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# Synthesis of GluN2A-selective NMDA receptor antagonists with an electron-rich aromatic B-ring

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## Abstract

Glutamatergic *N*-Methyl-D-aspartate (NMDA) receptors are heterotetrameric ion channels that can be comprised of different subunits. GluN2A subunit-containing NMDA receptors are associated with diseases like anxiety, depression, and schizophrenia. However, the exact contribution of these NMDA receptor subtypes is

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still unclear. To understand better the role of the GluN2A-containing receptors, novel ligands were designed. In co-crystallization with the isolated binding site, TCN-201 (**1**) and analogs adopt a U-shape conformation with parallel orientation of rings A and B. In order to increase the π/π-interactions between these rings, ring B of TCN-201 was replaced bioisosterically by different electron-rich thiazole, oxazole, and isoxazole heterocycles. The inhibitory activity was measured by two-electrode voltage clamp experiments with *Xenopus laevis* oocytes expressing GluN2A-containing NMDA receptors. It was found that **21c**, **31a**, **37a**, and **37b** were able to inhibit the ion channel. The isoxazole derivative **37b** was the most potent negative allosteric modulator displaying 40 % of the TCN-201 activity at a concentration of 10 µM.

**Keywords**

NMDA receptor; GluN2A selective antagonists; negative allosteric modulator; TCN-201; bioisosteric replacement; electron rich heterocycles; two-electrode voltage clamp; biological evaluation; electrophysiology.

## 1. Introduction

The *N*-methyl-D-aspartate (NMDA) receptor belongs to the group of ionotropic glutamate receptors and is mainly expressed in the central nervous system.[1] This ligand gated ion channel is one of the most intensively studied topics in medical research due to its complexity, its diversity and its participation in a variety of physiological processes and neurological disorders.[2] The name has been derived from *N*-methyl-D-aspartate (NMDA), which binds with high affinity and selectivity to it.[3] In addition to $Na^+$ and $K^+$ ions, the NMDA receptor is able to conduct high

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amounts of $Ca^{2+}$ ions leading to elevated intracellular $Ca^{2+}$ concentrations that are important for synaptic plasticity.[4] Highly enhanced NMDA receptor activity can lead to $Ca^{2+}$ overload and subsequent excitotoxicity. Consequently, overactivation of NMDA receptors is associated with neurodegenerative disorders including Alzheimer's, Huntington's, and Parkinson's disease.[5,6]

Seven different subunits form different heterotetrameric NMDA receptors: GluN1, GluN2A-D, and GluN3A-B subunits. A functional NMDA receptor is composed of two GluN1 and typically two GluN2 subunits.[7,8] While different genes encode for different GluN2 and GluN3 subunits, only one gene encodes for GluN1, which exists due to alternate splicing in 8 different variants GluN1a-h. Even though different genes encode the subunits, they share a common structure.[9,10] Each subunit comprises of an extracellular amino-terminal domain (ATD), the ligand-binding domain (LBD), a transmembrane domain (TMD) with three transmembrane helices and an intracellular C-terminal domain (CTD). The physiological gating properties of the NMDA receptor depend manly on the receptor forming GluN2 subunit.[11]

A particular feature of the NMDA receptor is the large number of binding sites for endogenous molecules as well as for drugs. For activation, NMDA receptors require the simultaneous binding of the two endogenous agonists (*S*)-glutamate and glycine together. A slight depolarization of the plasmic membrane is required for the relief of the pore blocking $Mg^{2+}$ ion to allow for ion flux through the activated receptor. The binding sites for the two agonists are located at the channel forming subunits GluN1 (glycine) and GluN2 ((*S*)-glutamate). Channel blockers such as phencyclidine (PCP binding site), $Mg^{2+}$ ions and ketamine are located within the channel pore.[12,13]

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Furthermore, several allosteric binding sites exist at the amino terminal domain including binding sites for $H^+$, spermidine and $Zn^{2+}$ ions.[14]

GluN2A subunit-containing NMDA receptors are highly expressed in the hippocampus and cerebral cortex and moderately expressed in the midbrain, cerebellum, striatum, and brainstem. Beside the mentioned pathophysiological processes, GluN2A-containing NMDA receptors are also involved in diseases like epilepsy, cerebral ischemia and schizophrenia. However, the GluN2A subunit is not only found in the brain but also in peripheral tissues. Makhro *et al*. reported the expression of NMDA receptor subunits in rat hearts. The expression of the GluN2A subunit was restricted to the atria. In the heart, activation of NMDA receptors is associated with tachycardia, sinus arrhythmia and ischemia.[15] Due to the lack of appropriate ligands and tool compounds, the exact role of the GluN2A subunit remains to be elucidated.

The first class of GluN2A-selective negative allosteric modulators (NAMs) was reported by Bettini *et al*. in 2010.[16] TCN-201 (**1**) represents the prototypical member of this class. (Figure 1) It inhibits glycine binding to the GluN1 subunit of the GluN1/GluN2A NMDA receptor (here GluN2A receptor) by binding at the interface between GluN1 and GluN2A subunits. With an $IC_{50}$ value of 109 nM TCN-201 inhibits the ion flux in HEK cells. TCN-201 does not inhibit GluN2B and GluN2D receptors. However, the potency of TCN-201 to inhibit GluN2A receptors decreases with increasing concentrations of extracellular glycine. At a concentration of 300 µM of glycine, TCN-201 could no longer inhibit the ion flux. Also, TCN-201 has very low solubility, which limits its use in biological studies.[17-19] In 2016, Volkmann *et al*.[19]

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reported two analogs of TCN-201 by replacing the phenyl ring in the middle of TCN-201 (ring B) by a pyrazine ring resulting in MPX-004 (**2**) and MPX-007 (**3**). (Figure 1) Both pyrazine derivatives **2** and **3** show higher activity ($IC_{50}$ = 79 nM and 27 nM), better solubility, and less glycine dependence when compared to TCN-201.

Systematic modification of the benzenesulfonamide part of **1** was reported recently. It was shown that a halogen atom in 3-position of the benzenesulfonamide part resulted in high potency at GluN2A receptors. The 3-bromo derivative **4** was 2.5-fold more potent than TCN-201 (**1**).[20] Modifications of the terminal benzoyl moiety of **1** led to the thiophen-2-ylcarbonyl derivative **5** with also 2.5-fold increased activity.[21]

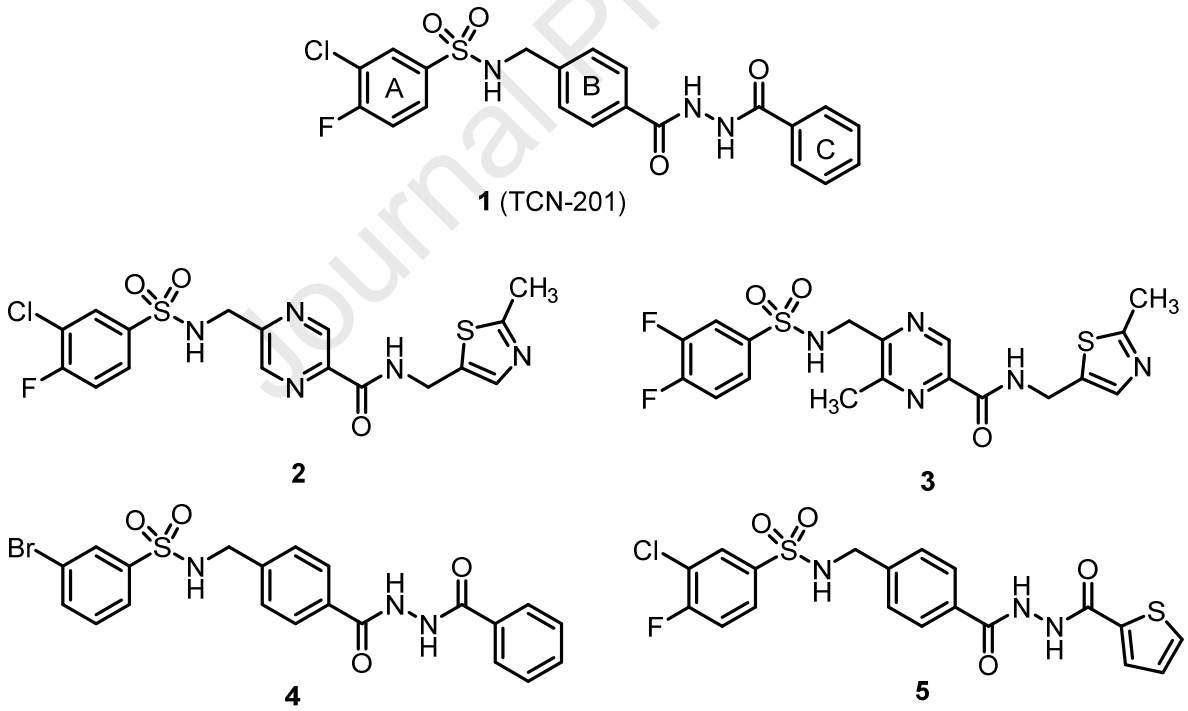


Figure 1: GluN2A selective NMDA ligands

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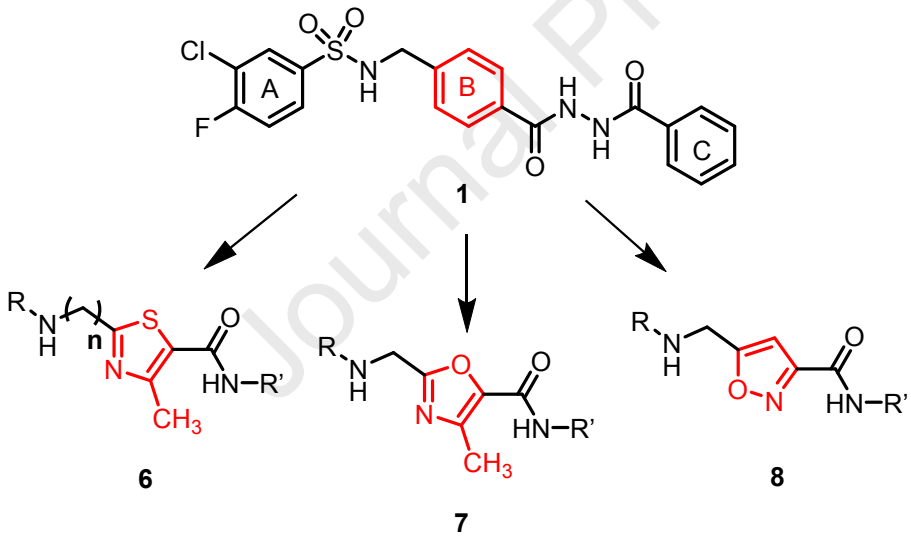
The X-ray crystal structure of the GluN1-GluN2A ligand binding domain together with the lead compound **1** shows a hairpin-like or U-shaped conformation of **1** within the binding pocket of the receptor. This U-shaped conformation of **1** is favored by π stacking of the benzene rings A and B. However, both benzene rings are electron deficient aromatic rings. Therefore, the idea came up to replace the benzene ring in the middle (ring B) by an electron-rich five membered heterocycle, which should increase the π stacking with ring A and at the end the interactions with the GluN2A receptor.[22] Herein, we wish to report the synthesis and pharmacological evaluation of novel analogs of TCN-201 (**1**) containing electron rich five-membered heterocycles thiazole, oxazole and isoxazole instead of benzene ring B, which should help to




better understand the role of ring B in NAM activity. (Figure 2)

Figure 2: GluN2A selective NMDA receptor antagonists designed by replacement of ring B of the lead compound **1** by thiazole (**6**), oxazole (**7**), and isoxazole (**8**) rings.

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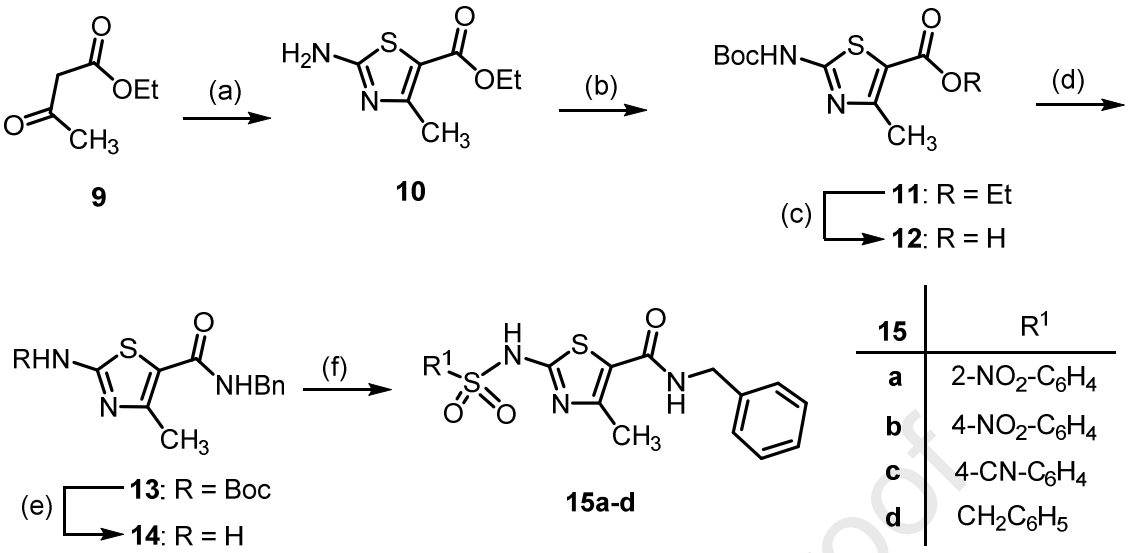


## 2. Synthesis

Scheme 1: Synthesis of aminothiazole-based TCN-201 analogs **15a-d**. Reagents and reaction conditions: (a) 1. NBS, THF : $H_2O$ 2 : 1, 0 °C to rt, 3 h; 2. thiourea, 80 °C, 2 h, 84 %. (b) $(Boc)_2O$, $Et_3N$, DMAP, THF, rt, 16 h, 87 %. (c) 2 M KOH, $CH_3OH$ : THF 1 : 1, rt, 16 h, 75 %. (d) $BnNH_3Cl$, COMU, DIPEA, THF; rt, 16 h, 77 %. (e) $F_3CCO_2H$, $CH_2Cl_2$, rt, 1 h, 85 %. (f) $R^1SO_2Cl$, NaH, THF, 0 °C to rt, overnight, **15a**: 75 %; **15b**: 67 %; **15c**: 76 %; **15d**: 84 %.

At first, TCN-201 analogs **15a**-**d** with a thiazole moiety as ring B were envisaged. The synthesis started with a two-step one-pot preparation of thiazolecarboxylate **10**.[23] At first ethyl acetoacetate (**9**) was converted with NBS into the corresponding 2-bromo derivative, which reacted with thiourea affording the thiazolecarboxylate **10** in 84 % yield. After introduction of the Boc-protective group, ester **11** was saponified by KOH to provide the acid **12**. Acid **12** was coupled with benzylamine in the presence of COMU leading to the N-benzylcarboxamide **13** in 85 % yield. Finally, the Boc-protective group was removed by $F_3CCO_2H$ to give the primary amine **14**,[24]

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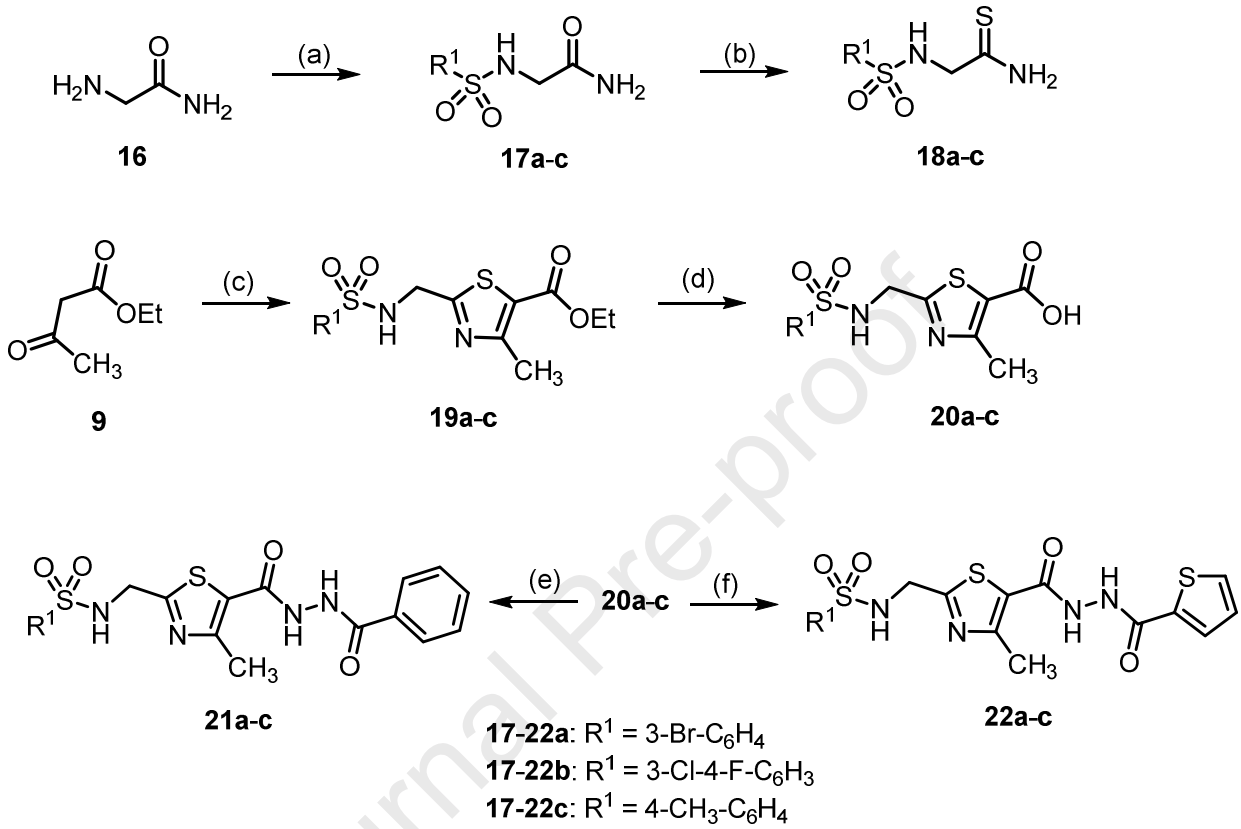
which allowed the introduction of various sulfonyl moieties at the very end of the synthesis providing diverse sulfonamides **15a**-**d**. (Scheme 1)




Scheme 2: Synthesis of (aminomethyl)thiazole-based TCN-201 analogs **21** and **22**. Reagents and reaction conditions: (a) $R^1SO_2Cl$, $Et_3N$, THF : $H_2O$ 5 : 1, 0 °C to rt, 3 h, **17a**: 68 %; **17b**: 95 %; **17c**: 67 %. (b) Lawesson's reagent, dioxane, 70 $^oC$, 27 h, **18a**: 95 %; **18b**: 76 %; **18c**: 86 %. (c) 1. NBS, THF : $H_2O$ 2 : 1, 0 °C to rt, 3 h; 2. **18a-c**, 80 °C, 16 h, **19a**: 42 %; **19b**: 56 %; **19c**: 71 %. (d) 2 M KOH, $CH_3OH$ : THF 1 : 1, rt, 16 h, **20a**: 81 %; **20b**: 75 %; **20c**: 79 %. (e) $PhC(=O)NHNH_2$, COMU, DIPEA, THF; rt, 16 h, **21a**: 78 %; **21b**: 69 %; **21c**: 66 %. (f) Thiophen-2-C(=O)$NHNH_2$, COMU, DIPEA, THF; rt, 16 h, **22a**: 72 %; **22b**: 66 %; **22c**: 73 %.

The first step in the synthesis of (aminomethyl)thiazole analogs **21** and **22** was the reaction of glycinamide (**16**) with different sulfonyl chlorides to obtain sulfonylated glycinamides **17a-c**, which were then converted into thioglycinamides **18a-c** upon

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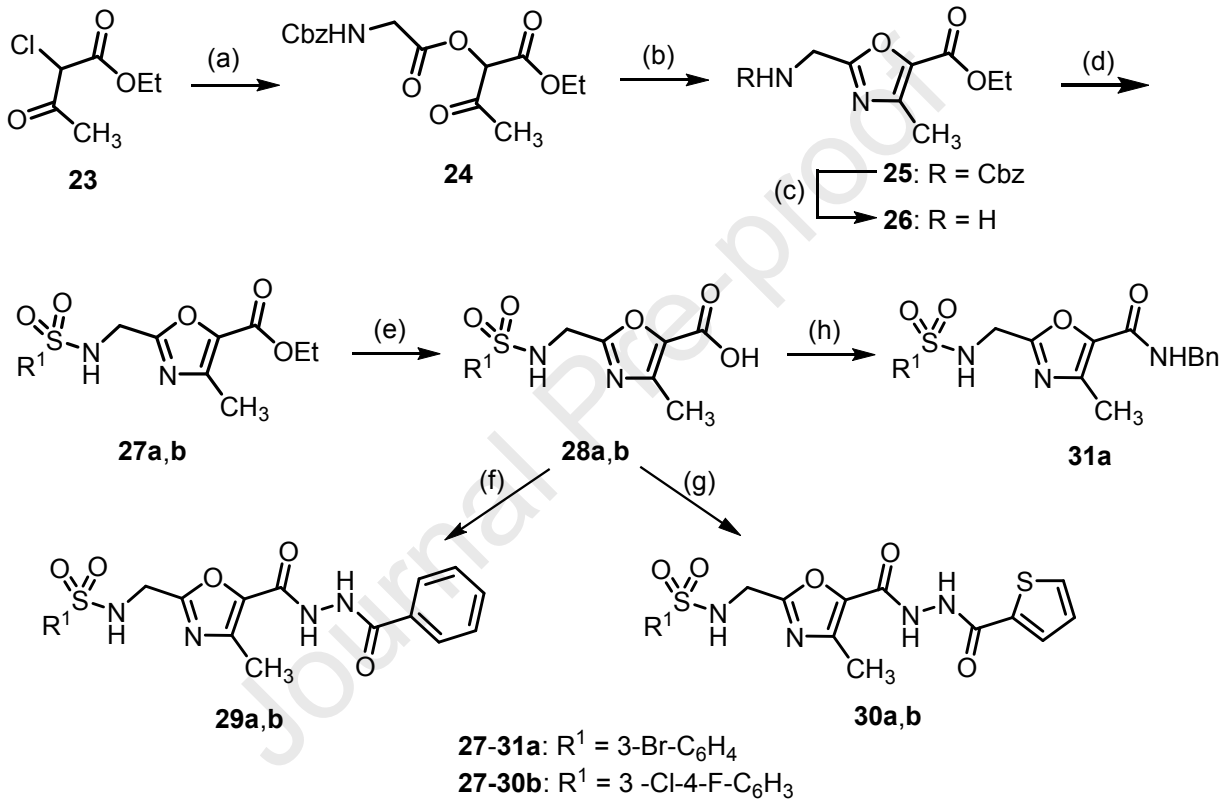
treatment with Lawesson's reagent. (Scheme 2) The one-pot reaction of ethyl acetoacetate (**9**), *N*-bromosuccinimide, and the sulfonylated thiogycinamides **18a-c** in water/THF led to thiazole-5-carboxylates **19a-c**. Saponification of ethyl esters **19a-c** with KOH provided acids **20a-c**, which were then coupled with benzoylhydrazine and thiophen-2-ylcarbonylhydrazine to afford the diacylhydrazine derivatives **21a**-**c** and **22a**-**c**. (Scheme 2)




Scheme 3: Synthesis of (aminomethyl)oxazole-based TCN-201 analogs **29**-**31**. Reagents and reaction conditions: (a) *N*-[(benzyloxy)carbonyl]glycine ($CbzNHCH_2CO_2H$), $Et_3N$, ethyl acetate, 0 °C to 70 °C, 16 h, 60 %. (b) $CH_3CO_2NH_4$, $CH_3CO_2H$, 135 °C, 16 h, 95 %. (c) $H_2$, Pd/C, THF : $CH_3OH$ 1 : 1, rt, 10 h, 78 %. (d) $R^1SO_2Cl$, $Et_3N$, THF, 0 °C to rt, 16 h; **27a**: 57 %; **27b**: 47 %. (e) 2 M KOH, THF : $CH_3OH$ 1 : 1, rt, 16 h; **28a**: 86 %; **28b**: 72 %. (f) $PhC(=O)NHNH_2$, COMU, DIPEA, THF, rt, 16 h; **29a**: 76 %; **29b**: 71 %. (g) Thiophen-2-$C(=O)NHNH_2$, COMU, DIPEA, THF; rt, 16 h, **30a**: 70 %; **30b**: 75 %. (h) $BnNH_2$, COMU, DIPEA, THF; rt, 16 h, 71 %.

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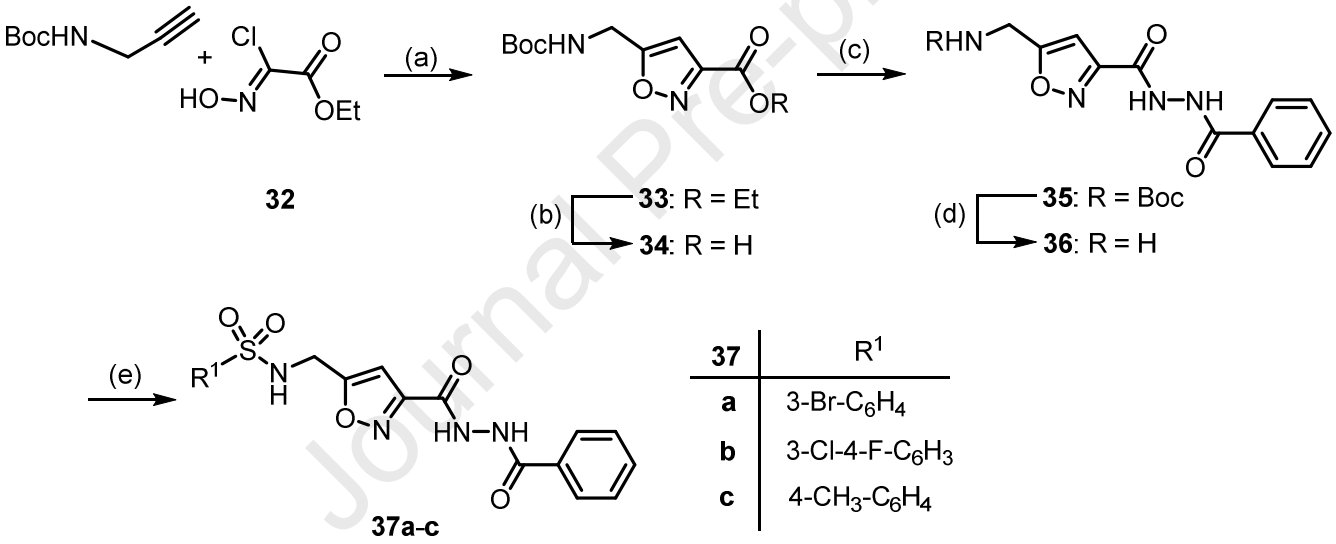
In the next series of GluN2A ligands, the S-atom of the thiazoles **21** and **22** was replaced by an O-atom (**29**-**31**). For the synthesis of oxazole derivatives **29**-**31** a nucleophilic substitution of α-chloro-β-ketoester **23** with Cbz-protected glycinate was performed providing the α-substituted β-ketoester **24**, which reacted with ammonium acetate to form oxazolecarboxylate **25**.[25] The primary amine **26** was obtained by hydrogenolytic removal of the Cbz-protective group of **25** using Pd/C as catalyst. Reaction of the primary amine **26** with two different sulfonyl chlorides provided sulfonamides **27a** and **27b**. After hydrolysis of the esters **27a** and **27b** with KOH, the free acids **28a** and **28b** were coupled with benzoylhydrazine, (thiophen-2-




| 37 | $R^1$ |
|---|---|
| **a** | 3-Br-$C_6H_4$ |
| **b** | 3-Cl-4-F-$C_6H_3$ |
| **c** | 4-$CH_3$-$C_6H_4$ |

ylcarbonyl)hydrazine and benzylamine to achieve the oxazole-based test compounds **29**-**31**. (Scheme 3)

Scheme 4: Synthesis of (aminomethyl)isoxazole-based TCN-201 analogs. Reagents and reaction conditions: (a) $Et_3N$, THF : $Et_2O$ 1 : 3, rt, 16 h, 69 %. (b) 2 M KOH, $CH_3OH$ : THF 1 : 1, rt, 16 h, 86 %. (c) benzoylhydrazine, COMU, DIPEA, THF; rt, 16 h, 75 %. (d) $F_3CCO_2H$, $CH_2Cl_2$, rt, 1h, 83 %. (e) $R^1SO_2Cl$, $Et_3N$, THF, 0 °C to rt, 16 h; **37a**: 66 %; **37b**: 67 %; **37c**: 64 %.

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The regioisomeric bioisosteric isoxazole derivatives **37** were prepared starting with a 1,3-dipolar cycloaddition of ethyl 2-chloro-2-(hydroxyimino)acetate (**32**) with *N*-Boc propargylamine.[26] The resulting ethyl isoxazolecarboxylate **33** was hydrolyzed to yield the acid **34**, which was coupled with benzoylhydrazine to obtain die diacylhydrazine derivative **35**. Subsequent removal the Boc protective group with $CF_3CO_2H$ provided the primary amine **36**, which was sulfonylated with three different sulfonyl chlorides to yield the isoxazole-based TCN-201 analogous test compounds **37a-c**. (Scheme 4)

Synthesis of (aminomethyl)thiazole and -oxazole TCN-201 analogs **21**,**22** and **29**,**30** involved the introduction of the acylhydrazine moiety as last step allowing facile diversification of rind C. The last step in the synthesis of aminothiazoles **15** and (aminomethyl)isoxazoles **37** was the sulfonylation of a primary amine allowing the facile diversification of the arylsulfonyl part (ring A) of TCN-201 analogs. All the acid-amine coupling reactions were performed in the presence of the coupling reagent COMU giving high yields, respectively.

## 3. Biological evaluation

The inhibitory activity of the synthesized test compounds was assessed electrophysiologically by two-electrode voltage clamp (TEVC) technique in *Xenopus laevis* oocytes expressing functional GluN1a-GluN2A NMDA receptors. In each experiment, 10 µM (*S*)-glutamate and 10 µM glycine were used to activate the NMDA receptor. The resulting cationic current was recorded and the changes of the current upon addition of the test compounds (c = 10 µM) were measured. The inhibition caused by the test compound (c = 10 µM) was normalized to the inhibition

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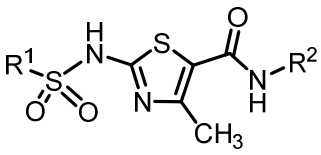
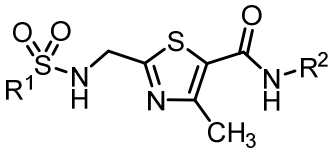
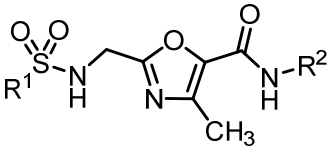
generated by TCN-201 (**1**, c = 10 µM) and is summarized in Table 1 for each compound.



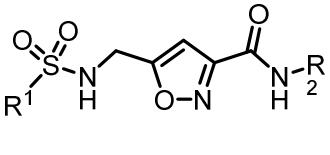
**15a-d**



**21,22a-c**



**29-31a,b**



**37a-c**

Table 1: Normalized GluN2A receptor inhibition $I_{norm}$ of the synthesized test compounds.

| compd | $R^1$ | $R^2$ | normalized Inhibition $I_{norm}$ ± SEM (%) |
|---|---|---|---|
| **1** (TCN-201) | - | - | 100 ± 0.06 % |
| **15a** | 2-$NO_2$-$C_6H_4$ | $CH_2C_6H_5$ | 8 ± 1.2 % |
| **15b** | 4-$NO_2$-$C_6H_4$ | $CH_2C_6H_5$ | 7 ± 0.2 % |
| **15c** | 4 CN-$C_6H_4$ | $CH_2C_6H_5$ | 6 ± 0.9 % |
| **15d** | $CH_2$-$C_6H_5$ | $CH_2C_6H_5$ | 7 ± 1.3 % |
| **21a** | 3-Br-$C_6H_4$ | NHC(=O)$C_6H_5$ | 7 ± 1.1 % |
| **21b** | 3-Cl-4-F-$C_6H_4$ | NHC(=O)$C_6H_5$ | 3 ± 0.7 % |
| **21c** | 4-$CH_3$-$C_6H_4$ | NHC(=O)$C_6H_5$ | 37 ± 5 % |
| **22a** | 3-Br-$C_6H_4$ | NHC(=O)thioph[#] | 2 ± 0.7 % |
| **22b** | 3-Cl-4-F-$C_6H_4$ | NHC(=O)thioph[#] | 2 ± 0.9 % |
| **22c** | 4-$CH_3$-$C_6H_4$ | NHC(=O)thioph[#] | 2 ± 1.2 % |
| **29a** | 3-Br-$C_6H_4$ | NHC(=O)$C_6H_5$ | -5 ± 3.9 % |
| **29b** | 3-Cl-4-F-$C_6H_4$ | NHC(=O)$C_6H_5$ | 13 ± 1.3 % |
| **30a** | 3-Br-$C_6H_4$ | NHC(=O)thioph[#] | 2 ± 0.8 % |

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| | | | |
|---|---|---|---|
| **30b** | 3-Cl-4-F-$C_6H_4$ | NHC(=O)thioph[#] | 14 ± 1.5 % |
| **31a** | 3-Br-$C_6H_4$ | $CH_2C_6H_5$ | 34 ± 1.9 % |
| **37a** | 3-Br-$C_6H_4$ | NHC(=O)$C_6H_5$ | 31 ± 1.4 % |
| **37b** | 3-Cl-4-F-$C_6H_4$ | NHC(=O)$C_6H_5$ | 40 ± 1.9 % |
| **37c** | 4-$CH_3$-$C_6H_4$ | NHC(=O)$C_6H_5$ | 12 ± 0.8 % |

[#] $_t$hioph = thiophen-2-yl

Relative percent inhibition of ion current measured by TEVC at GluN1a-GluN2A-expressing oocytes caused by test compounds (c = 10 µM) after activation by 10 µM (*S*)-glutamate and 10 µM glycine. Inhibition was normalized to the inhibition of 10 µM TCN-201 (**1**) (n = 3 in total) measured in the same experiment. Each compound was measured with three independent oocytes (n = 3).

The aminothiazole-based TCN-201 analogs **15a-d** displayed normalized inhibition of the GluN2A receptor ion channel lower than 10 % of the lead compound TCN-201 (**1**). Among the (aminomethyl)thiazole TCN-201 analogs, the tosyl derivative **21c** showed the highest normalized inhibition of 38 % ($p < 0.05$). All the other thiazoles revealed less than 7 % GluN2A receptor inhibition of TCN-201.

The aminomethyl oxazole and isoxazole derivatives **31a** (34 %), **37a** (29 %) and **37b** (40 %) showed the highest normalized GluN2A inhibition. The presence of a methyl group at the 4-position of the phenylsulfonyl moiety led to a considerable decrease in the normalized inhibition of isoxazole **37c** (11 %, $p < 0.05$) when compared with halogenated analogs **37a** and **37b**. This data synchronizes with the results published by Müller *et al*.[20] It can be concluded that halogen atoms at ring A increase the GluN2A inhibitory activity.

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In addition to the inhibition of GluN2A receptor associated ion channel, the affinity of the thiazoles **15**, **21**, and **22**, the oxazoles **29**-**31** and isoxazoles **37** towards the ifenprodil binding site of GluN2B NMDA receptors,[30,31] $\sigma_1$ und $\sigma_2$ receptors[32-34] was recorded in radioligand receptor binding assay. However, up to a concentration of 10 µM, the novel ligands did not interact with the ifenprodil binding site, $\sigma_1$ und $\sigma_2$ receptors. The low affinity towards the ifenprodil binding site and both σ receptor subtypes indicates high selectivity for the GluN2A receptor over these related receptors.

## 4. Conclusion

To analyze the effect of the bioisosteric replacement of ring B of the GluN2A inhibitor TCN-201 (**1**) by electron-rich aromatic rings on the negative allosteric modulation of GluN2A subunit-containing NMDA receptors, four different classes of compounds were designed and synthesized. The GluN2A channel blocking activity of the synthesized compounds was determined electrophysiologically by two-electrode voltage clamp technique. The oxazole and isoxazole derivatives **31a**, **37a** and **37b** have GluN2A NMDA receptor inhibitory activity in the range of 29-40 % of the lead compound TCN-201. However, none of the compounds reached the activity of TCN-201. In particular, the most potent ligands display high selectivity for GluN2A over GluN2B, $\sigma_1$ und $\sigma_2$ receptors.

## 5. Experimental part

### 5.1. Chemistry, general methods

Oxygen and moisture sensitive reactions were carried out under nitrogen, dried with silica gel with moisture indicator (orange gel, VWR, Darmstadt, Germany) and in dry

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glassware (Schlenk flask or Schlenk tube). Temperature was controlled with dry ice/acetone (-78 °C), ice/water (0 °C), Cryostat (Julabo TC100E-F, Seelbach, Germany), magnetic stirrer MR 3001 K (Heidolph, Schwalbach, Germany) or RCT CL (IKA, Staufen, Germany), together with temperature controller EKT HeiCon (Heidolph) or VT-5 (VWR) and PEG or silicone bath. All solvents were of analytical or technical grade quality. Demineralized water was used. $CH_2Cl_2$ was distilled from $CaH_2$; THF was distilled from sodium/benzophenone; MeOH was distilled from magnesium methanolate. Thin layer chromatography (tlc): tlc silica gel 60 $F_{254}$ on aluminum sheets (VWR). Flash chromatography (fc): Silica gel 60, 40–63 µm (VWR); parentheses include: diameter of the column (ø), length of the stationary phase (l), fraction size (v) and eluent. Automated flash chromatography: Isolera™ Spektra One (Biotage®); parentheses include: cartridge size, flow rate, eluent, fractions size was always 20 mL. Melting point: Melting point system MP50 (Mettler Toledo, Gießen, Germany), open capillary, uncorrected. MS: MicroTOFQII mass spectrometer (Bruker Daltonics, Bremen, Germany); deviations of the found exact masses from the calculated exact masses were 5 ppm or less; the data were analyzed with DataAnalysis® (Bruker Daltonics). NMR: NMR spectra were recorded in deuterated solvents on Agilent DD2 400 MHz and 600 MHz spectrometers (Agilent, Santa Clara CA, USA); chemical shifts ($\delta$) are reported in parts per million (ppm) against the reference substance tetramethylsilane and calculated using the solvent residual peak of the undeuterated solvent; coupling constants are given with 0.5 Hz resolution; assignment of $^{1}H$ and $^{13}C$ NMR signals was supported by 2-D NMR techniques where necessary.IR: FT/IR IR Affinity®-1 spectrometer (Shimadzu, Düsseldorf, Germany) using ATR technique.

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### 5.2. HPLC method for the determination of the purity

Equipment 1: Pump: L-7100, degasser: L-7614, autosampler: L-7200, UV detector: L-7400, interface: D-7000, data transfer: D-line, data acquisition: HSM-Software (all from Merck Hitachi, Darmstadt, Germany); Equipment 2: Pump: LPG-3400SD, degasser: DG-1210, autosampler: ACC-3000T, UV-detector: VWD-3400RS, interface: DIONEX UltiMate 3000, data acquisition: Chromeleon 7 (equipment and software from Thermo Fisher Scientific, Lauenstadt, Germany); column: LiChrospher® 60 RP-select B (5 µm), LiChroCART® 250-4 mm cartridge; flow rate: 1.0 mL/min; injection volume: 5.0 µL; detection at λ = 210 nm; solvents: A: demineralized water with 0.05 % (V/V) trifluoroacetic acid, B: $CH_3CN$ with 0.05 % (V/V) trifluoroacetic acid; gradient elution (% A): 0 - 4 min: 90 %; 4 - 29 min: gradient from 90 % to 0 %; 29 - 31 min: 0 %; 31 - 31.5min: gradient from 0 % to 90 %; 31.5 - 40 min: 90 %. Unless otherwise mentioned, the purity of all test compounds is greater than 95 %.

### 5.3. Synthetic procedures

The synthesis of the intermediates **10** – **14** is reported in references.[23, 24] The synthesis of the intermediates **24** and **25** is reported in reference.[25] The 1,3-dipolar cycloaddition to obtain **33** is described in referende.[26] Without any information about synthesis and properties compounds **17a** and **18a** can be found in databases https://pubchem.ncbi.nlm.nih.gov/compound/2556165 and https://pubchem.ncbi.nlm.nih.gov/compound/61127496, respectively. The synthesis of **17c** has already been mentioned in a patent,[32] but herein the procedure was optimized. For the synthesis of **18c**, Lawesson's reagent was used instead of $H_2S$,[33] herein.

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### 5.3.1. *N*-Benzyl-4-methyl-2-[(2-nitrophenyl)sulfonamido]thiazole-5-carboxamide (15a)

Primary amine **14**[23,24] (0.3 g, 1.2 mmol, 1.0 equiv.) was dissolved in THF (15 mL) and the solution was cooled to 0 °C. NaH (87 mg, 3.6 mmol, 3.0 equiv.) and a solution of 2-nitrobenzenesulfonyl chloride (0.27 g, 1.2 mmol, 1.0 equiv.) in THF (5 mL) were added. The reaction mixture was stirred overnight at room temperature. After completion of the transformation, the solvent was evaporated *in vacuo* and ethyl acetate (30 mL) and $H_2O$ (30 mL) were added. The organic layer was separated, and the aqueous layer was extracted with ethyl acetate (3 x 30 mL). The organic layer was dried ($Na_2SO_4$) and concentrated *in vacuo*. The residue was purified by FCC (Ø = 2 cm, l = 20 cm, V = 45 mL, $CH_2Cl_2$:$CH_3OH$ 95:5). $R_f$ = 0.45 ($CH_2Cl_2$:$CH_3OH$ 95:5). Pale yellow solid, mp 192 °C, yield 0.39 g (75 %). Purity (HPLC): 96.9 % ($t_R$ = 18.7 min). $C_{18}H_{16}N_4O_5S_2$ (432.5 g/mol). $^1$H NMR (400 MHz, $CD_3OD$): δ (ppm) = 2.42 (s, 3H, C*H*$_3$-B), 4.45 (s, 2H, CONHC*H*$_2$), 7.20 – 7.25 (m, 1H, 4-$H_C$), 7.28 – 7.32 (m, 4H, 2-$H_C$, 3-$H_C$, 5-$H_C$, 6-$H_C$), 7.70 – 7.75 (m, 3H, 4-$H_A$, 5-$H_A$, 6-$H_A$), 8.12 (dd, *J* = 7.3 / 2.3 Hz, 1H, 3-$H_A$). $^{13}$C NMR (101 MHz, $CD_3OD$): δ (ppm) = 11.9 (*C*$H_3$-B), 43.0 (CONH*C*$H_2$), 112.5 (C-5$_B$), 123.9 (C-5$_A$), 126.8 (C-4$_C$), 127.1 (2C, C-2$_C$, C-6$_C$), 128.1 (2C, C-3$_C$, C-5$_C$), 129.2 (C-3$_A$), 131.5 (C-4$_A$), 133.2 (2C, C-1$_A$, C-6$_A$), 134.4 (C-2$_A$), 138.4 (C-1$_C$), 139.1 (C-4$_B$), 161.4 (C-2$_B$), 167.1 (*C*ONHCH$_2$).

### 5.3.2. *N*-Benzyl-4-methyl-2-[(4-nitrophenyl)sulfonamido]thiazole-5-carboxamide (15b)

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Primary amine **14**[23,24] (0.3 g, 1.2 mmol, 1.0 equiv.) was dissolved in THF (15 mL) and the solution was cooled to 0 $^{o}$C. NaH (87 mg, 3.6 mmol, 3.0 equiv.) and a solution of 4-nitrobenzenesulfonyl chloride (0.27 g, 1.2 mmol, 1.0 equiv.) in THF (5 mL) were added. The reaction mixture was stirred overnight at room temperature. After completion of the transformation, the solvent was evaporated *in vacuo* and ethyl acetate (30 mL) and $H_2O$ (30 mL) were added. The organic layer was separated and the aqueous layer was extracted with ethyl acetate (3 x 30 mL). The organic layer was dried ($Na_2SO_4$) and concentrated *in vacuo*. The residue was purified by FCC (Ø = 2 cm, l = 20 cm, V = 40 mL, $CH_2Cl_2$:$CH_3OH$ 95:5). $R_f$ = 0.45 ($CH_2Cl_2$:$CH_3OH$ 95:5). Light yellow solid, mp 197 $^{o}$C, yield 0.35 g (67 %). $C_{18}H_{16}N_4O_5S_2$ (432.5 g/mol). $^{1}$H NMR (600 MHz, DMSO-$D_6$): δ (ppm) = 2.34 (s, 3H, C*H*$_3$-B), 4.34 (d, *J* = 6.0 Hz, 2H, CONHC*H*$_2$), 7.21 (t, *J* = 7.2 Hz, 1H, 4-$H_C$), 7.25 (d, *J* = 6.6 Hz, 2H, 2-$H_C$, 6-$H_C$), 7.30 (t, *J* = 7.3 Hz, 2H, 3-$H_C$, 5-$H_C$), 8.03 (d, *J* = 8.9 Hz, 2H, 2-$H_A$, 6-$H_A$), 8.34 (d, *J* = 8.9 Hz, 2H, 3-$H_A$, 5-$H_A$), 8.64 (t, *J* = 5.9 Hz, 1H, CON*H*$CH_2$), 13.18 (s, 1H, $SO_2$N*H*thiaz$_B$). $^{13}$C NMR (151 MHz, DMSO-$D_6$): δ (ppm) = 16.3 (*C*$H_3$-B), 45.8 (CONH*C*$H_2$), 115.3 (C-5$_B$) 127.7 (2C, C-3$_A$, C-5$_A$), 129.9 (C-4$_C$), 130.3 (2C, C-2$_A$, C-6$_A$), 130.4 (2C, C-2$_C$, C-6$_C$), 131.4 (2C, C-3$_C$, C-5$_C$), 142.2 (C-1$_C$), 142.3 (C-4$_B$), 150.4 (C-1$_A$), 152.6 (C-4$_A$), 163.1 (C-2$_B$), 169.5 (*C*ONHC$H_2$).

### 5.3.3. *N*-Benzyl-2-[(4-cyanophenyl)sulfonamido]-4-methylthiazole-5-carboxamide (15c)

Primary amine **14**[23,24] (0.1 g, 0.4 mmol, 1.0 equiv.) was dissolved in THF (10 mL) and the solution was cooled to 0 $^{o}$C. NaH (29 mg, 1.2 mmol, 3.0 equiv.) and a solution of 4-cyanobenzenesulfonyl chloride (80 mg, 0.4 mmol, 1.0 equiv.) in THF (5 mL) were added. The reaction mixture was stirred overnight at room temperature.

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After completion of the transformation, the solvent was evaporated *in vacuo* and ethyl acetate (30 mL) and $H_2O$ (30 mL) were added. The organic layer was separated, and the aqueous layer was extracted with ethyl acetate (3 x 30 mL). The organic layer was dried ($Na_2SO_4$) and concentrated *in vacuo*. The residue was purified by FCC (Ø = 1 cm, l = 15 cm, V = 20 mL, $CH_2Cl_2$:$CH_3OH$ 95:5). $R_f$ = 0.48 ($CH_2Cl_2$:$CH_3OH$ 95:5). Colorless solid, mp 200 $^oC$, yield 0.13 g (76 %). $C_{19}H_{16}N_4O_3S_2$ (432.5 g/mol). $^1H$ NMR (600 MHz, DMSO-$D_6$): δ (ppm) = 2.34 (s, 3H, C*H*$_3$-B), 4.33 (d, *J* = 5.9 Hz, 2H, CONHC*H*$_2$), 7.21 (t, *J* = 7.2 Hz, 1H, 4-$H_C$), 7.25 (d, *J* = 8.4 Hz, 2H, 2-$H_C$, 6-$H_C$), 7.30 (t, *J* = 8.1 Hz, 2H, 3-$H_C$, 5-$H_C$), 7.94 (d, *J* = 8.5 Hz, 2H, 3-$H_A$, 5-$H_A$), 8.01 (d, *J* = 8.6 Hz, 2H, 2-$H_A$, 6-$H_A$), 8.63 (t, *J* = 6.0 Hz, 1H, CON*H*$CH_2$), 13.15 (s, 1H, SO2N*H*thiaz$_B$). $^{13}C$ NMR (151 MHz, DMSO-$D_6$): δ (ppm) = 16.3 (*C*$H_3$-B), 45.8 (CONH*C*$H_2$), 115.2 (C-5$_B$), 117.8 (*C*≡N), 120.9 (C-1$_A$), 129.6 (2C, C-3$_A$, C-5$_A$), 130.0 (C-4$_C$), 130.4 (2C, C-2$_C$, C-6$_C$), 131.4 (2C, ,C-3$_C$, C-5$_C$), 136.5 (2C, C-2$_A$, C-6$_A$) 142.3 (C-4$_B$, C-1$_C$), 149.0 (C-4$_A$), 163.1 (C-2$_B$), 169.5 (*C*ONHC$H_2$).

### 5.3.4. *N*-Benzyl-4-methyl-2-[(phenylmethyl)sulfonamido]thiazole-5-carboxamide (15d)

Primary amine **14**[23,24] (10 mg, 0.04 mmol, 1.0 equiv.) was dissolved in THF (5 mL) and the solution was cooled to 0 $^oC$. NaH (0.2 mg, 0.1 mmol, 3.0 equiv.) and a solution of phenylmethanesulfonyl chloride (7 mg, 0.4 mmol, 1.0 equiv.) in THF (5 mL) were added. The reaction mixture was stirred overnight at room temperature. After completion of the transformation, the solvent was evaporated *in vacuo* and ethyl acetate (15 mL) and $H_2O$ (15 mL) were added. The organic layer was separated, and the aqueous layer was extracted with ethyl acetate (3 x 15 mL). The organic layer was dried ($Na_2SO_4$) and concentrated *in vacuo*. The residue was

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purified by FCC (Ø = 1 cm, l = 10 cm, V = 15 mL, $CH_2Cl_2$:$CH_3OH$ 95:5). $R_f$ = 0.45 ($CH_2Cl_2$:$CH_3OH$ 95:5). Colorless solid, mp 194 °C yield 14 mg (84 %). $C_{19}H_{19}N_3O_3S_2$ (432.5 g/mol). $^1$H NMR (600 MHz, DMSO-$D_6$): δ (ppm) = 2.34 (s, 3H, C*H*$_3$-B), 4.30 (d, *J* = 6.5 Hz, 2H, CONHC*H*$_2$), 4.31 (s, 2H, C*H*$_2$$SO_2$NH), 7.21 (t, *J* = 7.1 Hz, 1H, 4-$H_C$), 7.24 (d, *J* = 7.1 Hz, 2H, 2-$H_C$, 6-$H_C$), 7.27 – 7.31 (m, 5H, 3-$H_C$, 5-$H_C$, 3-$H_A$, 4-$H_A$, 5-$H_A$), 7.33 (dd, *J* = 7.9, 1.3 Hz, 2H, 2-$H_A$, 6-$H_A$), 8.51 (t, *J* = 5.9 Hz, 1H, CON*H*$CH_2$), 12.78 (s, 1H, $CH_2SO_2$N*H*). $^{13}$C NMR (151 MHz, DMSO-$D_6$): δ (ppm) = 16.23 (*C*$H_3$-B), 45.7 (CONH*C*$H_2$), 61.4 (*C*$H_2SO_2$NH), 113.9 (C-$5_B$), 129.9 (C-$4_C$), 130.3 (2C, C-$2_C$, C-$6_C$), 130.9 (C-$4_A$), 131.2 (2C, C-$3_C$, C-$5_C$) 131.4 (2C, C-$3_A$, C-$5_A$), 133.4 (2C, C-$1_A$, C-$1_C$), 134.0 (2C, C-$2_A$, C-$6_A$), 142.4 (C-$4_B$), 163.3 (C-$2_B$), 169.4 (*C*ONHC$H_2$).

### 5.3.5. 2-[(3-Bromophenyl)sulfonamido]acetamide (17a)

At 0 °C, $Et_3N$ (0.25 mL, 1.8 mmol, 2.0 equiv.) was added to a solution of **16** (0.10 g, 0.9 mmol, 1.0 equiv.) in DMF (25 mL). After 10 min, 3-bromobenzenesulfonyl chloride (0.13 g, 0.9 mmol, 1.0 equiv.) was added and the reaction mixture was stirred overnight at rt. After completion of the transformation, the solvent was evaporated *in vacuo* and $CH_2Cl_2$ (25 mL) and $H_2O$ (25 mL) were added. The organic layer was separated, and the aqueous layer was extracted with $CH_2Cl_2$ (3 x 25 mL). The organic layer was dried ($Na_2SO_4$) and concentrated *in vacuo*. The residue was purified by FCC (Ø = 2 cm, l = 20 cm, V = 20 mL, $CH_2Cl_2$/$CH_3OH$/$Et_3N$ 95:5:0.1). $R_f$ = 0.5 ($CH_2Cl_2$/$CH_3OH$ 9:1). Colorless solid, mp 186 °C, yield 0.18 g (68 %). $C_8H_9BrN_2O_3S$ (293.1 g/mol). $^1$H NMR (400 MHz, DMSO-$D_6$): δ (ppm) = 3.41 (s, 2H, $SO_2$NHC*H*$_2$), 7.06 (s, 1H, N-H), 7.28 (s, 1H, N-H), 7.53 (t, *J* = 7.9 Hz, 1H, 5-$H_{arom}$), 7.77 (ddd, *J* = 7.9 / 1.8 / 1.0 Hz, 1H, 6-$H_{arom}$), 7.83 (ddd, *J* = 8.0 / 2.1 / 1.0 Hz, 1H, 4-$H_{arom}$), 7.91 – 7.97 (m, 1H, 2-$H_{arom}$), 8.02 (s, 1H, $SO_2$N*H*$CH_2$).

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### 5.3.6. 2-[(3-Chloro-4-fluorophenyl)sulfonamido]acetamide (17b)

At 0 $^{o}$C, $Et_3N$ (0.25 mL, 1.8 mmol, 2.0 equiv.) was added to a solution of **16** (0.10 g, 0.9 mmol, 1.0 equiv.) in DMF (25 mL). After 10 min, 3-chloro-4-fluorobenzenesulfonyl chloride (0.13 g, 0.9 mmol, 1.0 equiv.) was added and the reaction mixture was stirred overnight at rt. After completion of the transformation, the solvent was evaporated *in vacuo* and $CH_2Cl_2$ (20 mL) and $H_2O$ (20 mL) were added. The organic layer was separated, and the aqueous layer was extracted with $CH_2Cl_2$ (3 x 25 mL). The organic layer was dried ($Na_2SO_4$) and concentrated *in vacuo*. The residue was purified by FCC (Ø = 2 cm, l = 15 cm, V = 25 mL, $CH_2Cl_2$/$CH_3OH$/$Et_3N$ 95:5:0.1). $R_f$ = 0.45 ($CH_2Cl_2$/$CH_3OH$ 9:1). Colorless solid, mp 184 $^{o}$C, yield 0.16 g (95 %). $C_8H_8ClFN_2O_3S$ (266.7 g/mol). $^1$H NMR (400 MHz, DMSO-$D_6$): δ (ppm) = 3.44 (s, 2H, $SO_2NHC\mathit{H_2}$), 7.06 (s, 1H, N-H), 7.29 (s, 1H, N-H), 7.62 (t, *J* = 8.9 Hz, 1H, 5-$H_{arom}$), 7.79 (ddd, *J* = 8.7 / 4.5 / 2.3 Hz, 1H, 6-$H_{arom}$), 7.98 (dd, *J* = 6.9 / 2.3 Hz, 1H, 2-$H_{arom}$), 8.05 (s, 1H, $SO_2N\mathit{H}CH_2$).

### 5.3.7. 2-[(4-Methylphenyl)sulfonamido]acetamide (17c)[32]

A solution of glycinamide **16** (0.10 g, 0.9 mmol, 1.0 equiv.) in THF:$H_2O$ (5:1, 30 mL) and $Et_3N$ (0.25 mL, 1.8 mmol, 2.0 equiv.) was cooled to 0 °C. After 10 min, p-TsCl (0.17 g, 0.9 mmol, 1.0 equiv.) was added and the reaction mixture was stirred overnight at rt. After completion of the transformation, the solvent was evaporated *in vacuo* and $CH_2Cl_2$ (25 mL) and $H_2O$ (25 mL) were added. The organic layer was separated, and the aqueous layer was extracted with $CH_2Cl_2$ (3 x 25 mL). The organic layer was dried ($Na_2SO_4$) and concentrated *in vacuo*. The residue was purified by FCC (Ø = 2 cm, l = 10 cm, V = 20 mL, $CH_2Cl_2$/$CH_3OH$/$Et_3N$ 95:5:0.1). $R_f$ =

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0.45 ($CH_2Cl_2$/$CH_3OH$ 9:1). Colorless solid, mp 180 $^oC$, yield 0.14 g (67 %). $C_9H_{12}N_2O_3S$ (228.3 g/mol). $^1H$ NMR (400 MHz, DMSO-$D_6$): δ (ppm) = 2.36 (s, 3H, C*H*$_3$), 3.31 (s, 2H, $SO_2NHCH_2$), 7.07 (s, 1H, N-H), 7.22 (s, 1H, N-H), 7.36 (d, *J* = 7.7 Hz, 2H, 3-$H_{arom}$, 5-$H_{arom}$), 7.67 (d, *J* = 8.3 Hz, 2H, 2-$H_{arom}$, 6-$H_{arom}$), 7.72 (s, 1H, $SO_2NHCH_2$).

### 5.3.8. 2-[(3-Bromophenyl)sulfonamido]thioacetamide (18a)

Compound **17a** (0.2 g, 0.6 mmol, 1.0 equiv.) and Lawesson's reagent (0.16 g, 0.3 mmol, 0.6 equiv.) were dissolved in dioxane (20 mL) and the mixture was heated to 70 $^oC$ for 27 h. After completion of transformation, the solvent was evaporated *in vacuo*. The residue was dissolved in saturated $NaHCO_3$ solution (20 mL) and the mixture was stirred for 30 min. The precipitated solid was filtered through a sintered funnel, washed with distilled $H_2O$ and dried. Colorless solid, mp 189 $^oC$, yield 0.20 g (95 %). $C_8H_9BrN_2O_2S_2$ (309.2 g/mol). $^1H$ NMR (400 MHz, DMSO-$D_6$): δ (ppm) = 3.70 (d, *J* = 6.2 Hz, 2H, $SO_2NHCH_2$), 7.54 (t, *J* = 7.9 Hz, 1H, 5-$H_{arom}$), 7.79 (ddd, *J* = 7.9 / 1.8 / 1.0 Hz, 1H, 6-$H_{arom}$), 7.85 (ddd, *J* = 8.0 / 2.1 / 1.0 Hz, 1H, 4-$H_{arom}$), 7.93 – 7.95 (m, 1H, 2-$H_{arom}$), 8.20 (t, *J* = 6.2 Hz, 1H, $SO_2NHCH_2$), 9.07 (s, 1H, N-H), 9.78 (s, 1H, N-H).

### 5.3.9. 2-[(3-Chloro-4-fluorophenyl)sulfonamido]thioacetamide (18b)

Compound **17b** (0.16 g, 0.6 mmol, 1.0 equiv.) and Lawesson's reagent (0.15 g, 0.3 mmol, 0.6 equiv.) were dissolved in dioxane (15 mL) and heated to 70 $^oC$ for 27 h. After completion of the transformation, the solvent was evaporated *in vacuo*. The residue was dissolved in saturated $NaHCO_3$ solution (20 mL) and the mixture was stirred for 30 min. The precipitated solid was filtered through a sintered funnel,

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washed with distilled $H_2O$ and dried. Colorless solid, mp 187 $^oC$, yield 0.13 g (76 %). $C_8H_8ClFN_2O_2S_2$ (282.7 g/mol). $^1H$ NMR (400 MHz, DMSO-$D_6$): δ (ppm) = 3.71 (s, 2H, $SO_2NHC\mathit{H_2}$), 7.61 (t, *J* = 8.9 Hz, 1H, 5-$H_{arom}$), 7.78 (ddd, *J* = 8.7 / 4.6 / 2.3 Hz, 1H, 6-$H_{arom}$), 7.96 (dd, *J* = 6.9 / 2.3 Hz, 1H, 2-$H_{arom}$). The signals for the N-H protons are not seen in the spectrum.

### 5.3.10. 2-[(4-Methylphenyl)sulfonamido]thioacetamide (18c)[33]

Compound **17c** (0.12 g, 0.5 mmol, 1.0 equiv.) and Lawesson's reagent (0.12 g, 0.3 mmol, 0.6 equiv.) were dissolved in dioxane (20 mL) and the mixture was heated at 70 $^oC$ for 27 h. After completion of the transformation, the solvent was evaporated *in vacuo*. The residue was dissolved in saturated $NaHCO_3$ solution (20 mL) and the mixture was stirred for 30 min at rt. The precipitated solid was filtered through a sintered funnel, washed with distilled $H_2O$ and dried. Light yellow solid, mp 189 $^oC$, yield 0.11 g (86 %). $C_9H_{12}N_2O_2S_2$ (244.3 g/mol). $^1H$ NMR (400 MHz, DMSO-$D_6$): δ (ppm) = 2.37 (s, 3H, $C\mathit{H_3}$), 3.61 (s, 2H, $SO_2NHC\mathit{H_2}$), 7.38 (d, *J* = 7.8 Hz, 2H, 3-$H_{arom}$, 5-$H_{arom}$), 7.66 (d, *J* = 8.3 Hz, 2H, 2-$H_{arom}$, 6-$H_{arom}$). The signals for the N-H protons are not seen in the spectrum.

### 5.3.11. Ethyl 2-[(3-bromophenyl)sulfonamidomethyl]-4-methylthiazole-5-carboxylate (19a)

*N*-Bromosuccinimide (1.77 g, 9.9 mmol, 1.2equiv.) was added to a mixture of **9** (1.05 mL, 8.3 mmol, 1.0 equiv.) in THF/$H_2O$ (2:1, 60 mL) at 0 $^oC$,. The mixture was stirred at rt for 3 h. **18a** (2.57 g, 8.3 mmol, 1.0 equiv.) was added and the mixture was heated to 80 $^oC$ for 16 h. THF was removed *in vacuo* and ethyl acetate (50 mL) was added. The organic layer was separated, and the aqueous layer was extracted with

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ethyl acetate (3 x 50 mL). The organic layer was dried ($Na_2SO_4$) and concentrated under reduced pressure. The residue was purified by FCC (Ø = 6 cm, l = 22 cm, V =100 mL, cyclohexane:ethyl acetate 7:3). $R_f$ = 0.68 (cyclohexane:ethyl acetate 1:1). Light brown oil, yield 1.5 g (42 %). $C_{14}H_{15}BrN_2O_4S_2$ (419.3 g/mol). $^1$H NMR (400 MHz, DMSO-$D_6$): δ (ppm) = 1.27 (t, *J* = 7.1 Hz, 3H, $CO_2CH_2CH_3$), 2.51 (s, 3H, $CH_3$-B), 4.23 (q, *J* = 7.1 Hz, 2H, $CO_2CH_2CH_3$), 4.35 (d, *J* = 6.3 Hz, 2H, $SO_2NHCH_2$), 7.51 (t, *J* = 7.9 Hz, 1H, 5-$H_A$), 7.77 (ddd, *J* = 7.8 / 1.8 / 1.0 Hz, 1H, 6-$H_A$), 7.82 (ddd, *J* = 8.0 / 2.0 / 1.0 Hz, 1H, 4-$H_A$), 7.86 (t, *J* = 1.8 Hz, 1H, 2-$H_A$), 8.85 (t, *J* = 6.3 Hz, 1H, $SO_2NHCH_2$). $^{13}$C NMR (151 MHz, DMSO-$D_6$): δ (ppm) = 17.2 ($CO_2CH_2CH_3$), 19.9 ($CH_3$-B), 47.1 ($SO_2NHCH_2$), 64.2 ($CO_2CH_2CH_3$), 124.8 (*C*-$5_B$), 125.2 (*C*-$3_A$), 128.7 (*C*-$6_A$), 131.9 (*C*-$2_A$), 134.5 (*C*-$5_A$), 138.5 (*C*-$4_A$), 145.5 (*C*-$1_A$), 162.2 (*C*-$4_B$), 164.4 (*C*=O), 174.4 (*C*-$2_B$).

### 5.3.12. Ethyl 2-[(3-chloro-4-fluorophenyl)sulfonamidomethyl]-4-methylthiazole-5-carboxylate (19b)

N-Bromosuccinimide (0.10 g, 0.48 mmol, 1.2equiv.) was added to a mixture of **9** (0.06 mL, 0.4 mmol, 1.0 equiv.) in THF/$H_2O$ (2:1, 5mL) at 0 $^o$C. The mixture was stirred at rt for 3 h and TLC showed the disappearance of ethyl acetoacetate. **18b** (0.13 g, 0.4 mmol, 1.0 equiv.) was added and the mixture was heated at 80 $^o$C for 16 h. THF was removed *in vacuo* and ethyl acetate (10mL) was added. The organic layer was separated, and the aqueous layer was extracted with ethyl acetate (3 x 10 ml). The organic layer was dried ($Na_2SO_4$) and concentrated under reduced pressure. The residue was purified by FCC (Ø = 2 cm, l = 22 cm, V = 30 mL, cyclohexane:ethylacetate 7:3). $R_f$ = 0.67 (cyclohexane:ethylacetate 1:1). Yellow oil, yield 0.10 g (56 %). $C_{14}H_{14}ClFN_2O_4S_2$ (392.8 g/mol). $^1$H NMR (400 MHz, DMSO-$D_6$):

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δ (ppm) = 1.26 (t, *J* = 7.1 Hz, 3H, $CO_2CH_2CH_3$), 2.51 (s, 3H, $CH_3$-B), 4.17 (d, *J* = 6.0 Hz, 0.6H, $SO_2NHCH_2$*), 4.24 (q, *J* = 7.1 Hz, 2H, $CO_2CH_2CH_3$), 4.37 (d, *J* = 6.3 Hz, 2H, $SO_2NHCH_2$), 7.52 (s, 0.18H, N-H*), 7.62 (t, *J* = 8.9 Hz, 1H, 5-$H_A$), 7.63 (t, *J* = 8.0 Hz, 0.07H, 5-$H_A$*), 7.69 (t, *J* = 8.9 Hz, 0.3H, 5-$H_A$*), 7.78 (ddd, *J* = 8.7 / 4.5 / 2.3 Hz, 1H, 6-$H_A$), 7.81 (ddd, *J* = 8.7 / 4.1 / 2.3 Hz, 0.07H, 6-$H_A$*), 7.85 (ddd, *J* = 8.7 / 4.5 / 2.3 Hz, 0.3H, 6-$H_A$*), 7.91 (dd, *J* = 6.8 / 2.3 Hz, 1H, 2-$H_A$), 7.98 (dd, *J* = 6.9 / 2.3 Hz, 0.09H, 2-$H_A$*), 8.03 (dd, *J* = 6.8 / 2.3 Hz, 0.3H, 2-$H_A$*), 8.71 (t, *J* = 6.0 Hz, 0.3H, $SO_2NHCH_2$*), 8.86 (t, *J* = 6.3 Hz, 1H, $SO_2NHCH_2$). The signals of **18b** were also seen in the spectra. The signals of **18b** are marked with asterisks. $^{13}$C NMR (101 MHz, DMSO-$D_6$): δ (ppm) = 14.5 ($CO_2CH_2CH_3$), 17.3 ($CH_3$-B), 30.8 (0.3C, $SO_2NHCH_2$*), 44.4 ($SO_2NHCH_2$), 61.5 ($CO_2CH_2CH_3$), 118.2 (d, *J* = 22.7 Hz, C-$5_A$), 121.2 (d, *J* = 18.0 Hz, C-$3_A$), 122.2 (C-$5_B$), 128.5 (d, *J* = 8.8 Hz, C-$6_A$), 129.6 (C-$2_A$), 130.0 (0.3C, C-$2_A$*), 138.5 (d, *J* = 3.8 Hz, C-$1_A$), 158.6 (d, *J* = 252.3 Hz, C-$4_A$), 159.5 (C-$4_B$), 161.7 ($CO_2CH_2CH_3$), 171.6 (C-$2_B$). The signals of **18b** were also seen in the spectra. The signals of **18b** are marked with asterisks.

### 5.3.13. Ethyl 4-methyl-2-{[(4-methylphenyl)sulfonamido]methyl}thiazole-5-carboxylate (19c)

N-Bromosuccinimide (89 mg, 0.5 mmol, 1.2equiv.) was added to a mixture of **9** (0.05 mL, 0.4 mmol, 1.0 equiv.) in THF/$H_2O$ (2:1, 5mL) at 0 $^oC$. The mixture was stirred at rt for 3 h and TLC showed the disappearance of ethyl acetoacetate. **18c** (0.10 g, 0.4 mmol, 1.0 equiv.) was added and the mixture was heated to 80 $^oC$ for 16 h. THF was removed *in vacuo* and ethyl acetate (10 mL) was added. The organic layer was separated, and the aqueous layer was extracted with ethyl acetate (3 x 10 mL). The organic layer was dried ($Na_2SO_4$) and concentrated under reduced pressure. The

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residue was purified by FCC (Ø = 1 cm, l = 20 cm, V = 25 mL, cyclohexane:ethylacetate 7:3). $R_f$ = 0.75 (cyclohexane:ethyl acetate 1:1). Yellow resin, yield 0.10 g (71 %). $C_{15}H_{18}N_2O_4S_2$ (354.4 g/mol). $^1$H NMR (400 MHz, DMSO-$D_6$): δ (ppm) = 1.27 (t, *J* = 7.1 Hz, 3H, $OCH_2CH_3$), 2.36 (s, 3H, $CH_3$-A), 2.53 (s, 3H, $CH_3$-B), 4.24 (d, *J* = 6.2 Hz, 2H,$SO_2NHCH_2$), 4.24 (q, *J* = 7.1 Hz, 2H, $OCH_2CH_3$), 7.37 (d, *J* = 7.8 Hz, 2H, 3-$H_A$, 5-$H_A$), 7.67 (d, *J* = 8.3 Hz, 2H, 2-$H_A$, 6-$H_A$), 8.61 (t, *J* = 6.3 Hz, 1H, $SO_2NHCH_2$). $^{13}$C NMR (151 MHz, DMSO-$D_6$): δ (ppm) = 17.2 ($OCH_2CH_3$), 20.1 ($CH_3$-B), 24.1 ($CH_3$-A), 47.2 ($SO_2NHCH_2$), 64.2 ($OCH_2CH_3$), 124.7 (C-$5_B$), 129.7 (2C, C-$2_A$, C-$6_A$), 132.8 (2C, C-$3_A$, C-$5_A$) 140.4 (C-$4_A$), 146.2 (C-$1_A$), 162.3 (C-$4_B$), 164.5 (*C*=O), 175.5 (C-$2_B$)

### 5.3.14. 2-[(3-Bromophenyl)sulfonamidomethyl]-4-methylthiazole-5-carboxylic acid (20a)

Ester **19a** (1.0 g, 2.3 mmol, 1.0 equiv.) was dissolved in a mixture of $CH_3OH$ and THF (1:1, 50 mL). Then, 2 M KOH (1.19 mL, 2.3 mmol, 1.0 equiv.) was added and the reaction mixture was stirred for 16 h at rt. After completion of the conversion, the solvent was evaporated *in vacuo*. 1 M HCl (4.0 mL) was added dropwise to the residue untill the pH was <7 and the precipitated solid was collected by filtration. $R_f$ = 0.32 ($CH_2Cl_2$:$CH_3OH$ 2:1). Colorless solid, mp 208 $^o$C, yield 0.74 g (81 %). $C_{12}H_{11}BrN_2O_4S_2$ (389.9 g/mol). $^1$H NMR (400 MHz, DMSO-d): δ (ppm) = 2.49 (s, 3H, $CH_3$), 4.33 (d, *J* = 6.3 Hz, 2H, $SO_2NHCH_2$), 7.51 (t, *J* = 7.9 Hz, 1H, 5-$H_A$), 7.76 (d, *J* = 8.1 Hz, 1H, 6-$H_A$), 7.82 (d, *J* = 8.1 Hz, 1H, 4-$H_A$), 7.86 (s, 1H, 2-$H_A$), 8.82 (t, *J* = 6.3 Hz, 1H, $SO_2NHCH_2$), 13.24 (s, 1H, $CO_2H$). $^{13}$C NMR (101 MHz, DMSO-$d_6$): δ (ppm) = 17.2 ($CH_3$-B), 44.4 ($SO_2NHCH_2$), 122.5 (*C*-$3_A$), 123.7 (*C*-$5_B$), 126.0 (*C*-$6_A$), 129.4

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(*C*-$2_A$), 131.8 (*C*-$5_A$), 135.8 (*C*-$4_A$), 142.9 (*C*-$1_A$), 158.8 (*C*-$4_B$), 163.2 (*C*=O), 170.9 (*C*-$2_B$).

### 5.3.15. 2-[(3-Chloro-4-fluorophenyl)sulfonamidomethyl]-4-methylthiazole-5-carboxylic acid (20b)

Ester **19b** (1.0 g, 2.5 mmol, 1.0 equiv.) was dissolved in a mixture of $CH_3OH$ and THF (1:1, 25 mL). Then, 2 M KOH (1.25 mL, 2.5 mmol, 1.0 equiv.) was added and the reaction mixture was stirred for 16 h at rt. After completion of the conversion, the solvent was evaporated *in vacuo*. 1 M HCl (4.0 mL) was added dropwise to the residue untill the pH was <7 and the precipitated solid was collected by filtration. $R_f$ = 0.30 ($CH_2Cl_2$:$CH_3OH$ 2:1). Colorless solid, mp 206 $^oC$, yield 0.69 g (75 %). $C_{12}H_{10}ClFN_2O_4S_2$ (364.8 g/mol). $^1H$ NMR (400 MHz, DMSO-$d_6$): δ (ppm) = 2.48 (s, 3H, C*H*$_3$), 4.35 (d, *J* = 6.3 Hz, 2H, $SO_2NHCH_2$), 7.61 (t, *J* = 8.9 Hz, 1H, 5-*H*$_A$), 7.77 (ddd, *J* = 8.7 / 4.5 / 2.3 Hz, 1H, 6-*H*$_A$), 7.90 (dd, *J* = 6.8 / 2.3 Hz, 1H, *2*-*H*$_A$), 8.83 (t, *J* = 6.3 Hz, 1H, $SO_2NHCH_2$), 13.25 (s, 1H, $CO_2H$). $^{13}C$ NMR (151 MHz, DMSO-$d_6$): δ (ppm) = 19.8 ($CH_3$-B), 47.1 ($SO_2NHCH_2$), 120.9 (d, *J* = 22.6 Hz, *C*-$5_A$), 123.6 (d, *J* = 18.5 Hz, *C*-$3_A$), 126.3 (*C*-$5_B$), 131.2 (d, *J* = 8.9 Hz, C-$6_A$), 132.3 (C-$2_A$), 141.1 (d, *J* = 3.5 Hz, C-$1_A$), 161.4 (*C*-$4_B$), 161.7 (d, *J* = 252.7 Hz, C-$4_A$), 165.9 (*C*=O), 173.4(*C*-$2_B$).

### 5.3.16. 4-Methyl-2-[(4-methylphenyl)sulfonamidomethyl]thiazole-5-carboxylic acid (20c)

Ester **19c** (1.0 g, 2.8 mmol, 1.0 equiv.) was dissolved in a mixture of $CH_3OH$ and THF (1:1, 30 mL). Then, 2 M KOH (1.41 mL, 2.7 mmol, 1.0 equiv.) was added and the reaction mixture was stirred for 16 h at rt. After completion of the transformation, the solvent was evaporated *in vacuo*. 1 M HCl (3.0 mL) was added dropwise to the

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residue untill the pH was <7 and the precipitated solid was collected by filtration. $R_f$ = 0.30 ($CH_2Cl_2$/$CH_3OH$ 2:1). Colorless solid, mp 199 $^oC$, yield 0.73 g (79 %). $C_{13}H_{14}N_2O_4S_2$ (326.4 g/mol). $^1H$ NMR (400 MHz, DMSO-$D_6$): δ (ppm) = 2.36 (s, 3H, C*H*$_3$-A), 2.51 (s, 3H, C*H*$_3$-B), 4.21 (d, *J* = 6.3 Hz, 2H, $SO_2NHCH_2$), 7.37 (d, 8.6 Hz, 2H, 3-$H_A$, 5-$H_A$), 7.67 (d, *J* = 8.3 Hz, 2H, 2-$H_A$, 6-$H_A$), 8.58 (t, *J* = 6.3 Hz, 1H, $SO_2NHCH_2$), 13.23 (s, 1H, $CO_2H$). $^{13}C$ NMR (151 MHz, DMSO-$d_6$): δ (ppm) = 19.9 ($CH_3$-B), 24.1 ($CH_3$-A), 47.2 ($SO_2NHCH_2$), 126.2 (C-$5_B$), 129.7 (2C, C-$2_A$, C-$6_A$), 132.8 (2C, C-$3_A$,C-$5_A$), 140.4 (C-$1_A$), 146.1 (C-$4_A$), 161.5 (C-$4_B$), 165.9 ($CO_2H$), 174.6 (C-$2_B$).

### 5.3.17. *N*-({5[(2-Benzoylhydrazin-1-yl)carbonyl]-4-methylthiazol-2-yl}methyl)-3-bromobenzenesulfonamide (21a)

Carboxylic acid **20a** (530 mg, 1.36 mmol, 1.0 equiv.), benzoylhydrazine (184 mg, 1.36 mmol, 1.0 equiv.) and COMU (640 mg, 1.49 mmol, 1.1 equiv.) were suspended in THF (15 mL). After addition of DIPEA (472 µL, 2.71 mmol, 2.0 equiv.) the resulting yellow solution was stirred at rt overnight. The solvent was removed under reduced pressure and water (30 mL) was added to the residue. The resulting yellow solution was stirred for 30 min at rt and ethyl acetate (30 mL) was added. The organic phase was separated and the aqueous layer was extracted with ethyl acetate (3 x 30 mL). The organic layer was dried ($Na_2SO_4$) and concentrated *in vacuo*. The residue was purified by FCC (Ø = 2 cm, l = 25 cm, V = 35 mL, $CH_2Cl_2$:$CH_3OH$ 97:3). $R_f$ = 0.66 ($CH_2Cl_2$:$CH_3OH$ 9:1). Colorless solid, mp 210 $^oC$, yield 530 mg (78 %). $C_{19}H_{17}BrN_4O_4S_2$ (509.4 g/mol). $^1H$ NMR (400 MHz, DMSO-$D_6$): δ (ppm) = 2.52 (s, 3H, C*H*$_3$-B), 4.36 (s, 2H, $SO_2NHCH_2$), 7.50 (t, *J* = 7.3 Hz, 2H, 3-$H_C$, 5-$H_C$), 7.52 – 7.63 (m, 2H, 5-$H_A$, 4-$H_C$), 7.81 (d, *J* = 8.6 Hz, 1H, 6-$H_A$), 7.85 (d, *J* = 8.0 Hz, 1H, 4-

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*H*$_A$), 7.90 (d, *J* = 7.0 Hz, 2H, 2-*H*$_C$, 6-*H*$_C$), 7.94 (t, *J* = 1.9 Hz, 1H, 2-*H*$_A$), 8.87 (s, 1H, $SO_2N$*H*$CH_2$), 10.25 (s, 1H, N*H*NHCOaryl), 10.51 (s, 1H, NHN*H*COaryl). $^{13}$C NMR (151 MHz, DMSO-$D_6$): δ (ppm) = 20.0 (*C*$H_3$-B), 47.0 ($SO_2NHCH_2$), 125.3 (*C*-3$_A$), 126.6 (*C*-5$_B$), 128.7 (*C*-6$_A$), 130.5 (2C, *C*-2$_C$, *C*-6$_C$), 131.6 (2C, *C*-3$_C$, *C*-5$_C$), 132.1 (*C*-2$_A$), 134.6 (*C*-5$_A$), 135.0 (*C*-4$_C$), 135.5 (*C*-1$_A$), 138.6 (*C*-4$_A$), 145.4 (*C*-1$_C$), 158.8 (*C*-4$_B$), 164.0 (NH*C*(=O)Thiaz$_B$), 168.8 (NH*C*(=O)Aryl), 171.9 (*C*-2$_B$).

### 5.3.18. *N*-({5-[(2-Benzoylhydrazin-1-yl)carbonyl]-4-methylthiazol-2-yl}methyl)-3-chloro-4-fluorobenzenesulfonamide (21b)

Carboxylic acid **20b** (0.65 g, 1.8 mmol, 1.0 equiv.), benzoylhydrazine (0.25 g, 1.8 mmol, 1.0 equiv.) and COMU (0.8 g, 1.9 mmol, 1.1 equiv.) were suspended in THF (25 mL). After addition of DIPEA (0.63 mL, 3.6 mmol, 2.0 equiv.). The resulting yellow solution was stirred at rt overnight. The solvent was removed under reduced pressure and water (30 mL) was added to the residue. The resulting yellow solution was stirred for 30 min at rt and ethyl acetate (30 mL) was added. The organic phase was separated, and the aqueous layer was extracted with ethyl acetate (3 x 30 mL). The organic layer was dried ($Na_2SO_4$) and concentrated *in vacuo*. The residue was purified by FCC (Ø = 2 cm, l = 15 cm, V = 20 mL, $CH_2Cl_2$:$CH_3OH$ 97:3). $R_f$ = 0.7 ($CH_2Cl_2$:$CH_3OH$ 9:1). Pale yellow solid, mp 198 $^{o}$C, yield 0.6 g (69 %). $C_{19}H_{16}ClFN_4O_4S_2$ (482.9 g/mol). $^1$H NMR (400 MHz, DMSO-$D_6$): δ (ppm) = 2.51 (s, 3H, C*H*$_3$), 4.37 (s, 2H, $SO_2NHCH_2$), 7.50 (t, *J* = 7.8 Hz, 2H, 3-*H*$_C$, 5-*H*$_C$), 7.56 – 7.61 (m, 1H, 4-H$_C$), 7.63 (t, *J* = 8.9 Hz, 1H, 5-H$_A$), 7.82 (ddd, *J* = 8.7 / 4.5 / 2.3 Hz, 1H, 6-H$_A$), 7.89 (d, *J* = 7.1 Hz, 2H, 2-*H*$_C$, 6-*H*$_C$), 7.98 (dd, *J* = 6.8 / 2.3 Hz, 1H, 2H$_A$), 8.87 (s, 1H, $SO_2N$*H*$CH_2$), 10.25 (s, 1H, N*H*NHCOAryl), 10.51 (s, 1H, NHN*H*COAryl). $^{13}$C NMR (151 MHz, DMSO-$D_6$): δ (ppm) = 20.0 (*C*$H_3$-B), 47.0 ($SO_2NHCH_2$), 121.0 (d, *J*

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= 22.2 Hz, C-$5_A$), 123.7 (d, *J* = 18.3 Hz, C-$3_A$), 126.6 (C-$5_B$), 130.5 (2C, C-$2_C$, C-$6_C$), 131.1 (d, *J* = 9.1 Hz, C-$6_A$), 131.6 (2C, C-$3_C$, C-$5_C$), 132.4 (C-$2_A$), 135.0 (C-$4_C$), 135.4 (C-$1_C$), 141.0 (d, *J* = 3.9 Hz, C-$1_A$), 158.7 (C-$4_B$), 161.8 (d, *J* = 251.6 Hz, C-$4_A$), 164.0 (NH*C*(=O)Thiaz$_B$), 168.8(NH*C*(=O)Aryl$_C$), 171.8 (C-$2_B$).

### 5.3.19. *N*-({5-[(2-Benzoylhydrazin-1-yl)carbonyl]thiazol-2-yl}methyl)-4-methylbenzenesulfonamide (21c)

Carboxylic acid **20c** (20 mg, 0.06 mmol, 1.0 equiv.), benzoylhydrazine (8.3 mg, 0.06 mmol, 1.0 equiv.) and COMU (29 mg, 0.067 mmol, 1.1 equiv.) were suspended in THF (5 mL). After addition of DIPEA (20 µL, 0.12 mmol, 2.0 equiv.), the resulting yellow solution was stirred at rt overnight. The solvent was removed under reduced pressure and water (10 mL) was added to the residue. The resulting yellow solution was stirred for 30 min at rt and ethyl acetate (10 mL) was added. The organic phase was separated, and the aqueous layer was extracted with ethyl acetate (3 x 10 mL). The organic layer was dried ($Na_2SO_4$) and concentrated *in vacuo*. The residue was purified by FCC (Ø = 2 cm, l = 15 cm, V = 15 mL, $CH_2Cl_2$:$CH_3OH$ 97:3). $R_f$ = 0.67 ($CH_2Cl_2$:$CH_3OH$ 9:1). Colorless solid, mp 194 $^oC$, yield 18 mg (66 %). $C_{20}H_{20}N_4O_4S_2$ (444.5 g/mol). $^1H$ NMR (400 MHz, DMSO-$D_6$): δ (ppm) = 2.38 (s, 3H, C*H*$_3$-A), 2.53 (s, 3H, C*H*$_3$-B), 4.25 (d, *J* = 6.3 Hz, 2H, $SO_2NHCH_2$), 7.39 (d, J = 8.0 Hz, 2H, 3-$H_A$, 5-$H_A$), 7.51 (t, *J* = 7.8 Hz, 2H, 3-$H_C$, 5-$H_C$), 7.58 (t, *J* = 7.5 Hz, 1H, 4-$H_C$), 7.71 (d, J = 8.3 Hz, 2H, 2-$H_A$, 6-$H_A$), 7.90 (d, *J* = 7.3 Hz, 2H, 2-$H_C$, 6-$H_C$), 8.63 (t, *J* = 6.4 Hz, 1H, $SO_2NHCH_2$), 10.26 (s, 1H, NHNHCOAryl), 10.52 (s, 1H, NHNHCOAryl). $^{13}C$ NMR (151 MHz, DMSO-$D_6$): δ (ppm) = 20.0 ($CH_3$-B), 24.1 ($CH_3$-A), 47.2 ($SO_2NHCH_2$), 126.5 (C-$5_B$), 129.7 (2C, C-$2_A$, C-$6_A$), 130.6 (2C, C-$2_C$, C-$6_C$), 131.6 (2C, C-$3_C$, C-$5_C$),

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132.8 (2C, C-$3_A$, C-$5_A$), 135.0 (C-$4_C$), 135.5 (C-$1_C$), 140.3 (C-$4_A$), 146.2 (C-$1_A$), 158.8 (C-$4_B$), 164.1 (NHC(=O)Thiaz$_B$), 168.8 (NHC(=O)Aryl$_C$), 172.8 (C-$2_B$).

### 5.3.20. 3-Bromo-*N*-({4-methyl-5-[2-(thiophen-2-ylcarbonyl)hydrazin-1-ylcarbonyl]thiazol-2-yl}methyl)benzenesulfonamide (22a)

Carboxylic acid **20a** (0.34 g, 0.9 mmol, 1.0 equiv.), thiophene-2-carbohydrazide (0.12 g, 0.9 mmol, 1.0 equiv.) and COMU (0.4 g, 1.0 mmol, 1.1 equiv.) were suspended in THF (15 mL). After addition of DIPEA (0.3 mL, 1.8 mmol, 2.0 equiv.), the resulting yellow solution was stirred at rt overnight. The solvent was removed under reduced pressure and water (25 mL) was added to the residue. The resulting yellow solution was stirred for 30 min at rt and ethyl acetate (25 mL) was added. The organic phase was separated, and the aqueous layer was extracted with ethyl acetate (3 x 25 mL). The organic layer was dried ($Na_2SO_4$) and concentrated *in vacuo*. The residue was purified by FCC (Ø = 4 cm, l = 10 cm, V = 35 mL, $CH_2Cl_2$:$CH_3OH$ 97:3). $R_f$ = 0.63 ($CH_2Cl_2$:$CH_3OH$ 9:1). Yellow solid, mp 201 °C, yield 0.29 g (66 %). $C_{17}H_{15}BrN_4O_4S_3$ (515.4 g/mol). $^1$H NMR (400 MHz, DMSO-$D_6$): δ (ppm) = 2.51 (s, 3H, C*H*$_3$-B), 4.35 (s, 2H, $SO_2NHCH_2$), 7.19 (dd, *J* = 5.0 / 3.8 Hz, 1H, 4-$H_C$), 7.54 (t, *J* = 7.9 Hz, 1H, 5-$H_A$), 7.80 (d, *J* = 8.2 Hz, 1H, 6-$H_A$), 7.81 – 7.88 (m, 3H, 4-$H_A$, 3-$H_C$, 5-$H_C$), 7.93 (t, *J* = 1.9 Hz, 1H, 2-$H_A$), 8.86 (s, 1H, $SO_2NHCH_2$), 10.25 (s, 1H, NHNHCOThio$_C$), 10.53 (s, 1H, NHNHCOThio$_C$). $^{13}$C NMR (151 MHz, DMSO-$D_6$): δ (ppm) = 20.0 ($CH_3$-B), 47.0 ($SO_2NHCH_2$), 125.3 (C-$3_A$), 126.4 (C-$5_B$), 128.7 (C-$6_A$), 131.3 (C-$4_C$), 132.1 (C-$2_A$), 132.2 (C-$5_C$), 134.6 (C-$5_A$), 134.9 (C-$3_C$), 138.6 (C-$4_A$), 140.2 (C-$2_C$), 145.4 (C-$1_A$), 158.9 (C-$4_B$), 163.8 (NHC(=O)aryl), 164.0 (NHC(=O)thiaz$_B$), 172.0 (C-$2_B$).

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### 5.3.21. 3-Chloro-4-fluoro-N-({4-methyl-5-[2-(thiophen-2-ylcarbonyl)hydrazin-1-carbonyl]thiazol-2-yl}methyl)benzenesulfonamide (22b)

Carboxylic acid **20b** (0.8 g, 2.2 mmol, 1.0 equiv.), thiophene-2-carbohydrazide (0.31 g, 2.2 mmol, 1.0 equiv.) and COMU (1.02 g, 2.4 mmol, 1.1 equiv.) were suspended in THF (35 mL). After addition of DIPEA (0.76 mL, 4.4 mmol, 2.0 equiv.), the resulting yellow solution was stirred at rt overnight. The solvent was removed under reduced pressure and water (50 mL) was added to the residue. The resulting yellow solution was stirred for 30 min at rt and ethyl acetate (50 mL) was added. The organic phase was separated, and the aqueous layer was extracted with ethyl acetate (3 x 50 mL). The organic layer was dried ($Na_2SO_4$) and concentrated *in vacuo*. The residue was purified by FCC (Ø = 4 cm, l = 20 cm, V = 75 mL, $CH_2Cl_2$:$CH_3OH$ 97:3). $R_f$ = 0.45 ($CH_2Cl_2$:$CH_3OH$ 95:5). Colorless solid, mp 196 °C, yield 0.73 g (73 %). $C_{17}H_{14}ClFN_4O_4S_3$ (488.9 g/mol). $^1$H NMR (400 MHz, DMSO-$D_6$): δ (ppm) = 2.50 (s, 3H, C*H*$_3$-B), 4.38 (d, *J* = 6.3 Hz, 2H, $SO_2NHCH_2$), 7.19 (dd, *J* = 5.0 / 3.7 Hz, 1H, 4-*H*$_C$), 7.63 (t, *J* = 8.9 Hz, 1H, 5-*H*$_A$), 7.79 – 7.83 (m, 1H, 6-*H*$_A$), 7.83 – 7.87 (m, 2H, 3-H$_C$, 5-H$_C$), 7.97 (dd, *J* = 6.8 / 2.3 Hz, 1H, 2-H$_A$), 8.88 (t, *J* = 6.3 Hz, 1H, $SO_2NHCH_2$), 10.26 (s, 1H, N*H*NHCOThio$_C$), 10.54 (s, 1H, NHN*H*COThio$_C$). $^{13}$C NMR (151 MHz, DMSO-$D_6$): δ (ppm) = 20.0 ($CH_3$-B), 47.0 ($SO_2NHCH_2$), 121.1 (d, *J* = 25.5 Hz, C-5$_A$), 123.9 (d, *J* = 19.1 Hz, C-3$_A$), 126.4 (C-5$_B$), 131.1 (d, *J* = 8.7 Hz, C-6$_A$), 131.3 (C-4$_C$), 132.2 (C-5$_C$), 132.3 (C-2$_A$), 134.9 (C-3$_C$), 140.2 (C-2$_C$), 141.0 (d, *J* = 3.2 Hz, C-1$_A$), 158.9 (C-4$_B$), 161.8 (d, *J* = 255.1 Hz, C-4$_A$), 163.8 (NH*C*(=O)Thio$_C$), 164.0 (NH*C*(=O)Thiaz$_B$), 171.8 (C-2$_B$).

### 5.3.22. 4-Methyl-*N*-({4-methyl-5-[2-(thiophen-2-ylcarbonyl)hydrazin-1-ylcarbonyl]thiazol-2-yl}methyl)benzenesulfonamide (22c)

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Carboxylic acid **20c** (0.64 g, 1.96 mmol, 1.0 equiv.), thiophene-2-carbohydrazide (0.28 g, 1.96 mmol, 1.0 equiv.) and COMU (0.92 g, 2.1 mmol, 1.1 equiv.) were suspended in THF (30 mL). After addition of DIPEA (0.68 mL, 3.9 mmol, 2.0 equiv.), the resulting yellow solution was stirred at rt overnight. The solvent was removed under reduced pressure and water (50 mL) was added to the residue. The resulting yellow solution was stirred for 30 min at rt and ethyl acetate (50 mL) was added. The organic phase was separated, and the aqueous layer was extracted with ethyl acetate (3 x 50 mL). The organic layer was dried ($Na_2SO_4$) and concentrated *in vacuo*. The residue was purified by FCC (Ø = 4 cm, l = 10 cm, V = 25 mL, $CH_2Cl_2$:$CH_3OH$ 97:3). $R_f$ = 0.58 ($CH_2Cl_2$:$CH_3OH$ 9:1). Colorless solid, mp 207 °C, yield 0.63 g (72 %). $C_{18}H_{18}N_4O_4S_3$ (450.6 g/mol). $^1$H NMR (400 MHz, DMSO-$D_6$): δ (ppm) = 2.37 (s, 3H, C*H*$_3$-A), 2.50 (s, 3H, C*H*$_3$-B), 4.22 (d, *J* = 4.3 Hz, 2H, $SO_2NHCH_2$), 7.18 (dd, *J* = 4.9 / 3.9 Hz, 1H, 4-$H_C$), 7.38 (d, *J* = 7.6 Hz, 2H, 3-$H_A$, 5-$H_A$), 7.69 (d, *J* = 8.3 Hz, 2H, 2-$H_A$, 6-$H_A$), 7.80 – 7.87 (m, 2H, 3-$H_C$, 5-$H_C$), 8.61 (t, *J* = 6.4 Hz, 1H, $SO_2NHCH_2$), 10.24 (s, 1H, N*H*NHCOThio$_C$), 10.52 (s, 1H, NHN*H*COThio$_C$). $^{13}$C NMR (151 MHz, DMSO-$D_6$): δ (ppm) = 17.3 (*C*$H_3$-B), 21.4 (*C*$H_3$-A), 44.5 ($SO_2NHCH_2$), 123.7 (C-5$_B$), 127.0 (2C, C-2$_A$, C-6$_A$), 128.6 (C-4$_C$), 129.5 (C-5$_C$), 130.2 (2C, C-3$_A$, C-5$_A$), 132.2 (C-3$_C$), 137.5 (C-2$_C$), 137.6 (C-1$_A$), 143.5 (C-4$_A$), 156.2 (C-4$_B$), 161.1 (NH*C*(=O)aryl), 161.4 (NH*C*(=O)thiaz$_B$), 170.2 (C-2$_B$).

### 5.3.23. Ethyl 2-{2-[(benzyloxy)carbonylamino]acetoxy}-3-oxobutanoate (24)[25]

Ethyl 2-chloroacetoacetate **23** (0.7 mL, 4.7 mmol, 1 equiv.) and $Et_3N$ (1.98 mL, 14.3 mmol, 3.0 equiv.) were added to a solution of *N*-[(benzyloxy)carbonyl]glycine (1.0 g, 4.7 mmol, 1 equiv.) in ethyl acetate (50 mL) at 0 °C. After stirring the solution at 0 °C for 30 min, the solution was heated to 70 °C for 16 h. After completion, $H_2O$ (30 mL)

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was added and the organic layer was separated. The organic layer was washed with 10 % citric acid and brine, dried ($Na_2SO_4$) and concentrated under reduced pressure. The product **24** was proceeded to the next step without further purification due to decomposition during the purification process. Brown oil (not purified), yield 1.21 g (60 %). $C_{16}H_{19}NO_7$ (337.3 g/mol)

### 5.3.24. Ethyl 2-{[(benzyloxy)carbonylamino]methyl}-4-methyloxazole-5-carboxylate (25)[25]

Ammonium acetate (1.21 g, 14.3 mmol, 3 equiv.) was added to a solution of unpurified **24** in acetic acid (40 mL) and the resulting brown solution was heated to 135 $^{o}$C for 16 h. The reaction mixture was cooled down and acetic acid was removed under reduced pressure. The residue was dissolved in ethyl acetate (50 mL) and water (50 mL) was added. The two phases were separated, and the aqueous layer was extracted with ethyl acetate (3 x 50 mL). The organic layer was dried ($Na_2SO_4$) and concentrated *in vacuo*. The residue was purified by FCC (Ø = 5 cm, l = 18 cm, V = 100 mL, cyclohexane/ethyl acetate 7:3). $R_f$ = 0.47 (cyclohexane/ethyl acetate 2:1). Yellow oil, yield 1.10 g (95 %). $C_{16}H_{18}N_2O_5$ (318.3 g/mol). $^1$H NMR (400 MHz, DMSO-$D_6$): δ (ppm) = 1.27 (t, *J* = 7.1 Hz, 3H, $CO_2CH_2C\mathit{H_3}$), 2.34 (s, 3H, $C\mathit{H_3}$), 4.28 (q, *J* = 7.1 Hz, 2H, $CO_2C\mathit{H_2}CH_3$), 4.33 (d, *J* = 6.0 Hz, 2H, $NHC\mathit{H_2}$), 5.04 (s, 2H, $PhC\mathit{H_2}O$), 7.12– 7.37 (m, 5H, $H_{arom}$), 8.00 (t, *J* = 6.0 Hz, 1H, $CON\mathit{H}CH_2$). $^{13}$C NMR (600 MHz, DMSO-$D_6$): δ (ppm) = 16.1 ($\mathit{C}H_3$), 17.2 ($COCH_2\mathit{C}H_3$), 41.0 ($CONH\mathit{C}H_2$), 63.9 ($CO_2\mathit{C}H_2CH_3$), 68.8 ($Ph\mathit{C}H_2CO$), 130.9 (C-$2_A$,C-$6_A$), 131.0 (C-$4_A$), 131.4 (C-$3_A$,C-$5_A$), 139.9 (C-$4_B$), 140.0 (C-$1_A$), 148.5 (C-$5_B$), 159.4 ($\mathit{C}ONHCH_2$), 161.0 ($\mathit{C}O_2CH_2CH_3$), 166.3 (C-$2_B$).

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### 5.3.25. Ethyl 2-(aminomethyl)-4-methyloxazole-5-carboxylate (26)

Pd/C (50 mg, 5 wt %) was added to a solution of **25** (1.0 g, 3.1 mmol, 1.0 equiv.) in THF/$CH_3OH$ (1:1, 12 mL) and the mixture was flushed thrice with $H_2$ gas. The flask was back-filled with $H_2$ (5 bar) and the reaction was carried out 10 h. Pd/C was removed by passing the reaction mixture through a Celite 545 bed. The filtrate was concentrated *in vacuo*. The residue was obtained as a pale-yellow oil, which was proceeded to the next step without purification. Pale yellow oil, yield 0.45 g (78 %). $C_8H_{12}N_2O_3$ (184.2 g/mol).

### 5.3.26. Ethyl 2-[(3-bromophenyl)sulfonamidomethyl]-4-methyloxazole-5-carboxylate (27a)

Primary amine **26** (1.0 g, 5.4 mmol, 1.0 equiv.) was dissolved in THF (50 mL) and the solution was cooled to 0 $^oC$. $Et_3N$ (2.2 mL, 16.2 mmol, 3.0 equiv.) and a solution of 3-bromobenzenesulfonyl chloride (0.8 mL, 5.4 mmol, 1.0 equiv.) in THF (5 mL) were added. The reaction mixture was stirred overnight at room temperature. After completion of the transformation, the solvent was evaporated *in vacuo* and ethyl acetate (50 mL) and $H_2O$ (50mL) were added. The organic layer was separated, and the aqueous layer was extracted with ethyl acetate (3 x 50 mL). The organic layer was dried ($Na_2SO_4$) and concentrated *in vacuo*. The residue was purified by FCC (Ø = 5 cm, l = 20 cm, V = 100 mL, cyclohexane/ethyl acetate 7:3). $R_f$ = 0.45 (cyclohexane/ethyl acetate 2:1). Yellow oil, yield 1.1 g (57 %). $C_{14}H_{15}BrN_2O_5S$ (403.3 g/mol). $^1H$ NMR (400 MHz, DMSO-$D_6$): δ (ppm) = 1.29 (t, *J* = 7.1 Hz, 3H, $CO_2CH_2CH_3$), 2.24 (s, 3H, $CH_3$), 4.27 (q, *J* = 7.1 Hz, 2H, $CO_2CH_2CH_3$), 4.28 (s, 2H, $SO_2NHCH_2$), 7.46 (td, *J* = 7.7 / 0.8 Hz, 1H, 5-$H_A$), 7.69 (ddd, *J* = 7.9 / 1.7 / 1.1 Hz, 1H, 6-$H_A$), 7.75 – 7.79 (m, 2H, 2-$H_A$, 4-$H_A$), 8.78 (s, 1H, $SO_2NHCH_2$). $^{13}C$ NMR (151

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MHz, DMSO-$D_6$): δ (ppm) = 15.9 (*C*$H_3$), 17.2 ($CO_2CH_2$*C*$H_3$), 42.4 ($SO_2NH$*C*$H_2$), 69.1 ($CO_2$*C*$H_2CH_3$), 125.0 (C-$3_A$), 128.6 (C-$6_A$), 131.8 (C-$2_A$), 134.3 (*C*-$5_A$), 138.2 (C-$4_A$), 140.0 (C-$4_B$), 145.4 (C-$1_A$), 148.1 (C-$5_B$), 160.6 (*C*=$O_{ester}$), 164.0 (C-$2_B$).

### 5.3.27. Ethyl 2-[(3-chloro-4-fluorophenyl)sulfonamidomethyl]-4-methyloxazole-5-carboxylate (27b)

Primary amine **26** (1.0 g, 5.4 mmol, 1.0 equiv.) was dissolved in THF (50 mL) and the solution was cooled to 0 $^oC$. $Et_3N$ (2.2 mL, 16.2 mmol, 3.0 equiv.) and a solution of 3-chloro-4-fluoro benzenesulfonyl chloride (0.76 mL, 5.4 mmol, 1.0 equiv.) in THF (5 mL) were added. The reaction mixture was stirred overnight at room temperature. After completion of the reaction, the solvent was evaporated *in vacuo* and ethyl acetate (50 mL) and $H_2O$ (50 mL) were added. The organic layer was separated, and the aqueous layer was extracted with ethyl acetate (3 x 50 mL). The organic layer was dried ($Na_2SO_4$) and concentrated *in vacuo*. The residue was purified by FCC (Ø = 6 cm, l = 22 cm, V = 100 mL, cyclohexane/ethyl acetate 7:3). $R_f$ = 0.43 (cyclohexane/ethyl acetate 2:1). Light yellow oil yield 0.97 g (47 %). $C_{14}H_{14}ClFN_2O_5S$ (376.8 g/mol). $^1$H NMR (400 MHz, DMSO-$D_6$): δ (ppm) = 1.27 (t, *J* = 7.1 Hz, 3H, $CO_2CH_2CH_3$), 2.23 (s, 3H, C*H*$_3$), 4.25 (q, *J* = 7.1 Hz, 2H, $CO_2CH_2CH_3$), 4.29 (s, 2H, $SO_2NHCH_2$), 7.56 (t, *J* = 8.9 Hz, 1H, 5-$H_A$), 7.68 (ddd, *J* = 7.5 / 2.3 / 1.2 Hz, 1H, 6-$H_A$), 7.78 (dd, *J* = 6.8 / 2.3 Hz, 1H, 2-$H_A$), 8.78 (s, 1H, $SO_2NHCH_2$). $^{13}$C NMR (151 MHz, DMSO-$D_6$): δ (ppm) = 15.8 (*C*$H_3$-B), 17.2 ($CO_2CH_2CH_3$), 42.4 ($SO_2NHCH_2$), 64.0 ($CO_2CH_2CH_3$), 120.8 (d, *J* = 22.5 Hz, C-$5_A$), 123.5 (d, *J* = 19.7 Hz, C-$3_A$), 131.0 (d, *J* = 9.0 Hz, C-$6_A$), 132.0 (C-$2_A$), 140.0 (C-$5_B$), 141.0 (d, *J* = 3.5 Hz, C-$1_A$), 148.1 (C-$4_B$), 160.6 (C-$2_B$), 161.5 (d, *J* = 253.7 Hz, C-$4_A$), 164.0 (C=O).

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### 5.3.28. 2-[(3-Bromophenyl)sulfonamidomethyl]-4-methyloxazole-5-carboxylic acid (28a)

Ester **27a** (1.0 g, 2.7 mmol, 1.0 equiv.) was dissolved in a mixture of $CH_3OH$ and THF (1:1, 50 mL) and 2 M KOH (1.33 mL, 2.7 mmol, 1.0 equiv.) was added. The reaction mixture was stirred for 16 h at rt. After completion of the conversion, the solvent was evaporated *in vacuo*. 1 M HCl (3.0 mL) was added dropwise to the residue untill the pH was <7 and the precipitated solid was collected by filtration. $R_f$ = 0.25 ($CH_2Cl_2$/$CH_3OH$ 2:1). Colorless solid, mp 202 $^oC$, yield 0.80 g (86 %). $C_{12}H_{11}BrN_2O_5S$ (375.2 g/mol). $^1H$ NMR (400 MHz, DMSO-$D_6$): δ (ppm) = 2.21 (s, 3H, C*H*$_3$), 4.23 (d, *J* = 6.3 Hz, 2H, $SO_2NHCH_2$), 7.45 (t, *J* = 8.1 Hz, 1H, 5-$H_A$), 7.68 (ddd, *J* = 7.9 / 1.8 / 1.1 Hz, 1H, 6-$H_A$), 7.75 – 7.79 (m, 2H, 2-$H_A$, 4-$H_A$), 8.73 (t, *J* = 6.3 Hz, 1H, $SO_2NHCH_2$), 13.37 (s, 1H, $CO_2H$). $^{13}C$ NMR (151 MHz, DMSO-$D_6$): δ (ppm) = 15.9 ($CH_3$), 43.2 ($SO_2NHCH_2$), 125.0 (C-3$_A$), 128.6 (C-6$_A$), 131.9 (C-2$_A$), 134.3 (C-5$_A$), 138.2 (C-4$_A$), 140.9 (C-4$_B$), 145.4(C-1$_A$), 147.3 (C-5$_B$), 162.1 ($CO_2H$), 163.6 (C-2$_B$).

### 5.3.29. 2-{[(3-Chloro-4-fluorophenyl)sulfonamido]methyl}-4-methyloxazole-5-carboxylic acid (28b)

Ester **27b** (0.5 g, 2.0 mmol, 1.0 equiv.) was dissolved in a mixture of $CH_3OH$ and THF (1:1, 20 mL) and 2 M KOH (1.03 mL, 2.0 mmol, 1.0 equiv.) was added. The reaction mixture was stirred for 16 h at rt. After completion of the conversion, the solvent was evaporated *in vacuo*. 1 M HCl (2.5 mL) was added dropwise to the residue untill the pH was < 7 and the precipitated solid was collected by filtration. $R_f$ = 0.20 ($CH_2Cl_2$/$CH_3OH$ 2:1). Yellow solid, mp 204 $^oC$, yield 0.36 g (72 %). $C_{12}H_{10}ClFN_2O_5S$ (348.7 g/mol). $^1H$ NMR (600 MHz, DMSO-$D_6$): δ (ppm) = 2.21 (s,

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3H, C*H*$_3$-B), 4.26 (d, *J* = 6.3 Hz, 2H, $SO_2NHCH_2$), 7.54 (t, *J* = 8.9 Hz, 1H, 5-$H_A$), 7.68 (ddd, *J* = 8.7 / 4.5 / 2.3 Hz, 1H, 6-$H_A$), 7.80 (dd, *J* = 6.8 / 2.3 Hz, 1H, 2-$H_A$), 8.75 (t, *J* = 6.3 Hz, 1H, $SO_2NHCH_2$), 13.41 (s, 1H, $CO_2H$). $^{13}$C NMR (151 MHz, DMSO-$D_6$): δ (ppm) = 15.8 ($CH_3$-B), 42.4 ($SO_2NHCH_2$), 120.8 (d, *J* = 21.5 Hz, C-5$_A$), 123.5 (d, *J* = 18.2 Hz, C-3$_A$), 131.1 (d, *J* = 9.2 Hz, C-6$_A$), 132.1 (C-2$_A$), 140.9 (C-5$_B$), 141.0 (d, *J* = 4.3 Hz, C-1$_A$), 147.3 (C-4$_B$), 161.6 (d, *J* = 253.1 Hz, C-4$_A$), 162.0 (C-2$_B$), 163.6 ($CO_2H$).

### 5.3.30. *N*-{[5-(2-benzoylhydrazin-1-carbonyl)-4-methyloxazol-]2-ylmethyl}-3-bromobenzenesulfonamide (29a)

Carboxylic acid **28a** (0.4 g, 1.07 mmol, 1.0 equiv.), benzoylhydrazine (0.14 g, 1.07 mmol, 1.0 equiv.) and COMU (0.5 g, 1.17 mmol, 1.1 equiv.) were suspended in THF (25 mL). After addition of DIPEA (0.37 mL, 2.14 mmol, 2.0 equiv.), the resulting yellow solution was stirred at rt overnight. The solvent was removed under reduced pressure and water (40 mL) was added to the residue. The resulting yellow solution was stirred for 30 min at rt and ethyl acetate (40 mL) was added. The organic phase was separated, and the aqueous layer was extracted with ethyl acetate (3 x 40 mL). The organic layer was dried ($Na_2SO_4$) and concentrated *in vacuo*. The residue was purified by FCC (Ø = 4 cm, l = 15 cm, V = 50 mL, $CH_2Cl_2$:$CH_3OH$ 97:3). $R_f$ = 0.65 ($CH_2Cl_2$:$CH_3OH$ 5:1). Colorless solid, mp 189 $^{o}$C, yield 0.41 g (76 %). $C_{19}H_{17}BrN_4O_5S$ (493.3 g/mol). $^1$H NMR (400 MHz, DMSO-$D_6$): δ (ppm) = 2.22 (s, 3H, C*H*$_3$-B), 4.25 (d, *J* = 5.5 Hz, 2H, $SO_2NHCH_2$), 7.48 – 7.55 (m, 3H, 5-$H_A$, 3-$H_C$, 5-$H_C$), 7.54 – 7.61 (m, 1H, 4-$H_C$), 7.78 (ddd, *J* = 7.9 / 1.8 / 1.0 Hz, 1H, 6-$H_A$), 7.81 (ddd, *J* = 8.0 / 2.0 / 1.0 Hz, 1H, 4-$H_A$), 7.86 (t, *J* = 1.8 Hz, 1H, 2-$H_A$), 7.89 (d, *J* = 7.1 Hz, 2H, 2-$H_C$, 6-$H_C$) 8.60 (t, *J* = 6.0 Hz, 1H, $SO_2NHCH_2$), 10.22 (s, 1H, NHN*H*COaryl), 10.45 (s,

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1H, N*H*NHCOaryl). $^{13}$C NMR (151 MHz, DMSO-$D_6$): δ (ppm) = 15.5 (*C*$H_3$-B), 42.5 ($SO_2NHCH_2$), 125.1 (C-$3_A$), 128.8 (C-$6_A$), 130.6 (2C, C-$2_C$, C-$6_C$), 131.6 (2C, C-$3_C$, C-$5_C$) 132.1 (C-$2_A$), 134.5 (C-$5_A$), 135.0 (C-$4_C$), 135.5 (C-$1_C$), 138.4 (C-$4_A$), 141.1 (C-$4_B$), 145.0 (C-$1_A$), 145.3 (C-$5_B$), 159.9 (NHC(=O)oxaz$_B$), 162.3 (C-$2_B$), 168.9 (NH*C*(=O)aryl$_C$).

### 5.3.31. *N*-{[5-(2-Benzoylhydrazin-1-ylcarbonyl)-4-methyloxazol]-2-ylmethyl}-3-chloro-4-fluorobenzenesulfonamide (29b)

Carboxylic acid **28b** (0.2 g, 0.6 mmol, 1.0 equiv.), benzoylhydrazine (80 mg, 0.6 mmol, 1.0 equiv.) and COMU (0.28 g, 0.66 mmol, 1.1 equiv.) were suspended in THF (20 mL). After addition of DIPEA (0.2 mL, 1.2 mmol, 2.0 equiv.), the resulting yellow solution was stirred at rt overnight. The solvent was removed under reduced pressure and water (20 mL) was added to the residue. The resulting yellow solution was stirred for 30 min at rt and ethyl acetate (20 mL) was added. The organic layer was separated, and the aqueous layer was extracted with ethyl acetate (3 x 20 mL). The organic layer was dried ($Na_2SO_4$) and concentrated *in vacuo*. The residue was purified by FCC (Ø = 2 cm, l = 10 cm, V = 30 mL, $CH_2Cl_2$:$CH_3OH$ 97:3). $R_f$ = 0.6 ($CH_2Cl_2$:$CH_3OH$ 2:1). Pale yellow solid, mp 191 $^{o}$C, yield 0.21 g (71 %). $C_{19}H_{16}ClFN_4O_5S$ (466.1 g/mol). $^1$H NMR (600 MHz, DMSO-$D_6$): δ (ppm) = 2.20 (s, 3H, C*H*$_3$-B), 4.27 (d, *J* = 6.1 Hz, 2H, $SO_2NHCH_2$), 7.51 (t, *J* = 7.7 Hz, 2H, 3-$H_C$, 5-$H_C$), 7.58 (t, *J* = 7.4 Hz, 1H, 4-$H_C$), 7.62 (t, *J* = 8.9 Hz, 1H, 5-$H_A$), 7.80 (ddd, *J* = 8.5 / 2.3 / 1.1 Hz, 1H, 6-$H_A$), 7.88 – 7.91 (m, 3H, 2-$H_A$, 4-$H_C$, 6-$H_C$), 8.62 (t, *J* = 6.1 Hz, 1H, $SO_2NHCH_2$), 10.26 (s, 1H, N*H*C(=O)oxaz$_B$), 10.47 (s, 1H, N*H*C(=O)aryl$_C$). $^{13}$C NMR (151 MHz, DMSO-$D_6$): δ (ppm) = 15.4 (*C*$H_3$-B), 42.6 ($SO_2NHCH_2$), 120.8 (d, *J* = 22.4 Hz,C-$5_A$), 123.6 (d, *J* = 19.0 Hz, C-$3_A$), 130.6 (2C, C-$2_C$, C-$6_C$), 131.3 (d, *J* = 8.9 Hz,

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C-$6_A$), 131.6 (2C, C-$3_C$, C-$5_C$), 132.4 (C-$2_A$), 135.1 (C-$4_C$) 135.5 (C-$1_C$), 140.6 (d, *J* = 3.7 Hz, C-$1_A$), 141.1 (C-$5_B$), 145.2 (C-$4_B$), 160.0 (NH*C*(=O)oxaz$_B$), 161.7 (d, *J* = 253.1 Hz, C-$4_A$), 162.3 (C-$2_B$), 168.9 (NH*C*(=O)aryl$_C$).

**5.3.32. 3-Bromo-*N*-({4-methyl-5-[2-(thiophen-2-ylcarbonyl)hydrazin-1-ylcarbonyl]oxazol-2-yl}methyl)benzenesulfonamide (30a)**

Carboxylic acid **28a** (0.3 g, 0.8 mmol, 1.0 equiv.), thiophene-2-carbohydrazide (0.11 g, 0.8 mmol, 1.0 equiv.) and COMU (0.37 g, 0.88 mmol, 1.1 equiv.) were suspended in THF (20 mL). After addition of DIPEA (0.28 mL, 1.6 mmol, 2.0 equiv.), the resulting yellow solution was stirred at rt overnight. The solvent was removed under reduced pressure and water (40 mL) was added to the residue. The resulting yellow solution was stirred for 30 min at rt and ethyl acetate (40 mL) was added. The organic phase was separated, and the aqueous layer was extracted with ethyl acetate (3 x 40 mL). The organic layer was dried ($Na_2SO_4$) and concentrated *in vacuo*. The residue was purified by FCC (Ø = 4 cm, l = 15 cm, V = 50 mL, $CH_2Cl_2$:$CH_3OH$ 96:4). $R_f$ = 0.5 ($CH_2Cl_2$:$CH_3OH$ 2:1). Colorless solid, mp 190 °C, yield 0.28 g (70 %). $C_{17}H_{15}BrN_4O_5S_2$ (499.3 g/mol). $^1$H NMR (600 MHz, DMSO-$D_6$): δ (ppm) = 2.21 (s, 3H, C*H*$_3$-B), 4.24 (s, 2H, $SO_2NHCH_2$), 7.20 (dd, *J* = 5.0 / 3.7 Hz, 1H, 4-$H_C$), 7.52 (t, *J* = 7.9 Hz, 1H, 5-$H_A$), 7.76 (ddd, *J* = 7.9 / 1.8 / 1.0 Hz, 1H, 6-$H_A$), 7.81 (ddd, *J* = 8.0 / 2.0 / 1.0 Hz, 1H, 4-$H_A$), 7.83 – 7.88 (m, 3H, 3-$H_C$ 5-$H_C$, 2-$H_A$), 8.60 (s, 1H, $SO_2NHCH_2$), 10.25 (s, 1H, NHC(=O)Oxaz$_B$), 10.46 (s, 1H, NHCOthiop$_C$). $^{13}$C NMR (151 MHz, DMSO-$D_6$): δ (ppm) = 12.8 ($CH_3$-B), 39.8 ($SO_2NHCH_2$), 122.4 (C-$3_A$), 126.1 (C-$6_A$), 128.6 (C-$4_C$), 129.5 (C-$3_C$), 129.6 (C-$2_A$), 131.8 (C-$5_A$), 132.2 (C-$5_C$), 135.7 (C-$4_A$), 137.6 (C-$2_C$), 138.4 (C-$5_B$), 142.4 (C-$1_A$), 142.8 (C-$4_B$), 157.4 (NHC(=O)Oxaz$_B$), 159.7 (C-$2_B$), 161.2 (NHC(=O)Thiop$_C$).

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### 5.3.33. 3-Chloro-4-fluoro-*N*-({4-methyl-5-[2-(thiophen-2-ylcarbonyl)hydrazin-1-ylcarbonyl]oxazol-2-yl}methyl)benzenesulfonamide (30b)

Carboxylic acid **28b** (0.15 g, 0.4 mmol, 1.0 equiv.), thiophene-2-carbohydrazide (61 mg, 0.4 mmol, 1.0 equiv.) and COMU (0.2 g, 0.44 mmol, 1.1 equiv.) were suspended in THF (15 mL). After addition of DIPEA (0.15 mL, 0.8 mmol, 2.0 equiv.), the resulting yellow solution was stirred at rt overnight. The solvent was removed under reduced pressure and water (20 mL) was added to the residue. The resulting yellow solution was stirred for 30 min at rt and ethyl acetate (20 mL) was added. The organic phase was separated, and the aqueous layer was extracted with ethyl acetate (3 x 20 mL). The organic layer was dried ($Na_2SO_4$) and concentrated *in vacuo*. The residue was purified by FCC (Ø = 2 cm, l = 15 cm, V = 30 mL, $CH_2Cl_2$:$CH_3OH$ 97:3). $R_f$ = 0.58 ($CH_2Cl_2$:$CH_3OH$ 2:1). Colorless solid, mp 195 °C, yield 0.15 g (75 %). $C_{17}H_{14}ClFN_4O_5S_2$ (472.0 g/mol). $^1$H NMR (600 MHz, DMSO-$D_6$): δ (ppm) = 2.20 (s, 3H, C*H*$_3$-B), 4.26 (d, *J* = 6.1 Hz, 2H, $SO_2NHCH_2$), 7.20 (dd, *J* = 5.0 / 3.7 Hz, 1H, 4-$H_C$), 7.61 (t, *J* = 8.9 Hz, 1H, 5-$H_A$), 7.79 (ddd, *J* = 8.7 / 2.4 / 1.2 Hz, 1H, 6-$H_A$), 7.83 – 7.87 (m, 2H, 3-$H_C$, 5-$H_C$), 7.89 (dd, *J* = 6.9 / 2.3 Hz, 1H, 2-$H_A$), 8.63 (t, *J* = 6.1 Hz, 1H, $SO_2NHCH_2$), 10.29 (s, 1H, N*H*C(=O)Oxaz$_B$), 10.49 (s, 1H, N*H*C(=O)Thio$_C$). $^{13}$C NMR (151 MHz, DMSO-$D_6$): δ (ppm) = 15.4 (*C*$H_3$-B), 42.6 ($SO_2NHCH_2$), 120.9 (d, *J* = 26.5 Hz, C-5$_A$), 123.7 (d, *J* = 20.6 Hz, C-3$_A$), 131.2 (d, *J* = 8.3 Hz, C-6$_A$), 131.3 (C-4$_C$), 132.3 (C-3$_C$), 132.4 (C-2$_A$), 134.9 (C-5$_C$), 140.2 (C-2$_C$), 140.6 (d, *J* = 3.8 Hz, C-1$_A$), 141.0 (C-5$_B$), 145.4 (C-4$_B$), 160.0 (NH*C*(=O)Oxaz$_B$), 161.7 (d, *J* = 257.5 Hz, C-4$_A$), 162.4 (C-2$_B$), 163.9 (NH*C*(=O)Thio$_C$).

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### 5.3.34. *N*-benzyl-2-{[(3-bromophenyl)sulfonamido]methyl}-4-methyloxazole-5-carboxamide (31a)

Carboxylic acid **28a** (0.4 g, 1.1 mmol, 1.0 equiv.), benzylamine HCl (0.15 g, 1.1 mmol, 1.0 equiv.) and COMU (0.5 g, 1.2 mmol, 1.1 equiv.) were suspended in THF (30 mL). After addition of DIPEA (0.4 mL, 2.1 mmol, 2.0 equiv.), the resulting yellow solution was stirred at rt overnight. The solvent was removed under reduced pressure and water (45 mL) was added to the residue. The resulting yellow solution was stirred for 30 min at rt and ethyl acetate (45 mL) was added. The organic phase was separated, and the aqueous layer was extracted with ethyl acetate (3 x 45 mL). The organic layer was dried ($Na_2SO_4$) and concentrated *in vacuo*. The residue was purified by FCC (Ø = 4 cm, l = 20 cm, V = 60 mL, $CH_2Cl_2$:$CH_3OH$ 98:2). $R_f$ = 0.45 ($CH_2Cl_2$:$CH_3OH$ 2:1). Pale yellow solid, mp 193 $^oC$, yield 0.35 g (71 %). $C_{19}H_{18}BrN_3O_4S$ (464.3 g/mol). $^1H$ NMR (400 MHz, DMSO-$D_6$): δ (ppm) = 2.18 (s, 3H, C$H_3$-B), 4.21 (d, *J* = 6.1 Hz, 2H, CONHC$H_2$), 4.38 (d, *J* = 6.1 Hz, 2H, $SO_2$NHC$H_2$), 7.23 (t, *J* = 7.1 Hz, 1H, 4-$H_C$), 7.28 (d, *J* = 6.6 Hz, 2H, 2-$H_C$, 6-$H_C$), 7.30 – 7.34 (m, 2H, 3-$H_C$, 5-$H_C$), 7.38 (t, *J* = 7.9 Hz, 1H, 5-$H_A$), 7.69 (ddd, *J* = 7.8 / 1.7 / 1.0 Hz, 1H, 6-$H_A$), 7.74 (ddd, *J* = 8.0 / 2.0 / 1.0 Hz, 1H, 4-$H_A$), 7.80 (t, *J* = 1.9 Hz, 1H, 2-$H_A$), 8.55 (t, *J* = 6.1 Hz, 1H, $SO_2$N*H*$CH_2$), 8.57 (t, *J* = 6.4 Hz, 1H, CON*H*$CH_2$). $^{13}C$ NMR (151 MHz, DMSO-$D_6$): δ (ppm) = 15.5 (*C*$H_3$-B), 42.6 (CONHC$H_2$), 45.0 ($SO_2$NHC$H_2$), 125.0 (C-$3_A$), 128.7 (C-$6_A$), 130.0 (C-$4_C$), 130.6 (2C, C-$2_C$, C-$6_C$), 131.4 (2C, C-$3_C$, C-$5_C$), 132.0 (C-$2_A$), 134.2 (C-$5_A$), 138.3 (C-$4_A$), 142.2 (C-$4_B$), 142.3 (C-$1_C$), 143.9 (C-$5_B$), 145.1 (C-$1_A$), 160.3 (*C*ONHC$H_2$), 161.5 (C-$2_B$).

### 5.3.35. Ethyl 5-{[(*tert*-butoxycarbonyl)amino]methyl}isoxazole-3-carboxylate (33)[29]

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Ethyl 2-chloro-2-(hydroxyimino)acetate **32** (0.1 g, 0.66 mmol, 1.0 equiv.) was dissolved in THF (15 mL). A solution of *N*-boc-propargylamine (0.11 g, 0.73 mmol, 1.1 equiv.) and $Et_3N$ (0.14 mL, 1.0 mmol, 1.5 equiv.) in $Et_2O$ was added and stirred at rt overnight. An aqueous solution of $NH_4Cl$ was added. The organic phase was separated, and the aqueous layer was extracted with ethyl acetate (3 x 40 mL). The organic layer was dried ($Na_2SO_4$) and concentrated *in vacuo*. The residue was purified by FCC (Ø = 2 cm, l = 10 cm, V = 20 mL, cyclohexane/ethyl acetate 7:3). $R_f$ = 0.25 (cyclohexane/ethyl acetate 8:2). Pale yellow oil, yield 0.12 g (69 %). $C_{12}H_{18}N_2O_5$ (270.3 g/mol). $^1$H NMR (600 MHz, DMSO-$D_6$): δ (ppm) = 1.29 (t, *J* = 7.1 Hz, 3H, $OCH_2C\mathit{H}_3$), 1.37 (s, 9H, $(\mathit{H}_3C)_3C$-O-C(=O)), 4.30 (d, *J* = 6.0 Hz, 2H, $C(=O)NHC\mathit{H}_2$), 4.33 (q, *J* = 7.1 Hz, 2H, $OC\mathit{H}_2CH_3$), 6.61 (s, 1H, 4-$H_{arom}$), 7.57 (t, *J* = 6.0 Hz, 1H, $C(=O)N\mathit{H}CH_2$). $^{13}$C NMR (151 MHz, DMSO-$D_6$): δ (ppm) = 17.0 ($OCH_2\mathit{C}H_3$), 31.2 (3C, $(\mathit{C}H_3)_3CO$), 39.1 ($CONHCH_2$), 64.9 ($O\mathit{C}H_2CH_3$), 81.7 ($(CH_3)_3\mathit{C}$-O-C(=O)), 105.2 (C-$4_B$), 158.6 (C-$5_B$), 159.1 (C-$3_B$), 162.4 ($\mathit{C}(=O)OCH_2CH_3$), 176.3 ($\mathit{C}ONHCH_2$).

### 5.3.36. 5-{[(tert-Butoxycarbonyl)amino]methyl}isoxazole-3-carboxylic acid (34)

Ester **33** (0.8 g, 2.9 mmol, 1.0 equiv.) was dissolved in a mixture of MeOH and THF (1:1, 30 mL) and 2 M KOH (1.5 mL, 2.9 mmol, 1.0 equiv.) was added. The reaction mixture was stirred for 16 h at rt. After completion of the conversion, the solvent was evaporated *in vacuo*. 1 M HCl (3.5 mL) was added dropwise to the residue untill the pH was < 7 and the precipitated solid was collected by filtration. $R_f$ = 0.23 ($CH_2Cl_2$/$CH_3OH$ 2:1). Colorless solid, mp 203 °C, yield 0.61 g (86 %). Purity (HPLC): 88.8 % ($t_R$ = 13.7 min). $C_{10}H_{14}N_2O_5$ (242.2 g/mol). $^1$H NMR (400 MHz, DMSO-$D_6$): δ (ppm) = 1.37 (s, 9H, $(\mathit{H}_3C)_3CO$), 4.29 (d, *J* = 6.0 Hz, 2H, $C(=O)NHC\mathit{H}_2$), 6.54 (s, 1H,

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$H_{arom}$), 7.55 (t, *J* = 6.0 Hz, 1H, C(=O)N*H*$CH_2$). A signal for the $CO_2H$ proton is not seen in the spectrum. $^{13}$C NMR (151 MHz, DMSO-$D_6$): δ (ppm) = 31.2 (3C, ($CH_3$)$_3$C), 39.1, C(=O)NH*C*$H_2$), 81.7 (($CH_3$)$_3$*C*O), 105.2 (C-$4_B$), 158.7 (C-$5_B$), 160.0 (C-$3_B$), 163.9 (*C*(=O)NHCH$_2$), 176.0 (*C*$O_2$H).

### 5.3.37. *tert*-Butyl {[3-(2-benzoylhydrazin-1-carbonyl)isoxazol-5-yl]methyl}carbamate (35)

Carboxylic acid **34** (0.25 g, 1.03 mmol, 1.0 equiv.), benzoylhydrazine (0.14 g, 1.03 mmol, 1.0 equiv.) and COMU (0.49 g, 1.13 mmol, 1.1 equiv.) were suspended in THF (30 mL). After addition of DIPEA (0.36 mL, 2.06 mmol, 2.0 equiv.), the resulting yellow solution was stirred at rt overnight. The solvent was removed under reduced pressure and water (30 mL) was added to the residue. The resulting yellow solution was stirred for 30 min at rt and ethyl acetate (30 mL) was added. The organic phase was separated, and the aqueous layer was extracted with ethyl acetate (3 x 30 mL). The organic layer was dried ($Na_2SO_4$) and concentrated *in vacuo*. The residue was purified by FCC (Ø = 3 cm, l = 20 cm, V = 50 mL, $CH_2Cl_2$:$CH_3OH$ 98:2). $R_f$ = 0.75 ($CH_2Cl_2$:$CH_3OH$ 9:1). Colorless solid, mp 211 $^{o}$C, yield 0.28 g (75 %). $C_{17}H_{20}N_4O_5$ (360.4 g/mol). $^1$H NMR (600 MHz, DMSO-$D_6$): δ (ppm) = 1.38 (s, 9H, (C*H*$_3$)$_3$C), 4.32 (d, *J* = 6.0 Hz, 2H, C(=O)NHC*H*$_2$), 6.64 (s, 1H, 4-$H_B$), 7.50 (t, *J* = 8.9, 2H, 3-$H_C$, 5-$H_C$), 7.58 (t, *J* = 7.4 Hz, 1H, 4-$H_C$), 7.60 (t, *J* = 6.0 Hz, 1H, N*H*$CH_2$), 7.88(dd, *J* = 7.0 / 1.0 Hz, 2H, 2-$H_C$, 6-$H_C$), 10.57 (s, 1H, N*H*COAryl$_C$), 10.77 (s, 1H, N*H*(C=O)Isox$_B$). $^{13}$C NMR (151 MHz, DMSO-$D_6$): δ (ppm) = 31.2 (3C, ($CH_3$)$_3$COC(=O)), 39.2 (C(=O)NH*C*$H_2$), 81.7 (($CH_3$)$_3$*C*OC(=O), 104.2 (C-$4_B$), 130.6 (2C, C-$2_C$, C-$6_C$), 131.7 (2C, C-$3_C$, C-$5_C$) 135.1 (C-$4_C$), 135.3 (C-$1_C$), 158.7 (C-$5_B$), 160.4 (C-$3_B$), 161.2 (NH*C*(=O)Isox$_B$), 168.6(NH*C*(=O)Aryl$_C$), 175.9 (($CH_3$)$_3$CO*C*(=O)).

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**5.3.38. 5-(Aminomethyl)-*N*'-benzoylisoxazole-3-carbohydrazide (36)**

A mixture of **35** (0.25 g, 0.69 mmol), $CH_2Cl_2$ (5 mL) and trifluoroacetic acid (5 mL) was stirred at rt for 1 h. After complete conversion monitored by TLC, the solvent was removed under reduced pressure. The residue was obtained as a solid which was then dissolved in ethyl acetate (10 mL) and $Et_3N$ (0.6 mL) was added to obtain the free amine. Water (10 mL) was added. The organic phase was separated, and the aqueous layer was extracted with ethyl acetate (3 x 10 mL). The organic layer was dried ($Na_2SO_4$) and concentrated *in vacuo*. The residue was obtained as a colorless oil, which was proceeded to the next step without purification. Colorless oil yield 0.15 g (83 %). $C_{12}H_{12}N_4O_3$ (260.3 g/mol).

**5.3.39. *N*-{[3-(2-Benzoylhydrazin-1-ylcarbonyl)isoxazol-5-yl]methyl}-3-bromobenzenesulfonamide (37a)**

Primary amine **36** (50 mg, 0.2 mmol, 1.0 equiv.) was dissolved in THF (5 mL) and the solution was cooled to 0 $^oC$. $Et_3N$ (60 µL, 0.6 mmol, 3.0 equiv.) and a solution of 3-bromobenzenesulfonyl chloride (58 mg, 0.2 mmol, 1.0 equiv.) in THF (5 mL) were added. The reaction mixture was stirred overnight at room temperature. After completion of the transformation, the solvent was evaporated *in vacuo* and ethyl acetate (10 mL) and $H_2O$ (10 mL) were added. The organic layer was separated, and the aqueous layer was extracted with ethyl acetate (3 x 10 mL). The organic layer was dried ($Na_2SO_4$) and concentrated *in vacuo*. The residue was purified by FCC (Ø = 2 cm, l = 15 cm, V = 35 mL, $CH_2Cl_2$:$CH_3OH$ 97:3). $R_f$ = 0.65 ($CH_2Cl_2$:$CH_3OH$ 9:1). Pale yellow solid, mp 215 $^oC$, yield 60 mg (66 %). $C_{18}H_{15}BrN_4O_5S$ (479.3 g/mol). $^1H$ NMR (600 MHz, DMSO-$D_6$): δ (ppm) = 4.34 (s, 2H,

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$SO_2NHC\mathit{H}_2$), 6.65 (s, 1H, 4-$H_B$), 7.50 (t, *J* = 7.6 Hz, 2H, 3-$H_C$, 5-$H_C$), 7.53 (t, *J* = 7.9 Hz, 1H, 5-$H_A$), 7.55 – 7.62 (m, 1H, 4-$H_C$), 7.78 (dd, *J* = 7.9 / 1.3 Hz, 1H, 6-$H_A$), 7.85 (ddd, *J* = 8.0 / 2.0 / 1.0 Hz, 1H, 4-$H_A$), 7.88 (d, *J* = 7.5 Hz, 2H, 2-$H_C$, 6-$H_C$), 7.92 (t, *J* = 1.9 Hz, 1H, 2-$H_A$). The signals for the N-H protons are not seen in the spectrum. $^{13}$C NMR (151 MHz, DMSO-$D_6$): δ (ppm) = 40.9 ($SO_2NHC\mathit{H}_2$), 105.6 (C-$4_B$), 125.3 (C-$3_A$), 128.6 (C-$6_A$), 130. 5 (2C, C-$2_C$, C-$6_C$), 131.6 (2C, C-$3_C$, C-$5_C$) 132.0 (C-$2_A$), 134.6 (C-$5_A$), 135.1 (C-$4_C$), 135.3 (C-$1_C$), 138.6 (C-$4_A$), 145.3 (C-$1_A$), 160.3 (C-$5_B$), 160.9 (C-$3_B$), 168.5 (NH*C*(=O)aryl$_C$), 173.5 (NH*C*(=O)Isox$_B$).

### 5.3.40. *N*-{[3-(2-Benzoylhydrazin-1-ylcarbonyl)isoxazol-5-yl]methyl}-3-chloro-4-fluorobenzenesulfonamide (37b)

Primary amine **36** (50 mg, 0.2 mmol, 1.0 equiv.) was dissolved in THF (5 mL) and the solution was cooled to 0 $^{o}$C. $Et_3N$ (60 µL, 0.6 mmol, 3.0 equiv.) and a solution of 4-chloro-3-fluorobenzenesulfonyl chloride (52 mg, 0.2 mmol, 1.0 equiv.) in THF (5 mL) were added. The reaction mixture was stirred overnight at room temperature. After completion of the transformation, the solvent was evaporated *in vacuo* and ethyl acetate (10 mL) and $H_2O$ (10 mL) were added. The organic layer was separated, and the aqueous layer was extracted with ethyl acetate (3 x 10 mL). The organic layer was dried ($Na_2SO_4$) and concentrated *in vacuo*. The residue was purified by FCC (Ø = 2 cm, l = 15 cm, V = 30 mL, $CH_2Cl_2$:$CH_3OH$ 97:3). $R_f$ = 0.65 ($CH_2Cl_2$:$CH_3OH$ 9:1). Pale yellow solid, mp 216 $^{o}$C, yield 58 mg (67 %). $C_{18}H_{14}ClFN_4O_5S$ (452.8 g/mol). $^1$H NMR (600 MHz, DMSO-$D_6$): δ (ppm) = 4.36 (s, 2H, $SO_2NHC\mathit{H}_2$), 6.66 (s, 1H, 4-$H_B$), 7.50 (t, *J* = 7.6 Hz, 2H, 3-$H_C$, 5-$H_C$), 7.56 – 7.60 (m, 1H, 4-$H_C$), 7.63 (t, *J* = 8.9 Hz, 1H, 5-$H_A$), 7.80 (ddd, *J* = 8.7 / 4.4 / 2.3 Hz, 1H, 6-$H_A$), 7.88 (d, *J* = 7.3, 2H, 2-$H_C$, 6-$H_C$), 7.96 (dd, *J* = 6.8 / 2.3 Hz, 1H, 2-$H_A$), 8.68 (s,

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1H, $SO_2NHCH_2$), 10.57 (s, 1H, N*H*CO$aryl_C$), 10.74 (s, 1H, N*H*CO$Isox_B$). $^{13}$C NMR (151 MHz, DMSO-$D_6$): δ (ppm) = 40.9 ($SO_2NHCH_2$), 105.6 (C-$4_B$), 121.2 (d, *J* = 26.1 Hz, C-$5_A$), 123.9 (d, *J* = 16.6 Hz, C-$3_A$), 130.6 (2C, C-$2_C$, C-$6_C$), 131.1 (d, *J* = 9.5 Hz, C-$6_A$), 131.6 (2C, C-$3_C$, C-$5_C$) 132.3 (C-$2_A$), 135.1 (C-$4_C$), 135.3 (C-$1_C$), 140.9 (d, *J* = 4.0 Hz, C-$1_A$), 160.4 (C-$5_B$), 160.9 (C-$3_B$), 161.8 (d, *J* = 255.3 Hz, C-$4_A$), 168.5 (NH*C*(=O)$aryl_C$), 173.5 (NH*C*(=O)$isox_B$).

### 5.3.41. *N*-{[3-(2-benzoylhydrazin-1-ylcarbonyl)isoxazol-5-yl]methyl}-4-methylbenzenesulfonamide (37c)

Primary amine **36** (50 mg, 0.2 mmol, 1.0 equiv.) was dissolved in THF (5 mL) and the solution was cooled to 0 $^{o}$C. $Et_3N$ (60 µL, 0.6 mmol, 3.0 equiv.) and a solution of p-TsCl (40 mg, 0.2 mmol, 1.0 equiv.) in THF (5 mL) were added. The reaction mixture was stirred overnight at room temperature. After completion of the transformation, the solvent was evaporated *in vacuo* and ethyl acetate (10 mL) and $H_2O$ (10 mL) were added. The organic layer was separated, and the aqueous layer was extracted with ethyl acetate (3 x 10 mL). The organic layer was dried ($Na_2SO_4$) and concentrated *in vacuo*. The residue was purified by FCC (Ø = 2 cm, l = 10 cm, V = 30 mL, $CH_2Cl_2$:$CH_3OH$ 97:3). $R_f$ = 0.62 ($CH_2Cl_2$:$CH_3OH$ 9:1). Light yellow solid, mp 212 $^{o}$C, yield 52 mg (64 %). Purity (HPLC): 92.5 % ($t_R$ = 16.2 min). $C_{19}H_{18}N_4O_5S$ (414.4 g/mol). $^1$H NMR (600 MHz, DMSO-$D_6$): δ (ppm) = 2.37 (s, 3H, C*H*$_3$-A), 4.24 (d, *J* = 5.9 Hz, 2H, $SO_2NHCH_2$), 6.59 (s, 1H, 4-$H_B$), 7.37 (d, *J* = 7.6 Hz, 2H, 3-$H_A$, 5-$H_A$), 7.50 (t, *J* = 7.6 Hz, 2H, 3-$H_C$, 5-$H_C$), 7.55 – 7.62 (m, 1H, 4-$H_C$), 7.67 (dd, *J* = 8.0 / 2.4 Hz, 2H, 2-$H_A$, 6-$H_A$), 7.85 – 7.91 (m, 2H, 2-$H_C$, 6-$H_C$), 8.40 (t, *J* = 6.1 Hz, 1H, $SO_2NHCH_2$), 10.55 (s, 1H, N*H*C(=O)$aryl_C$), 10.73 (s, 1H, N*H*C(=O)$Isox_B$). $^{13}$C NMR (151 MHz, DMSO-$D_6$): δ (ppm) = 24.1 ($CH_3$-A), 41.0 ($SO_2NHCH_2$), 105.4 (C-$4_B$),

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129.7 (2C, C-$2_A$, C-$6_A$) 130.6 (2C, C-$2_C$, C-$6_C$), 131.7 (2C, C-$3_C$,C-$5_C$) 132.8 (2C, C-$3_A$, C-$5_A$), 135.1 (C-$1_C$), 135.3 (C-$4_C$), 140.3 (C-$4_A$), 146.2 (C-$1_A$), 160.3 (C-$5_B$), 161.0 (C-$3_B$), 168.5 (NH*C*(=O)aryl$_C$), 173.8 (NH*C*(=O)Isox$_B$).

### 5.4. Pharmacological evaluation

#### 5.4.1. Two-electrode voltage clamp experiments

Procedures were as described before by Schreiber *et al*.[21] In brief, stage V defoliated *Xenopus laevis* oocytes were obtained from EcoCyte Bioscience (Castrop-Rauxel, Germany) and oocytes were injected with 0.8 ng GluN1a cRNA and 0.8 ng GluN2A cRNA *in-vitro* transcribed from linearized cDNA templates with Ambion T7 mMessage mMachine kit (Life Technologies, Darmstadt, Germany) using nanoliter injector 2000 (WPI, Berlin, Germany). Injected oocytes were stored for 3 days at 16 $^oC$ in Bath's solution containing (mmol/L): 88 NaCl, 1 KCl, 0.4 $CaCl_2$, 0.33 $Ca(NO_3)_2$, 0.6 $MgSO_4$, 5 Tris-HCl, 2.4 $NaHCO_3$ and supplemented with 80 mg/L theophylline, 63 mg/L penicillin, 40 mg/L streptomycin and 100 mg/L gentamycin.

TEVC recordings were conducted at room temperature using a Turbo Tec 10CX amplifier (NPI electronic, Tamm, Germany), NI USB 6221 DA/AD Interface (National Instruments, Austin, USA) and *GePulse* Software (Dr. Michael Pusch, Genova, Italy). The oocytes were recorded in $Ba^{2+}$-Ringer solution containing (mmol/L): 10 HEPES, 90 NaCl, 1 KCl, 1.5 $BaCl_2$ (pH was adjusted to 7.4 with 1 M NaOH) during measurements. The agonist solution contained 10 µM each of glycine and glutamate which was added from 100 mM stock solutions of glycine and glutamate prior experimentation. The test compound solutions were freshly prepared from 10 mM DMSO stock solutions. The recordings holding potential was -70 mV. The recording

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electrodes were backfilled with 3 M KCl and had resistances in the range of 0.5-1.5 MΩ.

The data were analyzed using constom software Ana (Dr. Michael Pusch, Genova, Italy) GraphPad Prism 8 and Origin. Inhibition was calculated by the following equation:

$$Inhibition = 1 - \frac{Ic\ - Ib}{Ia - Ib}$$

Where $Ic$ is the resting current in presence of the compound solution, $Ib$ is the holding current before adding agonist solution and $Ia$ is the current after adding agonist solution. The average inhibition of the compound was normalized to the average inhibition of **1** for comparing the activity of the compounds. The normalized inhibition $I_{norm}$ was calculated as follows:

$$I\ norm = \frac{Inhibition\ of\ compound\ at\ 10\ \mu M}{Inhibition\ of\ TCN - 201\ at\ 10\ \mu M}$$

Statistical significance of $I_{norm}$ was tested by application of One-way-ANOVA and post hoc mean comparison Tukey Test (See supporting information).

### 5.4.2. Receptor binding studies

The affinity towards the ifenprodil binding site of GluN2B subunit containing NMDA receptors was recorded as described in references.[27,28] Experimental details of the $\sigma_1$ and $\sigma_2$ assays are reported in references[29-31].

### Abbreviations

| | |
|---|---|
| COMU | (1-Cyano-1-ethoxycarbonylmethylenaminoxy)- |

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| | |
|---|---|
| | dimethylaminomorpholino-carbenium hexafluorophosphate |
| DIPEA | *N*,*N*-diisopropylethylamine |
| DMAP | 4-(*N*,*N*-dimethylamino)pyridine |
| LTD | Long term depression |
| LTP | Long term potentiation |
| NAM | Negative allosteric modulator |
| NBS | *N*-bromosuccinimide |
| SD | Standard deviation |
| SEM | Standard error of the mean |

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**Supporting Information**

The Supporting Information contains purity data of all test compounds, experimental procedures of receptor binding studies and $^{1}H$ and $^{13}C$ NMR spectra of prepared compounds.

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**Conflict of interests**

The authors declare no conflict of interest.

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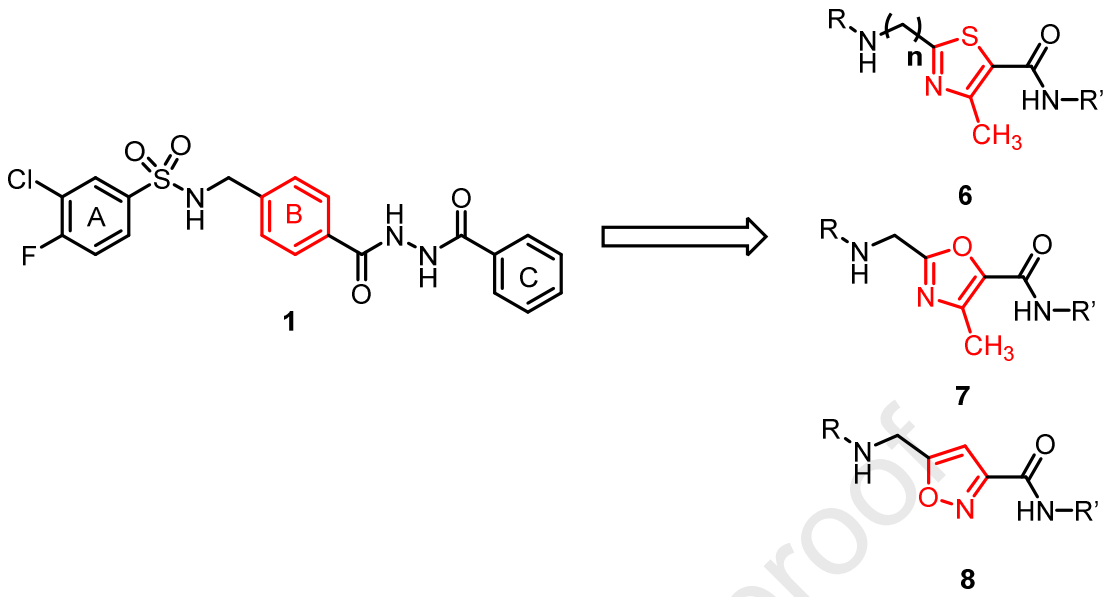


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Manuscript title

**Synthesis of GluN2A-selective NMDA receptor antagonists with an electron-rich aromatic B-ring**

Research Highlights

- NMDA receptors with GluN2A subunit are associated with various CNS diseases.
- TCN-201 adopts a U-shape structure in the binding pocket.
- Novel TCN-201 analogs with electron-rich ring B were synthesized.
- π/π-Interactions between the electron-rich and –poor aromatic rings should reinforce the U-shape structure.
- The inhibitor activity of the novel ligands was determined with two-electrode voltage clamp experiments.
- Oxazole and isoxazole analogs reach almost the activity of the lead compound TCN-201.

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To
Prof. Dr. Herve Galons
Editor in Chief
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**Submission of new manuscript**

**Synthesis of GluN2A-selective NMDA receptor antagonists with an electron-rich aromatic B-ring**

There is no conflict of interest.

Bernhard Wünsch

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**Declaration of interests**

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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