acidified to pH 4 with an aqueous solution of hydrogen chloride (6 M). Subsequently, the solvent was removed by condensation and the residue was purified by flash column chromatography (Ø = 4 cm, h = 15 cm, V = 15 mL, dichloromethane/methanol/acetic acid = 10/1/0.1) to give **52s** (230 mg, 0.43 mmol, 86% yield) as colorless solid.

m.p. = 289 °C;

TLC: R_f = 0.22 (dichloromethane/methanol/acetic acid = 10/1/0.1);

^{1}H NMR (500 MHz, DMSO-d_6): δ [ppm] = 0.93 – 1.13 (m, 2H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (1H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 1.14 – 1.32 (m, 3H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (1H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H)), 1.48 – 1.62 (m, 2H, NHCH(CH$_2$CH_2)$_2$CH$_2$ (1H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 1.63 – 1.73 (m, 2H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (1H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (1H)), 1.73 – 1.80 (m, 1H, NHCH(CH_2CH$_2$)$_2$CH$_2$), 2.18 (s br, 3H, ArCH_3), 3.55 – 3.66 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), 6.20 (s, 1H, NCHCO), 7.11 – 7.15 (m, 1H, 4''-H$_{m\text{-tolyl}}$), 7.19 (s br, 2H, ArNH_2), 7.21 – 7.25 (m, 2H, 3'''-H$_{\text{benzoic acid}}$, 5'''-H$_{\text{benzoic acid}}$), 7.59 (s br, 1H, CONH_2), 7.68 – 7.72 (m, 2H, 2'''-H$_{\text{benzoic acid}}$, 6'''-H$_{\text{benzoic acid}}$), 7.82 (s br, 1H, CONH_2), 8.12 (d, J = 7.7 Hz, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), 12.92 (s, 1H, CO$_2H$), the signals for 3 H$_{m\text{-tolyl}}$ cannot be observed in the spectrum;

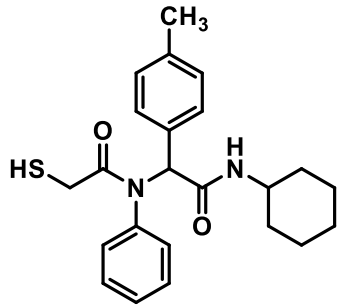
^{13}C NMR (126 MHz, DMSO-d_6): δ [ppm] = 24.4 (1C, NHCH(CH$_2$CH_2)$_2$CH$_2$), 24.6 (1C, NHCH(CH$_2$CH_2)$_2$CH$_2$), 25.1 (1C, NHCH(CH$_2$CH$_2$)$_2$CH_2), 32.1 (2C, NHCH(CH_2CH$_2$)$_2$CH$_2$), 48.0 (1C, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), 63.7 (1C, NCHCO), 122.8 (1C, C-5'$_{\text{isothiazole}}$), 128.7 (2C, C-2'''$_{\text{benzoic acid}}$, C-6'''$_{\text{benzoic acid}}$), 130.0 (1C, C-1'''$_{\text{benzoic acid}}$), 130.2 (1C, C-4''$_{m\text{-tolyl}}$), 130.5 (2C, C-3'''$_{\text{benzoic acid}}$, C-5'''$_{\text{benzoic acid}}$), 136.9 (1C, C-1''$_{m\text{-tolyl}}$), 138.5 (1C, C$_{m\text{-tolyl}}$), 139.9 (1C, C-4'''$_{\text{benzoic acid}}$), 146.4 (1C, C-3'$_{\text{isothiazole}}$), 151.3 (1C, C-4'$_{\text{isothiazole}}$), 162.3 (1C, ArCONCH), 163.9 (1C, ArCONH$_2$), 166.9 (1C, CO$_2$H), 167.9 (1C, CHCONHCH), the signals for ArCH$_3$ and 3 C$_{m\text{-tolyl}}$ cannot be observed in the spectrum;

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3467, 3386, 3353, 3189, 3040, 2933, 2853, 1721, 1675, 1651, 1623, 1562, 1534, 1489, 1449, 1415, 1376, 1364, 1341, 1322, 1292, 1251, 1218, 1191, 1179, 1150, 1111, 1074, 846, 802, 791, 752, 743, 718, 685, 663, 633, 553, 531, 502;

HRMS (m/z): [M+H]$^+$ calcd for $C_{27}H_{30}N_5O_5S$: 536.1962, found: 536.1985;

HPLC (method 1): t_R = 21.1 min, purity 97.0%.

***N*-Cyclohexyl-2-(2-mercapto-*N*-phenylacetamido)-2-(*p*-tolyl)acetamide (53)**



$C_{23}H_{28}N_2O_2S$

$M_r = 396.5$

Aniline (0.19 mL, 190 mg, 2.0 mmol) and 4-methylbenzaldehyde (0.24 mL, 240 mg, 2.0 mmol) were dissolved in dichloromethane (15 mL) and the solution was stirred at ambient temperature for 10 min. Then, sulfanylacetic acid (0.14 mL, 180 mg, 2.0 mmol) and isocyanocyclohexane (0.25 mL, 220 mg, 2.0 mmol) were added. After stirring the reaction mixture for 48 h at ambient temperature, the solvent was removed *in vacuo* and the residue was purified by flash column chromatography (Ø = 2 cm, h = 15 cm, V = 5 mL, dichloromethane/ethyl acetate = 20/1) to give **53a** (550 mg, 1.4 mmol, 69% yield) as colorless solid.

m.p. = 215 °C;

TLC: R_f = 0.27 (dichloromethane/ethyl acetate = 15/1);

^{1}H NMR (400 MHz, DMSO-d_6): δ [ppm] = 0.88 – 1.33 (m, 5H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 1.46 – 1.81 (m, 5H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 2.14 (s, 3H, ArCH_3), 2.58 (t, J = 6.8 Hz, 1H, *H*SCH$_2$CO), 2.94 (dd, J = 14.9/6.7 Hz, 1H, HSCH_2CO), 3.02 (dd, J = 14.9/6.9 Hz, 1H, HSCH_2CO), 3.51 – 3.64 (m, 1H, NHC*H*(CH$_2$CH$_2$)$_2$CH$_2$), 5.98 (s, 1H, NC*H*CO), 6.82 – 6.97 (m, 4H, 2"-H$_{p\text{-tolyl}}$, 3"-H$_{p\text{-tolyl}}$, 5"-H$_{p\text{-tolyl}}$, 6"-H$_{p\text{-tolyl}}$), 7.02 – 7.30 (m, 3H, H$_{phenyl}$), 7.91 (d, J = 7.7 Hz, 1H, N*H*CH(CH$_2$CH$_2$)$_2$CH$_2$), the signals for 2 H$_{phenyl}$ cannot be observed in the spectrum;

^{13}C NMR (151 MHz, DMSO-d_6): δ [ppm] = 20.6 (1C, Ar*C*H$_3$), 24.5 (1C, NHCH(CH$_2$*C*H$_2$)$_2$CH$_2$), 24.6 (1C, NHCH(CH$_2$*C*H$_2$)$_2$CH$_2$), 25.2 (1C, NHCH(CH$_2$CH$_2$)$_2$*C*H$_2$), 27.3 (1C, HS*C*H$_2$CO), 32.16 (1C, NHCH(*C*H$_2$CH$_2$)$_2$CH$_2$), 32.21 (1C, NHCH(*C*H$_2$CH$_2$)$_2$CH$_2$),

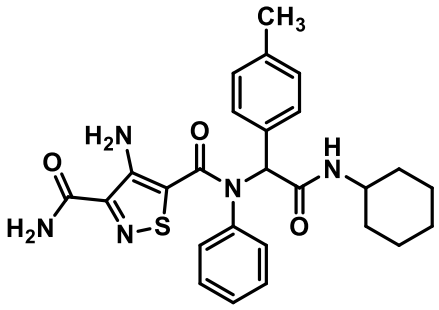
47.9 (1C, NH*C*H(CH$_2$CH$_2$)$_2$CH$_2$), 63.6 (1C, N*C*HCO), 127.8 (1C, C-4'$_{phenyl}$), 128.4 (4C, C-3"$_{p\text{-tolyl}}$, C-5"$_{p\text{-tolyl}}$, C$_{phenyl}$ (2C)), 130.0 (2C, C-2"$_{p\text{-tolyl}}$, C-6"$_{p\text{-tolyl}}$), 130.8 (2C, C$_{phenyl}$), 132.2 (1C, C-1"$_{p\text{-tolyl}}$), 136.7 (1C, C-4"$_{p\text{-tolyl}}$), 139.2 (1C, C-1'$_{phenyl}$), 168.6 (1C, CH*C*ONHCH), 168.9 (1C, HSCH$_2$*C*O);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3269, 3082, 2927, 2851, 1647, 1594, 1558, 1517, 1491, 1449, 1384, 1358, 1250, 1232, 1182, 1105, 978, 890, 827, 798, 699, 669, 629, 571, 527, 505;

HRMS (*m*/*z*): [M+H]$^+$ calcd for C$_{23}$H$_{29}$N$_2$O$_2$S: 397.1944, found: 397.1949;

HPLC (method 1): t$_R$ = 24.3 min, purity 96.9%.

4-Amino-*N*^{5}-[2-(cyclohexylamino)-2-oxo-1-(*p*-tolyl)ethyl]-*N*^{5}-phenylisothiazole-3,5-dicarboxamide (54a)



C$_{26}$H$_{29}$N$_5$O$_3$S

M$_r$ = 491.6

53a (400 mg, 1.0 mmol) was added in one portion to a solution of **25** (320 mg, 1.2 mmol) in acetone (100 mL). The mixture was cooled to 0 °C and morpholine (0.17 mL, 170 mg, 1.9 mmol) was added dropwise. After stirring the reaction mixture for 12 h at ambient temperature, the solvent was removed *in vacuo*. The residue was purified by flash column chromatography (Ø = 6 cm, h = 20 cm, V = 15 mL, dichloromethane/acetone = 10/1) and recrystallized from acetonitrile to give **54a** (320 mg, 0.66 mmol, 66% yield) as colorless solid.

m.p. = 217 °C;

TLC: R_f = 0.31 (dichloromethane/acetone = 10/1);

^{1}H NMR (500 MHz, DMSO-d_6): δ [ppm] = 0.91 – 1.02 (m, 1H, NHCH(C*H*$_2$CH$_2$)$_2$CH$_2$), 1.02 – 1.12 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$C*H*$_2$), 1.15 – 1.31 (m, 3H, NHCH(C*H*$_2$CH$_2$)$_2$CH$_2$ (1H),

NHCH(CH_2CH_2)$_2CH_2$ (2H)), 1.48 – 1.62 (m, 2H, NHCH(CH_2CH_2)$_2CH_2$ (1H), NHCH(CH_2CH_2)$_2CH_2$ (1H)), 1.62 – 1.73 (m, 2H, NHCH(CH_2CH_2)$_2CH_2$ (1H), NHCH(CH_2CH_2)$_2CH_2$ (1H)), 1.74 – 1.81 (m, 1H, NHCH(CH_2CH_2)$_2CH_2$), 2.15 (s, 3H, ArCH_3), 3.55 – 3.64 (m, 1H, NHCH(CH_2CH_2)$_2CH_2$), 6.12 (s, 1H, NCHCO), 6.89 – 6.99 (m, 4H, 2'''-H$_{p\text{-tolyl}}$, 3'''-H$_{p\text{-tolyl}}$, 5'''-H$_{p\text{-tolyl}}$, 6'''-H$_{p\text{-tolyl}}$), 7.18(s br, 2H, ArNH_2), 7.24 (s br, 2H, H$_{phenyl}$), 7.28 – 7.34 (m, 1H, 4''-H$_{phenyl}$), 7.58 (s br, 1H, CONH_2), 7.83 (s br, 1H, CONH_2), 8.00 (d, J = 7.7 Hz, 1H, NHCH(CH_2CH_2)$_2CH_2$), the signals for 2 H$_{phenyl}$ cannot be observed in the spectrum;

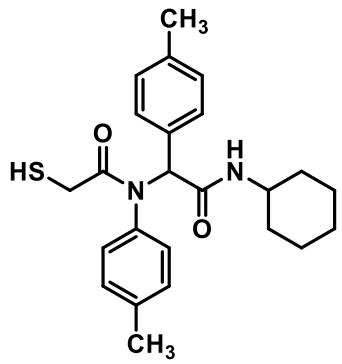
^{13}C NMR (126 MHz, DMSO-d_6): δ [ppm] = 20.6 (1C, ArCH_3), 24.4 (1C, NHCH(CH_2CH_2)$_2CH_2$), 24.6 (1C, NHCH(CH_2CH_2)$_2CH_2$), 25.2 (1C, NHCH(CH_2CH_2)$_2CH_2$), 32.1 (1C, NHCH(CH_2CH_2)$_2CH_2$), 32.2 (1C, NHCH(CH_2CH_2)$_2CH_2$), 48.0 (1C, NHCH(CH_2CH_2)$_2CH_2$), 63.7 (1C, NCHCO), 123.2 (1C, C-5'$_{isothiazole}$), 128.4 (2C, C-3'''$_{p\text{-tolyl}}$, C-5'''$_{p\text{-tolyl}}$), 128.9 (2C, C$_{phenyl}$), 129.5 (1C, C-4''$_{phenyl}$), 130.3 (2C, C-2'''$_{p\text{-tolyl}}$, C-6'''$_{p\text{-tolyl}}$), 131.8 (1C, C-1'''$_{p\text{-tolyl}}$), 133.3 (2C, C$_{phenyl}$), 136.9 1C, C-4'''$_{p\text{-tolyl}}$), 137.3 (1C, C-1''$_{phenyl}$), 146.5 (1C, C-3'$_{isothiazole}$), 151.2 (1C, C-4'$_{isothiazole}$), 162.2 (1C, ArCONCH), 163.9 (1C, ArCONH_2), 168.6 (1C, CHCONHCH);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3443, 3331, 3276, 2928, 2849, 1664, 1648, 1611, 1557, 1518, 1491, 1449, 1369, 1343, 1325, 1250, 1237, 1186, 1151, 1105, 1071, 976, 833, 761, 732, 700, 668, 631, 568, 531, 504;

HRMS (m/z): [M+H]$^+$ calcd for $C_{26}H_{30}N_5O_3S$: 492.2064, found: 492.2073;

HPLC (method 1): t_R = 24.1 min, purity 96.1%.

N-Cyclohexyl-2-[2-mercapto-*N*-(*p*-tolyl)acetamido]-2-(*p*-tolyl)acetamide (53b)



$C_{24}H_{30}N_2O_2S$

M_r = 410.6

4-Methylaniline (210 mg, 2.0 mmol) and 4-methylbenzaldehyde (0.24 mL, 240 mg, 2.0 mmol) were dissolved in dichloromethane (15 mL) and the solution was stirred at ambient temperature for 10 min. Then, sulfanylacetic acid (0.14 mL, 180 mg, 2.0 mmol) and isocyanocyclohexane (0.25 mL, 220 mg, 2.0 mmol) were added. After stirring the reaction mixture for 48 h at ambient temperature, the solvent was removed *in vacuo* and the residue was purified by flash column chromatography (Ø = 2 cm, h = 15 cm, V = 5 mL, petroleum ether/ethyl acetate = 4/1) to give **53b** (400 mg, 0.98 mmol, 49% yield) as colorless solid.

m.p. = 206 °C;

TLC: R_f = 0.26 (dichloromethane/ethyl acetate = 15/1);

^{1}H NMR (400 MHz, DMSO-d_6): δ [ppm] = 0.89 – 1.33 (m, 5H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 1.46 – 1.81 (m, 5H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 2.15 (s, 3H, ArCH_3), 2.18 (s, 3H, ArCH_3), 2.56 (t, J = 6.8 Hz, 1H, HSCH$_2$CO), 2.92 (dd, J = 14.9/6.7 Hz, 1H, HSCH_2CO), 3.00 (dd, J = 14.9/6.9 Hz, 1H, HSCH_2CO), 3.50 – 3.63 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), 5.97 (s, 1H, NCHCO), 6.82 – 7.22 (m, 6H, 2"-H$_{p\text{-tolyl}}$, 3"-H$_{p\text{-tolyl}}$, 5"-H$_{p\text{-tolyl}}$, 6"-H$_{p\text{-tolyl}}$, H$_{p\text{-tolyl}}$ (2H)), 7.89 (d, J = 7.6 Hz, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), the signals for 2 H$_{p\text{-tolyl}}$ cannot be observed in the spectrum;

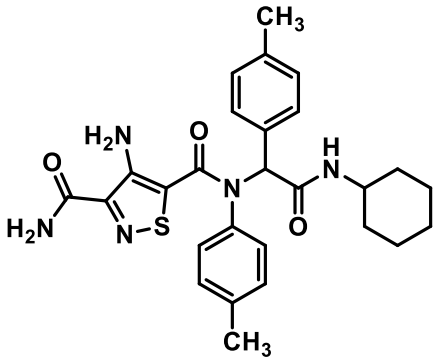
^{13}C NMR (101 MHz, DMSO-d_6): δ [ppm] = 20.56 (1C, ArCH$_3$), 20.61 (1C, ArCH$_3$), 24.5 (1C, NHCH(CH$_2$$CH_2$)$_2CH_2$), 24.6 (1C, NHCH(CH$_2$$CH_2$)$_2CH_2$), 25.2 (1C, NHCH(CH$_2CH_2$)$_2$$CH_2$), 27.3 (1C, HS$CH_2$CO), 32.15 (1C, NHCH($CH_2CH_2$)$_2CH_2$), 32.19 (1C, NHCH($CH_2CH_2$)$_2CH_2$), 47.9 (1C, NH$C$H(CH$_2CH_2$)$_2CH_2$), 63.4 (1C, N$C$HCO), 128.4 (2C, C-3"$_{p\text{-tolyl}}$, C-5"$_{p\text{-tolyl}}$), 128.9 (2C, C-3'$_{p\text{-tolyl}}$, C-5'$_{p\text{-tolyl}}$), 129.9 (2C, C-2"$_{p\text{-tolyl}}$, C-6"$_{p\text{-tolyl}}$), 130.5 (2C, C-2'$_{p\text{-tolyl}}$, C-6'$_{p\text{-tolyl}}$), 132.3 (1C, C-1"$_{p\text{-tolyl}}$), 136.7 (2C, C-1'$_{p\text{-tolyl}}$, C-4"$_{p\text{-tolyl}}$), 137.0 (1C, C-4'$_{p\text{-tolyl}}$), 168.5 (1C, CH$C$ONHCH), 169.0 (1C, HSCH$_2$$C$O);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3263, 3081, 3033, 2926, 2853, 1646, 1559, 1511, 1445, 1407, 1383, 1357, 1305, 1265, 1251, 1229, 1190, 1172, 1104, 1051, 1024, 978, 890, 840, 803, 737, 721, 669, 628, 595, 567, 529, 504;

HRMS (m/z): [M+H]$^+$ calcd for $C_{24}H_{31}N_2O_2S$: 411.2101, found: 411.2111;

HPLC (method 1): t_R = 25.2 min, purity 97.5%.

4-Amino-N^5-[2-(cyclohexylamino)-2-oxo-1-(*p*-tolyl)ethyl]-N^5-(*p*-tolyl)isothiazole-3,5-dicarboxamide (54b)



$C_{27}H_{31}N_5O_3S$

M_r = 505.6

53b (410 mg, 1.0 mmol) was added in one portion to a solution of **25** (320 mg, 1.2 mmol) in acetone (100 mL). The mixture was cooled to 0 °C and morpholine (0.17 mL, 170 mg, 1.9 mmol) was added dropwise. After stirring the reaction mixture for 12 h at ambient temperature, the solvent was removed *in vacuo*. The residue was purified by flash column chromatography (Ø = 6 cm, h = 20 cm, V = 15 mL, dichloromethane/methanol = 100/1) and recrystallized from acetonitrile to give **54b** (300 mg, 0.59 mmol, 59% yield) as colorless solid.

m.p. = 223 °C;

TLC: R_f = 0.30 (dichloromethane/acetone = 10/1);

^{1}H NMR (400 MHz, DMSO-d_6): δ [ppm] = 0.89 – 1.33 (m, 5H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 1.46 – 1.82 (m, 5H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 2.17 (s, 3H, ArCH_3), 2.26 (s, 3H, ArCH_3), 3.52 – 3.65 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), 6.11 (s, 1H, NCHCO), 6.89 – 7.01 (m, 4H, 2'''-H$_{p\text{-tolyl}}$, 3'''-H$_{p\text{-tolyl}}$, 5'''-H$_{p\text{-tolyl}}$, 6'''-H$_{p\text{-tolyl}}$), 7.05 (s br, 2H, H$_{p\text{-tolyl}}$), 7.15 (s br, 2H, ArNH_2), 7.57 (s br, 1H, CONH_2), 7.79 (s br, 1H, CONH_2), 7.96 (d, J = 7.6 Hz, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), the signals for 2 H$_{p\text{-tolyl}}$ cannot be observed in the spectrum;

^{13}C NMR (151 MHz, DMSO-d_6): δ [ppm] = 20.6 (1C, ArCH$_3$), 20.8 (1C, ArCH$_3$), 24.5 (1C, NHCH(CH$_2$$CH_2$)$_2CH_2$), 24.6 (1C, NHCH(CH$_2$$CH_2$)$_2CH_2$), 25.2 (1C, NHCH(CH$_2CH_2$)$_2$$CH_2$), 32.14 (1C, NHCH($CH_2CH_2$)$_2CH_2$), 32.20 (1C, NHCH($CH_2CH_2$)$_2CH_2$), 47.9 (1C, NH$C$H(CH$_2CH_2$)$_2CH_2$), 63.6 (1C, N$C$HCO), 123.2 (1C, C-5'$_{isothiazole}$), 128.5 (2C, C-3'''$_{p\text{-tolyl}}$, C-

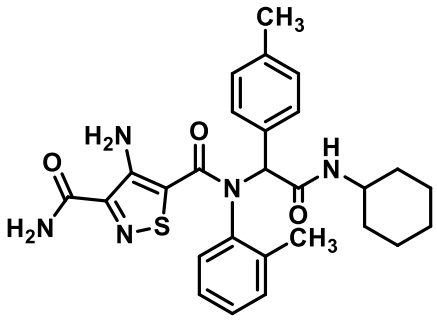
5'''(p-tolyl), 129.4 (2C, C-3''$_{p\text{-tolyl}}$, C-5''$_{p\text{-tolyl}}$), 130.3 (2C, C-2'''$_{p\text{-tolyl}}$, C-6'''$_{p\text{-tolyl}}$), 131.9 (1C, C-1'''$_{p\text{-tolyl}}$), 133.0 (2C, C-2''$_{p\text{-tolyl}}$, C-6''$_{p\text{-tolyl}}$), 134.7 (1C, C-1''$_{p\text{-tolyl}}$), 136.8 (1C, C-4'''$_{p\text{-tolyl}}$), 139.1 (1C, C-4''$_{p\text{-tolyl}}$), 146.4 (1C, C-3'$_{isothiazole}$), 151.1 (1C, C-4'$_{isothiazole}$), 162.4 (1C, Ar*C*ONCH), 163.9 (1C, Ar*C*ONH$_2$), 168.7 (1C, CH*C*ONHCH);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3454, 3338, 3274, 3084, 2923, 2850, 1672, 1646, 1614, 1561, 1510, 1493, 1449, 1368, 1346, 1327, 1263, 1249, 1238, 1186, 1152, 1106, 1072, 1023, 978, 838, 821, 805, 796, 759, 741, 676, 651, 603, 567, 531, 504;

HRMS (*m*/*z*): [M+H]$^+$ calcd for $C_{27}H_{32}N_5O_3S$: 506.2220, found: 506.2220;

HPLC (method 1): t_R = 24.8 min, purity 95.1%.

4-Amino-N^5-[2-(cyclohexylamino)-2-oxo-1-(*p*-tolyl)ethyl]-N^5-(*o*-tolyl)isothiazole-3,5-dicarboxamide (54c)



$C_{27}H_{31}N_5O_3S$

M_r = 505.6

2-Methylaniline (0.22 mL, 220 mg, 2.0 mmol) and 4-methylbenzaldehyde (0.24 mL, 240 mg, 2.0 mmol) were dissolved in dichloromethane (15 mL) and the solution was stirred at ambient temperature for 10 min. Then, sulfanylacetic acid (0.14 mL, 180 mg, 2.0 mmol) and isocyanocyclohexane (0.25 mL, 220 mg, 2.0 mmol) were added. After stirring the reaction mixture for 48 h at ambient temperature, the solvent was removed *in vacuo* and the residue was washed with acetonitrile.

A share (410 mg, 63%) of the obtained crude product (650 mg) was added in one portion to a solution of **25** (320 mg, 1.2 mmol) in acetone (100 mL). The mixture was cooled to 0 °C and morpholine (0.17 mL, 170 mg, 1.9 mmol) was added dropwise. After stirring the reaction mixture for 12 h at ambient temperature, the solvent was removed *in vacuo*. The residue was purified by flash column chromatography (Ø = 6 cm, h = 20 cm, V = 15 mL,

dichloromethane/ethyl acetate = 5/1) and recrystallized from acetonitrile to give **54c** (400 mg, 0.79 mmol, 63% yield) as colorless solid.

m.p. = 216 °C;

TLC: R_f = 0.27 (dichloromethane/ethyl acetate = 5/1);

^{1}H NMR (400 MHz, DMSO-d_6): δ [ppm] = 0.84 – 1.33 (m, 5H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 1.46 – 1.85 (m, 8H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H), C$H_{3,\ o\text{-tolyl}}$), 2.14 (s, 3H, C$H_{3,\ p\text{-tolyl}}$), 3.48 – 3.62 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), 6.07 (s, 1H, NCHCO), 6.84 – 6.94 (m, 2H, 3'''-H$_{p\text{-tolyl}}$, 5'''-H$_{p\text{-tolyl}}$), 6.94 – 7.06 (m, 3H, 2'''-H$_{p\text{-tolyl}}$, 6'''-H$_{p\text{-tolyl}}$, 3''-H$_{o\text{-tolyl}}$), 7.19 (s br, 2H, ArNH_2), 7.20 – 7.31 (m, 2H, 4''-H$_{o\text{-tolyl}}$, 5''-H$_{o\text{-tolyl}}$), 7.57 (s br, 1H, CONH_2), 7.81 (s br, 1H, CONH_2), 7.89 (d, J = 7.8 Hz, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), 7.95 – 8.01 (m, 1H, 6''-H$_{o\text{-tolyl}}$), the signals of the major rotamer are given;

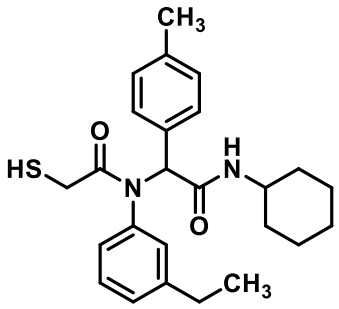
^{13}C NMR (101 MHz, DMSO-d_6): δ [ppm] = 17.7 (1C,CH$_{3,\ o\text{-tolyl}}$), 20.6 (1C, CH$_{3,\ p\text{-tolyl}}$), 24.4 (1C, NHCH(CH$_2$$CH_2$)$_2CH_2$), 24.6 (1C, NHCH(CH$_2$$CH_2$)$_2CH_2$), 25.2 (1C, NHCH(CH$_2CH_2$)$_2$$CH_2$), 32.1 (2C, NHCH($CH_2CH_2$)$_2CH_2$), 48.0 (1C, NH$C$H(CH$_2CH_2$)$_2CH_2$), 64.2 (1C, N$C$HCO), 122.6 (1C, C-5'$_{isothiazole}$), 126.9 (1C, C-5''$_{o\text{-tolyl}}$), 128.1 (2C, C-3'''$_{p\text{-tolyl}}$, C-5'''$_{p\text{-tolyl}}$), 130.1 (1C, C-4''$_{o\text{-tolyl}}$), 130.49 (1C, C-1'''$_{p\text{-tolyl}}$), 130.54 (2C, C-2'''$_{p\text{-tolyl}}$, C-6'''$_{p\text{-tolyl}}$), 130.9 (1C, C-3''$_{o\text{-tolyl}}$), 133.6 (1C, C-6''$_{o\text{-tolyl}}$), 136.0 (1C, C-1''$_{o\text{-tolyl}}$), 137.2 (1C, C-4'''$_{p\text{-tolyl}}$), 139.2 (1C, C-2''$_{o\text{-tolyl}}$), 146.5 (1C, C-3'$_{isothiazole}$), 151.3 (1C, C-4'$_{isothiazole}$), 162.3 (1C, Ar$C$ONCH), 163.9 (1C, Ar$CONH_2$), 168.9 (1C, CH$C$ONHCH);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3469, 3442, 3386, 3354, 3275, 3173, 2928, 2852, 1696, 1670, 1651, 1620, 1559, 1525, 1490, 1447, 1405, 1375, 1341, 1327, 1305, 1281, 1265, 1242, 1221, 1181, 1150, 1121, 1066, 1037, 974, 889, 832, 801, 760, 734, 684, 646, 630, 599, 561, 534, 494;

HRMS (m/z): [M+H]$^+$ calcd for $C_{27}H_{32}N_5O_3S$: 506.2220, found: 506.2225;

HPLC (method 1): t_R = 24.4 min, purity 95.9%.

_N_-Cyclohexyl-2-[_N_-(3-ethylphenyl)-2-mercaptoacetamido]-2-(_p_-tolyl)acetamide (53d)



$C_{25}H_{32}N_2O_2S$

$M_r = 424.6$

3-Ethylaniline (0.25 mL, 240 mg, 2.0 mmol) and 4-methylbenzaldehyde (0.24 mL, 240 mg, 2.0 mmol) were dissolved in dichloromethane (15 mL) and the solution was stirred at ambient temperature for 10 min. Then, sulfanylacetic acid (0.14 mL, 180 mg, 2.0 mmol) and isocyanocyclohexane (0.25 mL, 220 mg, 2.0 mmol) were added. After stirring the reaction mixture for 48 h at ambient temperature, the solvent was removed *in vacuo* and the residue was purified by flash column chromatography (Ø = 2 cm, h = 15 cm, V = 5 mL, petroleum ether/ethyl acetate = 4/1) to give **53d** (440 mg, 1.0 mmol, 52% yield) as colorless solid.

m.p. = 180 °C;

TLC: R_f = 0.46 (dichloromethane/methanol = 40/1);

^{1}H NMR (500 MHz, DMSO-d_6): δ [ppm] = 0.77 – 1.32 (m, 8H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H), ArCH$_2$CH_3), 1.48 – 1.79 (m, 5H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 2.14 (s, 3H, ArCH_3), 2.40 (s br, 2H, ArCH_2CH$_3$), 2.57 (t, J = 6.8 Hz, 1H, HSCH$_2$CO), 2.93 – 3.06 (m, 2H, HSCH_2CO), 3.52 – 3.62 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), 5.96 (s, 1H, NCHCO), 6.43 (s br, 1H, H$_{3\text{-ethylphenyl}}$), 6.85 – 6.93 (m, 4H, 2"-H$_{p\text{-tolyl}}$, 3"-H$_{p\text{-tolyl}}$, 5"-H$_{p\text{-tolyl}}$, 6"-H$_{p\text{-tolyl}}$), 6.93 – 6.99 (m, 1H, 4'-H$_{3\text{-ethylphenyl}}$), 7.07 (s br, 1H, H$_{3\text{-ethylphenyl}}$), 7.57 (s br, 1H, H$_{3\text{-ethylphenyl}}$), 7.82 (d, J = 7.7 Hz, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$);

^{13}C NMR (101 MHz, DMSO-d_6): δ [ppm] = 15.4 (1C, ArCH$_2$CH_3), 20.6 (1C, ArCH_3), 24.5 (1C, NHCH(CH$_2$CH_2)$_2$CH$_2$), 24.6 (1C, NHCH(CH$_2$CH_2)$_2$CH$_2$), 25.2 (1C, NHCH(CH$_2$CH$_2$)$_2$CH_2), 27.3 (1C, HSCH_2CO), 27.8 (1C, ArCH_2CH$_3$), 32.2 (2C, NHCH(CH_2CH$_2$)$_2$CH$_2$), 47.9 (1C, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), 63.6 (1C, NCHCO), 127.3 (1C, C-

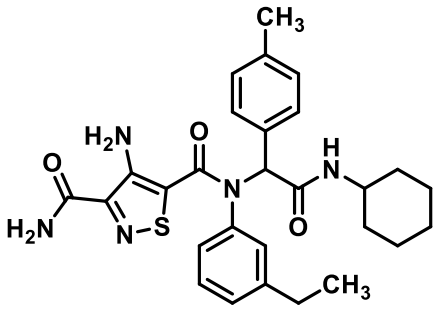
4'$_{3\text{-ethylphenyl}}$), 128.1 (1C, C$_{3\text{-ethylphenyl}}$), 128.2 (1C, C$_{3\text{-ethylphenyl}}$), 128.3 (2C, C-3"$_{p\text{-tolyl}}$, C-5"$_{p\text{-tolyl}}$), 130.0 (2C, C-2"$_{p\text{-tolyl}}$, C-6"$_{p\text{-tolyl}}$), 130.3 (1C, C$_{3\text{-ethylphenyl}}$), 132.2 (1C, C-1"$_{p\text{-tolyl}}$), 136.7 (1C, C-4"$_{p\text{-tolyl}}$), 139.1 (1C, C-1'$_{3\text{-ethylphenyl}}$), 144.0 (1C, C-3'$_{3\text{-ethylphenyl}}$), 168.5 (1C, CH*C*ONHCH), 168.8 (1C, HSCH$_2$*C*O);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3264, 3081, 2968, 2926, 2854, 1650, 1604, 1586, 1559, 1517, 1485, 1445, 1417, 1383, 1360, 1305, 1250, 1239, 1187, 1160, 1103, 1064, 965, 890, 818, 802, 740, 705, 668, 556, 505;

HRMS (*m*/*z*): [M+H]$^+$ calcd for $C_{25}H_{33}N_2O_2S$: 425.2257, found: 425.2262;

HPLC (method 1): t_R = 26.1 min, purity 99.3%.

4-Amino-*N*^{5}-[2-(cyclohexylamino)-2-oxo-1-(*p*-tolyl)ethyl]-*N*^{5}-(3-ethylphenyl)isothiazole-3,5-dicarboxamide (54d)



$C_{28}H_{33}N_5O_3S$

M_r = 519.7

53d (420 mg, 1.0 mmol) was added in one portion to a solution of **25** (320 mg, 1.2 mmol) in acetone (100 mL). The mixture was cooled to 0 °C and morpholine (0.17 mL, 170 mg, 1.9 mmol) was added dropwise. After stirring the reaction mixture for 12 h at ambient temperature, the solvent was removed *in vacuo*. The residue was purified by flash column chromatography (Ø = 6 cm, h = 20 cm, V = 15 mL, dichloromethane/ethyl acetate = 5/1) and recrystallized from acetonitrile to give **54d** (400 mg, 0.76 mmol, 76% yield) as colorless solid.

m.p. = 217 °C;

TLC: R_f = 0.27 (dichloromethane/ethyl acetate = 5/1);

^{1}H NMR (500 MHz, DMSO-*d*$_6$): δ [ppm] = 0.73 – 1.32 (m, 8H, NHCH(C*H*$_2$CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$C*H*$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH$_2$)$_2$C*H*$_2$ (1H), ArCH$_2$C*H*$_3$), 1.46 – 1.82 (m, 5H, NHCH(C*H*$_2$CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$C*H*$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH$_2$)$_2$C*H*$_2$ (1H)), 2.14 (s, 3H, ArC*H*$_3$), 2.22 – 2.62 (m br, 2H, ArC*H*$_2$CH$_3$), 3.53 – 3.66 (m, 1H, NHC*H*(CH$_2$CH$_2$)$_2$CH$_2$), 6.10 (s, 1H, NC*H*CO), 6.49 (s br, 1H, H$_{3\text{-ethylphenyl}}$), 6.82 – 7.02 (m, 4H, 2'''-H$_{p\text{-tolyl}}$, 3'''-H$_{p\text{-tolyl}}$, 5'''-H$_{p\text{-tolyl}}$, 6'''-H$_{p\text{-tolyl}}$), 7.10 (s br, 1H, H$_{3\text{-ethylphenyl}}$), 7.11 – 7.15 (m, 1H, 4''-H$_{3\text{-ethylphenyl}}$), 7.18 (s br, 2H, ArN*H*$_2$), 7.57 (s br, 1H, CON*H*$_2$), 7.66 (s br, 1H, H$_{3\text{-ethylphenyl}}$), 7.81 (s br, 1H, CON*H*$_2$), 7.98 (d, *J* = 7.7 Hz, 1H, N*H*CH(CH$_2$CH$_2$)$_2$CH$_2$), the signals of the major rotamer are given;

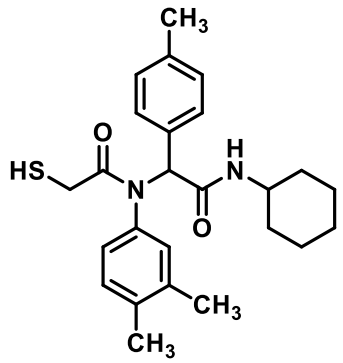
^{13}C NMR (151 MHz, DMSO-*d*$_6$): δ [ppm] = 15.5 (1C, ArCH$_2$*C*H$_3$), 20.6 (1C, Ar*C*H$_3$), 24.5 (1C, NHCH(CH$_2$*C*H$_2$)$_2$CH$_2$), 24.6 (1C, NHCH(CH$_2$*C*H$_2$)$_2$CH$_2$), 25.2 (1C, NHCH(CH$_2$CH$_2$)$_2$*C*H$_2$), 27.9 (1C, Ar*C*H$_2$CH$_3$), 32.2 (2C, NHCH(*C*H$_2$CH$_2$)$_2$CH$_2$), 47.9 (1C, NH*C*H(CH$_2$CH$_2$)$_2$CH$_2$), 63.7 (1C, N*C*HCO), 123.2 (1C, C-5'$_{\text{isothiazole}}$), 128.30 (1C, C$_{3\text{-ethylphenyl}}$), 128.35 (2C, C-3'''$_{p\text{-tolyl}}$, C-5'''$_{p\text{-tolyl}}$), 128.8 (1C, C$_{3\text{-ethylphenyl}}$), 129.0 (1C, C-4'$_{3\text{-ethylphenyl}}$), 130.3 (2C, C-2'''$_{p\text{-tolyl}}$, C-6'''$_{p\text{-tolyl}}$), 130.0 (1C, C$_{3\text{-ethylphenyl}}$), 131.9 (1C, C-1'''$_{p\text{-tolyl}}$), 132.8 (1C, C$_{3\text{-ethylphenyl}}$), 136.9 (1C, C-4'''$_{p\text{-tolyl}}$), 137.1 (1C, C-1''$_{3\text{-ethylphenyl}}$), 146.4 (1C, C-3'$_{\text{isothiazole}}$), 151.2 (1C, C-4'$_{\text{isothiazole}}$), 162.2 (1C, Ar*C*ONCH), 163.9 (1C, Ar*C*ONH$_2$), 168.6 (1C, CH*C*ONHCH);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3454, 3277, 2966, 2928, 2851, 1674, 1648, 1561, 1487, 1449, 1370, 1348, 1328, 1250, 1240, 1188, 1152, 1105, 1073, 1046, 890, 803, 760, 735, 707, 677, 558, 530, 504;

HRMS (*m*/*z*): [M+H]$^+$ calcd for C$_{28}$H$_{34}$N$_5$O$_3$S: 520.2377, found: 520.2370;

HPLC (method 1): t$_R$ = 25.7 min, purity 96.1%.

N-Cyclohexyl-2-[*N*-(3,4-dimethylphenyl)-2-mercaptoacetamido]-2-(*p*-tolyl)acetamide (53e)



C$_{25}$H$_{32}$N$_2$O$_2$S

M$_r$ = 424.6

3,4-Dimethylaniline (242 mg, 2.0 mmol) and 4-methylbenzaldehyde (0.24 mL, 240 mg, 2.0 mmol) were dissolved in dichloromethane (15 mL) and the solution was stirred at ambient temperature for 10 min. Then, sulfanylacetic acid (0.14 mL, 180 mg, 2.0 mmol) and isocyanocyclohexane (0.25 mL, 220 mg, 2.0 mmol) were added. After stirring the reaction mixture for 48 h at ambient temperature, the solvent was removed *in vacuo* and the residue was purified by flash column chromatography (Ø = 2 cm, h = 15 cm, V = 5 mL, dichloromethane/ethyl acetate = 20/1) to give **53e** (450 mg, 1.1 mmol, 53% yield) as colorless solid.

m.p. = 189 °C;

TLC: R_f = 0.47 (dichloromethane/ethyl acetate = 12/1);

^{1}H NMR (400 MHz, DMSO-*d*$_6$): δ [ppm] = 0.89 – 1.33 (m, 5H, NHCH(C*H*$_2$CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$C*H*$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH$_2$)$_2$C*H*$_2$ (1H)), 1.47 – 1.80 (m ,5H, NHCH(C*H*$_2$CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$C*H*$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH$_2$)$_2$C*H*$_2$ (1H)), 1.99 – 2.14 (m, 6H, Ar(C*H*$_3$)$_2$), 2.16 (s, 3H, ArC*H*$_3$), 2.55 (t, *J* = 6.8 Hz, 1H, *H*SCH$_2$CO), 2.93 (dd, *J* = 14.9/6.7 Hz, 1H, HSC*H*$_2$CO), 3.00 (dd, *J* = 14.9/6.9 Hz, 1H, HSC*H*$_2$CO), 3.50 – 3.63 (m, 1H, NHC*H*(CH$_2$CH$_2$)$_2$CH$_2$), 5.96 (s, 1H, NC*H*CO), 6.41 (s br, 1H, H$_{3,4\text{-dimethylphenyl}}$), 6.68 – 7.16 (m, 5H, 2''-H$_{p\text{-tolyl}}$, 3''-H$_{p\text{-tolyl}}$, 5''-H$_{p\text{-tolyl}}$, 6''-H$_{p\text{-tolyl}}$, H$_{3,4\text{-dimethylphenyl}}$ (1H)), 7.52 (s br, 1H, H$_{3,4\text{-dimethylphenyl}}$), 7.87 (d, *J* = 7.7 Hz, 1H, N*H*CH(CH$_2$CH$_2$)$_2$CH$_2$);

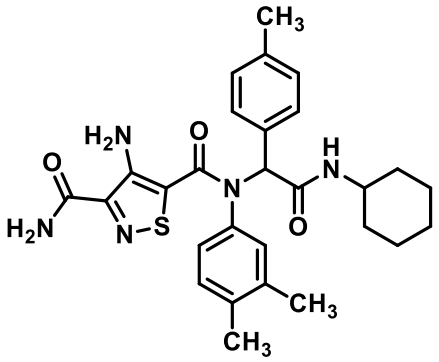
^{13}C NMR (101 MHz, DMSO-*d*$_6$): δ [ppm] = 18.9 (1C, Ar(*C*H$_3$)$_2$), 19.2 (1C, Ar(*C*H$_3$)$_2$), 20.6 (1C, Ar*C*H$_3$), 24.5 (1C, NHCH(CH$_2$*C*H$_2$)$_2$CH$_2$), 24.6 (1C, NHCH(CH$_2$*C*H$_2$)$_2$CH$_2$), 25.2 (1C, NHCH(CH$_2$CH$_2$)$_2$*C*H$_2$), 27.3 (1C, HS*C*H$_2$CO), 32.17 (1C, NHCH(*C*H$_2$CH$_2$)$_2$CH$_2$), 32.21 (1C, NHCH(*C*H$_2$CH$_2$)$_2$CH$_2$), 47.9 (1C, NH*C*H(CH$_2$CH$_2$)$_2$CH$_2$), 63.5 (1C, N*C*HCO), 127.9 (1C, C$_{3,4\text{-dimethylphenyl}}$), 128.4 (2C, C-3''$_{p\text{-tolyl}}$, C-5''$_{p\text{-tolyl}}$), 129.3 (1C, C$_{3,4\text{-dimethylphenyl}}$), 129.9 (2C, C-2''$_{p\text{-tolyl}}$, C-6''$_{p\text{-tolyl}}$), 131.5 (1C, C$_{3,4\text{-dimethylphenyl}}$), 132.4 (1C, C-1''$_{p\text{-tolyl}}$), 135.7 (1C, C$_{3,4\text{-dimethylphenyl}}$), 136.2 (1C, C$_{3,4\text{-dimethylphenyl}}$), 136.7 (1C, C-4''$_{p\text{-tolyl}}$), 136.9 (1C, C-1'$_{3,4\text{-dimethylphenyl}}$), 168.5 (1C, CH*C*ONHCH), 169.1 (1C, HSCH$_2$*C*O);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3298, 2923, 2851, 1650, 1608, 1550, 1517, 1503, 1449, 1374, 1250, 1226, 1184, 1107, 1026, 980, 891, 832, 787, 739, 721, 644, 633, 582, 552, 502;

HRMS (*m*/*z*): [M+H]$^+$ calcd for C$_{25}$H$_{33}$N$_2$O$_2$S: 425.2257, found: 425.2242;

HPLC (method 1): t_R = 25.8 min, purity 99.0%.

4-Amino-N^5-[2-(cyclohexylamino)-2-oxo-1-(*p*-tolyl)ethyl]-N^5-(3,4-dimethylphenyl)isothiazole-3,5-dicarboxamide (54e)



$C_{28}H_{33}N_5O_3S$

$M_r = 519.7$

53e (420 mg, 1.0 mmol) was added in one portion to a solution of **25** (320 mg, 1.2 mmol) in acetone (100 mL). The mixture was cooled to 0 °C and morpholine (0.17 mL, 170 mg, 1.9 mmol) was added dropwise. After stirring the reaction mixture for 12 h at ambient temperature, the solvent was removed *in vacuo*. The residue was purified by flash column chromatography (Ø = 6 cm, h = 20 cm, V = 15 mL, dichloromethane/acetone = 15/1) and recrystallized from acetonitrile to give **54e** (340 mg, 0.65 mmol, 65% yield) as colorless solid.

m.p. = 220 °C;

TLC: R_f = 0.36 (dichloromethane/acetone = 10/1);

^{1}H NMR (400 MHz, DMSO-d_6): δ [ppm] = 0.89 – 1.34 (m, 5H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 1.46 – 1.82 (m, 5H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 1.89 – 2.31 (m, 9H, Ar(CH_3)$_2$, ArCH_3), 3.51 – 3.65 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), 6.10 (s, 1H, NCHCO), 6.45 (s br, 1H, H$_{3,4\text{-dimethylphenyl}}$), 6.88 – 7.07 m, 5H, 2'''-H$_{p\text{-tolyl}}$, 3'''-H$_{p\text{-tolyl}}$, 5'''-H$_{p\text{-tolyl}}$, 6'''-H$_{p\text{-tolyl}}$, H$_{3,4\text{-dimethylphenyl}}$ (1H)), 7.15 (s br, 2H, ArNH_2), 7.40 – 7.73 (m, 2H, CONH_2 (1H), H$_{3,4\text{-dimethylphenyl}}$ (1H)), 7.78 (s br, 1H, CONH_2), 7.94 (d, J = 7.7 Hz, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$);

^{13}C NMR (126 MHz, DMSO-d_6): δ [ppm] = 19.1 (1C, Ar(*C*H$_3$)$_2$), 20.6 (1C, Ar*C*H$_3$), 24.4 (1C, NHCH(CH$_2$*C*H$_2$)$_2$CH$_2$), 24.6 (1C, NHCH(CH$_2$*C*H$_2$)$_2$CH$_2$), 25.2 (1C, NHCH(CH$_2$CH$_2$)$_2$*C*H$_2$), 32.1 (1C, NHCH(*C*H$_2$CH$_2$)$_2$CH$_2$), 32.2 (1C, NHCH(*C*H$_2$CH$_2$)$_2$CH$_2$), 47.9 (1C, NH*C*H(CH$_2$CH$_2$)$_2$CH$_2$), 63.6 (1C, N*C*HCO), 123.2 (1C, C-5'$_{\text{isothiazole}}$), 128.4 (2C, C-3'''$_{p\text{-tolyl}}$, C-

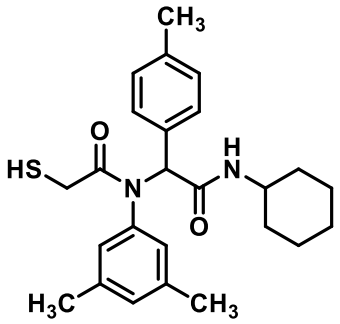
5'''$_{p\text{-tolyl}}$), 130.2 (2C, C-2'''$_{p\text{-tolyl}}$, C-6'''$_{p\text{-tolyl}}$), 130.3 (1C, $C_{3,4\text{-dimethylphenyl}}$), 132.0 (1C, C-1'''$_{p\text{-tolyl}}$), 133.9 (1C, $C_{3,4\text{-dimethylphenyl}}$), 134.8 (1C, $C_{3,4\text{-dimethylphenyl}}$), 136.8 (1C, C-4'''$_{p\text{-tolyl}}$), 137.7 (1C, $C_{3,4\text{-dimethylphenyl}}$), 146.3 (1C, C-3'$_{isothiazole}$), 151.1 (1C, C-4'$_{isothiazole}$), 162.5 (1C, Ar*C*ONCH), 163.9 (1C, Ar*C*ONH$_2$), 168.6 (1C, CH*C*ONHCH), the signals for Ar(*C*H$_3$)$_2$ (1C) and 2 $C_{3,4\text{-dimethylphenyl}}$ cannot be observed in the spectrum;

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3457, 3339, 3284, 3145, 2923, 2850, 1673, 1646, 1614, 1590, 1558, 1517, 1494, 1448, 1403, 1368, 1347, 1327, 1250, 1236, 1211, 1184, 1152, 1128, 1106, 1074, 1025, 821, 798, 758, 741, 718, 682, 652, 633, 552, 532, 502;

HRMS (*m*/*z*): [M+H]$^+$ calcd for $C_{28}H_{34}N_5O_3S$: 520.2377, found: 520.2384;

HPLC (method 1): t_R = 25.4 min, purity 96.4%.

N-Cyclohexyl-2-[*N*-(3,5-dimethylphenyl)-2-mercaptoacetamido]-2-(*p*-tolyl)acetamide (53f)



$C_{25}H_{32}N_2O_2S$

M_r = 424.6

3,5-Dimethylaniline (240 mg, 2.0 mmol) and 4-methylbenzaldehyde (0.24 mL, 240 mg, 2.0 mmol) were dissolved in dichloromethane (15 mL) and the solution was stirred at ambient temperature for 10 min. Then, sulfanylacetic acid (0.14 mL, 180 mg, 2.0 mmol) and isocyanocyclohexane (0.25 mL, 220 mg, 2.0 mmol) were added. After stirring the reaction mixture for 48 h at ambient temperature, the solvent was removed *in vacuo* and the residue was purified by flash column chromatography (Ø = 2 cm, h = 15 cm, V = 5 mL, dichloromethane/ethyl acetate = 20/1) to give **53f** (500 mg, 1.2 mmol, 59% yield) as colorless solid.

m.p. = 170 °C;

TLC: R_f = 0.39 (dichloromethane/ethyl acetate = 15/1);

^{1}H NMR (500 MHz, DMSO-d_6): δ [ppm] = 0.91 – 1.13 (m, 2H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (1H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 1.13 – 1.31 (m, 3H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (1H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H)), 1.47 – 1.79 (m, 5H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 2.09 (s br, 6H, Ar(CH_3)$_2$), 2.16 (s, 3H, ArCH_3), 2.55 (t, J = 6.8 Hz, 1H, HSCH$_2$CO), 2.98 (dd, J = 14.9/6.7 Hz, 1H, HSCH_2CO), 3.03 (dd, J = 14.9/6.9 Hz, 1H, HSCH_2CO), 3.51 – 3.61 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), 5.93 (s, 1H, NCHCO), 6.27 (s br, 1H, H$_{3,5\text{-dimethylphenyl}}$), 6.76 – 6.80 (m, 1H, 4'-H$_{3,5\text{-dimethylphenyl}}$), 6.88 – 6.96 (m, 4H, 2"-H$_{p\text{-tolyl}}$, 3"-H$_{p\text{-tolyl}}$, 5"-H$_{p\text{-tolyl}}$, 6"-H$_{p\text{-tolyl}}$), 7.37 (s br, 1H, H$_{3,5\text{-dimethylphenyl}}$), 7.86 (d, J = 7.7 Hz, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$);

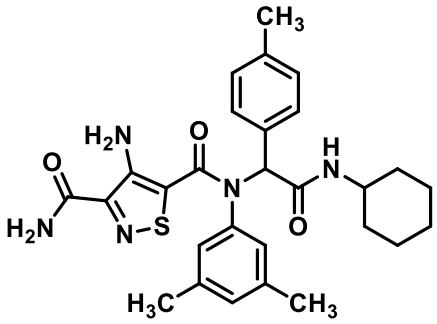
^{13}C NMR (101 MHz, DMSO-d_6): δ [ppm] = 20.6 (3C, ArCH$_3$, Ar(CH$_3$)$_2$), 24.5 (1C, NHCH(CH$_2$$CH_2$)$_2CH_2$), 24.6 (1C, NHCH(CH$_2$$CH_2$)$_2CH_2$), 25.2 (1C, NHCH(CH$_2CH_2$)$_2$$CH_2$), 27.3 (1C, HS$CH_2$CO), 32.18 (1C, NHCH($CH_2CH_2$)$_2CH_2$), 32.22 (1C, NHCH($CH_2CH_2$)$_2CH_2$), 47.9 (1C, NH$C$H(CH$_2CH_2$)$_2CH_2$), 63.7 (1C, N$C$HCO), 128.2 (4C, C-3"$_{p\text{-tolyl}}$, C-5"$_{p\text{-tolyl}}$, C-2'$_{3,5\text{-dimethylphenyl}}$, C-6'$_{3,5\text{-dimethylphenyl}}$), 129.1 (1C, C-4'$_{3,5\text{-dimethylphenyl}}$), 129.9 (2C, C-2"$_{p\text{-tolyl}}$, C-6"$_{p\text{-tolyl}}$), 132.3 (1C, C-1"$_{p\text{-tolyl}}$), 136.7 (1C, C-4"$_{p\text{-tolyl}}$), 137.3 (2C, C-3'$_{3,5\text{-dimethylphenyl}}$, C-5'$_{3,5\text{-dimethylphenyl}}$), 139.1 (1C, C-1'$_{3,5\text{-dimethylphenyl}}$), 168.4 (1C, CH$C$ONHCH), 168.8 (1C, HSCH$_2$$C$O);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3290, 2924, 2853, 2543, 1647, 1611, 1595, 1548, 1515, 1450, 1408, 1372, 1336, 1231, 1199, 1146, 1095, 1038, 985, 965, 929, 891, 863, 809, 750, 734, 710, 641, 631, 503;

HRMS (m/z): [M+H]$^+$ calcd for $C_{25}H_{33}N_2O_2S$: 425.2257, found: 425.2259;

HPLC (method 1): t_R = 26.0 min, purity 98.6%.

4-Amino-N^5-[2-(cyclohexylamino)-2-oxo-1-(*p*-tolyl)ethyl]-N^5-(3,5-dimethylphenyl)isothiazole-3,5-dicarboxamide (54f)



$C_{28}H_{33}N_5O_3S$

M_r = 519.7

53f (420 mg, 1.0 mmol) was added in one portion to a solution of **25** (320 mg, 1.2 mmol) in acetone (100 mL). The mixture was cooled to 0 °C and morpholine (0.17 mL, 170 mg, 1.9 mmol) was added dropwise. After stirring the reaction mixture for 12 h at ambient temperature, the solvent was removed *in vacuo*. The residue was purified by flash column chromatography (Ø = 6 cm, h = 20 cm, V = 15 mL, dichloromethane/acetone = 15/1) and recrystallized from acetonitrile to give **54f** (290 mg, 0.57 mmol, 57% yield) as colorless solid.

m.p. = 232 °C;

TLC: R_f = 0.33 (dichloromethane/acetone = 10/1);

^{1}H NMR (400 MHz, DMSO-d_6): δ [ppm] = 0.89 – 1.33 (m, 5H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 1.46 – 1.81 (m, 5H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 2.12 (s br, 6H, Ar(CH_3)$_2$), 2.17 (s, 3H, ArCH_3), 3.52 – 3.65 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), 6.07 (s, 1H, NCHCO), 6.34 (s br, 1H, H$_{3,5\text{-dimethylphenyl}}$), 6.84 – 7.01 (m, 5H, 2'''-H$_{p\text{-tolyl}}$, 3'''-H$_{p\text{-tolyl}}$, 5'''-H$_{p\text{-tolyl}}$, 6'''-H$_{p\text{-tolyl}}$, 4''-H$_{3,5\text{-dimethylphenyl}}$), 7.16 (s br, 2H, ArN$H_2$), 7.46 (s br, 1H, H$_{3,5\text{-dimethylphenyl}}$), 7.57 (s br, 1H, CON$H_2$), 7.78 (s br, 1H, CON$H_2$), 7.95 (d, J = 7.7 Hz, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$);

^{13}C NMR (101 MHz, DMSO-d_6): δ [ppm] = 20.6 (3C, ArCH$_3$, Ar(CH$_3$)$_2$), 24.5 (1C, NHCH(CH$_2$$CH_2$)$_2CH_2$), 24.6 (1C, NHCH(CH$_2$$CH_2$)$_2CH_2$), 25.2 (1C, NHCH(CH$_2CH_2$)$_2$$CH_2$), 32.17 (1C, NHCH($CH_2CH_2$)$_2CH_2$), 32.24 (1C, NHCH($CH_2CH_2$)$_2CH_2$), 48.0 (1C, NH$C$H(CH$_2CH_2$)$_2CH_2$), 63.7 (1C, N$C$HCO), 123.2 (1C, C-5'$_{\text{isothiazole}}$), 128.3 (2C, C-3'''$_{p\text{-tolyl}}$, C-

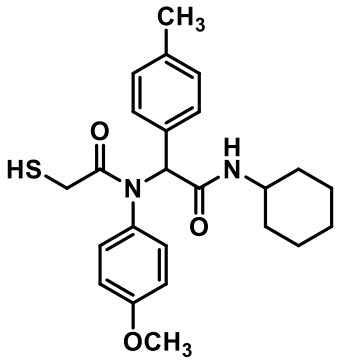
5'''$_{p\text{-tolyl}}$), 130.3 (2C, C-2'''$_{p\text{-tolyl}}$, C-6'''$_{p\text{-tolyl}}$), 130.67 (2C, C-2''$_{3,5\text{-dimethylphenyl}}$, C-6''$_{3,5\text{-dimethylphenyl}}$), 130.71 (1C, C-4''$_{3,5\text{-dimethylphenyl}}$), 131.9 (1C, C-1'''$_{p\text{-tolyl}}$), 136.9 (1C, $C_{arom.}$), 137.0 (1C, $C_{arom.}$), 138.0 (2C, C-3''$_{3,5\text{-dimethylphenyl}}$, C-5''$_{3,5\text{-dimethylphenyl}}$), 146.3 (1C, C-3'$_{isothiazole}$), 151.2 (1C, C-4'$_{isothiazole}$), 162.3 (1C, Ar*C*ONCH), 163.9 (1C, Ar*C*ONH$_2$), 168.6 (1C, CH*C*ONHCH);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3450, 3303, 3165, 2927, 2850, 1673, 1649, 1610, 1587, 1548, 1493, 1448, 1369, 1346, 1328, 1267, 1251, 1235, 1203, 1155, 1107, 1065, 848, 810, 756, 733, 710, 684, 530, 498;

HRMS (*m*/*z*): [M+H]$^+$ calcd for $C_{28}H_{34}N_5O_3S$: 520.2377, found: 520.2374;

HPLC (method 1): t_R = 25.7 min, purity 97.2%.

N-Cyclohexyl-2-[2-mercapto-*N*-(4-methoxyphenyl)acetamido]-2-(*p*-tolyl)acetamide (53g)



$C_{24}H_{30}N_2O_3S$

M_r = 426.6

4-Methoxyaniline (250 mg, 2.0 mmol) and 4-methylbenzaldehyde (0.24 mL, 240 mg, 2.0 mmol) were dissolved in dichloromethane (15 mL) and the solution was stirred at ambient temperature for 10 min. Then, sulfanylacetic acid (0.14 mL, 180 mg, 2.0 mmol) and isocyanocyclohexane (0.25 mL, 220 mg, 2.0 mmol) were added. After stirring the reaction mixture for 48 h at ambient temperature, the solvent was removed *in vacuo* and the residue was purified by flash column chromatography (Ø = 2 cm, h = 15 cm, V = 5 mL, dichloromethane/ethyl acetate = 20/1) to give **53g** (500 mg, 1.2 mmol, 59% yield) as colorless solid.

m.p. = 176 °C;

TLC: R_f = 0.39 (dichloromethane/ethyl acetate = 12/1);

^{1}H NMR (400 MHz, DMSO-d_6): δ [ppm] = 0.88 – 1.33 (m, 5H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 1.47 – 1.80 (m ,5H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 2.16 (s, 3H, ArCH_3), 2.56 (t, J = 6.8 Hz, 1H, HSCH$_2$CO), 2.94 (dd, J = 14.9/6.7 Hz, 1H, HSCH_2CO), 3.01 (dd, J = 14.9/6.9 Hz, 1H, HSCH_2CO), 3.51 – 3.62 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), 3.65 (s, 3H, ArOCH_3), 5.96 (s, 1H, NCHCO), 6.37 – 6.87 (m, 3H, H$_{4\text{-methoxyphenyl}}$), 6.87 – 6.92 (m, 2H, 2''-H$_{p\text{-tolyl}}$, 6''-H$_{p\text{-tolyl}}$), 6.92 – 6.97 (m, 2H, 3''-H$_{p\text{-tolyl}}$, 5''-H$_{p\text{-tolyl}}$), 7.72 (s br, 1H, H$_{4\text{-methoxyphenyl}}$), 7.88 (d, J = 7.7 Hz, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$);

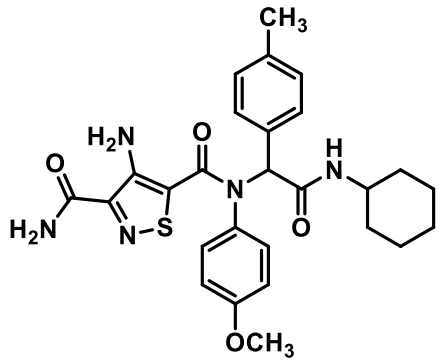
^{13}C NMR (101 MHz, DMSO-d_6): δ [ppm] = 20.6 (1C, ArCH$_3$), 24.5 (1C, NHCH(CH$_2$$CH_2$)$_2CH_2$), 24.6 (1C, NHCH(CH$_2$$CH_2$)$_2CH_2$), 25.2 (1C, NHCH(CH$_2CH_2$)$_2$$CH_2$), 27.3 (1C, HS$CH_2$CO), 32.17 (1C, NHCH(C$H_2CH_2$)$_2CH_2$), 32.21 (1C, NHCH(C$H_2CH_2$)$_2CH_2$), 47.9 (1C, NH$C$H(CH$_2CH_2$)$_2CH_2$), 55.1 (1C, ArO$CH_3$), 63.5 (1C, N$C$HCO), 113.5 (2C, C-3'$_{4\text{-methoxyphenyl}}$, C-5'$_{4\text{-methoxyphenyl}}$), 128.4 (2C, C-3''$_{p\text{-tolyl}}$, C-5''$_{p\text{-tolyl}}$), 130.0 (2C, C-2''$_{p\text{-tolyl}}$, C-6''$_{p\text{-tolyl}}$), 131.8 (1C, C-1'$_{4\text{-methoxyphenyl}}$), 131.9 (2C, C-2'$_{4\text{-methoxyphenyl}}$, C-6'$_{4\text{-methoxyphenyl}}$), 132.3 (1C, C-1''$_{p\text{-tolyl}}$), 136.7 (1C, C-4''$_{p\text{-tolyl}}$), 158.2 (1C, C-4'$_{4\text{-methoxyphenyl}}$), 168.6 (1C, CH$C$ONHCH), 169.3 (1C, HSCH$_2$$C$O);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3278, 2927, 2850, 1649, 1557, 1509, 1453, 1443, 1405, 1375, 1364, 1328, 1296, 1250, 1233, 1179, 1167, 1104, 1058, 1029, 977, 889, 840, 802, 742, 627, 598, 564, 540, 504;

HRMS (m/z): [M+H]$^+$ calcd for $C_{24}H_{31}N_2O_3S$: 427.2050, found: 427.2009;

HPLC (method 1): t_R = 24.2 min, purity 97.3%.

4-Amino-N^5-[2-(cyclohexylamino)-2-oxo-1-(*p*-tolyl)ethyl]-N^5-(4-methoxyphenyl)isothiazole-3,5-dicarboxamide (54g)



$C_{27}H_{31}N_5O_4S$

$M_r = 521.6$

53g (430 mg, 1.0 mmol) was added in one portion to a solution of **25** (320 mg, 1.2 mmol) in acetone (100 mL). The mixture was cooled to 0 °C and morpholine (0.17 mL, 170 mg, 1.9 mmol) was added dropwise. After stirring the reaction mixture for 12 h at ambient temperature, the solvent was removed *in vacuo*. The residue was purified by flash column chromatography (Ø = 6 cm, h = 20 cm, V = 15 mL, dichloromethane/acetone = 10/1) and recrystallized from acetonitrile to give **54g** (450 mg, 0.87 mmol, 87% yield) as colorless solid.

m.p. = 235 °C;

TLC: R_f = 0.22 (dichloromethane/acetone = 10/1);

^{1}H NMR (400 MHz, DMSO-d_6): δ [ppm] = 0.89 – 1.13 (m, 2H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (1H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 1.13 – 1.33 (m, 3H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (1H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H)), 1.47 – 1.81 (m, 5H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 2.17 (s, 3H, ArCH_3), 3.53 – 3.65 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), 3.71 (s, 3H, ArOCH_3), 6.10 (s, 1H, NCHCO), 6.64 (s br, 2H, H$_{4\text{-methoxyphenyl}}$), 6.79 – 7.02 (m, 5H, 2'''-H$_{p\text{-tolyl}}$, 3'''-H$_{p\text{-tolyl}}$, 5'''-H$_{p\text{-tolyl}}$, 6'''-H$_{p\text{-tolyl}}$, H$_{4\text{-methoxyphenyl}}$ (1H)), 7.16 (s br, 2H, ArNH_2), 7.57 (s br, 1H, CONH_2), 7.70 – 7.91 (m, 2H, CONH_2 (1H), H$_{4\text{-methoxyphenyl}}$ (1H)), 7.95 (d, J = 7.7 Hz, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$);

^{13}C NMR (126 MHz, DMSO-d_6): δ [ppm] = 20.6 (1C, ArCH$_3$), 24.4 (1C, NHCH(CH$_2$$CH_2$)$_2CH_2$), 24.6 (1C, NHCH(CH$_2$$CH_2$)$_2CH_2$), 25.2 (1C, NHCH(CH$_2CH_2$)$_2$$CH_2$), 32.1 (1C, NHCH($CH_2CH_2$)$_2CH_2$), 32.2 (1C, NHCH($CH_2CH_2$)$_2CH_2$), 47.9 (1C, NH$C$H(CH$_2CH_2$)$_2CH_2$), 55.3 (1C, ArO$CH_3$), 63.6 (1C, N$C$HCO), 114.0 (2C, C-3''$_{4\text{-methoxyphenyl}}$, C-5''$_{4\text{-methoxyphenyl}}$), 123.1 (1C, C-5'$_{\text{isothiazole}}$), 128.5 (2C, C-3'''$_{p\text{-tolyl}}$, C-5'''$_{p\text{-tolyl}}$), 129.8 (1C, C-1''$_{4\text{-methoxyphenyl}}$), 130.2 (2C, C-2'''$_{p\text{-tolyl}}$, C-6'''$_{p\text{-tolyl}}$), 132.0 (1C, C-1'''$_{p\text{-tolyl}}$), 134.5 (2C, C-2''$_{4\text{-methoxyphenyl}}$, C-6''$_{4\text{-methoxyphenyl}}$), 136.8 (1C, C-4'''$_{p\text{-tolyl}}$), 146.4 (1C, C-3'$_{\text{isothiazole}}$), 151.1 (1C, C-

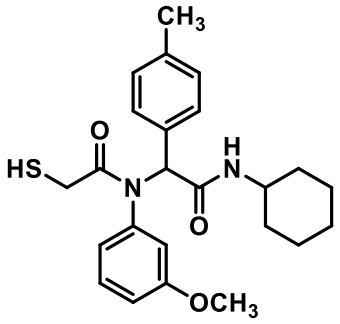
4'$_{isothiazole}$), 159.9 (1C, C-4"$_{4\text{-methoxyphenyl}}$), 162.6 (1C, Ar*C*ONCH), 163.9 (1C, Ar*C*ONH$_2$), 168.7 (1C, CH*C*ONHCH);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3451, 3335, 3262, 3083, 2928, 2850, 1668, 1644, 1613, 1561, 1509, 1471, 1445, 1405, 1366, 1351, 1328, 1301, 1249, 1240, 1183, 1170, 1152, 1105, 1073, 1027, 978, 838, 827, 805, 796, 761, 747, 678, 652, 635, 616, 602, 568, 536, 505;

HRMS (*m*/*z*): [M+H]$^+$ calcd for $C_{27}H_{32}N_5O_4S$: 522.2170, found: 522.2169;

HPLC (method 1): t_R = 24.0 min, purity 95.0%.

_N_-Cyclohexyl-2-[2-mercapto-_N_-(3-methoxyphenyl)acetamido]-2-(_p_-tolyl)acetamide (53h)



$C_{24}H_{30}N_2O_3S$

M_r = 426.6

3-Methoxyaniline (0.22 mL, 250 mg, 2.0 mmol) and 4-methylbenzaldehyde (0.24 mL, 240 mg, 2.0 mmol) were dissolved in dichloromethane (15 mL) and the solution was stirred at ambient temperature for 10 min. Then, sulfanylacetic acid (0.14 mL, 180 mg, 2.0 mmol) and isocyanocyclohexane (0.25 mL, 220 mg, 2.0 mmol) were added. After stirring the reaction mixture for 48 h at ambient temperature, the solvent was removed *in vacuo* and the residue was purified by flash column chromatography (Ø = 2 cm, h = 15 cm, V = 5 mL, dichloromethane/ethyl acetate = 20/1) to give **53h** (600 mg, 1.4 mmol, 70% yield) as colorless solid.

m.p. = 196 °C;

TLC: R_f = 0.62 (dichloromethane/ethyl acetate = 15/1);

^{1}H NMR (500 MHz, DMSO-d_6): δ [ppm] = 0.92 – 1.02 (m, 1H, NHCH(C*H*$_2$CH$_2$)$_2$CH$_2$), 1.02 – 1.12 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$C*H*$_2$), 1.13 – 1.31 (m, 3H, NHCH(C*H*$_2$CH$_2$)$_2$CH$_2$ (1H),

NHCH(CH_2CH_2)$_2CH_2$ (2H)), 1.48 – 1.79 (m, 5H, NHCH(CH_2CH_2)$_2CH_2$ (2H), NHCH(CH_2CH_2)$_2CH_2$ (2H), NHCH(CH_2CH_2)$_2CH_2$ (1H)), 2.15 (s, 3H, ArCH_3), 2.58 (t, J = 6.8 Hz, 1H, HSCH_2CO), 3.00 (dd, J = 14.9/6.7 Hz, 1H, HSCH_2CO), 3.07 (dd, J = 14.9/6.9 Hz, 1H, HSCH_2CO), 3.45 – 3.74 (m, 4H, NHCH(CH_2CH_2)$_2CH_2$, ArOCH_3), 5.97 (s, 1H, NCHCO), 6.32 (s br, 1H, $H_{3\text{-methoxyphenyl}}$), 6.68 – 6.74 (m, 1H, 4'-$H_{3\text{-methoxyphenyl}}$), 6.83 – 6.97 (m, 4H, 2"-$H_{p\text{-tolyl}}$, 3"-$H_{p\text{-tolyl}}$, 5"-$H_{p\text{-tolyl}}$, 6"-$H_{p\text{-tolyl}}$), 7.05 (s br, 1H, $H_{3\text{-methoxyphenyl}}$), 7.37 (s br, 1H, $H_{3\text{-methoxyphenyl}}$), 7.90 (d, J = 7.5 Hz, 1H, NHCH(CH_2CH_2)$_2CH_2$);

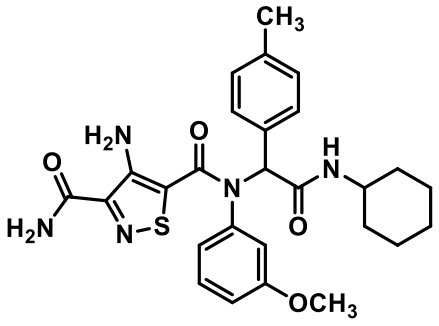
^{13}C NMR (126 MHz, DMSO-d_6): δ [ppm] = 20.6 (1C, ArCH_3), 24.4 (1C, NHCH(CH_2CH_2)$_2CH_2$), 24.6 (1C, NHCH(CH_2CH_2)$_2CH_2$), 25.2 (1C, NHCH(CH_2CH_2)$_2CH_2$), 27.2 (1C, HSCH_2CO), 32.2 (2C, NHCH(CH_2CH_2)$_2CH_2$), 47.9 (1C, NHCH(CH_2CH_2)$_2CH_2$), 55.0 (1C, ArOCH_3), 63.6 (1C, NCHCO), 113.8 (1C, C-4'$_{3\text{-methoxyphenyl}}$), 116.3 (1C, $C_{3\text{-methoxyphenyl}}$), 123.0 (1C, $C_{3\text{-methoxyphenyl}}$), 128.3 (2C, C-3"$_{p\text{-tolyl}}$, C-5"$_{p\text{-tolyl}}$), 128.9 (1C, $C_{3\text{-methoxyphenyl}}$), 130.0 (2C, C-2"$_{p\text{-tolyl}}$, C-6"$_{p\text{-tolyl}}$), 132.1 (1C, C-1"$_{p\text{-tolyl}}$), 136.7 (1C, C-4"$_{p\text{-tolyl}}$), 140.3 (1C, C-1'$_{3\text{-methoxyphenyl}}$), 158.9 (1C, C-3'$_{3\text{-methoxyphenyl}}$), 168.5 (1C, CH$C$ONHCH), 168.8 (1C, HS$CH_2C$O);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3274, 3081, 2928, 2850, 1650, 1601, 1584, 1557, 1486, 1373, 1358, 1232, 1206, 1175, 1039, 816, 732, 705, 651, 555, 505;

HRMS (m/z): [M+H]$^+$ calcd for $C_{24}H_{31}N_2O_3S$: 427.2050, found: 427.2044;

HPLC (method 1): t_R = 24.2 min, purity 97.5%.

4-Amino-N^5-[2-(cyclohexylamino)-2-oxo-1-(p-tolyl)ethyl]-N^5-(3-methoxyphenyl)isothiazole-3,5-dicarboxamide (54h)



$C_{27}H_{31}N_5O_4S$

M_r = 521.6

53h (430 mg, 1.0 mmol) was added in one portion to a solution of **25** (320 mg, 1.2 mmol) in acetone (100 mL). The mixture was cooled to 0 °C and morpholine (0.17 mL, 170 mg, 1.9 mmol) was added dropwise. After stirring the reaction mixture for 12 h at ambient temperature, the solvent was removed *in vacuo*. The residue was purified by flash column chromatography (Ø = 6 cm, h = 20 cm, V = 15 mL, dichloromethane/acetone = 15/1) and recrystallized from acetonitrile to give **54h** (460 mg, 0.89 mmol, 89% yield) as colorless solid.

m.p. = 250 °C;

TLC: R_f = 0.47 (dichloromethane/ethyl acetate = 4/1);

^{1}H NMR (400 MHz, DMSO-d_6): δ [ppm] = 0.90 – 1.13 (m, 2H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (1H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 1.13 – 1.33 (m, 3H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (1H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H)), 1.47 – 1.83 (m, 5H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 2.16 (s, 3H, ArCH_3), 3.45 – 3.75 (m, 4H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$, ArOCH_3), 6.11 (s, 1H, NCHCO), 6.85 – 6.91 (m, 1H, 4''-H$_{3\text{-methoxyphenyl}}$), 6.91 – 6.97 (m, 2H, 3'''-H$_{p\text{-tolyl}}$, 5'''-H$_{p\text{-tolyl}}$), 6.97 – 7.02 (m, 2H, 2'''-H$_{p\text{-tolyl}}$, 6'''-H$_{p\text{-tolyl}}$), 7.04 – 7.25 (m, 3H, ArNH_2, H$_{3\text{-methoxyphenyl}}$ (1H)), 7.59 (s br, 1H, CONH_2), 7.83 (s br, 1H, CONH_2), 8.00 (d, J = 7.7 Hz, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), the signals for 2 H$_{3\text{-methoxyphenyl}}$ cannot be observed in the spectrum;

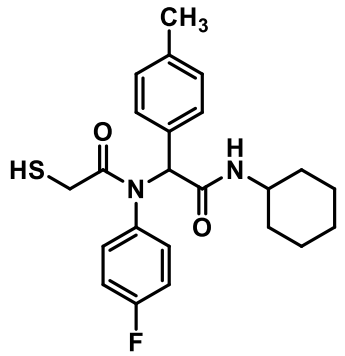
^{13}C NMR (126 MHz, DMSO-d_6): δ [ppm] = 20.6 (1C, ArCH$_3$), 24.4 (1C, NHCH(CH$_2$$CH_2$)$_2CH_2$), 24.6 (1C, NHCH(CH$_2$$CH_2$)$_2CH_2$), 25.2 (1C, NHCH(CH$_2CH_2$)$_2$$CH_2$), 32.2 (2C, NHCH($CH_2CH_2$)$_2CH_2$), 47.9 (1C, NH$C$H(CH$_2CH_2$)$_2CH_2$), 55.2 (1C, ArO$CH_3$), 63.7 (1C, N$C$HCO), 115.5 (1C, C-4''$_{3\text{-methoxyphenyl}}$), 118.7 (1C, C$_{3\text{-methoxyphenyl}}$), 123.0 (1C, C-5'$_{\text{isothiazole}}$), 125.5 (1C, C$_{3\text{-methoxyphenyl}}$), 128.4 (2C, C-3'''$_{p\text{-tolyl}}$, C-5'''$_{p\text{-tolyl}}$), 129.5 (1C, C-5''$_{3\text{-methoxyphenyl}}$), 130.3 (2C, C-2'''$_{p\text{-tolyl}}$, C-6'''$_{p\text{-tolyl}}$), 131.8 (1C, C-1'''$_{p\text{-tolyl}}$), 136.9 (1C, C-4'''$_{p\text{-tolyl}}$), 138.2 (1C, C-1''$_{3\text{-methoxyphenyl}}$), 146.4 (1C, C-3'$_{\text{isothiazole}}$), 151.2 (1C, C-4'$_{\text{isothiazole}}$), 159.4 (1C, C-3''$_{3\text{-methoxyphenyl}}$), 162.2 (1C, Ar$C$ONCH), 163.9 (1C, Ar$CONH_2$), 168.6 (1C, CH$C$ONHCH);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3448, 3267, 3080, 2928, 2850, 1671, 1646, 1582, 1559, 1485, 1449, 1369, 1346, 1327, 1283, 1252, 1208, 1178, 1039, 738, 706, 677, 555, 530, 505;

HRMS (m/z): [M+H]$^+$ calcd for $C_{27}H_{32}N_5O_4S$: 522.2170, found: 522.2177;

HPLC (method 1): t_R = 24.0 min, purity 96.1%.

_N_-Cyclohexyl-2-[_N_-(4-fluorophenyl)-2-mercaptoacetamido]-2-(_p_-tolyl)acetamide (53i)



$C_{23}H_{27}FN_2O_2S$

$M_r = 414.5$

4-Fluoroaniline (220 mg, 2.0 mmol) and 4-methylbenzaldehyde (0.24 mL, 240 mg, 2.0 mmol) were dissolved in dichloromethane (15 mL) and the solution was stirred at ambient temperature for 10 min. Then, sulfanylacetic acid (0.14 mL, 180 mg, 2.0 mmol) and isocyanocyclohexane (0.25 mL, 220 mg, 2.0 mmol) were added. After stirring the reaction mixture for 48 h at ambient temperature, the solvent was removed *in vacuo* and the residue was purified by flash column chromatography (Ø = 2 cm, h = 15 cm, V = 5 mL, dichloromethane/ethyl acetate = 15/1) to give **53i** (710 mg, 1.7 mmol, 86% yield) as colorless solid.

m.p. = 222 °C;

TLC: R_f = 0.26 (dichloromethane/ethyl acetate = 15/1);

^{1}H NMR (500 MHz, DMSO-d_6): δ [ppm] = 0.91 – 1.01 (m, 1H, NHCH(CH_2CH$_2$)$_2$CH$_2$), 1.01 – 1.12 (m, 1H, NHCH(CH$_2$CH_2)$_2$CH_2), 1.13 – 1.31 (m, 3H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (1H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H)), 1.48 – 1.78 (m, 5H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 2.16 (s, 3H, ArCH_3), 2.59 (t, J = 6.8 Hz, 1H, HSCH$_2$CO), 2.96 (dd, J = 14.9/6.7 Hz, 1H, HSCH_2CO), 3.04 (dd, J = 14.9/6.9 Hz, 1H, HSCH_2CO), 3.52 – 3.63 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), 5.98 (s, 1H, NCHCO), 6.72 (s br, 1H, H$_{4\text{-fluorophenyl}}$), 6.87 – 6.92 (m, 2H, 2''-H$_{p\text{-tolyl}}$, 6''-H$_{p\text{-tolyl}}$), 6.92 – 6.97 (m, 2H, 3''-H$_{p\text{-tolyl}}$, 5''-H$_{p\text{-tolyl}}$), 7.04 (s br, 2H, 3'-H$_{4\text{-fluorophenyl}}$, 5'-H$_{4\text{-fluorophenyl}}$), 7.87 (s br, 1H, H$_{4\text{-fluorophenyl}}$), 7.94 (d, J = 7.7 Hz, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$);

^{13}C NMR (126 MHz, DMSO-d_6): δ [ppm] = 20.6 (1C, ArCH$_3$), 24.4 (1C, NHCH(CH$_2$$CH_2$)$_2CH_2$), 24.6 (1C, NHCH(CH$_2$$CH_2$)$_2CH_2$), 25.2 (1C, NHCH(CH$_2CH_2$)$_2$$CH_2$),

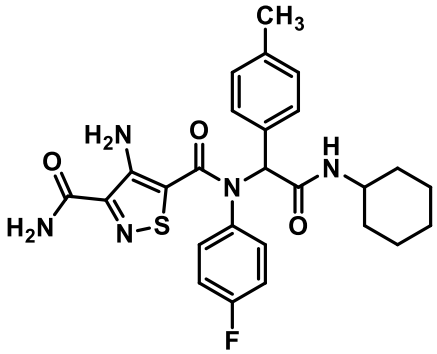
27.4 (1C, HS*C*H$_2$CO), 32.1 (1C, NHCH(*C*H$_2$CH$_2$)$_2$CH$_2$), 32.2 (1C, NHCH(*C*H$_2$CH$_2$)$_2$CH$_2$), 47.9 (1C, NH*C*H(CH$_2$CH$_2$)$_2$CH$_2$), 63.5 (1C, N*C*HCO), 115.1 (d, J = 22.1 Hz, 2C, C-3'$_{4\text{-fluorophenyl}}$, C-5'$_{4\text{-fluorophenyl}}$), 128.5 (2C, C-3"$_{p\text{-tolyl}}$, C-5"$_{p\text{-tolyl}}$), 130.0 (2C, C-2"$_{p\text{-tolyl}}$, C-6"$_{p\text{-tolyl}}$), 132.1 (1C, C-1"$_{p\text{-tolyl}}$), 133.0 (2C, C-2'$_{4\text{-fluorophenyl}}$, C-6'$_{4\text{-fluorophenyl}}$), 135.5 (1C, C-1'$_{4\text{-fluorophenyl}}$), 136.9 (1C, C-4"$_{p\text{-tolyl}}$), 161.0 (d, J = 244.2 Hz, 1C, C-4'$_{4\text{-fluorophenyl}}$), 168.6 (1C, CH*C*ONHCH), 169.0 (1C, HSCH$_2$*C*O);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3270, 3080, 2925, 2853, 1660, 1645, 1556, 1505, 1446, 1402, 1377, 1355, 1328, 1284, 1234, 1218, 1188, 1147, 1104, 1087, 1050, 1016, 978, 891, 843, 815, 784, 739, 707, 668, 640, 625, 591, 561, 532, 504;

HRMS (*m*/*z*): [M+H]$^+$ calcd for $C_{23}H_{28}FN_2O_2S$: 415.1850, found: 415.1855;

HPLC (method 1): t_R = 24.6 min, purity 97.8%.

4-Amino-*N*^{5}-[2-(cyclohexylamino)-2-oxo-1-(*p*-tolyl)ethyl]-*N*^{5}-(4-fluorophenyl)isothiazole-3,5-dicarboxamide (54i)



$C_{26}H_{28}FN_5O_3S$

M_r = 509.6

53i (410 mg, 1.0 mmol) was added in one portion to a solution of **25** (320 mg, 1.2 mmol) in acetone (100 mL). The mixture was cooled to 0 °C and morpholine (0.17 mL, 170 mg, 1.9 mmol) was added dropwise. After stirring the reaction mixture for 12 h at ambient temperature, the solvent was removed *in vacuo*. The residue was purified by flash column chromatography (Ø = 6 cm, h = 20 cm, V = 15 mL, dichloromethane/acetone = 10/1) and recrystallized from acetonitrile to give **54i** (320 mg, 0.63 mmol, 63% yield) as colorless solid.

m.p. = 250 °C;

TLC: R_f = 0.21 (dichloromethane/acetone = 10/1);

^{1}H NMR (500 MHz, DMSO-d_6): δ [ppm] = 0.91 – 1.02 (m, 1H, NHCH(CH_2CH$_2$)$_2$CH$_2$), 1.02 – 1.12 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$CH_2), 1.14 – 1.32 (m, 3H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (1H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H)), 1.48 – 1.62 (m, 2H, NHCH(CH$_2$CH_2)$_2$CH$_2$ (1H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 1.62 – 1.73 (m, 2H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (1H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (1H)), 1.73 – 1.81 (m, 1H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (1H)), 2.17 (s, 3H, ArCH_3), 3.55 – 3.64 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), 6.12 (s, 1H, NCHCO), 6.58 – 7.36 (m, 9H, 2'''-H$_{p\text{-tolyl}}$, 3'''-H$_{p\text{-tolyl}}$, 5'''-H$_{p\text{-tolyl}}$, 6'''-H$_{p\text{-tolyl}}$, ArNH_2, H$_{4\text{-fluorophenyl}}$ (3H)), 7.60 (s br, 1H, CONH_2), 7.72 – 8.14 (m, 3H, CONH_2 (1H), H$_{4\text{-fluorophenyl}}$ (1H), NHCH(CH$_2$CH$_2$)$_2$CH$_2$);

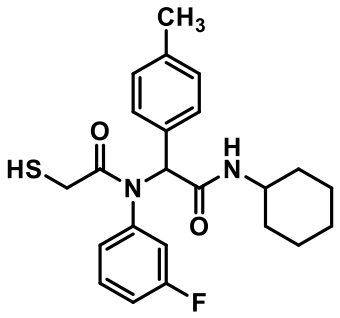
^{13}C NMR (101 MHz, DMSO-d_6): δ [ppm] = 20.6 (1C, ArCH$_3$), 24.4 (1C, NHCH(CH$_2$$CH_2$)$_2CH_2$), 24.6 (1C, NHCH(CH$_2$$CH_2$)$_2CH_2$), 25.2 (1C, NHCH(CH$_2CH_2$)$_2$$CH_2$), 32.1 (1C, NHCH($CH_2CH_2$)$_2CH_2$), 32.2 (1C, NHCH($CH_2CH_2$)$_2CH_2$), 48.0 (1C, NH$C$H(CH$_2CH_2$)$_2CH_2$), 63.6 (1C, N$C$HCO), 115.7 (d, J = 22.3 Hz, 2C, C-3"$_{4\text{-fluorophenyl}}$, C-5"$_{4\text{-fluorophenyl}}$), 122.7 (1C, C-5'$_{isothiazole}$), 128.5 (2C, C-3'''$_{p\text{-tolyl}}$, C-5'''$_{p\text{-tolyl}}$), 130.2 (2C, C-2'''$_{p\text{-tolyl}}$, C-6'''$_{p\text{-tolyl}}$), 131.7 (1C, C-1'''$_{p\text{-tolyl}}$), 133.7 (d, J = 2.5 Hz, 1C, C-1"$_{4\text{-fluorophenyl}}$), 135.6 (d, J = 9.3 Hz, 2C, C-2"$_{4\text{-fluorophenyl}}$, C-6"$_{4\text{-fluorophenyl}}$), 137.0 (1C, C-4'''$_{p\text{-tolyl}}$), 146.7 (1C, C-3'$_{isothiazole}$), 151.3 (1C, C-4'$_{isothiazole}$), 162.2 (1C, ArCONCH), 162.3 (d, J = 247.6 Hz, 1C, C-4"$_{4\text{-fluorophenyl}}$), 163.8 (1C, ArCONH$_2$), 168.6 (1C, CHCONHCH);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3455, 3340, 3279, 3080, 2928, 2850, 1672, 1646, 1609, 1558, 1504, 1447, 1371, 1346, 1325, 1308, 1289, 1250, 1239, 1219, 1187, 1150, 1105, 1091, 1072, 977, 842, 814, 761, 742, 674, 650, 632, 611, 567, 533, 504;

HRMS (m/z): [M+H]$^+$ calcd for $C_{26}H_{29}FN_5O_3S$: 510.1970, found: 510.1977;

HPLC (method 1): t_R = 24.4 min, purity 97.7%.

_N_-Cyclohexyl-2-[_N_-(3-fluorophenyl)-2-mercaptoacetamido]-2-(_p_-tolyl)acetamide (53j)



$C_{23}H_{27}FN_2O_2S$

$M_r = 414.5$

3-Fluoroaniline (220 mg, 2.0 mmol) and 4-methylbenzaldehyde (0.24 mL, 240 mg, 2.0 mmol) were dissolved in dichloromethane (15 mL) and the solution was stirred at ambient temperature for 10 min. Then, sulfanylacetic acid (0.14 mL, 180 mg, 2.0 mmol) and isocyanocyclohexane (0.25 mL, 220 mg, 2.0 mmol) were added. After stirring the reaction mixture for 48 h at ambient temperature, the solvent was removed *in vacuo* and the residue was purified by flash column chromatography (Ø = 2 cm, h = 15 cm, V = 5 mL, dichloromethane/ethyl acetate = 20/1) to give **53j** (630 mg, 1.5 mmol, 76% yield) as colorless solid.

m.p. = 239 °C;

TLC: R_f = 0.36 (dichloromethane/ethyl acetate = 15/1);

^{1}H NMR (500 MHz, DMSO-d_6): δ [ppm] = 0.91 – 1.02 (m, 1H, NHCH(CH_2CH$_2$)$_2$CH$_2$), 1.02 – 1.12 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$CH_2), 1.12 – 1.33 (m, 3H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (1H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H)), 1.47 – 1.80 (m, 5H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 2.15 (s, 3H, ArCH_3), 2.59 (t, J = 6.8 Hz, 1H, HSCH$_2$CO), 2.96 – 3.05 (m, 1H, HSCH_2CO), 3.09 04 (dd, J = 14.9/6.8 Hz, 1H, HSCH_2CO), 3.54 – 3.64 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), 6.00 (s, 1H, NCHCO), 6.87 – 6.97 (m, 4H, 2"-H$_{p\text{-tolyl}}$, 3"-H$_{p\text{-tolyl}}$, 5"-H$_{p\text{-tolyl}}$, 6"-H$_{p\text{-tolyl}}$), 6.97 – 7.04 (m, 1H, H$_{3\text{-fluorophenyl}}$), 7.18 (s br, 1H, H$_{3\text{-fluorophenyl}}$), 7.97 (d, J = 7.6 Hz, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), the signals for 2 H$_{3\text{-fluorophenyl}}$ cannot be observed in the spectrum, the signals of the major rotamer are given;

^{13}C NMR (151 MHz, DMSO-d_6): δ [ppm] = 20.6 (1C, ArCH$_3$), 24.5 (1C, NHCH(CH$_2$$CH_2$)$_2CH_2$), 24.7 (1C, NHCH(CH$_2$$CH_2$)$_2CH_2$), 25.2 (1C, NHCH(CH$_2CH_2$)$_2$$CH_2$), 27.4 (1C, HS$CH_2$CO), 32.21 (1C, NHCH($CH_2CH_2$)$_2CH_2$), 32.25 (1C, NHCH($CH_2CH_2$)$_2CH_2$),

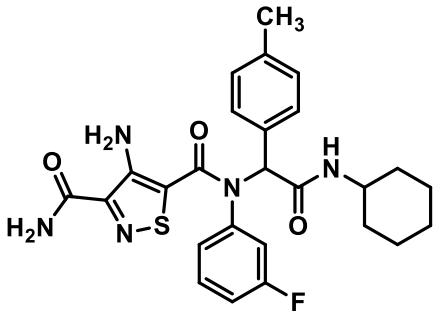
48.0 (1C, NH*C*H(CH$_2$CH$_2$)$_2$CH$_2$), 63.6 (1C, N*C*HCO), 128.5 (2C, C-3"$_{p\text{-tolyl}}$, C-5"$_{p\text{-tolyl}}$), 130.0 (2C, C-2"$_{p\text{-tolyl}}$, C-6"$_{p\text{-tolyl}}$), 131.9 (1C, C-1"$_{p\text{-tolyl}}$), 137.0 (1C, C-4"$_{p\text{-tolyl}}$), 168.6 (2C, CH*C*ONHCH, HSCH$_2$*C*O), the signals for 6 C$_{3\text{-fluorophenyl}}$ cannot be observed in the spectrum;

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3286, 3075, 2929, 2851, 1646, 1606, 1588, 1556, 1519, 1486, 1442, 1403, 1382, 1360, 1251, 1236, 1184, 1172, 1143, 1103, 1076, 980, 895, 882, 819, 781, 738, 708, 667, 645, 634, 591, 553, 505;

HRMS (*m*/*z*): [M+H]$^+$ calcd for $C_{23}H_{28}FN_2O_2S$: 415.1850, found: 415.1866;

HPLC (method 1): t_R = 24.7 min, purity 97.1%.

4-Amino-*N*^{5}-[2-(cyclohexylamino)-2-oxo-1-(*p*-tolyl)ethyl]-*N*^{5}-(3-fluorophenyl)isothiazole-3,5-dicarboxamide (54j)



$C_{26}H_{28}FN_5O_3S$

M_r = 509.6

53j (410 mg, 1.0 mmol) was added in one portion to a solution of **25** (320 mg, 1.2 mmol) in acetone (100 mL). The mixture was cooled to 0 °C and morpholine (0.17 mL, 170 mg, 1.9 mmol) was added dropwise. After stirring the reaction mixture for 12 h at ambient temperature, the solvent was removed *in vacuo*. The residue was purified by flash column chromatography (Ø = 6 cm, h = 20 cm, V = 15 mL, dichloromethane/acetone = 15/1) and recrystallized from acetonitrile to give **54j** (300 mg, 0.59 mmol, 59% yield) as colorless solid.

m.p. = 256 °C;

TLC: R_f = 0.32 (dichloromethane/acetone = 10/1);

^{1}H NMR (500 MHz, DMSO-d_6): δ [ppm] = 0.91 – 1.13 (m, 2H, NHCH(C*H*$_2$CH$_2$)$_2$CH$_2$ (1H), NHCH(CH$_2$CH$_2$)$_2$C*H*$_2$ (1H)), 1.14 – 1.33 (m, 3H, NHCH(C*H*$_2$CH$_2$)$_2$CH$_2$ (1H),

NHCH(CH_2CH_2)$_2CH_2$ (2H)), 1.48 – 1.63 (m, 2H, NHCH(CH_2CH_2)$_2CH_2$ (1H), NHCH(CH_2CH_2)$_2CH_2$ (1H)), 1.63 – 1.73 (m, 2H, NHCH(CH_2CH_2)$_2CH_2$ (1H), NHCH(CH_2CH_2)$_2CH_2$ (1H)), 1.73 – 1.81 (m, 1H, NHCH(CH_2CH_2)$_2CH_2$), 2.16 (s, 3H, ArCH_3), 3.56 – 3.65 (m, 1H, NHCH(CH_2CH_2)$_2CH_2$), 6.14 (s, 1H, NCHCO), 6.92 – 7.01 (m, 4H, 2'''-H$_{p\text{-tolyl}}$, 3'''-H$_{p\text{-tolyl}}$, 5'''-H$_{p\text{-tolyl}}$, 6'''-H$_{p\text{-tolyl}}$), 7.15 – 7.23 (m, 3H, ArNH_2, 4''-H$_{3\text{-fluorophenyl}}$), 7.28 (s br, 1H, 5''-H$_{3\text{-fluorophenyl}}$), 7.61 (s br, 1H, CONH_2), 7.86 (s br, 1H, CONH_2), 8.07 (d, J = 7.7 Hz, 1H, NHCH(CH_2CH_2)$_2CH_2$), the signals for 2''-H$_{3\text{-fluorophenyl}}$, 6''-H$_{3\text{-fluorophenyl}}$ cannot be observed in the spectrum;

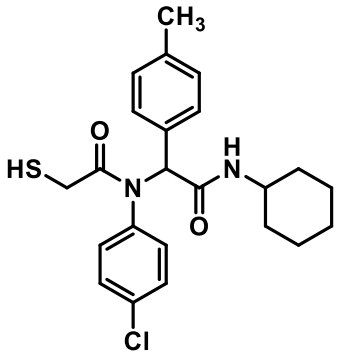
^{13}C NMR (151 MHz, DMSO-d_6): δ [ppm] = 20.6 (1C, ArCH_3), 24.5 (1C, NHCH(CH_2CH_2)$_2CH_2$), 24.6 (1C, NHCH(CH_2CH_2)$_2CH_2$), 25.2 (1C, NHCH(CH_2CH_2)$_2CH_2$), 32.1 (1C, NHCH(CH_2CH_2)$_2CH_2$), 32.2 (1C, NHCH(CH_2CH_2)$_2CH_2$), 48.0 (1C, NHCH(CH_2CH_2)$_2CH_2$), 63.7 (1C, NCHCO), 116.6 (d, J = 20.6 Hz, 1C, C-4''$_{3\text{-fluorophenyl}}$), 120.4 (d, J = 21.8 Hz, 1C, C-2''$_{3\text{-fluorophenyl}}$), 122.6 (1C, C-5'$_{isothiazole}$), 128.5 (2C, C-3'''$_{p\text{-tolyl}}$, C-5'''$_{p\text{-tolyl}}$), 129.8 (1C, C-6''$_{3\text{-fluorophenyl}}$), 130.3 (2C, C-2'''$_{p\text{-tolyl}}$, C-6'''$_{p\text{-tolyl}}$), 130.4 (d, J = 9.0 Hz, 1C, C-5''$_{3\text{-fluorophenyl}}$), 131.5 (1C, C-1'''$_{p\text{-tolyl}}$), 137.1 (1C, C-4'''$_{p\text{-tolyl}}$), 138.9 (d, J = 9.9 Hz, 1C, C-1''$_{3\text{-fluorophenyl}}$), 146.7 (1C, C-3'$_{isothiazole}$), 151.4 (1C, C-4'$_{isothiazole}$), 161.7 (d, J = 245.9 Hz, 1C, C-3''$_{3\text{-fluorophenyl}}$), 162.0 (1C, ArCONCH), 163.8 (1C, ArCONH_2), 168.6 (1C, CHCONHCH);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3450, 3285, 3078, 2930, 2850, 1665, 1646, 1561, 1487, 1445, 1371, 1346, 1327, 1253, 1186, 1151, 1104, 1070, 980, 894, 881, 821, 798, 758, 739, 706, 670, 554, 525, 505;

HRMS (m/z): [M+H]$^+$ calcd for $C_{26}H_{29}FN_5O_3S$: 510.1970, found: 510.1977;

HPLC (method 1): t_R = 26.0 min, purity 97.6%.

_N_-(4-Chlorophenyl)-_N_-[2-(cyclohexylamino)-2-oxo-1-(_p_-tolyl)ethyl]-2-mercaptoacetamide (53k)



$C_{23}H_{27}ClN_2O_2S$

$M_r = 431.0$

4-Chloroaniline (250 mg, 2.0 mmol) and 4-methylbenzaldehyde (0.24 mL, 240 mg, 2.0 mmol) were dissolved in dichloromethane (15 mL) and the solution was stirred at ambient temperature for 10 min. Then, sulfanylacetic acid (0.14 mL, 180 mg, 2.0 mmol) and isocyanocyclohexane (0.25 mL, 220 mg, 2.0 mmol) were added. After stirring the reaction mixture for 48 h at ambient temperature, the solvent was removed *in vacuo* and the residue was purified by flash column chromatography (Ø = 2 cm, h = 15 cm, V = 5 mL, dichloromethane/ethyl acetate = 20/1) to give **53k** (550 mg, 1.3 mmol, 64% yield) as colorless solid.

m.p. = 198 °C;

TLC: R_f = 0.36 (dichloromethane/ethyl acetate = 15/1);

^{1}H NMR (500 MHz, DMSO-d_6): δ [ppm] = 0.90 – 1.01 (m, 1H, NHCH(CH_2CH$_2$)$_2$CH$_2$), 1.01 – 1.13 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$CH_2), 1.13 – 1.31 (m, 3H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (1H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H)), 1.47 – 1.78 (m, 5H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 2.16 (s, 3H, ArCH_3), 2.61 (t, J = 6.7 Hz, 1H, HSCH$_2$CO), 2.96 (dd, J = 14.9/6.6 Hz, 1H, HSCH_2CO), 3.05 (dd, J = 14.9/6.8 Hz, 1H, HSCH_2CO), 3.53 – 3.62 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), 6.00 (s, 1H, NCHCO), 6.78 (s br, 1H, H$_{4\text{-chlorophenyl}}$), 6.87 – 6.93 (m, 2H, 2"-H$_{p\text{-tolyl}}$, 6"-H$_{p\text{-tolyl}}$), 6.93 – 6.99 (m, 2H, 3"-H$_{p\text{-tolyl}}$, 5"-H$_{p\text{-tolyl}}$), 7.23 (s br, 2H, H$_{4\text{-chlorophenyl}}$), 7.81 (s br, 1H, H$_{4\text{-chlorophenyl}}$), 7.97 (d, J = 7.7 Hz, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), the signals of the major rotamer are given;

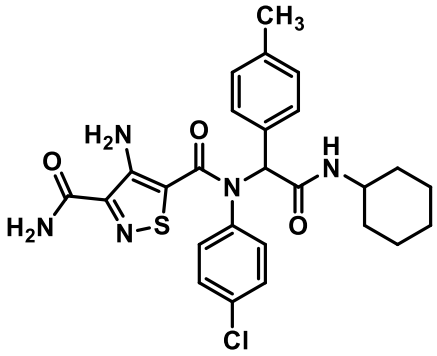
^{13}C NMR (101 MHz, DMSO-d_6): δ [ppm] = 20.6 (1C, Ar*C*H$_3$), 24.4 (1C, NHCH(CH$_2$*C*H$_2$)$_2$CH$_2$), 24.6 (1C, NHCH(CH$_2$*C*H$_2$)$_2$CH$_2$), 25.2 (1C, NHCH(CH$_2$CH$_2$)$_2$*C*H$_2$), 27.4 (1C, HS*C*H$_2$CO), 32.1 (1C, NHCH(*C*H$_2$CH$_2$)$_2$CH$_2$), 32.2 (1C, NHCH(*C*H$_2$CH$_2$)$_2$CH$_2$), 47.9 (1C, NH*C*H(CH$_2$CH$_2$)$_2$CH$_2$), 63.4 (1C, N*C*HCO), 128.4 (2C, C$_{4\text{-chlorophenyl}}$), 128.6 (2C, C-3"$_{p\text{-tolyl}}$, C-5"$_{p\text{-tolyl}}$), 129.9 (2C, C-2"$_{p\text{-tolyl}}$, C-6"$_{p\text{-tolyl}}$), 131.9 (1C, C-1"$_{p\text{-tolyl}}$), 132.3 (1C, C-4'$_{4\text{-chlorophenyl}}$), 132.7 (2C, C$_{4\text{-chlorophenyl}}$), 136.9 (1C, C-4"$_{p\text{-tolyl}}$), 138.2 (1C, C-1'$_{4\text{-chlorophenyl}}$), 168.5 (1C, CH*C*ONHCH), 168.8 (1C, HSCH$_2$*C*O);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3258, 3085, 2925, 2853, 1661, 1645, 1561, 1491, 1379, 1355, 1226, 1018, 978, 890, 842, 791, 755, 734, 670, 641, 626, 569, 533, 504;

HRMS (*m*/*z*): [M+H]$^+$ calcd for $C_{23}H_{28}{}^{35}ClN_2O_2S$: 431.1555, found: 431.1554;

HPLC (method 1): t_R = 25.5 min, purity 99.2%.

4-Amino-*N*^{5}-(4-chlorophenyl)-*N*^{5}-[2-(cyclohexylamino)-2-oxo-1-(*p*-tolyl)ethyl]isothiazole-3,5-dicarboxamide (54k)



$C_{26}H_{28}ClN_5O_3S$

M_r = 526.1

53k (430 mg, 1.0 mmol) was added in one portion to a solution of **25** (320 mg, 1.2 mmol) in acetone (100 mL). The mixture was cooled to 0 °C and morpholine (0.17 mL, 170 mg, 1.9 mmol) was added dropwise. After stirring the reaction mixture for 12 h at ambient temperature, the solvent was removed *in vacuo*. The residue was purified by flash column chromatography (Ø = 6 cm, h = 20 cm, V = 15 mL, dichloromethane/ethyl acetate = 10/1) and recrystallized from acetonitrile to give **54k** (380 mg, 0.72 mmol, 72% yield) as colorless solid.

m.p. = 263 °C;

TLC: R_f = 0.48 (dichloromethane/ethyl acetate = 5/1);

^{1}H NMR (500 MHz, DMSO-d_6): δ [ppm] = 0.92 – 1.02 (m, 1H, NHCH(CH_2CH$_2$)$_2$CH$_2$), 1.02 – 1.12 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$CH_2), 1.15 – 1.31 (m, 3H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (1H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H)), 1.48 – 1.62 (m, 2H, NHCH(CH$_2$CH_2)$_2$CH$_2$ (1H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 1.62 – 1.73 (m 2H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (1H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (1H)), 1.73 – 1.80 (m, 1H, NHCH(CH_2CH$_2$)$_2$CH$_2$), 2.18 (s, 3H, ArCH_3), 3.55 – 3.65 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), 6.14 (s, 1H, NCHCO), 6.93 – 7.00 (m, 4H, 2'''-H$_{p\text{-tolyl}}$, 3'''-H$_{p\text{-tolyl}}$, 5'''-H$_{p\text{-tolyl}}$, 6'''-H$_{p\text{-tolyl}}$), 7.19 (s br, 2H, ArNH_2), 7.33 (s br, 2H, H$_{4\text{-chlorophenyl}}$), 7.62 (s br, 1H, CONH_2), 7.87 (s br, 1H, CONH_2), 8.05 (d, J = 7.7 Hz, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), the signals for 2 H$_{4\text{-chlorophenyl}}$ cannot be observed in the spectrum;

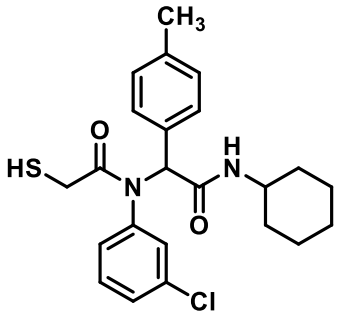
^{13}C NMR (126 MHz, DMSO-d_6): δ [ppm] = 20.6 (1C, ArCH$_3$), 24.4 (1C, NHCH(CH$_2$$CH_2$)$_2CH_2$), 24.6 (1C, NHCH(CH$_2$$CH_2$)$_2CH_2$), 25.2 (1C, NHCH(CH$_2CH_2$)$_2$$CH_2$), 32.1 (1C, NHCH($CH_2CH_2$)$_2CH_2$), 32.2 (1C, NHCH($CH_2CH_2$)$_2CH_2$), 48.0 (1C, NH$C$H(CH$_2CH_2$)$_2CH_2$), 63.6 (1C, N$C$HCO), 122.6 (1C, C-5'$_{\text{isothiazole}}$), 128.6 (2C, C-3'''$_{p\text{-tolyl}}$, C-5'''$_{p\text{-tolyl}}$), 128.9 (2C, C$_{4\text{-chlorophenyl}}$), 130.2 (2C, C-2'''$_{p\text{-tolyl}}$, C-6'''$_{p\text{-tolyl}}$), 131.6 (1C, C-1'''$_{p\text{-tolyl}}$), 134.2 (1C, C-4''$_{4\text{-chlorophenyl}}$), 135.1 (2C, C$_{4\text{-chlorophenyl}}$), 136.3 (1C, C-1''$_{4\text{-chlorophenyl}}$), 137.1 (1C, C-4'''$_{p\text{-tolyl}}$), 146.8 (1C, C-3'$_{\text{isothiazole}}$), 151.3 (1C, C-4'$_{\text{isothiazole}}$), 162.1 (1C, Ar$C$ONCH), 163.8 (1C, Ar$CONH_2$), 168.6 (1C, CH$C$ONHCH);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3459, 3342, 3267, 3176, 3080, 2923, 2850, 1672, 1645, 1610, 1559, 1517, 1488, 1448, 1401, 1370, 1345, 1326, 1263, 1249, 1238, 1185, 1150, 1088, 1071, 1017, 977, 840, 799, 747, 729, 678, 648, 632, 567, 520, 502;

HRMS (m/z): [M+H]$^+$ calcd for C$_{26}$H$_{29}$^{35}ClN$_5$O$_3$S: 526.1674, found: 526.1685;

HPLC (method 1): t_R = 25.2 min, purity 95.5%.

_N_-(3-Chlorophenyl)-_N_-[2-(cyclohexylamino)-2-oxo-1-(_p_-tolyl)ethyl]-2-mercaptoacetamide (53l)



$C_{23}H_{27}ClN_2O_2S$

M_r = 431.0

3-Chloroaniline (0.21 mL, 250 mg, 2.0 mmol) and 4-methylbenzaldehyde (0.24 mL, 240 mg, 2.0 mmol) were dissolved in dichloromethane (15 mL) and the solution was stirred at ambient temperature for 10 min. Then, sulfanylacetic acid (0.14 mL, 180 mg, 2.0 mmol) and isocyanocyclohexane (0.25 mL, 220 mg, 2.0 mmol) were added. After stirring the reaction mixture for 48 h at ambient temperature, the solvent was removed *in vacuo* and the residue was purified by flash column chromatography (Ø = 2 cm, h = 15 cm, V = 5 mL, dichloromethane/ethyl acetate = 20/1) to give **53l** (510 mg, 1.2 mmol, 59% yield) as colorless solid.

m.p. = 187 °C;

TLC: R_f = 0.40 (dichloromethane/ethyl acetate = 15/1);

^{1}H NMR (400 MHz, DMSO-d_6): δ [ppm] = 0.91 – 1.33 (m, 5H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 1.47 – 1.80 (m, 5H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)),

2.16 (s, 3H, ArCH_3), 2.62 (t, J = 6.8 Hz, 1H, HSCH$_2$CO), 2.94 – 3.05 (m, 1H, HSCH_2CO), 3.09 (dd, J = 14.9/6.8 Hz, 1H, HSCH_2CO), 3.53 – 3.65 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), 6.00 (s, 1H, NCHCO), 6.87 – 6.93 (m, 2H, 2"-H$_{p\text{-tolyl}}$, 6"-H$_{p\text{-tolyl}}$), 6.93 – 6.98 (m, 2H, 3"-H$_{p\text{-tolyl}}$, 5"-H$_{p\text{-tolyl}}$), 7.07 – 7.26 (m, 2H, H$_{3\text{-chlorophenyl}}$), 7.99 (d, J = 7.6 Hz, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), the signals for 2 H$_{3\text{-chlorophenyl}}$ cannot be observed in the spectrum;

^{13}C NMR (101 MHz, DMSO-d_6): δ [ppm] = 20.6 (1C, ArCH$_3$), 24.4 (1C, NHCH(CH$_2$$CH_2$)$_2CH_2$), 24.6 (1C, NHCH(CH$_2$$CH_2$)$_2CH_2$), 25.1 (1C, NHCH(CH$_2CH_2$)$_2$$CH_2$),

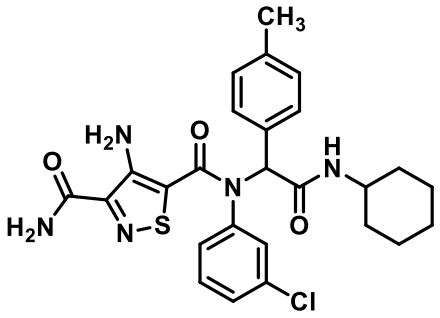
27.4 (1C, HS*C*H$_2$CO), 32.1 (1C, NHCH(*C*H$_2$CH$_2$)$_2$CH$_2$), 32.2 (1C, NHCH(*C*H$_2$CH$_2$)$_2$CH$_2$), 47.9 (1C, NH*C*H(CH$_2$CH$_2$)$_2$CH$_2$), 63.5 (1C, N*C*HCO), 127.9 (1C, $C_{3\text{-chlorophenyl}}$), 128.5 (2C, C-3"$_{p\text{-tolyl}}$, C-5"$_{p\text{-tolyl}}$), 129.7 (2C, $C_{3\text{-chlorophenyl}}$), 129.9 (2C, C-2"$_{p\text{-tolyl}}$, C-6"$_{p\text{-tolyl}}$), 130.9 (1C, $C_{3\text{-chlorophenyl}}$), 131.8 (1C, C-1"$_{p\text{-tolyl}}$), 132.4 (1C, $C_{3\text{-chlorophenyl}}$), 137.0 (1C, C-4"$_{p\text{-tolyl}}$), 140.6 (1C, C-1'$_{3\text{-chlorophenyl}}$), 168.4 (1C, *C*ON), 168.7 (1C, *C*ON);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3293, 3066, 2927, 2852, 1648, 1589, 1551, 1518, 1474, 1447, 1419, 1372, 1360, 1329, 1301, 1251, 1228, 1191, 1181, 1153, 1106, 1076, 1058, 978, 892, 836, 803, 791, 767, 748, 729, 704, 643, 631, 580, 547, 502;

HRMS (*m*/*z*): [M+H]$^+$ calcd for $C_{23}H_{28}{}^{35}ClN_2O_2S$: 431.1555, found: 431.1560;

HPLC (method 1): t_R = 25.4 min, purity 98.1%.

4-Amino-*N*^{5}-(3-chlorophenyl)-*N*^{5}-[2-(cyclohexylamino)-2-oxo-1-(*p*-tolyl)ethyl]isothiazole-3,5-dicarboxamide (54l)



$C_{26}H_{28}ClN_5O_3S$

M_r = 526.1

53l (430 mg, 1.0 mmol) was added in one portion to a solution of **25** (320 mg, 1.2 mmol) in acetone (100 mL). The mixture was cooled to 0 °C and morpholine (0.17 mL, 170 mg, 1.9 mmol) was added dropwise. After stirring the reaction mixture for 12 h at ambient temperature, the solvent was removed *in vacuo*. The residue was purified by flash column chromatography (Ø = 6 cm, h = 20 cm, V = 15 mL, dichloromethane/ethyl acetate = 10/1) and recrystallized from acetonitrile to give **54l** (400 mg, 0.75 mmol, 75% yield) as colorless solid.

m.p. = 246 °C;

TLC: R_f = 0.32 (dichloromethane/acetone = 10/1);

^{1}H NMR (500 MHz, DMSO-*d*$_6$): δ [ppm] = 0.91 – 1.32 (m, 5H, NHCH(C*H*$_2$CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$C*H*$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH$_2$)$_2$C*H*$_2$ (1H)), 1.47 – 1.81 (m, 5H, NHCH(C*H*$_2$CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$C*H*$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH$_2$)$_2$C*H*$_2$ (1H)), 2.17 (s, 3H, ArC*H*$_3$), 3.55 – 3.65 (m, 1H, NHC*H*(CH$_2$CH$_2$)$_2$CH$_2$), 6.14 (s, 1H, NC*H*CO), 6.86 – 7.01 (m, 5H, 2'''-H$_{p\text{-tolyl}}$, 3'''-H$_{p\text{-tolyl}}$, 5'''-H$_{p\text{-tolyl}}$, 6'''-H$_{p\text{-tolyl}}$, H$_{3\text{-chlorophenyl}}$ (1H)), 7.16 – 7.31 (m, 3H, ArN*H*$_2$, H$_{3\text{-chlorophenyl}}$ (1H)), 7.38 – 7.42 (m, 1H, H$_{3\text{-chlorophenyl}}$), 7.62 (s br, 1H, CON*H*$_2$), 7.87 (s br, 1H, CON*H*$_2$), 8.08 (d, *J* = 7.7 Hz, 1H, N*H*CH(CH$_2$CH$_2$)$_2$CH$_2$), the signal for 1 H$_{3\text{-chlorophenyl}}$ cannot be observed in the spectrum;

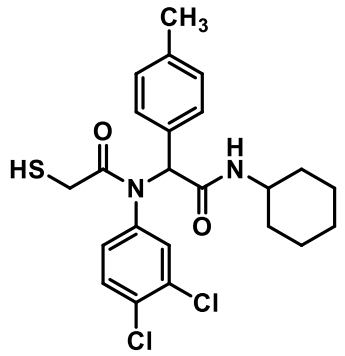
^{13}C NMR (126 MHz, DMSO-*d*$_6$): δ [ppm] = 20.6 (1C, Ar*C*H$_3$), 24.4 (1C, NHCH(CH$_2$*C*H$_2$)$_2$CH$_2$), 24.6 (1C, NHCH(CH$_2$*C*H$_2$)$_2$CH$_2$), 25.1 (1C, NHCH(CH$_2$CH$_2$)$_2$*C*H$_2$), 32.1 (1C, NHCH(*C*H$_2$CH$_2$)$_2$CH$_2$), 32.2 (1C, NHCH(*C*H$_2$CH$_2$)$_2$CH$_2$), 48.0 (1C, NH*C*H(CH$_2$CH$_2$)$_2$CH$_2$), 63.6 (1C, N*C*HCO), 122.5 (1C, C-5'$_{\text{isothiazole}}$), 128.5 (2C, C-3'''$_{p\text{-tolyl}}$, C-5'''$_{p\text{-tolyl}}$), 129.5 (1C, C$_{3\text{-chlorophenyl}}$), 130.2 (2C, C-2'''$_{p\text{-tolyl}}$, C-6'''$_{p\text{-tolyl}}$), 130.3 (1C, C$_{3\text{-chlorophenyl}}$), 131.5 (1C, C-1'''$_{p\text{-tolyl}}$), 132.1 (1C, C$_{3\text{-chlorophenyl}}$), 132.9 (1C, C-3''$_{3\text{-chlorophenyl}}$), 133.1 (1C, C$_{3\text{-chlorophenyl}}$), 137.2 (1C, C-4'''$_{p\text{-tolyl}}$), 138.8 (1C, C-1''$_{3\text{-chlorophenyl}}$), 146.8 (1C, C-3'$_{\text{isothiazole}}$), 151.4 (1C, C-4'$_{\text{isothiazole}}$), 161.9 (1C, Ar*C*ONCH), 163.8 (1C, Ar*C*ONH$_2$), 168.5 (1C, CH*C*ONHCH);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3454, 3339, 3280, 3189, 3068, 2928, 2850, 1675, 1645, 1614, 1584, 1559, 1518, 1492, 1473, 1448, 1423, 1398, 1370, 1344, 1326, 1264, 1250, 1237, 1183, 1151, 1106, 1074, 978, 891, 839, 799, 769, 752, 729, 704, 673, 549, 530, 503;

HRMS (*m*/*z*): [M+H]$^+$ calcd for C$_{26}$H$_{29}$^{35}ClN$_5$O$_3$S: 526.1674, found: 526.1669;

HPLC (method 1): t$_R$ = 25.1 min, purity 97.5%.

N-Cyclohexyl-2-[*N*-(3,4-dichlorophenyl)-2-mercaptoacetamido]-2-(*p*-tolyl)acetamide (53m)



C$_{23}$H$_{26}$Cl$_2$N$_2$O$_2$S

M$_r$ = 465.4

3,4-Dichloroaniline (320 mg, 2.0 mmol) and 4-methylbenzaldehyde (0.24 mL, 240 mg, 2.0 mmol) were dissolved in dichloromethane (15 mL) and the solution was stirred at ambient temperature for 10 min. Then, sulfanylacetic acid (0.14 mL, 180 mg, 2.0 mmol) and isocyanocyclohexane (0.25 mL, 220 mg, 2.0 mmol) were added. After stirring the reaction mixture for 48 h at ambient temperature, the solvent was removed *in vacuo* and the residue was purified by flash column chromatography (Ø = 2 cm, h = 15 cm, V = 5 mL, dichloromethane/ethyl acetate = 15/1) to give **53m** (600 mg, 1.3 mmol, 65% yield) as colorless solid.

m.p. = 166 °C;

TLC: R_f = 0.29 (dichloromethane/ethyl acetate = 15/1);

^{1}H NMR (500 MHz, DMSO-d_6): δ [ppm] = 0.91 – 1.13 (m, 2H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (1H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 1.13 – 1.32 (m, 3H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (1H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H)), 1.48 – 1.79 (m, 5H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 2.17 (s, 3H, ArCH_3), 2.58 – 2.68 (m, 1H, *H*SCH$_2$CO), 2.96 – 3.08 (m, 1H, HSCH_2CO), 3.13 (dd, J = 14.9/6.5 Hz, 1H, HSCH_2CO), 3.53 – 3.65 (m, 1H, NHC*H*(CH$_2$CH$_2$)$_2$CH$_2$), 6.02 (s, 1H, NC*H*CO), 6.89 – 6.95 (m, 2H, 2"-H$_{p\text{-tolyl}}$, 6"-H$_{p\text{-tolyl}}$), 6.96 – 7.01 (m, 2H, 3"-H$_{p\text{-tolyl}}$, 5"-H$_{p\text{-tolyl}}$), 7.43 (s br, 1H, H$_{3,4\text{-dichlorophenyl}}$), 8.04 (d, J = 7.3 Hz, 1H, N*H*CH(CH$_2$CH$_2$)$_2$CH$_2$), the signals for 2 H$_{3,4\text{-dichlorophenyl}}$ cannot be observed in the spectrum;

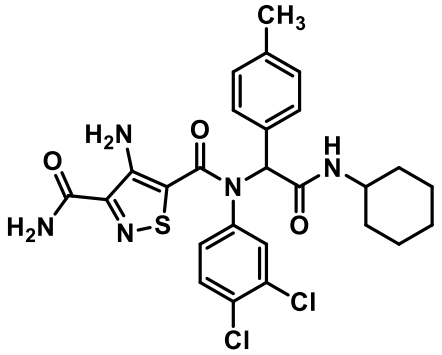
^{13}C NMR (126 MHz, DMSO-d_6): δ [ppm] = 20.6 (1C, Ar*C*H$_3$), 24.4 (1C, NHCH(CH$_2$*C*H$_2$)$_2$CH$_2$), 24.6 (1C, NHCH(CH$_2$*C*H$_2$)$_2$CH$_2$), 25.1 (1C, NHCH(CH$_2$CH$_2$)$_2$*C*H$_2$), 27.5 (1C, HS*C*H$_2$CO), 32.11 (1C, NHCH(*C*H$_2$CH$_2$)$_2$CH$_2$), 32.15 (1C, NHCH(*C*H$_2$CH$_2$)$_2$CH$_2$), 47.9 (1C, NH*C*H(CH$_2$CH$_2$)$_2$CH$_2$), 63.3 (1C, N*C*HCO), 128.6 (2C, C-3"$_{p\text{-tolyl}}$, C-5"$_{p\text{-tolyl}}$), 129.8 (2C, C-2"$_{p\text{-tolyl}}$, C-6"$_{p\text{-tolyl}}$), 130.0 (1C, C$_{3,4\text{-dichlorophenyl}}$), 130.4 (1C, C$_{3,4\text{-dichlorophenyl}}$), 131.3 (1C, C$_{3,4\text{-dichlorophenyl}}$), 131.7 (1C, C-1"$_{p\text{-tolyl}}$), 132.9 (1C, C$_{3,4\text{-dichlorophenyl}}$), 134.6 (1C, C$_{3,4\text{-dichlorophenyl}}$), 137.1 (1C, C-4"$_{p\text{-tolyl}}$), 139.2 (1C, C-1'$_{3,4\text{-dichlorophenyl}}$), 168.4 (1C, CH*C*ONHCH), 168.7 (1C, HSCH$_2$*C*O);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3260, 3083, 2927, 2853, 1661, 1644, 1560, 1516, 1469, 1451, 1365, 1251, 1229, 1189, 1151, 1129, 1101, 1059, 1032, 980, 888, 841, 811, 740, 713, 677, 644, 533, 503;

HRMS (*m*/*z*): [M+H]$^+$ calcd for C$_{23}$H$_{27}$^{35}Cl$_2$N$_2$O$_2$S: 465.1165, found: 465.1160;

HPLC (method 1): t_R = 26.4 min, purity 97.0%.

4-Amino-N^5-[2-(cyclohexylamino)-2-oxo-1-(*p*-tolyl)ethyl]-N^5-(3,4-dichlorophenyl)isothiazole-3,5-dicarboxamide (54m)



$C_{26}H_{27}Cl_2N_5O_3S$

M_r = 560.5

53m (470 mg, 1.0 mmol) was added in one portion to a solution of **25** (320 mg, 1.2 mmol) in acetone (100 mL). The mixture was cooled to 0 °C and morpholine (0.17 mL, 170 mg, 1.9 mmol) was added dropwise. After stirring the reaction mixture for 12 h at ambient temperature, the solvent was removed *in vacuo*. The residue was purified by flash column chromatography (Ø = 6 cm, h = 20 cm, V = 15 mL, dichloromethane/acetone = 15/1) and recrystallized from acetonitrile to give **54m** (400 mg, 0.71 mmol, 71% yield) as colorless solid.

m.p. = 249 °C;

TLC: R_f = 0.21 (dichloromethane/acetone = 15/1);

^{1}H NMR (500 MHz, DMSO-d_6): δ [ppm] = 0.93 – 1.13 (m, 2H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (1H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 1.14 – 1.32 (m, 3H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (1H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H)), 1.48 – 1.63 (m, 2H, NHCH(CH$_2$CH_2)$_2$CH$_2$ (1H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 1.63 – 1.73 (m, 2H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (1H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (1H)), 1.73 – 1.81 (m, 1H, NHCH(CH_2CH$_2$)$_2$CH$_2$), 2.19 (s, 3H, ArCH_3), 3.55 – 3.66 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), 6.15 (s, 1H, NCHCO), 6.96 – 7.04 (m, 4H, 2'''-H$_{p\text{-tolyl}}$, 3'''-H$_{p\text{-tolyl}}$, 5'''-H$_{p\text{-tolyl}}$, 6'''-H$_{p\text{-tolyl}}$), 7.21 (s br, 2H, ArNH_2), 7.54 (s br, 1H, H$_{3,4\text{-dichlorophenyl}}$), 7.65 (s br, 1H, CON$H_2$), 7.89 (s br, 1H, CON$H_2$), 8.12 (d, J = 7.7 Hz, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), the signals for 2 H$_{3,4\text{-dichlorophenyl}}$ cannot be observed in the spectrum;

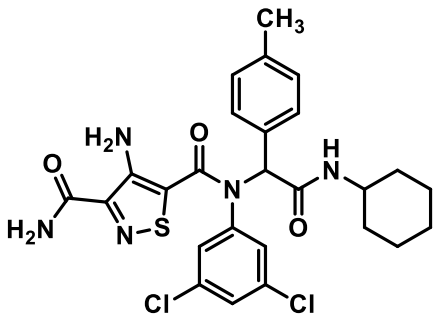
^{13}C NMR (151 MHz, DMSO-d_6): δ [ppm] = 20.6 (1C, Ar*C*H$_3$), 24.4 (1C, NHCH(CH$_2$*C*H$_2$)$_2$CH$_2$), 24.6 (1C, NHCH(CH$_2$*C*H$_2$)$_2$CH$_2$), 25.2 (1C, NHCH(CH$_2$CH$_2$)$_2$*C*H$_2$), 32.1 (1C, NHCH(*C*H$_2$CH$_2$)$_2$CH$_2$), 32.2 (1C, NHCH(*C*H$_2$CH$_2$)$_2$CH$_2$), 48.0 (1C, NH*C*H(CH$_2$CH$_2$)$_2$CH$_2$), 63.5 (1C, N*C*HCO), 122.1 (1C, C-5'$_{isothiazole}$), 128.7 (2C, C-3'''$_{p\text{-tolyl}}$, C-5'''$_{p\text{-tolyl}}$), 130.2 (2C, C-2'''$_{p\text{-tolyl}}$, C-6'''$_{p\text{-tolyl}}$), 130.7 (1C, C$_{3,4\text{-dichlorophenyl}}$), 131.0 (1C, C$_{3,4\text{-dichlorophenyl}}$), 131.4 (1C, C-1'''$_{p\text{-tolyl}}$), 132.3 (1C, C$_{3,4\text{-dichlorophenyl}}$), 133.6 (1C, C$_{3,4\text{-dichlorophenyl}}$), 135.1 (1C, C$_{3,4\text{-dichlorophenyl}}$), 137.4 (1C, C-4'''$_{p\text{-tolyl}}$), 137.5 (1C, C-1''$_{3,4\text{-dichlorophenyl}}$), 147.0 (1C, C-3'$_{isothiazole}$), 151.4 (1C, C-4'$_{isothiazole}$), 161.9 (1C, Ar*C*ONCH), 163.8 (1C, Ar*C*ONH$_2$), 168.5 (1C, CH*C*ONHCH);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3455, 3390, 3338, 3292, 2928, 2850, 1677, 1645, 1606, 1562, 1517, 1492, 1467, 1448, 1398, 1384, 1347, 1325, 1309, 1251, 1234, 1187, 1150, 1126, 1106, 1072, 1033, 980, 892, 841, 807, 792, 779, 755, 740, 712, 662, 635, 598, 547, 523, 503;

HRMS (*m*/*z*): [M+H]$^+$ calcd for C$_{26}$H$_{28}$^{35}Cl$_2$N$_5$O$_3$S: 560.1284, found: 560.1297;

HPLC (method 1): t_R = 26.1 min, purity 96.6%.

4-Amino-*N*^{5}-[2-(cyclohexylamino)-2-oxo-1-(*p*-tolyl)ethyl]-*N*^{5}-(3,5-dichlorophenyl)isothiazole-3,5-dicarboxamide (54n)



C$_{26}$H$_{27}$Cl$_2$N$_5$O$_3$S

M$_r$ = 560.5

3,5-Dichloroaniline (320 mg, 2.0 mmol) and 4-methylbenzaldehyde (0.24 mL, 240 mg, 2.0 mmol) were added to dichloromethane (15 mL) and the solution was stirred at ambient temperature for 10 min. Then, sulfanylacetic acid (0.14 mL, 180 mg, 2.0 mmol) and isocyanocyclohexane (0.25 mL, 220 mg, 2.0 mmol) were added. After stirring the reaction mixture for 48 h at ambient temperature, the solvent was removed *in vacuo* and the residue was washed with acetonitrile.

The obtained crude product was added in one portion to a solution of **25** (320 mg, 1.2 mmol) in acetone (100 mL). The mixture was cooled to 0 °C and morpholine (0.17 mL, 170 mg, 2.0 mmol) was added dropwise. After stirring the reaction mixture for 12 h at ambient temperature, the solvent was removed *in vacuo*. The residue was purified by flash column chromatography (Ø = 6 cm, h = 20 cm, V = 15 mL, dichloromethane/acetone = 15/1) and recrystallized from acetonitrile to give **54n** (290 mg, 0.51 mmol, 25% yield) as colorless solid.

m.p. = 253 °C;

TLC: R_f = 0.38 (dichloromethane/acetone = 10/1);

^{1}H NMR (400 MHz, DMSO-d_6): δ [ppm] = 0.92 – 1.14 (m, 2H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (1H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 1.14 – 1.34 (m, 3H, NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H), NHCH(CH_2CH$_2$)$_2$CH$_2$ (1H)), 1.47 – 1.65 (m, 2H, NHCH(CH$_2$CH_2)$_2$CH$_2$ (1H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 1.65 – 1.82 (m, 3H, NHCH(CH$_2$CH_2)$_2$CH$_2$ (1H), NHCH(CH_2CH$_2$)$_2$CH$_2$ (2H)), 2.19 (s, 3H, ArCH_3), 3.55 – 3.68 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), 6.14 (s, 1H, NCHCO), 6.97 – 7.05 (m, 4H, 2'''-H$_{p\text{-tolyl}}$, 3'''-H$_{p\text{-tolyl}}$, 5'''-H$_{p\text{-tolyl}}$, 6'''-H$_{p\text{-tolyl}}$), 7.22 (s br, 2H, ArNH_2), 7.63 (t, J = 1.9 Hz, 1H, 4''-H$_{3,5\text{-dichlorophenyl}}$), 7.65 (s br, 1H, CON$H_2$), 7.90 (s br, 1H, CON$H_2$), 8.15 (d, J = 7.7 Hz, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), the signals for 2''-H$_{3,5\text{-dichlorophenyl}}$ and 6''-H$_{3,5\text{-dichlorophenyl}}$ cannot be observed in the spectrum;

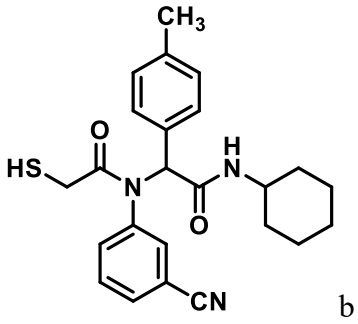
^{13}C NMR (101 MHz, DMSO-d_6): δ [ppm] = 20.6 (1C, ArCH$_3$), 24.4 (1C, NHCH(CH$_2$$CH_2$)$_2CH_2$), 24.6 (1C, NHCH(CH$_2$$CH_2$)$_2CH_2$), 25.1 (1C, NHCH(CH$_2CH_2$)$_2$$CH_2$), 32.1 (1C, NHCH($CH_2CH_2$)$_2CH_2$), 32.2 (1C, NHCH($CH_2CH_2$)$_2CH_2$), 48.0 (1C, NH$C$H(CH$_2CH_2$)$_2CH_2$), 63.6 (1C, N$C$HCO), 122.0 (1C, C-5'$_{\text{isothiazole}}$), 128.7 (2C, C-3'''$_{p\text{-tolyl}}$, C-5'''$_{p\text{-tolyl}}$), 129.2 (1C, C-4''$_{3,5\text{-dichlorophenyl}}$), 130.2 (2C, C-2'''$_{p\text{-tolyl}}$, C-6'''$_{p\text{-tolyl}}$), 131.2 (1C, C-1'''$_{p\text{-tolyl}}$), 132.1 (2C, C-2''$_{3,5\text{-dichlorophenyl}}$, C-6''$_{3,5\text{-dichlorophenyl}}$), 133.8 (2C, C-3''$_{3,5\text{-dichlorophenyl}}$, C-5''$_{3,5\text{-dichlorophenyl}}$), 137.4 (1C, C-4'''$_{p\text{-tolyl}}$), 139.8 (1C, C-1''$_{3,5\text{-dichlorophenyl}}$), 147.1 (1C, C-3'$_{\text{isothiazole}}$), 151.5 (1C, C-4'$_{\text{isothiazole}}$), 161.7 (1C, Ar$C$ONCH), 163.7 (1C, Ar$CONH_2$), 168.5 (1C, CH$C$ONHCH);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3450, 3336, 3292, 3182, 3076, 2928, 2850, 1677, 1647, 1615, 1562, 1518, 1494, 1438, 1396, 1371, 1342, 1325, 1307, 1288, 1251, 1236, 1181, 1152, 1118, 1098, 1078, 1060, 980, 891, 870, 841, 804, 753, 726, 704, 682, 658, 549, 530, 501;

HRMS (m/z): [M+H]$^+$ calcd for C$_{26}$H$_{28}$^{35}Cl$_2$N$_5$O$_3$S: 560.1284, found: 560.1293;

HPLC (method 1): t_R = 26.5 min, purity 97.2%.

***N*-(3-Cyanophenyl)-*N*-[2-(cyclohexylamino)-2-oxo-1-(*p*-tolyl)ethyl)-2-mercaptoacetamide (53o)**



b

$C_{24}H_{27}N_3O_2S$

M_r = 421.6

3-Aminobenzonitrile (240 mg, 2.0 mmol) and 4-methylbenzaldehyde (0.24 mL, 240 mg, 2.0 mmol) were dissolved in dichloromethane (15 mL) and the solution was stirred at ambient temperature for 10 min. Then, sulfanylacetic acid (0.14 mL, 180 mg, 2.0 mmol) and isocyanocyclohexane (0.25 mL, 220 mg, 2.0 mmol) were added. After stirring the reaction mixture for 48 h at ambient temperature, the solvent was removed *in vacuo* and the residue was purified by flash column chromatography (Ø = 2 cm, h = 15 cm, V = 5 mL, dichloromethane/ethyl acetate = 15/1) to give **53o** (460 mg, 1.1 mmol, 54% yield) as colorless solid.

m.p. = 205 °C;

TLC: R_f = 0.29 (dichloromethane/ethyl acetate = 15/1);

^{1}H NMR (500 MHz, DMSO-d_6): δ [ppm] = 0.92 – 1.02 (m, 1H, NHCH(CH_2CH$_2$)$_2$CH$_2$), 1.02 – 1.13 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$CH_2), 1.13 – 1.32 (m, 3H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (1H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H)), 1.48 – 1.62 (m, 2H, NHCH(CH$_2$CH_2)$_2$CH$_2$ (1H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 1.62 – 1.72 (m, 2H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (1H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (1H)), 1.72 – 1.80 (m, 1H, NHCH(CH_2CH$_2$)$_2$CH$_2$), 2.15 (s, 3H, ArCH_3), 2.58 – 2.68 (m, 1H, *H*SCH$_2$CO), 2.94 – 3.16 (m, 2H, HSCH_2CO), 3.54 – 3.65 (m, 1H, NHC*H*(CH$_2$CH$_2$)$_2$CH$_2$), 6.03 (s, 1H, NC*H*CO), 6.86 – 6.93 (m, 2H, 2"-H$_{p\text{-tolyl}}$, 6"-H$_{p\text{-tolyl}}$), 6.93 – 6.99 (m, 2H, 3"-H$_{p\text{-tolyl}}$, 5"-H$_{p\text{-tolyl}}$), 7.00 – 7.57 (m, 2H, H$_{3\text{-cyanophenyl}}$), 7.59 – 7.66 (m, 1H, 4'-H$_{3\text{-cyanophenyl}}$), 7.79 – 8.44 (m, 2H, N*H*CH(CH$_2$CH$_2$)$_2$CH$_2$, H$_{3\text{-cyanophenyl}}$ (1H));

^{13}C NMR (126 MHz, DMSO-*d*$_6$): δ [ppm] = 20.5 (1C, Ar*C*H$_3$), 24.4 (1C, NHCH(CH$_2$*C*H$_2$)$_2$CH$_2$), 24.6 (1C, NHCH(CH$_2$*C*H$_2$)$_2$CH$_2$), 25.1 (1C, NHCH(CH$_2$CH$_2$)$_2$*C*H$_2$), 27.6 (1C, HS*C*H$_2$CO), 32.11 (1C, NHCH(*C*H$_2$CH$_2$)$_2$CH$_2$), 32.15 (1C, NHCH(*C*H$_2$CH$_2$)$_2$CH$_2$), 47.9 (1C, NH*C*H(CH$_2$CH$_2$)$_2$CH$_2$), 63.3 (1C, N*C*HCO),

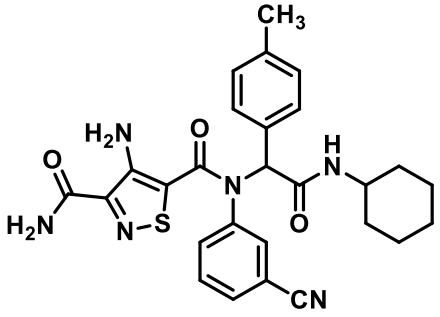
111.2 (1C, C-3'$_{3\text{-cyanophenyl}}$), 118.0 (1C, Ar*C*N), 128.6 (2C, C-3"$_{p\text{-tolyl}}$, C-5"$_{p\text{-tolyl}}$), 129.7 (1C, C$_{3\text{-cyanophenyl}}$), 129.8 (2C, C-2"$_{p\text{-tolyl}}$, C-6"$_{p\text{-tolyl}}$), 131.6 (1C, C-4'$_{3\text{-cyanophenyl}}$), 131.7 (1C, C-1"$_{p\text{-tolyl}}$), 134.7 (1C, C$_{3\text{-cyanophenyl}}$), 136.2 (1C, C$_{3\text{-cyanophenyl}}$), 137.1 (1C, C-4"$_{p\text{-tolyl}}$), 140.0 (1C, C-1'$_{3\text{-cyanophenyl}}$), 168.4 (1C, *C*ON), 168.6 (1C, *C*ON);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3298, 3061, 2928, 2853, 2234, 1646, 1598, 1580, 1551, 1518, 1481, 1451, 1429, 1372, 1360, 1330, 1309, 1251, 1232, 1182, 1167, 1107, 1053, 979, 892, 816, 805, 737, 705, 662, 645, 634, 596, 556, 503;

HRMS (*m*/*z*): [M+H]$^+$ calcd for C$_{24}$H$_{28}$N$_3$O$_2$S: 422.1897, found: 422.1892;

HPLC (method 1): t$_R$ = 23.9 min, purity 95.5%.

4-Amino-*N*^{5}-(3-cyanophenyl)-*N*^{5}-[2-(cyclohexylamino)-2-oxo-1-(*p*-tolyl)ethyl]isothiazole-3,5-dicarboxamide (54o)



C$_{27}$H$_{28}$N$_6$O$_3$S

M$_r$ = 516.6

53o (420 mg, 1.0 mmol) was added in one portion to a solution of **25** (320 mg, 1.2 mmol) in acetone (100 mL). The mixture was cooled to 0 °C and morpholine (0.17 mL, 170 mg, 1.9 mmol) was added dropwise. After stirring the reaction mixture for 12 h at ambient temperature, the solvent was removed *in vacuo*. The residue was purified by flash column chromatography (Ø = 6 cm, h = 20 cm, V = 15 mL, dichloromethane/acetone = 10/1) and recrystallized from acetonitrile to give **54o** (380 mg, 0.74 mmol, 74% yield) as colorless solid.

m.p. = 252 °C;

TLC: R_f = 0.23 (dichloromethane/acetone = 10/1);

^{1}H NMR (400 MHz, DMSO-d_6): δ [ppm] = 0.90 – 1.34 (m, 5H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 1.47 – 1.82 (m, 5H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 2.17 (s, 3H, ArCH_3), 3.56 – 3.68 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), 6.17 (s, 1H, NCHCO), 6.85 – 7.02 (m, 5H, 2'''-H$_{p\text{-tolyl}}$, 3'''-H$_{p\text{-tolyl}}$, 5'''-H$_{p\text{-tolyl}}$, 6'''-H$_{p\text{-tolyl}}$, H$_{3\text{-cyanophenyl}}$ (1H)), 7.22 (s br, 2H, ArNH_2), 7.46 (s br, 1H, H$_{3\text{-cyanophenyl}}$), 7.63 (s br, 1H, CONH_2), 7.78 – 7.83 (m, 1H, 4''-H$_{3\text{-cyanophenyl}}$), 7.87 (s br, 1H, CONH_2), 8.13 (d, J = 7.7 Hz, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), the signal for 1 H$_{3\text{-cyanophenyl}}$ cannot be observed in the spectrum;

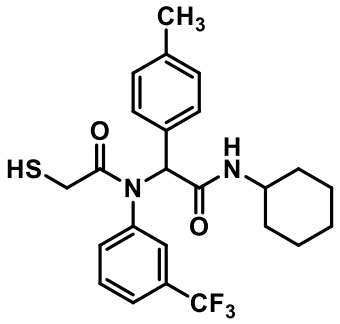
^{13}C NMR (126 MHz, DMSO-d_6): δ [ppm] = 20.5 (1C, ArCH$_3$), 24.4 (1C, NHCH(CH$_2$$CH_2$)$_2CH_2$), 24.6 (1C, NHCH(CH$_2$$CH_2$)$_2CH_2$), 25.1 (1C, NHCH(CH$_2CH_2$)$_2$$CH_2$), 32.1 (1C, NHCH($CH_2CH_2$)$_2CH_2$), 32.2 (1C, NHCH($CH_2CH_2$)$_2CH_2$), 48.0 (1C, NH$C$H(CH$_2CH_2$)$_2CH_2$), 63.5 (1C, N$C$HCO), 111.5 (1C, C-3''$_{3\text{-cyanophenyl}}$), 117.7 (1C, Ar$C$N), 122.2 (1C, C-5'$_{isothiazole}$), 128.7 (2C, C-3'''$_{p\text{-tolyl}}$, C-5'''$_{p\text{-tolyl}}$), 130.2 (2C, C-2'''$_{p\text{-tolyl}}$, C-6'''$_{p\text{-tolyl}}$), 130.3 (1C, C$_{3\text{-cyanophenyl}}$), 131.4 (1C, C-1'''$_{p\text{-tolyl}}$), 133.2 (1C, C-4''$_{3\text{-cyanophenyl}}$), 136.8 (1C, C$_{3\text{-cyanophenyl}}$), 137.3 (1C, C-4'''$_{p\text{-tolyl}}$), 138.4 (1C, C$_{3\text{-cyanophenyl}}$), 138.5 (1C, C$_{3\text{-cyanophenyl}}$), 147.0 (1C, C-3'$_{isothiazole}$), 151.4 (1C, C-4'$_{isothiazole}$), 161.8 (1C, Ar$C$ONCH), 163.7 (1C, Ar$CONH_2$), 168.5 (1C, CH$C$ONHCH);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3459, 3343, 3279, 3177, 3066, 2927, 2850, 2231, 1675, 1645, 1613, 1558, 1517, 1492, 1480, 1449, 1427, 1371, 1343, 1326, 1250, 1237, 1185, 1169, 1151, 1104, 1073, 980, 814, 799, 757, 737, 701, 678, 556,528, 503, 486;

HRMS (m/z): [M+H]$^+$ calcd for C$_{27}$H$_{29}$N$_6$O$_3$S: 517.2016, found: 517.2021;

HPLC (method 1): t_R = 23.7 min, purity 97.3%.

_N_-Cyclohexyl-2-{2-mercapto-_N_-[3-(trifluoromethyl)phenyl]acetamido}-2-(_p_-tolyl)acetamide (53p)



$C_{24}H_{27}F_3N_2O_2S$

$M_r = 464.5$

3-(Trifluoromethyl)aniline (320 mg, 2.0 mmol) and 4-methylbenzaldehyde (0.24 mL, 240 mg, 2.0 mmol) were dissolved in dichloromethane (15 mL) and the solution was stirred at ambient temperature for 10 min. Then, sulfanylacetic acid (0.14 mL, 180 mg, 2.0 mmol) and isocyanocyclohexane (0.25 mL, 220 mg, 2.0 mmol) were added. After stirring the reaction mixture for 48 h at ambient temperature, the solvent was removed *in vacuo* and the residue was purified by flash column chromatography (Ø = 2 cm, h = 15 cm, V = 5 mL, dichloromethane/acetone = 100/1) to give **53p** (560 mg, 1.2 mmol, 60% yield) as colorless solid.

m.p. = 168 °C;

TLC: R_f = 0.35 (dichloromethane/acetone = 50/1);

^{1}H NMR (500 MHz, DMSO-d_6): δ [ppm] = 0.91 – 1.02 (m, 1H, NHCH(CH_2CH$_2$)$_2$CH$_2$), 1.02 – 1.13 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$CH_2), 1.13 – 1.32 (m, 3H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (1H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H)), 1.48 – 1.72 (m, 4H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (1H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 1.72 – 7.80 (m, 1H, NHCH(CH_2CH$_2$)$_2$CH$_2$), 2.13 (s, 3H, ArCH_3), 2.60 – 2.69 (m, 1H, *H*SCH$_2$CO), 2.92 – 3.13 (m, 2H, HSCH_2CO), 3.55 – 3.65 (m, 1H, NHC*H*(CH$_2$CH$_2$)$_2$CH$_2$), 6.05 (s, 1H, NC*H*CO), 6.85 – 6.90 (m, 2H, 2"-H$_{p\text{-tolyl}}$, 6"-H$_{p\text{-tolyl}}$), 6.90 – 6.95 (m, 2H, 3"-H$_{p\text{-tolyl}}$, 5"-H$_{p\text{-tolyl}}$), 7.39 (s br, 1H, H$_{3\text{-(trifluoromethyl)phenyl}}$), 7.48 – 7.53 (m, 1H, H$_{3\text{-(trifluoromethyl)phenyl}}$), 7.79 – 8.45 (m, 2H, H$_{3\text{-(trifluoromethyl)phenyl}}$ (1H), N*H*CH(CH$_2$CH$_2$)$_2$CH$_2$), the signal for 1 H$_{3\text{-(trifluoromethyl)phenyl}}$ cannot be observed in the spectrum;

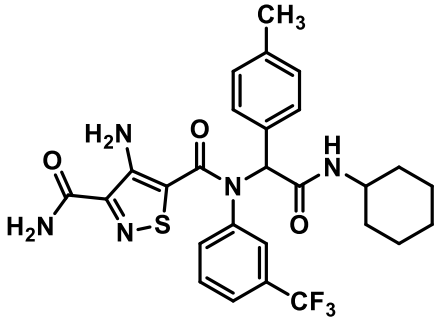
^{13}C NMR (101 MHz, DMSO-*d*$_6$): δ [ppm] = 20.5 (1C, Ar*C*H$_3$), 24.4 (1C, NHCH(CH$_2$*C*H$_2$)$_2$CH$_2$), 24.6 (1C, NHCH(CH$_2$*C*H$_2$)$_2$CH$_2$), 25.2 (1C, NHCH(CH$_2$CH$_2$)$_2$*C*H$_2$), 27.5 (1C, HS*C*H$_2$CO), 32.2 (2C, NHCH(*C*H$_2$CH$_2$)$_2$CH$_2$), 47.9 (1C, NH*C*H(CH$_2$CH$_2$)$_2$CH$_2$), 63.4 (1C, N*C*HCO), 124.5 (1C, C$_{3\text{-(trifluoromethyl)phenyl}}$), 127.9 (1C, C$_{3\text{-(trifluoromethyl)phenyl}}$), 128.5 (2C, C-3"$_{p\text{-tolyl}}$, C-5"$_{p\text{-tolyl}}$), 129.5 (1C, C$_{3\text{-(trifluoromethyl)phenyl}}$), 129.8 (2C, C-2"$_{p\text{-tolyl}}$, C-6"$_{p\text{-tolyl}}$), 131.8 (1C, C-1"$_{p\text{-tolyl}}$), 135.2 (1C, C$_{3\text{-(trifluoromethyl)phenyl}}$), 137.1 (1C, C-4"$_{p\text{-tolyl}}$), 139.9 (1C, C-1'$_{3\text{-(trifluoromethyl)phenyl}}$), 168.5 (1C, *C*ON), 168.7 (1C, *C*ON), the signals for Ar*C*F$_3$ and 1 C$_{3\text{-(trifluoromethyl)phenyl}}$ cannot be observed in the spectrum;

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3270, 3092, 2929, 2855, 1645, 1563, 1516, 1490, 1447, 1409, 1371, 1360, 1337, 1325, 1304, 1276, 1252, 1224, 1169, 1126, 1099, 1067, 981, 903, 840, 806, 739, 704, 685, 661, 610, 561, 529, 505;

HRMS (*m*/*z*): [M+H]$^+$ calcd for C$_{24}$H$_{28}$F$_3$N$_2$O$_2$S: 465.1818, found: 465.1813;

HPLC (method 1): t$_R$ = 25.9 min, purity 93.8%.

4-Amino-*N*^{5}-[2-(cyclohexylamino)-2-oxo-1-(*p*-tolyl)ethyl]-*N*^{5}-[3-(trifluoromethyl)phenyl]isothiazole-3,5-dicarboxamide (54p)



C$_{27}$H$_{28}$F$_3$N$_5$O$_3$S

M$_r$ = 559.6

53p (470 mg, 1.0 mmol) was added in one portion to a solution of **25** (320 mg, 1.2 mmol) in acetone (100 mL). The mixture was cooled to 0 °C and morpholine (0.17 mL, 170 mg, 1.9 mmol) was added dropwise. After stirring the reaction mixture for 12 h at ambient temperature, the solvent was removed *in vacuo*. The residue was purified by flash column chromatography (Ø = 6 cm, h = 20 cm, V = 15 mL, dichloromethane/acetone = 10/1) and recrystallized from acetonitrile to give **54p** (470 mg, 0.85 mmol, 85% yield) as colorless solid.

m.p. = 213 °C;

TLC: R_f = 0.41 (dichloromethane/acetone = 5/1);

^{1}H NMR (400 MHz, DMSO-d_6): δ [ppm] = 0.91 – 1.15 (m, 2H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (1H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 1.15 – 1.34 (m, 3H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (1H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H)), 1.47 – 1.63 (m, 2H, NHCH(CH$_2$CH_2)$_2$CH$_2$ (1H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 1.63 – 1.74 (m, 2H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (1H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (1H)), 1.74 – 1.83 (m, 1H, NHCH(CH_2CH$_2$)$_2$CH$_2$), 2.14 (s, 3H, ArCH_3), 3.56 – 3.67 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), 6.18 (s, 1H, NCHCO), 6.89 – 7.00 (m, 4H, 2'''-H$_{p\text{-tolyl}}$, 3'''-H$_{p\text{-tolyl}}$, 5'''-H$_{p\text{-tolyl}}$, 6'''-H$_{p\text{-tolyl}}$), 7.23 (s br, 2H, ArNH_2), 7.48 (s br, 1H, H$_{3\text{-(trifluoromethyl)phenyl}}$), 7.63 (s br, 1H, CON$H_2$), 7.66 – 7.71 (m, 1H, H$_{3\text{-(trifluoromethyl)phenyl}}$), 7.88 (s br, 1H, CON$H_2$), 8.13 (d, J = 7.7 Hz, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), the signals for 2 H$_{3\text{-(trifluoromethyl)phenyl}}$ cannot be observed in the spectrum;

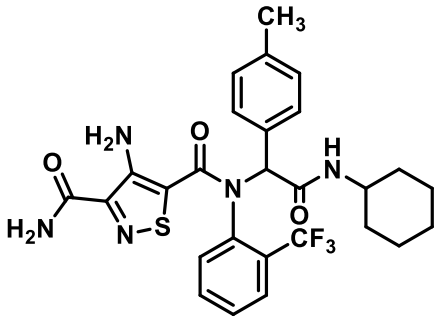
^{13}C NMR (101 MHz, DMSO-d_6): δ [ppm] = 20.5 (1C, ArCH$_3$), 24.4 (1C, NHCH(CH$_2$$CH_2$)$_2CH_2$), 24.6 (1C, NHCH(CH$_2$$CH_2$)$_2CH_2$), 25.2 (1C, NHCH(CH$_2CH_2$)$_2$$CH_2$), 32.18 (1C, NHCH($CH_2CH_2$)$_2CH_2$), 32.20 (1C, NHCH($CH_2CH_2$)$_2CH_2$), 48.0 (1C, NH$C$H(CH$_2CH_2$)$_2CH_2$), 63.6 (1C, N$C$HCO), 122.4 (1C, C-5'$_{isothiazole}$), 123.5 (q, J = 272.1 Hz, 1C, ArCF$_3$), 126.1 (1C, C$_{3\text{-(trifluoromethyl)phenyl}}$), 128.6 (2C, C-3'''$_{p\text{-tolyl}}$, C-5'''$_{p\text{-tolyl}}$), 130.1 (1C, C$_{3\text{-(trifluoromethyl)phenyl}}$), 130.2 (2C, C-2'''$_{p\text{-tolyl}}$, C-6'''$_{p\text{-tolyl}}$), 131.5 (1C, C-1'''$_{p\text{-tolyl}}$), 137.3 (1C, C-4'''$_{p\text{-tolyl}}$), 137.6 (1C, C$_{3\text{-(trifluoromethyl)phenyl}}$), 138.1 (1C, C-1''$_{3\text{-(trifluoromethyl)phenyl}}$), 147.0 (1C, C-3'$_{isothiazole}$), 151.5 (1C, C-4'$_{isothiazole}$), 161.9 (1C, Ar$C$ONCH), 163.8 (1C, Ar$CONH_2$), 168.6 (1C, CH$C$ONHCH), the signals for 2 C$_{3\text{-(trifluoromethyl)phenyl}}$ cannot be observed in the spectrum;

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3455, 3344, 3282, 3197, 3066, 2928, 2851, 1676, 1645, 1617, 1586, 1557, 1518, 1492, 1446, 1372, 1334, 1303, 1273, 1250, 1236, 1167, 1126, 1092, 1071, 979, 850, 841, 806, 758, 740, 702, 683, 655, 563, 531, 503;

HRMS (m/z): [M+H]$^+$ calcd for C$_{27}$H$_{29}$F$_3$N$_5$O$_3$S: 560.1938, found: 560.1933;

HPLC (method 1): t_R = 25.7 min, purity 98.1%.

4-Amino-N^5-[2-(cyclohexylamino)-2-oxo-1-(*p*-tolyl)ethyl]-N^5-[2-(trifluoromethyl)phenyl]isothiazole-3,5-dicarboxamide (54q)



$C_{27}H_{28}F_3N_5O_3S$

M_r = 559.6

2-(Trifluoromethyl)aniline (320 mg, 2.0 mmol) and 4-methylbenzaldehyde (0.24 mL, 240 mg, 2.0 mmol) were dissolved in dichloromethane (15 mL) and the solution was stirred at ambient temperature for 10 min. Then, sulfanylacetic acid (0.14 mL, 180 mg, 2.0 mmol) and isocyanocyclohexane (0.25 mL, 220 mg, 2.0 mmol) were added. After stirring the reaction mixture for 48 h at ambient temperature, the solvent was removed *in vacuo* and the residue was washed with acetonitrile.

A share (460 mg, 67%) of the obtained crude product (690 mg) was added in one portion to a solution of **25** (320 mg, 1.2 mmol) in acetone (100 mL). The mixture was cooled to 0 °C and morpholine (0.17 mL, 170 mg, 1.9 mmol) was added dropwise. After stirring the reaction mixture for 12 h at ambient temperature, the solvent was removed *in vacuo*. The residue was purified by flash column chromatography (Ø = 6 cm, h = 20 cm, V = 15 mL, dichloromethane/acetone = 10/1) and recrystallized from acetonitrile to give **54q** (310 mg, 0.55 mmol, 41% yield) as colorless solid.

m.p. = 179 °C;

TLC: R_f = 0.20 (dichloromethane/acetone = 10/1);

^{1}H NMR (500 MHz, DMSO-d_6): δ [ppm] = 1.00 – 1.30 (m, 5H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 1.48 – 1.80 (m, 5H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 2.31 (s, 3H, ArCH_3), 3.45 – 3.55 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), 5.99 (s, 1H, NCHCO), 6.90 (s br, 2H, ArNH_2), 7.19 – 7.24 (m, 2H, 3'''-H$_{p\text{-tolyl}}$, 5'''-H$_{p\text{-tolyl}}$), 7.37 – 7.43 (m, 3H, 2'''-H$_{p\text{-tolyl}}$, 6'''-H$_{p\text{-tolyl}}$),

7.77 (s br, 1H, CON*H*$_2$), 8.15 (s br, 1H, CON*H*$_2$), 8.20 (d, *J* = 7.8 Hz, 1H, N*H*CH(CH$_2$CH$_2$)$_2$CH$_2$), the signals for 4 H$_{2\text{-(trifluoromethyl)phenyl}}$ cannot be observed in the spectrum;

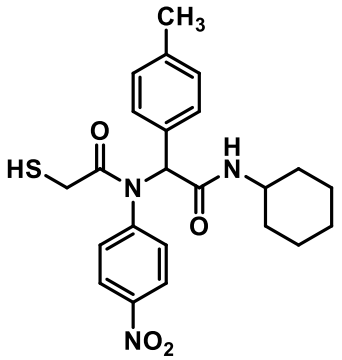
^{13}C NMR (101 MHz, DMSO-*d*$_6$): δ [ppm] = 20.7 (1C, Ar*C*H$_3$), 24.3 (1C, NHCH(CH$_2$*C*H$_2$)$_2$CH$_2$), 24.4 (1C, NHCH(CH$_2$*C*H$_2$)$_2$CH$_2$), 25.1 (1C, NHCH(CH$_2$CH$_2$)$_2$*C*H$_2$), 32.0 (1C, NHCH(*C*H$_2$CH$_2$)$_2$CH$_2$), 32.2 (1C, NHCH(*C*H$_2$CH$_2$)$_2$CH$_2$), 47.7 (1C, NH*C*H(CH$_2$CH$_2$)$_2$CH$_2$), 75.2 (1C, N*C*HCO), 120.1 (1C, C-5'$_{\text{isothiazole}}$), 127.2 (2C, C-2'''$_{p\text{-tolyl}}$, C-6'''$_{p\text{-tolyl}}$), 129.0 (2C, C-3'''$_{p\text{-tolyl}}$, C-5'''$_{p\text{-tolyl}}$), 132.7 (1C, C-1'''$_{p\text{-tolyl}}$), 138.1 (1C, C-4'''$_{p\text{-tolyl}}$), 148.8 (1C, C-3'$_{\text{isothiazole}}$), 149.8 (1C, C-4'$_{\text{isothiazole}}$), 160.2 (1C, Ar*C*ONCH), 163.5 (1C, Ar*C*ONH$_2$), 166.6 (1C, CH*C*ONHCH), the signals for 6 C$_{2\text{-(trifluoromethyl)phenyl}}$ and Ar*C*F$_3$ cannot be observed in the spectrum;

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3497, 3444, 3363, 3291, 2926, 2852, 1704, 1653, 1599, 1569, 1547, 1515, 1450, 1366, 1267, 1190, 1153, 1119, 1069, 1022, 963, 889, 793, 776, 761, 665;

HRMS (*m*/*z*): [M+H]$^+$ calcd for C$_{27}$H$_{29}$F$_3$N$_5$O$_3$S: 560.1938, found: 560.1976;

HPLC (method 1): t$_R$ = 22.6 min, purity 95.7%.

N-Cyclohexyl-2-[2-mercapto-*N*-(4-nitrophenyl)acetamido]-2-(*p*-tolyl)acetamide (53r)



C$_{23}$H$_{27}$N$_3$O$_4$S

M$_r$ = 441.5

4-Nitroaniline (280 mg, 2.0 mmol) and 4-methylbenzaldehyde (0.24 mL, 240 mg, 2.0 mmol) were dissolved in dichloromethane (15 mL) and the solution was stirred at ambient temperature for 10 min. Then, sulfanylacetic acid (0.14 mL, 180 mg, 2.0 mmol) and isocyanocyclohexane (0.25 mL, 220 mg, 2.0 mmol) were added. After stirring the reaction mixture for 48 h at ambient temperature, the solvent was removed *in vacuo* and the residue was purified by flash

column chromatography (Ø = 2 cm, h = 15 cm, V = 5 mL, petroleum ether/ethyl acetate = 4/1) to give **53r** (400 mg, 0.92 mmol, 46% yield) as colorless solid.

m.p. = 211 °C;

TLC: R_f = 0.27 (dichloromethane/ethyl acetate = 15/1);

^{1}H NMR (400 MHz, DMSO-d_6): δ [ppm] = 0.91 – 1.34 (m, 5H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 1.48 – 1.82 (m, 5H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 2.13 (s, 3H, ArCH_3), 2.59 – 2.70 (m, 1H, *H*SCH$_2$CO), 2.96 – 3.17 (m, 2H, HSCH_2CO), 3.55 – 3.67 (m, 1H, NHC*H*(CH$_2$CH$_2$)$_2$CH$_2$), 6.08 (s, 1H, NC*H*CO), 6.87 – 6.98 (m, 4H, 2"-H$_{p\text{-tolyl}}$, 3"-H$_{p\text{-tolyl}}$, 5"-H$_{p\text{-tolyl}}$, 6"-H$_{p\text{-tolyl}}$), 7.43 (s br, 1H, H$_{4\text{-nitrophenyl}}$), 8.00 – 8.05 (m, 1H, H$_{4\text{-nitrophenyl}}$), 8.07 (d, J = 7.7 Hz, 1H, N*H*CH(CH$_2$CH$_2$)$_2$CH$_2$), the signals for 2 H$_{4\text{-nitrophenyl}}$ cannot be observed in the spectrum;

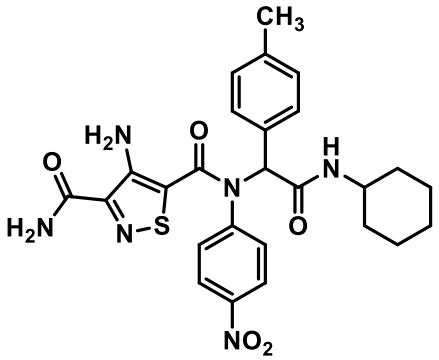
^{13}C NMR (101 MHz, DMSO-d_6): δ [ppm] = 20.5 (1C, Ar*C*H$_3$), 24.4 (1C, NHCH(CH$_2$*C*H$_2$)$_2$CH$_2$), 24.6 (1C, NHCH(CH$_2$*C*H$_2$)$_2$CH$_2$), 25.1 (1C, NHCH(CH$_2$CH$_2$)$_2$*C*H$_2$), 27.5 (1C, HS*C*H$_2$CO), 32.14 (1C, NHCH(*C*H$_2$CH$_2$)$_2$CH$_2$), 32.17 (1C, NHCH(*C*H$_2$CH$_2$)$_2$CH$_2$), 47.9 (1C, NH*C*H(CH$_2$CH$_2$)$_2$CH$_2$), 63.4 (1C, N*C*HCO), 122.7 (1C, C$_{4\text{-nitrophenyl}}$), 126.1 (1C, C$_{4\text{-nitrophenyl}}$), 128.7 (2C, C-3"$_{p\text{-tolyl}}$, C-5"$_{p\text{-tolyl}}$), 129.5 (1C, C$_{4\text{-nitrophenyl}}$), 129.8 (2C, C-2"$_{p\text{-tolyl}}$, C-6"$_{p\text{-tolyl}}$), 131.7 (1C, C-1"$_{p\text{-tolyl}}$), 137.2 (1C, C-4"$_{p\text{-tolyl}}$), 137.9 (1C, C$_{4\text{-nitrophenyl}}$), 140.3 (1C, C-1'$_{4\text{-nitrophenyl}}$), 147.3 (1C, C-4'$_{4\text{-nitrophenyl}}$), 168.4 (2C, CH*C*ONHCH, HSCH$_2$*C*O);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3300, 3082, 2927, 2854, 1645, 1531, 1480, 1452, 1421, 1372, 1352, 1303, 1271, 1250, 1230, 1192, 1180, 1161, 1108, 978, 891, 881, 828, 794, 759, 740, 726, 701, 628, 570, 535, 503;

HRMS (*m*/*z*): [M+H]$^+$ calcd for $C_{23}H_{28}N_3O_4S$: 442.1795, found: 442.1782;

HPLC (method 1): t_R = 24.5 min, purity 75.6%.

4-Amino-N^5-[2-(cyclohexylamino)-2-oxo-1-(*p*-tolyl)ethyl]-N^5-(4-nitrophenyl)isothiazole-3,5-dicarboxamide (54r)



$C_{26}H_{28}N_6O_5S$

M_r = 536.6

53r (220 mg, 0.50 mmol) was added in one portion to a solution of **25** (160 mg, 0.60 mmol) in acetone (50 mL). The mixture was cooled to 0 °C and morpholine (0.08 mL, 83 mg, 0.95 mmol) was added dropwise. After stirring the reaction mixture for 12 h at ambient temperature, the solvent was removed *in vacuo*. The residue was purified by flash column chromatography (Ø = 6 cm, h = 20 cm, V = 15 mL, dichloromethane/acetone = 10/1) and recrystallized from acetonitrile to give **54r** (200 mg, 0.37 mmol, 73% yield) as colorless solid.

m.p. = 234 °C;

TLC: R_f = 0.20 (dichloromethane/acetone = 10/1);

^{1}H NMR (500 MHz, DMSO-d_6): δ [ppm] = 0.93 – 1.03 (m, 1H, NHCH(CH_2CH$_2$)$_2$CH$_2$), 1.03 – 1.13 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$CH_2), 1.15 – 1.33 (m, 3H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (1H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H)), 1.49 – 1.63 (m, 2H, NHCH(CH$_2$CH_2)$_2$CH$_2$ (1H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 1.64 – 1.74 (m, 2H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (1H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (1H)), 1.75 – 1.82 (m, 1H, NHCH(CH_2CH$_2$)$_2$CH$_2$), 2.14 (s, 3H, ArCH_3), 3.58 – 3.68 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), 6.21 (s, 1H, NCHCO), 6.93 – 7.00 (m, 4H, 2'''-H$_{p\text{-tolyl}}$, 3'''-H$_{p\text{-tolyl}}$, 5'''-H$_{p\text{-tolyl}}$, 6'''-H$_{p\text{-tolyl}}$), 7.23 (s br, 2H, ArNH_2), 7.52 (s br, 1H, H$_{4\text{-nitrophenyl}}$), 7.64 (s br, 1H, CONH_2), 7.87 (s br, 1H, CONH_2), 8.16 (d, J = 7.7 Hz, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), 8.18 – 8.21 (m, 1H, H$_{4\text{-nitrophenyl}}$), the signals for 2 H$_{4\text{-nitrophenyl}}$ cannot be observed in the spectrum;

^{13}C NMR (151 MHz, DMSO-d_6): δ [ppm] = 20.6 (1C, ArCH$_3$), 24.4 (1C, NHCH(CH$_2$$CH_2$)$_2CH_2$), 24.6 (1C, NHCH(CH$_2$$CH_2$)$_2CH_2$), 25.2 (1C, NHCH(CH$_2CH_2$)$_2$$CH_2$),

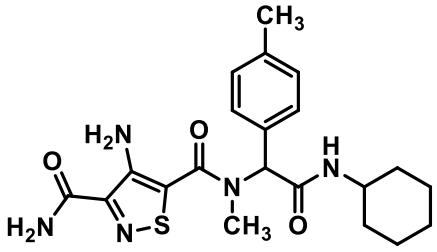
32.1 (1C, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), 32.2 (1C, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), 48.0 (1C, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), 63.6 (1C, NCHCO), 122.1 (1C, C-5'$_{isothiazole}$), 124.3 (1C, C$_{4\text{-nitrophenyl}}$), 128.0 (1C, C$_{4\text{-nitrophenyl}}$), 128.7 (2C, C-3'''$_{p\text{-tolyl}}$, C-5'''$_{p\text{-tolyl}}$), 130.2 (2C, C-2'''$_{p\text{-tolyl}}$, C-6'''$_{p\text{-tolyl}}$), 130.3 (1C, C$_{4\text{-nitrophenyl}}$), 131.3 (1C, C-1'''$_{p\text{-tolyl}}$), 137.4 (1C, C-4'''$_{p\text{-tolyl}}$), 138.7 (1C, C-1''$_{4\text{-nitrophenyl}}$), 140.2 (1C, C$_{4\text{-nitrophenyl}}$), 147.5 (1C, C$_{4\text{-nitrophenyl}}$), 147.1 (1C, C-3'$_{isothiazole}$), 151.5 (1C, C-4'$_{isothiazole}$), 161.8 (1C, ArCONCH), 163.7 (1C, ArCONH$_2$), 168.6 (1C, CHCONHCH);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3454, 3340, 3279, 3197, 3077, 2926, 2850, 1677, 1644, 1619, 1557, 1531, 1493, 1450, 1372, 1349, 1325, 1250, 1237, 1182, 1153, 1100, 1072, 979, 879, 828, 801, 756, 734, 720, 697, 530, 503, 449;

HRMS (m/z): [M+H]$^+$ calcd for $C_{26}H_{29}N_6O_5S$: 537.1915, found: 537.1918;

HPLC (method 1): t_R = 24.2 min, purity 95.2%.

4-Amino-N^5-[2-(cyclohexylamino)-2-oxo-1-(p-tolyl)ethyl]-N^5-methylisothiazole-3,5-dicarboxamide (54s)



$C_{21}H_{27}N_5O_3S$

M_r = 429.5

A 2 M solution of methanamine in methanol (1.0 mL, 2.0 mmol) and 4-methylbenzaldehyde (0.24 mL, 240 mg, 2.0 mmol) were added to dichloromethane (15 mL) and the solution was stirred at ambient temperature for 10 min. Then, sulfanylacetic acid (0.14 mL, 180 mg, 2.0 mmol) and isocyanocyclohexane (0.25 mL, 220 mg, 2.0 mmol) were added. After stirring the reaction mixture for 48 h at ambient temperature, the solvent was removed *in vacuo* and the residue was washed with acetonitrile.

A share (330 mg, 93%) of the obtained crude product (360 mg) was added in one portion to a solution of **25** (320 mg, 1.2 mmol) in acetone (100 mL). The mixture was cooled to 0 °C and morpholine (0.17 mL, 170 mg, 2.0 mmol) was added dropwise. After stirring the reaction mixture for 12 h at ambient temperature, the solvent was removed *in vacuo*. The residue was

purified by flash column chromatography (Ø = 6 cm, h = 20 cm, V = 15 mL, dichloromethane/acetone = 15/1) and recrystallized from acetonitrile to give **54s** (180 mg, 0.43 mmol, 23% yield) as colorless solid.

m.p. = 213 °C;

TLC: R_f = 0.41 (dichloromethane/acetone = 5/1);

^{1}H NMR (500 MHz, DMSO-d_6): δ [ppm] = 1.03 – 1.34 (m, 5H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 1.50 – 1.59 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$CH_2), 1.60 – 1.83 (m, 4H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H)), 2.31 (s, 3H, ArCH_3), 2.91 (s, 3H, NCH_3), 3.57 – 3.70 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), 6.05 (s br, 1H, NCHCO), 6.79 (s br, 2H, ArNH_2), 7.13 – 7.19 (m, 2H, 2'''-H$_{p\text{-tolyl}}$, 6'''-H$_{p\text{-tolyl}}$), 7.19 – 7.24 (m, 2H, 3'''-H$_{p\text{-tolyl}}$, 5'''-H$_{p\text{-tolyl}}$), 7.69 (s br, 1H, CONH_2), 8.08 (s br, 1H, CONH_2), 8.14 (d, J = 7.8 Hz, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$);

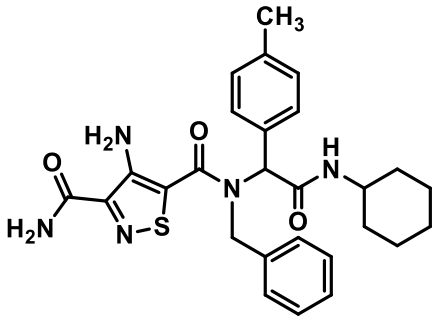
^{13}C NMR (126 MHz, DMSO-d_6): δ [ppm] = 20.7 (1C, ArCH$_3$), 24.5 (1C, NHCH(CH$_2$$CH_2$)$_2CH_2$), 24.6 (1C, NHCH(CH$_2$$CH_2$)$_2CH_2$), 25.2 (1C, NHCH(CH$_2CH_2$)$_2$$CH_2$), 32.2 (1C, NHCH($CH_2CH_2$)$_2CH_2$), 32.3 (1C, NHCH($CH_2CH_2$)$_2CH_2$), 33.2 (1C, N$CH_3$), 47.8 (1C, NH$C$H(CH$_2CH_2$)$_2CH_2$), 58.9 (1C, N$C$HCO), 128.8 (2C, C-2'''$_{p\text{-tolyl}}$, C-6'''$_{p\text{-tolyl}}$), 129.2 (2C, C-3'''$_{p\text{-tolyl}}$, C-5'''$_{p\text{-tolyl}}$), 133.0 (1C, C-1'''$_{p\text{-tolyl}}$), 137.3 (1C, C-4'''$_{p\text{-tolyl}}$), 147.8 (1C, C-3'$_{\text{isothiazole}}$), 163.4 (1C, Ar$C$ONCH), 163.9 (1C, Ar$CONH_2$), 168.0 (CH$C$ONHCH), the signals for C-4'$_{\text{isothiazole}}$ and C-5'$_{\text{isothiazole}}$ cannot be observed in the spectrum;

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3463, 3344, 3252, 2921, 2852, 1655, 1608, 1580, 1560, 1514, 1492, 1475, 1451, 1378, 1352, 1317, 1245, 1196, 1149, 1086, 879, 835, 755, 669, 556, 529, 498;

HRMS (m/z): [M+H]$^+$ calcd for $C_{21}H_{28}N_5O_3S$: 430.1907, found: 430.1896;

HPLC (method 1): t_R = 21.9 min, purity 97.9%.

4-Amino-N^5-benzyl-N^5-[2-(cyclohexylamino)-2-oxo-1-(*p*-tolyl)ethyl]isothiazole-3,5-dicarboxamide (54t)



$C_{27}H_{31}N_5O_3S$

$M_r = 505.6$

Benzylamine (0.22 mL, 210 mg, 2.0 mmol) and 4-methylbenzaldehyde (0.24 mL, 240 mg, 2.0 mmol) were dissolved in dichloromethane (15 mL) and the solution was stirred at ambient temperature for 10 min. Then, sulfanylacetic acid (0.14 mL, 180 mg, 2.0 mmol) and isocyanocyclohexane (0.25 mL, 220 mg, 2.0 mmol) were added. After stirring the reaction mixture for 48 h at ambient temperature, the solvent was removed *in vacuo* and the residue was washed with acetonitrile.

A share (410 mg, 77%) of the obtained crude product (530 mg) was added in one portion to a solution of **25** (320 mg, 1.2 mmol) in acetone (100 mL). The mixture was cooled to 0 °C and morpholine (0.17 mL, 170 mg, 1.9 mmol) was added dropwise. After stirring the reaction mixture for 12 h at ambient temperature, the solvent was removed *in vacuo*. The residue was purified by flash column chromatography (Ø = 6 cm, h = 20 cm, V = 15 mL, dichloromethane/acetone = 10/1) and recrystallized from acetonitrile to give **54t** (400 mg, 0.79 mmol, 51% yield) as colorless solid.

m.p. = 193 °C;

TLC: R_f = 0.24 (dichloromethane/acetone = 10/1);

^{1}H NMR (500 MHz, DMSO-d_6): δ [ppm] = 0.99 – 1.17 (m, 3H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 1.17 – 1.29 (m, 2H, NHCH(CH$_2$CH_2)$_2$CH$_2$), 1.47 – 1.56 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$CH_2), 1.57 – 1.74 (m, 4H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H)), 2.21 (s, 3H, ArCH_3), 3.50 – 3.60 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), 4.56 (d, J = 17.6 Hz, 1H, NCH_2Ph), 4.86 (d, J = 17.6 Hz, 1H, NCH_2Ph), 6.02 (s, 1H, NCHCO),

6.73 (s br, 2H, ArN*H*$_2$), 6.96 – 7.00 (m, 2H, 2''-H$_{phenyl}$, 6''-H$_{phenyl}$), 7.03 – 7.17 (m, 7H, 3''-H$_{phenyl}$, 4''-H$_{phenyl}$, 5''-H$_{phenyl}$, 2'''-H$_{p\text{-}tolyl}$, 3'''-H$_{p\text{-}tolyl}$, 5'''-H$_{p\text{-}tolyl}$, 6'''-H$_{p\text{-}tolyl}$), 7.66 (s br, 1H, CON*H*$_2$), 8.00 (s br, 1H, CON*H*$_2$), 8.11 (d, *J* = 7.7 Hz, 1H, N*H*CH(CH$_2$CH$_2$)$_2$CH$_2$);

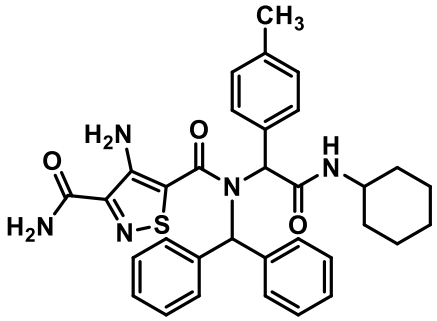
^{13}C NMR (126 MHz, DMSO-*d*$_6$): δ [ppm] = 20.6 (1C, Ar*C*H$_3$), 24.4 (1C, NHCH(CH$_2$*C*H$_2$)$_2$CH$_2$), 24.5 (1C, NHCH(CH$_2$*C*H$_2$)$_2$CH$_2$), 25.1 (1C, NHCH(CH$_2$CH$_2$)$_2$*C*H$_2$), 31.9 (1C, NHCH(*C*H$_2$CH$_2$)$_2$CH$_2$), 32.1 (1C, NHCH(*C*H$_2$CH$_2$)$_2$CH$_2$), 47.8 (1C, NH*C*H(CH$_2$CH$_2$)$_2$CH$_2$), 49.0 (1C, N*C*H$_2$Ph), 62.8 (1C, N*C*HCO), 125.5 (1C, C-5'$_{isothiazole}$), 126.3 (3C, C-2''$_{phenyl}$, C-4''$_{phenyl}$, C-6''$_{phenyl}$), 127.7 (2C, C-3''$_{phenyl}$, C-5''$_{phenyl}$), 128.8 (2C, C-3'''$_{p\text{-}tolyl}$, C-5'''$_{p\text{-}tolyl}$), 129.3 (2C, C-2'''$_{p\text{-}tolyl}$, C-6'''$_{p\text{-}tolyl}$), 132.4 (1C, C-1'''$_{p\text{-}tolyl}$), 137.3 (1C, C-4'''$_{p\text{-}tolyl}$), 138.1 (1C, C-1''$_{phenyl}$), 147.5 (1C, C-3'$_{isothiazole}$), 148.7 (1C, C-4'$_{isothiazole}$), 163.7 (1C, Ar*C*ONCH), 164.3 (1C, Ar*C*ONH$_2$), 168.1 (1C, CH*C*ONHCH);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3450, 3329, 3276, 2924, 2850, 1668, 1645, 1603, 1557, 1516, 1491, 1450, 1405, 1350, 1267, 1249, 1237, 1211, 1187, 1141, 1103, 1064, 1026, 983, 966, 940, 890, 839, 807, 792, 729, 695, 662, 557, 524, 503;

HRMS (*m*/*z*): [M+H]$^+$ calcd for C$_{27}$H$_{32}$N$_5$O$_3$S: 506.2220, found: 506.2221;

HPLC (method 1): t$_R$ = 24.0 min, purity 95.9%.

4-Amino-*N*^{5}-benzhydryl-*N*^{5}-[2-(cyclohexylamino)-2-oxo-1-(*p*-tolyl)ethyl]isothiazole-3,5-dicarboxamide (54u)



C$_{33}$H$_{35}$N$_5$O$_3$S

M$_r$ = 581.7

Benzhydrylamine (0.37 mL, 390 mg, 2.1 mmol) and 4-methylbenzaldehyde (0.24 mL, 240 mg, 2.0 mmol) were dissolved in dichloromethane (15 mL) and the solution was stirred at ambient temperature for 10 min. Then, sulfanylacetic acid (0.14 mL, 180 mg, 2.0 mmol) and isocyanocyclohexane (0.25 mL, 220 mg, 2.0 mmol) were added. After stirring the reaction

mixture for 48 h at ambient temperature, the solvent was removed *in vacuo* and the residue was washed with acetonitrile.

A share (490 mg, 61%) of the obtained crude product (790 mg) was added in one portion to a solution of **25** (320 mg, 1.2 mmol) in acetone (100 mL). The mixture was cooled to 0 °C and morpholine (0.17 mL, 170 mg, 1.9 mmol) was added dropwise. After stirring the reaction mixture for 12 h at ambient temperature, the solvent was removed *in vacuo*. The residue was purified by flash column chromatography (Ø = 6 cm, h = 20 cm, V = 15 mL, dichloromethane/acetone = 10/1) and recrystallized from acetonitrile to give **54u** (410 mg, 0.70 mmol, 57% yield) as colorless oil.

TLC: R_f = 0.27 (dichloromethane/acetone = 10/1);

^{1}H NMR (500 MHz, DMSO-d_6): δ [ppm] = 1.01 – 1.19 (m, 3H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 1.23 – 1.36 (m, 2H, NHCH(CH$_2$CH_2)$_2$CH$_2$), 1.49 – 1.57 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$CH_2), 1.57 – 1.69 (m, 2H, NHCH(CH$_2$CH_2)$_2$CH$_2$), 1.69 – 1.76 (m, 1H, NHCH(CH_2CH$_2$)$_2$CH$_2$), 1.76 – 1.83 (m, 1H, NHCH(CH_2CH$_2$)$_2$CH$_2$), 2.11 (s, 3H, ArCH_3), 3.67 – 3.77 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), 5.60 (s, 1H, NCHCO), 6.14 (s, 1H, NCHPh$_2$), 6.33 (s br, 2H, ArNH_2), 6.77 – 6.83 (m, 2H, 3'''-H$_{p\text{-tolyl}}$, 5'''-H$_{p\text{-tolyl}}$), 6.89 – 6.94 (m, 2H, 2'''-H$_{p\text{-tolyl}}$, 6'''-H$_{p\text{-tolyl}}$), 6.98 – 7.10 (m, 5H, 2''-H$_{phenyl}$ (1H), 3''-H$_{phenyl}$ (1H), 4''-H$_{phenyl}$ (1H), 5''-H$_{phenyl}$ (1H), 6''-H$_{phenyl}$ (1H)), 7.13 – 7.19 (m, 1H, 4''-H$_{phenyl}$), 7.19 – 7.25 (m, 2H, 3''-H$_{phenyl}$ (1H), 5''-H$_{phenyl}$ (1H)), 7.41 – 7.47 (m, 2H, 2''-H$_{phenyl}$ (1H), 6''-H$_{phenyl}$ (1H)), 7.64 (s br, 1H, CONH_2), 7.70 (s br, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), 8.03 (s br, 1H, CONH_2);

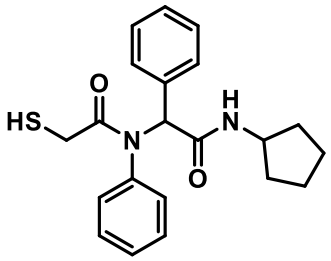
^{13}C NMR (126 MHz, DMSO-d_6): δ [ppm] = 20.5 (1C, ArCH$_3$), 24.3 (1C, NHCH(CH$_2$$CH_2$)$_2CH_2$), 24.4 (1C, NHCH(CH$_2$$CH_2$)$_2CH_2$), 25.1 (1C, NHCH(CH$_2CH_2$)$_2$$CH_2$), 31.9 (1C, NHCH($CH_2CH_2$)$_2CH_2$), 32.0 (1C, NHCH($CH_2CH_2$)$_2CH_2$), 47.8 (1C, NH$C$H(CH$_2CH_2$)$_2CH_2$), 64.8 (1C, N$C$HCO), 65.4 (1C, N$CHPh_2$), 126.3 (1C, C-4''$_{phenyl}$), 126.4 (1C, C-4''$_{phenyl}$), 127.36 (2C, C-3''$_{phenyl}$ (1C), C-5''$_{phenyl}$ (1C)), 127.44 (2C, C-3''$_{phenyl}$ (1C), C-5''$_{phenyl}$ (1C)), 127.9 (1C, C-5'$_{isothiazole}$), 128.3 (2C, C-3'''$_{p\text{-tolyl}}$, C-5'''$_{p\text{-tolyl}}$), 128.6 (2C, C-2''$_{phenyl}$ (1C), C-6''$_{phenyl}$ (1C)), 129.3 (4C, C-2'''$_{p\text{-tolyl}}$, C-6'''$_{p\text{-tolyl}}$, C-2''$_{phenyl}$ (1C), C-6''$_{phenyl}$ (1C)), 132.4 (1C, C-1'''$_{p\text{-tolyl}}$), 136.9 (1C, C-4'''$_{p\text{-tolyl}}$), 139.9 (1C, C-1''$_{phenyl}$), 140.8 (1C, C-1''$_{phenyl}$), 147.7 (1C, C-3'$_{isothiazole}$), 148.3 (1C, C-4'$_{isothiazole}$), 163.3 (1C, ArCON), 163.7 (1C, ArCON), 168.5 (1C, CHCONHCH);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3462, 3426, 3347, 2922, 2853, 1660, 1608, 1572, 1509, 1450, 1420, 1378, 1325, 1300, 1288, 1245, 1189, 1145, 1115, 1079, 1066, 1032, 922, 863, 827, 809, 793, 778, 745, 721, 701, 681, 650, 539, 515;

HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{33}H_{36}N_5O_3S$: 582.2533, found: 582.2534;

HPLC (method 1): t_R = 26.9 min, purity 98.8%.

***N*-Cyclopentyl-2-(2-mercapto-*N*-phenylacetamido)-2-phenylacetamide (55a)**



$C_{21}H_{24}N_2O_2S$

M_r = 368.5

Aniline (0.18 mL, 190 mg, 2.0 mmol) and benzaldehyde (0.20 mL, 210 mg, 2.0 mmol) were dissolved in dichloromethane (15 mL) and the solution was stirred at ambient temperature for 10 min. Then, sulfanylacetic acid (0.14 mL, 180 mg, 2.0 mmol) and isocyanocyclopentane (0.22 mL, 190 mg, 2.0 mmol) were added. After stirring the reaction mixture for 48 h at ambient temperature, the solvent was removed *in vacuo* and the residue was purified by flash column chromatography (Ø = 2 cm, h = 15 cm, V = 5 mL, dichloromethane/ethyl acetate = 20/1 → 15/1) to give **55a** (430 mg, 1.2 mmol, 58% yield) as colorless solid.

m.p. = 199 °C;

TLC: R_f = 0.27 (dichloromethane/ethyl acetate = 15/1);

^{1}H NMR (400 MHz, DMSO-d_6): δ [ppm] = 1.19 – 1.27 (m, 1H, NHCH(CH_2CH$_2$)$_2$), 1.39 – 1.54 (m, 4H, NHCH(CH_2CH$_2$)$_2$ (1H), NHCH(CH$_2$CH_2)$_2$ (3H)), 1.54 – 1.64 (m, 1H, NHCH(CH$_2$CH_2)$_2$), 1.70 – 1.85 (m, 2H, NHCH(CH_2CH$_2$)$_2$), 2.59 (t, *J* = 6.9 Hz, 1H, *H*SCH$_2$CO), 2.97 (dd, *J* = 14.9/6.7 Hz, 1H, HSCH_2CO), 3.04 (dd, *J* = 14.9/7.0 Hz, 1H, HSCH_2CO), 3.99 – 4.08 (m, 1H, NHC*H*(CH$_2$CH$_2$)$_2$), 6.02 (s, 1H, NC*H*CO), 6.86 – 7.36 (m, 10H, H_{phenyl}), 8.06 (d, *J* = 7.0 Hz, 1H, N*H*CH(CH$_2$CH$_2$)$_2$);

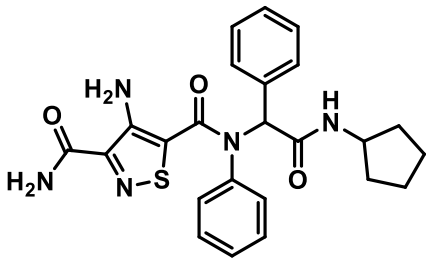
^{13}C NMR (101 MHz, DMSO-*d*$_6$): δ [ppm] = 23.4 (1C, NHCH(CH$_2$*C*H$_2$)$_2$), 23.5 (1C, NHCH(CH$_2$*C*H$_2$)$_2$), 27.3 (1C, HS*C*H$_2$CO), 31.8 (1C, NHCH(*C*H$_2$CH$_2$)$_2$), 32.1 (1C, NHCH(*C*H$_2$CH$_2$)$_2$), 50.6 (1C, NH*C*H(CH$_2$CH$_2$)$_2$), 63.8 (1C, N*C*HCO), 127.6 (1C, C$_{phenyl}$), 127.8 (3C, C$_{phenyl}$), 128.4 (2C, C$_{phenyl}$), 130.0 (2C, C$_{phenyl}$), 130.8 (2C, C$_{phenyl}$), 135.1 (1C, C$_{phenyl}$), 139.2 (1C, C$_{phenyl}$), 168.8 (1C, C=O), 168.9 (1C, C=O);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3269, 3064, 2959, 2869, 1649, 1594, 1556, 1492, 1453, 1422, 1386, 1368, 1313, 1231, 1183, 1157, 1073, 1027, 1003, 970, 920, 822, 803, 767, 727, 696, 626, 584, 566, 542, 518, 486;

HRMS (*m*/*z*): [M+Na]$^+$ calcd for C$_{21}$H$_{24}$N$_2$NaO$_2$S: 391.1451, found: 391.1458;

HPLC (method 1): t$_R$ = 22.5 min, purity 72.6%.

4-Amino-*N*^{5}-[2-(cyclopentylamino)-2-oxo-1-phenylethyl]-*N*^{5}-phenylisothiazole-3,5-dicarboxamide (57a)



C$_{24}$H$_{25}$N$_5$O$_3$S

M$_r$ = 463.6

55a (370 mg, 1.0 mmol) was added in one portion to a solution of **25** (320 mg, 1.2 mmol) in acetone (100 mL). The mixture was cooled to 0 °C and morpholine (0.17 mL, 170 mg, 2.0 mmol) was added dropwise. After stirring the reaction mixture for 12 h at ambient temperature, the solvent was removed *in vacuo*. The residue was purified by flash column chromatography (Ø = 6 cm, h = 20 cm, V = 15 mL, dichloromethane/acetone = 10/1) and recrystallized from acetonitrile to give **57a** (300 mg, 0.65 mmol, 65% yield) as colorless solid.

m.p. = 261 °C;

TLC: R_f = 0.20 (dichloromethane/acetone = 10/1);

^{1}H NMR (400 MHz, DMSO-d_6): δ [ppm] = 1.17 – 1.29 (m, 1H, NHCH(CH_2CH$_2$)$_2$), 1.37 – 1.66 (m, 5H, NHCH(CH_2CH$_2$)$_2$ (1H), NHCH(CH$_2$CH_2)$_2$), 1.69 – 1.89 (m, 2H, NHCH(CH_2CH$_2$)$_2$), 4.00 – 4.11 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$), 6.16 (s, 1H, NCHCO), 6.98 – 7.39 (m, 12H, H$_{arom.}$, ArNH_2), 7.60 (s br, 1H, CONH_2), 7.85 (s br, 1H, CONH_2), 8.18 (d, J = 7.0 Hz, 1H, NHCH(CH$_2$CH$_2$)$_2$);

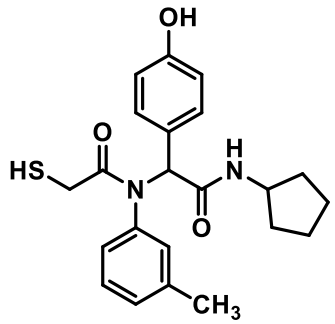
^{13}C NMR (101 MHz, DMSO-d_6): δ [ppm] = 23.45 (1C, NHCH(CH$_2$$CH_2$)$_2$), 23.52 (1C, NHCH(CH$_2$$CH_2$)$_2$), 31.7 (1C, NHCH($CH_2CH_2$)$_2$), 32.2 (1C, NHCH($CH_2CH_2$)$_2$), 50.6 (1C, NH$C$H(CH$_2CH_2$)$_2$), 64.0 (1C, N$C$HCO), 123.1 (1C, C-5'$_{isothiazole}$), 127.78 (1C, C$_{phenyl}$), 127.84 (2C, C$_{phenyl}$), 128.9 (2C, C$_{phenyl}$), 129.5 (1C, C$_{phenyl}$), 130.4 (2C, C$_{phenyl}$), 133.3 (2C, C$_{phenyl}$), 134.8 (1C, C$_{phenyl}$), 137.2 (1C, C$_{phenyl}$), 146.5 (1C, C-3'$_{isothiazole}$), 151.3 (1C, C-4'$_{isothiazole}$), 162.2 (1C, Ar$C$ONCH), 163.9 (1C, Ar$CONH_2$), 168.9 (1C, CH$C$ONHCH);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3456, 3340, 3271, 3092, 3063, 2954, 2865, 1668, 1648, 1609, 1561, 1490, 1452, 1406, 1375, 1355, 1339, 1311, 1291, 1244, 1183, 1148, 1073, 1035, 1003, 978, 848, 794, 759, 729, 697, 677, 642, 593, 578, 553, 513;

HRMS (m/z): [M+H]$^+$ calcd for C$_{24}$H$_{26}$N$_5$O$_3$S: 464.1751, found: 464.1741;

HPLC (method 1): t_R = 22.4 min, purity 95.0%.

N-Cyclopentyl-2-(4-hydroxyphenyl)-2-[2-mercapto-*N*-(*m*-tolyl)acetamido]acetamide (55b)



C$_{22}$H$_{26}$N$_2$O$_3$S

M_r = 398.5

3-Methylaniline (0.22 mL, 210 mg, 2.0 mmol) and 4-hydroxybenzaldehyde (240 mg, 2.0 mmol) were dissolved in dichloromethane (15 mL) and the solution was stirred at ambient temperature for 10 min. Then, sulfanylacetic acid (0.14 mL, 180 mg, 2.0 mmol) and isocyanocyclopentane

(0.22 mL, 190 mg, 2.0 mmol) were added. After stirring the reaction mixture for 48 h at ambient temperature, the solvent was removed *in vacuo* and the residue was purified by flash column chromatography (Ø = 4 cm, h = 15 cm, V = 15 mL, dichloromethane/ethyl acetate = 10/1) to give **55b** (580 mg, 1.5 mmol, 73% yield) as colorless solid.

m.p. = 193 °C;

TLC: R_f = 0.22 (dichloromethane/ethyl acetate = 10/1);

^{1}H NMR (500 MHz, DMSO-d_6): δ [ppm] = 1.18 – 1.26 (m, 1H, NHCH(CH_2CH$_2$)$_2$), 1.38 – 1.54 (m, 4H, NHCH(CH$_2$CH_2)$_2$ (3H), NHCH(CH_2CH$_2$)$_2$ (1H)), 1.55 – 1.65 (m, 1H, NHCH(CH$_2$CH_2)$_2$), 1.68 – 1.85 (m, 2H, NHCH(CH_2CH$_2$)$_2$), 2.17 (s br, 3H, ArCH_3), 2.55 (t, J = 6.8 Hz, 1H, HSCH$_2$CO), 2.96 (dd, J = 14.9/6.6 Hz, 1H, HSCH_2CO), 3.01 (dd, J = 14.9/7.0 Hz, 1H, HSCH_2CO), 3.96 – 4.06 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$), 5.88 (s, 1H, NCHCO), 6.20 – 6.68 (m, 3H, 3"-H$_{4\text{-hydroxyphenyl}}$, 5"-H$_{4\text{-hydroxyphenyl}}$, H$_{m\text{-tolyl}}$ (1H)), 6.76 – 6.83 (m, 2H, 2"-H$_{4\text{-hydroxyphenyl}}$, 6"-H$_{4\text{-hydroxyphenyl}}$), 6.92 – 6.99 (m, 1H, 4'-H$_{m\text{-tolyl}}$), 7.03 (s br, 1H, H$_{m\text{-tolyl}}$), 7.57 (s br, 1H, H$_{m\text{-tolyl}}$), 7.93 (d, J = 7.0 Hz, 1H, NHCH(CH$_2$CH$_2$)$_2$), 9.28 (s, 1H, ArOH);

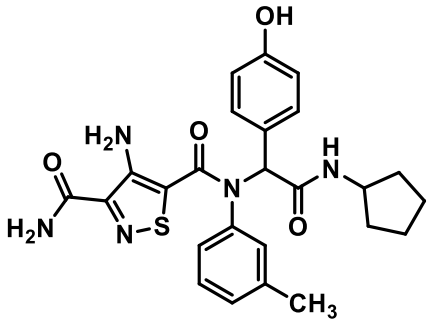
^{13}C NMR (101 MHz, DMSO-d_6): δ [ppm] = 20.7 (1C, ArCH$_3$), 23.45 (1C, NHCH(CH$_2$$CH_2$)$_2$), 23.51 (1C, NHCH(CH$_2$$CH_2$)$_2$), 27.4 (1C, HS$CH_2$CO), 31.8 (1C, NHCH($CH_2CH_2$)$_2$), 32.2 (1C, NHCH($CH_2CH_2$)$_2$), 50.5 (1C, NH$C$H(CH$_2CH_2$)$_2$), 63.4 (1C, N$C$HCO), 114.5 (2C, C-3"$_{4\text{-hydroxyphenyl}}$, C-5"$_{4\text{-hydroxyphenyl}}$), 125.3 (1C, C-1"$_{4\text{-hydroxyphenyl}}$), 127.8 (1C, C$_{m\text{-tolyl}}$), 128.0 (1C, C$_{m\text{-tolyl}}$), 128.3 (1C, C$_{m\text{-tolyl}}$), 131.2 (2C, C-2"$_{4\text{-hydroxyphenyl}}$, C-6"$_{4\text{-hydroxyphenyl}}$), 131.4 (1C, C$_{m\text{-tolyl}}$), 137.6 (1C, C-3'$_{m\text{-tolyl}}$), 139.2 (1C, C-1'$_{m\text{-tolyl}}$), 156.6 (1C, C-4"$_{4\text{-hydroxyphenyl}}$), 168.7 (1C, HSCH$_2$$C$O), 169.3 (1C, CH$C$ONHCH);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3298, 3185, 3067, 2965, 2865, 1659, 1626, 1616, 1599, 1585, 1547, 1518, 1487, 1458, 1422, 1383, 1304, 1272, 1240, 1189, 1175, 1157, 1107, 1092, 1055, 937, 864, 835, 819, 787, 744, 701, 641, 590, 573, 550, 510, 490, 455, 443, 402;

HRMS (m/z): [M+H]$^+$ calcd for $C_{22}H_{27}N_2O_3S$: 399.1737, found: 399.1738;

HPLC (method 1): t_R = 20.4 min, purity 78.4%.

4-Amino-N^5-[2-(cyclopentylamino)-1-(4-hydroxyphenyl)-2-oxoethyl]-N^5-(*m*-tolyl)isothiazole-3,5-dicarboxamide (57b)



$C_{25}H_{27}N_5O_4S$

M_r = 493.6

55b (400 mg, 1.0 mmol) was added in one portion to a solution of **25** (320 mg, 1.2 mmol) in acetone (100 mL). The mixture was cooled to 0 °C and morpholine (0.17 mL, 170 mg, 1.9 mmol) was added dropwise. After stirring the reaction mixture for 12 h at ambient temperature, the solvent was removed *in vacuo*. The residue was purified by flash column chromatography (Ø = 6 cm, h = 20 cm, V = 15 mL, dichloromethane/methanol = 10/1) to give **57b** (310 mg, 0.62 mmol, 62% yield) as colorless solid.

m.p. = 252 °C;

TLC: R_f = 0.22 (dichloromethane/methanol = 10/1);

^{1}H NMR (400 MHz, DMSO-d_6): δ [ppm] = 1.16 – 1.30 (m, 1H, NHCH(CH_2CH$_2$)$_2$), 1.36 – 1.68 (m, 5H, NHCH(CH$_2$CH_2)$_2$, NHCH(CH_2CH$_2$)$_2$ (1H)), 1.68 – 1.89 (m, 2H, NHCH(CH_2CH$_2$)$_2$), 2.21 (s br, 3H, ArCH_3), 3.98 – 4.09 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$), 6.01 (s, 1H, NCHCO), 6.36 – 6.68 (m, 3H, 3'''-H$_{4\text{-hydroxyphenyl}}$, 5'''-H$_{4\text{-hydroxyphenyl}}$, H$_{m\text{-tolyl}}$ (1H)), 6.81 – 6.91 (m, 2H, 2'''-H$_{4\text{-hydroxyphenyl}}$, 6'''-H$_{4\text{-hydroxyphenyl}}$), 6.96 – 7.31(m, 4H, ArNH_2, H$_{m\text{-tolyl}}$ (2H)), 7.52 – 7.76 (m, 2H, CONH_2 (1H), H$_{m\text{-tolyl}}$ (1H)), 7.82 (s br, 1H, CONH_2), 8.05 (d, J = 7.0 Hz, 1H, NHCH(CH$_2$CH$_2$)$_2$), 9.33 (s, 1H, ArOH);

^{13}C NMR (126 MHz, DMSO-d_6): δ [ppm] = 23.4 (1C, NHCH(CH$_2$CH_2)$_2$), 23.5 (1C, NHCH(CH$_2$CH_2)$_2$), 31.7 (1C, NHCH(CH_2CH$_2$)$_2$), 32.2 (1C, NHCH(CH_2CH$_2$)$_2$), 50.5 (1C, NHCH(CH$_2$CH$_2$)$_2$), 63.5 (1C, NCHCO), 114.6 (2C, C-3'''$_{4\text{-hydroxyphenyl}}$, C-5'''$_{4\text{-hydroxyphenyl}}$), 123.3 (1C, C-5'$_{\text{isothiazole}}$), 124.8 (1C, C-1'''$_{4\text{-hydroxyphenyl}}$), 128.6 (1C, C$_{m\text{-tolyl}}$), 129.9 (1C, C$_{m\text{-tolyl}}$), 130.2 (1C, C$_{m\text{-tolyl}}$), 131.5 (2C, C-2'''$_{4\text{-hydroxyphenyl}}$, C-6'''$_{4\text{-hydroxyphenyl}}$), 133.8 (1C, C$_{m\text{-tolyl}}$), 137.2 (1C, C-

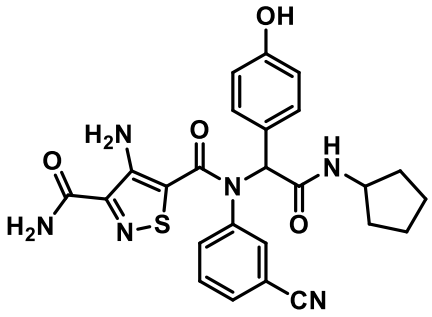
1''$_{m\text{-tolyl}}$), 146.4 (1C, C-3'$_{isothiazole}$), 151.1 (1C, C-4'$_{isothiazole}$), 156.8 (1C, C-4'''$_{4\text{-hydroxyphenyl}}$), 162.1 (1C, Ar*C*ONCH), 163.9 (1C, Ar*C*ONH$_2$), 169.4 (1C, CH*C*ONHCH), the signals for Ar*C*H$_3$ and 1 C$_{m\text{-tolyl}}$ cannot be observed in the spectrum;

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3465, 3400, 3353, 3175, 3962, 3871, 1673, 1662, 1623, 1611, 1579, 1556, 1524, 1515, 1487, 1442, 1405, 1363, 1335, 1274, 1242, 1217, 1190, 1172, 1145, 1076, 989, 933, 875, 852, 830, 802, 735, 712, 684, 668, 639, 584, 552, 531, 521, 509, 488;

HRMS (*m*/*z*): [M+H]$^+$ calcd for $C_{25}H_{28}N_5O_4S$: 494.1857, found: 494.1860;

HPLC (method 1): t_R = 20.3 min, purity 97.2%.

4-Amino-*N*^{5}-(3-cyanophenyl)-*N*^{5}-[2-(cyclopentylamino)-1-(4-hydroxyphenyl)-2-oxoethyl]isothiazole-3,5-dicarboxamide (57c)



$C_{25}H_{24}N_6O_4S$

M_r = 504.6

3-Aminobenzonitrile (240 mg, 2.0 mmol) and 4-hydroxybenzaldehyde (240 mg, 2.0 mmol) were dissolved in dichloromethane (15 mL) and the solution was stirred at ambient temperature for 10 min. Then, sulfanylacetic acid (0.14 mL, 180 mg, 2.0 mmol) and isocyanocyclopentane (0.22 mL, 190 mg, 2.0 mmol) were added. After stirring the reaction mixture for 48 h at ambient temperature, the solvent was removed *in vacuo* and the residue was washed with acetonitrile.

A share (410 mg, 53%) of the obtained crude product (770 mg) was added in one portion to a solution of **25** (320 mg, 1.2 mmol) in acetone (100 mL). The mixture was cooled to 0 °C and morpholine (0.17 mL, 170 mg, 1.9 mmol) was added dropwise. After stirring the reaction mixture for 12 h at ambient temperature, the solvent was removed *in vacuo*. The residue was purified by flash column chromatography (Ø = 6 cm, h = 20 cm, V = 15 mL,

dichloromethane/acetone = 5/1) and recrystallized from acetonitrile to give **57c** (340 mg, 0.67 mmol, 63% yield) as colorless solid.

m.p. = 288 °C;

TLC: R_f = 0.25 (dichloromethane/acetone = 5/1);

^{1}H NMR (500 MHz, DMSO-d_6): δ [ppm] = 1.20 – 1.29 (m, 1H, NHCH(CH_2CH$_2$)$_2$), 1.40 – 1.55 (m, 4H, NHCH(CH_2CH$_2$)$_2$ (1H), NHCH(CH$_2$CH_2)$_2$ (3H)), 1.55 – 1.67 (m, 1H, NHCH(CH$_2$CH_2)$_2$), 1.72 – 1.88 (m, 2H, NHCH(CH_2CH$_2$)$_2$), 4.01 – 4.10 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$), 6.08 (s, 1H, NCHCO), 6.50 – 6.55 (m, 2H, 3'''-H$_{4\text{-hydroxyphenyl}}$, 5'''-H$_{4\text{-hydroxyphenyl}}$), 6.84 – 6.89 (m, 2H, 2'''-H$_{4\text{-hydroxyphenyl}}$, 6'''-H$_{4\text{-hydroxyphenyl}}$), 7.22 (s br, 2H, ArN$H_2$), 7.48 (s br, 1H, H$_{3\text{-cyanophenyl}}$), 7.64 (s br, 1H, CON$H_2$), 7.79 – 7.84 (m, 1H, 4''-H$_{3\text{-cyanophenyl}}$), 7.88 (s br, 1H, CONH_2), 8.19 (d, J = 7.0 Hz, 1H, NHCH(CH$_2$CH$_2$)$_2$), 9.43 (s, 1H, ArOH), the signals for 2 H$_{3\text{-cyanophenyl}}$ cannot be observed in the spectrum;

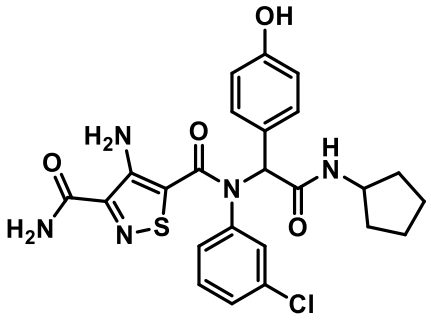
^{13}C NMR (101 MHz, DMSO-d_6): δ [ppm] = 23.5 (1C, NHCH(CH$_2$$CH_2$)$_2$), 23.6 (1C, NHCH(CH$_2$$CH_2$)$_2$), 31.8 (1C, NHCH($CH_2CH_2$)$_2$), 32.3 (1C, NHCH($CH_2CH_2$)$_2$), 50.6 (1C, NH$C$H(CH$_2CH_2$)$_2$), 63.5 (1C, N$C$HCO), 111.5 (1C, C-3''$_{3\text{-cyanophenyl}}$), 114.9 (2C, C-3'''$_{4\text{-hydroxyphenyl}}$, C-5'''$_{4\text{-hydroxyphenyl}}$), 117.9 (1C, Ar$C$N), 122.4 (1C, C-5'$_{\text{isothiazole}}$), 124.3 (1C, C-1'''$_{4\text{-hydroxyphenyl}}$), 130.3 (1C, C$_{3\text{-cyanophenyl}}$), 131.5 (2C, C-2'''$_{4\text{-hydroxyphenyl}}$, C-6'''$_{4\text{-hydroxyphenyl}}$), 133.2 (1C, C-4''$_{3\text{-cyanophenyl}}$), 136.9 (1C, C$_{3\text{-cyanophenyl}}$), 138.5 (1C, C$_{3\text{-cyanophenyl}}$), 138.6 (1C, C$_{3\text{-cyanophenyl}}$), 147.0 (1C, C-3'$_{\text{isothiazole}}$), 151.4 (1C, C-4'$_{\text{isothiazole}}$), 157.1 (1C, C-4'''$_{4\text{-hydroxyphenyl}}$), 161.8 (1C, Ar$C$ONCH), 163.8 (1C, Ar$CONH_2$), 169.3 (1C, CH$C$ONHCH);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3469, 3413, 3396, 3357, 3175, 3059, 2961, 2871, 2232, 1675, 1665, 1626, 1610, 1586, 1576, 1557, 1524, 1515, 1489, 1478, 1441, 1426, 1403, 1364, 1328, 1278, 1237, 1212, 1173, 1140, 1105, 1078, 933, 872, 851, 834, 820, 801, 762, 737, 709, 683, 669, 642, 595, 561, 533, 519, 505, 482, 451, 422;

HRMS (m/z): [M+H]$^+$ calcd for $C_{25}H_{25}N_6O_4S$: 505.1653, found: 505.1685;

HPLC (method 1): t_R = 20.8 min, purity 96.4%.

4-Amino-N^5-(3-chlorophenyl)-N^5-[2-(cyclopentylamino)-1-(4-hydroxyphenyl)-2-oxoethyl]isothiazole-3,5-dicarboxamide (57d)



$C_{24}H_{24}ClN_5O_4S$

M_r = 514.0

3-Chloroaniline (0.21 mL, 260 mg, 2.0 mmol) and 4-hydroxybenzaldehyde (240 mg, 2.0 mmol) were dissolved in dichloromethane (15 mL) and the solution was stirred at ambient temperature for 10 min. Then, sulfanylacetic acid (0.14 mL, 180 mg, 2.0 mmol) and isocyanocyclopentane (0.22 mL, 190 mg, 2.0 mmol) were added. After stirring the reaction mixture for 48 h at ambient temperature, the solvent was removed *in vacuo* and the residue was washed with acetonitrile.

A share (420 mg, 62%) of the obtained crude product (670 mg) was added in one portion to a solution of **25** (320 mg, 1.2 mmol) in acetone (100 mL). The mixture was cooled to 0 °C and morpholine (0.17 mL, 170 mg, 1.9 mmol) was added dropwise. After stirring the reaction mixture for 12 h at ambient temperature, the solvent was removed *in vacuo*. The residue was purified by flash column chromatography (Ø = 6 cm, h = 20 cm, V = 15 mL, dichloromethane/acetone = 5/1) and recrystallized from acetonitrile to give **57d** (480 mg, 0.93 mmol, 75% yield) as colorless solid.

m.p. = 279 °C;

TLC: R_f = 0.26 (dichloromethane/acetone = 5/1);

^{1}H NMR (500 MHz, DMSO-d_6): δ [ppm] = 1.19 – 1.29 (m, 1H, NHCH(CH_2CH$_2$)$_2$), 1.39 – 1.55 (m, 4H, NHCH(CH_2CH$_2$)$_2$ (1H), NHCH(CH$_2$CH_2)$_2$ (3H)), 1.55 – 1.66 (m, 1H, NHCH(CH$_2$CH_2)$_2$), 1.71 – 1.88 (m, 2H, NHCH(CH_2CH$_2$)$_2$), 4.00 – 4.09 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$), 6.05 (s, 1H, NCHCO), 6.50 – 6.54 (m, 2H, 3'''-H$_{4\text{-hydroxyphenyl}}$, 5'''-H$_{4\text{-hydroxyphenyl}}$), 6.85 – 6.90 (m, 2H, 2'''-H$_{4\text{-hydroxyphenyl}}$, 6'''-H$_{4\text{-hydroxyphenyl}}$), 7.19 (s br, 2H, ArN$H_2$),

7.27 (s br, 1H, $H_{3\text{-chlorophenyl}}$), 7.38 – 7.44 (m, 1H, 4''-$H_{3\text{-chlorophenyl}}$), 7.62 (s br, 1H, CON$H_2$), 7.87 (s br, 1H, CON$H_2$), 8.14 (d, J = 7.0 Hz, 1H, NHCH(CH_2CH_2)$_2$), 9.40 (s, 1H, ArOH), the signals for 2 $H_{3\text{-chlorophenyl}}$ cannot be observed in the spectrum;

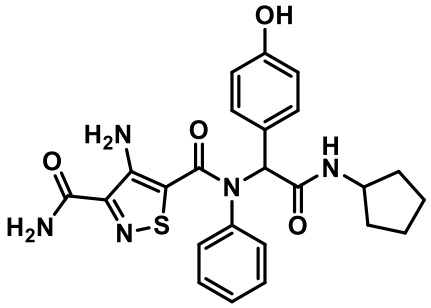
^{13}C NMR (151 MHz, DMSO-d_6): δ [ppm] = 23.5 (1C, NHCH(CH_2CH_2)$_2$), 23.6 (1C, NHCH(CH_2CH_2)$_2$), 31.8 (1C, NHCH(CH_2CH_2)$_2$), 32.3 (1C, NHCH(CH_2CH_2)$_2$), 50.6 (1C, NHCH(CH_2CH_2)$_2$), 63.5 (1C, NCHCO), 114.8 (2C, C-3'''$_{4\text{-hydroxyphenyl}}$, C-5'''$_{4\text{-hydroxyphenyl}}$), 122.6 (1C, C-5'$_{\text{isothiazole}}$), 124.5 (1C, C-1'''$_{4\text{-hydroxyphenyl}}$), 129.5 (1C, C-4''$_{3\text{-chlorophenyl}}$), 130.3 (1C, $C_{3\text{-chlorophenyl}}$), 131.5 (2C, C-2'''$_{4\text{-hydroxyphenyl}}$, C-6'''$_{4\text{-hydroxyphenyl}}$), 132.2 (1C, $C_{3\text{-chlorophenyl}}$), 132.8 (1C, C-3''$_{3\text{-chlorophenyl}}$), 133.2 (1C, $C_{3\text{-chlorophenyl}}$), 138.9 (1C, C-1''$_{3\text{-chlorophenyl}}$), 146.8 (1C, C-3'$_{\text{isothiazole}}$), 151.4 (1C, C-4'$_{\text{isothiazole}}$), 157.0 (1C, C-4'''$_{4\text{-hydroxyphenyl}}$), 161.9 (1C, ArCONCH), 163.8 (1C, ArCONH$_2$), 169.3 (1C, CHCONHCH);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3466, 3404, 3355, 3173, 2950, 2872, 1675, 1661, 1625, 1612, 1580, 1557, 1526, 1515, 1488, 1473, 1443, 1404, 1363, 1333, 1275, 1234, 1218, 1173, 1142, 1102, 1076, 844, 819, 795, 744, 731, 708, 686, 665, 641, 554, 532, 510, 489, 466, 442, 425;

HRMS (m/z): [M+H]$^+$ calcd for $C_{24}H_{25}{}^{35}ClN_5O_4S$: 514.1310, found: 514.1318;

HPLC (method 1): t_R = 20.8 min, purity 95.6%.

4-Amino-N^5-[2-(cyclopentylamino)-1-(4-hydroxyphenyl)-2-oxoethyl]-N^5-phenylisothiazole-3,5-dicarboxamide (57e)



$C_{24}H_{25}N_5O_4S$

M_r = 479.6

Aniline (0.18 mL, 190 mg, 2.0 mmol) and 4-hydroxybenzaldehyde (240 mg, 2.0 mmol) were dissolved in dichloromethane (15 mL) and the solution was stirred at ambient temperature for 10 min. Then, sulfanylacetic acid (0.14 mL, 180 mg, 2.0 mmol) and isocyanocyclopentane (0.22 mL, 190 mg, 2.0 mmol) were added. After stirring the reaction mixture for 48 h at

ambient temperature, the solvent was removed *in vacuo* and the residue was washed with acetonitrile.

A share (380 mg, 77%) of the obtained crude product (500 mg) was added in one portion to a solution of **25** (320 mg, 1.2 mmol) in acetone (100 mL). The mixture was cooled to 0 °C and morpholine (0.17 mL, 170 mg, 1.9 mmol) was added dropwise. After stirring the reaction mixture for 12 h at ambient temperature, the solvent was removed *in vacuo*. The residue was purified by flash column chromatography (Ø = 6 cm, h = 20 cm, V = 15 mL, dichloromethane/acetone = 5/1) and recrystallized from acetonitrile to give **57e** (390 mg, 0.82 mmol, 53% yield) as colorless solid.

m.p. = 290 °C;

TLC: R_f = 0.29 (dichloromethane/acetone = 5/1);

^{1}H NMR (500 MHz, DMSO-d_6): δ [ppm] = 1.18 – 1.29 (m, 1H, NHCH(CH_2CH$_2$)$_2$), 1.38 – 1.67 (m, 5H, NHCH(CH_2CH$_2$)$_2$ (1H), NHCH(CH$_2$CH_2)$_2$), 1.68 – 1.89 (m, 2H, NHCH(CH_2CH$_2$)$_2$), 3.98 – 4.10 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$), 6.05 (s, 1H, NCHCO), 6.43 – 6.53 (m, 2H, 3'''-H$_{4\text{-hydroxyphenyl}}$, 5'''-H$_{4\text{-hydroxyphenyl}}$), 6.81 – 6.90 (m, 2H, 2'''-H$_{4\text{-hydroxyphenyl}}$, 6'''-H$_{4\text{-hydroxyphenyl}}$), 7.06 – 7.43 (m, 5H, ArNH_2, H$_{phenyl}$ (3H)), 7.58 (s br, 1H, CONH_2), 7.83 (s br, 1H, CONH_2), 8.06 (d, J = 7.0 Hz, 1H, NHCH(CH$_2$CH$_2$)$_2$), 9.33 (s, 1H, ArOH), the signals for 2 H$_{phenyl}$ cannot be observed in the spectrum;

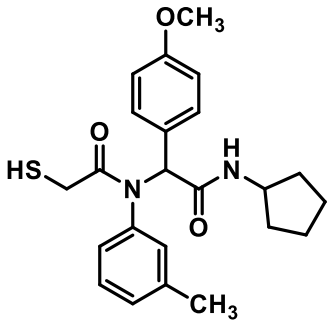
^{13}C NMR (101 MHz, DMSO-d_6): δ [ppm] = 23.5 (1C, NHCH(CH$_2$$CH_2$)$_2$), 23.6 (1C, NHCH(CH$_2$$CH_2$)$_2$), 31.8 (1C, NHCH($CH_2CH_2$)$_2$), 32.3 (1C, NHCH($CH_2CH_2$)$_2$), 50.6 (1C, NH$C$H(CH$_2CH_2$)$_2$), 63.6 (1C, N$C$HCO), 114.7 (2C, C-3'''$_{4\text{-hydroxyphenyl}}$, C-5'''$_{4\text{-hydroxyphenyl}}$), 123.3 (1C, C-5'$_{isothiazole}$), 124.8 (1C, C-1'''$_{4\text{-hydroxyphenyl}}$), 128.9 (2C, C$_{phenyl}$), 129.5 (1C, C-4''$_{phenyl}$), 131.6 (2C, C-2'''$_{4\text{-hydroxyphenyl}}$, C-6'''$_{4\text{-hydroxyphenyl}}$), 133.4 (2C, C$_{phenyl}$), 137.4 (1C, C-1''$_{phenyl}$), 146.5 (1C, C-3'$_{isothiazole}$), 151.2 (1C, C-4'$_{isothiazole}$), 156.8 (1C, C-4'''$_{4\text{-hydroxyphenyl}}$), 162.2 (1C, Ar$C$ONCH), 164.0 (1C, Ar$CONH_2$), 169.5 (1C, CH$C$ONHCH);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3469, 3399, 3358, 3173, 2965, 2870, 1675, 1659, 1624, 1612, 1583, 1557, 1524, 1514, 1491, 1445, 1403, 1365, 1333, 1274, 1235, 1218, 1183, 1172, 1141, 1105, 1081, 1025, 876, 838, 815, 803, 781, 767, 743, 730, 705, 685, 669, 642, 541, 530, 507, 492, 454, 424;

HRMS (m/z): [M+H]$^+$ calcd for C$_{24}$H$_{26}$N$_5$O$_4$S: 480.1700, found: 480.1699;

HPLC (method 1): t_R = 19.7 min, purity 95.2%.

_N_-Cyclopentyl-2-[2-mercapto-_N_-(_m_-tolyl)acetamido]-2-(4-methoxyphenyl)acetamide (55f)



$C_{23}H_{28}N_2O_3S$

$M_r = 412.5$

3-Methylaniline (0.22 mL, 210 mg, 2.0 mmol) and 4-methoxybenzaldehyde (0.24 mL, 270 mg, 2.0 mmol) were dissolved in dichloromethane (15 mL) and the solution was stirred at ambient temperature for 10 min. Then, sulfanylacetic acid (0.14 mL, 180 mg, 2.0 mmol) and isocyanocyclopentane (0.22 mL, 190 mg, 2.0 mmol) were added. After stirring the reaction mixture for 48 h at ambient temperature, the solvent was removed *in vacuo* and the residue was purified by flash column chromatography (Ø = 4 cm, h = 15 cm, V = 15 mL, dichloromethane/ethyl acetate = 25/1) to give **55f** (370 mg, 0.90 mmol, 45% yield) as colorless solid.

m.p. = 171 °C;

TLC: R_f = 0.39 (dichloromethane/ethyl acetate = 15/1);

^{1}H NMR (500 MHz, DMSO-d_6): δ [ppm] = 1.17 – 1.26 (m, 1H, NHCH(CH_2CH$_2$)$_2$), 1.38 – 1.54 (m, 4H, NHCH(CH$_2$CH_2)$_2$ (3H), NHCH(CH_2CH$_2$)$_2$ (1H)), 1.55 – 1.65 (m, 1H, NHCH(CH$_2$CH_2)$_2$), 1.69 – 1.85 (m, 2H, NHCH(CH_2CH$_2$)$_2$), 2.16 (s br, 3H, ArCH_3), 2.55 (t, J = 6.8 Hz, 1H, HSCH$_2$CO), 2.97 (dd, J = 14.9/6.7 Hz, 1H, HSCH_2CO), 3.02 (dd, J = 14.9/7.0 Hz, 1H, HSCH_2CO), 3.63 (s, 3H, ArOCH_3), 3.98 – 4.06 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$), 5.94 (s, 1H, NCHCO), 6.48 (s br, 1H, H$_{m\text{-tolyl}}$), 6.61 – 6.73 (m, 2H, 3"-H$_{4\text{-methoxyphenyl}}$, 5"-H$_{4\text{-methoxyphenyl}}$), 6.85 – 7.25 (m, 4H, 2"-H$_{4\text{-methoxyphenyl}}$, 6"-H$_{4\text{-methoxyphenyl}}$, H$_{m\text{-tolyl}}$ (2H)), 7.58 (s br, 1H, H$_{m\text{-tolyl}}$), 7.97 (d, J = 7.0 Hz, 1H, NHCH(CH$_2$CH$_2$)$_2$);

^{13}C NMR (101 MHz, DMSO-d_6): δ [ppm] = 20.7 (1C, ArCH$_3$), 23.45 (1C, NHCH(CH$_2$$CH_2$)$_2$), 23.51 (1C, NHCH(CH$_2$$CH_2$)$_2$), 27.4 (1C, HS$CH_2$CO), 31.8 (1C, NHCH($CH_2CH_2$)$_2$), 32.2 (1C, NHCH($CH_2CH_2$)$_2$), 50.5 (1C, NH$C$H(CH$_2CH_2$)$_2$), 54.9 (1C, ArO$CH_3$), 63.3 (1C, N$C$HCO),

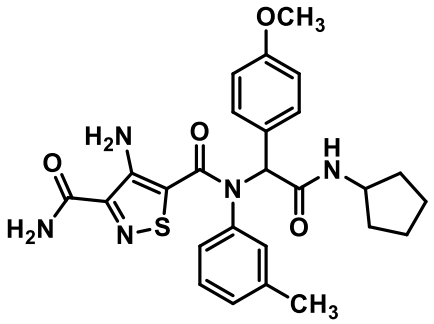
113.1 (2C, C-3"$_{4\text{-methoxyphenyl}}$, C-5"$_{4\text{-methoxyphenyl}}$), 127.1 (1C, C-1"$_{4\text{-methoxyphenyl}}$), 127.8 (1C, C$_{m\text{-tolyl}}$), 128.1 (1C, C$_{m\text{-tolyl}}$), 128.4 (1C, C$_{m\text{-tolyl}}$), 131.3 (2C, C-2"$_{4\text{-methoxyphenyl}}$, C-6"$_{4\text{-methoxyphenyl}}$), 131.4 (1C, C$_{m\text{-tolyl}}$), 137.7 (1C, C-3'$_{m\text{-tolyl}}$), 139.1 (1C, C-1'$_{m\text{-tolyl}}$), 158.5 (1C, C-4"$_{4\text{-methoxyphenyl}}$), 168.8 (1C, HSCH$_2$*C*O), 169.1 (1C, CH*C*ONHCH);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3280, 3085, 2954, 2866, 2838, 2558, 1647, 1608, 1586, 1558, 1513, 1488, 1454, 1443, 1410, 1373, 1306, 1242, 1179, 1112, 1092, 1069, 1033, 980, 895, 859, 831, 791, 742, 708, 673, 626, 555, 528, 501, 468, 449, 430;

HRMS (*m*/*z*): [M+H]$^+$ calcd for $C_{23}H_{29}N_2O_3S$: 413.1893, found: 413.1895;

HPLC (method 1): t_R = 23.1 min, purity 73.3%.

4-Amino-*N*^{5}-[2-(cyclopentylamino)-1-(4-methoxyphenyl)-2-oxoethyl]-*N*^{5}-(*m*-tolyl)isothiazole-3,5-dicarboxamide (57f)



$C_{26}H_{29}N_5O_4S$

M_r = 507.6

55f (410 mg, 1.0 mmol) was added in one portion to a solution of **25** (320 mg, 1.2 mmol) in acetone (100 mL). The mixture was cooled to 0 °C and morpholine (0.17 mL, 170 mg, 1.9 mmol) was added dropwise. After stirring the reaction mixture for 12 h at ambient temperature, the solvent was removed *in vacuo*. The residue was purified by flash column chromatography (Ø = 6 cm, h = 20 cm, V = 15 mL, dichloromethane/acetone = 10/1) to give **57f** (300 mg, 0.59 mmol, 59% yield) as colorless solid.

m.p. = 259 °C;

TLC: R_f = 0.25 (dichloromethane/acetone = 10/1);

^{1}H NMR (400 MHz, DMSO-d_6): δ [ppm] = 1.17 – 1.27 (m, 1H, NHCH(CH_2CH$_2$)$_2$), 1.39 – 1.55 (m, 4H, NHCH(CH$_2$CH_2)$_2$ (3H), NHCH(CH_2CH$_2$)$_2$ (1H)), 1.55 – 1.67 (m, 1H, NHCH(CH$_2$CH_2)$_2$), 1.69 – 1.89 (m, 2H, NHCH(CH_2CH$_2$)$_2$), 2.20 (s br, 3H, ArCH_3), 3.64 (s, 3H, ArOCH_3), 3.99 – 4.09 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$), 6.06 (s, 1H, NCHCO), 6.57 (s br, 1H, H$_{m\text{-tolyl}}$), 6.63 – 6.77 (m, 2H, 3'''-H$_{4\text{-methoxyphenyl}}$, 5'''-H$_{4\text{-methoxyphenyl}}$), 6.89 – 7.29 (m, 6H, 2'''-H$_{4\text{-methoxyphenyl}}$, 6'''-H$_{4\text{-methoxyphenyl}}$, ArN$H_2$, H$_{m\text{-tolyl}}$ (2H)), 7.44 – 7.79 (m, 2H, CONH_2 (1H), H$_{m\text{-tolyl}}$ (1H)), 7.83 (s br, 1H, CONH_2), 8.09 (d, J = 7.0 Hz, 1H, NHCH(CH$_2$CH$_2$)$_2$);

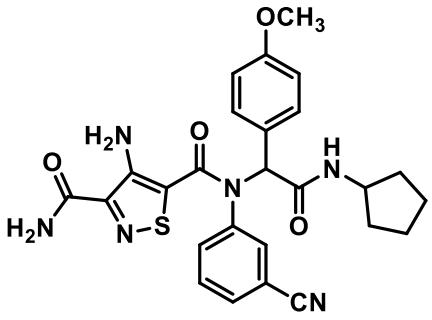
^{13}C NMR (126 MHz, DMSO-d_6): δ [ppm] = 20.7 (1C, ArCH$_3$), 23.45 (1C, NHCH(CH$_2$$CH_2$)$_2$), 23.52 (1C, NHCH(CH$_2$$CH_2$)$_2$), 31.8 (1C, NHCH(C$H_2CH_2$)$_2$), 32.2 (1C, NHCH(C$H_2CH_2$)$_2$), 50.6 (1C, NH$C$H(CH$_2CH_2$)$_2$), 55.0 (1C, ArO$CH_3$), 63.4 (1C, N$C$HCO), 113.2 (2C, C-3'''$_{4\text{-methoxyphenyl}}$, C-5'''$_{4\text{-methoxyphenyl}}$), 123.2 (1C, C-5'$_{\text{isothiazole}}$), 126.6 (1C, C-1'''$_{4\text{-methoxyphenyl}}$), 128.6 (1C, C$_{m\text{-tolyl}}$), 130.0 (1C, C$_{m\text{-tolyl}}$), 130.2 (1C, C$_{m\text{-tolyl}}$), 131.6 (2C, C-2'''$_{4\text{-methoxyphenyl}}$, C-6'''$_{4\text{-methoxyphenyl}}$), 133.7 (1C, C$_{m\text{-tolyl}}$), 137.1 (1C, C-1''$_{m\text{-tolyl}}$), 138.3 (1C, C-3''$_{m\text{-tolyl}}$), 146.4 (1C, C-3'$_{\text{isothiazole}}$), 151.2 (1C, C-4'$_{\text{isothiazole}}$), 158.7 (1C, C-4'''$_{4\text{-methoxyphenyl}}$), 162.2 (1C, Ar$C$ONCH), 163.9 (1C, Ar$CONH_2$), 169.2 (1C, CH$C$ONHCH);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3463, 3394, 3348, 3189, 2965, 2869, 1686, 1672, 1612, 1556, 1512, 1485, 1443, 1410, 1376, 1355, 1338, 1304, 1292, 1276, 1258, 1245, 1195, 1178, 1150, 1114, 1071, 1029, 972, 928, 897, 877, 847, 827, 801, 789, 772, 758, 739, 709, 684, 665, 628, 562, 535, 502, 460, 440, 422;

HRMS (m/z): [M+H]$^+$ calcd for C$_{26}$H$_{30}$N$_5$O$_4$S: 508.2013, found: 508.2021;

HPLC (method 1): t_R = 22.9 min, purity 98.1%.

4-Amino-N^5-(3-cyanophenyl)-N^5-[2-(cyclopentylamino)-1-(4-methoxyphenyl)-2-oxoethyl]isothiazole-3,5-dicarboxamide (57g)



C$_{26}$H$_{26}$N$_6$O$_4$S

M$_r$ = 518.6

3-Aminobenzonitrile (240 mg, 2.0 mmol) and 4-methoxybenzaldehyde (0.24 mL, 270 mg, 2.0 mmol) were dissolved in dichloromethane (15 mL) and the solution was stirred at ambient temperature for 10 min. Then, sulfanylacetic acid (0.14 mL, 180 mg, 2.0 mmol) and isocyanocyclopentane (0.22 mL, 190 mg, 2.0 mmol) were added. After stirring the reaction mixture for 48 h at ambient temperature, the solvent was removed *in vacuo* and the residue was washed with acetonitrile.

A share (420 mg, 53%) of the obtained crude product (790 mg) was added in one portion to a solution of **25** (320 mg, 1.2 mmol) in acetone (100 mL). The mixture was cooled to 0 °C and morpholine (0.17 mL, 170 mg, 1.9 mmol) was added dropwise. After stirring the reaction mixture for 12 h at ambient temperature, the solvent was removed *in vacuo*. The residue was purified by flash column chromatography (Ø = 6 cm, h = 20 cm, V = 15 mL, dichloromethane/acetone = 10/1) and recrystallized from acetonitrile to give **57g** (410 mg, 0.78 mmol, 73% yield) as colorless solid.

m.p. = 233 °C;

TLC: R_f = 0.24 (dichloromethane/acetone = 10/1);

^{1}H NMR (500 MHz, DMSO-d_6): δ [ppm] = 1.20 – 1.28 (m, 1H, NHCH(CH_2CH$_2$)$_2$), 1.41 – 1.54 (m, 4H, NHCH(CH_2CH$_2$)$_2$ (1H), NHCH(CH$_2$CH_2)$_2$ (3H)), 1.55 – 1.66 (m, 1H, NHCH(CH$_2$CH_2)$_2$), 1.72 – 1.89 (m, 2H, NHCH(CH_2CH$_2$)$_2$), 3.65 (s, 3H, ArOCH_3), 4.02 – 4.11 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$), 6.14 (s, 1H, NCHCO), 6.70 – 6.75 (m, 2H, 3'''-H$_{4\text{-methoxyphenyl}}$, 5'''-H$_{4\text{-methoxyphenyl}}$), 6.97 – 7.02 (m, 2H, 2'''-H$_{4\text{-methoxyphenyl}}$, 6'''-H$_{4\text{-methoxyphenyl}}$), 7.23 (s br, 2H, ArN$H_2$), 7.48 (s br, 1H, H$_{3\text{-cyanophenyl}}$), 7.64 (s br, 1H, CON$H_2$), 7.80 – 7.84 (m, 1H, 4''-H$_{3\text{-cyanophenyl}}$), 7.89 (s br, 1H, CONH_2), 8.23 (d, J = 7.0 Hz, 1H, NHCH(CH$_2$CH$_2$)$_2$), the signals for 2 H$_{3\text{-cyanophenyl}}$ cannot be observed in the spectrum;

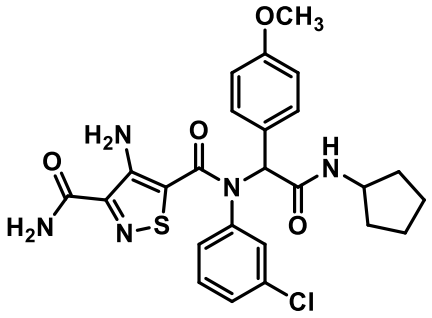
^{13}C NMR (101 MHz, DMSO-d_6): δ [ppm] = 23.5 (1C, NHCH(CH$_2$$CH_2$)$_2$), 23.6 (1C, NHCH(CH$_2$$CH_2$)$_2$), 31.8 (1C, NHCH($CH_2CH_2$)$_2$), 32.3 (1C, NHCH($CH_2CH_2$)$_2$), 50.7 (1C, NH$C$H(CH$_2CH_2$)$_2$), 55.1 (1C, ArO$CH_3$), 63.3 (1C, N$C$HCO), 111.6 (1C, C-3''$_{3\text{-cyanophenyl}}$), 113.5 (2C, C-3'''$_{4\text{-methoxyphenyl}}$, C-5'''$_{4\text{-methoxyphenyl}}$), 117.8 (1C, Ar$C$N), 122.3 (1C, C-5'$_{\text{isothiazole}}$), 126.1 (1C, C-1'''$_{4\text{-methoxyphenyl}}$), 130.4 (1C, C$_{3\text{-cyanophenyl}}$), 131.6 (2C, C-2'''$_{4\text{-methoxyphenyl}}$, C-6'''$_{4\text{-methoxyphenyl}}$), 133.2 (1C, C-4''$_{3\text{-cyanophenyl}}$), 136.9 (1C, C$_{3\text{-cyanophenyl}}$), 138.47 (1C, C$_{3\text{-cyanophenyl}}$), 138.55 (1C, C$_{3\text{-cyanophenyl}}$), 147.0 (1C, C-3'$_{\text{isothiazole}}$), 151.5 (1C, C-4'$_{\text{isothiazole}}$), 158.9 (1C, C-4'''$_{4\text{-methoxyphenyl}}$), 161.8 (1C, Ar$C$ONCH), 163.8 (1C, Ar$CONH_2$), 169.2 (1C, CH$C$ONHCH);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3467, 3433, 3396, 3351, 3209, 3080, 2961, 2846, 2237, 1686, 1670, 1620, 1568, 1514, 1490, 1448, 1427, 1407, 1378, 1327, 1308, 1250, 1183, 1168, 1144, 1116, 1068, 1025, 847, 832, 798, 757, 740, 705, 682, 657, 613, 575, 523, 493, 469, 454, 422;

HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{26}H_{27}N_6O_4S$: 519.1809, found: 519.1775;

HPLC (method 1): t_R = 22.0 min, purity 95.4%.

4-Amino-*N*^{5}-(3-chlorophenyl)-*N*^{5}-[2-(cyclopentylamino)-1-(4-methoxyphenyl)-2-oxoethyl]isothiazole-3,5-dicarboxamide (57h)



$C_{25}H_{26}ClN_5O_4S$

M_r = 528.0

3-Chloroaniline (0.21 mL, 260 mg, 2.0 mmol) and 4-methoxybenzaldehyde (0.24 mL, 270 mg, 2.0 mmol) were dissolved in dichloromethane (15 mL) and the solution was stirred at ambient temperature for 10 min. Then, sulfanylacetic acid (0.14 mL, 180 mg, 2.0 mmol) and isocyanocyclopentane (0.22 mL, 190 mg, 2.0 mmol) were added. After stirring the reaction mixture for 48 h at ambient temperature, the solvent was removed *in vacuo* and the residue was washed with acetonitrile.

A share (430 mg, 72%) of the obtained crude product (600 mg) was added in one portion to a solution of **25** (320 mg, 1.2 mmol) in acetone (100 mL). The mixture was cooled to 0 °C and morpholine (0.17 mL, 170 mg, 1.9 mmol) was added dropwise. After stirring the reaction mixture for 12 h at ambient temperature, the solvent was removed *in vacuo*. The residue was purified by flash column chromatography (Ø = 6 cm, h = 20 cm, V = 15 mL, dichloromethane/acetone = 15/1) and recrystallized from acetonitrile to give **57h** (450 mg, 0.85 mmol, 59% yield) as colorless solid.

m.p. = 254 °C;

TLC: R_f = 0.28 (dichloromethane/acetone = 10/1);

^{1}H NMR (400 MHz, DMSO-d_6): δ [ppm] = 1.17 – 1.29 (m, 1H, NHCH(CH_2CH$_2$)$_2$), 1.38 – 1.68 (m, 5H NHCH(CH$_2$CH_2)$_2$, NHCH(CH_2CH$_2$)$_2$ (1H)), 1.70 – 1.90 (m, 2H NHCH(CH_2CH$_2$)$_2$), 3.65 (s, 3H, ArOCH_3), 4.00 – 4.11 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$), 6.10 (s, 1H, NCHCO), 6.69 – 6.76 (m, 2H, 3'''-H$_{4\text{-methoxyphenyl}}$, 5'''-H$_{4\text{-methoxyphenyl}}$), 6.97 – 7.04 (m, 2H, 2'''-H$_{4\text{-methoxyphenyl}}$, 6'''-H$_{4\text{-methoxyphenyl}}$), 7.14 – 7.36 (m, 3H, ArNH_2, H$_{3\text{-chlorophenyl}}$ (1H)), 7.38 – 7.45 (m, 1H, 4''-H$_{3\text{-chlorophenyl}}$), 7.64 (s br, 1H, CONH_2), 7.89 (s br, 1H, CONH_2), 8.19 (d, J = 7.0 Hz, 1H, NHCH(CH$_2$CH$_2$)$_2$), the signals for 2 H$_{3\text{-chlorophenyl}}$ cannot be observed in the spectrum;

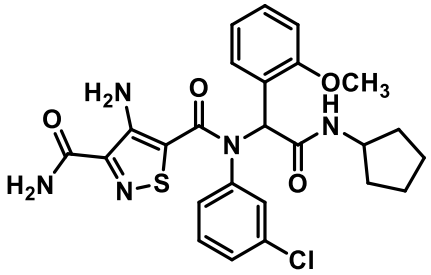
^{13}C NMR (126 MHz, DMSO-d_6): δ [ppm] = 23.4 (1C, NHCH(CH$_2$CH_2)$_2$), 23.5 (1C, NHCH(CH$_2$CH_2)$_2$), 31.7 (1C, NHCH(CH_2CH$_2$)$_2$), 32.2 (1C, NHCH(CH_2CH$_2$)$_2$), 50.6 (1C, NHCH(CH$_2$CH$_2$)$_2$), 55.0 (1C, ArOCH_3), 63.3 (1C, NCHCO), 113.4 (2C, C-3'''$_{4\text{-methoxyphenyl}}$, C-5'''$_{4\text{-methoxyphenyl}}$), 122.5 (1C, C-5'$_{\text{isothiazole}}$), 126.2 (1C, C-1'''$_{4\text{-methoxyphenyl}}$), 129.5 (1C, C-4''$_{3\text{-chlorophenyl}}$), 130.3 (1C, C$_{3\text{-chlorophenyl}}$), 131.5 (2C, C-2'''$_{4\text{-methoxyphenyl}}$, C-6'''$_{4\text{-methoxyphenyl}}$), 132.1 (1C, C$_{3\text{-chlorophenyl}}$), 132.9 (1C, C-3''$_{3\text{-chlorophenyl}}$), 133.2 (1C, C$_{3\text{-chlorophenyl}}$), 138.8 (1C, C-1''$_{3\text{-chlorophenyl}}$), 146.8 (1C, C-3'$_{\text{isothiazole}}$), 151.3 (1C, C-4'$_{\text{isothiazole}}$), 158.8 (1C, C-4'''$_{4\text{-methoxyphenyl}}$), 161.9 (1C, Ar$C$ONCH), 163.8 (1C, Ar$CONH_2$), 169.2 (1C, CH$C$ONHCH);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3466, 3424, 3397, 3351, 3186, 2960, 2868, 2837, 1685, 1673, 1621, 1562, 1513, 1487, 1470, 1445, 1426, 1407, 1375, 1359, 1328, 1307, 1297, 1250, 1180, 1145, 1112, 1100, 1072, 1031, 1001, 904, 864, 840, 820, 801, 792, 771, 747, 733, 706, 684, 663, 646, 618, 534, 502,480, 461, 443, 423;

HRMS (m/z): [M+H]$^+$ calcd for C$_{25}$H$_{27}$^{35}ClN$_5$O$_4$S: 528.1467, found: 528.1437;

HPLC (method 1): t_R = 23.2 min, purity 97.5%.

4-Amino-N^5-(3-chlorophenyl)-N^5-[2-(cyclopentylamino)-1-(2-methoxyphenyl)-2-oxoethyl]isothiazole-3,5-dicarboxamide (57i)



$C_{25}H_{26}ClN_5O_4S$

M_r = 528.0

3-Chloroaniline (0.21 mL, 260 mg, 2.0 mmol) and 2-methoxybenzaldehyde (0.24 mL, 270 mg, 2.0 mmol) were dissolved in dichloromethane (15 mL) and the solution was stirred at ambient temperature for 10 min. Then, sulfanylacetic acid (0.14 mL, 180 mg, 2.0 mmol) and isocyanocyclopentane (0.22 mL, 190 mg, 2.0 mmol) were added. After stirring the reaction mixture for 48 h at ambient temperature, the solvent was removed *in vacuo* and the residue was washed with acetonitrile.

A share (430 mg, 70%) of the obtained crude product (620 mg) was added in one portion to a solution of **25** (320 mg, 1.2 mmol) in acetone (100 mL). The mixture was cooled to 0 °C and morpholine (0.17 mL, 170 mg, 1.9 mmol) was added dropwise. After stirring the reaction mixture for 12 h at ambient temperature, the solvent was removed *in vacuo*. The residue was purified by flash column chromatography (Ø = 6 cm, h = 20 cm, V = 15 mL, dichloromethane/acetone = 15/1) and recrystallized from acetonitrile to give **57i** (500 mg, 0.95 mmol, 67% yield) as colorless solid.

m.p. = 233 °C;

TLC: R_f = 0.31 (dichloromethane/acetone = 10/1);

^{1}H NMR (400 MHz, DMSO-d_6): δ [ppm] = 1.24 – 1.35 (m, 1H, NHCH(CH_2CH$_2$)$_2$), 1.40 – 1.67 (m, 5H, NHCH(CH$_2$CH_2)$_2$, NHCH(CH_2CH$_2$)$_2$ (1H)), 1.73 – 1.89 (m, 2H, NHCH(CH_2CH$_2$)$_2$), 3.83 (s, 3H, ArOCH_3), 4.04 – 4.15 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$), 6.37 (s, 1H, NCHCO), 6.64 – 6.70 (m, 1H, 5'''-H$_{2\text{-methoxyphenyl}}$), 6.75 – 6.80 (m, 1H, 6'''-H$_{2\text{-methoxyphenyl}}$), 6.85 – 6.91 (m, 1H, 3'''-H$_{2\text{-methoxyphenyl}}$), 7.12 – 7.19 (m, 1H, 4'''-H$_{2\text{-methoxyphenyl}}$), 7.21 (s br, 2H, ArN$H_2$), 7.34 – 7.39 (m, 1H, 4''-H$_{3\text{-chlorophenyl}}$), 7.62 (s br, 1H, CONH_2), 7.86 (s br, 1H, CONH_2), 8.24 (d,

J = 7.0 Hz, 1H, N*H*CH(CH_2CH_2)$_2$), the signals for 3 $H_{3\text{-chlorophenyl}}$ cannot be observed in the spectrum;

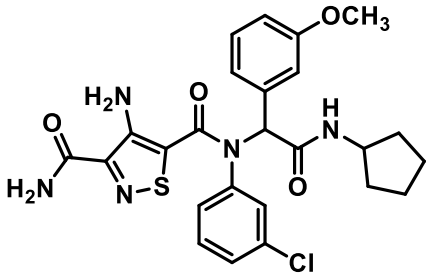
^{13}C NMR (101 MHz, DMSO-d_6): δ [ppm] = 23.46 (1C, NHCH(CH_2*C*H_2)$_2$), 23.49 (1C, NHCH(CH_2*C*H_2)$_2$), 31.6 (1C, NHCH(*C*H_2CH_2)$_2$), 32.3 (1C, NHCH(*C*H_2CH_2)$_2$), 50.7 (1C, NH*C*H(CH_2CH_2)$_2$), 55.3 (1C, ArO*C*H_3), 58.7 (1C, N*C*HCO), 110.5 (1C, C-3'''$_{\text{2-methoxyphenyl}}$), 119.9 (1C, C-5'''$_{\text{2-methoxyphenyl}}$), 122.5 (1C, C-5'$_{\text{isothiazole}}$), 122.6 (1C, C-1'''$_{\text{2-methoxyphenyl}}$), 129.6 (1C, $C_{arom.}$), 129.8 (1C, $C_{arom.}$), 129.9 (1C, $C_{arom.}$), 130.4 (1C, $C_{arom.}$), 138.7 (1C, C-1''$_{\text{3-chlorophenyl}}$), 146.9 (1C, C-3'$_{\text{isothiazole}}$), 151.3 (1C, C-4'$_{\text{isothiazole}}$), 157.1 (1C, C-2'''$_{\text{2-methoxyphenyl}}$), 161.8 (1C, Ar*C*ONCH), 163.8 (1C, Ar*C*ONH_2), 169.1 (1C, CH*C*ONHCH), the signals for 3 $C_{arom.}$ cannot be observed in the spectrum;

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3464, 3347, 3306, 3075, 2950, 1678, 1654, 1606, 1565, 1550, 1489, 1451, 1422, 1405, 1373, 1352, 1337, 1323, 1303, 1247, 1195, 1167, 1140, 1112, 1096, 1071, 1057, 1021, 1003, 972, 880, 864, 831, 797, 755, 742, 711, 673, 660, 584, 565, 529, 482, 463, 412;

HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{25}H_{27}{}^{35}ClN_5O_4S$: 528.1467, found: 528.1451;

HPLC (method 1): t_R = 23.7 min, purity 95.2%.

4-Amino-N^5-(3-chlorophenyl)-N^5-[2-(cyclopentylamino)-1-(3-methoxyphenyl)-2-oxoethyl]isothiazole-3,5-dicarboxamide (57j)



$C_{25}H_{26}ClN_5O_4S$

M_r = 528.0

3-Chloroaniline (0.21 mL, 260 mg, 2.0 mmol) and 3-methoxybenzaldehyde (0.24 mL, 270 mg, 2.0 mmol) were dissolved in dichloromethane (15 mL) and the solution was stirred at ambient temperature for 10 min. Then, sulfanylacetic acid (0.14 mL, 180 mg, 2.0 mmol) and isocyanocyclopentane (0.22 mL, 190 mg, 2.0 mmol) were added. After stirring the reaction

mixture for 48 h at ambient temperature, the solvent was removed *in vacuo* and the residue was washed with acetonitrile.

A share (430 mg, 82%) of the obtained crude product (530 mg) was added in one portion to a solution of **25** (320 mg, 1.2 mmol) in acetone (100 mL). The mixture was cooled to 0 °C and morpholine (0.17 mL, 170 mg, 1.9 mmol) was added dropwise. After stirring the reaction mixture for 12 h at ambient temperature, the solvent was removed *in vacuo*. The residue was purified by flash column chromatography (Ø = 6 cm, h = 20 cm, V = 15 mL, dichloromethane/acetone = 15/1) and recrystallized from acetonitrile to give **57j** (470 mg, 0.89 mmol, 54% yield) as colorless solid.

m.p. = 238 °C;

TLC: R_f = 0.29 (dichloromethane/acetone = 10/1);

^{1}H NMR (400 MHz, DMSO-d_6): δ [ppm] = 1.21 – 1.31 (m, 1H, NHCH(CH_2CH$_2$)$_2$), 1.40 – 1.56 (m, 4H, NHCH(CH$_2$CH_2)$_2$ (3H), NHCH(CH_2CH$_2$)$_2$ (1H)), 1.56 – 1.67 (m, 1H, NHCH(CH$_2$CH_2)$_2$), 1.72 – 1.90 (m, 2H, NHCH(CH_2CH$_2$)$_2$), 3.62 (s, 3H, ArOCH_3), 4.02 – 4.12 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$), 6.13 (s, 1H, NCHCO), 6.64 – 6.74 (m, 3H, 2'''-H$_{3\text{-methoxyphenyl}}$, 4'''-H$_{3\text{-methoxyphenyl}}$, 6'''-H$_{3\text{-methoxyphenyl}}$), 7.07 (t, J = 7.8 Hz, 1H, 5'''-H$_{3\text{-methoxyphenyl}}$), 7.16 – 7.33 (m, 3H, ArNH_2, H$_{3\text{-chlorophenyl}}$ (1H)), 7.38 – 7.44 (m, 1H, 4''-H$_{3\text{-chlorophenyl}}$), 7.62 (s br, 1H, CONH_2), 7.86 (s br, 1H, CONH_2), 8.23 (d, J = 7.0 Hz, 1H, NHCH(CH$_2$CH$_2$)$_2$), the signals for 2 H$_{3\text{-chlorophenyl}}$ cannot be observed in the spectrum;

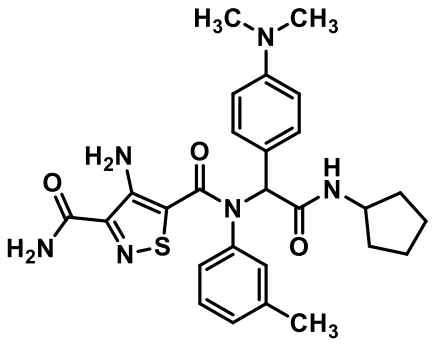
^{13}C NMR (101 MHz, DMSO-d_6): δ [ppm] = 23.46 (1C, NHCH(CH$_2$$CH_2$)$_2$), 23.52 (1C, NHCH(CH$_2$$CH_2$)$_2$), 31.7 (1C, NHCH($CH_2CH_2$)$_2$), 32.2 (1C, NHCH($CH_2CH_2$)$_2$), 50.7 (1C, NH$C$H(CH$_2CH_2$)$_2$), 55.0 (1C, ArO$CH_3$), 63.9 (1C, N$C$HCO), 114.0 (1C, C-4'''$_{3\text{-methoxyphenyl}}$), 115.7 (1C, C$_{3\text{-methoxyphenyl}}$), 122.4 (1C, C-5'$_{\text{isothiazole}}$), 122.5 (1C, C$_{3\text{-methoxyphenyl}}$), 129.0 (1C, C-5'''$_{3\text{-methoxyphenyl}}$), 129.5 (1C, C-4''$_{3\text{-chlorophenyl}}$), 130.3 (1C, C$_{3\text{-chlorophenyl}}$), 132.1 (1C, C$_{3\text{-chlorophenyl}}$), 132.8 (1C, C-3''$_{3\text{-chlorophenyl}}$), 133.2 (1C, C$_{3\text{-chlorophenyl}}$), 135.8 (1C, C-1'''$_{3\text{-methoxyphenyl}}$), 138.7 (1C, C-1''$_{3\text{-chlorophenyl}}$), 146.8 (1C, C-3'$_{\text{isothiazole}}$), 151.4 (1C, C-4'$_{\text{isothiazole}}$), 158.8 (1C, C-3'''$_{3\text{-methoxyphenyl}}$), 161.9 (1C, Ar$C$ONCH), 163.8 (1C, Ar$CONH_2$), 168.8 (1C, CH$C$ONHCH);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3456, 3339, 3261, 3063, 2958, 2869, 2835, 1673, 1645, 1612, 1583, 1559, 1489, 1472, 1450, 1397, 1368, 1339, 1316, 1267, 1240, 1212, 1151, 1097, 1073, 1049, 980, 888, 794, 775, 752, 725, 696, 674, 662, 594, 574, 528, 505, 471, 440, 417;

HRMS (m/z): [M+H]$^+$ calcd for C$_{25}$H$_{27}$^{35}ClN$_5$O$_4$S: 528.1467, found: 528.1490;

HPLC (method 1): t_R = 23.4 min, purity 98.4%.

4-Amino-*N*^{5}-{2-(cyclopentylamino)-1-[4-(dimethylamino)phenyl]-2-oxoethyl}-*N*^{5}-(*m*-tolyl)isothiazole-3,5-dicarboxamide (57k)



$C_{27}H_{32}N_6O_3S$

M_r = 520.7

3-Methylaniline (0.22 mL, 210 mg, 2.0 mmol) and 4-(dimethylamino)benzaldehyde (300 mg, 2.0 mmol) were dissolved in dichloromethane (15 mL) and the solution was stirred at ambient temperature for 10 min. Then, sulfanylacetic acid (0.14 mL, 180 mg, 2.0 mmol) and isocyanocyclopentane (0.22 mL, 190 mg, 2.0 mmol) were added. After stirring the reaction mixture for 48 h at ambient temperature, the solvent was removed

A share (430 mg, 60%) of the obtained crude product (710 mg) was added in one portion to a solution of **25** (320 mg, 1.2 mmol) in acetone (100 mL). The mixture was cooled to 0 °C and morpholine (0.17 mL, 170 mg, 1.9 mmol) was added dropwise. After stirring the reaction mixture for 12 h at ambient temperature, the solvent was removed *in vacuo*. The residue was purified by flash column chromatography (Ø = 6 cm, h = 20 cm, V = 15 mL, dichloromethane/acetone = 10/1) and recrystallized from acetonitrile to give **57k** (410 mg, 0.79 mmol, 66% yield) as colorless solid.

m.p. = 246 °C;

TLC: R_f = 0.19 (dichloromethane/acetone = 10/1);

^{1}H NMR (400 MHz, DMSO-d_6): δ [ppm] = 1.17 – 1.28 (m, 1H, NHCH(CH_2CH$_2$)$_2$), 1.39 – 1.67 (m, 5H NHCH(CH$_2$CH_2)$_2$, NHCH(CH_2CH$_2$)$_2$ (1H)), 1.69 – 1.88 (m, 2H, NHCH(CH_2CH$_2$)$_2$), 2.18 (s br, 3H, ArCH_3), 2.78 (s, 6H, ArN(CH_3)$_2$), 3.99 – 4.09 (m, 1H,

NHC*H*(CH_2CH_2)$_2$), 6.01 (s, 1H, NC*H*CO), 6.41 – 6.49 (m, 2H, 3'''-$H_{4\text{-(dimethylamino)phenyl}}$, 5'''-$H_{4\text{-(dimethylamino)phenyl}}$), 6.82 – 6.90 (m, 2H, 2'''-$H_{4\text{-(dimethylamino)phenyl}}$, 6'''-$H_{4\text{-(dimethylamino)phenyl}}$), 7.00 – 7.27 (m, 4H, ArNH_2, $H_{m\text{-tolyl}}$ (2H)), 7.56 (s br, 1H, CONH_2), 7.79 (s br, 1H, CONH_2), 7.96 (d, J = 7.0 Hz, 1H, N*H*CH(CH_2CH_2)$_2$), the signals for 2 $H_{m\text{-tolyl}}$ cannot be observed in the spectrum;

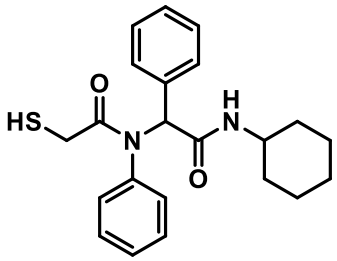
^{13}C NMR (101 MHz, DMSO-d_6): δ [ppm] = 20.7 (1C, Ar*C*H_3), 23.5 (1C, NHCH(CH_2*C*H_2)$_2$), 23.6 (1C, NHCH(CH_2*C*H_2)$_2$), 31.8 (1C, NHCH(*C*H_2CH_2)$_2$), 32.3 (1C, NHCH(*C*H_2CH_2)$_2$), 39.9 (2C, ArN(*C*H_3)$_2$), 50.5 (1C, NH*C*H(CH_2CH_2)$_2$), 63.6 (1C, N*C*HCO), 111.4 (2C, C-3'''$_{4\text{-(dimethylamino)phenyl}}$, C-5'''$_{4\text{-(dimethylamino)phenyl}}$), 121.6 (1C, C-1'''$_{4\text{-(dimethylamino)phenyl}}$), 123.4 (1C, C-5'$_{isothiazole}$), 128.6 (1C, C$_{m\text{-tolyl}}$), 129.9 (1C, C$_{m\text{-tolyl}}$), 130.2 (1C, C$_{m\text{-tolyl}}$), 131.0 (2C, C-2'''$_{4\text{-(dimethylamino)phenyl}}$, C-6'''$_{4\text{-(dimethylamino)phenyl}}$), 133.8 (1C, C$_{m\text{-tolyl}}$), 137.3 (1C, C-1''$_{m\text{-tolyl}}$), 138.2 (1C, C-3''$_{m\text{-tolyl}}$), 146.4 (1C, C-3'$_{isothiazole}$), 149.7 (1C, C-4'''$_{4\text{-(dimethylamino)phenyl}}$), 151.1 (1C, C-4'$_{isothiazole}$), 162.2 (1C, Ar*C*ONCH), 163.9 (1C, Ar*C*ONH_2), 169.5 (1C, CH*C*ONHCH);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3463, 3423, 3385, 3348, 3193, 2949, 2864, 2803, 1681, 1671, 1613, 1556, 1521, 1484, 1445, 1410, 1375, 1332, 1253, 1223, 1204, 1190, 1168, 1126, 1062, 945, 816, 801, 774, 738, 706, 683, 664, 644, 620, 552, 522, 501, 439, 421;

HRMS (*m*/*z*): [M+Na]$^+$ calcd for $C_{27}H_{32}N_6NaO_3S$: 543.2149, found: 543.2139;

HPLC (method 1): t_R = 18.9 min, purity 95.5%.

N-Cyclohexyl-2-(2-mercapto-*N*-phenylacetamido)-2-phenylacetamide (56a)



$C_{22}H_{26}N_2O_2S$

M_r = 382.5

Aniline (0.18 mL, 190 mg, 2.0 mmol) and benzaldehyde (0.20 mL, 210 mg, 2.0 mmol) were dissolved in dichloromethane (15 mL) and the solution was stirred at ambient temperature for 10 min. Then, sulfanylacetic acid (0.14 mL, 180 mg, 2.0 mmol) and isocyanocyclohexane (0.25 mL, 220 mg, 2.0 mmol) were added. After stirring the reaction mixture for 48 h at ambient temperature, the solvent was removed *in vacuo* and the residue was purified by flash

column chromatography (Ø = 4 cm, h = 15 cm, V = 15 mL, dichloromethane/ethyl acetate = 18/1) to give **56a** (440 mg, 1.1 mmol, 57% yield) as colorless solid.

m.p. = 201 °C;

TLC: R_f = 0.27 (dichloromethane/ethyl acetate = 15/1);

^{1}H NMR (500 MHz, DMSO-d_6): δ [ppm] = 0.92 – 1.13 (m, 2H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (1H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 1.13 – 1.32 (m, 3H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (1H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H)), 1.48 – 1.80 (m, 5H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 2.59 (t, J = 6.8 Hz, 1H, HSCH$_2$CO), 2.96 (dd, J = 14.9/6.7 Hz, 1H, HSCH_2CO), 3.03 (dd, J = 14.9/6.9 Hz, 1H, HSCH_2CO), 3.54 – 3.64 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), 6.03 (s, 1H, NCHCO), 6.98 – 7.06 (m, 2H, H$_{phenyl}$), 7.06 – 7.25 (m, 6H, H$_{phenyl}$), 7.97 (d, J = 7.7 Hz, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), the signals for 2 H$_{phenyl}$ cannot be observed in the spectrum;

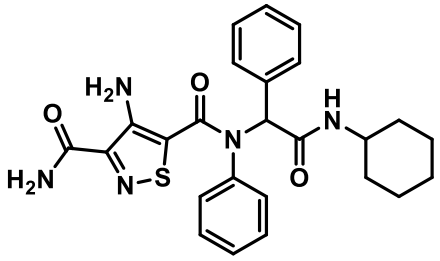
^{13}C NMR (151 MHz, DMSO-d_6): δ [ppm] = 24.4 (1C, NHCH(CH$_2$$CH_2$)$_2CH_2$), 24.6 (1C, NHCH(CH$_2$$CH_2$)$_2CH_2$), 25.2 (1C, NHCH(CH$_2CH_2$)$_2$$CH_2$), 27.3 (1C, HS$CH_2$CO), 32.1 (1C, NHCH($CH_2CH_2$)$_2CH_2$), 32.2 (1C, NHCH($CH_2CH_2$)$_2CH_2$), 47.9 (1C, NH$C$H(CH$_2CH_2$)$_2CH_2$), 63.9 (1C, N$C$HCO), 127.6 (1C, C$_{phenyl}$), 127.8 (3C, C$_{phenyl}$), 128.4 (2C, C$_{phenyl}$), 130.1 (2C, C$_{phenyl}$), 130.8 (2C, C$_{phenyl}$), 135.2 (1C, C$_{phenyl}$), 139.2 (1C, C$_{phenyl}$), 168.4 (1C, CH$C$ONHCH), 168.9 (1C, HSCH$_2$$C$O);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3253, 3085, 2926, 2853, 2550, 1643, 1595, 1565, 1491, 1449, 1406, 1384, 1366, 1344, 1317, 1286, 1251, 1240, 1230, 1188, 1154, 1106, 1075, 1054, 1037, 1026, 1004, 978, 966, 920, 891, 807, 769, 746, 734, 697, 659, 641, 573, 555, 513, 441, 416;

HRMS (m/z): [M+H]$^+$ calcd for C$_{22}$H$_{27}$N$_2$O$_2$S: 383.1788, found: 383.1782;

HPLC (method 1): t$_R$ = 23.5 min, purity 78.6%.

4-Amino-N^5-[2-(cyclohexylamino)-2-oxo-1-phenylethyl]-N^5-phenylisothiazole-3,5-dicarboxamide (58a)



$C_{25}H_{27}N_5O_3S$

$M_r = 477.6$

56a (380 mg, 1.0 mmol) was added in one portion to a solution of **25** (320 mg, 1.2 mmol) in acetone (100 mL). The mixture was cooled to 0 °C and morpholine (0.17 mL, 170 mg, 1.9 mmol) was added dropwise. After stirring the reaction mixture for 12 h at ambient temperature, the solvent was removed *in vacuo*. The residue was purified by flash column chromatography (Ø = 4 cm, h = 20 cm, V = 15 mL, dichloromethane/methanol = 50/1) to give **58a** (270 mg, 0.57 mmol, 57% yield) as colorless solid.

m.p. = 269 °C;

TLC: R_f = 0.27 (dichloromethane/methanol = 40/1);

^{1}H NMR (400 MHz, DMSO-d_6): δ [ppm] = 0.90 – 1.37 (m, 5H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 1.46 – 1.84 (m, 5H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 3.52 – 3.68 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), 6.17 (s, 1H, NCHCO), 6.95 – 7.36 (m, 10H, H$_{phenyl}$ (8H), ArNH_2), 7.57 (s, 1H, CONH_2), 7.82 (s, 1H, CONH_2), 8.05 (d, J = 7.3 Hz, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), the signals for 2 H$_{phenyl}$ cannot be observed in the spectrum;

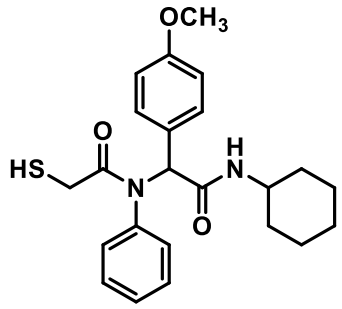
^{13}C NMR (101 MHz, DMSO-d_6): δ [ppm] = 24.4 (1C, NHCH(CH$_2$CH_2)$_2$CH$_2$), 24.6 (1C, NHCH(CH$_2$CH_2)$_2$CH$_2$), 25.2 (1C, NHCH(CH$_2$CH$_2$)$_2$CH_2), 32.1 (1C, NHCH(CH_2CH$_2$)$_2$CH$_2$), 32.2 (1C, NHCH(CH_2CH$_2$)$_2$CH$_2$), 48.0 (1C, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), 64.0 (1C, NCHCO), 123.1 (1C, C-5'$_{isothiazole}$), 127.79 (1C, C$_{phenyl}$), 127.85 (2C, C$_{phenyl}$), 128.9 (2C, C$_{phenyl}$), 129.5 (1C, C$_{phenyl}$),130.4 (2C, C$_{phenyl}$), 133.3 (2C, C$_{phenyl}$), 134.9 (1C, C$_{phenyl}$), 137.2 (1C, C$_{phenyl}$), 146.5 (1C, C-3'$_{isothiazole}$), 151.3 (1C, C-4'$_{isothiazole}$), 162.3 (1C, ArCONCH), 163.9 (1C, ArCONH$_2$), 168.5 (1C, CHCONHCH);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3453, 3337, 3261, 3087, 3061, 2926, 2852, 1665, 1645, 1609, 1562, 1492, 1450, 1369, 1353, 1339, 1251, 1241, 1189, 1151, 1107, 1073, 978, 758, 729, 698, 667, 573, 554, 533, 512, 411;

HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{25}H_{28}N_5O_3S$: 478.1907, found: 478.1913;

HPLC (method 1): t_R = 23.4 min, purity 97.8%.

***N*-Cyclohexyl-2-(2-mercapto-*N*-phenylacetamido)-2-(4-methoxyphenyl)acetamide (56b)**



$C_{23}H_{28}N_2O_3S$

M_r = 412.5

Aniline (0.18 mL, 190 mg, 2.0 mmol) and 4-methoxybenzaldehyde (0.24 mL, 270 mg, 2.0 mmol) were dissolved in dichloromethane (15 mL) and the solution was stirred at ambient temperature for 10 min. Then, sulfanylacetic acid (0.14 mL, 180 mg, 2.0 mmol) and isocyanocyclohexane (0.25 mL, 220 mg, 2.0 mmol) were added. After stirring the reaction mixture for 48 h at ambient temperature, the solvent was removed *in vacuo* and the residue was purified by flash column chromatography (Ø = 4 cm, h = 15 cm, V = 15 mL, dichloromethane/ethyl acetate = 18/1) to give **56b** (460 mg, 1.1 mmol, 56% yield) as colorless solid.

m.p. = 201 °C;

TLC: R_f = 0.30 (dichloromethane/ethyl acetate = 15/1);

^{1}H NMR (500 MHz, DMSO-d_6): δ [ppm] = 0.92 – 1.02 (m, 1H, NHCH(CH_2CH$_2$)$_2$CH$_2$), 1.02 – 1.12 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$CH_2), 1.14 – 1.32 (m, 3H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (1H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H)), 1.48 – 1.72 (m, 4H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (1H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 1.72 – 1.79 (m, 1H,

NHCH(C*H*$_2$CH$_2$)$_2$CH$_2$), 2.54 – 2.60 (m, 1H, *H*SCH$_2$CO), 2.89 – 3.05 (m, 2H, HSC*H*$_2$CO), 3.53 – 3.61 (m, 1H, NHC*H*(CH$_2$CH$_2$)$_2$CH$_2$), 3.62 (s, 3H, ArOC*H*$_3$), 5.96 (s, 1H, NC*H*CO), 6.63 – 6.68 (m, 2H, 3"-H$_{4\text{-methoxyphenyl}}$, 5"-H$_{4\text{-methoxyphenyl}}$), 6.89 – 6.95 (m, 2H, 2"-H$_{4\text{-methoxyphenyl}}$, 6"-H$_{4\text{-methoxyphenyl}}$), 7.04 – 7.31 (m, 3H, H$_{phenyl}$), 7.89 (d, *J* = 7.7 Hz, 1H, N*H*CH(CH$_2$CH$_2$)$_2$CH$_2$), the signals for 2 H$_{phenyl}$ cannot be observed in the spectrum;

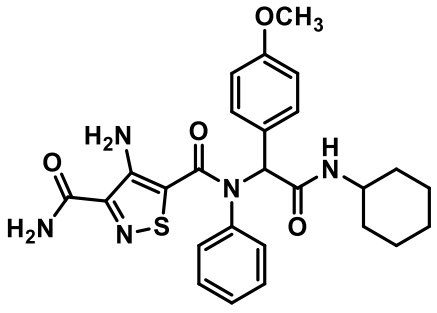
^{13}C NMR (126 MHz, DMSO-*d*$_6$): δ [ppm] = 24.4 (1C, NHCH(CH$_2$*C*H$_2$)$_2$CH$_2$), 24.6 (1C, NHCH(CH$_2$*C*H$_2$)$_2$CH$_2$), 25.2 (1C, NHCH(CH$_2$CH$_2$)$_2$*C*H$_2$), 27.3 (1C, HS*C*H$_2$CO), 32.15 (1C, NHCH(*C*H$_2$CH$_2$)$_2$CH$_2$), 32.20 (1C, NHCH(*C*H$_2$CH$_2$)$_2$CH$_2$), 47.9 (1C, NH*C*H(CH$_2$CH$_2$)$_2$CH$_2$), 54.9 (1C, ArO*C*H$_3$), 63.3 (1C, N*C*HCO), 113.2 (2C, C-3"$_{4\text{-methoxyphenyl}}$, C-5"$_{4\text{-methoxyphenyl}}$), 127.0 (1C, C-1"$_{4\text{-methoxyphenyl}}$), 127.8 (1C, C-4'$_{phenyl}$), 128.4 (2C, C$_{phenyl}$), 130.8 (2C, C$_{phenyl}$), 131.3 (2C, C-2"$_{4\text{-methoxyphenyl}}$, C-6"$_{4\text{-methoxyphenyl}}$), 139.3 (1C, C-1'$_{phenyl}$), 158.5 (1C, C-4"$_{4\text{-methoxyphenyl}}$), 168.7 (1C, *C*ON), 168.8 (1C, *C*ON);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3276, 3075, 2927, 2852, 2832, 1648, 1613, 1593, 1555, 1514, 1492, 1450, 1404, 1383, 1356, 1328, 1306, 1290, 1248, 1230, 1178, 1152, 1104, 1043, 977, 890, 836, 801, 790, 754, 738, 698, 662, 637, 627, 571, 543, 522, 490, 476, 455, 433, 393;

HRMS (*m*/*z*): [M+Na]$^+$ calcd for C$_{23}$H$_{28}$N$_2$NaO$_3$S: 435.1713, found: 435.1717;

HPLC (method 1): t$_R$ = 23.2 min, purity 91.9%.

4-Amino-*N*^{5}-[2-(cyclohexylamino)-1-(4-methoxyphenyl)-2-oxoethyl]-*N*^{5}-phenylisothiazole-3,5-dicarboxamide (58b)



C$_{26}$H$_{29}$N$_5$O$_4$S

M$_r$ = 507.6

56b (410 mg, 1.0 mmol) was added in one portion to a solution of **25** (320 mg, 1.2 mmol) in acetone (100 mL). The mixture was cooled to 0 °C and morpholine (0.17 mL, 170 mg, 1.9 mmol) was added dropwise. After stirring the reaction mixture for 12 h at ambient

temperature, the solvent was removed *in vacuo*. The residue was purified by flash column chromatography (Ø = 6 cm, h = 20 cm, V = 15 mL, dichloromethane/acetone = 15/1) and recrystallized from acetonitrile to give **58b** (400 mg, 0.79 mmol, 79% yield) as colorless solid.

m.p. = 232 °C;

TLC: R_f = 0.30 (dichloromethane/acetone = 10/1);

^{1}H NMR (500 MHz, DMSO-d_6): δ [ppm] = 092 – 1.02 (m, 1H, NHCH(CH_2CH$_2$)$_2$CH$_2$), 1.02 – 1.13 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$CH_2), 1.16 – 1.32 (m, 3H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (1H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H)), 1.49 – 1.62 (m, 2H, NHCH(CH$_2$CH_2)$_2$CH$_2$ (1H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 1.62 – 1.74 (m, 2H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (1H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (1H)), 1.74 – 1.81 (m, 1H, NHCH(CH_2CH$_2$)$_2$CH$_2$), 3.55 – 3.63 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), 3.63 (s, 3H, ArOCH_3), 6.10 (s, 1H, NCHCO), 6.65 – 6.69 (m, 2H, 3'''-H$_{4\text{-methoxyphenyl}}$, 5'''-H$_{4\text{-methoxyphenyl}}$), 6.96 – 7.01 (m, 2H, 2'''-H$_{4\text{-methoxyphenyl}}$, 6'''-H$_{4\text{-methoxyphenyl}}$), 7.06 – 7.36 (m, 5H, ArNH_2, H$_{phenyl}$ (3H)), 7.57 (s br, 1H, CONH_2), 7.82 (s br, 1H, CONH_2), 7.98 (d, J = 7.7 Hz, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), the signals for 2 H$_{phenyl}$ cannot be observed in the spectrum;

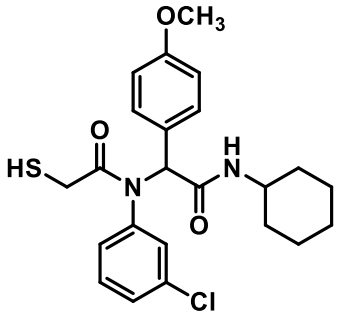
^{13}C NMR (126 MHz, DMSO-d_6): δ [ppm] = 24.5 (1C, NHCH(CH$_2$$CH_2$)$_2CH_2$), 24.6 (1C, NHCH(CH$_2$$CH_2$)$_2CH_2$), 25.2 (1C, NHCH(CH$_2CH_2$)$_2$$CH_2$), 32.16 (1C, NHCH(*C*H$_2CH_2$)$_2CH_2$), 32.23 (1C, NHCH(*C*H$_2CH_2$)$_2CH_2$), 48.0 (1C, NH*C*H(CH$_2CH_2$)$_2CH_2$), 54.9 (1C, ArO*C*H$_3$), 63.4 (1C, N*C*HCO), 113.2 (2C, C-3'''$_{4\text{-methoxyphenyl}}$, C-5'''$_{4\text{-methoxyphenyl}}$), 123.2 (1C, C-5'$_{isothiazole}$), 126.7 (1C, C-1'''$_{4\text{-methoxyphenyl}}$), 128.9 (2C, C$_{phenyl}$), 129.5 (1C, C-4''$_{phenyl}$), 131.6 (2C, C-2'''$_{4\text{-methoxyphenyl}}$, C-6'''$_{4\text{-methoxyphenyl}}$), 133.3 (2C, C$_{phenyl}$), 137.3 (1C, C-1''$_{phenyl}$), 146.5 (1C, C-3'$_{isothiazole}$), 151.2 (1C, C-4'$_{isothiazole}$), 158.6 (1C, C-4'''$_{4\text{-methoxyphenyl}}$), 162.2 (1C, Ar*C*ONCH), 163.9 (1C, Ar*C*ONH$_2$), 168.8 (1C, CH*C*ONHCH);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3481, 3446, 3331, 3285, 2927, 2849, 1664, 1650, 1614, 1597, 1560, 1541, 1487, 1447, 1369, 1344, 1322, 1309, 1269, 1247, 1227, 1196, 1150, 1105, 1071, 983, 958, 892, 868, 851, 803, 792, 755, 741, 710, 666, 578, 562, 529, 516, 506, 459, 444, 411;

HRMS (*m*/*z*): [M+H]$^+$ calcd for $C_{26}H_{30}N_5O_4S$: 508.2013, found: 508.2028;

HPLC (method 1): t_R = 23.1 min, purity 95.9%.

***N*-(3-Chlorophenyl)-*N*-[2-(cyclohexylamino)-1-(4-methoxyphenyl)-2-oxoethyl]-2-mercaptoacetamide (56c)**



$C_{23}H_{27}ClN_2O_3S$

M_r = 447.0

3-Chloroaniline (0.21 mL, 260 mg, 2.0 mmol) and 4-methoxybenzaldehyde (0.24 mL, 270 mg, 2.0 mmol) were dissolved in dichloromethane (15 mL) and the solution was stirred at ambient temperature for 10 min. Then, sulfanylacetic acid (0.14 mL, 180 mg, 2.0 mmol) and isocyanocyclohexane (0.25 mL, 220 mg, 2.0 mmol) were added. After stirring the reaction mixture for 48 h at ambient temperature, the solvent was removed *in vacuo* and the residue was purified by flash column chromatography (Ø = 4 cm, h = 15 cm, V = 15 mL, dichloromethane/ethyl acetate = 15/1) to give **56c** (520 mg, 1.2 mmol, 58% yield) as colorless solid.

m.p. = 158 °C;

TLC: R_f = 0.25 (dichloromethane/ethyl acetate = 15/1);

^{1}H NMR (400 MHz, DMSO-d_6): δ [ppm] = 0.90 – 1.33 (m, 5H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 1.48 – 1.81 (m, 5H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 2.63 (t, J = 6.7 Hz, 1H, HSCH$_2$CO), 2.94 – 3.04 (m, 1H, HSCH_2CO), 3.08 (dd, J = 14.9/6.7 Hz, 1H, HSCH_2CO), 3.52 – 3.63 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), 3.64 (s, 3H, ArOCH_3), 5.98 (s, 1H, NCHCO), 6.67 – 6.74 (m, 2H, 3"-H$_{4\text{-methoxyphenyl}}$, 5"-H$_{4\text{-methoxyphenyl}}$), 6.90 – 6.97 (m, 2H, 2"-H$_{4\text{-methoxyphenyl}}$, 6"-H$_{4\text{-methoxyphenyl}}$), 7.07 – 7.28 (m, 2H, H$_{3\text{-chlorophenyl}}$), 8.00 (d, J = 7.5 Hz, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), the signals for 2 H$_{3\text{-chlorophenyl}}$ cannot be observed in the spectrum;

^{13}C NMR (101 MHz, DMSO-d_6): δ [ppm] = 24.4 (1C, NHCH(CH$_2$CH_2)$_2$CH$_2$), 24.6 (1C, NHCH(CH$_2$CH_2)$_2$CH$_2$), 25.2 (1C, NHCH(CH$_2$CH$_2$)$_2$CH_2), 27.4 (1C, HSCH_2CO), 32.15 (1C,

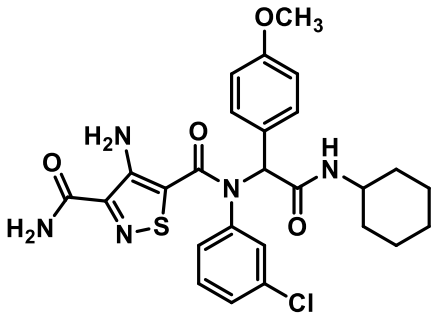
NHCH(*C*H$_2$CH$_2$)$_2$CH$_2$), 32.21 (1C, NHCH(*C*H$_2$CH$_2$)$_2$CH$_2$), 47.9 (1C, NH*C*H(CH$_2$CH$_2$)$_2$CH$_2$), 55.0 (1C, ArO*C*H$_3$), 63.2 (1C, N*C*HCO), 113.3 (2C, C-3"$_{4\text{-methoxyphenyl}}$, C-5"$_{4\text{-methoxyphenyl}}$), 126.7 (1C, C-1"$_{4\text{-methoxyphenyl}}$), 127.9 (1C, C$_{3\text{-chlorophenyl}}$), 129.8 (2C, C$_{3\text{-chlorophenyl}}$), 130.9 (1C, C$_{3\text{-chlorophenyl}}$), 131.2 (2C, C-2"$_{4\text{-methoxyphenyl}}$, C-6"$_{4\text{-methoxyphenyl}}$), 132.4 (1C, C$_{3\text{-chlorophenyl}}$), 140.7 (1C, C-1'$_{3\text{-chlorophenyl}}$), 158.7 (1C, C-4"$_{4\text{-methoxyphenyl}}$), 168.6 (2C, *C*ON);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3258, 3076, 3006, 2924, 2851, 2835, 2561, 1658, 1642, 1614, 1588, 1560, 1514, 1474, 1452, 1441, 1408, 1370, 1333, 1310, 1249, 1231, 1180, 1105, 1075, 1037, 1001, 979, 889, 841, 820, 806, 788, 768, 753, 738, 706, 684, 643, 627, 583, 557, 522, 497, 452, 434, 417;

HRMS (*m*/*z*): [M+H]$^+$ calcd for $C_{23}H_{28}{}^{35}ClN_2O_3S$: 447.1504, found: 447.1470;

HPLC (method 1): t_R = 24.4 min, purity 75.9%.

4-Amino-*N*^{5}-(3-chlorophenyl)-*N*^{5}-[2-(cyclohexylamino)-1-(4-methoxyphenyl)-2-oxoethyl]isothiazole-3,5-dicarboxamide (58c)



$C_{26}H_{28}ClN_5O_4S$

M_r = 542.1

56c (450 mg, 1.0 mmol) was added in one portion to a solution of **25** (320 mg, 1.2 mmol) in acetone (100 mL). The mixture was cooled to 0 °C and morpholine (0.17 mL, 170 mg, 1.9 mmol) was added dropwise. After stirring the reaction mixture for 12 h at ambient temperature, the solvent was removed *in vacuo*. The residue was purified by flash column chromatography (Ø = 6 cm, h = 20 cm, V = 15 mL, dichloromethane/acetone = 13/1) and recrystallized from acetonitrile to give **58c** (470 mg, 0.86 mmol, 86% yield) as colorless solid.

m.p. = 249 °C;

TLC: R_f = 0.26 (dichloromethane/acetone = 10/1);

^{1}H NMR (400 MHz, DMSO-d_6): δ [ppm] = 0.91 – 1.34 (m, 5H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 1.47 – 1.82 (m, 5H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 3.55 – 3.64 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), 3.65 (s, 3H, ArOCH_3), 6.11 (s, 1H, NCHCO), 6.68 – 6.75 (m, 2H, 3'''-H$_{4\text{-methoxyphenyl}}$, 5'''-H$_{4\text{-methoxyphenyl}}$), 6.98 – 7.04 (m, 2H, 2'''-H$_{4\text{-methoxyphenyl}}$, 6'''-H$_{4\text{-methoxyphenyl}}$), 7.14 – 7.33 (m, 3H, ArNH_2, H$_{3\text{-chlorophenyl}}$ (1H)), 7.38 – 7.44 (m, 1H, 4''-H$_{3\text{-chlorophenyl}}$), 7.61 (s br, 1H, CONH_2), 7.86 (s br, 1H, CONH_2), 8.06 (d, J = 7.7 Hz, 1H, NHCH(CH$_2$CH$_2$)$_2$), the signals for 2 H$_{3\text{-chlorophenyl}}$ cannot be observed in the spectrum;

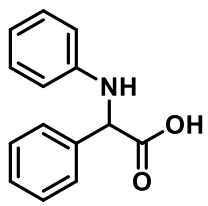
^{13}C NMR (151 MHz, DMSO-d_6): δ [ppm] = 24.4 (1C, NHCH(CH$_2$$CH_2$)$_2CH_2$), 24.6 (1C, NHCH(CH$_2$$CH_2$)$_2CH_2$), 25.1 (1C, NHCH(CH$_2CH_2$)$_2$$CH_2$), 32.1 (1C, NHCH($CH_2CH_2$)$_2CH_2$), 32.2 (1C, NHCH($CH_2CH_2$)$_2CH_2$), 48.0 (1C, NH$C$H(CH$_2CH_2$)$_2CH_2$), 54.9 (1C, ArO$CH_3$), 63.2 (1C, N$C$HCO), 113.2 (2C, C-3'''$_{4\text{-methoxyphenyl}}$, C-5'''$_{4\text{-methoxyphenyl}}$), 122.6 (1C, C-5'$_{\text{isothiazole}}$), 126.3 (1C, C-1'''$_{4\text{-methoxyphenyl}}$), 129.4 (1C, C-4''$_{3\text{-chlorophenyl}}$), 130.1 (1C, C$_{3\text{-chlorophenyl}}$), 131.4 (2C, C-2'''$_{4\text{-methoxyphenyl}}$, C-6'''$_{4\text{-methoxyphenyl}}$), 131.9 (1C, C$_{3\text{-chlorophenyl}}$), 132.9 (1C, C-3''$_{3\text{-chlorophenyl}}$), 133.2 (1C, C$_{3\text{-chlorophenyl}}$), 138.7 (1C, C-1''$_{3\text{-chlorophenyl}}$), 146.6 (1C, C-3'$_{\text{isothiazole}}$), 151.3 (1C, C-4'$_{\text{isothiazole}}$), 158.8 (1C, C-4'''$_{4\text{-methoxyphenyl}}$), 161.9 (1C, Ar$C$ONCH), 163.7 (1C, Ar$CONH_2$), 168.7 (1C, CH$C$ONHCH);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3460, 3342, 3271, 3083, 2924, 2851, 1675, 1644, 1610, 1563, 1515, 1494, 1471, 1444, 1425, 1372, 1349, 1331, 1311, 1249, 1182, 1151, 1105, 1074, 1041, 979, 891, 841, 792, 769, 756, 736, 705, 676, 557, 532, 505, 441, 433, 421;

HRMS (m/z): [M+H]$^+$ calcd for C$_{26}$H$_{29}$^{35}ClN$_5$O$_4$S: 542.1623, found: 542.1597;

HPLC (method 1): t_R = 24.2 min, purity 95.1%.

2-Phenyl-2-(phenylamino)acetic acid (62)



C$_{14}$H$_{13}$NO$_2$

Mr = 227.3

Aniline hydrochloride (4.9 g, 38 mmol) was dissolved in water (38 mL) and the mixture was cooled to 0 °C. A solution of potassium cyanide (4.0 g, 61 mmol) in water (19 mL) was added dropwise. Under vigorous stirring, benzaldehyde (4.0 g, 38 mmol) was added and the mixture was stirred for 1 h at 0 °C. Then petroleum ether (50 mL) was added and the mixture was stirred for 12 h at ambient temperature. After phase separation, the aqueous phase was extracted with ethyl acetate (3× 100 mL). The combined organic phases were washed with water (200 mL) and brine (200 mL), dried with sodium sulfate, filtered, and the solvent was removed *in vacuo*. The residue was washed with petroleum ether.

Hydrogen peroxide (30 %, 20 mL) was added dropwise to the mixture of a share (4.2 g, 85%) of the obtained crude product (5.1 g) and potassium carbonate (1.4 g, 10 mmol) in dimethyl sulfoxide (25 mL) at 0 °C. After stirring for 2 h, the resulting precipitate was filtered and dried *in vacuo*.

A share (2.2 g, 53%) of the obtained crude product (4.2 g) and sodium hydroxide (2.4 g, 60 mmol) were dissolved in a mixture of methanol and water (4:1) (100 mL) and the mixture was stirred at 90 °C for 6 h. After cooling, the mixture was concentrated and diluted with water (100 mL) and the resulting mixture was extracted with ethyl acetate (100 mL). The aqueous phase was acidified to pH 4 with an aqueous solution of hydrogen chloride (6 M) and extracted with dichloromethane (3× 100 mL). The combined organic layers were washed with brine (100 mL), dried with sodium sulfate, filtered, and the solvent was removed *in vacuo* to give **62** as colorless solid (2.1 g, 9.2 mmol, 54 % yield).

m.p. = 204 °C (decomposition);

TLC: R_f = 0.70 (dichloromethane/methanol = 10/1);

^{1}H NMR (400 MHz, DMSO-d_6): δ [ppm] = 5.09 (s, 1H, NHC*H*CO), 6.19 (br, 1H, N*H*CHCO), 6.55 (dd, J = 7.8, 6.7 Hz, 1H, H_{phenyl}), 6.72 – 6.62 (m, 2H, H_{phenyl}), 7.09 – 6.98 (m, 2H, H_{phenyl}), 7.33 – 7.24 (m, 1H, H_{phenyl}), 7.36 (t, J = 7.4 Hz, 2H, H_{phenyl}), 7.56 – 7.48 (m, 2H, H_{phenyl}), 12.86 (br, 1H, COO*H*);

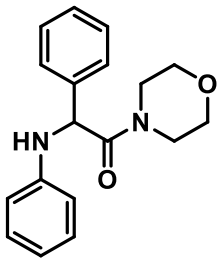
^{13}C NMR (101 MHz, DMSO-d_6): δ [ppm] = 59.8 (1C, CO*C*HNH), 110.2 (1C, C_{phenyl}), 113.09 (1C, C_{phenyl}), 116.61 (1C, C_{phenyl}), 127.5 (2C, C_{phenyl}), 127.8 (1C, C_{phenyl}), 128.5 (2C, C_{phenyl}), 128.8 (1C, C_{phenyl}), 138.6 (1C, C_{phenyl}), 147.0 (1C, C_{phenyl}), 173.0 (1C, *C*OOH);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3060, 2991, 2781, 2718, 2635, 2312, 2016, 1953, 1892, 1571, 1492, 1455, 1414, 1379, 1316, 1297, 1245, 1198, 1186, 1075, 1024, 1001, 924, 895, 873, 773, 755, 737, 722, 690, 618, 566, 530, 487, 455;

HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{14}H_{14}NO_2$: 228.1019, found: 228.1027;

HPLC (method 1): t_R = 20.2 min, purity 97.3 %.

1-Morpholino-2-phenyl-2-(phenylamino)ethan-1-one (63)



$C_{18}H_{20}N_2O_2$

M_r = 296.4

DIPEA (0.52 mL, 390 mg, 3.0 mmol) and COMU (1.3 g, 3.0 mmol) were added to an ice-cooled solution of **62** (680 mg, 3.0 mmol) in acetonitrile (40 mL) and the mixture was stirred for 10 min at 0 °C. Then, morpholine (0.26 mL, 260 mg, 3.0 mmol) and DIPEA (0.52 mL, 390 mg, 3.0 mmol) were added. After stirring the reaction mixture at ambient temperature for 8 h, the volatiles were removed *in vacuo*. The residue was dissolved in dichloromethane (100 mL) and the organic solvent was washed with water (50 mL) and brine (50 mL), dried over sodium sulfate, filtered, and removed *in vacuo*. The residue was purified by flash column chromatography (Ø = 6 cm, h = 20 cm, V = 30 mL, dichloromethane/methanol = 80/1) to give **63** (480 mg, 1.6 mmol, 54% yield) as colorless solid.

m.p. = 86 °C;

TLC: R_f = 0.62 (dichloromethane/methanol = 20/1);

^{1}H NMR (400 MHz, DMSO-d_6): δ [ppm] = 3.19 – 3.30 (m, 1H, N(CH_2CH_2)$_2$O), 3.31 – 3.63 (m, 6H, N(CH_2CH_2)$_2$O (3H), N(CH_2CH_2)$_2$O (3H)), 3.72 – 3.85 (m, 1H, N(CH_2CH_2)$_2$O), 5.62 (d, *J* = 8.6 Hz, 1H, NHC*H*CO), 6.12 (d, *J* = 8.6 Hz, 1H, N*H*CHCO), 6.49 – 6.56 (m, 1H, 4'-H_{phenyl}), 6.70 – 6.77 (m, 2H, 2'-H_{phenyl}, 6'-H_{phenyl}), 6.99 – 7.07 (m, 2H, 3'-H_{phenyl}, 5'-H_{phenyl}), 7.23 – 7.29 (m, 1H, 4''-H_{phenyl}), 7.30 – 7.37 (m, 2H, 3''-H_{phenyl}, 5''-H_{phenyl}), 7.47 – 7.52 (m, 2H, 2''-H_{phenyl}, 6''-H_{phenyl});

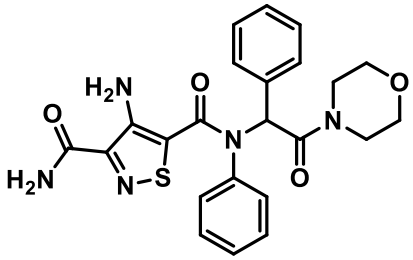
^{13}C NMR (126 MHz, DMSO-d_6): δ [ppm] = 42.3 (1C, N(CH$_2$CH$_2$)$_2$O), 45.5 (1C, N(CH$_2$CH$_2$)$_2$O), 55.7 (1C, NHCHCO), 65.9 (1C, N(CH$_2$$CH_2$)$_2$O), 66.0 (1C, N(CH$_2$$CH_2$)$_2$O), 113.2 (2C, C-2'$_{phenyl}$, C-6'$_{phenyl}$), 116.4 (1C, C-4'$_{phenyl}$), 127.5 (1C, C-4"$_{phenyl}$), 127.8 (2C, C-2"$_{phenyl}$, C-6"$_{phenyl}$), 128.4 (2C, C-3"$_{phenyl}$, C-5"$_{phenyl}$), 128.7 (2C, C-3'$_{phenyl}$, C-5'$_{phenyl}$), 138.8 (1C, C-1"$_{phenyl}$), 146.9 (1C, C-1'$_{phenyl}$), 169.2 (1C, CH$C$ON);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3400, 2967, 2919, 2850, 1632, 1601, 1579, 1505, 1469, 1422, 1386, 1358, 1340, 1319, 1298, 1270, 1252, 1178, 1161, 1131, 1111, 1080, 1070, 1035, 1011, 993, 968, 884, 864, 840, 812, 746, 709, 701, 691, 646, 631, 609, 589, 539, 503, 487, 434;

HRMS (m/z): [M+H]$^+$ calcd for $C_{18}H_{21}N_2O_2$: 297.1598, found: 297.1615;

HPLC (method 1): t_R = 19.5 min, purity 96.5%.

4-Amino-N^5-(2-morpholino-2-oxo-1-phenylethyl)-N^5-phenylisothiazole-3,5-dicarboxamide (66)



$C_{23}H_{23}N_5O_4S$

M_r = 465.5

Triethylamine (0.34 mL, 240 mg, 2.4 mmol) and chloroacetyl chloride (0.19 mL, 270 mg, 2.4 mmol) were added to an ice-cooled solution of **63** (590 mg, 2.0 mmol) in dichloromethane (40 mL) and the reaction mixture was stirred for 12 h at ambient temperature. Then, the organic solvent was washed with a saturated aqueous solution of ammonium chloride (2× 40 mL), a saturated aqueous solution of sodium bicarbonate (2× 40 mL), and brine (40 mL), dried over sodium sulfate, filtered, and removed *in vacuo*.

A share (370 mg, 93%) of the obtained crude product (400 mg) was dissolved in acetone (20 mL). Finely ground thiourea (91 mg, 1.2 mmol) was added and the resulting mixture was stirred for 16 h at ambient temperature. After removal of the solvent, the residue was treated with ethyl acetate (50 mL). Under vigorous stirring, a solution of sodium sulfite (250 mg, 2.0 mmol) in water (50 mL) was added and the mixture was stirred for 3 h at ambient

temperature. The organic phase was washed with water (50 mL) and brine (50 mL), dried over sodium sulfate, filtered, and the solvent was removed *in vacuo*. The residue was washed with acetonitrile.

A share (190 mg, 90%) of the obtained crude product (210 mg) was added in one portion to a solution of **25** (160 mg, 0.60 mmol) in acetone (50 mL). The solution was cooled to 0 °C and morpholine (0.09 mL, 87 mg, 1.0 mmol) was added. After stirring the mixture for 12 h at ambient temperature, the solvent was removed *in vacuo*. The residue was purified by flash column chromatography (Ø = 4 cm, h = 20 cm, V = 15 mL, dichloromethane/acetone = 3/1) and recrystallized from acetonitrile to give **66** (120 mg, 0.26 mmol, 16% yield) as yellowish solid.

m.p. = 235 °C;

TLC: R_f = 0.26 (dichloromethane/acetone = 5/2);

^{1}H NMR (400 MHz, DMSO-*d*$_6$): δ [ppm] = 2.85 – 2.95 (m, 1H, N(CH$_2$C*H*$_2$)$_2$O), 3.13 – 3.24 (m, 1H, N(C*H*$_2$CH$_2$)$_2$O), 3.38 – 3.55 (m, 4H, N(C*H*$_2$CH$_2$)$_2$O (2H), N(CH$_2$C*H*$_2$)$_2$O (2H)), 3.56 – 3.70 (m, 2H, N(C*H*$_2$CH$_2$)$_2$O (1H), N(CH$_2$C*H*$_2$)$_2$O (1H)), 6.60 (s, 1H, NC*H*CO), 6.70 (s br, 1H, H$_{phenyl}$), 6.99 – 7.47 (m, 10H, H$_{phenyl}$ (8H), ArN*H*$_2$), 7.59 (s br, 1H, CON*H*$_2$), 7.83 (s br, 1H, CON*H*$_2$), 7.92 (s br, 1H, H$_{phenyl}$);

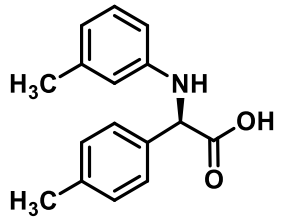
^{13}C NMR (101 MHz, DMSO-*d*$_6$): δ [ppm] = 42.3 (1C, N(*C*H$_2$CH$_2$)$_2$O), 45.7 (1C, N(*C*H$_2$CH$_2$)$_2$O), 62.0 (1C, N*C*HCO), 65.4 (1C, N(CH$_2$*C*H$_2$)$_2$O), 66.0 (1C, N(CH$_2$*C*H$_2$)$_2$O), 122.8 (1C, C-5'$_{isothiazole}$), 128.3 (3C, C$_{phenyl}$), 129.0 (2C, C$_{phenyl}$), 129.7 (1C, C$_{phenyl}$), 130.5 (2C, C$_{phenyl}$), 132.9 (1C, C$_{phenyl}$), 133.3 (2C, C$_{phenyl}$), 137.1 (1C, C$_{phenyl}$), 146.5 (1C, C-3'$_{isothiazole}$), 151.3 (1C, C-4'$_{isothiazole}$), 162.1 (1C, Ar*C*ONCH), 163.9 (1C, Ar*C*ONH$_2$), 167.8 (1C, CH*C*ON);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3456, 3343, 3196, 2967, 2922, 2850, 1667, 1647, 1613, 1555, 1490, 1441, 1399, 1374, 1360, 1325, 1279, 1268, 1233, 1179, 1149, 1113, 1070, 1033, 1002, 858, 838, 798, 781, 763, 745, 707, 677, 648, 636, 577, 546, 518, 507, 494, 452, 430, 415;

HRMS (*m*/*z*): [M+H]$^+$ calcd for C$_{23}$H$_{24}$N$_5$O$_4$S: 466.1544, found: 466.1525;

HPLC (method 1): t_R = 20.2 min, purity 96.5%.

(*R*)-2-(*p*-Tolyl)-2-(*m*-tolylamino)acetic acid (77)



$C_{16}H_{17}NO_2$

Mr = 255.3

(1*S*)-1-(4-methoxyphenyl)ethanamine hydrochloride (7.5 g, 50 mmol) was dissolved in methanol (100 mL) and the mixture was cooled to 0 °C. A solution of potassium cyanide (4.0 g, 60 mmol) in water (50 mL) was added dropwise. Under vigorous stirring, 4-methylbenzaldehyde (5.9 mL, 6.0 g, 50 mmol) was added and the mixture was stirred for 1 h at 0 °C. After stirring for 12 h at ambient temperature, the resulting precipitate was filtered and purified by recrystallization with petroleum ether to give colorless needle-like crystalline solid.

The solid was dissolved in 6 M hydrochloric acid (100 mL) and the mixture was stirred at 90 °C for 6 h. After cooling, the resulting precipitate was filtered and dried *in vacuo*. Then a Schlenk tube was charged with a share (4.02 g, 80%) of the obtained crude product (5.03 g), 3-Iodotoluene (4.36 g, 20 mmol), caesium carbonate (19.5 g, 60 mmol), and copper(I) iodide (570 mg, 3.0 mmol). Under N_2 atmosphere, DMSO (50 mL) was added by syringe. The tube was sealed and the mixture was stirred at ambient temperature for 24 h. Then, the reaction mixture was diluted with 200 mL of water. Under cooling, 6 M hydrochloric acid was added to adjust the pH to 4. Then, the aqueous phase was extracted with ethyl acetate (3×100 mL). The combined organic phases were washed with water (200 mL) and brine (200 mL), dried with sodium sulfate, filtered, and the solvent was removed *in vacuo*. The residue was purified by flash column chromatography (Ø = 8 cm, h = 30 cm, V = 30 mL, petroleum ether/ethyl acetate = 2/1) to give **77** (4.1 g, 16.3 mmol, 41% yield) as colorless solid.

m.p. = 190 °C (decomposition);

TLC: R_f = 0.71 (dichloromethane/methanol = 10/1);

^{1}H NMR (400 MHz, DMSO-d_6): δ [ppm] = 2.15 (s, 3H, C$H_{3,m\text{-tolyl}}$), 2.28 (s, 3H, C$H_{3,p\text{-tolyl}}$), 3.41 (br, 2H, COO*H*, COCHN*H*), 5.02 (s, 1H, NHC*H*CO), 6.34 – 6.48 (m, 2H, 4'-H$_{m\text{-tolyl}}$, 6'-

$H_{m\text{-tolyl}}$), 6.50 (t, *J* = 1.9 Hz, 1H, 2'-$H_{m\text{-tolyl}}$), 6.92 (t, *J* = 7.7 Hz, 1H, 5'-$H_{m\text{-tolyl}}$), 7.16 (d, *J* = 7.9 Hz, 2H, 3"-$H_{p\text{-tolyl}}$, 5"-$H_{p\text{-tolyl}}$), 7.39 (d, *J* = 7.9 Hz, 2H, 2"-$H_{p\text{-tolyl}}$, 6"-$H_{p\text{-tolyl}}$);

^{13}C NMR (101 MHz, DMSO-d_6): δ [ppm] = 20.6 (1C, $CH_{3,m\text{-tolyl}}$), 21.3 (1C, $CH_{3,p\text{-tolyl}}$), 59.4 (1C, CO*C*HNH), 110.2 (1C, C-4'$_{m\text{-tolyl}}$), 113.7 (1C, C-2'$_{m\text{-tolyl}}$), 117.4 (1C, C-6'$_{m\text{-tolyl}}$), 127.3 (2C, C-2"$_{p\text{-tolyl}}$, C-6"$_{p\text{-tolyl}}$), 128.5 (1C, C-5'$_{m\text{-tolyl}}$), 128.9 (2C, C-3"$_{p\text{-tolyl}}$, C-5"$_{p\text{-tolyl}}$), 135.5 (1C, C-1"$_{p\text{-tolyl}}$), 136.8 (1C, C-4"$_{p\text{-tolyl}}$), 137.6 (1C, C-3'$_{m\text{-tolyl}}$), 146.9 (1C, C-1'$_{m\text{-tolyl}}$), 173.1 (1C, *C*OOH);

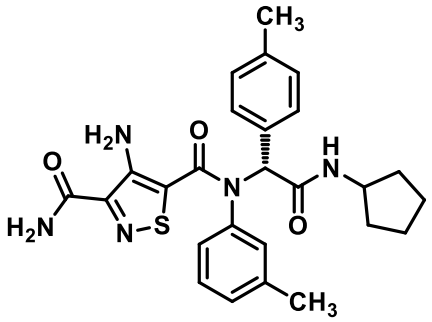
IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3021, 2917, 2865, 2539, 1719, 1605, 1589, 1509, 1489, 1436, 1377, 1324, 1307, 1256, 1181, 1127, 769, 725, 691, 503, 440, 404;

HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{16}H_{18}NO_2$: 256.1332, found: 256.1354;

HPLC (method 1): t_R = 15.3 min, purity 90.5%;

Chiral HPLC (method 3): t_R = 26.0 min, % ee = 95.6 %.

(*R*)-4-Amino-N^5-(2-(cyclopentylamino)-2-oxo-1-(*p*-tolyl)ethyl)-N^5-(*m*-tolyl)isothiazole-3,5-dicarboxamide ((*R*)-20)



$C_{26}H_{29}N_5O_3S$

M_r = 491.6

DIPEA (0.87 mL, 650 mg, 5 mmol) and COMU (2.2 g, 5.0 mmol) were added to an ice-cooled solution of **77** (1.28 g, 5 mmol) in acetonitrile (100 mL) and the mixture was stirred for 10 min at 0 °C. Then, cyclopentylamine (0.49 mL, 425 mg, 5.0 mmol) and DIPEA (0.87 mL, 650 mg, 5 mmol) were added. After stirring the reaction mixture at 0 °C for 12 h, the volatiles were removed *in vacuo*. The residue was dissolved in dichloromethane (150 mL) and the organic solvent was washed with water (50 mL) and brine (50 mL), dried over sodium sulfate, filtered, and removed *in vacuo*. The residue was purified by flash column chromatography (Ø = 6 cm, h = 20 cm, V = 30 mL, dichloromethane/methanol = 80/1) to give **78** (1.3 g, 4.1 mmol) as colorless solid.

Triethylamine (0.56 mL, 0.4 g, 4 mmol) and chloroacetyl chloride (0.33 mL, 0.45 g, 4 mmol) were added to an ice-cooled solution of **78** (1.28 g, 4 mmol) in dichloromethane (100 mL) and the reaction mixture was stirred for 12 h at 0 °C. Then, the organic solvent was washed with a saturated aqueous solution of ammonium chloride (2× 50 mL), a saturated aqueous solution of sodium bicarbonate (50 mL), and brine (50 mL), dried over sodium sulfate, filtered, and removed *in vacuo*.

A share (1.4 g, 93 %) of the obtained crude product (1.5 g) was dissolved in acetone (150 mL). Finely ground thiourea (265 mg, 3.5 mmol) was added and the resulting mixture was stirred for 16 h at ambient temperature. After removal of the solvent, the residue was treated with ethyl acetate (200 mL). Under vigorous stirring, a solution of sodium sulfite (0.44 g, 3.5 mmol) in water (100 mL) was added and the mixture was stirred for 3 h at ambient temperature. The organic phase was washed with water (200 mL) and brine (100 mL), dried over sodium sulfate, filtered, and the solvent was removed *in vacuo*. The residue was washed with acetonitrile.

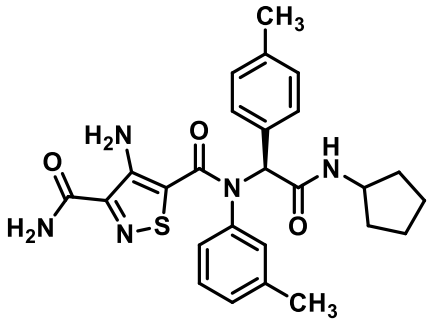
A share (792 mg, 97 %) of the obtained crude product (817 mg) was added in one portion to a solution of **25** (533 mg, 2 mmol) in acetone (250 mL). The solution was cooled to 0 °C and morpholine (0.25 mL, 217 mg, 2 mmol) was added dropwise. After stirring the mixture for 12 h at ambient temperature, the solvent was removed *in vacuo*. The residue was purified by flash column chromatography (Ø = 6 cm, h = 20 cm, V = 15 mL, dichloromethane/acetone = 10/1) and recrystallized from acetonitrile to give (*R*)-**20** (505 mg, 1.03 mmol, 23 % yield) as yellowish solid.

TLC, **^{1}H NMR, ^{13}C NMR**, **IR**, **HRMS** and **HPLC** data are same as **20**;

m.p. = 241 °C;

Chiral HPLC (method 2): t_R = 10.8 min, % ee = 97.2 %.

(*S*)-4-Amino-N^5-(2-(cyclopentylamino)-2-oxo-1-(*p*-tolyl)ethyl)-N^5-(*m*-tolyl)isothiazole-3,5-dicarboxamide ((*S*)-20)



$C_{26}H_{29}N_5O_3S$

M_r = 491.6

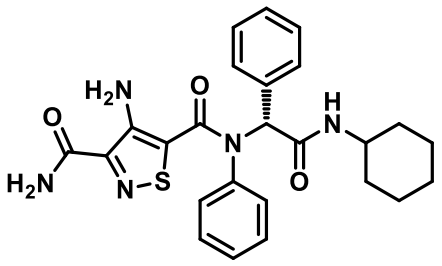
Referring to the synthesis method of compound **77** and compound (*R*)-**20**, (1*R*)-1-(4-methoxyphenyl)ethanamine hydrochloride was used as raw material to react under the above reaction conditions, and corresponding post-processing was performed to give (*S*)-**20** (505 mg, 0.97 mmol, 18 % yield) as yellowish solid.

TLC, **^{1}H NMR, ^{13}C NMR**, **IR**, **HRMS** and **HPLC** data are same as **20**.

m.p. = 243 °C;

Chiral HPLC (method 2): t_R = 7.21 min, % ee = 97.0 %.

(*R*)-4-Amino-N^5-[2-(cyclohexylamino)-2-oxo-1-phenylethyl]-N^5-phenylisothiazole-3,5-dicarboxamide ((*R*)-58a)



$C_{25}H_{27}N_5O_3S$

M_r = 477.6

A Schlenk tube was charged with (*R*)-2-amino-2-phenylacetic acid (3.02 g, 20 mmol), 3-Iodotoluene (4.36 g, 20 mmol), caesium carbonate (9.75 g, 30 mmol), and copper(I) iodide (570 mg, 3.0 mmol). Under N_2 atmosphere, DMSO (50 mL) was added by syringe. The tube was sealed and the mixture was stirred at ambient temperature for 24 h. Then, the reaction mixture

was diluted with 200 mL of water. Under cooling, 6 M hydrochloric acid was added to adjust the pH to 4. Then, the aqueous phase was extracted with ethyl acetate (3×100 mL). The combined organic phases were washed with water (200 mL) and brine (200 mL), dried with sodium sulfate, filtered, and the solvent was removed *in vacuo*. The residue was purified by flash column chromatography (Ø = 8 cm, h = 30 cm, V = 30 mL, petroleum ether/ethyl acetate = 2/1) to give **82** (3.2 g, 14.1 mmol) as colorless solid.

DIPEA (0.87 mL, 650 mg, 5 mmol) and COMU (2.2 g, 5.0 mmol) were added to an ice-cooled solution of **82** (1.14 g, 5 mmol) in acetonitrile (100 mL) and the mixture was stirred for 10 min at 0 °C. Then, cyclohexylamine (0.57 mL, 495 mg, 5.0 mmol) and DIPEA (0.87 mL, 650 mg, 5 mmol) were added. After stirring the reaction mixture at 0 °C for 12 h, the volatiles were removed *in vacuo*. The residue was dissolved in dichloromethane (150 mL) and the organic solvent was washed with water (50 mL) and brine (50 mL), dried over sodium sulfate, filtered, and removed *in vacuo*. The residue was purified by flash column chromatography (Ø = 6 cm, h = 20 cm, V = 30 mL, dichloromethane/methanol = 80/1) to give **83** (1.3 g, 4.3 mmol) as colorless solid.

Triethylamine (0.56 mL, 0.4 g, 4 mmol) and chloroacetyl chloride (0.33 mL, 0.45 g, 4 mmol) were added to an ice-cooled solution of **83** (1.23 g, 4 mmol) in dichloromethane (100 mL) and the reaction mixture was stirred for 12 h at 0 °C. Then, the organic solvent was washed with a saturated aqueous solution of ammonium chloride (2× 50 mL), a saturated aqueous solution of sodium bicarbonate (50 mL), and brine (50 mL), dried over sodium sulfate, filtered, and removed *in vacuo*.

A share (1.34 g, 97 %) of the obtained crude product (1.34 g) was dissolved in acetone (150 mL). Finely ground thiourea (265 mg, 3.5 mmol) was added and the resulting mixture was stirred for 16 h at ambient temperature. After removal of the solvent, the residue was treated with ethyl acetate (200 mL). Under vigorous stirring, a solution of sodium sulfite (0.44 g, 3.5 mmol) in water (100 mL) was added and the mixture was stirred for 3 h at ambient temperature. The organic phase was washed with water (200 mL) and brine (100 mL), dried over sodium sulfate, filtered, and the solvent was removed *in vacuo*. The residue was washed with acetonitrile.

A share (764 mg, 92 %) of the obtained crude product (830 mg) was added in one portion to a solution of **25** (533 mg, 2 mmol) in acetone (250 mL). The solution was cooled to 0 °C and morpholine (0.25 mL, 217 mg, 2 mmol) was added dropwise. After stirring the mixture for 12 h at ambient temperature, the solvent was removed *in vacuo*. The residue was purified by flash column chromatography (Ø = 6 cm, h = 20 cm, V = 15 mL, dichloromethane/acetone = 10/1)

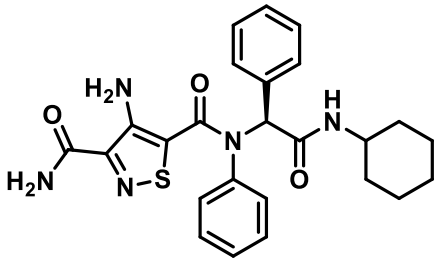
and recrystallized from acetonitrile to give (*R*)-**58a** (479 mg, 1.00 mmol, 17 % yield) as cololess solid.

TLC, **^{1}H NMR, ^{13}C NMR**, **IR**, **HRMS** and **HPLC** data are same as **58a**.

m.p. = 259 °C;

Chiral HPLC (method 2): t_R = 8.06 min, % ee = 96.5 %.

(*S*)-4-Amino-*N*^{5}-[2-(cyclohexylamino)-2-oxo-1-phenylethyl]-*N*^{5}-phenylisothiazole-3,5-dicarboxamide ((*S*)-58a)



$C_{25}H_{27}N_5O_3S$

M_r = 477.6

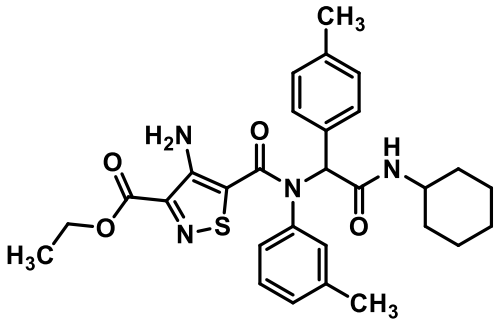
Referring to the synthesis method of compound (*R*)-**58a**, (*S*)-2-amino-2-phenylacetic acid was used as raw material to react under the above reaction conditions, and corresponding post-processing was performed to give (*S*)-**58a** (392 mg, 0.82 mmol, 11 % yield) as colorless solid.

TLC, **^{1}H NMR, ^{13}C NMR**, **IR**, **HRMS** and **HPLC** data are same as **58a**.

m.p. = 260 °C;

Chiral HPLC (method 2): t_R = 10.30 min, % ee = 97.1 %.

Ethyl 4-amino-5-{[2-(cyclohexylamino)-2-oxo-1-(*p*-tolyl)ethyl](*m*-tolyl)carbamoyl}isothiazole-3-carboxylate (87)



$C_{29}H_{34}N_4O_4S$

$M_r = 534.7$

48a (410 mg, 1.0 mmol) was added in one portion to a solution of ethyl (*E*)-2-cyano-2-[(tosyloxy)imino]acetate (360 mg, 1.2 mmol) in acetone (100 mL). The mixture was cooled to 0 °C and morpholine (0.17 mL, 170 mg, 1.9 mmol) was added dropwise. After stirring the reaction mixture for 12 h at ambient temperature, the solvent was removed *in vacuo*. The residue was purified by flash column chromatography (Ø = 6 cm, h = 20 cm, V = 15 mL, petroleum ether/ethyl acetate = 4/1) and recrystallized from acetonitrile to give **87** (490 mg, 0.92 mmol, 92% yield) as colorless solid.

m.p. = 220 °C;

TLC: R_f = 0.25 (petroleum ether/ethyl acetate = 4/1);

^{1}H NMR (400 MHz, $CDCl_3$): δ [ppm] = 0.93 – 1.19 (m, 3H, NHCH(C*H*$_2$CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH$_2$)$_2$C*H*$_2$ (1H)), 1.23 – 1.43 (m, 5H, NHCH(CH$_2$C*H*$_2$)$_2$CH$_2$ (2H), CO$_2$CH$_2$C*H*$_3$), 1.51 – 1.73 (m, 3H, NHCH(CH$_2$C*H*$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH$_2$)$_2$C*H*$_2$ (1H)), 1.79 – 1.91 (m, 1H, NHCH(C*H*$_2$CH$_2$)$_2$CH$_2$), 1.91 – 2.02 (m, 1H, NHCH(C*H*$_2$CH$_2$)$_2$CH$_2$), 2.03 – 2.42 (m, 6H, ArC*H*$_3$), 3.78 – 3.90 (m, 1H, NHC*H*(CH$_2$CH$_2$)$_2$CH$_2$), 4.36 (q, *J* = 7.1 Hz, 2H, CO$_2$C*H*$_2$CH$_3$), 5.61 (d, *J* = 8.1 Hz, 1H, N*H*CH(CH$_2$CH$_2$)$_2$CH$_2$), 6.04 (s, 1H, NC*H*CO), 6.46 (s br, 1H, H$_{m\text{-tolyl}}$), 6.95 – 7.08 (m, 6H, 2'''-H$_{p\text{-tolyl}}$, 3'''-H$_{p\text{-tolyl}}$, 5'''-H$_{p\text{-tolyl}}$, 6'''-H$_{p\text{-tolyl}}$, ArN*H*$_2$), 7.12 – 7.18 (m, 1H, 4''-H$_{m\text{-tolyl}}$), 7.57 (s br, 1H, H$_{m\text{-tolyl}}$), the signal for 1 H$_{m\text{-tolyl}}$ cannot be observed in the spectrum;

^{13}C NMR (126 MHz, $CDCl_3$): δ [ppm] = 14.3 (1C, CO$_2$CH$_2$*C*H$_3$), 21.2 (2C, Ar*C*H$_3$), 24.9 (1C, NHCH(CH$_2$*C*H$_2$)$_2$CH$_2$), 25.0 (1C, NHCH(CH$_2$*C*H$_2$)$_2$CH$_2$), 25.6 (1C, NHCH(CH$_2$CH$_2$)$_2$*C*H$_2$), 32.97 (1C, NHCH(*C*H$_2$CH$_2$)$_2$CH$_2$), 33.02 (1C, NHCH(*C*H$_2$CH$_2$)$_2$CH$_2$), 48.9 (1C,

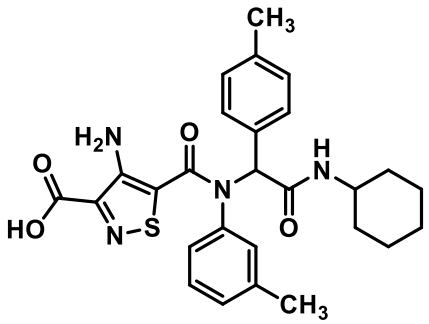
NH*C*H(CH$_2$CH$_2$)$_2$CH$_2$), 61.6 (1C, CO$_2$*C*H$_2$CH$_3$), 65.8 (1C, N*C*HCO), 125.8 (1C, C-5'$_{isothiazole}$), 129.3 (2C, C-3'''$_{p\text{-tolyl}}$, C-5'''$_{p\text{-tolyl}}$), 129.7 (1C, C$_{m\text{-tolyl}}$), 130.4 (2C, C-2'''$_{p\text{-tolyl}}$, C-6'''$_{p\text{-tolyl}}$), 130.8 (1C, C$_{m\text{-tolyl}}$), 131.3 (1C, C-1'''$_{p\text{-tolyl}}$), 133.4 (1C, C$_{m\text{-tolyl}}$), 137.8 (1C, C-1''$_{m\text{-tolyl}}$), 138.6 (1C, C-4'''$_{p\text{-tolyl}}$), 143.4 (1C, C-3'$_{isothiazole}$), 153.0 (1C, C-4'$_{isothiazole}$), 162.1 (1C, *C*O$_2$CH$_2$CH$_3$), 163.5 (1C, Ar*C*ONCH), 168.7 (1C, CH*C*ONHCH), the signals for 2 C$_{m\text{-tolyl}}$ cannot be observed in the spectrum;

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3461, 3338, 3293, 2989, 2929, 2850, 1691, 1648, 1600, 1557, 1519, 1493, 1446, 1383, 1363, 1349, 1298, 1252, 1238, 1192, 1142, 1106, 1075, 1015, 978, 890, 874, 850, 815, 795, 771, 748, 733, 707, 669, 649, 632, 608, 579, 548, 525, 503, 468, 403;

HRMS (*m*/*z*): [M+H]$^+$ calcd for C$_{29}$H$_{35}$N$_4$O$_4$S: 535.2374, found: 535.2387;

HPLC (method 1): t_R = 28.5 min, purity 97.8%.

4-Amino-5-{[2-(cyclohexylamino)-2-oxo-1-(*p*-tolyl)ethyl](*m*-tolyl)carbamoyl}isothiazole-3-carboxylic acid (88)



C$_{27}$H$_{30}$N$_4$O$_4$S

M$_r$ = 506.6

Lithium hydroxide (48 mg, 2.0 mmol) and **87** (270 mg, 0.50 mmol) were dissolved in a mixture of water (20 mL) and methanol (20 mL). After stirring the reaction mixture at ambient temperature overnight, the mixture was concentrated and diluted with water (40 mL). The mixture was acidified to pH 4 with an aqueous solution of hydrogen chloride (6 M) and the solvent was removed *in vacuo*. The residue was purified by flash column chromatography (Ø = 4 cm, h = 15 cm, V = 15 mL, dichloromethane/methanol/acetic acid = 10/1/0.1) to give **88** (180 mg, 0.36 mmol, 72% yield) as colorless solid.

m.p. = 229 °C;

TLC: R_f = 0.29 (dichloromethane/methanol/acetic acid = 10/1/0.1);

^{1}H NMR (600 MHz, DMSO-d_6): δ [ppm] = 0.93 – 1.01 (m, 1H, NHCH(CH_2CH$_2$)$_2$CH$_2$), 1.03 – 1.12 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$CH_2), 1.15 – 1.30 (m, 3H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (1H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H)), 1.50 – 1.56 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$CH_2), 1.56 – 1.62 (m, 1H, NHCH(CH$_2$CH_2)$_2$CH$_2$), 1.62 – 1.72 (m, 2H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (1H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (1H)), 1.73 – 1.80 (m, 1H, NHCH(CH_2CH$_2$)$_2$CH$_2$), 1.97 – 2.35 (m, 6H, ArCH_3), 3.54 – 3.63 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), 6.09 (s, 1H, NCHCO), 6.92 – 6.98 (m, 4H, 2'''-H$_{p\text{-tolyl}}$, 3'''-H$_{p\text{-tolyl}}$, 5'''-H$_{p\text{-tolyl}}$, 6'''-H$_{p\text{-tolyl}}$), 7.12 – 7.16 (m, 1H, 4''-H$_{m\text{-tolyl}}$), 7.98 (d, J = 7.8 Hz, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), the signals for 3 H$_{m\text{-tolyl}}$, ArNH_2, and CO$_2H$ cannot be observed in the spectrum;

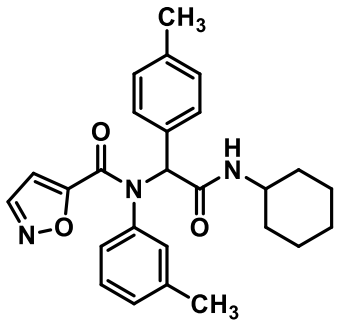
^{13}C NMR (151 MHz, DMSO-d_6): δ [ppm] = 20.6 (2C, ArCH$_3$), 24.5 (1C, NHCH(CH$_2C$H$_2$)$_2$CH$_2$), 24.6 (1C, NHCH(CH$_2C$H$_2$)$_2$CH$_2$), 25.2 (1C, NHCH(CH$_2$CH$_2$)$_2C$H$_2$), 32.17 (1C, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), 32.23 (1C, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), 48.0 (1C, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), 63.8 (1C, NCHCO), 124.0 (1C, C-5'$_{isothiazole}$), 128.4 (2C, C-3'''$_{p\text{-tolyl}}$, C-5'''$_{p\text{-tolyl}}$), 128.8 (1C, C$_{m\text{-tolyl}}$), 130.1 (1C, C$_{m\text{-tolyl}}$), 130.2 (1C, C$_{m\text{-tolyl}}$), 130.3 (2C, C-2'''$_{p\text{-tolyl}}$, C-6'''$_{p\text{-tolyl}}$), 131.8 (1C, C-1'''$_{p\text{-tolyl}}$), 133.7 (1C, C$_{m\text{-tolyl}}$), 136.9 (1C, C-4'''$_{p\text{-tolyl}}$), 137.1 (1C, C-1''$_{m\text{-tolyl}}$), 138.4 (1C, C$_{m\text{-tolyl}}$), 144.6 (1C, C-3'$_{isothiazole}$), 152.1 (1C, C-4'$_{isothiazole}$), 162.2 (1C, ArCONCH), 163.1 (1C, CO$_2$H), 168.6 (1C, CHCONHCH);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3486, 3336, 3295, 3067, 2930, 2850, 1727, 1677, 1650, 1614, 1557, 1518, 1489, 1450, 1382, 1341, 1322, 1251, 1236, 1192, 1140, 1102, 1075, 1044, 978, 890, 875, 856, 815, 793, 762, 734, 706, 660, 632, 611, 578, 548, 535, 504;

HRMS (m/z): [M+H]$^+$ calcd for C$_{27}$H$_{31}$N$_4$O$_4$S: 507.2061, found: 507.2067;

HPLC (method 1): t_R = 25.5 min, purity 95.8%.

***N*-[2-(Cyclohexylamino)-2-oxo-1-(*p*-tolyl)ethyl]-*N*-(*m*-tolyl)isoxazole-5-carboxamide (89a)**



$C_{26}H_{29}N_3O_3$

$M_r = 431.5$

3-Methylaniline (0.22 mL, 210 mg, 2.0 mmol) and 4-methylbenzaldehyde (0.24 mL, 240 mg, 2.0 mmol) were dissolved in dichloromethane (15 mL) and the solution was stirred at ambient temperature for 10 min. Then, 1,2-oxazole-5-carboxylic acid (230 mg, 2.0 mmol) and isocyanocyclohexane (0.25 mL, 220 mg, 2.0 mmol) were added. After stirring the reaction mixture for 48 h at ambient temperature, the solvent was removed *in vacuo* and the residue was purified by flash column chromatography (Ø = 4 cm, h = 15 cm, V = 15 mL, dichloromethane/acetone = 20/1) to give **89a** (470 mg, 1.1 mmol, 54% yield) as colorless solid.

m.p. = 189 °C;

TLC: R_f = 0.28 (dichloromethane/acetone = 20/1);

^{1}H NMR (500 MHz, DMSO-d_6): δ [ppm] = 0.93 – 1.14 (m, 2H, NHCH(CH_2CH$_2$)$_2$CH$_2$, (1H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 1.14 – 1.32 (m, 3H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (1H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H)), 1.48 – 1.73 (m, 4H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (1H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 1.73 – 1.81 (m, 1H, NHCH(CH_2CH$_2$)$_2$CH$_2$), 2.10 (s br, 3H, ArCH_3), 2.17 (s, 3H, ArCH_3), 3.56 – 3.65 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), 5.59 (s br, 1H, 4'-H$_{isoxazole}$), 6.09 (s, 1H, NCHCO), 6.93 – 7.04 (m, 6H, 2'''-H$_{p\text{-tolyl}}$, 3'''-H$_{p\text{-tolyl}}$, 5'''-H$_{p\text{-tolyl}}$, 6'''-H$_{p\text{-tolyl}}$, H$_{m\text{-tolyl}}$ (2H)), 8.04 (d, J = 7.8 Hz, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), 8.41 (d, J = 1.9 Hz, 1H, 3'-H$_{isoxazole}$), the signals for 2 H$_{m\text{-tolyl}}$ cannot be observed in the spectrum;

^{13}C NMR (101 MHz, DMSO-d_6): δ [ppm] = 20.6 (2C, ArCH$_3$), 24.5 (1C, NHCH(CH$_2$$CH_2$)$_2CH_2$), 24.6 (1C, NHCH(CH$_2$$CH_2$)$_2CH_2$), 25.2 (1C, NHCH(CH$_2CH_2$)$_2$$CH_2$), 32.2 (2C, NHCH($CH_2CH_2$)$_2CH_2$), 48.0 (1C, NH$C$H(CH$_2CH_2$)$_2CH_2$), 64.3 (1C, N$C$HCO), 106.5

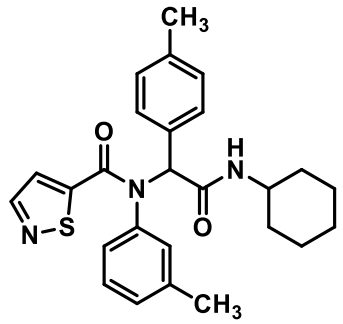
(1C, C-4'$_{isoxazole}$), 128.0 (2C, C$_{m\text{-tolyl}}$), 128.4 (2C, C-3'''$_{p\text{-tolyl}}$, C-5'''$_{p\text{-tolyl}}$), 128.9 (1C, C$_{m\text{-tolyl}}$), 130.3 (2C, C-2'''$_{p\text{-tolyl}}$, C-6'''$_{p\text{-tolyl}}$), 131.3 (1C, C$_{arom.}$), 131.4 (1C, C$_{arom.}$), 137.1 (1C, C-4'''$_{p\text{-tolyl}}$), 137.6 (1C, C-3''$_{m\text{-tolyl}}$), 138.3 (1C, C-1''$_{m\text{-tolyl}}$), 150.6 (1C, C-3'$_{isoxazole}$), 157.0 (1C, Ar*C*ONCH), 161.9 (1C, C-5'$_{isoxazole}$), 168.0 (1C, CH*C*ONHCH);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3291, 3075, 2929, 2850, 1649, 1605, 1587, 1566, 1555, 1518, 1488, 1467, 1450, 1384, 1357, 1324, 1308, 1251, 1238, 1200, 1191, 1152, 1105, 1047, 991, 978, 936, 918, 906, 890, 817, 805, 765, 752, 735, 708, 664, 647, 632, 624, 581, 549, 505, 459, 446, 426;

HRMS (*m*/*z*): [M+H]$^+$ calcd for $C_{26}H_{30}N_3O_3$: 432.2282, found: 432.2285;

HPLC (method 1): t_R = 24.7 min, purity 98.1%.

N-[2-(Cyclohexylamino)-2-oxo-1-(*p*-tolyl)ethyl]-*N*-(*m*-tolyl)isothiazole-5-carboxamide (89b)



$C_{26}H_{29}N_3O_2S$

M_r = 447.6

3-Methylaniline (0.22 mL, 210 mg, 2.0 mmol) and 4-methylbenzaldehyde (0.24 mL, 240 mg, 2.0 mmol) were dissolved in dichloromethane (15 mL) and the solution was stirred at ambient temperature for 10 min. Then, 1,2-thiazole-5-carboxylic acid (260 mg, 2.0 mmol) and isocyanocyclohexane (0.25 mL, 220 mg, 2.0 mmol) were added. After stirring the reaction mixture for 48 h at ambient temperature, the solvent was removed *in vacuo* and the residue was purified by flash column chromatography (Ø = 4 cm, h = 15 cm, V = 15 mL, dichloromethane/ethyl acetate = 15/1) to give **89b** (450 mg, 1.0 mmol, 50% yield) as colorless solid.

m.p. = 165 °C;

TLC: R_f = 0.32 (dichloromethane/ethyl acetate = 15/1);

^{1}H NMR (400 MHz, DMSO-d_6): δ [ppm] = 0.92 – 1.14 (m, 2H, NHCH(CH_2CH$_2$)$_2$CH$_2$, (1H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 1.14 – 1.33 (m, 3H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (1H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H)), 1.47 – 1.82 (m, 5H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 1.94 – 2.31 (m, 6H, ArCH_3), 3.54 – 3.66 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), 6.12 (s, 1H, NCHCO), 6.92 – 7.01 (m, 4H, 2'''-H$_{p\text{-tolyl}}$, 3'''-H$_{p\text{-tolyl}}$, 5'''-H$_{p\text{-tolyl}}$, 6'''-H$_{p\text{-tolyl}}$), 7.07 – 7.11 (m, 1H, 4''-H$_{m\text{-tolyl}}$), 7.13 (d, J = 1.8 Hz, 1H, 4'-H$_{\text{isothiazole}}$), 8.01 (d, J = 7.8 Hz, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), 8.35 (d, J = 1.8 Hz, 1H, 3'-H$_{\text{isothiazole}}$), the signals for 3 H$_{m\text{-tolyl}}$ cannot be observed in the spectrum;

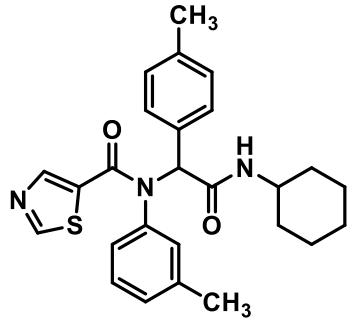
^{13}C NMR (126 MHz, DMSO-d_6): δ [ppm] = 20.6 (2C, ArCH$_3$), 24.4 (1C, NHCH(CH$_2$$CH_2$)$_2CH_2$), 24.6 (1C, NHCH(CH$_2$$CH_2$)$_2CH_2$), 25.1 (1C, NHCH(CH$_2CH_2$)$_2$$CH_2$), 32.1 (1C, NHCH($CH_2CH_2$)$_2CH_2$), 32.2 (1C, NHCH($CH_2CH_2$)$_2CH_2$), 48.0 (1C, NH$C$H(CH$_2CH_2$)$_2CH_2$), 64.5 (1C, N$C$HCO), 127.9 (1C, C-4'$_{\text{isothiazole}}$), 128.4 (2C, C-3'''$_{p\text{-tolyl}}$, C-5'''$_{p\text{-tolyl}}$), 128.5 (1C, C$_{m\text{-tolyl}}$), 129.0 (1C, C$_{m\text{-tolyl}}$), 129.7 (1C, C$_{m\text{-tolyl}}$), 130.3 (2C, C-2'''$_{p\text{-tolyl}}$, C-6'''$_{p\text{-tolyl}}$), 131.5 (1C, C-1'''$_{p\text{-tolyl}}$), 132.4 (1C, C$_{m\text{-tolyl}}$), 137.0 (1C, C-4'''$_{p\text{-tolyl}}$), 138.0 (1C, C-1''$_{m\text{-tolyl}}$), 138.1 (1C, C-3''$_{m\text{-tolyl}}$), 156.9 (1C, C-3'$_{\text{isothiazole}}$), 159.2 (1C, Ar$C$ONCH), 160.3 (1C, C-5'$_{\text{isothiazole}}$), 168.2 (1C, CH$C$ONHCH);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3465, 3293, 3016, 2970, 2930, 2850, 1739, 1648, 1631, 1604, 1587, 1552, 1518, 1490, 1448, 1371, 1365, 1261, 1251, 1230, 1217, 1207, 1107, 1093, 978, 891, 843, 835, 818, 810, 760, 741, 734, 708, 666, 640, 628, 587, 547, 527, 516, 504;

HRMS (m/z): [M+H]$^+$ calcd for C$_{26}$H$_{30}$N$_3$O$_2$S: 448.2053, found: 448.2019;

HPLC (method 1): t_R = 25.6 min, purity 98.9%.

***N*-[2-(Cyclohexylamino)-2-oxo-1-(*p*-tolyl)ethyl]-*N*-(*m*-tolyl)thiazole-5-carboxamide (89c)**



C$_{26}$H$_{29}$N$_3$O$_2$S

M$_r$ = 447.6

3-Methylaniline (0.22 mL, 210 mg, 2.0 mmol) and 4-methylbenzaldehyde (0.24 mL, 240 mg, 2.0 mmol) were dissolved in dichloromethane (15 mL) and the solution was stirred at ambient temperature for 10 min. Then, 1,3-thiazole-5-carboxylic acid (260 mg, 2.0 mmol) and isocyanocyclohexane (0.25 mL, 220 mg, 2.0 mmol) were added. After stirring the reaction mixture for 48 h at ambient temperature, the solvent was removed *in vacuo* and the residue was purified by flash column chromatography (Ø = 4 cm, h = 15 cm, V = 15 mL, dichloromethane/acetone = 10/1) to give **89c** (570 mg, 1.3 mmol, 64% yield) as colorless solid.

m.p. = 167 °C;

TLC: R_f = 0.21 (dichloromethane/acetone = 10/1);

^{1}H NMR (400 MHz, DMSO-*d*$_6$): δ [ppm] = 0.91 – 1.14 (m, 2H, NHCH(C*H*$_2$CH$_2$)$_2$CH$_2$, (1H), NHCH(CH$_2$CH$_2$)$_2$C*H*$_2$ (1H)), 1.14 – 1.33 (m, 3H, NHCH(C*H*$_2$CH$_2$)$_2$CH$_2$ (1H), NHCH(CH$_2$C*H*$_2$)$_2$CH$_2$ (2H)), 1.48 – 1.80 (m, 5H, NHCH(C*H*$_2$CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$C*H*$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH$_2$)$_2$C*H*$_2$ (1H)), 2.04 – 2.24 (m, 6H, ArC*H*$_3$), 3.53 – 3.65 (m, 1H, NHC*H*(CH$_2$CH$_2$)$_2$CH$_2$), 6.11 (s, 1H, NC*H*CO), 6.92 – 7.00 (m, 4H, 2'''-H$_{p\text{-tolyl}}$, 3'''-H$_{p\text{-tolyl}}$, 5'''-H$_{p\text{-tolyl}}$, 6'''-H$_{p\text{-tolyl}}$), 7.00 – 7.12 (m, 2H, H$_{m\text{-tolyl}}$), 7.25 (s, 1H, H$_{\text{thiazole}}$), 7.97 (d, *J* = 7.8 Hz, 1H, N*H*CH(CH$_2$CH$_2$)$_2$CH$_2$), 9.00 (s, 1H, H$_{\text{thiazole}}$), the signals for 2 H$_{m\text{-tolyl}}$ cannot be observed in the spectrum;

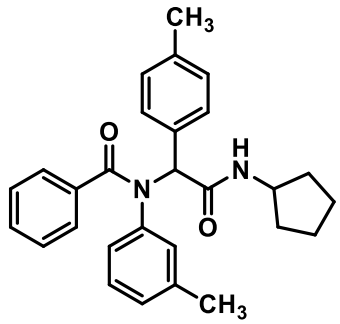
^{13}C NMR (101 MHz, DMSO-*d*$_6$): δ [ppm] = 20.6 (2C, Ar*C*H$_3$), 24.5 (1C, NHCH(CH$_2$*C*H$_2$)$_2$CH$_2$), 24.6 (1C, NHCH(CH$_2$*C*H$_2$)$_2$CH$_2$), 25.2 (1C, NHCH(CH$_2$CH$_2$)$_2$*C*H$_2$), 32.16 (1C, NHCH(*C*H$_2$CH$_2$)$_2$CH$_2$), 32.20 (1C, NHCH(*C*H$_2$CH$_2$)$_2$CH$_2$), 47.9 (1C, NH*C*H(CH$_2$CH$_2$)$_2$CH$_2$), 64.4 (1C, N*C*HCO), 128.3 (3C, C-3'''$_{p\text{-tolyl}}$, C-5'''$_{p\text{-tolyl}}$, 1 C$_{m\text{-tolyl}}$), 128.8 (1C, C$_{m\text{-tolyl}}$), 129.3 (1C, C$_{m\text{-tolyl}}$), 130.3 (2C, C-2'''$_{p\text{-tolyl}}$, C-6'''$_{p\text{-tolyl}}$), 131.7 (1C, C-1'''$_{p\text{-tolyl}}$), 132.2 (1C, C$_{m\text{-tolyl}}$), 133.7 (1C, C-5'$_{\text{thiazole}}$), 136.9 (1C, C-4'''$_{p\text{-tolyl}}$), 138.0 (1C, C-3''$_{m\text{-tolyl}}$), 138.7 (1C, C-1''$_{m\text{-tolyl}}$), 146.7 (1C, C$_{\text{thiazole}}$), 158.6 (1C, C$_{\text{thiazole}}$), 160.2 (1C, Ar*C*ONCH), 168.4 (1C, CH*C*ONHCH);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3293, 2925, 2850, 1649, 1621, 1601, 1548, 1497, 1385, 1240, 1188, 1111, 853, 815, 738, 708, 600, 541;

HRMS (*m*/*z*): [M+H]$^+$ calcd for $C_{26}H_{30}N_3O_2S$: 448.2053, found: 448.2001;

HPLC (method 1): t_R = 24.6 min, purity 98.7%.

***N*-[2-(Cyclopentylamino)-2-oxo-1-(*p*-tolyl)ethyl]-*N*-(*m*-tolyl)benzamide (89d)**



$C_{28}H_{30}N_2O_2$

$M_r = 426.6$

3-Methylaniline (0.22 mL, 210 mg, 2.0 mmol) and 4-methylbenzaldehyde (0.24 mL, 240 mg, 2.0 mmol) were dissolved in dichloromethane (15 mL) and the solution was stirred at ambient temperature for 10 min. Then, benzoic acid (240 mg, 2.0 mmol) and isocyanocyclopentane (0.22 mL, 190 mg, 2.0 mmol) were added. After stirring the reaction mixture for 48 h at ambient temperature, the solvent was removed *in vacuo* and the residue was purified by flash column chromatography (Ø = 4 cm, h = 15 cm, V = 15 mL, dichloromethane/acetone = 15/1) to give **59d** (390 mg, 0.92 mmol, 46% yield) as colorless solid.

m.p. = 199 °C;

TLC: R_f = 0.22 (dichloromethane/acetone = 15/1);

^{1}H NMR (400 MHz, DMSO-d_6): δ [ppm] = 1.22 – 1.34 (m, 1H, NHCH(CH_2CH$_2$)$_2$), 1.36 – 1.65 (m, 5H, NHCH(CH$_2$CH_2)$_2$, NHCH(CH_2CH$_2$)$_2$ (1H)), 1.72 – 1.86 (m, 2H, NHCH(CH_2CH$_2$)$_2$), 1.99 (s, 3H, C$H_{3,\ m\text{-tolyl}}$), 2.18 (s, 3H, C$H_{3,\ p\text{-tolyl}}$), 4.02 – 4.13 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$), 6.18 (s, 1H, NCHCO), 6.69 – 6.74 (m, 1H, H$_{m\text{-tolyl}}$), 6.74 – 6.83 (m, 2H, H$_{m\text{-tolyl}}$), 6.90 (s br, 1H, H$_{m\text{-tolyl}}$), 6.94 – 7.03 (m, 4H, 2'''-H$_{p\text{-tolyl}}$, 3'''-H$_{p\text{-tolyl}}$, 5'''-H$_{p\text{-tolyl}}$, 6'''-H$_{p\text{-tolyl}}$), 7.12 – 7.23 (m, 5H, 2'-H$_{\text{phenyl}}$, 3'-H$_{\text{phenyl}}$, 4'-H$_{\text{phenyl}}$, 5'-H$_{\text{phenyl}}$, 6'-H$_{\text{phenyl}}$), 8.04 (d, J = 7.1 Hz, 1H, CONHCH);

^{13}C NMR (101 MHz, DMSO-d_6): δ [ppm] = 20.6 (2C, ArCH$_3$), 23.48 (1C, NHCH(CH$_2$$CH_2$)$_2$), 23.51 (1C, NHCH(CH$_2$$CH_2$)$_2$), 31.8 (1C, NHCH(C$H_2CH_2$)$_2$), 32.2 (1C, NHCH(C$H_2CH_2$)$_2$), 50.6 (1C, NH$C$H(CH$_2CH_2$)$_2$), 63.9 (1C, N$C$HCO), 127.2 (1C, C$_{m\text{-tolyl}}$), 127.3 (1C, C$_{m\text{-tolyl}}$), 127.5 (2C, C$_{\text{phenyl}}$), 127.9 (2C, C$_{\text{phenyl}}$), 128.1 (1C, C$_{m\text{-tolyl}}$), 128.4 (2C, C-3'''$_{p\text{-tolyl}}$, C-5'''$_{p\text{-tolyl}}$), 128.9 (1C, C-4'$_{\text{phenyl}}$), 130.0 (2C, C-2'''$_{p\text{-tolyl}}$, C-6'''$_{p\text{-tolyl}}$), 131.4 (1C, C$_{m\text{-tolyl}}$), 132.5 (1C, C-1'''$_{p\text{-tolyl}}$),

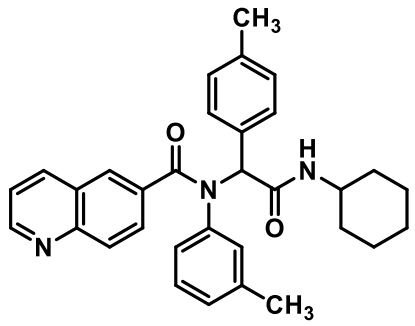
136.6 (1C, $C_{arom.}$), 136.7 (1C, $C_{arom.}$), 137.0 (1C, C-1'$_{phenyl}$), 140.2 (1C, C-1"$_{m\text{-}tolyl}$), 169.1 (1C, CH*C*ONH), 169.8 (1C, Ph*C*ONCH);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3299, 3058, 2957, 2867, 1650, 1605, 1576, 1551, 1516, 1489, 1445, 1361, 1331, 1309, 1247, 1189, 1143, 1114, 1090, 1077, 1030, 978, 925, 891, 815, 792, 767, 739, 707, 695, 658, 640, 632, 551, 521, 502, 476, 453;

HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{28}H_{31}N_2O_2$: 427.2380, found: 427.2391;

HPLC (method 1): t_R = 25.0 min, purity 96.6%.

***N*-[2-(cyclohexylamino)-2-oxo-1-(*p*-tolyl)ethyl]-*N*-(*m*-tolyl)quinoline-6-carboxamide (89e)**



$C_{32}H_{33}N_3O_2$

M_r = 491.6

3-Methylaniline (0.22 mL, 210 mg, 2.0 mmol) and 4-methylbenzaldehyde (0.24 mL, 240 mg, 2.0 mmol) were dissolved in dichloromethane (15 mL) and the solution was stirred at ambient temperature for 10 min. Then, quinoline-6-carboxylic acid (350 mg, 2.0 mmol) and isocyanocyclohexane (0.25 mL, 220 mg, 2.0 mmol) were added. After stirring the reaction mixture for 48 h at ambient temperature, the solvent was removed *in vacuo* and the residue was purified by flash column chromatography (Ø = 4 cm, h = 15 cm, V = 15 mL, dichloromethane/acetone = 10/1) to give **89e** (580 mg, 1.2 mmol, 59% yield) as colorless solid.

m.p. = 263 °C;

TLC: R_f = 0.27 (dichloromethane/acetone = 10/1);

^{1}H NMR (400 MHz, DMSO-d_6): δ [ppm] = 0.97 – 1.35 (m, 5H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 1.49 – 1.82 (m, 5H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 1.96 (s,

3H, C$H_{3,\ m\text{-tolyl}}$), 2.18 (s, 3H, C$H_{3,\ p\text{-tolyl}}$), 3.60 – 3.72 (m, 1H, NHCH(CH_2CH_2)$_2CH_2$), 6.25 (s, 1H, NCHCO), 6.65 – 6.70 (m, 1H, $H_{m\text{-tolyl}}$), 6.76 (t, J = 7.6 Hz, 1H, $H_{m\text{-tolyl}}$), 6.83 (s br, 1H, $H_{m\text{-tolyl}}$), 6.92 – 7.10 (m, 5H, 2'''-$H_{p\text{-tolyl}}$, 3'''-$H_{p\text{-tolyl}}$, 5'''-$H_{p\text{-tolyl}}$, 6'''-$H_{p\text{-tolyl}}$, $H_{m\text{-tolyl}}$ (1H)), 7.48 (dd, J = 8.3/4.2 Hz, 1H, 3'-$H_{quinoline}$), 7.55 (dd, J = 8.8/1.8 Hz, 1H, 7'-$H_{quinoline}$), 7.76 (d, J = 8.8 Hz, 1H, 8'-$H_{quinoline}$), 7.88 (d, J = 1.7 Hz, 1H, 5'-$H_{quinoline}$), 8.00 (d, J = 7.7 Hz, 1H, CONHCH), 8.23 – 8.28 (m, 1H, 4'-$H_{quinoline}$), 8.85 (dd, J = 4.2/1.7 Hz, 1H, 2'-$H_{quinoline}$);

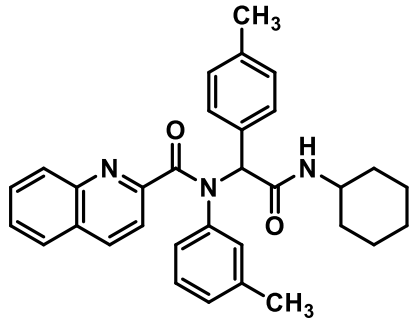
^{13}C NMR (101 MHz, DMSO-d_6): δ [ppm] = 20.5 (1C, ArCH$_3$), 20.6 (1C, ArCH$_3$), 24.5 (1C, NHCH(CH_2CH_2)$_2CH_2$), 24.6 (1C, NHCH(CH_2CH_2)$_2CH_2$), 25.2 (1C, NHCH(CH_2CH_2)$_2CH_2$), 32.17 (1C, NHCH(CH_2CH_2)$_2CH_2$), 32.25 (1C, NHCH(CH_2CH_2)$_2CH_2$), 48.0 (1C, NHCH(CH_2CH_2)$_2CH_2$), 64.1 (1C, NCHCO), 121.9 (1C, C-3'$_{quinoline}$), 126.8 (1C, C-4a'$_{quinoline}$), 127.3 (1C, C$_{m\text{-tolyl}}$), 127.4 (1C, C$_{m\text{-tolyl}}$), 128.00 (1C, C$_{quinoline}$), 128.05 (1C, C$_{quinoline}$), 128.2 (1C, C$_{m\text{-tolyl}}$), 128.4 (2C, C-3'''$_{p\text{-tolyl}}$, C-5'''$_{p\text{-tolyl}}$), 128.5 (1C, C-7'$_{quinoline}$), 130.1 (2C, C-2'''$_{p\text{-tolyl}}$, C-6'''$_{p\text{-tolyl}}$), 131.6 (1C, C$_{m\text{-tolyl}}$), 132.4 (1C, C-1'''$_{p\text{-tolyl}}$), 135.0 (1C, C-6'$_{quinoline}$), 136.4 (1C, C-4'$_{quinoline}$), 136.8 (2C, C-3''$_{m\text{-tolyl}}$, C-4'''$_{p\text{-tolyl}}$), 140.0 (1C, C-1''$_{m\text{-tolyl}}$), 147.1 (1C, C-8a'$_{quinoline}$), 151.4 (1C, C-2'$_{quinoline}$), 168.6 (1C, CHCONH), 169.3 (1C, ArCONCH);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3279, 3060, 2925, 2852, 1645, 1605, 1587, 1555, 1516, 1488, 1453, 1429, 1364, 1347, 1306, 1251, 1238, 1187, 1151, 1127, 1103, 1059, 1030, 978, 892, 843, 821, 804, 779, 758, 742, 708, 649, 634, 620, 601, 592, 575, 550, 521, 504, 478, 454, 421;

HRMS (m/z): [M+H]$^+$ calcd for $C_{32}H_{34}N_3O_2$: 492.2646, found: 492.2643;

HPLC (method 1): t_R = 21.9 min, purity 98.8%.

***N*-[2-(cyclohexylamino)-2-oxo-1-(*p*-tolyl)ethyl]-*N*-(*m*-tolyl)quinoline-2-carboxamide (89f)**



$C_{32}H_{33}N_3O_2$

M_r = 491.6

3-Methylaniline (0.22 mL, 210 mg, 2.0 mmol) and 4-methylbenzaldehyde (0.24 mL, 240 mg, 2.0 mmol) were dissolved in dichloromethane (15 mL) and the solution was stirred at ambient temperature for 10 min. Then, quinoline-2-carboxylic acid (350 mg, 2.0 mmol) and isocyanocyclohexane (0.25 mL, 220 mg, 2.0 mmol) were added. After stirring the reaction mixture for 48 h at ambient temperature, the solvent was removed *in vacuo* and the residue was purified by flash column chromatography (Ø = 4 cm, h = 15 cm, V = 15 mL, dichloromethane/acetone = 10/1) to give **89f** (590 mg, 1.2 mmol, 60% yield) as colorless solid.

m.p. = 296 °C;

TLC: R_f = 0.22 (dichloromethane/acetone = 10/1);

^{1}H NMR (400 MHz, DMSO-d_6): δ [ppm] = 0.96 – 1.35 (m, 5H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 1.48 – 1.84 (m, 5H, NHCH(CH_2CH$_2$)$_2$CH$_2$ (2H), NHCH(CH$_2$CH_2)$_2$CH$_2$ (2H), NHCH(CH$_2$CH$_2$)$_2$CH_2 (1H)), 1.93 (s, 3H, C$H_{3,\ m\text{-tolyl}}$), 2.18 (s, 3H, C$H_{3,\ p\text{-tolyl}}$), 3.57 – 3.73 (m, 1H, NHCH(CH$_2$CH$_2$)$_2$CH$_2$), 6.25 (s, 1H, NCHCO), 6.57 – 6.66 (m, 1H, H$_{m\text{-tolyl}}$), 6.66 – 6.75 (m, 1H, H$_{m\text{-tolyl}}$), 6.84 (s br, 1H, H$_{m\text{-tolyl}}$), 6.95 – 7.10 (m, 5H, 2'''-H$_{p\text{-tolyl}}$, 3'''-H$_{p\text{-tolyl}}$, 5'''-H$_{p\text{-tolyl}}$, 6'''-H$_{p\text{-tolyl}}$, H$_{m\text{-tolyl}}$ (1H)), 7.41 (d, J = 8.4 Hz, 1H, 3'-H$_{\text{quinoline}}$), 7.52 – 7.59 (m, 1H, 6'-H$_{\text{quinoline}}$), 7.66 – 7.74 (m, 1H, 7'-H$_{\text{quinoline}}$), 7.78 – 7.89 (m, 2H, 5'-H$_{\text{quinoline}}$, 8'-H$_{\text{quinoline}}$), 8.02 (s br, 1H, CONH), 8.20 (d, J = 8.2 Hz, 1H, 4'-H$_{\text{quinoline}}$);

^{13}C NMR (101 MHz, DMSO-d_6): δ [ppm] = 20.5 (1C, ArCH$_3$), 20.6 (1C, ArCH$_3$), 24.5 (1C, NHCH(CH$_2$$CH_2$)$_2CH_2$), 24.6 (1C, NHCH(CH$_2$$CH_2$)$_2CH_2$), 25.2 (1C, NHCH(CH$_2CH_2$)$_2$$CH_2$), 32.15 (1C, NHCH($CH_2CH_2$)$_2CH_2$), 32.22 (1C, NHCH($CH_2CH_2$)$_2CH_2$), 48.0 (1C, NH$C$H(CH$_2CH_2$)$_2CH_2$), 63.6 (1C, N$C$HCO), 120.0 (1C, C-3'$_{\text{quinoline}}$), 126.8 (1C, C-4a'$_{\text{quinoline}}$), 127.0 (1C, C$_{m\text{-tolyl}}$), 127.2 (1C, C-6'$_{\text{quinoline}}$), 127.3 (1C, C$_{m\text{-tolyl}}$), 127.8 (1C, C-5'$_{\text{quinoline}}$), 128.2 (1C, C$_{m\text{-tolyl}}$), 128.4 (2C, C-3'''$_{p\text{-tolyl}}$, C-5'''$_{p\text{-tolyl}}$), 128.7 (1C, C-8'$_{\text{quinoline}}$), 129.9 (1C, C-7'$_{\text{quinoline}}$), 130.0 (2C, C-2'''$_{p\text{-tolyl}}$, C-6'''$_{p\text{-tolyl}}$), 131.8 (1C, C$_{m\text{-tolyl}}$), 132.1 (1C, C-1'''$_{p\text{-tolyl}}$), 136.1 (1C, C-4'$_{\text{quinoline}}$), 136.3 (1C, C-3''$_{m\text{-tolyl}}$), 136.8 (1C, C-4'''$_{p\text{-tolyl}}$), 139.1 (1C, C-1''$_{m\text{-tolyl}}$), 146.1 (1C, C-8a'$_{\text{quinoline}}$), 155.0 (1C, C-2'$_{\text{quinoline}}$), 168.2 (1C, CON), 168.4 (1C, CON);

IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3292, 3064, 2928, 2851, 1641, 1606, 1588, 1547, 1517, 1501, 1488, 1454, 1427, 1385, 1374, 1363, 1336, 1315, 1299, 1252, 1236, 1209, 1190, 1168, 1142, 1110, 1089, 1039, 991, 970, 934, 889, 874, 838, 818, 801, 776, 765, 733, 706, 677, 642, 631, 620, 589, 571, 549, 498, 477, 460;

HRMS (m/z): [M+H]$^+$ calcd for C$_{32}$H$_{34}$N$_3$O$_2$: 492.2646, found: 492.2648;

HPLC (method 1): t_R = 25.5 min, purity 99.3%.

5.3 Biological evaluation

5.3.1 Culture of cells and viruses

Cell-culture assays were performed with Madin Darby canine kidney (MDCK) cells (Friedrich Löffler Institute, Riems, Germany), with Eagle's minimal essential medium (EMEM) supplemented with 2 µg/ml trypsin, 2 mM L-glutamine, 1% nonessential amino acids, 100 U/mL penicillin, and 100 U/mL streptomycin.

Influenza viruses A/WSN/33 (WSN33; subtype H1N1[112]), A/Jena/8178/09 (8178/98; subtype A(H1N1)pdm09[111]), A/Hong Kong/1/33 (HK/33; subtype H3N2; Schaper and Brümmer GmbH & Co. KG, Salzgitter, Germany), A/1519-19 (subtype A(H1N1)pdm09[114]), and A/2588-19 (subtype H3N2[114]) were propagated and titrated in MDCK cells. Aliquots were stored at -80 °C.

5.3.2 Cytotoxicity

Cytotoxicity of synthesized compounds were analyzed in confluent MDCK cell monolayers as published with some modifications[113].

MDCK cells were seeded at 3×10^5 cells/mLwell in 96-well plates for 48 h. After removal of the growth medium, serial half-log dilutions of compound in test medium were added. The cells were continually incubated for 3 days at 37 °C under a 5% CO_2 atmosphere. Then, the supernatant was aspirated and the cell monolayers were washed three times with physiological sodium chloride solution to remove death cells. And the cells were fixed and stained in one step with 0.03% crystal violet (w/v) in 20% methanol for 10 min. After six further automated washings with water, the stained monolayers were treated for 20 min with lysis buffer (0.8979 g of sodium citrate and 1.25 ml of 1 N HCl in 98.05 ml 47.5% ethanol) to elute the crystal violet. The absorbance was measured at a wavelength of 550/630 nm. The 50% cytotoxic concentrations (CC_{50}) were calculated from the mean dose–response curves of three assays each with two parallels.

5.3.3 Cytopathic effect inhibitory assays

Cytopathic effect was performed as previously described with some modification[113].

The cytopathic effect (CPE) inhibitory assay was carried out in 2-day-old confluent monolayers of MDCK cells in 96-well plates. After the aspiration of the cell growth medium, 50 µL of drug solution diluted in the test medium (the same concentrations as used in the cytotoxicity assay) and a constant multiplicity of infection (MOI) of the respective test virus in a volume of 50 mL

of the appropriate test medium were added to the cell monolayers. Six wells of noninfected and six wells of infected cells without the test compound served as cell and virus control, respectively, on each plate. To calibrate the assay, the 50 and 100% plaque inhibitory concentrations of reference compounds (each three wells) were included as positive control in each microtiter plate. Plates were incubated at 37 °C in a humidified atmosphere with 5% CO_2. The development of cytopathic effect was monitored by light microscopy. Scoring the inhibition of the virus-induced CPE, untreated infected control showed maximum cytopathic effect and the reference compound-treated wells a 50 or 100% protection. At this time point, the cell monolayers were fixed and stained with a 0.03% crystal violet solution in 2% ethanol and 3% formalin in water. The percentage of antiviral activities of the test compounds were calculated using the following equation:

antiviral activity = [(mean optical density of six cell controls−mean optical density of six virus controls) / (optical density of test−mean optical density of six virus controls)] × 100%.

Based on these results, the 50% cytopathic effect inhibitory concentration (IC_{50}) was calculated.

5.3.4 Plaque reductions assay

Plaque reduction assays were performed in MDCK cells as described previously[113]. Briefly, after removal of the cell growth medium, confluent 2-day-old cell monolayer in 12-well plates (Greiner AG, Kremsmünster, Austria) were inoculated with 0.5 ml of the respective virus suspension in test medium in the absence or presence of serial half-log dilutions (0.316, 1, 3.16, and 10 µg/mL) of the test compounds. After adsorption at 37 °C for 1 h, the inoculum was aspirated and 1 ml of the respective test medium containing 0.4% agar with or without the appropriate concentrations of the drugs were added. Three untreated virus controls and one uninfected untreated cell control were included in all assays. Each compound concentration was tested in duplicate. The tests were incubated at 37 °C for 48 h until plaques appeared and then fixed and stained with a solution of 0.4% crystal violet in a mixture of formalin (3% v/v) and ethanol (1.67% v/v) in water overnight. Plaques were counted over a light box after removal of the agar overlay. The average plaque count from compound treated wells at each concentration was plotted against the average plaque count of three untreated virus infected wells. The concentration required to reduce the plaque number by 50% (IC_{50}) was calculated from the mean dose–response curves of at least three plaque reduction assays.

5.3.5 Evaluation of binding affinity *via* GCI

The binding kinetics were performed on a Creoptix®WAVEsystem apparatus using a PCH-NTA chip. The GCI kinetic measurements were performed in 20 mM HEPES buffer (pH 7.0), supplemented with 150 mM NaCl, 2 mM TCEP, 0.2% DMSO, and 0.005% Tween.

Nucleozin, previously reported to bind to NP, was used as a positive control. Purified NP was diluted to 100 μg/mL with buffer and immobilized on an activated PCH-NTA chip with EDCI/NHS. A total of 7000 response units (RU) of NP were immobilized over 7 minutes. Subsequently, any remaining active sites were neutralized with 1 M ethanolamine. Compounds were dissolved in running buffer at concentrations that allowed total solubilization. Different concentrations of the compounds and nucleozin were injected. Dilutions were performed as to reach same DMSO concentration as the running buffer. The analysis protocol included 10 startup measurements, a blank measurement between each run, and three DMSO corrections (at the beginning, middle, and end), utilizing samples at five two-fold dilutions (10 μM, 5 μM, 2.5 μM, 1.25 μM, 0.625 μM). Each sample was analyzed in ascending order of concentration and then in the reverse order. The K_D values were determined using a steady-state affinity model provided by the Creoptix®WAVEsystem analysis software.

6.0 APPENDIX

6.1 Hazards and precautionary statements

Compound	GHS code	Hazard (H) statement	Precautionary (P) statement
(*R*)-(+)-1-(4-Methoxyphenyl)ethylamine	05, 07	H302, H314, H317, H412	P261, P273, P280, P301+P312, P303+P361+P353, P305+P351+P338
(*S*)-(-)-1-(4-Methoxyphenyl)ethylamine	05	H314	P280, P305+P351+P338, P310
1,1'-Carbonyldiimidazole	05, 07, 08	H302, H314, H360D	P260, P280, P301+P312, P303+P361+P353, P304+P340+P310, P305+P351+P338
1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide	06, 08, 09	H302, H311, H315, H317, H373, H410	P260, P273, P280, P301+P312, P302+P352+P312, P314
1-Hydroxybenzotriazole	01, 07	H203, H319, H412	P210
1-Isocyanobutane	02, 07	H225, H302+H312+H332	P210, P233, P280, P301+P312, P303+P361+P353, P304+P340+P312
2-(Trifluoromethyl)aniline	07, 08, 09	H302, H315, H317, H319, H373, H411	P280, P301+P330+P331, P312, P302+P352, P333+P313, P337+P313
2-Chloroacetyl chloride	06, 08, 05, 09	H290, H301, H311, H331, H372, H314, H400	P280, P301+P330+P331, P304+P340, P305+P351+P338, P310, P303+P361+P353
2-Chlorobenzaldehyde	05	H314	P280, P305+P351+P338, P310

Compound	GHS code	Hazard (H) statement	Precautionary (P) statement
2-Isocyano-2-methylpropane	02, 06	H225, H330	P210, P304+P340+P310
2-Methoxybenzaldehyde	07	H315, H319, H335	P261, P264, P271, P280, P302+P352, P305+P351+P338
2-Methoxyethylamine	02, 05, 07	H225, H302, H332, H302+H332, H314	P210, P280, P301+P312+P330, P303+P361+P353, P304+P340+P312, P305+P351+P338+P310
2-Methylaniline	06, 08, 09	H301, H331, H301+H331, H319, H350, H410	P201, P202, P273, P301+P310, P304+P340+P311, P305+P351+P338
2-Methylbenzaldehyde	07	H302, H315, H319, H335	P280, P301+P330+P331, P312, P302+P352, P304+P340, P337+P313
2-Sulfanylacetic acid	06, 05	H301, H311, H331, H301+H311+H331, H314	P280, P301+P330+P331, P302+P352, P304+P340, P305+P351+P338, P308+P310
3-(Trifluoromethyl)aniline	06, 08, 05, 09	H302, H312, H330, H315, H318, H335, H373, H411	P280, P301+P330+P331, P302+P352, P304+P340, P305+P351+P338, P332+P313, P310
3,4-Dichloroaniline	06, 05, 09	H301, H311, H331, H301+H311+H331, H317, H318, H410	P273, P280, P301+P310+P330, P302+P352+P312, P304+P340+P311, P305+P351+P338+P310

Compound	GHS code	Hazard (H) statement	Precautionary (P) statement
3,4-Dimethylaniline	06, 08, 09	H301, H311, H331, H301+H311+H331, H373, H411	P273, P280, P301+P310+P330, P302+P352+P312, P304+P340+P311, P314
3,5-Dichloroaniline	06, 08, 09	H301, H311, H331, H373, H410	P273, P280, P301+P310+P330, P302+P352+P312, P314
3,5-Dimethylaniline	06, 08, 09	H301, H311, H331, H301+H311+H331, H373, H411	P260, P280, P301+P330+P331+P310, P302+P352+P312, P304+P340+P311, P403+P233
3,5-Dimethylbenzaldehyde	07	H227, H315, H319, H335	P305+P351+P338
3-Aminobenzonitrile	07	H302, H312, H332, H302+H312+H332, H317	P280
3-Chloroaniline	06, 08, 09	H301, H311, H331, H301+H311+H331, H373, H410	P273, P280, P301+P310+P330, P302+P352+P312, P304+P340+P311, P314
3-Chlorobenzaldehyde	07	H315, H319, H335	P261, P264, P271, P280, P302+P352, P305+P351+P338
3-Ethylaniline	06, 08	H301, H311, H331, H301+H311+H331, H373	P260, P280, P301+P310, P302+P352, P304+P340, P312
3-Fluoroaniline	05, 07	H302, H315, H318, H335	P261, P280, P305+P351+P338
3-Iodotoluene	07	H315, H319, H335	P261, P264, P271, P280, P302+P352, P305+P351+P338

Compound	GHS code	Hazard (H) statement	Precautionary (P) statement
3-Methoxyaniline	06, 08, 09	H311, H302, H332, H302+H332, H373, H410	P280, P301+P330+P331, P312, P302+P352, P304+P340
3-Methoxybenzaldehyde	07	H315, H319, H335	P261, P264, P271, P280, P302+P352, P305+P351+P338
3-Methylaniline	06, 08, 09	H301, H311, H331, H301+H311+H331, H373, H410	P273, P280, P301+P310+P330, P302+P352+P312, P304+P340+P311, P314
3-Methylbenzaldehyde	07	H227, H302, H315, H319, H335	Not a dangerous substance according to GHS.
4-(Dimethylamino)benzaldehyde	07	H317	P210e, P261, P280a, P305+P351+P338, P405, P501a
4-(Trifluoromethoxy)benzaldehyde	07	H315, H319, H335, H227	P210e, P280a, P405, P501a, P261, P305+P351+P338
4-(Trifluoromethyl)benzaldehyde	07	H302, H315, H319	P264, P270, P280, P301+P312, P302+P352, P305+P351+P338
4-Bromobenzaldehyde	07	H302, H315, H317, H319, H335	P261, P264, P280, P301+P312, P302+P352, P305+P351+P338
4-Chloroaniline	06, 08, 09	H301, H311, H317, H331, H350, H410	P201, P280, P302+P352, P304+P340, P308+P313, P273
4-Chlorobenzaldehyde	07, 09	H302, H315, H317, H319, H411	P261, P273, P280, P301+P312, P302+P352, P305+P351+P338

Compound	GHS code	Hazard (H) statement	Precautionary (P) statement
4-Chlorobenzaldehyde	07, 09	H302, H315, H317, H319, H411	P261, P273, P280, P301+P312, P302+P352, P305+P351+P338
4-Cyanobenzaldehyde	07	H302+H312+H332	P261, P264, P280, P301+P312, P302+P352+P312, P304+P340+P312
4-Dimethylaminopyridine	06, 08, 05, 09	H301, H331, H301+H331, H310, H315, H318, H370, H370, H411	P262, P273, P280, P301+P310, P302+P352+P312, P305+P351+P338
4-Ethylbenzaldehyde	07	H227, H315, H319	P280g, P305+P351+P338, P210, P280, P370+P378, P403+P235, P501
4-Fluoroaniline	05, 08, 07, 09	H302, H314, H373, H410	P280, P301+P330+P331, P305+P351+P338, P310, P303+P361+P353
4-Fluorobenzaldehyde	02, 07	H226, H315, H319, H335	P210, P233, P240, P241, P303+P361+P353, P305+P351+P338
4-Hydroxybenzaldehyde	05	H318	P280, P305+P351+P338
4-Methoxyaniline	06, 08, 09	H300, H310, H330, H300+H310+H330, H373, H400	P260, P284, P280, P301+P310+P330, P302+P352+P310, P361+P364, P304+P340+P310
4-Methoxybenzaldehyde	02, 05, 07, 08	H412	P273, P501

Compound	GHS code	Hazard (H) statement	Precautionary (P) statement
4-Methylaniline	06, 08, 09	H301, H311, H331, H301+H311+H331, H317, H319, H351, H410	P273, P280, P301+P310, P302+P352+P312, P304+P340+P311, P305+P351+P338
4-Methylbenzaldehyde	07	H302, H315, H319	P264, P280, P301+P312, P302+P352, P305+P351+P338, P332+P313
4-Methylbenzenesulfonyl chloride	05, 07	H290, H315, H317, H318	P234, P261, P264, P280, P302+P352, P305+P351+P338
4-Nitroaniline	06, 08	H301, H311, H331, H373, H412	P273, P280, P304+P340, P302+P352, P308+P310
Acetaldehyde	02, 08, 07	H224, H319, H335, H341, H350	P202, P210, P233, P305+P351+P338, P308+P313, P403+P233
Acetic acid	02, 05	H226, H314	P210, P280, P301+P330+P331, P303+P361+P353, P305+P351+P338
Acetone	02, 07	H225, H319, H336	P210, P233, P240, P241, P242, P305+P351+P338
Acetone-d_6	02, 07	H225, H319, H336	P210, P233, P240, P241, P242, P305+P351+P338
Acetonitrile	02, 07	H225, H302, H312, H332, H302+H312+H332, H319	P210, P280, P301+P312, P303+P361+P353, P304+P340+P312, P305+P351+P338

Compound	GHS code	Hazard (H) statement	Precautionary (P) statement
Ammonium Hydroxide	05, 07, 09	H314, H335, H410	P261, P271, P273, P280, P303+P361+P353, P305+P351+P338
Aniline	06, 08, 05, 09	H301, H311, H331, H317, H318, H341, H351, H372, H372, H410	P273, P280, P301+P310, P302+P352+P312, P304+P340+P311, P305+P351+P338
Benzaldehyde	07	H302, H332, H319, H335	P280, P301+P310
Benzhydrylamine	07	H302, H315, H319, H335	P261, P264, P270, P301+P312-P302+P352, P305+P351+P338
Benzyl isocyanide	07	H302+H312+H332	P261, P264, P280, P301+P312, P302+P352+P312, P304+P340+P312
Benzylamine	05, 07	H302, H312, H314	P280, P301+P330+P331, P303+P361+P353, P305+P351+P338, P310
Caesium carbonate	05, 08	H318, H361f, H373	P201, P202, P260, P280, P305+P351+P338, P308+P313
COMU	07	H315, H319, H335	P261, P280a, P304+P340, P305+P351+P338, P405, P501a
Copper(I) iodide	05, 07, 09	H302, H315, H317, H318, H410	P273, P280, P301+P312+P330, P302+P352, P305+P351+P338+P310
Cuminaldehyde	07	H302	P264, P270, P301+P312, P501

Compound	GHS code	Hazard (H) statement	Precautionary (P) statement
Cyanoacetamide	07	H302, H315, H319, H335	P261, P264, P270, P301+P312, P302+P352, P305+P351+P338
Cyclobutylamine	02, 05	H225, H314	P210, P233, P240, P280, P303+P361+P353, P305+P351+P338
Cyclohexanecarboxaldehyde	02, 07	H226, H315, H319, H335	P210, P302+P352, P305+P351+P338
Cyclohexylamine	02, 06, 05, 08	H226, H301, H311, H314, H361	P210, P280, P301+P330+P331, P303+P361+P353, P305+P351+P338, P310
Cyclopentylamine	02, 06, 05	H225, H300, H331, H314, H317, H412	P210, P280, P301+P330+P331, P303+P361+P353, P304+P340+P310, P305+P351+P338
Cyclopropylamine	02, 05, 07	H225, H302, H314	P210, P233, P280, P301+P312, P303+P361+P353, P305+P351+P338
Dichloromethane	08, 07	H315, H319, H336, H351	P201, P302+P352, P305+P351+P338, P308+P313
Dicyclohexylcarbodiimide	06, 05	H302, H311, H317, H318	P280, P301+P312+P330, P302+P352+P312, P305+P351+P338+P310
Diisopropylethylamine	02, 06, 05	H225, H302, H331, H318, H335	P210, P280, P301+P312+P330, P304+P340+P311, P305+P351+P338+P310

Compound	GHS code	Hazard (H) statement	Precautionary (P) statement
Dimethyl sulfoxide	08	H227, H320, H373	P210e, P280a, P370+P378a, P403+P235, P501a, P260, P264, P305+P351+P338+P337+P313, P314-P501
Dimethyl sulfoxide-d_6	07	H227	P210e, P280a, P370+P378a, P403+P235, P501a
Ethanol	02, 07	H225, H319	P210, P233, P240, P241, P242, P305+P351+P338
Ethyl 2-sulfanylacetate	06	H301, H315, H317, H319, H335	P261, P264, P280, P301+P310, P302+P352, P305+P351+P338
Ethyl Acetate	02, 07	H225, H319, H336	P210, P233, P240, P305+P351+P338, P403+P235
Ethyl cyanoglyoxylate-2-oxime	06	H301	P264, P270, P301+P310a, P321, P405, P501a
Hydrochloric acid	04, 05, 06	H280, H331, H314	P260, P280, P303+P361+P353+P315, P304+P340+P315, P305+P351+P338+P315, P403, P405
Hydrogen peroxide	03, 05, 07	H271, H302, H332, H302+H332, H314, H335	P220, P261, P280, P302+P352+P310, P305+P351+P338, P312
Iodobenzene	07	H302	P264, P270, P301+P312, P501

Compound	GHS code	Hazard (H) statement	Precautionary (P) statement
Isocyanobenzene	06	H331, H301, H311	P280, P302+P352, P312, P322, P361, P363, P405, P501, P261, P271, P304+P340, P311, P321, P403+P233, P405, P501, P264, P270, P301+P310, P321, P330, P405, P501
Isocyanocyclohexane	06	H301+H311+H331	P261, P264, P280, P301+P310, P302+P352+P312, P304+P340+P311
Isocyanocyclopentane	07	H319, H302+H312+H332, H315	P264, P280, P305+P351+P338, P337+P313P, P264, P280, P302+P352, P321, P332+P313, P362
Lithium hydroxide	07, 05	H302, H314	P280, P305+P351+P338, P303+P361+P353, P301+P330+P331, P310
Methanol	02, 06, 08	H225, H301, H311, H331, H301+H311+H331, H370	P210, P233, P280, P301+P310, P303+P361+P353, P304+P340+P311
Methyl 4-formylbenzoate	07	H315, H319	P264, P280, P302+P352+P332+P313+P362+P364-P305+P351+P338+P337+P313
Methylamine	02, 04, 06, 05	H220, H280, H331, H315, H318, H335	P210, P280, P304+P340+P311, P305+P351+P338, P403+P233, P410

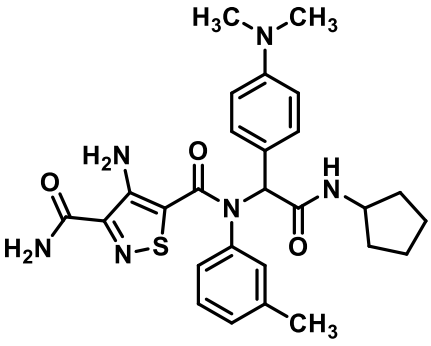
Compound	GHS code	Hazard (H) statement	Precautionary (P) statement
Morpholine	02, 05, 06, 08	H226, H302, H311, H314, H331, H361	P210, P280, P301+P312, P303+P361+P353, P304+P340+P310, P305+P351+P338
N,N'-Diisopropylcarbodiimide	02, 05, 06, 08	H226, H317, H318, H330, H334	P210, P233, P280, P303+P361+P353, P304+P340+P310, P305+P351+P338
N,N'-Dimethylformamide	02, 08, 07	H226, H312, H332, H312+H332, H319, H360	P201, P210, P302+P352, P305+P351+P338, P308+P313
Piperidine	02, 06, 05	H225, H302, H311, H331, H311+H331, H314	P210, P280, P301+P312, P303+P361+P353, P304+P340+P310, P305+P351+P338
Potassium carbonate	07	H315, H319, H335	P261, P264, P271, P280, P302+P352, P305+P351+P338
Potassium cyanide	06, 08, 05, 09	H290, H300, H310, H330, H300+H310+H330, H372, H372, H410	P262, P273, P280, P302+P352+P310, P304+P340+P310, P314
Sodium hydroxide	05	H290, H314	P280, P301+P330+P331, P305+P351+P338, P308+P310
Sodium nitrite	03, 06, 09	H272, H301, H319, H400	P210, P220, P264, P273, P301+P310, P305+P351+P338

Compound	GHS code	Hazard (H) statement	Precautionary (P) statement
Sodium Sulfite	07	H315, H319, H335	P233, P260, P261, P264, P271, P280, P302+P352, P304, P304+P340, P305+P351+P338, P312, P321, P332+P313, P337+P313, P340, P362, P403, P403+P233, P405, P501
Tetrahydrofuran	02, 07, 08	H225, H302, H319, H335, H336, H351	P210, P280, P301+P312+P330, P305+P351+P338, P370+P378, P403+P235
Thionyl dichloride	06, 05	H302, H331, H314, H335	P280, P301+P330+P331, P304+P340, P305+P351+P338, P308+P310
Thiourea	08, 07, 09	H302, H351, H361, H411	P201, P280, P301+P310, P304+P340, P310, P273
Triethylamine	02, 06, 05	H225, H302, H311, H331, H311+H331, H314, H335	P210, P280, P301+P312, P303+P361+P353, P304+P340+P311, P305+P351+P338+P310

Legend:

GHS code	Pictogram	GHS code	Pictogram
01	 Explosive	06	 Corrosive
02	 Flammable	07	 Harmful
03	 Oxidizing	08	 Health hazard
04	 Compressed Gas	09	 Environmental hazard
05	 Corrosive		

6.2 Compound index



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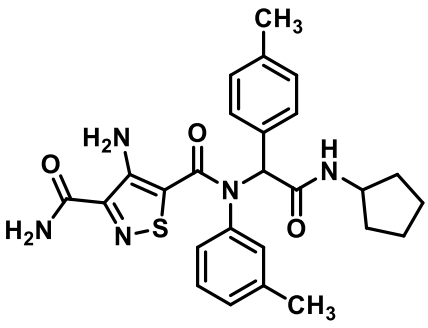

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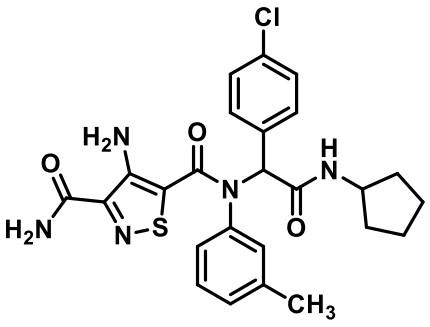

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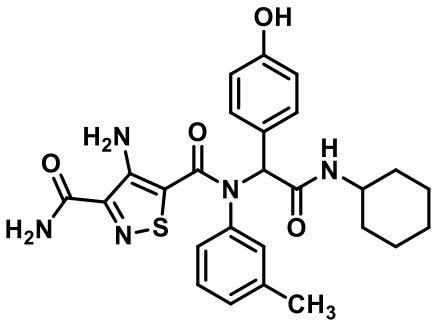

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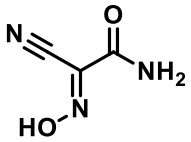

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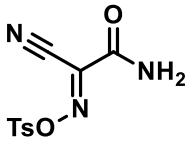

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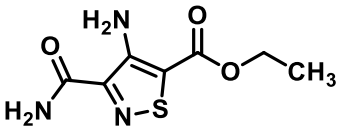

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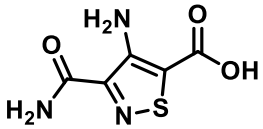

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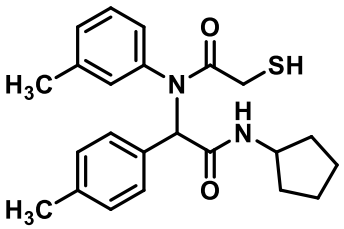

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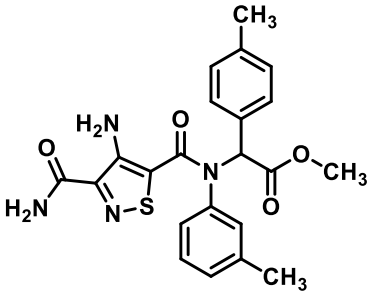

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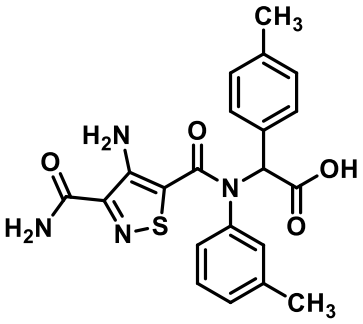

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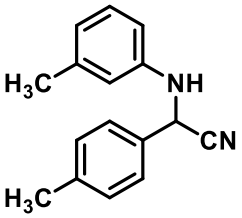

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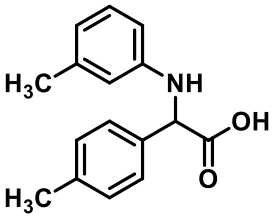

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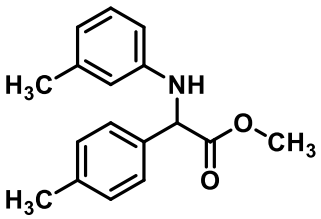

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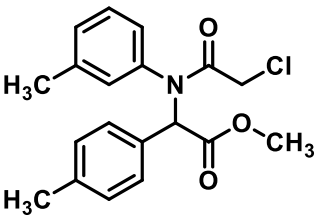

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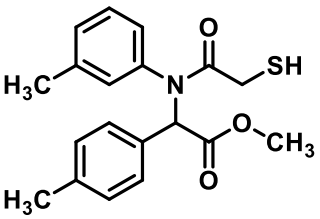

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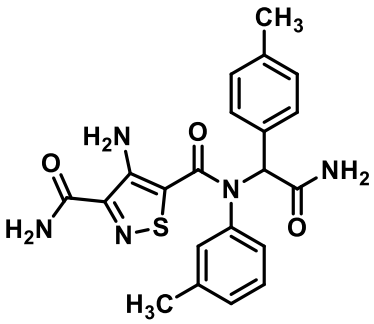

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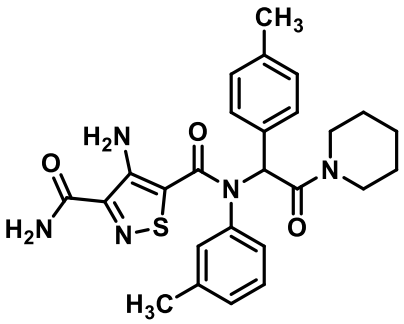

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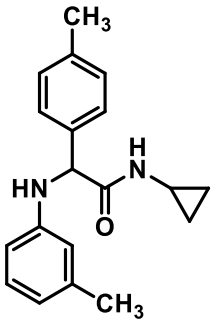

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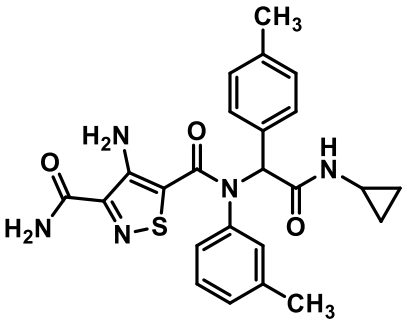

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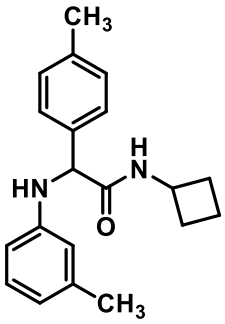

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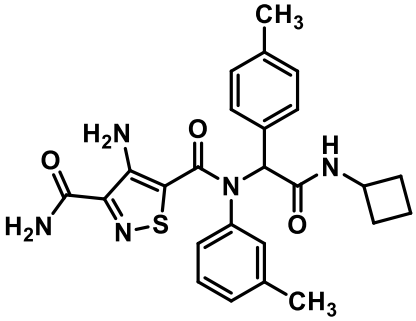

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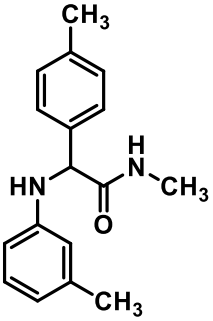

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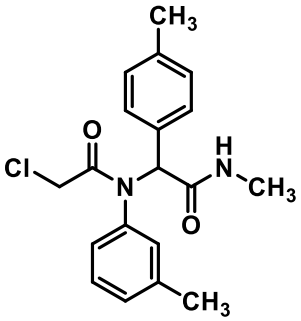

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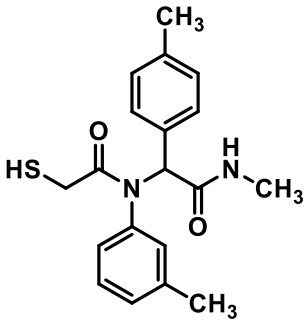

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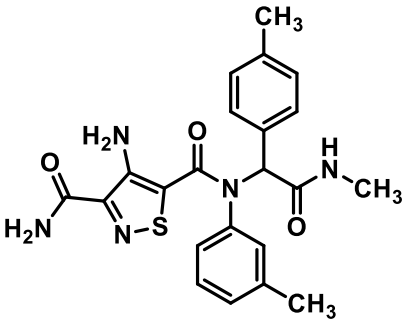

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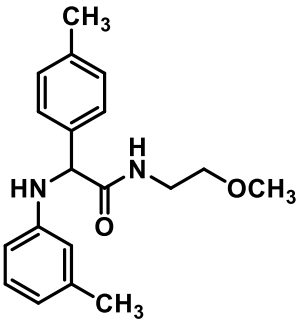

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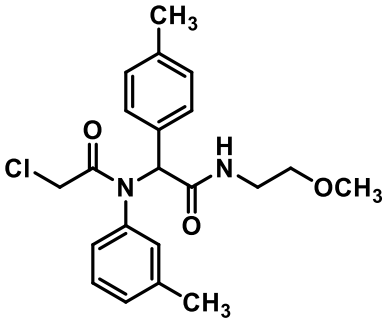

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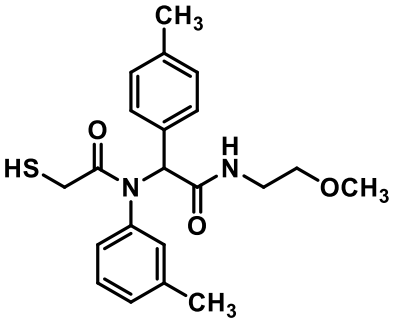

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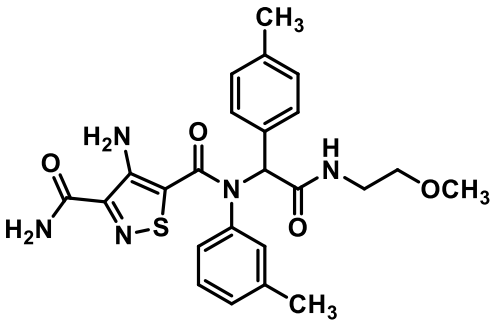

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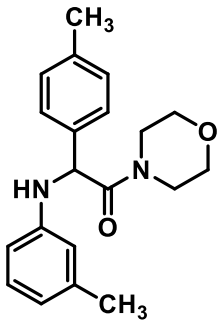

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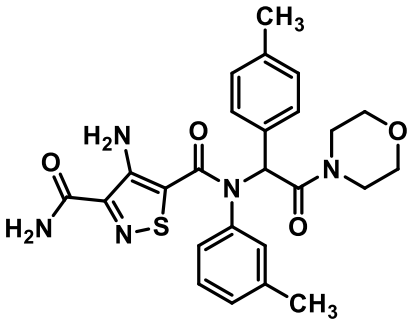

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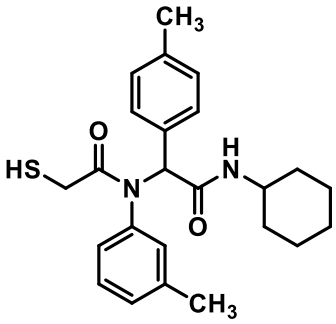

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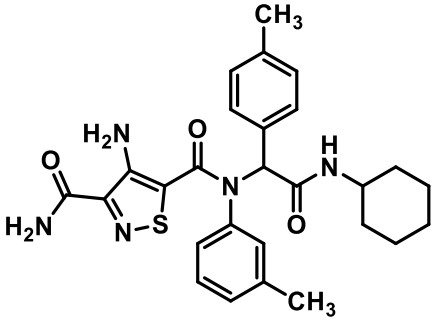

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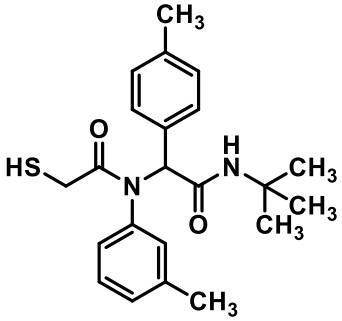

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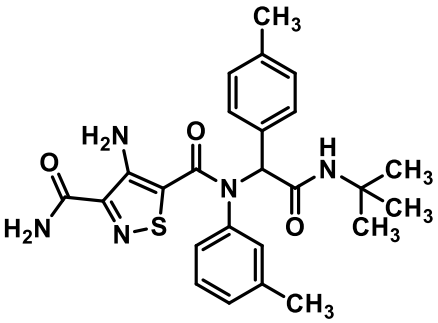

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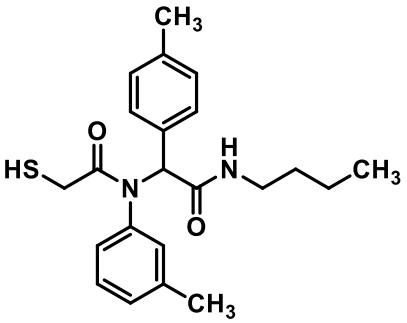

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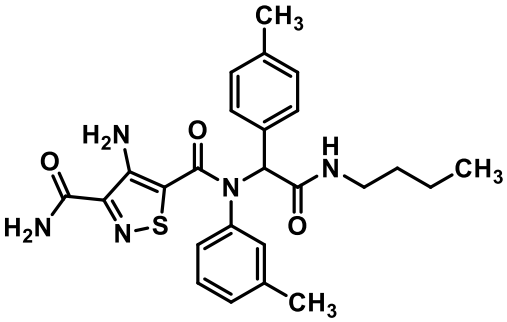

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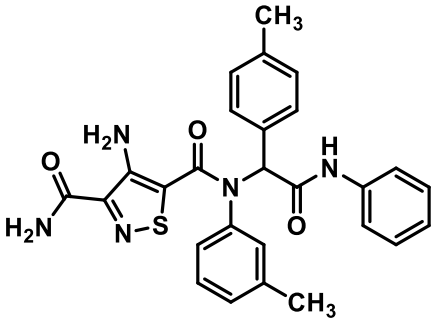

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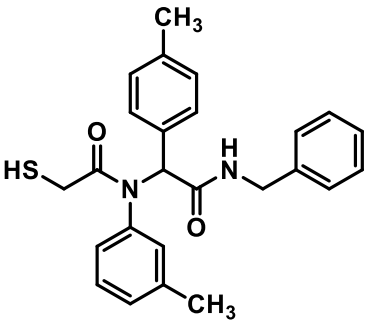

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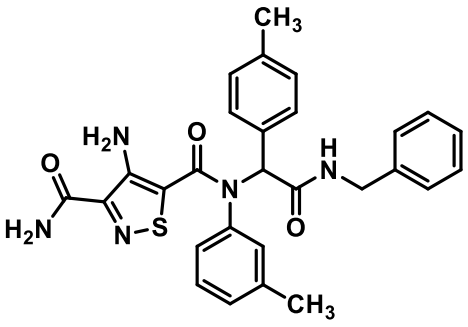

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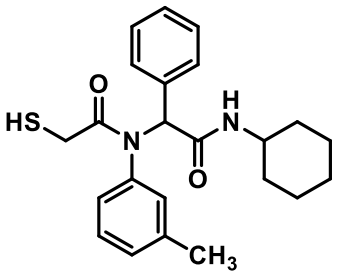

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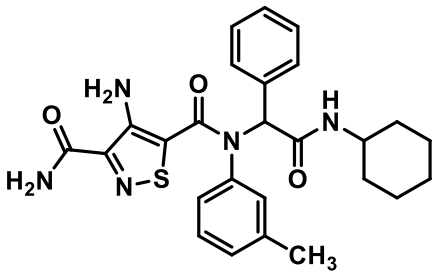

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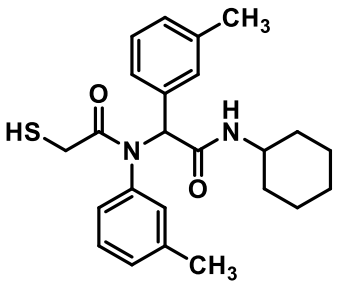

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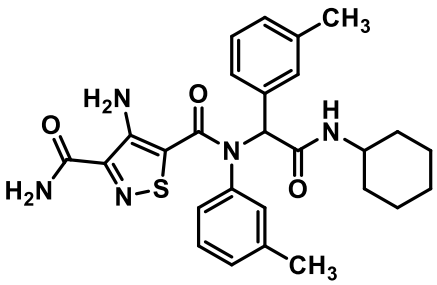

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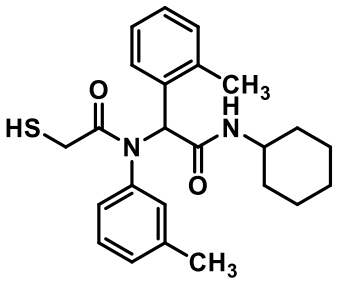

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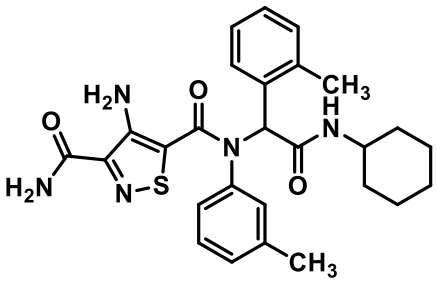

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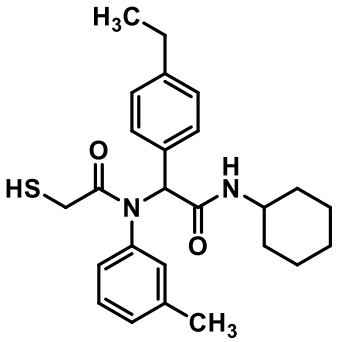

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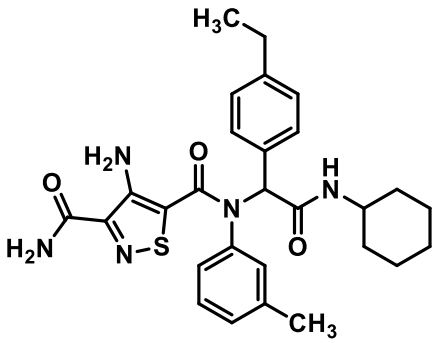

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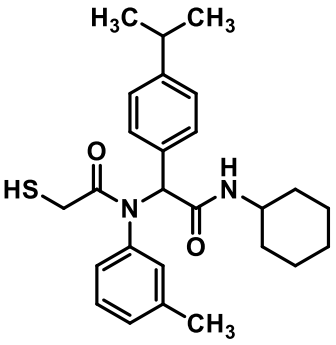

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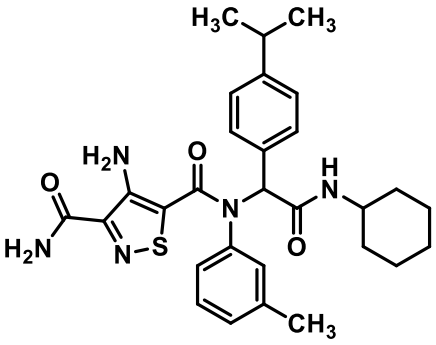

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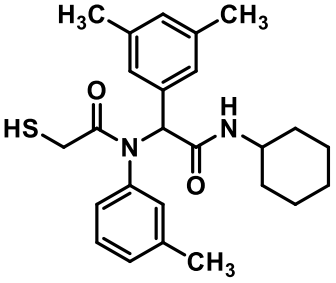

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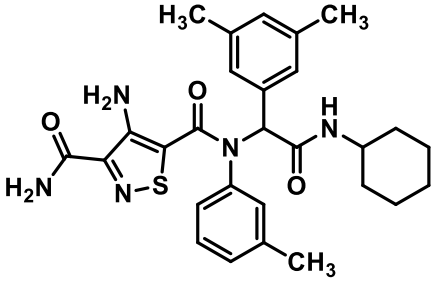

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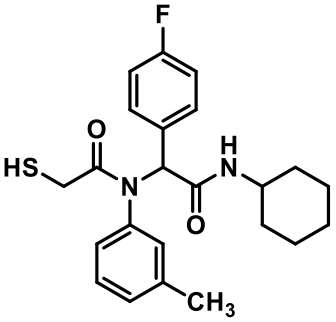

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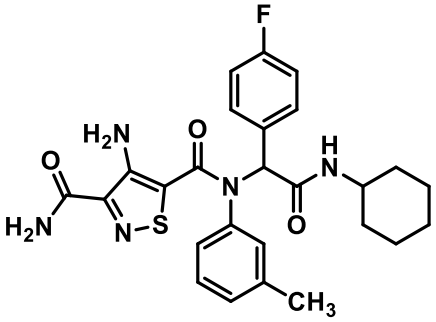

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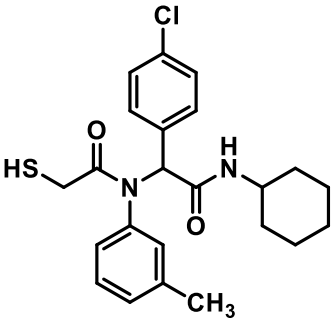

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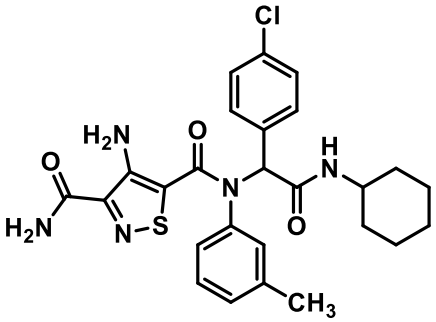

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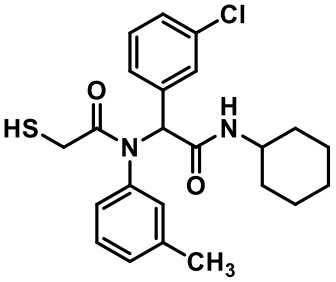

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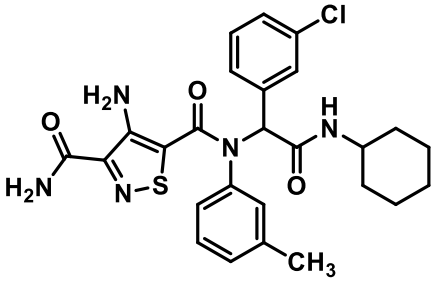

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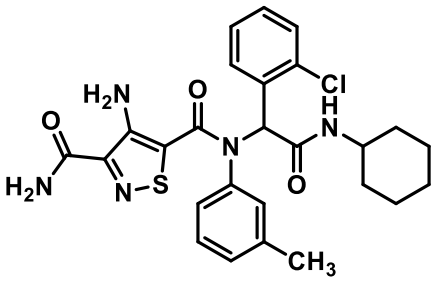

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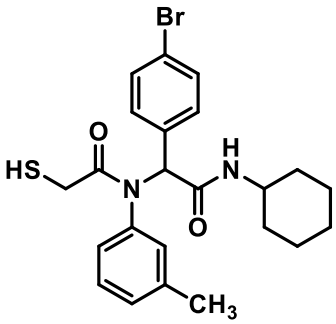

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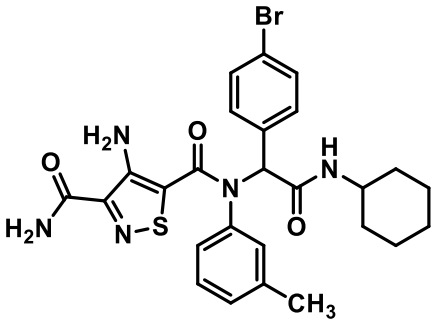

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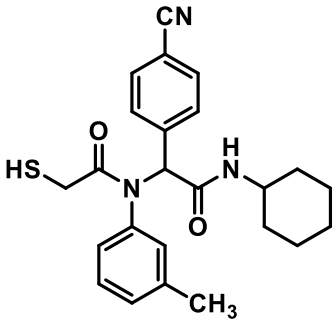

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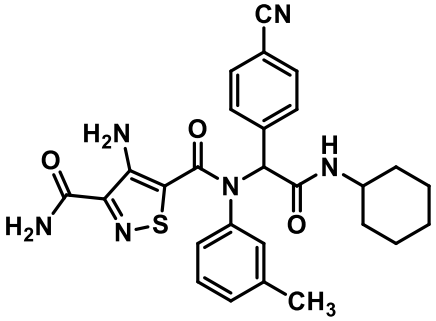

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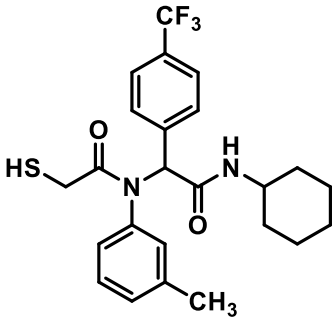

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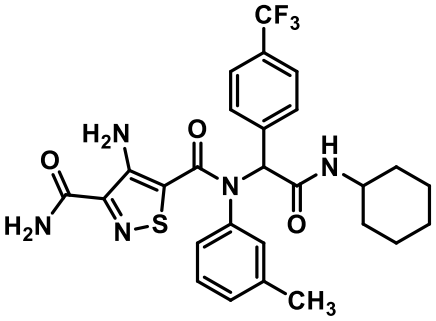

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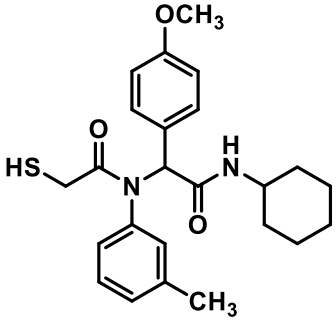

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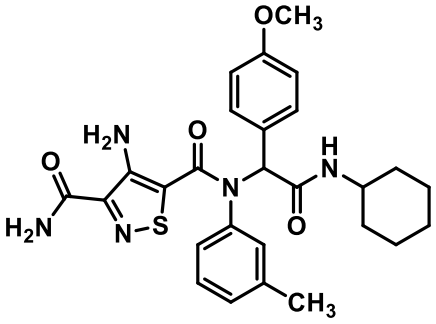

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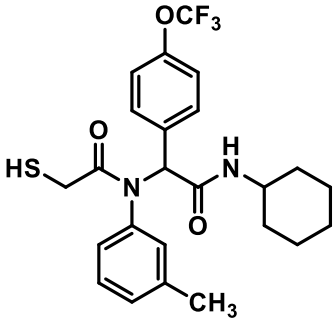

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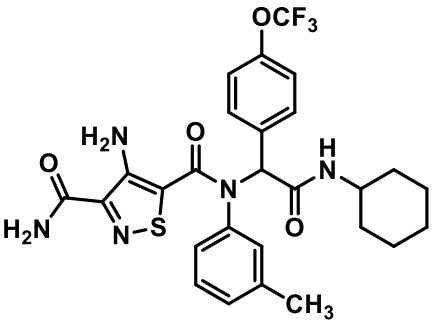

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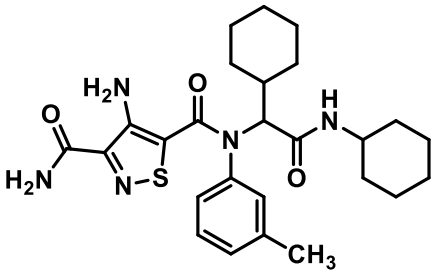

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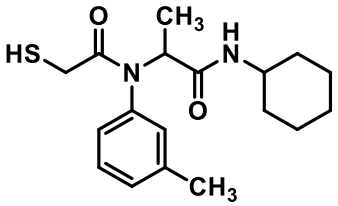

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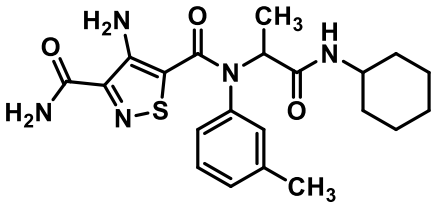

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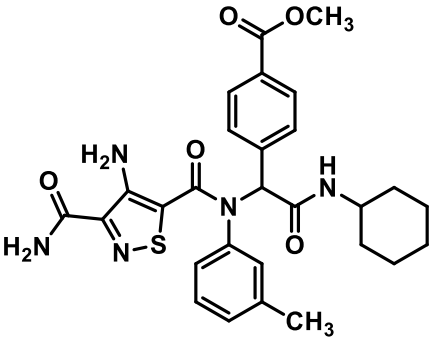

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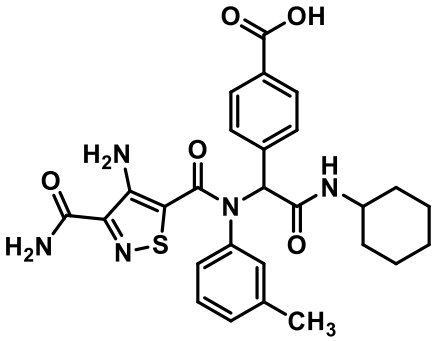

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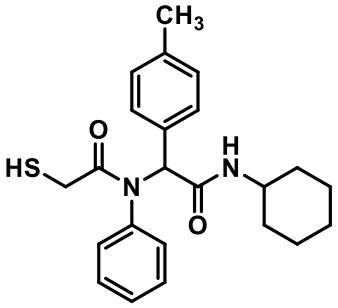

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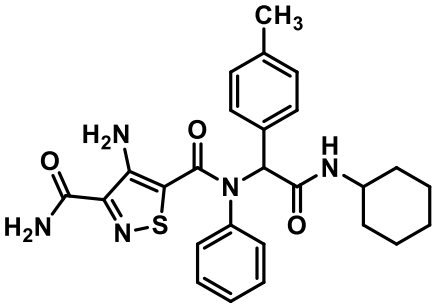

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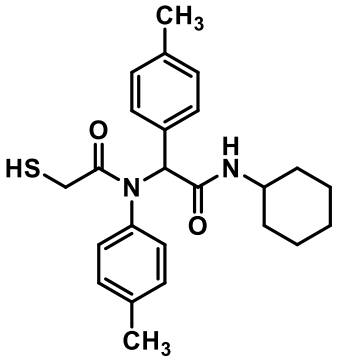

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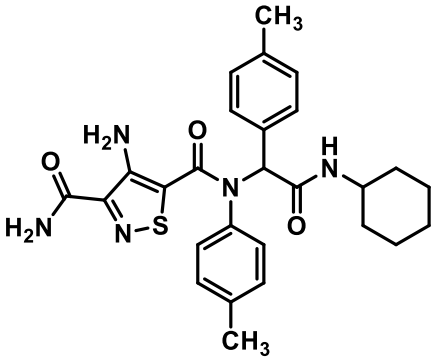

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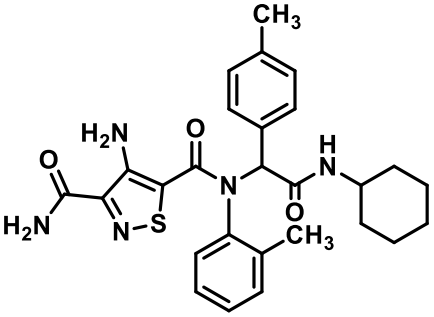

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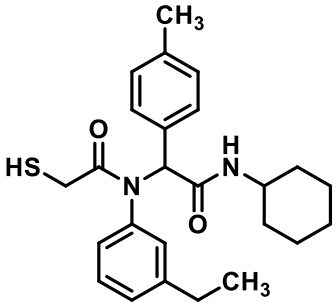

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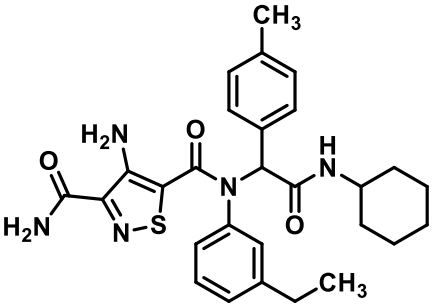

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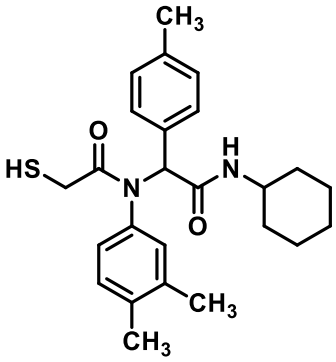

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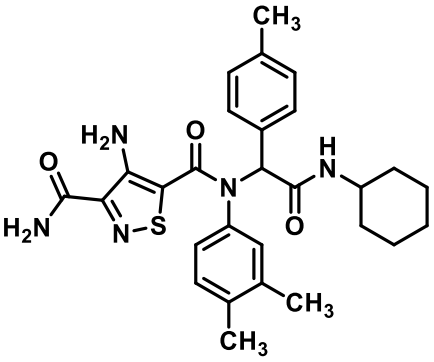

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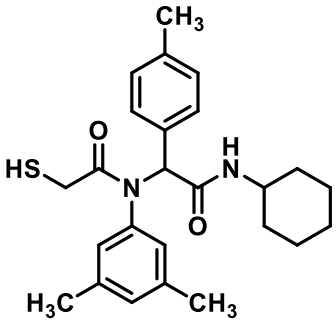

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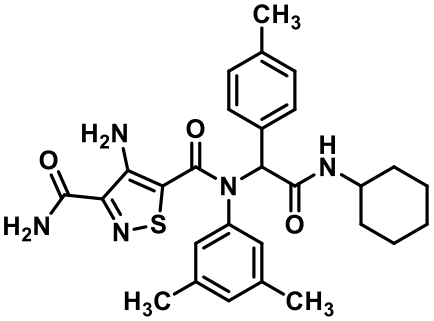

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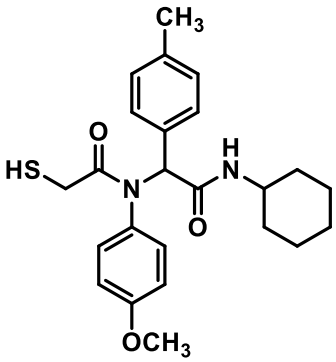

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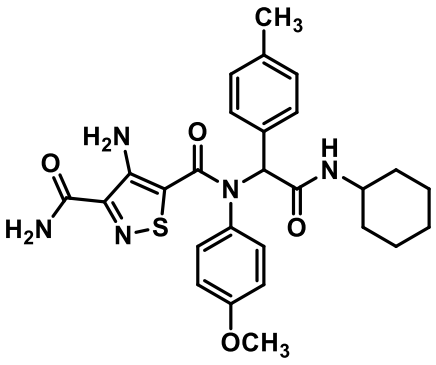

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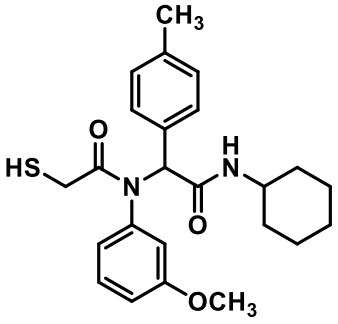

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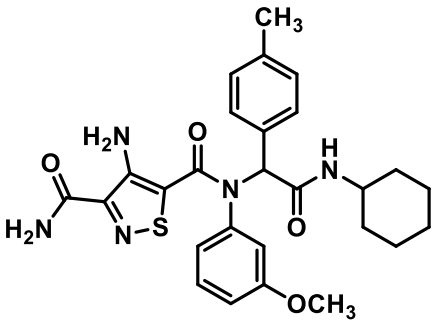

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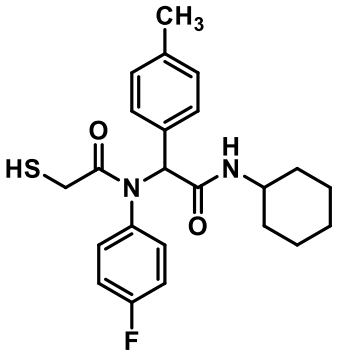

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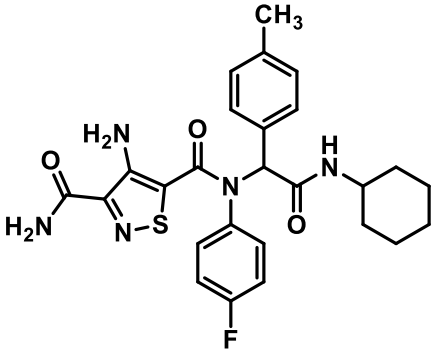

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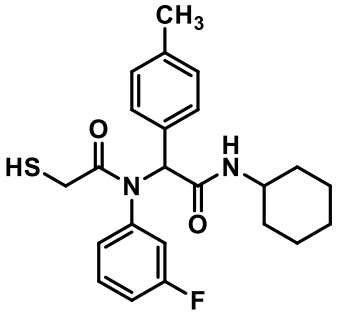

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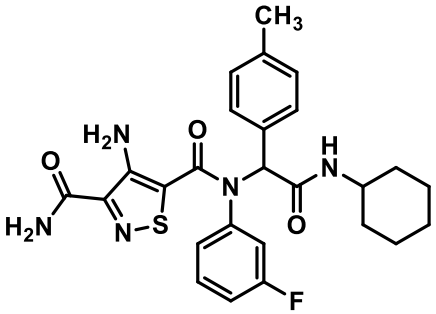

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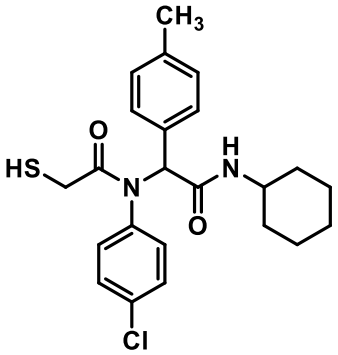

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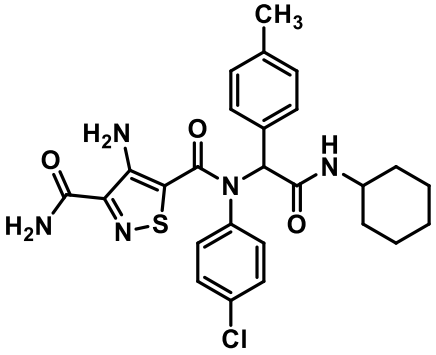

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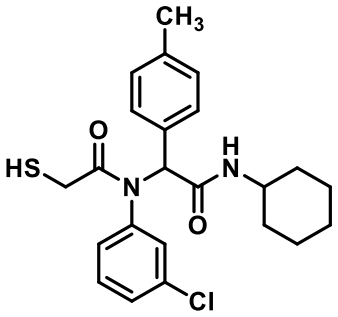

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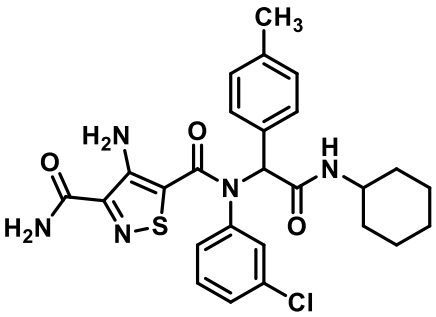

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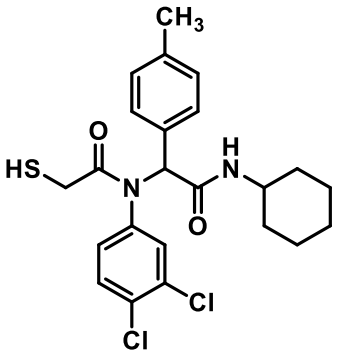

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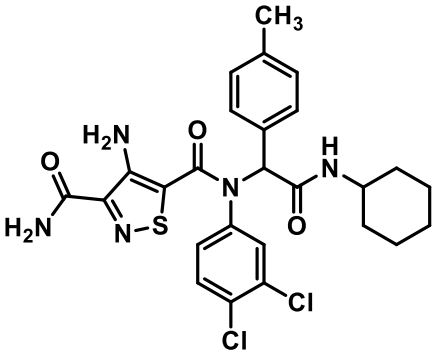

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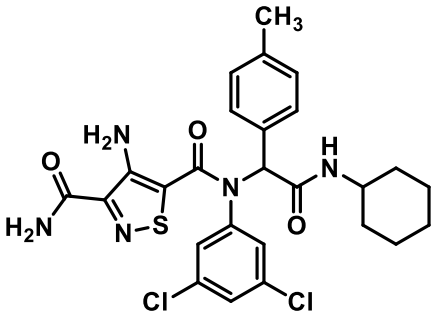

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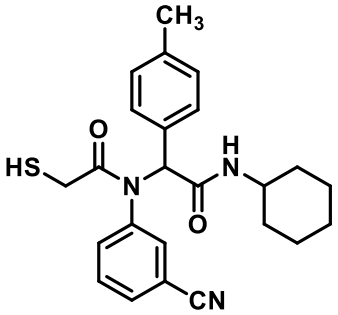

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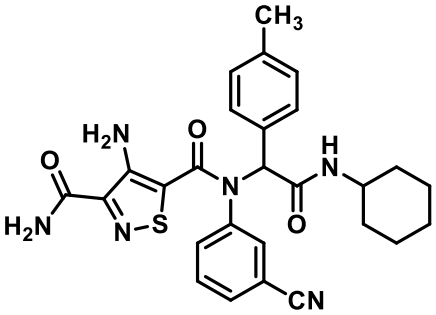

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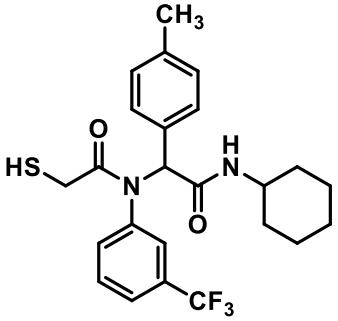

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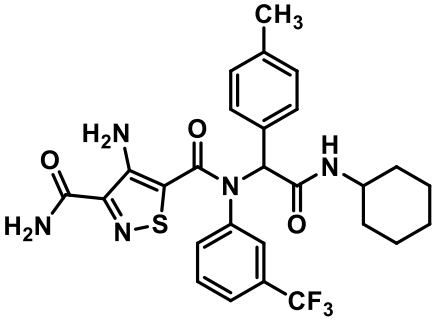

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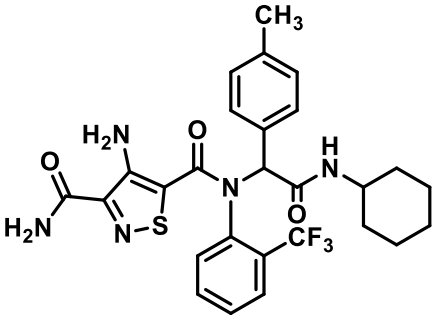

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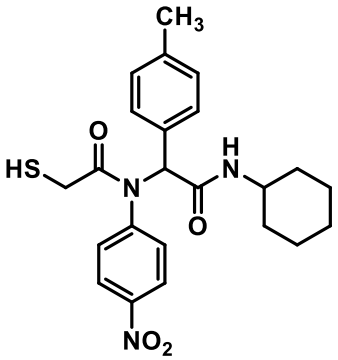

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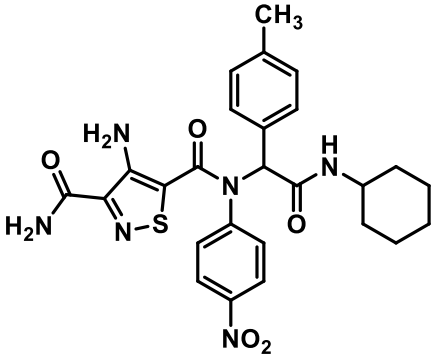

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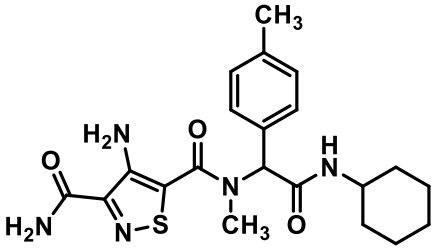

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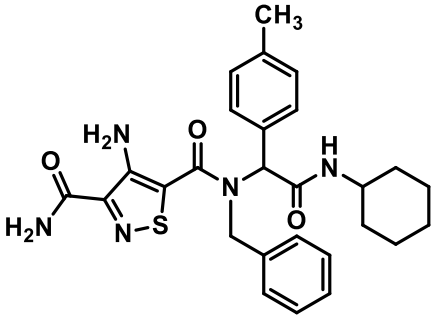

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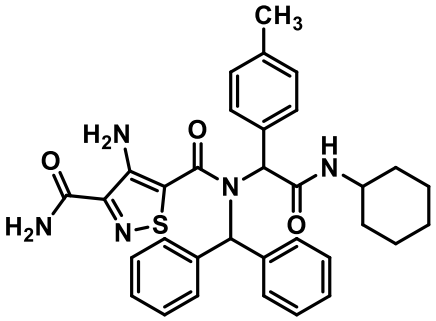

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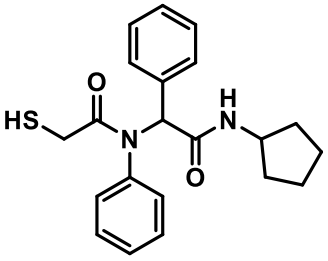

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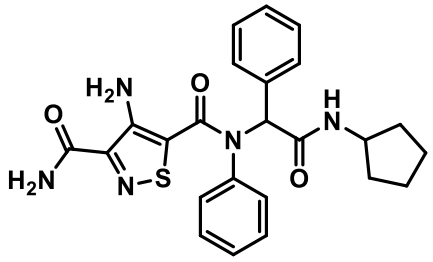

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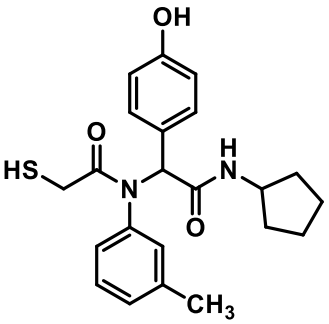

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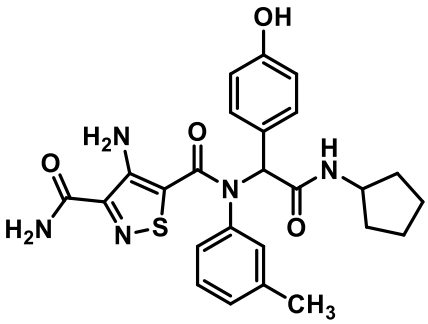

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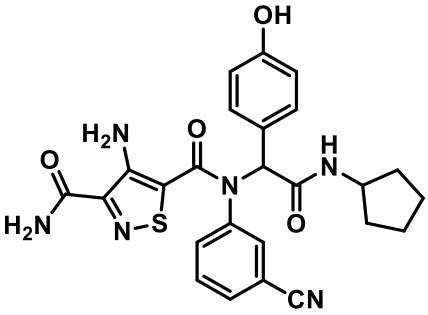

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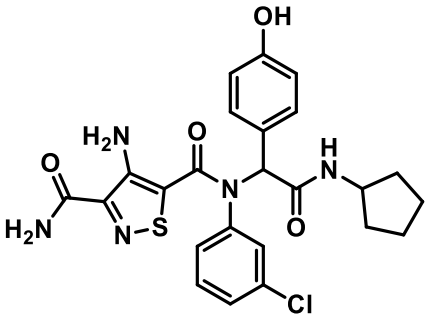

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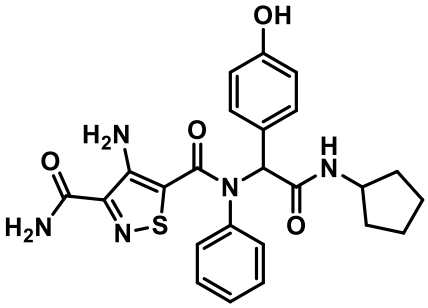

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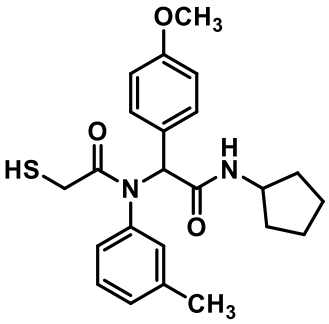

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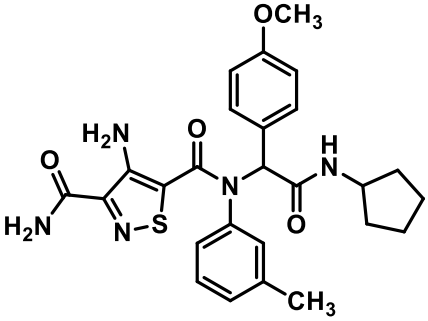

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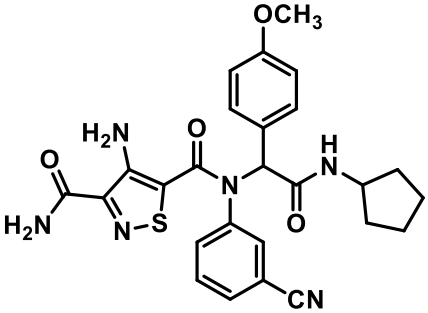

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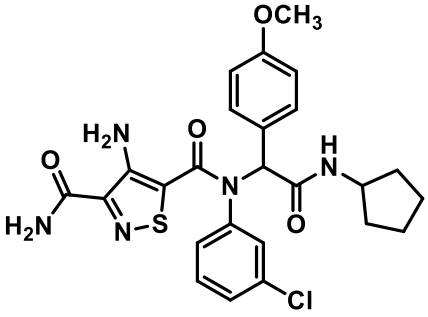

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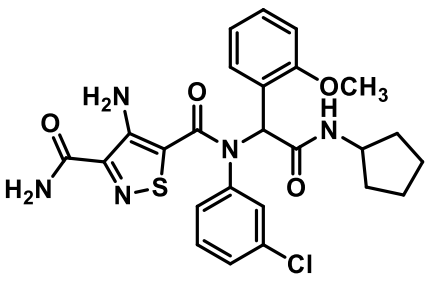

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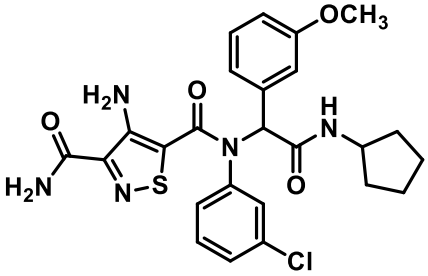

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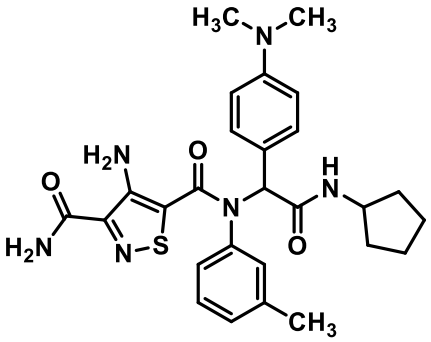

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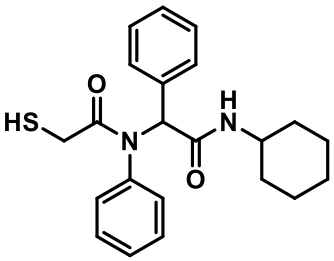

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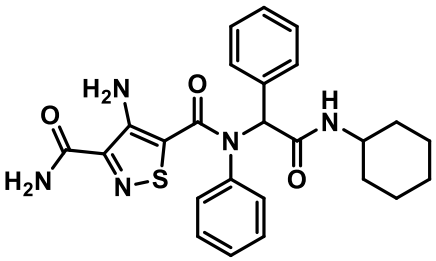

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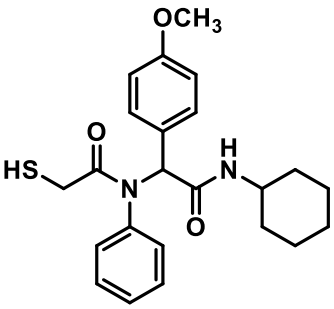

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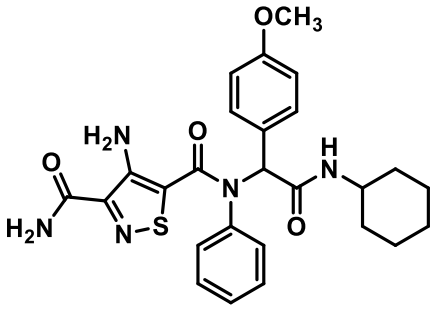

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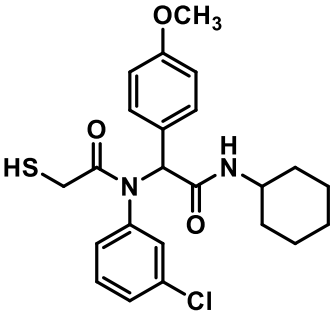

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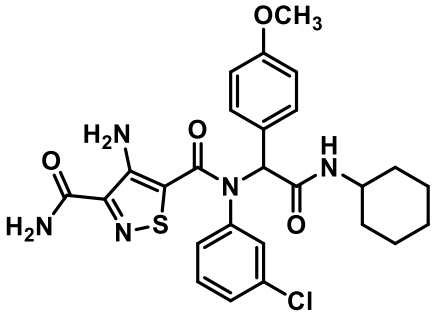

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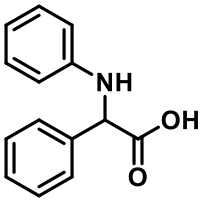

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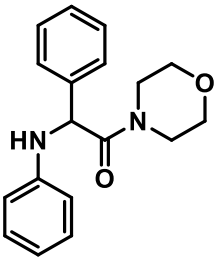

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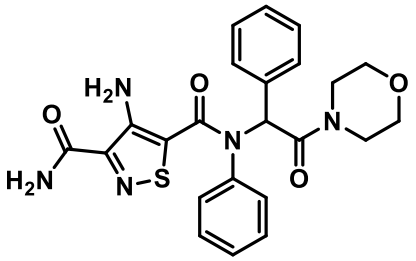

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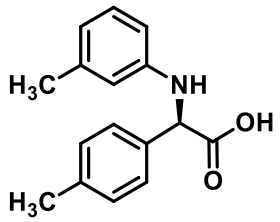

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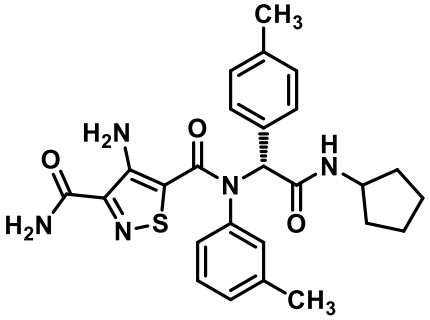

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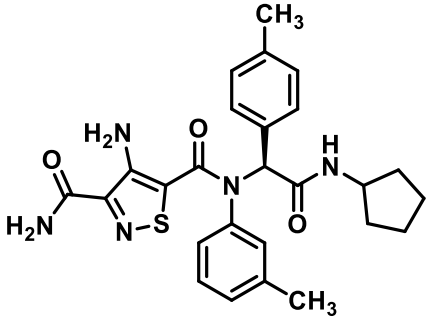

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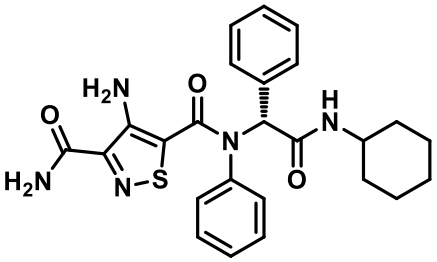

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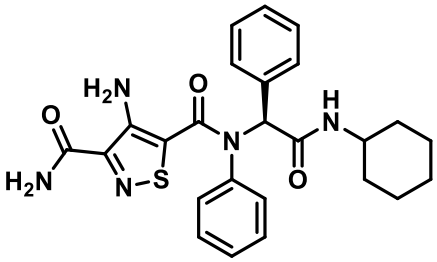

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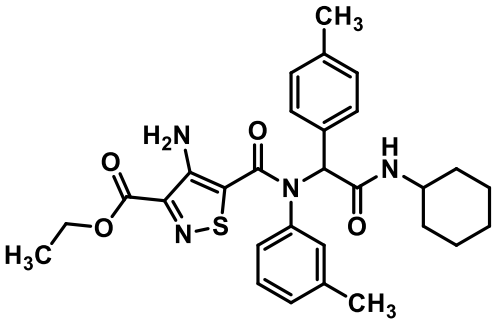

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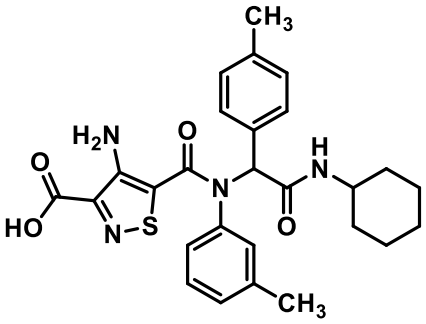

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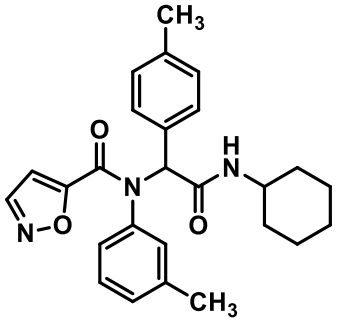
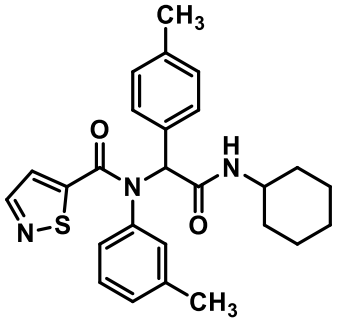
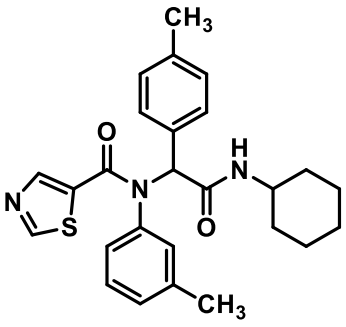


89a



89b



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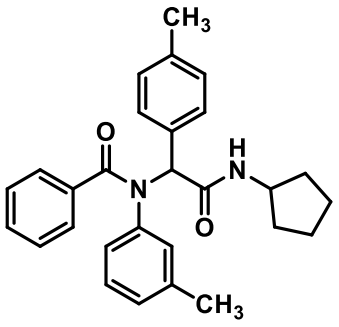

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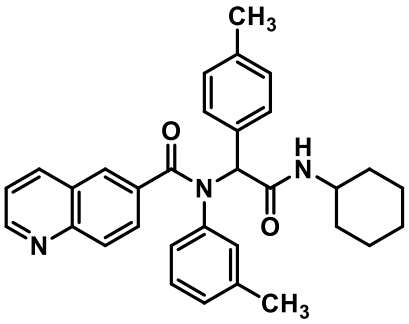

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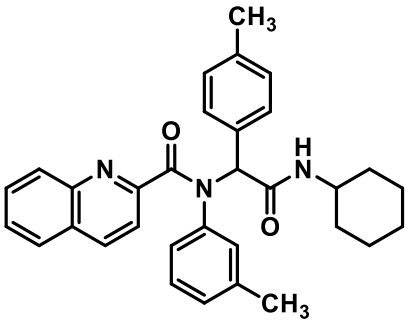

89f

6.3 References

1. Bridges, C. B.; Kuehnert, M. J.; Hall, C. B., Transmission of influenza: implications for control in health care settings. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* **2003,** *37* (8), 1094-101.
2. Morens, D. M.; Taubenberger, J. K., Making Universal Influenza Vaccines: Lessons From the 1918 Pandemic. *The Journal of infectious diseases* **2019,** *219* (Suppl_1), S5-s13.
3. Babazadeh, A.; Mohseni Afshar, Z.; Javanian, M.; Mohammadnia-Afrouzi, M.; Karkhah, A.; Masrour-Roudsari, J.; Sabbagh, P.; Koppolu, V.; Vasigala, V. K.; Ebrahimpour, S., Influenza Vaccination and Guillain-Barré Syndrome: Reality or Fear. *Journal of translational internal medicine* **2019,** *7* (4), 137-142.
4. Hedlund, M.; Larson, J. L.; Fang, F., Antiviral strategies for pandemic and seasonal influenza. *Viruses* **2010,** *2* (8), 1766-1781.
5. Stuart-Harris, C. J. B. M. B., Epidemiology of influenza in man. **1979,** *35* (1), 3-8.
6. Webster, R. G.; Bean, W. J.; Gorman, O. T.; Chambers, T. M.; Kawaoka, Y., Evolution and ecology of influenza A viruses. *Microbiological reviews* **1992,** *56* (1), 152-79.
7. Nicholson, K. G.; Wood, J. M.; Zambon, M., Influenza. *Lancet (London, England)* **2003,** *362* (9397), 1733-45.
8. Paules, C.; Subbarao, K., Influenza. *Lancet (London, England)* **2017,** *390* (10095), 697-708.
9. Nakagawa, H.; Noma, H.; Kotake, O.; Motohashi, R.; Yasuda, K.; Shimura, M., Optic neuritis and acute anterior uveitis associated with influenza A infection: a case report. *International medical case reports journal* **2017,** *10*, 1-5.
10. Nayak, J.; Hoy, G.; Gordon, A., Influenza in Children. *Cold Spring Harbor perspectives in medicine* **2021,** *11* (1).
11. Nair, H.; Brooks, W. A.; Katz, M.; Roca, A.; Berkley, J. A.; Madhi, S. A.; Simmerman, J. M.; Gordon, A.; Sato, M.; Howie, S.; Krishnan, A.; Ope, M.; Lindblade, K. A.; Carosone-Link, P.; Lucero, M.; Ochieng, W.; Kamimoto, L.; Dueger, E.; Bhat, N.; Vong, S.; Theodoratou, E.; Chittaganpitch, M.; Chimah, O.; Balmaseda, A.; Buchy, P.; Harris, E.; Evans, V.; Katayose, M.; Gaur, B.; O'Callaghan-Gordo, C.; Goswami, D.; Arvelo, W.; Venter, M.; Briese, T.; Tokarz, R.; Widdowson, M. A.; Mounts, A. W.; Breiman, R. F.; Feikin, D. R.; Klugman, K. P.; Olsen, S. J.; Gessner, B. D.; Wright, P. F.; Rudan, I.; Broor, S.; Simões, E. A.; Campbell, H., Global burden of respiratory infections due to seasonal influenza in

young children: a systematic review and meta-analysis. *Lancet (London, England)* **2011,** *378* (9807), 1917-30.

12. Quigley, E., Influenza therapies: vaccines and antiviral drugs. *Drug Discovery Today* **2006,** *11* (11), 478-480.
13. Javanian, M.; Barary, M.; Ghebrehewet, S.; Koppolu, V.; Vasigala, V.; Ebrahimpour, S., A brief review of influenza virus infection. *Journal of medical virology* **2021,** *93* (8), 4638-4646.
14. Uyeki, T. M.; Hui, D. S.; Zambon, M.; Wentworth, D. E.; Monto, A. S., Influenza. *Lancet (London, England)* **2022,** *400* (10353), 693-706.
15. Sullivan, S. J. T. L., Challenges in reducing influenza-associated mortality. **2018,** *391* (10127), 1242-1244.
16. WHO Influenza fact sheet. https://www.who.int/en/news-room/fact-sheets/detail/influenza-(seasonal).
17. Guan, Y.; Vijaykrishna, D.; Bahl, J.; Zhu, H.; Wang, J.; Smith, G. J. J. P.; cell, The emergence of pandemic influenza viruses. **2010,** *1*, 9-13.
18. Potter, C. W., A history of influenza. *Journal of applied microbiology* **2001,** *91* (4), 572-9.
19. Simonsen, L.; Clarke, M. J.; Schonberger, L. B.; Arden, N. H.; Cox, N. J.; Fukuda, K., Pandemic versus epidemic influenza mortality: a pattern of changing age distribution. *The Journal of infectious diseases* **1998,** *178* (1), 53-60.
20. Neumann, G.; Noda, T.; Kawaoka, Y., Emergence and pandemic potential of swine-origin H1N1 influenza virus. *Nature* **2009,** *459* (7249), 931-939.
21. Mills, C. E.; Robins, J. M.; Lipsitch, M. J. N., Transmissibility of 1918 pandemic influenza. **2004,** *432* (7019), 904-906.
22. Peiris, J. S.; de Jong, M. D.; Guan, Y., Avian influenza virus (H5N1): a threat to human health. *Clinical microbiology reviews* **2007,** *20* (2), 243-67.
23. Schrauwen, E. J.; Fouchier, R. A. J. E. m.; infections, Host adaptation and transmission of influenza A viruses in mammals. **2014,** *3* (1), 1-10.
24. Smith, G. J.; Vijaykrishna, D.; Bahl, J.; Lycett, S. J.; Worobey, M.; Pybus, O. G.; Ma, S. K.; Cheung, C. L.; Raghwani, J.; Bhatt, S. J. N., Origins and evolutionary genomics of the 2009 swine-origin H1N1 influenza A epidemic. **2009,** *459* (7250), 1122-1125.
25. Shapshak, P.; Chiappelli, F.; Somboonwit, C.; Sinnott, J., The influenza pandemic of 2009: lessons and implications. *Molecular diagnosis & therapy* **2011,** *15* (2), 63-81.

26. Neumann, G.; Kawaoka, Y., The first influenza pandemic of the new millennium. *Influenza and other respiratory viruses* **2011,** *5* (3), 157-66.
27. WHO Virus detection graphs. https://www.who.int/teams/global-influenza-programme/surveillance-and-monitoring/influenza-surveillance-outputs.
28. Fouchier, R. A.; Munster, V.; Wallensten, A.; Bestebroer, T. M.; Herfst, S.; Smith, D.; Rimmelzwaan, G. F.; Olsen, B.; Osterhaus, A. D., Characterization of a novel influenza A virus hemagglutinin subtype (H16) obtained from black-headed gulls. *Journal of virology* **2005,** *79* (5), 2814-22.
29. Boechat Fda, C.; Sacramento, C. Q.; Cunha, A. C.; Sagrillo, F. S.; Nogueira, C. M.; Fintelman-Rodrigues, N.; Santos-Filho, O.; Riscado, C. S.; Forezi Lda, S.; Faro, L. V.; Brozeguini, L.; Marques, I. P.; Ferreira, V. F.; Souza, T. M.; de Souza, M. C., 1,2,3-Triazolyl-4-oxoquinolines: A feasible beginning for promising chemical structures to inhibit oseltamivir-resistant influenza A and B viruses. *Bioorganic & medicinal chemistry* **2015,** *23* (24), 7777-84.
30. El-Shesheny, R.; Feeroz, M. M.; Krauss, S.; Vogel, P.; McKenzie, P.; Webby, R. J.; Webster, R. G. J. E. m.; infections, Replication and pathogenic potential of influenza A virus subtypes H3, H7, and H15 from free-range ducks in Bangladesh in mammals. **2018,** *7* (1), 1-13.
31. Krug, R. M.; Aramini, J. M., Emerging antiviral targets for influenza A virus. *Trends in pharmacological sciences* **2009,** *30* (6), 269-77.
32. Schrauwen, E. J.; de Graaf, M.; Herfst, S.; Rimmelzwaan, G. F.; Osterhaus, A. D.; Fouchier, R. A. J. E. j. o. c. m.; diseases, i., Determinants of virulence of influenza A virus. **2014,** *33*, 479-490.
33. Paules, C. I.; Fauci, A. S., Influenza Vaccines: Good, but We Can Do Better. *The Journal of infectious diseases* **2019,** *219* (Suppl_1), S1-s4.
34. te Velthuis, A. J. W.; Fodor, E., Influenza virus RNA polymerase: insights into the mechanisms of viral RNA synthesis. *Nature Reviews Microbiology* **2016,** *14* (8), 479-493.
35. Huang, Q.; Sivaramakrishna, R. P.; Ludwig, K.; Korte, T.; Böttcher, C.; Herrmann, A. J. B. e. B. A.-B., Early steps of the conformational change of influenza virus hemagglutinin to a fusion active state: stability and energetics of the hemagglutinin. **2003,** *1614* (1), 3-13.

36. Massari, S.; Goracci, L.; Desantis, J.; Tabarrini, O., Polymerase Acidic Protein-Basic Protein 1 (PA-PB1) Protein-Protein Interaction as a Target for Next-Generation Anti-influenza Therapeutics. *Journal of medicinal chemistry* **2016,** *59* (17), 7699-718.
37. von Itzstein, M., The war against influenza: discovery and development of sialidase inhibitors. *Nature Reviews Drug Discovery* **2007,** *6* (12), 967-974.
38. Wang, C.; Lamb, R. A.; Pinto, L. H., Activation of the M2 ion channel of influenza virus: a role for the transmembrane domain histidine residue. *Biophysical journal* **1995,** *69* (4), 1363-71.
39. Portela, A.; Digard, P. J. J. o. g. v., The influenza virus nucleoprotein: a multifunctional RNA-binding protein pivotal to virus replication. **2002,** *83* (4), 723-734.
40. Massari, S.; Goracci, L.; Desantis, J.; Tabarrini, O. J. J. o. m. c., Polymerase acidic protein–basic protein 1 (PA–PB1) protein–protein interaction as a target for next-generation anti-influenza therapeutics. **2016,** *59* (17), 7699-7718.
41. Fodor, E.; Te Velthuis, A. J. W., Structure and Function of the Influenza Virus Transcription and Replication Machinery. *Cold Spring Harbor perspectives in medicine* **2020,** *10* (9).
42. Byrd-Leotis, L.; Cummings, R. D.; Steinhauer, D. A., The Interplay between the Host Receptor and Influenza Virus Hemagglutinin and Neuraminidase. *International journal of molecular sciences* **2017,** *18* (7).
43. Pflug, A.; Guilligay, D.; Reich, S.; Cusack, S. J. N., Structure of influenza A polymerase bound to the viral RNA promoter. **2014,** *516* (7531), 355-360.
44. Reich, S.; Guilligay, D.; Pflug, A.; Malet, H.; Berger, I.; Crépin, T.; Hart, D.; Lunardi, T.; Nanao, M.; Ruigrok, R. W. J. N., Structural insight into cap-snatching and RNA synthesis by influenza polymerase. **2014,** *516* (7531), 361-366.
45. Basler, C. F., Influenza viruses: basic biology and potential drug targets. *Infectious disorders drug targets* **2007,** *7* (4), 282-93.
46. Chauhan, R. P.; Gordon, M. L., An overview of influenza A virus genes, protein functions, and replication cycle highlighting important updates. *Virus Genes* **2022,** *58* (4), 255-269.
47. Wu, W.; Li, R.; Li, X.; He, J.; Jiang, S.; Liu, S.; Yang, J. J. V., Quercetin as an antiviral agent inhibits influenza A virus (IAV) entry. **2015,** *8* (1), 6.
48. Chen, Q.; Guo, Y. J. A. I. D., Influenza viral hemagglutinin peptide inhibits influenza viral entry by shielding the host receptor. **2016,** *2* (3), 187-193.

49. Sieczkarski, S. B.; Whittaker, G. R., Viral entry. *Current topics in microbiology and immunology* **2005,** *285*, 1-23.
50. Stegmann, T., Membrane fusion mechanisms: the influenza hemagglutinin paradigm and its implications for intracellular fusion. *Traffic (Copenhagen, Denmark)* **2000,** *1* (8), 598-604.
51. Pinto, L. H.; Lamb, R. A., The M2 proton channels of influenza A and B viruses. *The Journal of biological chemistry* **2006,** *281* (14), 8997-9000.
52. Helenius, A. J. C., Unpacking the incoming influenza virus. **1992,** *69* (4), 577-578.
53. Das, K. J. J. o. m. c., Antivirals targeting influenza A virus. **2012,** *55* (14), 6263-6277.
54. Wu, W. W. H.; Sun, Y.-H. B.; Panté, N., Nuclear import of influenza A viral ribonucleoprotein complexes is mediated by two nuclear localization sequences on viral nucleoprotein. *Virology Journal* **2007,** *4* (1), 49.
55. Wu, W. W.; Weaver, L. L.; Panté, N., Ultrastructural analysis of the nuclear localization sequences on influenza A ribonucleoprotein complexes. *Journal of molecular biology* **2007,** *374* (4), 910-6.
56. Walker, A. P.; Fodor, E., Interplay between Influenza Virus and the Host RNA Polymerase II Transcriptional Machinery. *Trends in microbiology* **2019,** *27* (5), 398-407.
57. Li, M. L.; Rao, P.; Krug, R. M., The active sites of the influenza cap-dependent endonuclease are on different polymerase subunits. *The EMBO journal* **2001,** *20* (8), 2078-86.
58. Plotch, S. J.; Bouloy, M.; Ulmanen, I.; Krug, R. M., A unique cap(m7GpppXm)-dependent influenza virion endonuclease cleaves capped RNAs to generate the primers that initiate viral RNA transcription. *Cell* **1981,** *23* (3), 847-58.
59. Arranz, R.; Coloma, R.; Chichón, F. J.; Conesa, J. J.; Carrascosa, J. L.; Valpuesta, J. M.; Ortín, J.; Martín-Benito, J., The Structure of Native Influenza Virion Ribonucleoproteins. **2012,** *338* (6114), 1634-1637.
60. Samji, T., Influenza A: understanding the viral life cycle. *The Yale journal of biology and medicine* **2009,** *82* (4), 153-9.
61. Neumann, G.; Hughes, M. T.; Kawaoka, Y., Influenza A virus NS2 protein mediates vRNP nuclear export through NES-independent interaction with hCRM1. *The EMBO journal* **2000,** *19* (24), 6751-8.
62. Boulo, S.; Akarsu, H.; Ruigrok, R. W.; Baudin, F., Nuclear traffic of influenza virus proteins and ribonucleoprotein complexes. *Virus research* **2007,** *124* (1-2), 12-21.

63. Chen, B. J.; Lamb, R. A., Mechanisms for enveloped virus budding: can some viruses do without an ESCRT? *Virology* **2008,** *372* (2), 221-32.
64. Ferraris, O.; Lina, B. J. J. o. C. V., Mutations of neuraminidase implicated in neuraminidase inhibitors resistance. **2008,** *41* (1), 13-19.
65. Gamblin, S. J.; Skehel, J. J. J. J. o. b. c., Influenza hemagglutinin and neuraminidase membrane glycoproteins. **2010,** *285* (37), 28403-28409.
66. Moeller, A.; Kirchdoerfer, R. N.; Potter, C. S.; Carragher, B.; Wilson, I. A., Organization of the influenza virus replication machinery. *Science (New York, N.Y.)* **2012,** *338* (6114), 1631-4.
67. Ye, Q.; Krug, R. M.; Tao, Y. J., The mechanism by which influenza A virus nucleoprotein forms oligomers and binds RNA. *Nature* **2006,** *444* (7122), 1078-82.
68. Ng, A. K.; Zhang, H.; Tan, K.; Li, Z.; Liu, J. H.; Chan, P. K.; Li, S. M.; Chan, W. Y.; Au, S. W.; Joachimiak, A.; Walz, T.; Wang, J. H.; Shaw, P. C., Structure of the influenza virus A H5N1 nucleoprotein: implications for RNA binding, oligomerization, and vaccine design. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* **2008,** *22* (10), 3638-47.
69. Chenavas, S.; Estrozi, L. F.; Slama-Schwok, A.; Delmas, B.; Di Primo, C.; Baudin, F.; Li, X.; Crépin, T.; Ruigrok, R. W. H., Monomeric Nucleoprotein of Influenza A Virus. *PLOS Pathogens* **2013,** *9* (3), e1003275.
70. Tang, Y. S.; Xu, S.; Chen, Y. W.; Wang, J. H.; Shaw, P. C., Crystal structures of influenza nucleoprotein complexed with nucleic acid provide insights into the mechanism of RNA interaction. *Nucleic acids research* **2021,** *49* (7), 4144-4154.
71. Eisfeld, A. J.; Neumann, G.; Kawaoka, Y., At the centre: influenza A virus ribonucleoproteins. *Nature reviews. Microbiology* **2015,** *13* (1), 28-41.
72. Baudin, F.; Bach, C.; Cusack, S.; Ruigrok, R. W., Structure of influenza virus RNP. I. Influenza virus nucleoprotein melts secondary structure in panhandle RNA and exposes the bases to the solvent. *The EMBO journal* **1994,** *13* (13), 3158-65.
73. Ortega, J.; Martín-Benito, J.; Zürcher, T.; Valpuesta, J. M.; Carrascosa, J. L.; Ortín, J., Ultrastructural and functional analyses of recombinant influenza virus ribonucleoproteins suggest dimerization of nucleoprotein during virus amplification. *Journal of virology* **2000,** *74* (1), 156-63.
74. Biswas, S. K.; Boutz, P. L.; Nayak, D. P., Influenza virus nucleoprotein interacts with influenza virus polymerase proteins. *Journal of virology* **1998,** *72* (7), 5493-501.

75. Medcalf, L.; Poole, E.; Elton, D.; Digard, P., Temperature-sensitive lesions in two influenza A viruses defective for replicative transcription disrupt RNA binding by the nucleoprotein. *Journal of virology* **1999,** *73* (9), 7349-56.
76. Amorim, M. J.; Bruce, E. A.; Read, E. K.; Foeglein, A.; Mahen, R.; Stuart, A. D.; Digard, P., A Rab11- and microtubule-dependent mechanism for cytoplasmic transport of influenza A virus viral RNA. *Journal of virology* **2011,** *85* (9), 4143-56.
77. Bui, M.; Wills, E. G.; Helenius, A.; Whittaker, G. R., Role of the influenza virus M1 protein in nuclear export of viral ribonucleoproteins. *Journal of virology* **2000,** *74* (4), 1781-6.
78. Akarsu, H.; Burmeister, W. P.; Petosa, C.; Petit, I.; Müller, C. W.; Ruigrok, R. W.; Baudin, F., Crystal structure of the M1 protein-binding domain of the influenza A virus nuclear export protein (NEP/NS2). *The EMBO journal* **2003,** *22* (18), 4646-55.
79. Wu, W. W.; Panté, N., The directionality of the nuclear transport of the influenza A genome is driven by selective exposure of nuclear localization sequences on nucleoprotein. *Virol J* **2009,** *6*, 68.
80. Newcomb, L. L.; Kuo, R. L.; Ye, Q.; Jiang, Y.; Tao, Y. J.; Krug, R. M., Interaction of the influenza a virus nucleocapsid protein with the viral RNA polymerase potentiates unprimed viral RNA replication. *Journal of virology* **2009,** *83* (1), 29-36.
81. Fodor, E., The RNA polymerase of influenza a virus: mechanisms of viral transcription and replication. *Acta virologica* **2013,** *57* (2), 113-22.
82. Yang, F.; Pang, B.; Lai, K. K.; Cheung, N. N.; Dai, J.; Zhang, W.; Zhang, J.; Chan, K. H.; Chen, H.; Sze, K. H.; Zhang, H.; Hao, Q.; Yang, D.; Yuen, K. Y.; Kao, R. Y., Discovery of a Novel Specific Inhibitor Targeting Influenza A Virus Nucleoprotein with Pleiotropic Inhibitory Effects on Various Steps of the Viral Life Cycle. *Journal of virology* **2021,** *95* (9).
83. Wang, J.; Cady, S. D.; Balannik, V.; Pinto, L. H.; DeGrado, W. F.; Hong, M. J. J. o. t. A. C. S., Discovery of spiro-piperidine inhibitors and their modulation of the dynamics of the M2 proton channel from influenza A virus. **2009,** *131* (23), 8066-8076.
84. De Clercq, E. J. N. r. D. d., Antiviral agents active against influenza A viruses. **2006,** *5* (12), 1015-1025.
85. Hu, Y.; Sneyd, H.; Dekant, R.; Wang, J., Influenza A Virus Nucleoprotein: A Highly Conserved Multi-Functional Viral Protein as a Hot Antiviral Drug Target. *Current topics in medicinal chemistry* **2017,** *17* (20), 2271-2285.

86. Gerritz, S. W.; Cianci, C.; Kim, S.; Pearce, B. C.; Deminie, C.; Discotto, L.; McAuliffe, B.; Minassian, B. F.; Shi, S.; Zhu, S.; Zhai, W.; Pendri, A.; Li, G.; Poss, M. A.; Edavettal, S.; McDonnell, P. A.; Lewis, H. A.; Maskos, K.; Mörtl, M.; Kiefersauer, R.; Steinbacher, S.; Baldwin, E. T.; Metzler, W.; Bryson, J.; Healy, M. D.; Philip, T.; Zoeckler, M.; Schartman, R.; Sinz, M.; Leyva-Grado, V. H.; Hoffmann, H. H.; Langley, D. R.; Meanwell, N. A.; Krystal, M., Inhibition of influenza virus replication via small molecules that induce the formation of higher-order nucleoprotein oligomers. *Proceedings of the National Academy of Sciences of the United States of America* **2011,** *108* (37), 15366-71.

87. Su, C. Y.; Cheng, T. J.; Lin, M. I.; Wang, S. Y.; Huang, W. I.; Lin-Chu, S. Y.; Chen, Y. H.; Wu, C. Y.; Lai, M. M.; Cheng, W. C.; Wu, Y. T.; Tsai, M. D.; Cheng, Y. S.; Wong, C. H., High-throughput identification of compounds targeting influenza RNA-dependent RNA polymerase activity. *Proceedings of the National Academy of Sciences of the United States of America* **2010,** *107* (45), 19151-6.

88. Kao, R. Y.; Yang, D.; Lau, L.-S.; Tsui, W. H. W.; Hu, L.; Dai, J.; Chan, M.-P.; Chan, C.-M.; Wang, P.; Zheng, B.-J.; Sun, J.; Huang, J.-D.; Madar, J.; Chen, G.; Chen, H.; Guan, Y.; Yuen, K.-Y., Identification of influenza A nucleoprotein as an antiviral target. *Nature Biotechnology* **2010,** *28* (6), 600-605.

89. Amorim, M.-J.; Read, E. K.; Dalton, R. M.; Medcalf, L.; Digard, P., Nuclear Export of Influenza A Virus mRNAs Requires Ongoing RNA Polymerase II Activity. **2007,** *8* (1), 1-11.

90. Pang, B.; Cheung, N. N.; Zhang, W.; Dai, J.; Kao, R. Y.; Zhang, H.; Hao, Q., Structural Characterization of H1N1 Nucleoprotein-Nucleozin Binding Sites. *Scientific reports* **2016,** *6*, 29684.

91. Cheng, H.; Wan, J.; Lin, M.-I.; Liu, Y.; Lu, X.; Liu, J.; Xu, Y.; Chen, J.; Tu, Z.; Cheng, Y.-S. E.; Ding, K., Design, Synthesis, and in Vitro Biological Evaluation of 1H-1,2,3-Triazole-4-carboxamide Derivatives as New Anti-influenza A Agents Targeting Virus Nucleoprotein. *Journal of medicinal chemistry* **2012,** *55* (5), 2144-2153.

92. Liao, J.; Cheng, H.; Wan, J.; Chen, P.; Li, Y.; Ding, K.; Tortorella, M. D.; Tu, Z.; Zhang, Y. J. O. J. o. M. C., Evaluation of Benzamide Derivatives as New Influenza A Nucleoprotein Inhibitors. **2016,** *6* (03), 43.

93. Chan, W. H.; Ng, A. K.; Robb, N. C.; Lam, M. K.; Chan, P. K.; Au, S. W.; Wang, J. H.; Fodor, E.; Shaw, P. C., Functional analysis of the influenza virus H5N1

nucleoprotein tail loop reveals amino acids that are crucial for oligomerization and ribonucleoprotein activities. *Journal of virology* **2010,** *84* (14), 7337-45.

94. Shen, Y. F.; Chen, Y. H.; Chu, S. Y.; Lin, M. I.; Hsu, H. T.; Wu, P. Y.; Wu, C. J.; Liu, H. W.; Lin, F. Y.; Lin, G.; Hsu, P. H.; Yang, A. S.; Cheng, Y. S.; Wu, Y. T.; Wong, C. H.; Tsai, M. D., E339...R416 salt bridge of nucleoprotein as a feasible target for influenza virus inhibitors. *Proceedings of the National Academy of Sciences of the United States of America* **2011,** *108* (40), 16515-20.
95. Dilly, S.; Fotso Fotso, A.; Lejal, N.; Zedda, G.; Chebbo, M.; Rahman, F.; Companys, S.; Bertrand, H. C.; Vidic, J.; Noiray, M.; Alessi, M.-C.; Tarus, B.; Quideau, S.; Riteau, B.; Slama-Schwok, A., From Naproxen Repurposing to Naproxen Analogues and Their Antiviral Activity against Influenza A Virus. *Journal of medicinal chemistry* **2018,** *61* (16), 7202-7217.
96. Lejal, N.; Tarus, B.; Bouguyon, E.; Chenavas, S.; Bertho, N.; Delmas, B.; Ruigrok, R. W. H.; Primo, C. D.; Slama-Schwok, A., Structure-Based Discovery of the Novel Antiviral Properties of Naproxen against the Nucleoprotein of Influenza A Virus. **2013,** *57* (5), 2231-2242.
97. Zheng, W.; Fan, W.; Zhang, S.; Jiao, P.; Shang, Y.; Cui, L.; Mahesutihan, M.; Li, J.; Wang, D.; Gao, G. F.; Sun, L.; Liu, W., Naproxen Exhibits Broad Anti-influenza Virus Activity in Mice by Impeding Viral Nucleoprotein Nuclear Export. *Cell Reports* **2019,** *27* (6), 1875-1885.e5.
98. Tarus, B.; Bertrand, H.; Zedda, G.; Di Primo, C.; Quideau, S.; Slama-Schwok, A., Structure-based design of novel naproxen derivatives targeting monomeric nucleoprotein of Influenza A virus. *Journal of biomolecular structure & dynamics* **2015,** *33* (9), 1899-912.
99. Kakisaka, M.; Sasaki, Y.; Yamada, K.; Kondoh, Y.; Hikono, H.; Osada, H.; Tomii, K.; Saito, T.; Aida, Y., A Novel Antiviral Target Structure Involved in the RNA Binding, Dimerization, and Nuclear Export Functions of the Influenza A Virus Nucleoprotein. *PLoS Pathog* **2015,** *11* (7), e1005062.
100. Makau, J. N.; Watanabe, K.; Otaki, H.; Mizuta, S.; Ishikawa, T.; Kamatari, Y. O.; Nishida, N., A Quinolinone Compound Inhibiting the Oligomerization of Nucleoprotein of Influenza A Virus Prevents the Selection of Escape Mutants. *Viruses* **2020,** *12* (3).
101. Huang, F.; Chen, J.; Zhang, J.; Tan, L.; Lu, G.; Luo, Y.; Pan, T.; Liang, J.; Li, Q.; Luo, B.; Zhang, H.; Lu, G., Identification of a novel compound targeting the nuclear export of influenza A virus nucleoprotein. **2018,** *22* (3), 1826-1839.

102. Sethy, B.; Hsieh, C.-F.; Lin, T.-J.; Hu, P.-Y.; Chen, Y.-L.; Lin, C.-Y.; Tseng, S.-N.; Horng, J.-T.; Hsieh, P.-W., Design, Synthesis, and Biological Evaluation of Itaconic Acid Derivatives as Potential Anti-Influenza Agents. *Journal of medicinal chemistry* **2019,** *62* (5), 2390-2403.
103. White, K. M.; Abreu, P., Jr.; Wang, H.; De Jesus, P. D.; Manicassamy, B.; García-Sastre, A.; Chanda, S. K.; DeVita, R. J.; Shaw, M. L., Broad Spectrum Inhibitor of Influenza A and B Viruses Targeting the Viral Nucleoprotein. *ACS Infectious Diseases* **2018,** *4* (2), 146-157.
104. Asinex.com Screening Libraries. https://www.asinex.com/screening-libraries-(all-libraries).
105. Ugi, I. In *Versuche mit isonitrilen*, Angewandte Chemie-International Edition, WILEY-V CH VERLAG GMBH MUHLENSTRASSE 33-34, D-13187 BERLIN, GERMANY: 1959; pp 386-386.
106. Marcaccini, S.; Torroba, T., The use of the Ugi four-component condensation. *Nature Protocols* **2007,** *2* (3), 632-639.
107. Alvim, H. G. O.; da Silva Júnior, E. N.; Neto, B. A. D., What do we know about multicomponent reactions? Mechanisms and trends for the Biginelli, Hantzsch, Mannich, Passerini and Ugi MCRs. *RSC Advances* **2014,** *4* (97), 54282-54299.
108. Rocha, R. O.; Rodrigues, M. O.; Neto, B. A. D., Review on the Ugi Multicomponent Reaction Mechanism and the Use of Fluorescent Derivatives as Functional Chromophores. *ACS Omega* **2020,** *5* (2), 972-979.
109. Abbracchio, M.; Eberini, I.; Parravicini, C.; Martini, C.; Trincavelli, M. L.; Daniele, S., Gpr17-modulating compounds, diagnostic and therapeutic uses thereof. Google Patents: 2015.
110. Gewald, K.; Bellmann, P. J. L. A. d. C., Synthese und Reaktionen von 4-Aminoisothiazolen. **1979,** *1979* (10), 1534-1546.
111. Walther, E.; Xu, Z.; Richter, M.; Kirchmair, J.; Grienke, U.; Rollinger, J. M.; Krumbholz, A.; Saluz, H. P.; Pfister, W.; Sauerbrei, A.; Schmidtke, M., Dual Acting Neuraminidase Inhibitors Open New Opportunities to Disrupt the Lethal Synergism between Streptococcus pneumoniae and Influenza Virus. *Frontiers in microbiology* **2016,** *7*, 357.
112. Schade, D.; Kotthaus, J.; Riebling, L.; Kotthaus, J.; Müller-Fielitz, H.; Raasch, W.; Hoffmann, A.; Schmidtke, M.; Clement, B., Zanamivir Amidoxime- and N-

Hydroxyguanidine-Based Prodrug Approaches to Tackle Poor Oral Bioavailability. *Journal of pharmaceutical sciences* **2015,** *104* (9), 3208-19.

113. Schmidtke, M.; Schnittler, U.; Jahn, B.; Dahse, H.; Stelzner, A., A rapid assay for evaluation of antiviral activity against coxsackie virus B3, influenza virus A, and herpes simplex virus type 1. *Journal of virological methods* **2001,** *95* (1-2), 133-43.
114. Döring, K.; Langeder, J.; Duwe, S.; Tahir, A.; Grienke, U.; Rollinger, J. M.; Schmidtke, M., Insights into the direct anti-influenza virus mode of action of Rhodiola rosea. *Phytomedicine : international journal of phytotherapy and phytopharmacology* **2022,** *96*, 153895.
115. Naim, S. S.; Sharma, S., A Convenient Synthesis of 1,2,5-Trisubstituted 4,5-Dihydroimidazoles and 1,3-Diazaspiro [4.4(5)]alk-2-enes from N-Alkylideneanilines. *Synthesis* **1992,** *1992* (07), 664-666.
116. Katritzky, A. R.; Pilarski, B.; Urogdi, L., Efficient Conversion of Nitriles to Amides with Basic Hydrogen Peroxide in Dimethyl Sulfoxide. *Synthesis* **1989,** *1989* (12), 949-950.
117. Albericio, F.; El-Faham, A., Choosing the Right Coupling Reagent for Peptides: A Twenty-Five-Year Journey. *Organic Process Research & Development* **2018,** *22* (7), 760-772.
118. Hu, L.; Xu, S.; Zhao, Z.; Yang, Y.; Peng, Z.; Yang, M.; Wang, C.; Zhao, J., Ynamides as Racemization-Free Coupling Reagents for Amide and Peptide Synthesis. *Journal of the American Chemical Society* **2016,** *138* (40), 13135-13138.
119. Vargas-Berenguel, A.; Ortega-Caballero, F.; Santoyo-González, F.; García-López, J. J.; Giménez-Martínez, J. J.; García-Fuentes, L.; Ortiz-Salmerón, E., Dendritic Galactosides Based on a β-Cyclodextrin Core for the Construction of Site-Specific Molecular Delivery Systems: Synthesis and Molecular Recognition Studies. **2002,** *8* (4), 812-827.
120. Ayman El-Faham; Subirã³S Funosas, R.; Rafel Prohens; Fernando Albericio, COMU: A Safer and More Effective Replacement for Benzotriazole-Based Uronium Coupling Reagents. **2009,** *15*, 9404-9416.
121. Sperry, J. B.; Minteer, C. J.; Tao, J.; Johnson, R.; Duzguner, R.; Hawksworth, M.; Oke, S.; Richardson, P. F.; Barnhart, R.; Bill, D. R.; Giusto, R. A.; Weaver, J. D., III, Thermal Stability Assessment of Peptide Coupling Reagents Commonly Used in Pharmaceutical Manufacturing. *Organic Process Research & Development* **2018,** *22* (9), 1262-1275.

122. Wang, Q.; Wang, D.-X.; Wang, M.-X.; Zhu, J., Still Unconquered: Enantioselective Passerini and Ugi Multicomponent Reactions. *Accounts of Chemical Research* **2018,** *51* (5), 1290-1300.
123. Zhang, J.; Yu, P.; Li, S.-Y.; Sun, H.; Xiang, S.-H.; Wang, J.; Houk, K. N.; Tan, B., Asymmetric phosphoric acid–catalyzed four-component Ugi reaction. **2018,** *361* (6407), eaas8707.
124. Pérez-Fuertes, Y.; Taylor, J. E.; Tickell, D. A.; Mahon, M. F.; Bull, S. D.; James, T. D., Asymmetric Strecker Synthesis of α-Arylglycines. *The Journal of Organic Chemistry* **2011,** *76* (15), 6038-6047.
125. Stout, D. M.; Black, L. A.; Matier, W. L., Asymmetric Strecker synthesis: isolation of pure enantiomers and mechanistic implications. *The Journal of Organic Chemistry* **1983,** *48* (26), 5369-5373.
126. Dawei Ma; Qian Cai; Hui Zhang, Mild Method for Ullmann Coupling Reaction of Amines and Aryl Halides. *Organic Letters* **2003,** *5* (14), 2453-2455.
127. Luo, S.; De Brabander, J. K., Ligand-free copper-catalyzed coupling of α-amino acids with N-Boc-2-iodoanilines for the synthesis of enantiopure 3-substituted dihydroquinoxalinones. *Tetrahedron Letters* **2015,** *56* (23), 3179-3182.
128. Ma, D.; Zhang, Y.; Yao, J.; Wu, S.; Tao, F., Accelerating Effect Induced by the Structure of α-Amino Acid in the Copper-Catalyzed Coupling Reaction of Aryl Halides with α-Amino Acids. Synthesis of Benzolactam-V8. *Journal of the American Chemical Society* **1998,** *120* (48), 12459-12467.
129. Fulmer, G. R.; Miller, A. J. M.; Sherden, N. H.; Gottlieb, H. E.; Nudelman, A.; Stoltz, B. M.; Bercaw, J. E.; Goldberg, K. I., NMR Chemical Shifts of Trace Impurities: Common Laboratory Solvents, Organics, and Gases in Deuterated Solvents Relevant to the Organometallic Chemist. *Organometallics* **2010,** *29* (9), 2176-2179.
130. Blessing, R. H., An empirical correction for absorption anisotropy. *Acta crystallographica. Section A, Foundations of crystallography* **1995,** *51 (Pt 1)*, 33-8.
131. Busing, W. R.; Levy, H. A. J. A. C., High-speed computation of the absorption correction for single-crystal diffraction measurements. **1957,** *10* (3), 180-182.
132. Dolomanov, O. V.; Bourhis, L. J.; Gildea, R. J.; Howard, J. A.; Puschmann, H. J. J. o. a. c., OLEX2: a complete structure solution, refinement and analysis program. **2009,** *42* (2), 339-341.
133. Sheldrick, G. M. J. A. C. S. A. F.; Advances, SHELXT–Integrated space-group and crystal-structure determination. **2015,** *71* (1), 3-8.

134. Spek, A. L. J. A. C. S. D. B. C., Structure validation in chemical crystallography. **2009,** *65* (2), 148-155.

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DECLARATION ON OATH

I hereby declare on oath that this doctoral dissertation was written independently and solely on my own based on the original work of my PhD and has not been used other than the acknowledged resources and aids.

Hamburg 17.11.2023

Place, Date

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