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FULL PAPER

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Design, synthesis, and biological evaluation of 1-ethyl-3-(thiazol-2-yl)urea derivatives as *Escherichia coli* DNA gyrase inhibitors

Tihomir Tomašič[1] | Michaela Barančoková[1] | Nace Zidar[1] | Janez Ilaš[1] | Päivi Tammela[2] | Danijel Kikelj[1]

[1] Faculty of Pharmacy, University of Ljubljana, Ljubljana, Slovenia

[2] Division of Pharmaceutical Biosciences, Faculty of Pharmacy, University of Helsinki, Helsinki, Finland

Correspondence
Dr. Tihomir Tomašič, Faculty of Pharmacy, University of Ljubljana, Aškerčeva cesta 7, Ljubljana 1000, Slovenia.
Email: tihomir.tomasic@ffa.uni-lj.si

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Abstract

Discovery of novel DNA gyrase B inhibitors remains an attractive field in the search for new antibacterial drugs to overcome the known bacterial resistance mechanisms. In the present study, we designed and synthesized novel ethylurea derivatives of 4,5,6,7-tetrahydrobenzo[1,2-*d*]thiazole-2,6-diamine, 2-(2-aminothiazol-4-yl)acetic acid, and benzo[1,2-*d*]thiazole-2,6-diamine and evaluated their *Escherichia coli* DNA gyrase inhibition. The most potent DNA gyrase inhibitors in the prepared library of compounds were benzo[1,2-*d*]thiazoles **32–34**, **36**, and **37** with IC_{50} values in the low micromolar range. The most promising inhibitors identified were evaluated against selected Gram-positive and Gram-negative bacterial strains. Compound **33** showed a MIC of 50 μM against an *E. coli* efflux pump-defective strain, which suggests that efflux decreases the on-target concentrations of these compounds.

KEYWORDS
antibacterial, DNA gyrase, ethylurea, inhibitor, thiazole

1 | INTRODUCTION

Antibacterial drug resistance is a serious threat to human health, with bacteria such as *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, and *Enterobacter* spp. (i.e., the "ESKAPE" bacteria) becoming multidrug resistant against the presently available antibiotics.[1] To successfully fight infections with these "bad bugs" in the future, there is the continuous need for development of novel antibacterial agents that are not liable to the existing resistance mechanisms.

DNA gyrase and topoisomerase IV are type IIA bacterial topoisomerases that are involved in modulation of DNA topology during DNA replication, transcription, and recombination. As these enzymes are essential for cell viability and are conserved across all bacteria but are not found in human cells, this makes them attractive targets for antibacterial drug discovery.[2]

DNA gyrase and topoisomerase IV are heterotetrameric proteins, with DNA gyrase composed of two GyrA and two GyrB subunits (A_2B_2), and topoisomerase IV of two ParC and ParE subunits (C_2E_2). The GyrB and ParE subunits have the ATPase activities which are necessary for providing the energy for the supercoiling and decatenation reactions of the catalytic GyrA and ParC subunits. The aminocoumarin antibiotic novobiocin interacts with the ATP binding sites of GyrB and ParE, but it was withdrawn from the market because of its toxicity and lack of efficacy.[3] In recent years, several structural classes of GyrB and ParE inhibitors have been discovered through high-throughput screening, virtual screening, and *de-novo* design, in both industry and academia.[3–5] Structure-based optimization of hits has resulted in potent GyrB and/or

Abbreviations: EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; GyrA, DNA gyrase subunit A; GyrB, DNA gyrase subunit B; HOBt, 1-hydroxybenzotriazole; NMM, *N*-methylmorpholine; ParC, topoisomerase IV subunit A; ParE, topoisomerase IV subunit B; RA, residual activity.

ParE inhibitors that have antibacterial activities mainly against Gram-positive bacteria, such as the cyclothialidine,[6] ethylurea,[7–14] pyrazolopyridone,[15,16] and pyrrolamide[17–19] classes (Figure 1). Rare examples of GyrB and ParE inhibitors with antibacterial activities against Gram-negative bacteria include pyrrolopyrimidines,[20] pyrimidinoindoles,[21] and some pyrrolamides[18] (Figure 1). However, despite extensive efforts to develop new ATPase GyrB/ParE inhibitors, none has progressed beyond phase 1 clinical trials.[3]

We have recently designed and optimized several structural classes of pyrrolamide-based DNA gyrase and topoisomerase IV inhibitors, which were based on the 4,5,6,7-tetrahydrobenzo[1,2-*d*]-thiazole-2,6-diamine,[22,23] benzo[1,2-*d*]thiazole-2,6-diamine,[24] 4-aminopiperidine,[25] and aniline[26,27] scaffolds. In all of them, the 4,5-dibromopyrrolamide moiety was shown to be an important structural feature, as it is necessary to achieve potent *Escherichia coli* DNA gyrase inhibition. The 5,7-diarylbenzo[1,2-*d*]thiazole ethylureas (Figure 1) have already been reported to be potent DNA gyrase inhibitors and to have broad-spectrum Gram-positive antibacterial activities.[13,14] We describe here the design and synthesis of novel thiazole-based ethylureas, and the evaluation of their *E. coli* DNA gyrase inhibition and antibacterial activities against selected Gram-positive and Gram-negative bacteria.

2 | RESULTS AND DISCUSSION

2.1 | Design

The crystal structures of DNA gyrase in complexes with ethylurea-based inhibitors[12] show that their Asp73 (*E. coli* numbering) side-chain carboxylate forms two hydrogen bonds with the two NH groups of the ethylurea moiety, while the nitrogen atom of the central scaffold forms an additional hydrogen bond with a conserved water molecule (Figure 2). With this hydrogen bonding motif, ethylurea-based DNA gyrase inhibitors mimic the binding of the adenine ring of ATP, while additional interactions are formed with the amino acids of the lipophilic floor of the ATP binding site and with the Arg76 and Arg136 side-chains.[3,4]

Based on the described binding mode, we have designed novel DNA gyrase B inhibitors bearing ethylurea moiety at position 2 of the thiazole moiety of the 4,5,6,7-tetrahydrobenzo[1,2-*d*]thiazole-2,6-diamine (Figure 2, **5–15**), 2-(2-aminothiazol-4-yl)acetic acid (Figure 2, **23–26**), and benzo[1,2-*d*]thiazole-2,6-diamine (Figure 2, **30–38**) central scaffolds. Additional substituents were introduced to interact with Arg76 and/or Arg136 side chains to improve binding affinity of the designed compounds. The design of all three of these series of compounds was supported by molecular docking to the *E. coli* DNA gyrase ATP binding site, which predicted the characteristic hydrogen bonding network that is shown in Figure 2.

2.2 | Chemistry

The synthesis of the 4,5,6,7-tetrahydrobenzo[1,2-*d*]thiazole-2,6-diamine derivatives **5–15** is outlined in Scheme 1. The enantiomerically pure starting (*S*)-4,5,6,7-tetrahydrobenzo[1,2-*d*]thiazole-2,6-diamine (**1**) was synthesized according to the previously reported procedure.[22,28] In the first step, the 6-amino group was selectively protected as a *tert*-butylcarbamate using di-*tert*-butyl dicarbonate (Boc_2O) in tetrahydrofuran, to obtain **2**, and then the 2-amino group was reacted with ethyl isocyanate in chloroform to yield ethylurea **3**. The Boc protecting group of **3** was removed by acidolysis with HCl gas generated *in situ* by the slow addition of acetyl chloride to methanol. The hydrochloride of **4** was obtained first, and was converted to its free

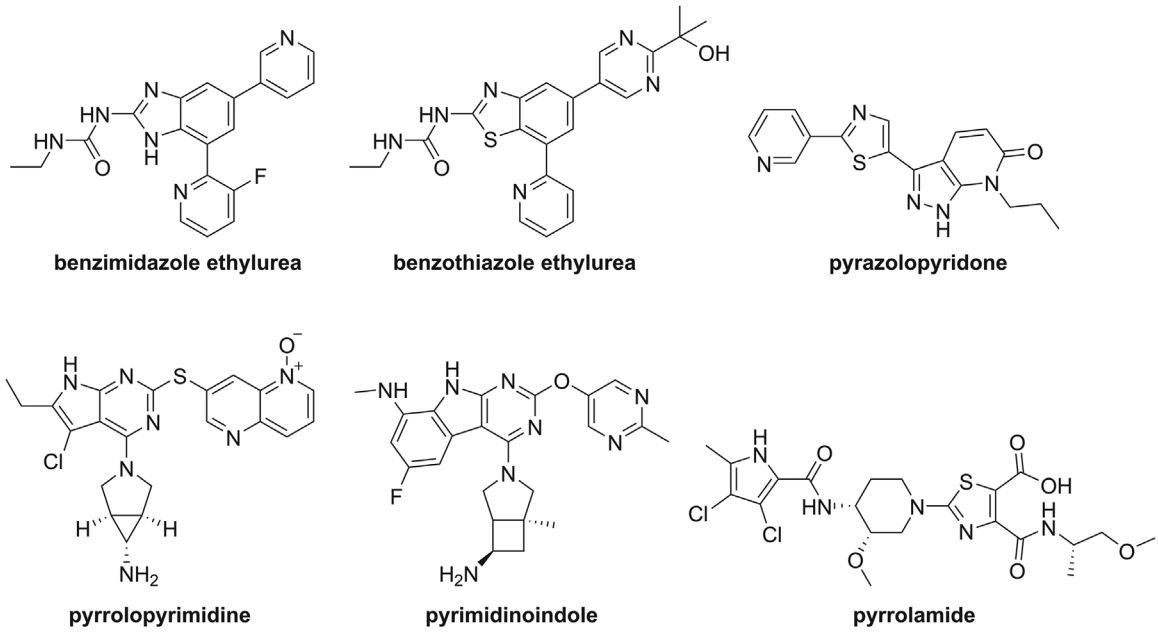


FIGURE 1 Representative synthetic GyrB and/or ParE inhibitors

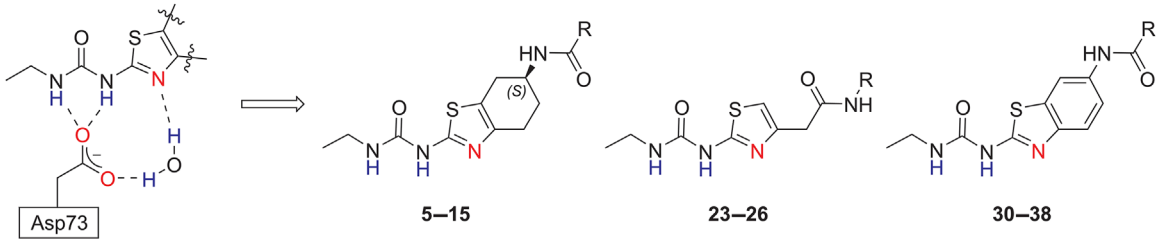
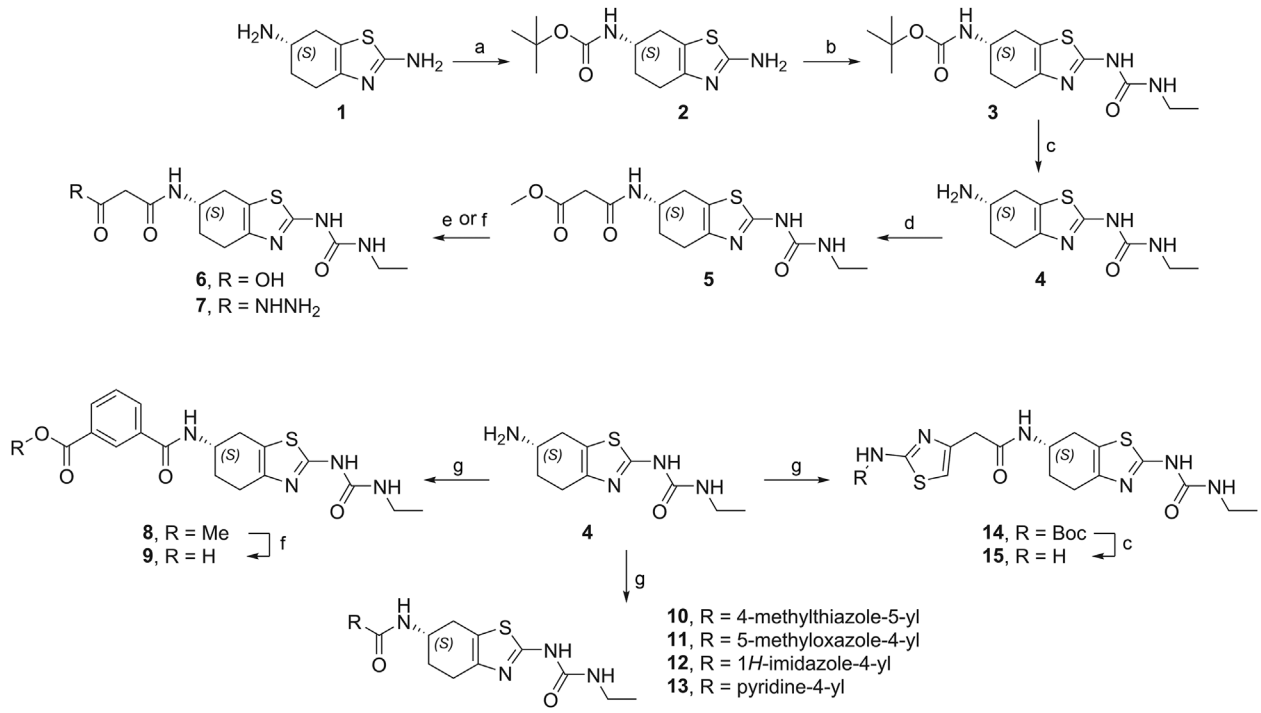


FIGURE 2 The hydrogen bonds of the thiazole-based ethylureas in the ATP binding site of DNA gyrase (*E. coli* numbering) and designed novel thiazole-based ethylurea DNA gyrase B inhibitors

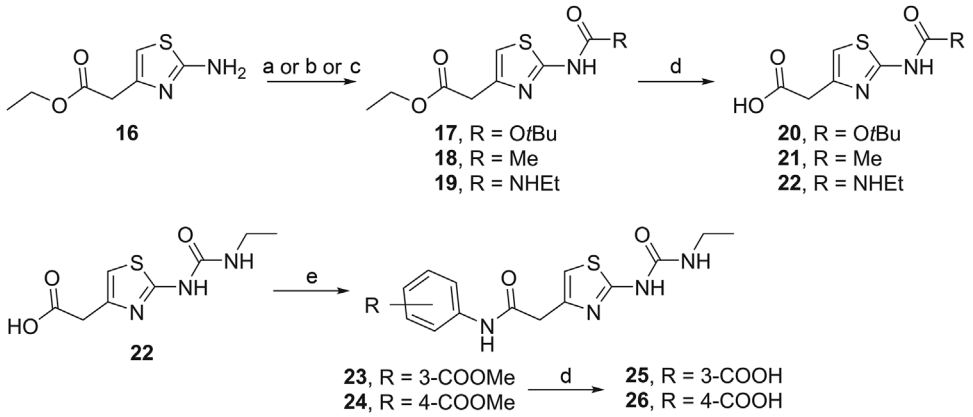
base as this offered higher yields under weakly alkaline conditions of the following amide-bond formation step than seen for the reaction with the hydrochloride of **4**. Acylation of **4** with methyl malonyl chloride in 1,4-dioxane at room temperature yielded ester **5**, which was hydrolyzed to its acid derivative **6** under alkaline conditions, and converted to hydrazide **7** by ester hydrazinolysis. Amides **8** and **10–14** were prepared by coupling of amine **4** with the corresponding carboxylic acids, using EDC/HOBt coupling reagents. Benzoic acid derivative **9** was prepared by alkaline hydrolysis of ester **8**, while acidolysis of the *tert*-butylcarbamate **14** resulted in amine **15**.

The designed 2-(2-aminothiazol-4-yl)acetic acid derivatives **23–26** were prepared according to the synthetic procedures presented in Scheme 2. Amine **16** was converted to *tert*-butylcarbamate **17** using Boc_2O, to acetamide **18** using acetyl chloride, and to ethylurea **19** using ethyl isocyanate. Compounds **17–19** were then hydrolyzed under alkaline conditions, to their carboxylic acid counterparts **20–22**. Carboxylic acid **22** was coupled in the following step to methyl 3-aminobenzoate and methyl 4-aminobenzoate using EDC and HOBt, to obtain compounds **23** and **24**. Their carboxylic acid derivatives **25** and **26** were prepared by alkaline hydrolysis.

Benzo[1,2-*d*]thiazoles **30–38** were synthesized as summarized in Scheme 3. In the first step, ethylurea **28** was prepared from amine **27** and ethyl isocyanate in toluene. Reduction of the nitro group of **28** by catalytic hydrogenation provided amine **29**, which was then used for the further synthesis. The 6-amino group of **29** was acylated with ethyl oxalyl chloride to provide ester **30**, which was hydrolyzed to acid **31** using 1 M NaOH. The coupling of amine **29** with 2-(2-aminothiazol-4-yl)acetic acids **20–22** (Scheme 2) yielded compounds **32–34**. In addition, amine **35**, which was obtained by Boc deprotection of **33**, was acylated with methyl malonyl chloride to



SCHEME 1 Synthesis of compounds **6–13**. Reagents and conditions: a) Boc_2O, THF, r.t., 18 h. b) Ethyl isocyanate, $CHCl_3$, r.t., 18 h. c) Acetyl chloride, MeOH, 0°C, 1 h, then r.t., 18 h. d) Methyl malonyl chloride, Et_3N, 1,4-dioxane, r.t., 18 h. e) **6**: 1 M NaOH, MeOH/H_2O, r.t., 24 h. f) **7**: Hydrazine hydrate, EtOH, 80°C, 18 h. g) Carboxylic acid, EDC, HOBt, NMM, DMF, r.t., 24 h



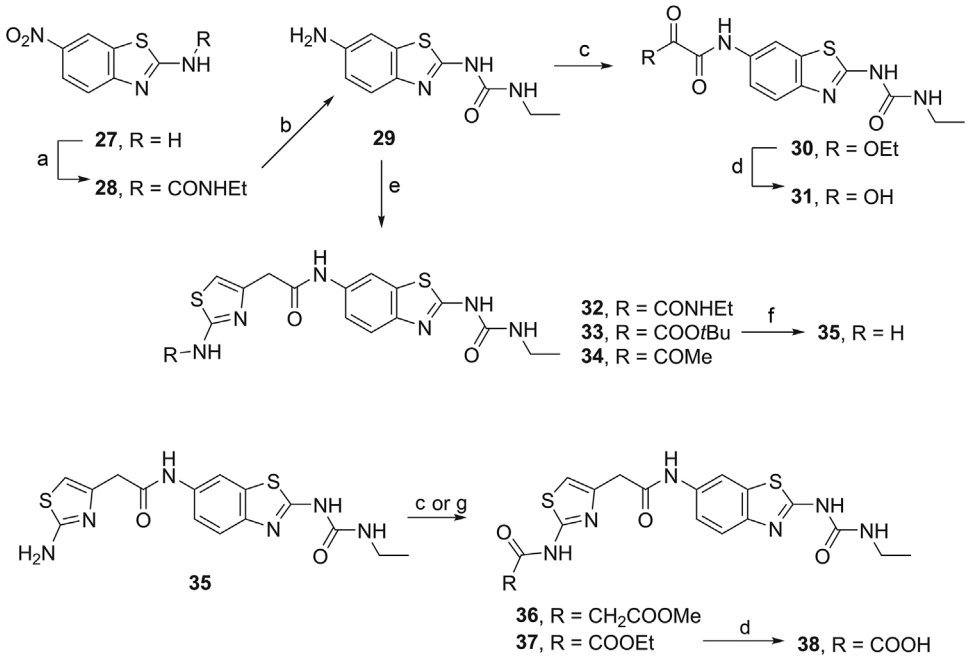
SCHEME 2 Synthesis of compounds **17–26**. Reagents and conditions: a) **17**: Boc_2O, 4-dimethylaminopyridine, CH_2Cl_2, r.t., 18 h. b) **18**: Acetyl chloride, Et_3N, CH_2Cl_2, r.t., 4 h. c) **19**: Ethyl isocyanate, $CHCl_3$, 50°C, 18 h. d) 2 M NaOH, MeOH/H_2O, r.t., 18 h. e) Amine, EDC, HOBt, NMM, DMF, r.t., 24 h

give **36** and ethyl oxalyl chloride to give **37**. Hydrolysis of **37** then gave carboxylic acid **38**.

2.3 | Biological evaluation

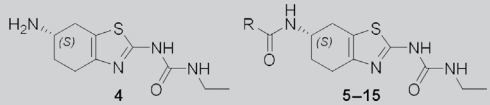
Compounds **4–15**, **23–26**, and **30–38** were tested for *E. coli* DNA gyrase inhibition using an *in vitro* DNA gyrase supercoiling assay (Tables 1–3). These data are presented as residual activities of the *E. coli* DNA gyrase at 100 or 10 μM of the tested compounds, or as IC_{50} values for the more active compounds, where the residual activities were <50% at 100 μM of the tested compounds. The selected final compounds were then screened for their antibacterial activities at 50 μM against two Gram-positive and two Gram-negative bacteria strains: *S. aureus* (ATCC 25923) and *Enterococcus faecalis* (ATCC 29212), and *E. coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853), respectively. In addition, the compounds that showed IC_{50} values less than 10 μM against *E. coli* DNA gyrase were tested against two *E. coli* mutant strains: *E. coli* JD17464, an *lpxC* deletion mutant with a defective outer membrane due to impaired lipid A biosynthesis; and *E. coli* JW5503, which has a defective efflux pump due to deletion of a *tolC* gene. The data from these antibacterial activity evaluations are presented in Tables 1, 3, and 4.

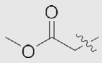
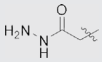
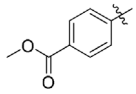
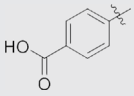
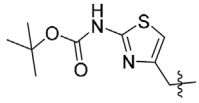
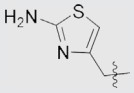
The *in vitro E. coli* DNA gyrase inhibitory activities of the 4,5,6,7-tetrahydrobenzo[1,2-*d*]thiazoles **4–15** and 2-(2-aminothiazol-4-yl)-



SCHEME 3 Synthesis of compounds **28–38**. Reagents and conditions. a) Ethyl isocyanate, Et_3N, toluene, reflux, 24 h. b) H_2, Pd/C, EtOH/DMF, r.t., 48 h. c) Ethyl oxalyl chloride, Et_3N, 1,4-dioxane, r.t., 18 h. d) 1 M NaOH, MeOH/H_2O, r.t., 18 h. e) Carboxylic acid, EDC, HOBt, NMM, DMF, r.t., 18 h. f) Acetyl chloride, MeOH, 0°C, 1 h, then r.t., 18 h. g) Methyl malonyl chloride, Et_3N, 1,4-dioxane, r.t., 18 h

TABLE 1 Inhibition of *E. coli* DNA gyrase and antibacterial activity of 4,5,6,7-tetrahydrobenzo[1,2-*d*]thiazoles **4–15**

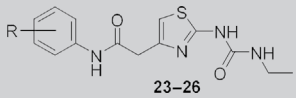


Compound	R	*E. coli* DNA gyrase inhibition		Growth inhibition at 50 μM (%)			
		IC_{50} (μM)	Residual activity[a] (%)	*S. aureus* ATCC 25923	*E. faecalis* ATCC 25212	*E. coli* ATCC 25922	*P. aeruginosa* ATCC 27853
4			100	n.t.[b]	n.t.	n.t.	n.t.
5			100	n.t.	n.t.	n.t.	n.t.
6			100	n.t.	n.t.	n.t.	n.t.
7			100	n.t.	n.t.	n.t.	n.t.
8			69	n.t.	n.t.	n.t.	n.t.
9			99	n.t.	n.t.	n.t.	n.t.
10			65	6	14	8	3
11		610 ± 20		n.t.	n.t.	n.t.	n.t.
12		900 ± 50		n.t.	n.t.	n.t.	n.t.
13			65	8	14	3	2
14		350 ± 30		4	20	7	8
15			79	0	18	6	0
Novobiocin		0.17					
Ciprofloxacin (MIC)[c]				1.5 μM	3.0 μM	0.05 μM	3.0 μM

[a]Percentage residual activity of the enzyme at 100 μM compound.
[b]n.t., not tested.
[c]Data for ciprofloxacin bacterial growth inhibition given as MICs.

acetic acid derivatives **23–26** are presented in Tables 1 and 2. The results show that these compounds are poor *E. coli* DNA gyrase inhibitors, since only compounds **11**, **12**, and **14** inhibited the enzyme at high micromolar concentrations. In contrast, some of the benzo[1,2-*d*]thiazoleureas **30–38** (Table 3) showed low micromolar *E. coli* DNA gyrase inhibition (IC_{50} values between 3.9 and 8.3 μM). Comparison of the partially saturated 4,5,6,7-tetrahydrobenzo[1,2-*d*]thiazole **14** (IC_{50} = 350 μM; Table 1) and unsaturated benzo[1,2-*d*]thiazole **33** (IC_{50} = 3.9 μM; Table 3) suggests the importance of the cation-π interactions between the Arg76 side-chain and the phenyl ring of these inhibitors (Figure 3). When the ethylurea moiety interacts with Asp73 in the DNA gyrase ATP binding site, the fused tetrahydrobenzene ring of compounds **5–15** is positioned under the Glu50-Arg76 salt bridge. Thus the cation-π interactions between Arg76 and the inhibitors cannot be formed, which results in less potent inhibitory activities. A similar phenomenon was reported previously in a comparison of the

TABLE 2 Inhibition of *E. coli* DNA gyrase by 2-(2-aminothiazol-4-yl)-acetic acids **23–26**



23–26

Compound	R	*E. coli* DNA gyrase residual activity[a] (%)
23	3-COOMe	98
24	4-COOMe	91
25	3-COOH	74
26	4-COOH	83

These compounds were not tested for growth inhibition of S. aureus ATCC 25923, *E. faecalis* ATCC 25212, *E. coli* ATCC 25922, and *P. aeruginosa* ATCC 27853.

[a]Percentage residual activity of the enzyme at 100 μM compound.

inhibitory activities of 4,5,6,7-tetrahydrobenzo[1,2-*d*]thiazoles[23] and benzo[1,2-*d*]thiazoles[24] with a pyrrolamide moiety at position 2. The lack of this cation-π interaction is probably also the reason for the inactivity of the 2-(2-aminothiazol-4-yl)acetic acid derivatives **23–26** (Table 2).

All of the tested compounds were only weakly active or were inactive at 50 μM against Gram-negative *E. coli* and *P. aeruginosa* and Gram-positive *S. aureus* and *E. faecalis* (Tables 1 and 3). One of the possible reasons for these weak antibacterial activities against these bacteria might be their low micromolar DNA gyrase inhibition. Some previously reported ethylurea-based compounds were shown to have nanomolar DNA gyrase inhibition and antibacterial activity against Gram-positive bacteria.[7–14] However, these compounds usually lack activity against Gram-negative bacteria, which is often a consequence of the efflux of these inhibitors. Similarly, in the present study, the improved MIC values against the *E. coli* mutant strain JW5503 for inhibitor **33** (MIC = 50 μM) (Table 4) suggests that these compounds are efflux pump substrates. Furthermore, as no improvements in the MICs against *E. coli* JD17464 were seen (Table 4), the efflux of these compounds and not their insufficient penetration through the cell wall appears to be the major reason for the absence of their antibacterial activity.

3 | CONCLUSIONS

A focused library of novel 4,5,6,7-tetrahydrobenzo[1,2-*d*]thiazole-, 2-(2-aminothiazol-4-yl)acetic acid-, and benzo[1,2-*d*]thiazole-based ethylurea derivatives was designed and prepared as potential DNA gyrase B inhibitors. The molecular docking studies showed hydrogen bond network formed with Asp73, structural water molecule and Arg136 in the ATP-binding site of *E. coli* DNA gyrase. The results of the *in vitro E. coli* DNA gyrase supercoiling assay showed that the most potent of these compounds were benzo[1,2-*d*]thiazoles **32–34**, **36**, and **37** with IC_{50} values in the low micromolar range, while 4,5,6,7-tetrahydrobenzo[1,2-*d*]-thiazole and 2-(2-aminothiazol-4-yl)acetic acid derivatives were only weakly active or inactive. Evaluation of the antibacterial activities showed weak inhibition of bacterial growth at 50 μM against *S. aureus*, *E. faecalis*, *E. coli*, and *P. aeruginosa*. In both Gram-positive and Gram-negative bacteria this could be the result of only micromolar enzyme inhibition and/or poor penetration through the bacterial cell wall. In addition, the *in vitro* assays on the *E. coli* efflux-pump-deficient strain (JW5503) indicated that these compounds are efflux pump substrates in *E. coli*, which will result in reduced cytoplasmic concentrations and hence poor antibacterial activity against Gram-negative strains. Nevertheless, these structure–activity relationships provide a basis for further optimization of this structural class of DNA gyrase inhibitors.

4 | EXPERIMENTAL

4.1 | Chemistry

4.1.1 | General procedures

Chemicals were obtained from Acros Organics (Geel, Belgium), Sigma–Aldrich (St. Louis, MO, USA), and TCI Europe N.V. (Zwijndrecht, Belgium) and used without further purification. Analytical TLC was performed on silica gel Merck 60 F_{254} plates (0.25 mm), using visualization with UV light and spray reagents. Column chromatography was carried out on silica gel 60 (particle size 240–400 mesh). HPLC analyses were performed on Agilent Technologies 1100 instrument with G1365B UV-VIS detector, G1316A thermostat and G1313A autosampler using Agilent Eclipse Plus C18 column (5 μm, 4.6 × 150 mm) using Method A: mobile phase: 0.1% trifluoroacetic acid in water (A) and acetonitrile (B); gradient: 2 min 95% A, 90% A to 10% A in 12 min, 10% A to 5% A in 1 min, then 5 min 5% A; flow rate 1.0 mL/min; injection volume: 10 μL. All tested compounds were ≥95% pure by HPLC. Melting points were determined on a Reichert hot stage microscope and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded at 400 and 100 MHz, respectively, on a Bruker AVANCE III 400 spectrometer (Bruker Corporation, Billerica, MA, USA) in DMSO-d_6 or $CDCl_3$ solutions, with TMS as the internal standard. Mass spectra were obtained using a VG Analytical Autospec Q mass spectrometer (Fisons, VG Analytical, Manchester, UK). Optical rotations were measured on a PerkinElmer 241 MC polarimeter. The reported values for specific rotation are average values of five successive measurements using an integration time of 5 s.

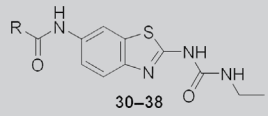
The InChI codes of the investigated compounds together with some biological activity data are provided as Supporting Information.

4.1.2 | Synthetic procedures

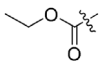
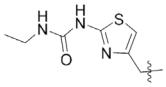
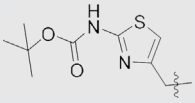
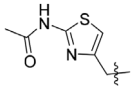
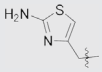
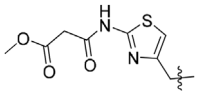
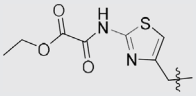
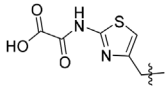
tert-Butyl (*S*)-(2-amino-4,5,6,7-tetrahydrobenzo[1,2-*d*]thiazol-6-yl)carbamate (2)

A solution of (*S*)-4,5,6,7-tetrahydrobenzo[1,2-*d*]thiazole-2,6-diamine (**1**) (3.002 g, 17.8 mmol) in tetrahydrofuran (THF) (100 mL) was cooled to 0°C on an ice bath. Then a solution of di-*tert*-butyl dicarbonate

TABLE 3 Inhibition of *E. coli* DNA gyrase and antibacterial activity of benzo[1,2-*d*]thiazoles **30–38**



30–38

Compound	R	*E. coli* DNA gyrase inhibition		Growth inhibition at 50 μM (%)			
		IC_{50} (μM)	Residual activity[a] (%)	*S. aureus* ATCC 25923	*E. faecalis* ATCC 25212	*E. coli* ATCC 25922	*P. aeruginosa* ATCC 27853
30			82	4	6	0	16
31			79	0	7	0	0
32		7.0 ± 0.5		15	27	10	17
33		3.9 ± 0.1		64	51	15	35
34		5.6 ± 0.2		12	30	5	20
35			91	n.t.[b]	n.t.	n.t.	n.t.
36		6.9 ± 0.2		25	16	1	1
37		8.3 ± 0.1		0	19	0	14
38			75	0	2	0	0
Novobiocin		0.17					
Ciprofloxacin (MIC)[c]				1.5 μM	3.0 μM	0.050 μM	3.0 μM

[a]Residual activity of the enzyme at 10 μM of compound.
[b]n.t., not tested.
[c]Data for ciprofloxacin bacterial growth inhibition given as MICs.

(Boc_2O) (4.068 g, 18.7 mmol) in THF (30 mL) was added dropwise over 20 min. The reaction mixture was stirred at room temperature overnight. The solvent was removed under reduced pressure and the crude product dissolved in ethyl acetate (100 mL). Organic phase was successively washed with saturated aqueous $NaHCO_3$ solution (100 mL) and brine (100 mL), dried over Na_2SO_4, filtered and the solvent removed under reduced pressure. Product was used without further purification. Yield: 4.79 g (100%); white solid; mp 140–142°C; $[\alpha]_D$ −40.3 (*c* 0.26, MeOH); ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.38 (s, 9H, $C(CH_3)_3$), 1.54–1.65 (m, 1H, H_A-7), 1.80–1.86 (m, 1H, H_B-7), 2.31–2.47 (m, 3H, H_A-4, H-5), 2.68 (dd, 1H, J_1 = 14.8 Hz, J_2 = 5.5 Hz, H_B-4), 3.57–3.69 (m, 1H, CHNH), 6.64 (s, 2H, NH_2), 6.94 (d, 1H, *J* = 7.9 Hz, NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 24.9, 28.2 (3C), 28.87, 28.94, 46.8, 77.6, 112.4, 144.1, 154.9, 166.1 ppm.

tert-Butyl (*S*)-(2-(3-ethylureido)-4,5,6,7-tetrahydrobenzo[1,2-*d*]-thiazol-6-yl)carbamate (3)

A solution of **2** (4.460 g, 16.6 mmol) in chloroform (100 mL) was cooled to 0°C on an ice bath under an argon atmosphere. Then ethyl isocyanate (1.44 mL, 18.2 mmol) was added dropwise over 20 min. The reaction mixture was stirred at room temperature overnight. Reaction mixture was transferred to the separating funnel and successively washed with saturated aqueous $NaHCO_3$ solution (2 × 25 mL) and

TABLE 4 Inhibition of *E. coli* DNA gyrase and antibacterial activities of compounds **32**–**34**, **36**, and **37** against wild-type *E. coli* and two mutant *E. coli* strains

		Growth inhibition			
		E. coli ATCC 25922	*E. coli* JW5503		*E. coli* JD17464
Compound	*E. coli* DNA gyrase IC_{50} (μM)	(%)[a]	MIC (μM)	(%)[a]	(%)[a]
32	7.0 ± 0.5	10		0	2
33	3.9 ± 0.1	15	50		37
34	5.6 ± 0.2	5		4	15
36	6.9 ± 0.2	1		0	1
37	8.3 ± 0.1	0		0	6
Ciprofloxacin (MIC)[b]		0.050 μM	0.015 μM		0.12 μM

[a]Percentage growth inhibition at 50 μM;
[b]Data for ciprofloxacin bacterial growth inhibition given as MICs.

brine (100 mL), dried over Na_2SO_4, filtered and the solvent removed under reduced pressure. Crude product was purified by column chromatography using dichloromethane/methanol (30:1) as eluent. Yield: 2.29 g (40.7%); white solid; mp 183–185°C; $[\alpha]_D$ −15.6 (*c* 0.22, MeOH); ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.05 (t, 3H, J = 7.2 Hz, $CH_2C\underline{H_3}$), 1.40 (s, 9H, 3 × CH_3), 1.61–1.71 (m, 1H, H_A-7), 1.86–1.94 (m, 1H, H_B-7), 2.38–2.47 (m, 1H, H_A-5), 2.55–2.71 (m, 2H, H_B-5, H_A-4), 2.81 (dd, 1H, J_1 = 15.7 Hz, J_2 = 5.0 Hz, H_B-4), 3.09–3.16 (m, 2H, $C\underline{H_2}CH_3$), 3.58–3.76 (m, 1H, C<u>H</u>NH), 6.50 (t, 1H, J = 5.0 Hz, N<u>H</u>CH_2CH_3), 6.99 (d, 1H, J = 7.9 Hz, N<u>H</u>CH), 10.11 (s, 1H, N<u>H</u>CO) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 15.2, 24.8, 28.2 (3C), 28.5, 28.9, 34.1, 46.4, 77.6, 117.4, 144.8, 153.6, 155.0, 157.6 ppm; HRMS (ESI^+) *m/z* for $C_{15}H_{24}N_4O_3S$ ($[M+H]^+$): calcd. 341.1647, found 341.1652.

(*S*)-1-(6-Amino-4,5,6,7-tetrahydrobenzo[1,2-*d*]thiazol-2-yl)-3-ethylurea (4)

Methanol (30 mL) was cooled on an ice bath and then acetyl chloride (4.80 mL, 67.4 mmol) was added dropwise. The mixture was stirred at 0°C for 30 min and then solution of **3** (2.292 g, 6.74 mmol) in methanol (20 mL) was added. Reaction mixture was stirred at 0°C for 1 h and then at room temperature overnight. The solvent was evaporated under reduced pressure, crude product dissolved in a small volume of water and then 1 M NaOH was added to reach pH ~ 12. Off-white precipitate (1.615 g, 99.8%) was filtered off and dried. mp 121–123°C; $[\alpha]_D$ −34.5 (*c* 0.24, MeOH); ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.05 (t, 3H, J = 7.2 Hz, $CH_2C\underline{H_3}$), 1.48–1.59 (m, 1H, H_A-7), 1.82–1.89 (m, 1H, H_B-7), 2.25–2.32 (m, 1H, H_A-5), 2.44–2.60 (m, 2H, H_A-4, H_B-5, signal partially overlapped with DMSO-d_5), 2.77 (dd, 1H, J_1 = 15.6 Hz, J_2 = 4.8 Hz, H_B-4), 3.01–3.07 (m, 1H, C<u>H</u>NH), 3.10–3.16 (m, 2H, $C\underline{H_2}CH_3$), 6.58 (s, 1H, N<u>H</u>CH_2CH_3) ppm; signals for NH_2 and NHCO are not seen in the spectrum; ^{13}C NMR (100 MHz, DMSO-d_6): δ 15.7, 25.1, 32.2, 32.5, 34.5, 47.9, 118.3, 143.3, 154.3, 158.0 ppm; HRMS (ESI^+) *m/z* for $C_{10}H_{16}N_4OS$ ($[M+H]^+$): calcd. 241.1123, found 241.1117.

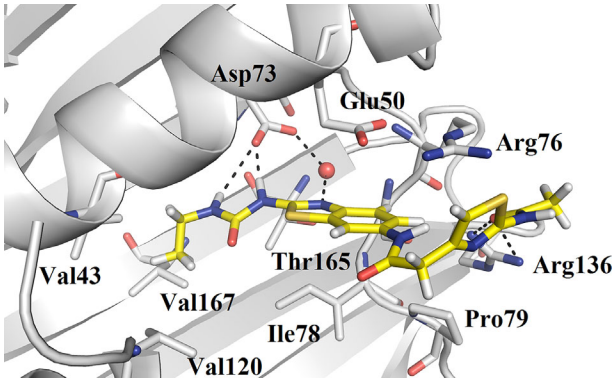


FIGURE 3 The docking binding mode of inhibitor **34** (in yellow) in the ATP binding site of *E. coli* DNA gyrase B (in gray, PDB code: 4DUH[29]). The ligand and the neighboring protein side-chains are shown as stick models, colored according to the chemical atom type (blue, N; red, O; orange, S; green, Cl). Water molecule is presented as a red sphere. Hydrogen bonds are indicated by black dotted lines

Methyl (*S*)-3-((2-(3-ethylureido)-4,5,6,7-tetrahydrobenzo[1,2-*d*]-thiazol-6-yl)amino)-3-oxopropanoate (5)

To a solution of **4** (0.159 g, 0.66 mmol) and Et_3N (0.138 mL, 0.99 mmol) in 1,4-dioxane (30 mL), methyl 3-chloro-3-oxopropanoate (0.092 mL, 0.86 mmol) was added dropwise under an argon atmosphere. Reaction mixture was stirred at room temperature for 18 h. Solvent was removed under reduced pressure and to the crude product ethyl acetate (20 mL) was added. The precipitate was filtered off, washed with ethyl acetate (5 mL), dried and then purified by flash column chromatography using dichloromethane/methanol (40:1) as eluent. Yield: 0.130 g (57.7%); off-white solid; mp 198–200°C; $[\alpha]_D$ −10.1 (*c* 0.21, MeOH); ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.05 (t, 3H, J = 7.2 Hz, $CH_2C\underline{H_3}$), 1.71–1.80 (m, 1H, H_A-7), 1.84–1.92 (m, 1H, H_B-7), 2.42–2.46 (m, 1H, H_A-4), 2.56–2.63 (m, 2H, H_A-5, H_B-4), 2.87 (dd, 1H, J_1 = 16.0 Hz, J_2 = 5.5 Hz, H_B-5), 3.09–3.16 (m, 2H, $C\underline{H_2}CH_3$), 3.25 (s, 2H, $COCH_2CO$), 3.62 (s, 3H, $COOCH_3$), 3.99–4.06 (m, 1H, C<u>H</u>NH), 6.76 (br s, 1H, N<u>H</u>CH_2CH_3), 8.18 (d, 1H, J = 7.6 Hz, CON<u>H</u>CH), 10.14 (br s, 1H, NHCO) ppm; HRMS (ESI^-) *m/z* for $C_{14}H_{19}N_4O_4S$ ($[M-H]^-$): calcd. 339.1127, found 339.1121; HPLC: method A, t_r 9.07 min (100% at 254 nm).

(*S*)-3-((2-(3-Ethylureido)-4,5,6,7-tetrahydrobenzo[1,2-*d*]thiazol-6-yl)amino)-3-oxopropanoic acid (6)

To a solution of **5** (0.037 g, 0.11 mmol) in methanol (10 mL), 1 M NaOH (0.44 mL, 0.44 mmol) was added and the reaction mixture stirred at room temperature for 3 h. Methanol was evaporated under reduced pressure and reaction mixture extracted with ethyl acetate (10 mL). Water phase was acidified with 1 M HCl to pH ~ 2, precipitate was filtered off and purified by flash column chromatography using dichloromethane/methanol/acetic acid (5:1:0.1) as eluent. Yield: 0.021 g (59.2%); off-white solid; mp 130–132°C; $[\alpha]_D$ −12.6 (*c* 0.23, MeOH); ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.05 (t, 3H, J = 7.2 Hz, $CH_2C\underline{H_3}$), 1.70–1.80 (m, 1H, H_A-7), 1.84–1.90 (m, 1H, H_B-7), 2.41–2.45 (m, 1H, H_A-4), 2.56–2.68 (m, 2H, H_A-5, H_B-4), 2.87 (dd, 1H, J_1 = 15.9 Hz, J_2 = 4.9 Hz, H_B-5), 3.09–3.16 (m, 2H, $C\underline{H_2}CH_3$), 3.22 (s, 2H, $COCH_2CO$), 3.99–4.12 (m, 1H, C<u>H</u>NH), 6.54 (t, 1H, J = 5.4 Hz, N<u>H</u>CH_2CH_3), 8.20 (d, 1H, J = 7.5 Hz, CON<u>H</u>CH), 10.17 (br s, 1H, NHCO) ppm; signal for COOH not seen in the spectrum; HRMS (ESI$^-$) *m/z* for $C_{13}H_{17}N_4O_4S$ ([M−H]$^-$): calcd. 325.0971, found 325.0977; HPLC: method A, t_r 8.59 min (95.1% at 254 nm).

(*S*)-*N*-(2-(3-Ethylureido)-4,5,6,7-tetrahydrobenzo[1,2-*d*]thiazol-6-yl)-3-hydrazinyl-3-oxopropanamide (7)

To a solution of **5** (0.080 g, 0.24 mmol) in ethanol (4 mL), hydrazine monohydrate (0.143 mL, 2.40 mmol) was added and the reaction mixture stirred at 80°C overnight. Ethanol was evaporated under reduced pressure and the residue purified by flash column chromatography using dichloromethane/methanol (9:1) as eluent. Yield: 0.075 g (93.8%); gray solid; mp 241–243°C; $[\alpha]_D$ −16.3 (*c* 0.20, MeOH); ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.05 (t, 3H, J = 7.1 Hz, $CH_2C\underline{H_3}$), 1.70–1.80 (m, 1H, H_A-7), 1.83–1.90 (m, 1H, H_B-7), 2.40–2.45 (m, 1H, H_A-4), 2.58–2.63 (m, 2H, H_A-5, H_B-4), 2.87 (dd, 1H, J_1 = 15.4 Hz, J_2 = 4.5 Hz, H_B-5), 2.98 (s, 2H, $COCH_2CO$), 3.09–3.16 (m, 2H, $C\underline{H_2}CH_3$), 3.97–4.06 (m, 1H, C<u>H</u>NH), 4.24 (br s, 2H, NHN<u>H</u>$_2$), 6.52 (t, 1H, J = 4.7 Hz, N<u>H</u>CH_2CH_3), 8.12 (d, 1H, J = 7.6 Hz, CON<u>H</u>CH), 9.08 (s, 1H, N<u>H</u>NH_2), 10.17 (br s, 1H, NHCO) ppm; HRMS (ESI$^-$) *m/z* for $C_{13}H_{19}N_6O_3S$ ([M−H]$^-$): calcd. 339.1239, found 339.1234; HPLC: method A, t_r 7.99 min (100% at 254 nm).

4.1.3 | General procedure A

A solution of carboxylic acid (1 mmol) in *N*,*N*-dimethylformamide (10 mL) was cooled to 0°C and then EDC (1.2 mmol) and HOBt (1.3 mmol) were added. pH was adjusted to 8 with *N*-methylmorpholine and the reaction mixture stirred for 20 min at 0°C. Then amine (1 mmol) was added and reaction mixture stirred overnight at room temperature. The solvent was evaporated *in vacuo* and the oily residue dissolved in ethyl acetate (30 mL) and washed successively with 10% citric acid (2 × 30 mL), saturated aqueous $NaHCO_3$ solution (2 × 30 mL), and brine (30 mL). The organic phase was dried over Na_2SO_4, filtered and the solvent evaporated under reduced pressure.

Methyl (*S*)-3-((2-(3-ethylureido)-4,5,6,7-tetrahydrobenzo[1,2-*d*]-thiazol-6-yl)carbamoyl)benzoate (8)

Compound was prepared from **4** (0.195 g, 0.81 mmol) and 3-(methoxycarbonyl)benzoic acid (0.146 g, 0.81 mmol) according to the general procedure **A**. Crude product was purified by column chromatography using dichloromethane/methanol (20:1) as eluent. Yield: 0.138 g (42.2%); yellowish solid; mp 174–176°C; $[\alpha]_D$ −12.6 (*c* 0.30, DMF); ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.06 (t, 3H, J = 7.1 Hz, $CH_2C\underline{H_3}$), 1.86–1.91 (m, 1H, H_A-7), 2.00–2.03 (m, 1H, H_B-7), 2.63–2.70 (m, 3H, H_A-5, H-4), 2.95 (dd, 1H, J_1 = 15.3 Hz, J_2 = 5.2 Hz, H_B-5), 3.12–3.18 (m, 2H, $C\underline{H_2}CH_3$), 3.90 (s, 3H, $COOCH_3$), 4.20–4.29 (m, 1H, C<u>H</u>NH), 6.52 (t, 1H, J = 5.6 Hz, N<u>H</u>CH_2CH_3), 7.64 (t, 1H, J = 7.8 Hz, Ar-H-5), 8.10–8.15 (m, 2H, Ar-H-4, Ar-H-6), 8.46 (t, 1H, J = 1.6 Hz, Ar-H-2), 8.73 (d, 1H, J = 7.7 Hz, CON<u>H</u>CH), 10.14 (s, 1H, NHCO) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 15.2, 24.9, 28.2, 28.6, 34.1, 46.2, 52.3, 117.4, 128.0, 128.9, 129.7, 131.6, 132.1, 135.1, 142.9, 153.7, 157.5, 164.9, 165.8 ppm; HRMS (ESI$^-$) *m/z* for $C_{19}H_{21}N_4O_4S$ ([M−H]$^-$): calcd. 401.1284, found 401.1286; HPLC: method A, t_r 10.89 min (98.7% at 254 nm).

(*S*)-3-((2-(3-Ethylureido)-4,5,6,7-tetrahydrobenzo[1,2-*d*]thiazol-6-yl)carbamoyl)benzoic acid (9)

To a solution of **8** (0.138 g, 0.34 mmol) in methanol (5 mL), 1 M NaOH (1.02 mL, 1.02 mmol) was added and the reaction mixture stirred at room temperature overnight. Methanol was evaporated under reduced pressure and reaction mixture extracted with ethyl acetate (10 mL). Water phase was acidified with 1 M HCl to pH ~ 2. Precipitate was filtered off and dried. Water phase was extracted with ethyl acetate (3 × 20 mL), combined organic phases were washed with brine, dried over Na_2SO_4, filtered and the solvent evaporated under reduced pressure. Yield: 0.132 g (99.1%); pink solid; mp 220–222°C; $[\alpha]_D$ −16.3 (*c* 0.29, MeOH); ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.07 (t, 3H, J = 7.2 Hz, $CH_2C\underline{H_3}$), 1.85–1.93 (m, 1H, H_A-7), 1.99–2.05 (m, 1H, H_B-7), 2.61–2.73 (m, 3H, H_A-5, H-4), 2.97 (dd, 1H, J_1 = 15.5 Hz, J_2 = 4.9 Hz, H_B-5), 3.14–3.18 (m, 2H, $C\underline{H_2}CH_3$), 4.22–4.30 (m, 1H, C<u>H</u>NH), 7.14 (br s, 1H, N<u>H</u>CH_2CH_3), 7.61 (t, 1H, J = 7.7 Hz, Ar-H-5), 8.08–8.12 (m, 2H, Ar-H-4, Ar-H-6), 8.44 (s, 1H, Ar-H-2), 8.74 (d, 1H, J = 7.5 Hz, CON<u>H</u>CH), 10.99 (br s, 1H, NHCO) ppm; signal for COOH not seen in the spectrum; ^{13}C NMR (100 MHz, DMSO-d_6): δ 14.9, 22.7, 27.5, 27.9, 34.3, 45.4, 118.3, 128.1, 128.7, 130.8, 131.7, 131.8, 134.8, 137.0, 152.7, 159.9, 165.2, 166.9 ppm; HRMS (ESI$^-$) *m/z* for $C_{18}H_{19}N_4O_4S$ ([M−H]$^-$): calcd. 387.1127, found 387.1118; HPLC: method A, t_r 9.95 min (98.4% at 254 nm).

(*S*)-*N*-(2-(3-Ethylureido)-4,5,6,7-tetrahydrobenzo[1,2-*d*]thiazol-6-yl)-4-methylthiazole-5-carboxamide (10)

Compound was prepared from **4** (0.084 g, 0.35 mmol) and 4-methylthiazole-5-carboxylic acid (0.050 g, 0.35 mmol) according to the general procedure **A**. Crude product was purified by column chromatography using dichloromethane/methanol (20:1) as eluent. Yield: 0.092 g (72.0%); yellow solid; mp 198–200°C; $[\alpha]_D$ −8.0 (*c* 0.26, MeOH); ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.05 (t, 3H, J = 7.1 Hz, $CH_2C\underline{H_3}$), 1.80–1.90 (m, 1H, H_A-7), 1.96–2.03 (m, 1H, H_B-7), 2.56 (s,

3H, CH_3), 2.58–2.69 (m, 3H, H_A-5, H-4), 2.92 (dd, 1H, J_1 = 15.3 Hz, J_2 = 4.5 Hz, H_B-5), 3.09–3.16 (m, 2H, CH_2CH_3), 4.11–4.21 (m, 1H, CHNH), 6.73 (s, 1H, $NHCH_2CH_3$), 8.37 (d, 1H, J = 7.7 Hz, CONHCH), 9.05 (s, 1H, Ar-H), 10.25 (s, 1H, NHCO) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 15.2, 16.7, 24.8, 28.0, 28.4, 34.0, 46.2, 117.1, 126.3, 142.8, 153.4, 153.9, 154.2, 157.5, 160.9 ppm; HRMS (ESI^+) m/z for $C_{15}H_{19}N_5O_2S_2$ ($[M+H]^+$): calcd. 366.1058, found 366.1058; HPLC: method A, t_r 9.56 min (100% at 254 nm).

(*S*)-*N*-(2-(3-Ethylureido)-4,5,6,7-tetrahydrobenzo[1,2-*d*]thiazol-6-yl)-5-methylisoxazole-4-carboxamide (11)

Compound was prepared from **4** (0.082 g, 0.34 mmol) and 4-methylisoxazole-4-carboxylic acid (0.050 g, 0.34 mmol) according to the general procedure **A**. Crude product was purified by column chromatography using dichloromethane/methanol (40:1) as eluent. Yield: 0.030 g (21.8%); yellow solid; mp 160–162°C; $[\alpha]_D$ −10.0 (*c* 0.19, DMF); ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.05 (t, 3H, J = 7.2 Hz, CH_2CH_3), 1.65–1.72 (m, 1H, H_A-7), 1.76–1.93 (m, 1H, H_B-7), 1.99 (s, 3H, CH_3), 2.54–2.64 (m, 3H, H_A-5, H-4), 2.92 (dd, 1H, J_1 = 16.1 Hz, J_2 = 4.8 Hz, H_B-5), 3.09–3.16 (m, 2H, CH_2CH_3), 3.52–3.61 (m, 1H, CHNH), 6.77 (s, 1H, $NHCH_2CH_3$), 6.98 (d, 1H, J = 6.0 Hz, CONHCH), 8.41 (s, 1H, Ar-H), 10.27 (s, 1H, NHCO) ppm; HRMS (ESI^+) m/z for $C_{15}H_{19}N_5O_3S$ ($[M+H]^+$): calcd. 350.1287, found 350.1286; HPLC: method A, t_r 11.09 min (95.0% at 254 nm).

(*S*)-*N*-(2-(3-Ethylureido)-4,5,6,7-tetrahydrobenzo[1,2-*d*]thiazol-6-yl)-1H-imidazole-4-carboxamide (12)

Compound was prepared from **4** (0.108 g, 0.45 mmol) and 1*H*-imidazole-4-carboxylic acid (0.050 g, 0.45 mmol) according to the general procedure **A**. Crude product was purified by column chromatography using dichloromethane/methanol (9:1) as eluent. Yield: 0.005 g (3.4%); yellow solid; mp 180–182°C; $[\alpha]_D$ −18.3 (*c* 0.20, MeOH); ^{1}H NMR (400 MHz, MeOD-d_4): δ 1.19 (t, 3H, J = 7.2 Hz, CH_2CH_3), 1.97–2.04 (m, 1H, H_A-7), 2.12–2.19 (m, 1H, H_B-7), 2.68–2.82 (m, 3H, H_A-5, H-4), 3.08 (dd, 1H, J_1 = 15.5 Hz, J_2 = 5.6 Hz, H_B-5), 3.23–3.31 (m, 2H, CH_2CH_3), 4.37–4.43 (m, 1H, CHNH), 7.70 (d, 1H, J = 1.0 Hz, Ar-H), 7.74 (d, 1H, J = 1.0 Hz, Ar-H) ppm; signals for NH groups are not seen in the spectrum; HRMS (ESI^+) m/z for $C_{14}H_{18}N_6O_2S$ ($[M+H]^+$): calcd. 335.1290, found 335.1297; HPLC: method A, t_r 8.48 min (100% at 254 nm).

(*S*)-*N*-(2-(3-Ethylureido)-4,5,6,7-tetrahydrobenzo[1,2-*d*]thiazol-6-yl)isonicotinamide (13)

Compound was prepared from **4** (0.82 g, 0.34 mmol) and pyridine-4-carboxylic acid (0.042 g, 0.34 mmol) according to the general procedure **A**. Crude product was purified by column chromatography using dichloromethane/methanol (20:1) as eluent. Yield: 0.013 g (11.0%); white solid; mp 125–127°C; $[\alpha]_D$ −6.3 (*c* 0.18, DMF); ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.06 (t, 3H, J = 7.2 Hz, CH_2CH_3), 1.82–1.92 (m, 1H, H_A-7), 1.98–2.05 (m, 1H, H_B-7), 2.61–2.69 (m, 3H, H_A-5, H-4), 2.95 (dd, 1H, J_1 = 15.3 Hz, J_2 = 4.8 Hz, H_B-5), 3.10–3.17 (m, 2H, CH_2CH_3), 4.17–4.27 (m, 1H, CHNH), 6.51 (t, 1H, J = 5.5 Hz, $NHCH_2CH_3$), 7.77 (dd, 2H, J_1 = 4.4 Hz, J_2 = 1.7 Hz, Ar-H), 8.73 (dd, 2H, J_1 = 4.4 Hz, J_2 = 1.7 Hz, Ar-H), 8.77 (d, 1H, J = 7.7 Hz, CHNH), 10.14 (s, 1H, NHCO) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 15.2, 24.5, 28.1, 28.5, 34.1, 46.2, 117.2, 121.4 (2C), 141.5, 150.1 (2C), 153.7, 157.7, 157.9, 164.4 ppm; HRMS (ESI^+) m/z for $C_{16}H_{19}N_5O_2S$ ($[M+H]^+$): calcd. 346.1338, found 346.1330; HPLC: method A, t_r 8.65 min (95.1% at 254 nm).

tert-Butyl (*S*)-(4-(2-((2-(3-ethylureido)-4,5,6,7-tetrahydrobenzo[1,2-*d*]thiazol-6-yl)amino)-2-oxoethyl)thiazol-2-yl)carbamate (14)

Compound was prepared from **4** (0.067 g, 0.28 mmol) and 2-(2-((*tert*-butoxycarbonyl)amino)thiazol-4-yl)acetic acid (0.071 g, 0.28 mmol) according to the general procedure **A**. Yield: 0.098 g (74.2%); yellow solid; mp 140–142°C; $[\alpha]_D$ −6.0 (*c* 0.27, MeOH); ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.05 (t, 3H, J = 7.2 Hz, CH_2CH_3), 1.48 (s, 9H, $C(CH_3)_3$), 1.68–1.79 (m, 1H, H_A-7), 1.84–1.94 (m, 1H, H_B-7), 2.39–2.47 (m, 1H, H_A-4), 2.54–2.70 (m, 2H, H_B-4, H_A-5), 2.86 (dd, 1H, J_1 = 16.1 Hz, J_2 = 5.5 Hz, H_B-5), 3.08–3.17 (m, 2H, CH_2CH_3), 3.42 (s, 2H, Ar-CH_2), 3.95–4.02 (m, 1H, CHNH), 6.51 (t, 1H, J = 5.6 Hz, $NHCH_2CH_3$), 6.81 (s, 1H, Ar-H), 8.11 (d, 1H, J = 7.5 Hz, CHNH), 10.12 (s, 1H, NHCO), 11.39 (s, 1H, NHCO) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 15.3, 28.2 (3C), 29.0, 35.0, 39.1, 44.8, 55.4, 66.9, 77.2, 109.8, 115.6, 116.1, 117.5, 143.2, 152.4, 154.6, 159.8, 161.2 ppm; HRMS (ESI^+) m/z for $C_{20}H_{28}N_6O_4S_2$ ($[M+H]^+$): calcd. 481.1692, found 481.1685; HPLC: method A, t_r 11.28 min (100% at 254 nm).

(*S*)-2-(2-Aminothiazol-4-yl)-*N*-(2-(3-ethylureido)-4,5,6,7-tetrahydrobenzo[1,2-*d*]thiazol-6-yl)acetamide (15)

Methanol (5 mL) was cooled on an ice bath and then acetyl chloride (0.108 mL, 1.52 mmol) was added dropwise. The mixture was stirred at 0°C for 30 min and then solution of **14** (0.073 g, 0.15 mmol) in methanol (5 mL) was added. Reaction mixture was stirred at 0°C for 1 h and then at room temperature overnight. Then solvent was evaporated under reduced pressure and crude product purified by column chromatography using dichloromethane/methanol (9:1) as eluent. Yield: 0.032 g (55.2%); yellow solid; mp 120–122°C; $[\alpha]_D$ −5.7 (*c* 0.22, MeOH); ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.03 (t, 3H, J = 7.2 Hz, CH_2CH_3), 1.67–1.78 (m, 1H, H_A-7), 1.84–1.93 (m, 1H, H_B-7), 2.53–2.60 (m, 3H, H-4, H_A-5), 2.84 (dd, 1H, J_1 = 15.2 Hz, J_2 = 5.3 Hz, H_B-5), 3.06–3.12 (m, 2H, CH_2CH_3), 3.26 (s, 2H, Ar-CH_2), 3.92–4.00 (m, 1H, CHNH), 6.21 (s, 1H, $NHCH_2CH_3$), 6.89 (s, 2H, NH_2), 7.99 (s, 1H, Ar-H), 8.14 (d, 1H, J = 7.7 Hz, CHNH), 11.08 (br s, 1H, NHCO) ppm; HRMS (ESI^+) m/z for $C_{15}H_{20}N_6O_2S_2$ ($[M+H]^+$): calcd. 381.1167, found 381.1162; HPLC: method A, t_r 8.60 min (96.5% at 254 nm).

Ethyl 2-(2-((*tert*-butoxycarbonyl)amino)thiazol-4-yl)acetate (17)

To a solution of **16** (1.077 g, 5.78 mmol) and catalytic amount of 4-dimethylaminopyridine in dichloromethane (50 mL) a solution of di-*tert*-butyl dicarbonate (Boc_2O) (1.640 g, 7.52 mmol) in dichloromethane (20 mL) was added dropwise over 20 min. The reaction mixture was stirred at room temperature overnight. The solvent was removed under reduced pressure and the crude product dissolved in ethyl acetate (100 mL). Organic phase was successively washed with

saturated aqueous $NaHCO_3$ solution (100 mL) and brine (100 mL), dried over Na_2SO_4, filtered, and the solvent removed under reduced pressure. Product was used in the next step without further purification. Yield: 1.655 g (100%); yellow solid; mp 128–130°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.18 (t, 3H, J = 7.1 Hz, $CH_2C\underline{H}_3$), 1.48 (s, 9H, $C(CH_3)_3$), 3.64 (d, 2H, J = 0.7 Hz, CH_2), 4.07 (q, 2H, J = 7.1 Hz, $OC\underline{H}_2CH_3$), 6.92 (s, 1H, Ar-H), 11.44 (br s, 1H, NHCO) ppm; MS (ESI$^-$) m/z for $C_{12}H_{17}N_2O_4S$ ([M–H]$^-$): 285 (100).

Ethyl 2-(2-acetamidothiazol-4-yl)acetate (18)

A solution of **16** (1.066 g, 5.73 mmol) and triethylamine (1.040 mL, 7.46 mmol) in dichloromethane (50 mL) was cooled to 0°C on an ice bath under an argon atmosphere. Then acetyl chloride (0.450 mL, 6.33 mmol) was added dropwise. The reaction mixture was stirred at room temperature for 4 h. Organic phase was successively washed with 10% citric acid (2 × 20 mL), saturated aqueous $NaHCO_3$ solution (2 × 20 mL) and brine (20 mL), dried over Na_2SO_4, filtered and the solvent removed under reduced pressure. Crude product was crystallized from a mixture of ethyl acetate/hexane (1:2). Yield: 0.591 g (45.3%); yellow solid; mp 112–114°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.18 (t, 3H, J = 7.1 Hz, $CH_2C\underline{H}_3$), 2.12 (s, 3H, $COCH_3$), 3.68 (s, 2H, CH_2), 4.08 (q, 2H, J = 7.1 Hz, $OC\underline{H}_2CH_3$), 6.95 (s, 1H, Ar-H), 12.14 (br s, 1H, NHCO) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 14.6, 22.9, 37.1, 60.8, 110.6, 144.0, 158.1, 168.8, 170.5 ppm; MS (ESI$^-$) m/z for $C_9H_{11}N_2O_3S$ ([M–H]$^-$): 227 (100).

Ethyl 2-(2-(3-ethylureido)thiazol-4-yl)acetate (19)

A solution of **16** (4.214 g, 22.7 mmol) in chloroform (100 mL) was cooled to 0°C on an ice bath under an argon atmosphere. Then ethyl isocyanate (2.87 mL, 36.2 mmol) was added dropwise over 20 min. The reaction mixture was stirred at 50°C overnight. Reaction mixture was transferred to the separating funnel and successively washed with saturated aqueous $NaHCO_3$ solution (2 × 25 mL) and brine (100 mL), dried over Na_2SO_4, filtered and the solvent removed under reduced pressure. Crude product was purified by column chromatography using ethyl acetate/*n*-hexane (1:6) as eluent. Yield: 2.665 g (45.8%); white solid; mp 152–154°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.06 (t, 3H, J = 7.2 Hz, $NHCH_2C\underline{H}_3$), 1.18 (t, 3H, J = 7.1 Hz, $OCH_2C\underline{H}_3$), 3.09–3.17 (m, 2H, $NHC\underline{H}_2CH_3$), 3.60 (s, 2H, CH_2), 4.08 (q, 2H, J = 7.1 Hz, $OC\underline{H}_2CH_3$), 6.45 (br s, 1H, $N\underline{H}CH_2CH_3$), 6.76 (s, 1H, Ar-H), 10.44 (br s, 1H, NHCO) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 14.5, 15.7, 34.6, 37.2, 60.7, 109.0, 143.6, 154.2, 160.3, 170.6 ppm; MS (ESI$^-$) m/z for $C_{10}H_{14}N_3O_3S$ ([M–H]$^-$): 256 (100).

2-(2-((*tert*-Butoxycarbonyl)amino)thiazol-4-yl)acetic acid (20)

To a solution of **17** (1.655 g, 5.79 mmol) in methanol (20 mL), 2 M NaOH (11.6 mL, 23.2 mmol) was added and the reaction mixture stirred at room temperature overnight. Reaction mixture was then stirred at 50°C for additional 2 h. Methanol was evaporated under reduced pressure and reaction mixture extracted with ethyl acetate (2 × 20 mL). Water phase was acidified with concentrated HCl to pH ~ 2. Precipitate was filtered off and dried. Yield: 0.822 g (55.1%); yellow solid; mp 160–162°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.48 (s, 9H, $C(CH_3)_3$), 3.55 (s, 2H, CH_2), 6.89 (s, 1H, Ar-H), 11.42 (br s, 1H, NHCO), 12.35 (s, 1H, COOH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 28.4 (3C), 37.3, 81.5, 110.0, 144.9, 159.7, 159.8, 172.1 ppm; MS (ESI$^-$) m/z for $C_{10}H_{13}N_2O_4S$ ([M–H]$^-$): 257 (100).

2-(2-Acetamidothiazol-4-yl)acetic acid (21)

To a solution of **19** (0.591 g, 2.59 mmol) in methanol (20 mL), 2 M NaOH (5.2 mL, 10.4 mmol) was added and the reaction mixture stirred at room temperature overnight. Reaction mixture was then stirred at 50°C for additional 2 h. Methanol was evaporated under reduced pressure and reaction mixture extracted with ethyl acetate (2 × 20 mL). Water phase was acidified with concentrated HCl to pH ~ 2. Water phase was extracted with ethyl acetate (3 × 50 mL), combined organic phases washed with brine (100 mL), dried over Na_2SO_4, filtered and the solvent removed under reduced pressure. Yield: 0.138 g (26.6%); yellowish solid; mp 211–213°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.12 (s, 3H, $COCH_3$), 3.58 (s, 2H, CH_2), 6.92 (s, 1H, Ar-H), 12.12 (br s, 1H, NHCO), 12.36 (br s, 1H, COOH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 22.9, 37.3, 110.3, 144.6, 157.9, 168.8, 172.1 ppm; MS (ESI$^+$) m/z for $C_7H_8N_2O_3SNa$ ([M+Na]$^+$): 223 (100).

2-(2-(3-Ethylureido)thiazol-4-yl)acetic acid (22)

To a solution of **19** (2.645 g, 10.3 mmol) in ethanol (20 mL), 2 M NaOH (20.6 mL, 41.2 mmol) was added and the reaction mixture stirred at room temperature overnight. Ethanol was evaporated under reduced pressure and reaction mixture extracted with ethyl acetate (2 × 25 mL). Water phase was acidified with concentrated HCl to pH ~ 2. Precipitate was filtered off and dried. Yield: 2.308 g (97.9%); white solid; mp 173–175°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.06 (t, 3H, J = 7.2 Hz, $NHCH_2C\underline{H}_3$), 3.10–3.17 (m, 2H, $NHC\underline{H}_2CH_3$), 3.51 (s, 2H, CH_2), 6.45 (t, 1H, J = 4.8 Hz, $N\underline{H}CH_2CH_3$), 6.74 (s, 1H, Ar-H), 10.41 (br s, 1H, NHCO), 12.29 (s, 1H, COOH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 15.2, 34.0, 36.6, 108.4, 142.9, 153.8, 159.6, 171.5 ppm. MS (ESI$^+$) m/z for $C_8H_{11}N_3O_3SNa$ ([M+Na]$^+$): 252 (100).

Methyl 3-(2-(2-(3-ethylureido)thiazol-4-yl)acetamido)benzoate (23)

Compound was prepared from **22** (0.076 g, 0.33 mmol) and methyl 3-aminobenzoate (0.050 g, 0.33 mmol) according to the general procedure **A**. Crude product was purified by column chromatography using dichloromethane/methanol (40:1) as eluent. Yield: 0.030 g (25.0%); yellow solid; mp 140–142°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.05 (t, 3H, J = 7.2 Hz, $NHCH_2C\underline{H}_3$), 3.10–3.17 (m, 2H, $NHC\underline{H}_2CH_3$), 3.62 (s, 2H, CH_2), 3.85 (s, 3H, $COOCH_3$), 6.44 (t, 1H, J = 5.2 Hz, $N\underline{H}CH_2CH_3$), 6.78 (s, 1H, thiazole-H), 7.46 (t, 1H, J = 8.0 Hz, Ar-H-5), 7.64 (ddd, 1H, J_1 = 8.0 Hz, J_2 = 2.0 Hz, J_3 = 1.1 Hz, Ar-H-4/6), 7.85 (ddd, 1H, J_1 = 8.0 Hz, J_2 = 2.0 Hz, J_3 = 1.1 Hz, Ar-H-4/6), 8.29 (t, 1H, J = 2.0 Hz, Ar-H-2), 10.35 (s, 1H, NHCO), 10.40 (s, 1H, NHCO) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 15.2, 34.1, 52.2, 108.2, 119.5, 123.5, 123.8, 129.2, 130.1, 139.5, 144.4, 153.7, 159.7, 159.8, 166.1, 168.3 ppm; HRMS (ESI$^-$) m/z for $C_{16}H_{17}N_4O_4S$ ([M–H]$^-$): calcd. 361.0971, found 361.0975; HPLC: method A, t_r 11.16 min (100% at 254 nm).

Methyl 4-(2-(2-(3-ethylureido)thiazol-4-yl)acetamido)benzoate (24)

Compound was prepared from **22** (0.200 g, 0.87 mmol) and methyl 4-aminobenzoate (0.132 g, 0.87 mmol) according to the general procedure **A**. Crude product was purified by column chromatography using dichloromethane/methanol (20:1) as eluent. Yield: 0.066 g (20.9%); orange solid; mp 122–124°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.04 (t, 3H, J = 7.2 Hz, NHCH$_2$C<u>H$_3$</u>), 3.10–3.16 (m, 2H, NHC<u>H$_2$</u>CH$_3$), 3.65 (s, 2H, CH$_2$), 3.83 (s, 3H, COOCH$_3$), 6.43 (t, 1H, J = 5.3 Hz, N<u>H</u>CH$_2$CH$_3$), 6.78 (s, 1H, thiazole-H), 7.75 (d, 2H, J = 8.8 Hz, Ar-H), 7.92 (d, 2H, J = 8.8 Hz, Ar-H), 10.42 (s, 1H, NHCO), 10.48 (s, 1H, NHCO) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 15.2, 34.1, 51.9, 108.3, 118.4 (2C), 123.8, 130.3 (2C), 143.6, 144.2, 153.7, 159.7 (2C), 165.8, 168.6 ppm; HRMS (ESI$^-$) m/z for $C_{16}H_{17}N_4O_4S$ ([M–H]$^-$): calcd. 361.0971, found 361.0977; HPLC: method A, t_r 11.19 min (100% at 254 nm).

3-(2-(2-(3-Ethylureido)thiazol-4-yl)acetamido)benzoic acid (25)

To a solution of **23** (0.015 g, 0.041 mmol) in methanol (5 mL), 2 M NaOH (0.062 mL, 0.12 mmol) was added and the reaction mixture stirred at room temperature overnight. Methanol was evaporated under reduced pressure and water phase acidified with concentrated HCl to pH ~ 2. Precipitate was filtered off and dried. Yield: 0.012 g (83.2%); brown solid; mp 154–156°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.04 (t, 3H, J = 7.2 Hz, NHCH$_2$C<u>H$_3$</u>), 3.10–3.16 (m, 2H, NHC<u>H$_2$</u>CH$_3$), 3.63 (s, 2H, CH$_2$), 6.56 (t, 1H, J = 5.1 Hz, N<u>H</u>CH$_2$CH$_3$), 6.78 (s, 1H, thiazole-H), 7.43 (t, 1H, J = 7.9 Hz, Ar-H-5), 7.62 (d, 1H, J = 7.9 Hz, Ar-H-4/6), 7.84 (d, 1H, J = 7.9 Hz, Ar-H-4/6), 8.25 (s, 1H, Ar-H-2), 10.35 (s, 1H, NHCO), 10,48 (s, 1H, NHCO), 12.97 (br s, 1H, COOH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 15.2, 34.1, 108.2, 119.7, 123.1, 124.0, 129.0, 131.2, 137.9, 139.4, 144.0, 153.6, 159.8, 167.1, 168.2 ppm; HRMS (ESI$^-$) m/z for $C_{15}H_{15}N_4O_4S$ ([M–H]$^-$): calcd. 347.0814, found 347.0818; HPLC: method A, t_r 9.99 min (100% at 254 nm).

4-(2-(2-(3-Ethylureido)thiazol-4-yl)acetamido)benzoic acid (26)

To a solution of **24** (0.039 g, 0.11 mmol) in methanol (5 mL), 2 M NaOH (0.16 mL, 0.32 mmol) was added and the reaction mixture stirred at room temperature overnight. Methanol was evaporated under reduced pressure and water phase acidified with concentrated HCl to pH ~ 2. Precipitate was filtered off and dried. Yield: 0.028 g (74.7%); yellow solid; mp 248–250°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.05 (t, 3H, J = 7.2 Hz, NHCH$_2$C<u>H$_3$</u>), 3.11–3.17 (m, 2H, NHC<u>H$_2$</u>CH$_3$), 3.68 (s, 2H, CH$_2$), 6.68 (t, 1H, J = 5.8 Hz, N<u>H</u>CH$_2$CH$_3$), 6.81 (s, 1H, thiazole-H), 7.73 (d, 2H, J = 8.9 Hz, Ar-H), 7.89 (d, 2H, J = 8.9 Hz, Ar-H), 10.51 (s, 1H, NHCO), 10.61 (br s, 1H, NHCO) ppm; signal for COOH not seen in the spectrum; ^{13}C NMR (100 MHz, DMSO-d_6): δ 15.1, 34.2, 108.8, 118.3 (2C), 125.1, 130.4 (2C), 142.2, 143.2, 153.4, 160.3, 166.9, 168.2 ppm; HRMS (ESI$^-$) m/z for $C_{15}H_{15}N_4O_4S$ ([M–H]$^-$): calcd. 347.0814, found 347.0804; HPLC: method A, t_r 9.91 min (100% at 254 nm).

1-Ethyl-3-(6-nitrobenzo[1,2-*d*]thiazol-2-yl)urea (28)

To a suspension of **27** (4.88 g, 25.0 mmol) in toluene (100 mL), triethyl amine (3.49 mL, 25.0 mmol) and ethyl isocyanate (1.98 mL, 25.0 mmol) were added and the reaction mixture stirred under reflux overnight. The reaction mixture was then cooled on an ice bath, precipitate filtered off and dried to obtain **28** as a yellow solid. Yield: 5.75 g (86.0%); ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.10 (t, 3H, J = 7.2 Hz, NHCH$_2$C<u>H$_3$</u>), 3.17–3.24 (m, 2H, NHC<u>H$_2$</u>CH$_3$), 6.80 (s, 1H, N<u>H</u>CH$_2$CH$_3$), 7.74 (d, 1H, J = 8.9 Hz, Ar-H-4), 8.21 (dd, 1H, J_1 = 8.9 Hz, J_2 = 2.5 Hz, Ar-H-5), 8.94 (d, 1H, J = 2.5 Hz, Ar-H-7), 11.20 (s, 1H, NHCO) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 15.0, 34.4, 118.5, 119.4, 121.6, 132.2, 142.2, 153.4, 154.3 165.3 ppm.

1-(6-Aminobenzo[1,2-*d*]thiazol-2-yl)-3-ethylurea (29)

Compound **28** (1.512 g, 5.68 mmol) was dissolved in a mixture of ethanol (100 mL) and *N*,*N*-dimethylformamide (30 mL), Pd-C (150 mg) was added and the reaction mixture was stirred under hydrogen atmosphere for 48 h. The catalyst was filtered off and the solvent removed under reduced pressure. Crude product was purified by column chromatography using dichloromethane/methanol (20:1) as eluent. Yield: 0.411 g (30.6%); brown solid; mp 198–200°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.07 (t, 3H, J = 7.2 Hz, NHCH$_2$C<u>H$_3$</u>), 3.13–3.20 (m, 2H, NHC<u>H$_2$</u>CH$_3$), 5.03 (s, 2H, NH$_2$), 6.63 (dd, 1H, J_1 = 8.5 Hz, J_2 = 2.2 Hz, Ar-H-5), 6.68 (t, 1H, J = 5.8 Hz, N<u>H</u>CH$_2$CH$_3$), 6.94 (d, 1H, J = 2.2 Hz, Ar-H-7), 7.29 (d, 1H, J = 8.5 Hz, Ar-H-4), 10.28 (s, 1H, NHCO) ppm; MS (ESI$^+$) m/z for $C_{10}H_{12}N_4OSNa$ ([M+Na]$^+$): 259 (100).

Ethyl 2-((2-(3-ethylureido)benzo[1,2-*d*]thiazol-6-yl)amino)-2-oxoacetate (30)

To a solution of **29** (0.100 g, 0.42 mmol) and Et$_3$N (0.147 mL, 1.1 mmol) in 1,4-dioxane (20 mL), ethyl 2-chloro-2-oxoacetate (0.057 mL, 0.51 mmol) was added dropwise under an argon atmosphere. Reaction mixture was stirred at room temperature for 18 h. The precipitate was filtered off, solvent was removed under reduced pressure and the crude product purified by flash column chromatography using dichloromethane/methanol (20:1) as eluent. Yield: 0.015 g (10.5%); yellow solid; mp 253–255°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.10 (t, 3H, J = 7.2 Hz, NHCH$_2$C<u>H$_3$</u>), 1.33 (t, 3H, J = 7.1 Hz, COOCH$_2$C<u>H$_3$</u>), 3.15–3.22 (m, 2H, NHC<u>H$_2$</u>CH$_3$), 4.32 (q, 2H, J = 7.1 Hz, COOC<u>H$_2$</u>CH$_3$), 6.71 (t, 1H, J = 6.0 Hz, N<u>H</u>CH$_2$CH$_3$), 7.59 (d, 1H, J = 8.7 Hz, Ar-H-4), 7.67 (dd, 1H, J_1 = 8.7 Hz, J_2 = 1.9 Hz, Ar-H-5), 8.21 (d, 1H, J = 1.9 Hz, Ar-H-7), 10.68 (s, 1H, NHCO), 10.88 (s, 1H, NHCO) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 13.8, 15.1, 34.2, 62.3, 113.0, 119.3, 119.5, 131.7, 132.5, 145.9, 155.3, 159.6, 160.7, 168.4 ppm; HRMS (ESI$^-$) m/z for $C_{14}H_{15}N_4O_4S$ ([M–H]$^-$): calcd. 335.0814, found 335.0810; HPLC: method A, t_r 11.06 min (95.9% at 254 nm).

2-((2-(3-Ethylureido)benzo[1,2-*d*]thiazol-6-yl)amino)-2-oxoacetic acid (31)

To a solution of **30** (0.010 g, 0.030 mmol) in methanol (3 mL), 1 M NaOH (0.060 mL, 0.060 mmol) was added and the reaction mixture stirred at room temperature overnight. Then the acidic ion exchange resin Amberlite® IR120 H was added for neutralization (pH ~ 6). After stirring of mixture for 10 min, the resin was filtered off, washed with methanol and the solvent removed *in vacuo*. Yield: 0.008 g (86.9%); yellow solid; mp >300°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.09 (t, 3H,

J = 7.2 Hz, NHCH$_2$C$\underline{H}_3$), 3.15–3.22 (m, 2H, NHC$\underline{H}_2$CH$_3$), 6.73 (t, 1H, J = 5.6 Hz, N$\underline{H}$CH$_2$CH$_3$), 7.57 (d, 1H, J = 8.7 Hz, Ar-H-4), 7.69 (dd, 1H, J_1 = 8.7 Hz, J_2 = 2.0 Hz, Ar-H-5), 8.31 (d, 1H, J = 2.0 Hz, Ar-H-7), 10.67 (s, 1H, NHCO), 10.78 (s, 1H, NHCO) ppm, signal for COOH not seen in the spectrum; ^{13}C NMR (100 MHz, DMSO-d_6): δ 15.1, 34.2, 112.6, 116.5, 119.1, 119.4, 125.7, 131.6, 132.8, 159.5, 162.2, 163.3 ppm; HRMS (ESI$^-$) m/z for $C_{12}H_{11}N_4O_4S$ ([M−H]$^-$): calcd. 307.0501, found 307.0499; HPLC: method A, t_r 9.36 min (100% at 254 nm).

N-(2-(3-Ethylureido)benzo[1,2-*d*]thiazol-6-yl)-2-(2-(3-ethylureido)thiazol-4-yl)acetamide (32)

Compound was prepared from **22** (0.097 g, 0.42 mmol) and **29** (0.100 g, 0.42 mmol) according to the general procedure **A**. Crude product was crystallized from a mixture of dichloromethane and methanol (20:1). Yield: 0.066 g (34.7%); brown solid; mp 250–252°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.05 (t, 3H, J = 7.2 Hz, NHCH$_2$C$\underline{H}_3$), 1.09 (t, 3H, J = 7.2 Hz, NHCH$_2$C$\underline{H}_3$), 3.10–3.21 (m, 4H, 2 × NHC$\underline{H}_2$CH$_3$), 3.62 (s, 2H, CH$_2$), 6.45 (t, 1H, J = 5.4 Hz, N$\underline{H}$CH$_2$CH$_3$), 6.71 (t, 1H, J = 4.5 Hz, N$\underline{H}$CH$_2$CH$_3$), 6.77 (s, 1H, thiazole-H), 7.46 (dd, 1H, J_1 = 8.7 Hz, J_2 = 1.9 Hz, Ar-H-5), 7.54 (d, 1H, J = 8.7 Hz, Ar-H-4), 8.21 (d, 1H, J = 1.9 Hz, Ar-H-7), 10.20 (s, 1H, NHCO), 10.41 (s, 1H, NHCO), 10.61 (s, 1H, NHCO) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 15.1, 15.2, 34.1, 34.2, 40.1, 108.0, 111.3, 118.0, 119.5, 131.7, 134.4, 144.6, 145.0, 145.1, 153.7, 158.9, 162.3, 167.8 ppm; HRMS (ESI$^-$) m/z for $C_{18}H_{20}N_7O_3S_2$ ([M−H]$^-$): calcd. 446.1096, found 446.1076; HPLC: method A, t_r 10.44 min (97.2% at 254 nm).

tert-Butyl (4-(2-((2-(3-ethylureido)benzo[1,2-*d*]thiazol-6-yl)-amino)-2-oxoethyl)thiazol-2-yl)carbamate (33)

Compound was prepared from **20** (0.108 g, 0.42 mmol) and **29** (0.100 g, 0.42 mmol) according to the general procedure **A**. Crude product was crystallized from a mixture of dichloromethane and methanol (20:1). Yield: 0.085 g (42.1%); yellow solid; mp 172–174°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.09 (t, 3H, J = 7.2 Hz, NHCH$_2$C$\underline{H}_3$), 1.48 (s, 9H, C(CH$_3$)$_3$), 3.15–3.22 (m, 2H, NHC$\underline{H}_2$CH$_3$), 3.66 (s, 2H, CH$_2$), 6.73 (t, 1H, J = 4.7 Hz, N$\underline{H}$CH$_2$CH$_3$), 6.92 (s, 1H, thiazole-H), 7.46 (dd, 1H, J_1 = 8.7 Hz, J_2 = 2.0 Hz, Ar-H-5), 7.54 (d, 1H, J = 8.7 Hz, Ar-H-4), 8.20 (d, 1H, J = 2.0 Hz, Ar-H-7), 10.21 (s, 1H, NHCO), 10.63 (s, 1H, NHCO), 11.44 (s, 1H, NHCO) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 15.1, 27.9 (3C), 34.2, 40.4, 80.9, 109.3, 111.3, 118.0, 119.5, 131.7, 134.4, 145.1, 145.2, 152.8, 153.7, 158.9, 159.3, 167.7 ppm; HRMS (ESI$^-$) m/z for $C_{20}H_{23}N_6O_4S_2$ ([M−H]$^-$): calcd. 475.1222, found 475.1213. HPLC: method A, t_r 10.54 min (96.1% at 254 nm).

2-(2-Acetamidothiazol-4-yl)-*N*-(2-(3-ethylureido)benzo[1,2-*d*]-thiazol-6-yl)acetamide (34)

Compound was prepared from **21** (0.077 g, 0.39 mmol) and **29** (0.091 g, 0.39 mmol) according to the general procedure **A**. Crude product was crystallized from a mixture of dichloromethane and methanol (20:1). Yield: 0.043 g (26.7%); yellow solid; mp 263–265°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.09 (t, 3H, J = 7.2 Hz, NHCH$_2$C$\underline{H}_3$), 2.11 (s, 3H, COCH$_3$), 3.15–3.21 (m, 2H, NHC$\underline{H}_2$CH$_3$), 3.70 (s, 2H, CH$_2$), 6.70 (t, 1H, J = 5.0 Hz, N$\underline{H}$CH$_2$CH$_3$), 6.95 (s, 1H, thiazole-H), 7.46 (dd, 1H, J_1 = 8.7 Hz, J_2 = 2.0 Hz, Ar-H-5), 7.54 (d, 1H, J = 8.7 Hz, Ar-H-4), 8.20 (d, 1H, J = 2.0 Hz, Ar-H-7), 10.23 (s, 1H, NHCO), 10.63 (s, 1H, NHCO), 12.11 (s, 1H, NHCO) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 15.1, 22.4, 34.2, 40.1, 109.7, 111.3, 118.0, 119.5, 131.8, 134.4, 145.0, 145.2, 153.6, 157.6, 158.9, 167.7, 168.3 ppm; HRMS (ESI$^-$) m/z for $C_{17}H_{17}N_6O_3S_2$ ([M−H]$^-$): calcd. 417.0804, found 417.0797; HPLC: method A, t_r 10.40 min (100% at 254 nm).

2-(2-Aminothiazol-4-yl)-*N*-(2-(3-ethylureido)benzo[1,2-*d*]-thiazol-6-yl)acetamide (35)

Methanol (10 mL) was cooled on an ice bath and then acetyl chloride (0.860 mL, 12.1 mmol) was added dropwise. The mixture was stirred at 0°C for 30 min and then solution of **33** (0.192 g, 0.40 mmol) in methanol (5 mL) was added. Reaction mixture was stirred at 0°C for 1 h and then at room temperature overnight. Then solvent was evaporated under reduced pressure and crude product dissolved in water (5 mL). Water phase was extracted with ethyl acetate (3 × 20 mL), combined organic phases washed with brine (30 mL), dried over Na_2SO_4, filtered and the solvent removed under reduced pressure. Yield: 0.130 g (86.7%); yellow solid; mp 135–137°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.08 (t, 3H, J = 7.2 Hz, NHCH$_2$C$\underline{H}_3$), 3.13–3.20 (m, 2H, NHC$\underline{H}_2$CH$_3$), 3.60 (s, 2H, CH$_2$), 5.03 (s, 2H, NH$_2$), 6.63 (dd, 1H, J_1 = 8.6 Hz, J_2 = 2.2 Hz, Ar-H-5), 6.68 (t, 1H, J = 5.6 Hz, N$\underline{H}$CH$_2$CH$_3$), 6.92 (s, 1H, thiazole-H), 6.94 (d, 1H, J = 2.2 Hz, Ar-H-7), 7.28 (d, 1H, J = 8.6 Hz, Ar-H-4), 10.28 (s, 1H, NHCO) ppm, signal for NHCO not seen in the spectrum; HRMS (ESI$^-$) m/z for $C_{15}H_{16}N_6O_2S_2$ ([M−H]$^-$): calcd. 375.0698, found 375.0691; HPLC: method A, t_r 10.05 min (98.2% at 254 nm).

Methyl 3-((4-(2-((2-(3-ethylureido)benzo[1,2-*d*]thiazol-6-yl)-amino)-2-oxoethyl)thiazol-2-yl)amino)-3-oxopropanoate (36)

A solution of **35** (0.100 g, 0.27 mmol) and triethylamine (0.092 mL, 0.67 mmol) in 1,4-dioxane (10 mL) was cooled to 0°C on an ice bath under an argon atmosphere. Then methyl 3-chloro-3-oxopropanoate (0.035 mL, 0.32 mmol) was added dropwise. The reaction mixture was stirred at room temperature overnight. Then solvent was evaporated under reduced pressure and the residue dissolved in ethyl acetate (20 mL). Organic phase was successively washed with 1% citric acid (2 × 10 mL), saturated aqueous $NaHCO_3$ solution (2 × 10 mL) and brine (10 mL), dried over Na_2SO_4, filtered and the solvent removed under reduced pressure. Crude product purified by flash column chromatography using dichloromethane/methanol (20:1) as eluent. Yield: 0.016 g (12.6%); yellow solid; mp 129–131°C; ^{1}H NMR (400 MHz, MeOD-d_4): δ 1.23 (t, 3H, J = 7.2 Hz, NHCH$_2$C$\underline{H}_3$), 3.31–3.33 (m, 2H, NHC$\underline{H}_2$CH$_3$; signal partially overlapped with solvent residual peak), 3.74 (s, 3H, CH$_3$), 3.77 (s, 2H, CH$_2$), 3.80 (s, 2H, CH$_2$), 7.00 (s, 1H, thiazole-H), 7.45 (dd, 1H, J_1 = 8.7 Hz, J_2 = 2.1 Hz, Ar-H-5), 7.59 (d, 1H, J = 8.7 Hz, Ar-H-4), 7.28 (d, 1H, J = 2.1 Hz, Ar-H-7) ppm, signals for NH groups not seen in the spectrum; HRMS (ESI$^+$) m/z for $C_{19}H_{21}N_6O_5S_2$ ([M+H]$^+$): calcd. 477.1015, found 477.1026; HPLC: method A, t_r 10.76 min (100% at 254 nm).

Ethyl 2-((4-(2-((2-(3-ethylureido)benzo[1,2-*d*]thiazol-6-yl)-amino)-2-oxoethyl)thiazol-2-yl)amino)-2-oxoacetate (37)

A solution of **35** (0.200 g, 0.53 mmol) and triethylamine (0.185 mL, 1.33 mmol) in 1,4-dioxane (20 mL) was cooled to 0°C on an ice bath under an argon atmosphere. Then ethyl 2-chloro-2-oxoacetate (0.071 mL, 0.64 mmol) was added dropwise. The reaction mixture was stirred at room temperature overnight. Then solvent was evaporated under reduced pressure and crude product purified by flash column chromatography using dichloromethane/methanol (30:1) as eluent. Yield: 0.048 g (19.0%); yellow solid; mp 141–143°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.09 (t, 3H, J = 7.2 Hz, NHCH$_2$C<u>H$_3$</u>), 1.31 (t, 3H, J = 7.1 Hz, COOCH$_2$C<u>H$_3$</u>), 3.15–3.22 (m, 2H, NHC<u>H$_2$</u>CH$_3$), 3.77 (s, 2H, CH$_2$), 4.30 (q, 2H, J = 7.1 Hz, COOC<u>H$_2$</u>CH$_3$), 6.70 (t, 1H, J = 4.9 Hz, N<u>H</u>CH$_2$CH$_3$), 7.16 (s, 1H, thiazole-H), 7.47 (dd, 1H, J_1 = 8.7 Hz, J_2 = 2.0 Hz, Ar-H-5), 7.55 (d, 1H, J = 8.7 Hz, Ar-H-4), 7.28 (d, 1H, J = 2.0 Hz, Ar-H-7), 10.25 (s, 1H, NHCO), 10.63 (s, 1H, NHCO), 12.99 (s, 1H, NHCO) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 13.7, 15.1, 34.2, 40.1, 62.5, 111.4, 111.7, 118.1, 119.5, 131.8, 134.3, 145.2, 145.7, 153.6, 158.9, 167.4, 167.5, 175.1, 179.6 ppm; HRMS (ESI$^+$) m/z for $C_{19}H_{21}N_6O_5S_2$ ([M+H]$^+$): calcd. 477.1015, found 477.1009; HPLC: method A, t_r 11.25 min (95.1% at 254 nm).

2-((4-(2-((2-(3-Ethylureido)benzo[1,2-*d*]thiazol-6-yl)amino)-2-oxoethyl)thiazol-2-yl)amino)-2-oxoacetic acid (38)

To a solution of **37** (0.025 g, 0.053 mmol) in methanol (5 mL), 1 M NaOH (0.11 mL, 0.11 mmol) was added and the reaction mixture stirred at room temperature overnight. Then the acidic ion exchange resin Amberlite® IR120 H was added for neutralization (pH ~ 6). After stirring of mixture for 10 min, the resin was filtered off, washed with methanol and the solvent removed *in vacuo*. Yield: 0.017 g (72.3%); orange solid; mp >300°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.06 (t, 3H, J = 7.2 Hz, NHCH$_2$C<u>H$_3$</u>), 3.10–3.17 (m, 2H, NHC<u>H$_2$</u>CH$_3$), 3.68 (s, 2H, CH$_2$), 6.96 (s, 1H, thiazole-H), 7.19 (br s, 1H, N<u>H</u>CH$_2$CH$_3$), 7.28–7.37 (m, 2H, Ar-H-4, Ar-H-5), 8.03 (s, 1H, Ar-H-7), 10.11 (s, 1H, NHCO) ppm; signals for NH groups are not seen in the spectrum; ^{13}CNMR (100 MHz, DMSO-d_6): δ 15.2, 34.2, 40.1, 109.8, 111.5, 118.0, 119.5, 131.8, 135.4, 145.1, 145.5, 152.8, 156.5, 159.8, 164.6, 167.6, 170.5 ppm; HRMS (ESI$^+$) m/z for $C_{17}H_{15}N_6O_5S_2$ ([M–H]$^-$): calcd. 447.0545, found 447.0532; HPLC: method A, t_r 9.64 min (95.2% at 254 nm).

4.2 | Molecular modeling

4.2.1 | Ligand and protein preparation

Three-dimensional models of designed compounds were built in ChemBio3D Ultra 13.0.[30] Their geometries were optimized using MMFF94[31] force field and partial atomic charges were added. Energy was minimized until the gradient value was smaller than 0.001 kcal/(mol Å). The optimized structure was further refined with GAMESS interface in ChemBio3D Ultra 13.0 using the semiempirical PM3 method, QA optimization algorithm and Gasteiger Hückel charges for all atoms for 100 steps.[30] Molecular docking calculations were performed using FlexX,[32,33] as available in LeadIT,[34] running on four octal core AMD Opteron CPU processors, 16 GB RAM, two 750 GB hard drives, running 64-bit Scientific Linux 6.0. Receptor was prepared in a LeadIT graphical user interface using the Receptor wizard. Amino acid residues within a radius of 7 Å around the ligand from the X-ray structure (PDB entry: 4DUH[29]) were defined as the binding site. Hydrogen atoms were added to the binding site residues and correct tautomers and protonation states were assigned. Water molecules, except HOH614, and the ligand were deleted from the crystal structure.

The FlexX molecular docking program, as available in LeadIT,[34] was used for ligand docking. A hybrid algorithm (enthalpy and entropy driven ligand binding) was used to place the "base fragment". The maximum number of solutions per iteration and the maximum number of solutions per fragmentation parameter values were increased to 1000, while other parameters were set at their default values. In order to validate our docking protocol, crystal structure ligand was docked into the defined ATP-binding site of *E. coli* GyrB using the above described docking parameters. The protocol was able to reproduce the binding of the crystal structure ligand with an RMSD value of 1.2 Å, which highlights the docking protocol as suitable for binding mode studies of the designed DNA gyrase inhibitors that were docked using the same settings as used for docking protocol validation. Proposed binding modes and scoring function scores of the top five highest scored docking poses per ligand were evaluated and the highest ranked binding pose was used for graphical representation in PyMOL.[35]

4.3 | Biological activity assays

4.3.1 | *In vitro* inhibitory activity screening and determination of IC_{50} values on *E. coli* DNA gyrase

The assay for determining IC_{50} values (Inspiralis) was performed on black streptavidin-coated 96-well microtiter plates (Thermo Scientific Pierce). The plate was first rehydrated with the wash buffer supplied (20 mM Tris-HCl (pH 7.6), 137 mM NaCl, 0.01% (w/v) BSA, 0.05% (v/v) Tween 20). Biotinylated oligonucleotide in wash buffer was immobilized onto the wells. The excess of oligonucleotide was then washed off and the enzyme assay carried out in the wells (5 min). The final reaction volume of 30 µL in buffer (35 mM Tris-HCl (pH 7.5); 24 mM KCl; 4 mM $MgCl_2$; 2 mM DTT; 1.8 mM spermidine; 1 mM ATP; 6.5% (w/v) glycerol; 0.1 mg/mL albumin) contained 1.5 U of DNA gyrase from *E. coli*, 0.75 µg of relaxed pNO1 plasmid, and 3 µL of inhibitors solution in 10% DMSO and 0.008% Tween® 20. Reactions were incubated for 30 min at 37°C and, after addition of the TF buffer (50 mM NaOAc (pH 5.0), 50 mM NaCl and 50 mM $MgCl_2$), which terminated the enzymatic reaction, for another 30 min at room temperature to allow triplex formation (biotin–oligonucleotide–plasmid). The unbound plasmid was then washed off using TF buffer, and a solution of SybrGOLD stain in T10 buffer (10 mM Tris-HCl (pH 8.0) and 1 mM EDTA) was

added. After mixing, the fluorescence (excitation, 485 nm; emission, 535 nm) was read using a BioTek Synergy H4 microplate reader. Preliminary screening was performed at inhibitor concentrations of 100 and 10 µM. For the most potent compounds, IC_{50} was determined with seven concentrations of the inhibitors. IC_{50} values were calculated using GraphPad Prism software and represent the concentration of inhibitor where the residual activity of the enzyme is 50% in three independent measurements; the final result is given as their average value. Novobiocin (IC_{50} = 0.17 µM) was used as a positive control.

4.3.2 | Determination of antibacterial activity

Clinical control strains of *E. faecalis* (Gram positive, ATCC 29212), *S. aureus* (Gram positive, ATCC 25923), *E. coli* (Gram negative, ATCC 25922), and *P. aeruginosa* (Gram negative, ATCC 27853), were obtained from Microbiologics Inc. (St. Cloud, Minnesota, USA). Single-gene knock-out mutant strains of *E. coli*, JW5503 (*tolC* knock-out)[36] and JD17464 (*lpxC* knock-out), were obtained from the NBRP-*E.coli* collection at National Institute of Genetics (NIG, Japan). Antimicrobial testing was carried out by using the broth microdilution method in 96-well plate format according to the CLSI guidelines.[37] Briefly, bacterial suspensions yielding final inoculum of 5×10^5 CFU/mL were prepared into cation-adjusted Mueller Hinton broth (Becton Dickinson, Franklin Lakes, NJ, USA) and mixed on the plate with test compound solution diluted into assay media. After incubating the plate for 24 h at 37°C, absorbance values were measured at 620 nm and used for evaluating the antimicrobial effects of test compounds by comparing to untreated controls and expressed as percentage inhibition of growth. Ciprofloxacin was used as a positive control on every assay plate (minimum inhibitory concentration [MIC] against *E. faecalis*, *S. aureus*, *E. coli*, and *P. aeruginosa*, was 3.0, 1.5, 0.05, and 3.0 µM, respectively). All compounds were initially assayed at final concentration of 50 µM (n = 3). For compound **33** that displayed ≥80% inhibition at 50 µM, MIC (lowest concentration that inhibited the growth by ≥90%) was determined by carrying out dose-response experiments at eight different concentrations.

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CONFLICT OF INTEREST

The authors have declared no conflict of interest.

ORCID

Tihomir Tomašič http://orcid.org/0000-0001-5534-209X

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