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Discovery of Benzothiazole Scaffold-Based DNA Gyrase B Inhibitors

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ABSTRACT. Bacterial DNA gyrase and topoisomerase IV control the topological state of DNA during replication and are validated targets for antibacterial drug discovery. Starting from our recently reported 4,5,6,7-tetrahydrobenzo[1,2-*d*]thiazole-based DNA gyrase B inhibitors, we replaced their central core with benzothiazole-2,6-diamine scaffold and interchanged substituents in positions 2 and 6. This resulted in equipotent nanomolar inhibitors of DNA gyrase from *Escherichia coli* displaying improved inhibition of *Staphylococcus aureus* DNA gyrase and topoisomerase IV from both bacteria. Compound **27** was the most balanced inhibitor of DNA gyrase and topoisomerase IV both from *E. coli* and *S. aureus*. The crystal structure of the 2-((2-(4,5-dibromo-1*H*-pyrrole-2-carboxamido)benzothiazol-6-yl)amino)-2-oxoacetic acid (**24**) in complex with *E. coli* DNA gyrase B revealed the binding mode of the inhibitor in the ATP-binding pocket. Only some compounds possessed weak antibacterial activity against Gram-positive bacteria. These results provide a basis for structure-based optimization towards dual DNA gyrase and topoisomerase IV inhibitors with antibacterial activity.

INTRODUCTION

In the middle of the 20th century, antibiotics were considered to be a powerful discovery that made infectious diseases a problem of the past. Today, it is clear that some of the antibacterial compounds discovered during the last century were only a short-term solution for the treatment of infectious diseases because bacterial resistance to these drugs is increasing and treatments are becoming less effective.^{1,2} Although the discovery of antibiotics is a very important achievement for mankind, the greatest challenges in antibacterial research are yet to come. There is a need for new antibacterial compounds that have new mechanisms of action with activity against resistant bacterial strains, and the search for new antibacterial agents remains a significant challenge for the future.

Bacterial DNA gyrase is a topoisomerase type II enzyme that has attracted attention since its discovery in 1976, when it was first isolated from *Escherichia coli*³ and identified as a target of the already known aminocoumarin class of antibacterial compounds. In the 1990s, when the crystal structure of the N-terminal fragment of DNA gyrase had already been solved,⁴ topoisomerase IV, a bacterial enzyme with a similar structure, function, and inhibitor susceptibility to DNA gyrase was identified.^{5,6}

DNA gyrase and topoisomerase IV catalyze changes in DNA topology by breaking and rejoining double stranded DNA. In particular, DNA gyrase introduces negative supercoils in DNA in front of the replication fork, while topoisomerase IV is important for decatenation during DNA replication. Thus, both enzymes are involved in very important processes during DNA replication and are essential for cell viability. Both enzymes are ATP-fueled heterotetrameric proteins; DNA gyrase is composed of two A subunits (GyrA) and two B subunits (GyrB), whereas topoisomerase IV consists of two C subunits (ParC) and two E

subunits (ParE), which are homologous to GyrA and GyrB, respectively.⁷ The GyrA and ParC subunits are involved in DNA transit, while the GyrB and ParE subunits contain an ATP-binding domain.⁸ Type II topoisomerase is also found in eukaryotic cells, but unlike the prokaryotic enzymes, eukaryotic topoisomerase II is homodimeric. This difference in structure makes selective targeting of prokaryotic topoisomerase II possible.⁷ The similarities in the structure of DNA gyrase and topoisomerase IV offer an exceptional opportunity for the dual targeting of these enzymes by new antibacterial compounds, thereby reducing the probability of bacteria to develop target-based resistance against them.⁹ Thus, the selectivity of targeting prokaryotic topoisomerase II, the potential of dual targeting, and the well-known structure of these enzymes makes both DNA gyrase and topoisomerase IV attractive targets in the challenging search for new antibacterial compounds.⁹⁻¹⁷

DNA gyrase and topoisomerase IV have been recognized as targets of aminocoumarin and quinolone classes of antibacterial compounds. However, these two classes of inhibitors target different parts of the enzymes. Well known and widely used quinolones target GyrA and ParC subunits, while aminocoumarin class inhibitors target GyrB and ParE subunits. Novobiocin (Figure 1), a natural product aminocoumarin class inhibitor of DNA gyrase and topoisomerase IV discovered in the mid-1950s, targets ATP-binding sites at the GyrB and ParE subunits. It is the only GyrB inhibitor to be used in clinical practice, but despite its good pharmacokinetic profile, it was withdrawn from the market primarily due to its toxicity and also due to the high rate of resistance development. Coumermycin A1 and clorobiocin (Figure 1) are two other aminocoumarin natural products that showed good inhibitory activities against DNA gyrase B, but they never reached the market because of their undesirable toxicity profiles. Numerous studies toward the development of improved novobiocin analogues were unsuccessful, mainly

because of their undesirable pharmacokinetic profile and lack of inhibitory or microbiological activity. Thus, novobiocin is still the only GyrB inhibitor that was used clinically and also the only GyrB inhibitor that has progressed beyond Phase I clinical trials.¹⁸

Figure 1. Structures of the DNA gyrase B inhibitors novobiocin and clorobiocin.

Figure 2. Examples of dual inhibitors of GyrB and ParE possessing (a) benzimidazole (b) bithiazole, (c) thiazolopyridine, (d) pyrrolopyrimidine, (e) imidazopyridine, and (f) benzothiazole scaffolds.

Determination of the crystal structure of DNA gyrase B in complex with adenylyl 5'-(β,γ -imido)-triphosphate (ADPNP) in 1991 and later with other known inhibitors of DNA gyrase B^{18,19,20} provided significant information about the most important inhibitor interactions in the protein ATP-binding site. This information created a solid basis for the structure-based design of

inhibitors over the years and led to discovery of several new scaffolds that had good potential for inhibition of DNA gyrase and topoisomerase IV. Over the years, using scaffold hopping approaches²¹ and exploiting opportunities offered to medicinal chemistry by aromatic heterocycles,²² benzimidazole ureas,²³ bithiazoles,²⁴ thiazolopyridines,²⁵ pyrrolopyrimidines,²⁶ imidazopyridines,²⁷ benzothiazoles^{28,29} (Figure 2) and other structural types¹⁸ have been studied as dual inhibitors of GyrB and ParE subunits of bacterial gyrase and topoisomerase IV.^{9,14}

RESULTS AND DISCUSSION

Design. Our research group has recently reported the discovery of novel structural classes of DNA gyrase inhibitors based on 4,5,6,7-tetrahydrobenzo[1,2-*d*]thiazole³⁰ and *N*-phenyl-4,5-dibromopyrrolamide³¹ scaffolds inspired by the marine natural product oroidin (Figure 3). We obtained the crystal structure of **02** in complex with *E. coli* GyrB and demonstrated that the 4,5-dibromo-1*H*-pyrrole-2-carboxamide moiety of **01** and **02** bound to the ATP binding site of GyrB participates in a hydrogen bond with the Asp73 carboxylate group and a conserved water molecule, whereas the terminal carboxylic acid moiety is involved in interactions with Arg76 and/or Arg136.^{30,31} Based on the crystal structure of the *E. coli* GyrB - **02** complex and potent inhibition of *E. coli* DNA gyrase by 4,5,6,7-tetrahydrobenzo[1,2-*d*]thiazole class inhibitors in conjunction with their weaker inhibition of *Staphylococcus aureus* DNA gyrase and *E. coli* and *S. aureus* topoisomerase IV, we examined the effects of different structural modifications of the parent compound **01** on the inhibition of both enzymes. First, the 4,5,6,7-tetrahydrobenzo[1,2-*d*]thiazole moiety was replaced by a planar aromatic benzothiazole scaffold to enable additional cation - π interaction of the benzene ring with Arg76 within the Glu50-Arg76 salt bridge, which is not present in the 4,5,6,7-tetrahydrobenzo[1,2-*d*] series and was shown to be important for the

strong inhibition of DNA gyrase B.¹⁸ The benzothiazole scaffold additionally made possible the substitution pattern which proved to be successful in the 4,5,6,7-tetrahydrobenzo[1,2-*d*]thiazole series.³⁰ Second, because our docking experiments (Figure 4) suggested similar binding modes of compounds incorporating the pyrrole-2-carboxamido moiety at positions 6 (Series A) and 2 (Series B) of the central heterocyclic core, series B analogues of the parent compound **01** were prepared in addition to series A compounds. Third, the dibromopyrrole moiety was replaced by a smaller dichloropyrrole moiety to possibly improve multiple inhibition against *S. aureus* DNA gyrase and *E. coli* and *S. aureus* topoisomerase IV because these three enzymes possess a smaller hydrophobic pocket compared to that of *E. coli* DNA gyrase.³⁰ Finally, the effect of the length of the ester or carboxylic acid moieties on the multiple inhibition of DNA gyrase and topoisomerase IV was evaluated. As demonstrated below, this resulted in an improved inhibitor series that possesses inhibitory activity against topoisomerase IV from both bacterial strains. The crystal structure of series B compound **24** in complex with *E. coli* DNA gyrase B revealed the binding mode of the studied benzothiazole class of compounds, thus providing a solid basis for structure-based design and optimization of this promising class of ATP-binding site targeting DNA gyrase B inhibitors.

Figure 3. Design of series A and B benzothiazole-2,6-diamine-based DNA gyrase B inhibitors.

Figure 4. The binding mode of a) inhibitor **24** (in *green* sticks) and b) inhibitor **13** (in *orange* sticks) in the ATP-binding site of *E. coli* DNA gyrase B (PDB code: 4DUH) as predicted by docking with LeadIT. The ligand and neighboring protein side chains are shown as stick models, colored according to the chemical atom type ($C_{\text{ligand } 24}$ in *green*, $C_{\text{ligand } 13}$ in *orange*, C_{GyrB} in *gray*, N in *blue*, O in *red*, S in *yellow*, and Br in *brown*). Hydrogen bonds with their lengths in Å are indicated as *black* dotted lines. Figure was prepared by PyMOL.

Chemistry. The synthesis of the series A compounds with the pyrrole-2-carboxamido group attached at position 6 of the benzothiazole-2,6-diamine scaffold is presented in Scheme 1. Acylation of **1** with appropriate acyl chloride (ethyl oxalyl chloride, methyl malonyl chloride or methyl succinyl chloride) in the presence of triethylamine in 1,4-dioxane at room temperature afforded compounds **2**, **3** and **4**. The reduction of the nitro group of **2-4** using catalytic hydrogenation in the presence of 10% Pd/C in methanol or ethanol yielded amines **5**, **6** and **7**. These were acylated with freshly prepared 4,5-dibromo-1*H*-pyrrole-2-carbonyl chloride or 4,5-dichloro-1*H*-pyrrole-2-carbonyl chloride in the presence of pyridine in dichloromethane to obtain esters **8-12**. Compounds **8** and **10** were hydrolyzed with 1 M sodium hydroxide in methanol to produce carboxylic acids **13** and **14**. All attempts to hydrolyze malonic acid derivatives **9** and **12**

were unsuccessful due to decarboxylation that, surprisingly, did not compromise the hydrolysis of the series B esters **18-23** (Scheme 2) and related 4,5,6,7-tetrahydrobenzo[1,2-*d*]thiazole analogs.³⁰

Scheme 1. Synthesis of Series A compounds^a

^aReagents and conditions: (a) ethyl oxalyl chloride (for **2**), methyl malonyl chloride (for **3**) or methyl succinyl chloride (for **4**), Et₃N, 1,4-dioxane, rt, 4 h; (b) H₂, 10% Pd/C, EtOH (for **5**) or MeOH (for **6**, **7**), rt, 24 h; (c) *i*) 4,5-dibromo-1*H*-pyrrole-2-carboxylic acid (for **8**, **9** and **10**) or 4,5-dichloro-1*H*-pyrrole-2-carboxylic acid (for **11** and **12**), oxalyl chloride, DCM, rt, 15 h, then *ii*) **5** (for **8** and **11**), **6** (for **9** and **12**) or **7** (for **10**), pyridine, DCM, rt, 2 h; (d) 1 M NaOH, MeOH, rt, 24 h.

Synthesis of compounds from series B started from the commercially available 6-nitrobenzothiazol-2-amine (**1**), which was catalytically hydrogenated to benzothiazole-2,6-diamine (**15**) in a Parr hydrogenator at 2.1 bar pressure, using ethanol as a solvent and 10% Pd/C as a catalyst. The diamine **15** was further coupled with 2,2,2-trichloro-1-(4,5-dibromo-1*H*-pyrrol-2-yl)ethan-1-one or 2,2,2-trichloro-1-(4,5-dichloro-1*H*-pyrrol-2-yl)ethan-1-one in the presence of

Na₂CO₃ as a base in *N,N*-dimethylformamide at 80 °C to obtain pyrrolamides **16** and **17**. Acylation of **16** and **17** with ethyl oxalyl chloride, methyl malonyl chloride or methyl succinyl chloride at room temperature in the presence of triethylamine in 1,4-dioxane afforded esters **18-23**, which were hydrolyzed to carboxylic acids **24-29** upon treatment with 1 M sodium hydroxide in methanol (Scheme 2).

Scheme 2. Synthesis of Series B Compounds^a

^aReagents and conditions: (a) H₂ (2.1 bar), 10% Pd/C, EtOH, rt, 6 h; (b) 2,2,2-trichloro-1-(4,5-dibromo-1*H*-pyrrol-2-yl)ethan-1-one (for **16**) or 2,2,2-trichloro-1-(4,5-dichloro-1*H*-pyrrol-2-yl)ethan-1-one (for **17**), DMF, Na₂CO₃, 80 °C, 5 h; (c) ethyl oxalyl chloride (for **18** and **21**), methyl malonyl chloride (for **19** and **22**), methyl succinyl chloride (for **20** and **23**), Et₃N, 1,4-dioxane, rt, 12 h; (d) 1 M NaOH, MeOH, rt, 24 h.

***In vitro* inhibition of DNA gyrase and topoisomerase IV.** Synthesized benzothiazole derivatives were evaluated for their inhibitory activity against DNA gyrase and topoisomerase IV from both *E. coli* and *S. aureus* in supercoiling and decatenation assays, respectively. The inhibitory activities of the tested compounds are presented in Tables 1 and 2 as IC₅₀ values.

Inhibitory activities against DNA gyrase

In the series A compounds, in which the 4,5-dichloro- or 4,5-dibromopyrrolamido moiety is attached at the position 6 of the benzothiazole ring, the most favorable acyl substituent at position 2 proved to be the oxalyl group, both in the ester and the free carboxylic acid form as demonstrated by the most potent inhibitor **13** ($IC_{50} = 38$ nM) of *E. coli* DNA gyrase among compounds in the Series A (Table 1). The dichloro ester analogues **11** and **12** displayed weaker inhibition of *E. coli* DNA gyrase than the corresponding dibromo analogues, so the respective carboxylic acids were not prepared. The inhibitory potency of dibromo ethyl oxalate **8** equaled that of the 4,5,6,7-tetrahydrobenzo[1,2-*d*]thiazole analogue ($IC_{50} = 100$ nM) while the methylmalonate and methylsuccinate esters **9** and **10** were found to be twofold and ninefold weaker inhibitors of *E. coli* DNA gyrase than the corresponding 4,5,6,7-tetrahydrobenzo[1,2-*d*]thiazole analogues.³⁰ The carboxylic acid inhibitors **13** ($IC_{50} = 38$ nM) and **14** ($IC_{50} = 57$ nM) were equipotent with the corresponding 4,5,6,7-tetrahydrobenzo[1,2-*d*]thiazole analogues (oxalyl analogue: $IC_{50} = 58$ nM; succinyl analogue: $IC_{50} = 49$ nM).³⁰ Compounds from the Series A did not display a substantial *S. aureus* DNA gyrase inhibition. (Table 1).

From the inhibitory activities of series B compounds **16** and **17**, shown in Table 2 it can be concluded that the 4,5-dibromo-1*H*-pyrrolamide moiety attached at position 2 on the benzothiazole ring contributes to the affinity for the ATP-binding site of *E. coli* DNA GyrB more than the 4,5-dichloro-1*H*-pyrrolamide moiety attached at the same position. This trend was also observed for the activities of other compounds from Series B containing the 4,5-dibromo-1*H*-pyrrolamide moiety (c.f. **18** vs. **21** and **20** vs. **23**, **24** vs. **27** and **26** vs. **29**), with the exception of the malonyl pairs **19** and **22** and **25** and **28**, in which a trend of stronger inhibition by the 4,5-

Table 1. Inhibition of *E. coli* and *S. aureus* DNA gyrase and topoisomerase IV by the benzothiazole compounds containing the pyrrole-2-carboxamido moiety at position 6 on the benzothiazole core (Series A).

<p style="text-align: center;">8 - 14</p>							
compound	X	n	R	DNA gyrase		topoisomerase IV	
				IC ₅₀ [μM]		IC ₅₀ [μM]	
				<i>E. coli</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>S. aureus</i>
novobiocin	-	-	-	0.17	0.040	11	27
8	Br	0	Et	0.081 ± 0.05	> 100	> 100	> 100
9	Br	1	Me	0.24 ± 0.02	> 100	> 100	> 100
10	Br	2	Me	0.87 ± 0.21	> 100	> 100	> 100
11	Cl	0	Et	1.8 ± 0.4	> 100	> 100	> 100
12	Cl	1	Me	7.9 ± 2.1	> 100	> 100	> 100
13	Br	0	H	0.038 ± 0.001	> 100	5.4 ± 1.0	2.5 ± 0.2
14	Br	2	H	0.057 ± 0.018	> 100	4.5 ± 0.7	2.2 ± 0.3

dichloro-1*H*-pyrrolamide compounds was observed. On the contrary, the differences between the dibromopyrrole and dichloropyrrole compounds were not straightforward for inhibition of DNA gyrase from *S. aureus*. Ethyl oxalyl, methyl malonyl and methyl succinyl moieties attached at position 6 of the benzothiazole ring improved the inhibitory activities of the dichloropyrrole compounds **21-23** but not of the dibromopyrrole compounds **18-20** against *E. coli* DNA gyrase, with the methyl malonyl derivative **22** (IC₅₀ = 0.22 μM) being the most potent inhibitor in this ester series. In the carboxylic acid series (compounds **24-29**) the measured IC₅₀ values for

inhibition of DNA gyrase from *E. coli* were all in the nanomolar range, due to the contribution of the carboxylate group interaction with Arg136. The most potent inhibitors in the dibromopyrrole carboxylic acid series were succinyl and oxalyl derivatives **26** ($IC_{50} = 33$ nM) and **24** ($IC_{50} = 58$ nM), while for the dichloropyrrole compounds the most potent inhibitor was oxalyl compound **27** with an IC_{50} value of 87 nM. The affinity of compound **24** for DNA gyrase B from *E. coli* was further confirmed by surface plasmon resonance (SPR) (for experimental details see Ref. 29) with a K_d of 0.23 μ M. The inhibition of *S. aureus* DNA gyrase was observed only for the dibromopyrrole carboxylic acid **25** ($IC_{50} = 5.6$ μ M) and dichloropyrrole carboxylic acids **27-29**, with IC_{50} values in the range of 0.51 to 94 μ M. Generally, all of the compounds from Series B displayed weaker inhibition of *S. aureus* DNA gyrase compared to *E. coli* gyrase. It is worth noting the low micromolar activity of malonyl compounds **25** in the dibromo series ($IC_{50} = 5.6$ μ M) and **28** in the dichloro series ($IC_{50} = 8.5$ μ M) against *S. aureus* gyrase. The nanomolar activity of the dichloro oxalyl derivative **27** ($IC_{50} = 0.51$ μ M) in comparison to dibromo analogue **24** ($IC_{50} > 100$ μ M), as well as to malonyl analogue **28** ($IC_{50} = 8.5$ μ M) and succinyl analogue **29** ($IC_{50} = 94$ μ M) is also noteworthy, indicating that the carboxylic acid moiety and small changes in the length of the acyl moiety (c.f. **24** vs. **25**, **27** vs. **28**) are important for the binding of Series B compounds to the ATP-binding site of *S. aureus* DNA gyrase. In addition, substitution in the pyrrole ring (c.f. **24** vs. **27**) is important for the orientation of the whole molecule in the binding pocket of *S. aureus* DNA gyrase. The main difference between the compounds from the Series B (pyrrole-2-carboxamido group at position 2) and Series A (pyrrole-2-carboxamido group at position 6) is that the compounds in series B exhibited better inhibitory activity against *S. aureus* DNA gyrase, while their inhibitory activity against *E. coli* DNA gyrase remained in the nanomolar range.

Table 2. Inhibition of *E. coli* and *S. aureus* DNA gyrase and topoisomerase IV by the benzothiazole compounds containing the pyrrole-2-carboxamido moiety at position 2 on the benzothiazole core (Series B).

<div style="display: flex; justify-content: space-around; align-items: center;"> <div style="text-align: center;"> <p>16, 17</p> </div> <div style="text-align: center;"> <p>18-29</p> </div> </div>							
compound	X	n	R	DNA gyrase		topoisomerase IV	
				IC ₅₀ [μM]		IC ₅₀ [μM]	
				<i>E. coli</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>S. aureus</i>
novobiocin	-	-	-	0.17	0.040	11	27
16	Br	-	-	2.0 ± 0.2	> 100	> 100	> 100
17	Cl	-	-	> 100	> 100	> 100	> 100
18	Br	0	Et	29 ± 6	> 100	> 100	> 100
19	Br	1	Me	2.0 ± 0.4	> 100	> 100	> 100
20	Br	2	Me	7.0 ± 0.9	> 100	> 100	> 100
21	Cl	0	Et	> 100	> 100	> 100	> 100
22	Cl	1	Me	0.22 ± 0.02	> 100	> 100	> 100
23	Cl	2	Me	35 ± 12	> 100	> 100	> 100
24	Br	0	H	0.058 ± 0.031	> 100	13 ± 3	10 ± 2
25	Br	1	H	0.14 ± 0.06	5.6 ± 2.6	45 ± 5	17 ± 3
26	Br	2	H	0.033 ± 0.025	> 100	7.2 ± 1.3	26 ± 1
27	Cl	0	H	0.087 ± 0.001	0.51 ± 0.23	1.8 ± 0.8	1.7 ± 0.8
28	Cl	1	H	0.12 ± 0.02	8.5 ± 1	5.19 ± 4.7	2.4 ± 1.3
29	Cl	2	H	0.43 ± 0.11	94 ± 25	5.3 ± 0.4	28 ± 4

Inhibitory activities against topoisomerase IV

Generally, the inhibitory potencies of most of the Series B compounds against topoisomerase IV were lower than those against DNA gyrase for the *E. coli* enzymes but for the *S. aureus* enzymes, the IC₅₀ values for the inhibition of both enzymes were more comparable (Tables 1 and 2). Inhibition of topoisomerase IV by most of the active compounds (**13**, **14**, and **24-29**) was better than or comparable to that of novobiocin (IC₅₀ (*E. coli*) = 11 μM; IC₅₀ (*S. aureus*) = 27 μM). The esters **8-12** and **18-23** did not show any promising activity, which indicates that a carboxylic acid moiety is required for the binding of the compounds in the ATP-binding site of topoisomerase IV. All of the final carboxylic acids (**13**, **14**, and **24-29**) from both series A and B exhibited low micromolar activities against topoisomerase IV from both *E. coli* and *S. aureus*.

Compounds **25**, **27** and **28** were found to be especially interesting for further studies because they showed promising inhibitory activities against all four tested enzymes. In particular, 4,5-dichloropyrrole-based benzothiazole inhibitor **27** exhibited well-balanced multiple inhibition of all four enzymes in the nanomolar and low micromolar range (0.087 – 1.8 μM).

Antibacterial activity. All of the compounds from series A and series B were tested against two Gram-positive (*Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212) and two Gram-negative (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853) bacterial strains. In general, the compounds did not show significant antibacterial activities against either Gram-positive or Gram-negative bacteria. The only notable activities were observed against Gram-positive *E. faecalis* for compounds **25** and **11** which showed 59% and 47% inhibition of the growth of *E. faecalis* at a 50 μM concentration after 24 h incubation, respectively. None of the final compounds showed significant antibacterial activity against the tested Gram-negative bacteria. Because a possible reason for the absence of the antibacterial activity of compounds against Gram-negative bacteria could be their affinity for efflux pumps,

we tested the compounds **17-19**, **21-25**, **27** and **29** in $\Delta tolC$ *E. coli*, an efflux deficient strain derived from wild-type *E. coli* ECM1194 that lacks a functional *tolC* gene that encodes the essential outer membrane component of the multicomponent efflux pump (for experimental details see Ref. 30). We found that compound **25** exhibited more potent antibacterial activity (MIC < 16 $\mu\text{g/mL}$) in $\Delta tolC$, than in the wild type *E. coli* strain that contained efflux pump (MIC > 256 $\mu\text{g/mL}$). These results may explain the weak antibacterial activity of **25** and similar compounds against both Gram-negative bacterial strains tested, with only 12% inhibition of the growth of *E. coli* and 11% inhibition of the growth of *P. aeruginosa* after 24 h incubation.

X-Ray crystallography and molecular modeling. A detailed binding mode of the series B inhibitor **24** in the ATP-binding site of the 43 kDa N-terminal fragment of the *E. coli* DNA gyrase B (residues Gly15 to Thr392) was revealed from the X-ray co-crystal structure, which was solved to a resolution of 2.83 Å (PDB code: 5L3J). The electron density for most of the ligand is well defined, but is missing for the terminal carboxylate group (Figure 5), which can probably be attributed to its flexibility in the binding site. Similar situation was observed in the co-crystal structure of *E. coli* DNA gyrase in complex with *N*-phenyl-4,5-dibromopyrrolamide inhibitor, where the mobility of the inhibitor in the binding site was also shown through molecular dynamics simulation.³¹ In contrast, the high resolution crystal structure of bithiazole inhibitor in complex with *E. coli* DNA gyrase (PDB entry: 4DUH) revealed a hydrogen bond between the inhibitor and Gly101 carbonyl oxygen, which stabilized the Gly97-Ser108 flexible loop, decreased ligand flexibility and enhanced chances for obtaining a high resolution structure.²⁴ Lack of the structural feature in this series of the benzothiazole inhibitors, enabling hydrogen bonding with amino residues of the Gly97-Ser108 flexible loop, thus probably also

contributed to the higher mobility of ligand in the binding site and consequently to the lower crystal structure resolution of *E. coli* DNA gyrase – **24** complex.

The orientation and conformation of the terminal carboxylate group of **24** was hence modeled in two different ways (Figure 5; model 1, model 2) and is located in the vicinity of Arg76 and Arg136, which are usually in contact with DNA gyrase B inhibitors.^{9,18} However, the electron density for the Arg76 side chain is missing in the experimental data and no hydrogen bonds can be predicted. The 4,5-dibromopyrrolamide moiety of **24** is bound to the adenine binding pocket, where a hydrogen bond between the pyrrole NH group and Asp73 side chain is formed. However, due to the X-ray structure resolution of 2.83 Å, only a few water molecules were modeled based on the electron density and the conserved water molecule was not observed in the structure. Therefore, a hydrogen bond between the inhibitor carbonyl group and Asp73 side chain bridged by this water molecule could not be observed. The 4,5-dibromopyrrole moiety forms additional hydrophobic contacts with Val43, Asn46, Val71, Val120, Thr165 and Val167, while the benzothiazole scaffold forms hydrophobic interactions with Glu50, Gly77, Ile78 and Pro79.

Possible binding modes of DNA gyrase inhibitors of the benzothiazole-2,6-diamine series A and B were also studied following our recently described docking protocol,³⁰ using LeadIT and the high resolution structure of *E. coli* DNA gyrase in complex with a bithiazole inhibitor.²⁴ Comparison of the predicted (Figure 4a) and experimental binding mode (Figure 5a) of **24** in the

Figure 5. X-ray crystal structure of *E. coli* DNA gyrase B in complex with ligand **24** (PDB code: 5L3J): a) model 1; b) model 2. The ligand and neighboring protein side chains are shown as stick models, that are colored according to the chemical atom type (C_{ligand} in *green* for model 1 and in *yellow* for model 2, C_{GyrB} in *gray*, N in *blue*, O in *red*, S in *yellow*, and Br in *brown*). The ligand molecule is shown superimposed with the refined 2Fo–Fc electron density map contoured at 1.0 σ . Hydrogen bonds are indicated as *black* dotted lines. Figure was prepared by PyMOL.

E. coli DNA gyrase ATP-binding site shows similar conformation of the ligand, while its position is slightly different. The docking predicts the formation of hydrogen bonds between the terminal oxalate moiety and Arg76 and Arg136 side chains that could not be observed in the X-ray structure (Figure 5). Such hydrogen bonds are predicted for all oxalic (**8**, **11**, **13**, **18**, **21**, **24** and **27**), malonic (**9**, **12**, **19**, **22**, **25**, and **28**) and succinic acid (**10**, **14**, **20**, **23**, **26** and **29**) derivatives in both series A and series B. As an example, the predicted binding mode of series A compound **13**, which has the opposite substitution as **24**, is presented in Figure 4b.

CONCLUSION

In summary, replacement of the central core of 4,5,6,7-tetrahydrobenzo[1,2-*d*]thiazole-based DNA gyrase B inhibitors²⁹ with a planar benzothiazole-2,6-diamine scaffold and moving the 4,5-

dihalopyrrole-2-carbonyl group from *N*-6 to *N*-2 of the central core resulted in two series of nanomolar inhibitors. These compounds were nearly equipotent with the 4,5,6,7-tetrahydrobenzo[1,2-*d*]thiazole compounds as inhibitors of *E. coli* DNA gyrase but displayed improved inhibition of *S. aureus* DNA gyrase and topoisomerase IV from both bacteria. A crystal structure of 2-((2-(4,5-dibromo-1*H*-pyrrole-2-carboxamido)benzothiazol-6-yl)amino)-2-oxoacetic acid (**24**) in complex with *E. coli* DNA gyrase B solved to a resolution of 2.83 Å revealed the binding mode of the inhibitor in the ATP-binding pocket of the enzyme. In general, no significant antibacterial activity against both Gram-negative and Gram-positive bacteria was observed, most likely due to efflux of inhibitors. Some of the compounds possessed only weak antibacterial activity against Gram-positive *E. faecalis*. The results of this study provide a good starting point for structure-based design of improved ATP-competitive DNA gyrase and topoisomerase IV inhibitors with antibacterial activity. In this respect, compounds **25**, **27** and **28** are especially interesting for further studies because they showed promising well balanced multiple inhibition in the nanomolar/low micromolar range against DNA gyrase and topoisomerase IV both in *E. coli* and *S. aureus*.

EXPERIMENTAL SECTION

Chemistry. The chemicals obtained from Sigma-Aldrich (St. Louis, MO, USA), Acros Organics (Geel, Belgium), and Appollo Scientific (Stockport, UK) were used without further purification unless otherwise stated. Analytical TLC was performed on 0.25 mm silica gel Merck 60 F₂₅₄ plates, using visualization with UV light and spray reagents. Column chromatography was carried out on Merck silica gel 60 (particle size 240-400 mesh). Melting points were

determined on a Reichert hot stage microscope and are uncorrected. ^1H NMR and ^{13}C spectra were recorded at 400 and 100 MHz, respectively, on a Bruker AVANCE III spectrometer in $\text{DMSO-}d_6$ or CDCl_3 solution with TMS as an internal standard at room temperature. IR spectra were obtained using a Thermo Nicolet Nexus 470 ESP FT-IR spectrometer. Mass spectra were recorded on a VG Analytical Autospec Q mass spectrometer. HPLC analyses were run on an Agilent Technologies 1100 instrument (Agilent Technologies, Santa Clara, CA, USA) with a G1365B UV-VIS detector, a G1316A thermostat, and a G1313A autosampler using a Phenomenex Luna 5- μm C18 column (4.6 mm $\times$ 150 mm or 4.6 mm $\times$ 250 mm, Phenomenex, Torrance, CA, USA) and a flow rate of 1.0 mL/min. The eluent consisted of 0.1 % trifluoroacetic acid in water (A) and methanol (B); gradient, 90 % A to 10 % A in 20 min, then 5 min 10 % A; flow rate 1.0 mL/min; injection volume, 10 μL . All tested compounds had a purity of at least 95% by HPLC and passed the PAINS filter (see below).

General procedure A. Synthesis of compound 2, 3 and 4.

6-Nitro-benzothiazol-2-amine (**1**) (1.95 g, 10.0 mmol) was dissolved in 1,4-dioxane (100 mL), triethylamine (2.09 mL, 15.0 mmol) was added and the mixture was cooled down to 0 $^\circ\text{C}$. Ethyl oxalyl chloride (1.68 mL, 15.0 mmol), methyl malonyl chloride (1.61 mL, 15.0 mmol) or methyl succinyl chloride (1.85 mL, 15.0 mmol) were added dropwise to the mixture and the reaction mixture was stirred for additional 3 h at room temperature, whereupon saturated NaHCO_3 solution was added to quench the reaction. The reaction mixture was concentrated in vacuo, dissolved in ethyl acetate and the solution was washed with 3 $\times$ 10 mL saturated NaHCO_3 , 3 $\times$ 10 mL 10% citric acid, 3 $\times$ 10 mL water and 3 $\times$ 10 mL brine. If the product precipitated between the ethyl acetate and water phase it was collected by filtration. The ethyl acetate phase

was dried over sodium sulfate and concentrated. The combined crude products were recrystallized from ethyl acetate.

Ethyl 2-((6-nitrobenzo[d]thiazol-2-yl)amino)-2-oxoacetate (2). Prepared according to general procedure A from compound **1** (2.00 g, 10.2 mmol); yellow crystals, yield 2.56 g (85 %); mp 225-228 °C; IR (ATR) ν 3267, 3096, 1699, 1573, 1541, 1513, 1471, 1441, 1412, 1377, 1346, 1333, 1289, 1270, 1154, 1124, 1041, 1011, 959, 903, 857, 841, 828, 742, 719 cm^{-1} ; ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 1.34 (t, 3H, $J = 7.1$ Hz, CH_3CH_2), 4.34 (q, 2H, $J = 7.1$ Hz, CH_3CH_2), 7.97 (d, 1H, $^3J = 8.9$ Hz, Ar-H4), 8.31 (dd, 1H, $^3J = 8.9$ Hz, $^4J = 2.4$ Hz, Ar-H5), 9.12 (dd, 1H, $^4J = 2.4$ Hz), 13.64 (s br, 1H, CONH) ppm; ^{13}C NMR (100 MHz, $\text{DMSO-}d_6$) δ 13.71, 62.85, 119.19, 120.96, 121.83, 132.27, 143.30, 152.86, 157.43, 158.48, 163.03 ppm; HRMS (ESI^+) m/z for $\text{C}_{11}\text{H}_8\text{N}_3\text{O}_5\text{S}$ ($[\text{M-H}]^-$): calcd. 294.0185, found 294.0189.

Methyl 3-((6-nitrobenzo[d]thiazol-2-yl)amino)-3-oxopropanoate (3). Prepared according to general procedure A from compound **1** (1.55 g, 7.94 mmol); yellow crystals, yield 1.60 g (68 %); mp 199-202 °C; IR (ATR) ν 2978, 1733, 1665, 1609, 1575, 1548, 1509, 1475, 1446, 1434, 1412, 1330, 1280, 1235, 1202, 1168, 1152, 1125, 1045, 1018, 977, 900, 834, 807, 746, 720 cm^{-1} ; ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 3.68 (s, 3H, OCH_3), 3.82 (s, 2H, COCH_2CO), 7.93 (d, 1H, $^3J = 8.9$ Hz, Ar-H4), 8.30 (dd, 1H, $^3J = 8.9$ Hz, $^4J = 2.4$ Hz, Ar-H5), 9.09 (d, 1H, $^4J = 2.4$ Hz, Ar-H7), 13.19 (s br, 1H, CONH) ppm; ^{13}C NMR (100 MHz, $\text{DMSO-}d_6$) δ 42.21, 52.27, 119.11, 120.75, 121.77, 132.13, 143.06, 153.27, 163.04, 165.95, 167.16 ppm; HRMS (ESI^+) m/z for $\text{C}_{11}\text{H}_{10}\text{N}_3\text{O}_5\text{S}$ ($[\text{M+H}]^+$): calcd. 296.0341, found 296.0343.

Methyl 4-((6-nitrobenzo[d]thiazol-2-yl)amino)-4-oxobutanoate (4). Prepared according to general procedure A from compound **1** (2.00 g, 10.2 mmol); yellow crystals, yield 2.00 g (63 %);

mp 231-234 °C; IR (ATR) ν 3259, 3107, 2967, 2923, 2362, 1719, 1667, 1576, 1538, 1508, 1440, 1430, 1336, 1272, 1212, 1154, 1132, 1071, 1044, 1016, 997, 954, 908, 829 cm^{-1} ; ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 2.70 (m, 2H, COCH_2), 2.83 (m, 2H, COCH_2), 3.62 (s, 3H, OCH_3), 7.88 (d, 1H, $^3J = 8.9$ Hz, Ar-H4), 8.27 (dd, 1H, $^3J = 8.9$ Hz, $^4J = 2.4$ Hz, Ar-H5), 9.03 (d, 1H, $^4J = 2.4$ Hz, Ar-H7), 12.86 (s br, 1H, CONH) ppm; ^{13}C NMR (100 MHz, $\text{DMSO-}d_6$) δ 27.83, 30.08, 51.52, 119.00, 120.51, 121.72, 132.11, 142.89, 153.45, 163.30, 171.84, 172.50 ppm; HRMS (ESI^+) m/z for $\text{C}_{12}\text{H}_{12}\text{N}_3\text{O}_5\text{S}$ ($[\text{M}+\text{H}]^+$): calcd. 310.0498, found 310.0495.

General procedure B. Synthesis of compounds 5, 6 and 7. Catalytic hydrogenation of compound **2** (1.00 mmol in 10 mL methanol) and compounds **3** and **4** (1.00 mmol in 10 mL ethanol) were performed in the presence of 10% Pd/C (0.030 g) as a catalyst at room temperature and atmospheric pressure for 24 h. At the end of the reaction, the reaction mixture was filtered and the filtrate was concentrated to give the crude products which were recrystallized from methanol (compound **5**) or ethanol (compounds **6**, **7**).

Ethyl 2-((6-aminobenzo[d]thiazol-2-yl)amino)-2-oxoacetate (5). Prepared according to general procedure B from compound **2** (0.850 g, 2.88 mmol); greenish crystals, yield 0.698 g (91 %); mp 147-150 °C; IR (ATR) ν 3268, 2988, 2362, 1733, 1688, 1604, 1549, 1464, 1393, 1367, 1296, 1258, 1160, 1112, 1058, 1011, 964, 907, 865, 812, 802 cm^{-1} ; ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 1.31 (t, 3H, $J = 7.1$ Hz, CH_2CH_3), 4.31 (q, 2H, $J = 7.1$ Hz, CH_2CH_3), 5.34 (s br, 2H, NH_2), 6.76 (dd, 1H, $^3J = 8.6$ Hz, $^4J = 2.1$ Hz, Ar-H5), 7.05 (d, 1H, $^4J = 2.1$ Hz, Ar-H7), 7.47 (d, 1H, $J = 8.6$ Hz, Ar-H4), 12.82 (s br, 1H, CONH); ^{13}C NMR (100 MHz, $\text{DMSO-}d_6$): δ 13.74, 62.51, 103.94, 114.88, 120.88, 132.99, 138.48, 146.31, 152.84, 156.77, 159.41 ppm; HRMS (ESI^+) m/z for $\text{C}_{11}\text{H}_{12}\text{N}_3\text{O}_3\text{S}$ ($[\text{M}+\text{H}]^+$): calcd. 266.0599, found 266.0602.

Methyl 3-((6-aminobenzo[d]thiazol-2-yl)amino)-3-oxopropanoate (6). Prepared according to general procedure B from compound **3** (1.50 g, 5.08 mmol); brownish crystals, yield 0.825 g (61 %); mp 162-165 °C; IR (ATR) ν 3381, 2958, 2361, 2342, 1726, 1683, 1608, 1555, 1464, 1433, 1408, 1331, 1310, 1285, 1252, 1233, 1212, 1175, 1141, 1057, 1015, 978, 909, 866, 780 cm^{-1} ; ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 3.62 (s, 2H, COCH_2CO), 3.67 (s, 3H, OCH_3), 5.24 (s br, 2H, NH_2), 6.72 (dd, 1H, $^3J = 8.6$ Hz, $^4J = 2.1$ Hz, Ar-H5), 7.01 (d, 1H, $^4J = 2.1$ Hz, Ar-H7), 7.42 (d, 1H, $^3J = 8.6$ Hz, Ar-H4), 12.23 (s br, 1H, CONH) ppm; ^{13}C NMR (100 MHz, $\text{DMSO-}d_6$) δ 42.10, 52.14, 103.97, 114.49, 120.99, 132.92, 139.43, 145.91, 152.51, 164.41, 167.51 ppm; HRMS (ESI^+) m/z for $\text{C}_{11}\text{H}_{12}\text{N}_3\text{O}_3\text{S}$ ($[\text{M}+\text{H}]^+$): calcd. 266.0599, found 266.0606.

Methyl 4-((6-aminobenzo[d]thiazol-2-yl)amino)-4-oxobutanoate (7). Prepared according to general procedure B from compound **4** (0.700 g, 2.26 mmol); brownish crystals, yield 0.440 g (70 %); mp 208-211 °C; IR (ATR) ν 3201, 2988, 2947, 2362, 1727, 1687, 1607, 1575, 1556, 1472, 14737, 1422, 1387, 1311, 1271, 1232, 1151, 1081, 1060, 999, 983, 915, 898 cm^{-1} ; ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 2.64 (m, 2H, COCH_2), 2.72 (m, 2H, COCH_2), 3.61 (s, 3H, OCH_3), 5.16 (s br, 2H, NH_2), 6.70 (dd, 1H, $^3J = 8.6$ Hz, $^4J = 2.3$ Hz, Ar-H5), 6.99 (d, 1H, $^4J = 2.3$ Hz, Ar-H7), 7.40 (d, 1H, $^3J = 8.6$ Hz, Ar-H4), 12.07 (s, 1H, CONH) ppm; ^{13}C NMR (100 MHz, $\text{DMSO-}d_6$) δ 28.00, 29.81, 51.43, 104.03, 114.34, 120.78, 132.85, 139.56, 145.68, 152.87, 170.26, 172.59 ppm; HRMS (ESI^+) m/z for $\text{C}_{12}\text{H}_{14}\text{N}_3\text{O}_3\text{S}$ ($[\text{M}+\text{H}]^+$): calcd. 280.0756, found 280.0758.

General procedure C. Synthesis of compounds 8-12. To a solution of 4,5-dibromo-1H-pyrrole-2-carboxylic acid (for **8**, **9** and **10**) (0.250 g, 0.930 mmol) or 4,5-dichloro-1H-pyrrole-2-carboxylic acid (for **11** and **12**) (0.167 g, 0.930 mmol) in anhydrous dichloromethane (20 mL), oxalyl chloride (0.46 mL of 2M solution in methylene chloride, 0.93 mmol) was added and the

reaction mixture was stirred for 15 h. The solvent was removed under reduced pressure whereupon dichloromethane (15 mL), pyridine (5 mL) appropriate amine (1.00 mmol) were added to the residue and stirred for 2 h at room temperature. The reaction mixture was concentrated and dissolved in ethyl acetate (50 mL) and the solution washed successively with 1M HCl (10 mL), NaHCO₃ (10 mL), water (10 mL) and brine (10 mL). The ethyl acetate phase was dried over sodium sulfate and concentrated. The crude products were recrystallized from methanol.

Ethyl 2-((6-(4,5-dibromo-1H-pyrrole-2-carboxamido)benzo[d]thiazol-2-yl)amino)-2-oxoacetate (**8**). Prepared according to general procedure C from amine **5** (0.220 g, 0.829 mmol) and 4,5-dibromo-1H-pyrrole-2-carbonyl chloride (0.357 g, 1.24 mmol); yellow crystals, yield 0.220 g (51 %); mp 252-255 °C; IR (ATR) ν 3122, 2971, 2362, 1745, 1703, 1640, 1609, 1574, 1546, 1521, 1460, 1434, 1410, 1332, 1312, 1251, 1232, 1187, 1163, 1107, 1042, 1014, 984, 974, 961, 869, 824, 810 cm⁻¹; ¹H NMR (400 MHz, DMSO-*d*₆) δ 1.33 (t, 3H, *J* = 7.1 Hz, CH₂CH₃), 4.33 (q, 2H, *J* = 7.1 Hz, CH₂CH₃), 7.26 (d, 1H, ⁴*J* = 2.7 Hz, pyrrole-H3), 7.72 (dd, 1H, ³*J* = 8.8 Hz, ⁴*J* = 2.0 Hz, Ar-H5), 7.79 (d, 1H, ³*J* = 8.8 Hz, Ar-H4), 8.44 (d, 1H, ⁴*J* = 2.0 Hz, Ar-H7), 10.07 (s, 1H, NHCO), 12.96 (s br, 1H, NHCOCO), 13.17 (s br, 1H, pyrrole-NH) ppm; ¹³C NMR (100 MHz, DMSO-*d*₆) δ 13.75, 62.68, 98.17, 106.02, 112.49, 113.86, 119.78, 120.69, 127.79, 131.98, 135.35, 144.25, 157.29, 159.03 ppm (2 carbonyl signals not seen due to broadening); HRMS (ESI⁺) *m/z* for C₁₆H₁₁Br₂N₄O₄S ([M-H]⁺): calcd. 512.8868, found 512.8882; HPLC: *t*_r = 20.94 min (96.3 % at 220 nm).

Methyl 3-((6-(4,5-dibromo-1H-pyrrole-2-carboxamido)benzo[d]thiazol-2-yl)amino)-3-oxopropanoate (**9**). Prepared according to general procedure C from compound **6** (0.200 g, 0.754 mmol) and 4,5-dibromo-1H-pyrrole-2-carbonyl chloride (0.325 g, 1.13 mmol); yellow crystals,

yield 0.225 g (58 %); mp > 340 °C; IR (ATR) ν 3199, 2988, 2361, 1722, 1691, 1643, 1611, 1575, 1549, 1523, 1468, 1436, 1410, 1370, 1329, 1370, 1254, 1225, 1180, 1148, 1067, 1016, 973, 869, 815 cm^{-1} ; ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 3.68 (s, 5H, OCH_3 , COCH_2CO), 7.26 (d, 1H, $^4J = 2.7$ Hz, pyrrole-H3), 7.68 (dd, 1H, $^3J = 8.8$ Hz, $^4J = 2.0$ Hz, Ar-H5), 7.74 (d, 1H, $^3J = 8.8$ Hz, Ar-H4), 8.39 (d, 1H, $^4J = 2.0$ Hz, Ar-H7), 10.02 (s, 1H, NHCO), 12.54 (s, 1H, NHCOCH_2), 12.94 (d, 1H, $^4J = 2.7$ Hz, pyrrole-NH) ppm; ^{13}C NMR (100 MHz, $\text{DMSO-}d_6$): δ 42.16, 52.21, 98.15, 105.93, 112.4, 113.78, 119.51, 120.63, 127.84, 131.85, 134.89, 144.61, 156.61, 157.24, 165.05, 167.38 ppm; HRMS (ESI $^-$) m/z for $\text{C}_{16}\text{H}_{11}\text{Br}_2\text{N}_4\text{O}_4\text{S}$ ($[\text{M-H}]^-$): calcd. 512.8868, found 512.8879; HPLC: $t_r = 19.07$ min (97.1 % at 220 nm).

Methyl 4-((6-(4,5-dibromo-1H-pyrrole-2-carboxamido)benzo[d]thiazol-2-yl)amino)-4-oxobutanoate (10). Prepared according to general procedure C from compound **7** (0.318 g, 1.14 mmol) and 4,5-dibromo-1H-pyrrole-2-carbonyl chloride (0.491 g, 1.71 mmol); yellow crystals, yield 0.260 g (43 %); mp. 254-257 °C; IR (ATR) ν 3198, 2988, 2361, 1701, 1647, 1616, 1578, 1547, 1521, 1473, 1443, 1408, 1357, 1332, 1279, 1259, 1223, 1181, 1154, 1066, 983, 970, 954, 920, 867 cm^{-1} ; ^1H NMR (400 MHz, $\text{DMSO-}d_6$): δ 2.67 (m, 2H, CH_2CO), 2.78 (m, 2H, CH_2CO), 3.62 (s, 3H, OCH_3), 7.25 (d, 1H, $^4J = 2.7$ Hz, pyrrole-H3), 7.66 (dd, 1H, $^3J = 8.8$ Hz, $^4J = 2.0$ Hz, Ar-H5), 7.72 (d, 1H, $^3J = 8.8$ Hz, Ar-H4), 8.36 (d, 1H, $^4J = 2.0$ Hz, Ar-H7), 10.00 (s, 1H, NHCO), 12.38 (s, 1H, NHCOCH_2), 12.93 (d, 1H, $^4J = 2.7$ Hz, pyrrole-NH) ppm; ^{13}C NMR (100 MHz, $\text{DMSO-}d_6$) δ 27.94, 29.92, 51.47, 98.14, 105.88, 112.40, 113.75, 119.38, 120.41, 127.87, 131.81, 134.66, 144.72, 156.92, 157.23, 170.92, 172.56 ppm; HRMS (ESI $^-$) m/z for $\text{C}_{17}\text{H}_{13}\text{Br}_2\text{N}_4\text{O}_4\text{S}$ ($[\text{M-H}]^-$): calcd. 526.9024, found 526.9035; HPLC: $t_r = 20.39$ min (97.1 % at 220 nm).

Ethyl 2-((6-(4,5-dichloro-1H-pyrrole-2-carboxamido)benzo[d]thiazol-2-yl)amino)-2-oxoacetate (**11**). Prepared according to general procedure C from compound **5** (0.250 g, 0.942 mmol); yellow crystals, yield 0.230 g (57 %); mp 272-275 °C; IR (ATR) ν 3410, 3173, 2986, 1752, 1705, 1654, 1608, 1579, 1545, 1521, 1465, 1412, 1339, 1285, 1231, 1188, 1146, 1015, 961, 868, 812, 747 cm^{-1} ; ^1H NMR (400 MHz, $\text{DMSO-}d_6$): δ 1.33 (t, 3H, $J = 7.1$ Hz, CH_2CH_3), 4.33 (q, 2H, $J = 7.1$ Hz, CH_2CH_3), 7.23 (s, 1H, pyrrole-H3), 7.72 (dd, 1H, $^3J = 8.87$ Hz, $^4J = 2.0$ Hz, Ar-H5), 7.79 (d, 1H, $J = 8.8$ Hz, Ar-H4), 8.44 (d, 1H, $J = 2.0$ Hz, Ar-H7), 10.08 (s, 1H, NHCO), 13.01 (s br, 1H, NHCOCH_2), 13.18 (s br, 1H, pyrrole-NH) ppm; ^{13}C NMR (100 MHz, $\text{DMSO-}d_6$) δ 13.75, 62.68, 108.28, 110.93, 112.58, 116.11, 119.83, 120.72, 124.52, 131.96, 135.28, 143.59, 157.42, 159.05 ppm (2 carbonyl signals not seen due to broadening). HRMS (ESI $^-$) m/z for $\text{C}_{16}\text{H}_{11}\text{Cl}_2\text{N}_4\text{O}_4\text{S}$ ($[\text{M-H}]^-$): calcd 424.9878, found 424.9872; HPLC: $t_r = 20.73$ min (96.2 % at 220 nm).

Methyl 3-((6-(4,5-dichloro-1H-pyrrole-2-carboxamido)benzo[d]thiazol-2-yl)amino)-3-oxopropanoate (**12**). Prepared according to general procedure C from compound **6** (0.220 g, 0.829 mmol); yellow crystals, yield 0.195 g (55 %); mp. 332-335 °C; IR (ATR) ν 3285, 3184, 3041, 2988, 1725, 1691, 1637, 1614, 1575, 1548, 1526, 1461, 1413, 1381, 1254, 1234, 1210, 1177, 1149, 1082, 1019, 978, 869, 817, 761, 724, 674 cm^{-1} ; ^1H NMR (400 MHz, $\text{DMSO-}d_6$): δ 3.68 (s, 5H, CH_2 , OCH_3), 7.22 (d, 1H, $^3J = 2.8$ Hz, pyrrole-H3), 7.68 (dd, 1H, $^3J = 8.8$ Hz, $^4J = 2.0$ Hz, Ar-H5), 7.74 (d, 1H, $^3J = 8.8$ Hz, Ar-H4), 8.38 (d, 1H, $J = 2.0$ Hz, Ar-H7), 10.04 (s, 1H, NHCO), 12.54 (s br, 1H, NHCOCH_2), 12.98 (d, 1H, $^4J = 2.8$ Hz, pyrrole-NH) ppm; ^{13}C NMR (100 MHz, $\text{DMSO-}d_6$) δ 42.17, 52.20, 108.25, 110.86, 112.53, 116.03, 119.56, 120.62, 124.55, 131.81, 134.83, 144.64, 156.66, 157.37, 165.07, 167.38 ppm. HRMS (ESI $^-$) m/z for

$\text{C}_{16}\text{H}_{11}\text{Cl}_2\text{N}_4\text{O}_4\text{S}$ ($[\text{M}-\text{H}]^-$): calcd. 424.9878, found 424.9880; HPLC: $t_r = 19.58$ min (96.4 % at 220 nm).

General procedure D. Synthesis of compounds 13, 14 and 24-29. Hydrolysis of esters **8**, **10** and **18-23** (1.00 mmol) was performed with 1M NaOH (2.00 mL, 2.00 mmol) in methanol (10 mL) at room temperature overnight. After evaporation of the methanol, the reaction mixture was acidified with 1M HCl to pH 3 and the precipitated carboxylic acids were collected by filtration.

2-((6-(4,5-Dibromo-1H-pyrrole-2-carboxamido)benzo[d]thiazol-2-yl)amino)-2-oxoacetic acid (13). Prepared according to general procedure D from compound **8** (0.105 g, 0.203 mmol); brownish crystals, yield 0.051 g (51 %); mp 238-241 °C; IR (ATR) ν 3117, 1703, 1656, 1613, 1547, 1518, 1478, 1411, 1388, 1293, 1225, 1161, 927, 907, 864, 813, 739, 689 cm^{-1} ; ^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ 7.27 (d, 1H, $J = 2.8$ Hz, pyrrole-H3), 7.71 (dd, 1H, $^3J = 8.7$ Hz, $^4J = 2.0$ Hz, Ar-H5), 7.78 (d, 1H, $^3J = 8.7$ Hz, Ar-H4), 8.44 (d, 1H, $^4J = 2.0$ Hz, Ar-H7), 10.06 (s, 1H, NHCO), 12.94 (s br, 1H, NHCOCO), 12.95 (d, 1H, $^4J = 2.8$ Hz, pyrrole-NH) ppm, signal for COOH not observed; ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$) δ 98.17, 105.97, 112.40, 113.89, 119.68, 120.85, 127.81, 132.09, 135.27, 144.41, 156.28, 157.27, 158.40, 160.69 ppm; HRMS (ESI) m/z for $\text{C}_{14}\text{H}_7\text{Br}_2\text{N}_4\text{O}_4\text{S}$ ($[\text{M}-\text{H}]^-$): calcd 484.8555, found 484.8550; HPLC: $t_r = 17.02$ min (97.4 % at 220 nm).

4-((6-(4,5-Dibromo-1H-pyrrole-2-carboxamido)benzo[d]thiazol-2-yl)amino)-4-oxobutanoic acid (14). Prepared according to general procedure D from compound **10** (0.110 g, 0.207 mmol); brownish crystals, yield 0.039 g (36 %); mp. 285-288 °C; IR (ATR) ν 3203, 2971, 1660, 1615, 1574, 1547, 1515, 1472, 1405, 1367, 1333, 1311, 1284, 1254, 1215, 1159, 1066, 1010, 973, 917, 867, 844, 805, 743, 715 cm^{-1} ; ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 2.59 (m, 2H, CH_2CO), 2.73 (m,

2H, CH₂CO), 7.25 (d, 1H, $J = 2.7$ Hz, pyrrole-H3), 7.66 (dd, 1H, $^3J = 8.8$ Hz, $^4J = 2.0$ Hz, Ar-H5), 7.71 (d, 1H, $^3J = 8.8$ Hz, Ar-H4), 8.36 (d, 1H, $^4J = 2.0$ Hz, Ar-H7), 10.00 (s, 1H, NHCO), 12.35 (s br, 2H, NHCOCH₂. COOH), 12.93 (d, 1H, $^4J = 2.8$ Hz, pyrrole-NH) ppm; ¹³C NMR (100 MHz, DMSO-*d*₆) δ 28.25, 30.06, 98.14, 105.87, 112.40, 113.75, 119.38, 120.38, 127.87, 131.79, 134.63, 144.73, 156.97, 157.22, 171.18, 173.53 ppm; HRMS (ESI) m/z for C₁₆H₁₁Br₂N₄O₄S ([M-H]⁻): calcd. 512.8868, found 512.8881; HPLC: $t_r = 17.00$ min (95.2 % at 220 nm).

Benzo[d]thiazole-2,6-diamine (15). 10% Pd/C (0.195 g) was added to a solution of 6-nitrobenzo[d]thiazol-2-amine (**1**) (1.95 g, 10.0 mmol) in ethanol (100 mL) and hydrogenated in Parr hydrogenator at 2.1 bar pressure at room temperature for 6 h. The reaction mixture was filtered through celite and cotton and the filtrate was evaporated under reduced pressure to give benzo[d]thiazole-2,6-diamine (**15**) (1.59 g, 96 % yield). ¹H NMR (400 MHz, DMSO-*d*₆) δ 4.79 (s br, 2H, 6-NH₂), 6.45 (dd, 1H, $^3J = 8.4$ Hz, $^4J = 2.2$ Hz, Ar-H5), 6.81 (d, 1H, $^4J = 2.2$ Hz, Ar-H7), 6.93 (s br, 2H, 2-NH₂), 7.03 (d, 1H, $J = 8.4$ Hz, Ar-H4) ppm.

General procedure E. Synthesis of compounds 16 and 17. Benzo[d]thiazole-2,6-diamine (**15**) (0.714 g, 4.32 mmol) and Na₂CO₃ (0.458 g, 4.32 mmol) were dissolved in *N,N*-dimethylformamide (30 mL) and the mixture was heated to 80 °C. 2,2,2-Trichloro-1-(4,5-dibromo-1*H*-pyrrol-2-yl)ethan-1-one (1.60 g, 4.32 mmol) or 2,2,2-trichloro-1-(4,5-dichloro-1*H*-pyrrol-2-yl)ethan-1-one (1.22 g, 4.32 mmol) was added and the mixture stirred for 5 h at 80 °C. Solvent was removed under reduced pressure and the residue was recrystallized from EtOAc to obtain compound **16** or **17**.

N-(6-Aminobenzo[d]thiazol-2-yl)-4,5-dibromo-1*H*-pyrrole-2-carboxamide (**16**). Prepared according to general procedure E from **15** and 2,2,2-trichloro-1-(4,5-dibromo-1*H*-pyrrol-2-yl)ethan-1-one; yellow crystals, yield 1.35 g (75 %); mp 269-271 °C; IR (ATR) ν 2971, 2362, 1687, 1649, 1611, 1556, 1470, 1405, 1381, 1318, 1298, 1276, 1244, 1174, 1137, 1057, 991, 974, 938, 914, 865, 823 cm⁻¹; ¹H NMR (400 MHz, DMSO-*d*₆): δ 5.23 (s, 2H, 6-NH₂), 6.73 (dd, 1H, ³*J* = 8.6, ⁴*J* = 2.0 Hz, Ar-H5), 7.03 (d, 1H, ⁴*J* = 2.0 Hz, Ar-H7), 7.43 (d, 1H, ³*J* = 8.6 Hz, Ar-H4), 7.45 (s, 1H, pyrrole-H3), 12.18 (s br, 1H, NH), 13.12 (s br, 1H, NH) ppm; ¹³C NMR (100 MHz, DMSO-*d*₆): δ 98.83, 103.98, 107.94, 114.44, 115.49, 120.59, 126.03, 133.01, 139.44, 145.82, 153.35, 156.82 ppm, HRMS (ESI⁻) *m/z* for C₁₂H₇Br₂N₄OS ([M-H]⁻): calcd 412.8707, found 412.8708; HPLC: *t*_r = 17.34 min (100 % at 254 nm).

N-(6-Aminobenzo[d]thiazol-2-yl)-4,5-dichloro-1*H*-pyrrole-2-carboxamide (**17**). Prepared according to general procedure E from **15** and 2,2,2-trichloro-1-(4,5-dichloro-1*H*-pyrrol-2-yl)ethan-1-one; yellow crystals, yield 1.10 g (78 %); mp 275-278 °C; IR (ATR) ν 3126, 2988, 2362, 1659, 1614, 1553, 1473, 1419, 1392, 1327, 1295, 1278, 1182, 1132, 1057, 1020, 992, 937, 862, 824, 724 cm⁻¹; ¹H NMR (400 MHz, DMSO-*d*₆) δ 5.22 (s, 2H, 6-NH₂), 6.73 (dd, 1H, ³*J* = 8.6, ⁴*J* = 2.0 Hz, Ar-H5), 7.03 (d, 1H, ⁴*J* = 2.0 Hz, Ar-H7), 7.42 (s, 1H, pyrrole-H3), 7.43 (d, 1H, ³*J* = 8.6 Hz, Ar-H4), 12.19 (s br, 1H, NH), 13.22 (s br, 1H, NH) ppm; ¹³C NMR (100 MHz, DMSO-*d*₆) δ 104.00, 108.90, 112.58, 114.46, 117.76, 120.58, 122.72, 132.99, 139.41, 145.80, 153.37, 156.99 ppm; HRMS (ESI⁻) *m/z* for C₁₂H₇Cl₂N₄OS ([M-H]⁻): calcd 324.9718, found 324.9709; HPLC: *t*_r = 17.09 min (97.3 % at 254 nm).

General procedure F. Synthesis of compounds 18-23. To a solution of compound **16** (0.220 g, 0.529 mmol) (for **18**, **19**, **20**) or compound **17** (0.173 g, 0.529 mmol) (for **21**, **22**, **23**) and triethylamine (0.10 mL, 0.79 mmol) in 1,4-dioxane (20 mL) cooled to 0 °C, ethyl oxalyl chloride

(0.089 mL, 0.79 mmol), methyl malonyl chloride (0.084 mL, 0.79 mmol) or methyl succinyl chloride (0.097 mL, 0.79 mmol) were added dropwise and the reaction mixture was stirred for additional 3 h at room temperature. Saturated NaHCO₃ solution was added to quench the reaction. The mixture was concentrated, dissolved in ethyl acetate (25 mL) and the solution was washed with saturated NaHCO₃ solution (3 × 10 mL), 10% citric acid (3 × 10 mL), water (3 × 10 mL) and brine (3 × 10 mL). If the product precipitated between the ethyl acetate and water phase it was collected by filtration. The ethyl acetate phase was dried over sodium sulfate and concentrated in vacuo. The crude products were recrystallized from ethyl acetate.

Ethyl 2-((2-(4,5-dibromo-1H-pyrrole-2-carboxamido)benzo[d]thiazol-6-yl)amino)-2-oxoacetate (**18**). Prepared according to general procedure F from compound **16** (0.220 g, 0.529 mmol) and ethyl oxalyl chloride (0.089 mL, 0.79 mmol); yellow crystals, yield 0.230 g (84 %); mp 273-276 °C; IR (ATR) ν 3333, 3297, 3125, 2362, 1711. 1655, 1610, 1560, 1520, 1470, 1437, 1409, 1382, 1324, 1303, 1277, 1228, 1179, 1112, 1059, 1014, 998, 979, 880, 861, 836, 815, 805 cm⁻¹; ¹H NMR (400 MHz, DMSO-*d*₆ + TFA) δ 1.33 (t, 3H, *J* = 7.1 Hz, CH₂CH₃), 4.32 (q, 2H, *J* = 7.1 Hz, CH₂CH₃), 7.51 (s, 1H, pyrrole-H3) 7.72 (d, 1H, ³*J* = 8.8 Hz, Ar-H4), 7.77 (dd, 1H, ³*J* = 8.8 Hz, ⁴*J* = 2.0 Hz, Ar-H5), 8.41 (d, 1H, ⁴*J* = 2.0 Hz, Ar-H7), 10.97 (s, 1H, NHCOCO), 12.62 (s br, 1H, NH), 13.27 (s br, 1H, *J* = Hz, NH) ppm; ¹³C NMR (100 MHz, DMSO-*d*₆): δ 13.83, 62.42, 99.04, 108.55, 113.09, 116.10, 119.83, 120.33, 125.61, 131.90, 133.35., 145.64, 155.46, 157.03, 157.95, 160.66 ppm; HRMS (ESI⁻) *m/z* for C₁₆H₁₁Br₂N₄O₄S ([M-H]⁻): calcd 512.8868, found 512.8864; HPLC: *t*_r = 21.97 min (98.7 % at 254 nm).

Methyl 3-((2-(4,5-dibromo-1H-pyrrole-2-carboxamido)benzo[d]thiazol-6-yl)amino)-3-oxopropanoate (**19**). Prepared according to general procedure F from compound **16** (0.434 g, 1.04 mmol) and methyl malonyl chloride (0.18 mL, 1.6 mmol); yellow crystals, yield 0.330 g

(61 %); mp. 261-263 °C; IR (ATR) ν 3309, 2988, 2362, 1708, 1657, 1614, 1579, 1538, 1472, 1440, 1384, 1359, 1318, 1297, 1278, 1262, 1214, 1172, 1103, 1057, 996, 977, 940, 906, cm^{-1} ; ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 3.52 (s, 2H, COCH_2CO), 3.67 (s, 3H, OCH_3), 7.50 (s br, 1H, pyrrole-H3), 7.53 (dd, 1H, $^3J = 8.7$ Hz, $^4J = 1.8$ Hz, Ar-H5), 7.70 (d, 1H, $^3J = 8.7$ Hz, Ar-H4), 8.31 (d, 1H, $^4J = 1.8$ Hz, Ar-H7), 10.43 (s, 1H, NHCOCH_2), 12.57 (s br, 1H, NH), 13.24 (s br, 1H, NH) ppm; ^{13}C NMR (100 MHz, $\text{DMSO-}d_6$): δ 43.42, 51.97, 99.00, 108.50, 111.48, 115.97, 118.51, 120.38, 125.67, 132.11, 134.81, 144.81, 157.01, 157.44, 163.91, 168.13 ppm; HRMS (ESI $^-$) m/z for $\text{C}_{16}\text{H}_{11}\text{Br}_2\text{N}_4\text{O}_4\text{S}$ ($[\text{M-H}]^-$): calcd 512.8868, found 512.8854; HPLC: $t_r = 20.59$ min (99.4 % at 254 nm).

Methyl 4-((2-(4,5-dibromo-1H-pyrrole-2-carboxamido)benzo[d]thiazol-6-yl)amino)-4-oxobutanoate (20). Prepared according to general procedure F from compound **16** (0.125 g, 0.300 mmol) and succinyl chloride (0.050 mL, 0.45 mmol). Yellow crystals, yield: 0.055 g (34 %); mp 139-141 °C; IR (ATR) ν 3364, 1718, 1660, 1612, 1578, 1533, 1470, 1436, 1408, 1382, 1316, 1276, 1205, 1170 cm^{-1} ; ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 28.46, 30.79, 51.36, 99.00, 108.46, 111.14, 115.93, 118.39, 120.36, 125.69, 132.04, 135.32, 144.43, 157.09, 157.44, 169.74, 172.86 ppm 2.59-2.68 (m, 4H, $\text{COCH}_2\text{CH}_2\text{CO}$), 3.61 (s, 3H, OCH_3), 7.51-7.54 (m, 2H, pyrrole-H3, Ar-H5), 7.69 (d, 1H, $^3J = 8.1$ Hz, Ar-H4), 8.32 (d, 1H, $^4J = 1.8$ Hz, Ar-H7), 10.19 (s, 1H, NHCOCH_2), 12.56 (s, 1H, NH), 13.23 (s, 1H, NH) ppm; ^{13}C NMR (100 MHz, $\text{DMSO-}d_6$) δ 43.42, 51.97, 99.00, 108.50, 111.48, 115.97, 118.51, 120.38, 125.67, 132.11, 134.81, 144.81, 157.01, 157.44, 163.91, 168.13 ppm; HRMS (ESI $^+$) m/z for $\text{C}_{17}\text{H}_{15}\text{Br}_2\text{N}_4\text{O}_4\text{S}$ ($[\text{M+H}]^+$): calcd 528.9181, found 528.9190; HPLC: $t_r = 20.70$ min (100% at 254 nm).

Ethyl 2-((2-(4,5-dichloro-1H-pyrrole-2-carboxamido)benzo[d]thiazol-6-yl)amino)-2-oxoacetate (21). Prepared according to general procedure F from compound **17** (0.150 g, 0.458 mmol) and ethyl oxalyl chloride (0.077 mL, 0.69 mmol); yellow crystals, yield 0.155 g (79 %);

mp 274-277 °C; IR (ATR) ν 3313, 2988, 2362, 1724, 1703, 1652, 1609, 1568, 1528, 1478, 1428, 1399, 1369, 1323, 1306, 1279, 1230, 1191, 1115, 1064, 1016, 956, 882, 852 cm^{-1} ; ^1H NMR (400 MHz, $\text{DMSO-}d_6$ + TFA) δ 1.33 (t, 3H, $J = 7.1$ Hz, CH_2CH_3), 4.32 (q, 2H, $J = 7.1$ Hz, CH_2CH_3), 7.49 (s, 1H, pyrrole-H3) 7.73 (d, 1H, $^3J = 8.7$ Hz, Ar-H4), 7.78 (dd, 1H, $^3J = 8.7$ Hz, $^4J = 2.0$ Hz, Ar-H5), 8.41 (d, 1H, $^4J = 2.0$ Hz, Ar-H7), 10.97 (s, 1H, COCONH), 12.66 (s br, 1H, NH), 13.35 (s br, 1H, NH) ppm; ^{13}C NMR (100 MHz, $\text{DMSO-}d_6$): δ 13.82, 62.43, 109.10, 113.12, 113.19, 118.34, 119.85, 120.39, 122.32, 131.91, 133.35, 145.64, 155.48, 157.25, 158.01, 160.64 ppm; HRMS (ESI $^-$) m/z for $\text{C}_{16}\text{H}_{11}\text{Cl}_2\text{N}_4\text{O}_4\text{S}$ ($[\text{M-H}]^-$): calcd 424.9878, found 424.9889; HPLC: t_r = 21.59 min (96.3 % at 254 nm).

Methyl 3-((2-(4,5-dichloro-1H-pyrrole-2-carboxamido)benzo[d]thiazol-6-yl)amino)-3-oxopropanoate (**22**). Prepared according to general procedure F from compound **17** (0.150 g, 0.458 mmol) and methyl malonyl chloride (0.074 mL, 0.69 mmol); yellow crystals, yield 0.120 g (61 %); mp. 281-284 °C; IR (ATR) ν 3343, 2988, 2361, 1705, 1662, 1617, 1582, 1565, 1543, 1474, 1434, 1411, 1393, 1360, 1326, 1281, 1264, 1224, 1179, 1108, 1057, 1021, 1002, 946, 879 cm^{-1} ; ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 3.56 (s, 2H, COCH_2CO), 3.66 (s, 3H, OCH_3), 7.46 (s br, 1H, pyrrole-H3), 7.58 (dd, 1H, $^3J = 8.7$ Hz, $^4J = 1.9$ Hz, Ar-H5), 7.70 (d, 1H, $^3J = 8.7$ Hz, Ar-H4), 8.33 (d, 1H, $^4J = 1.9$ Hz, Ar-H7), 10.68 (s, 1H, NHCOCH_2), 12.64 (s br, 1H, NH), 13.35 (s br, 1H, NH) ppm; ^{13}C NMR (100 MHz, $\text{DMSO-}d_6$): δ 43.33, 51.93, 108.99, 111.36, 113.14, 118.28, 118.49, 120.27, 122.51, 131.99, 134.99, 144.69, 157.31, 157.42, 163.99, 168.21 ppm; HRMS (ESI $^-$) m/z for $\text{C}_{16}\text{H}_{11}\text{Cl}_2\text{N}_4\text{O}_4\text{S}$ ($[\text{M-H}]^-$): calcd 424.9878, found 424.9890; HPLC: t_r = 20.40 min (96.1 % at 254 nm).

Methyl 4-((2-(4,5-dichloro-1H-pyrrole-2-carboxamido)benzo[d]thiazol-6-yl)amino)-4-oxobutanoate (**23**). Prepared according to general procedure F from compound **17** (0.150 g,

0.458 mmol) and methyl succinyl chloride (0.084 mL, 0.69 mmol); yellow crystals, yield 0.110 g (54 %); mp 293-296 °C; IR (ATR) ν 3363, 3118, 1720, 1655, 1611, 1545, 1472, 1419, 1393, 1328, 1295, 1277, 1234, 1209, 1186, 1141, 1038, 1019, 990, 873, 848, 816, 796, 741 cm^{-1} ; ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 2.64 (m, 4H, $\text{CH}_2\text{CH}_2\text{CO}$), 3.61 (s, 3H, OCH_3), 7.48 (s br, 1H, pyrrole-H3), 7.53 (dd, 1H, $^3J = 8.8$ Hz, $^4J = 2.0$ Hz, Ar-H5), 7.68 (d, 1H, $J = 8.8$ Hz, Ar-H4), 8.32 (d, 1H, $^4J = 2.0$ Hz, Ar-H7), 10.20 (s, 1H, CONHCH_2), 12.57 (s br, 1H, NH), 13.30 (s br, 1H, NH) ppm; ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$): δ 28.46, 30.80, 51.34, 109.01, 111.07, 113.14, 118.14, 118.39, 120.19, 122.51, 131.92, 135.44, 144.14, 157.11, 157.34, 169.78, 172.85 ppm; HRMS (ESI^-) m/z for $\text{C}_{17}\text{H}_{13}\text{Cl}_2\text{N}_4\text{O}_4\text{S}$ ($[\text{M}-\text{H}]^-$): calcd 439.0035, found 439.0040; HPLC: $t_r = 20.85$ (97.8 % at 254 nm).

2-((2-(4,5-Dibromo-1H-pyrrole-2-carboxamido)benzo[d]thiazol-6-yl)amino)-2-oxoacetic acid (24). Prepared by alkaline hydrolysis of ethyl ester **18** (0.080 g, 0.15 mmol) according to general procedure D; yield 0.040 g (53 %); mp 276-279 °C; IR (ATR) ν 3112, 2925, 1653, 1610, 1555, 1470, 1375, 1320, 1278, 1172, 977, 868, 809, 738, 547 cm^{-1} ; ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 7.51 (s br, 1H, pyrrole-H3), 7.73 (d, 1H, $^3J = 8.7$ Hz, Ar-H4), 7.79 (dd, 1H, $^3J = 8.7$ Hz, $^4J = 1.6$ Hz, Ar-H5), 8.43 (d, 1H, $^4J = 1.6$ Hz, Ar-H7), 10.89 (s, 1H, COCONH), 12.62 (s br, 1H, NH), 13.25 (s br, 1H, NH), 14.15 (s br, 1H, COOH) ppm; ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$) δ 99.02, 108.57, 112.81, 114.96, 119.67, 120.25, 125.69, 131.85, 133.65, 145.38, 156.79, 157.16, 157.95, 162.13 ppm; HRMS (ESI^-) m/z for $\text{C}_{14}\text{H}_7\text{Br}_2\text{N}_4\text{O}_4\text{S}$ ($[\text{M}-\text{H}]^-$): calcd 484.8555, found 484.8543; HPLC: $t_r = 20.19$ min (95.9 % at 254 nm).

3-((2-(4,5-Dibromo-1H-pyrrole-2-carboxamido)benzo[d]thiazol-6-yl)amino)-3-oxopropanoic acid (25). Prepared by alkaline hydrolysis of methyl ester **19** (0.090 g, 0.17 mmol) according to general procedure D; brownish crystals, yield 0.035 g (40 %); mp 252-255 °C; IR (ATR) ν 3106,

2958, 2362, 1683, 1615, 1587, 1558, 1470, 1372, 1316, 1241, 1166, 1143, 992, 977, 940, 865, 806, 702 cm^{-1} ; ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 3.42 (s, 2H, COCH_2CO), 7.50 (d, 1H, $J = 2.3$ Hz, pyrrole-H3), 7.53 (dd, 1H, $^3J = 8.7$ Hz, $^4J = 1.6$ Hz, Ar-H5), 7.70 (d, 1H, $J = 8.7$ Hz, Ar-H4), 8.32 (d, 1H, $^4J = 1.6$ Hz, Ar-H7), 10.33 (s, 1H, NHCOCH_2), 12.60 (s br, 2H, NH, COOH), 13.23 (s br, 1H, NH) ppm; ^{13}C NMR (100 MHz, $\text{DMSO-}d_6$): δ 43.92, 99.00, 108.46, 111.38, 115.96, 118.48, 120.28, 125.73, 132.04, 134.98, 144.54, 157.12, 157.45, 164.49, 169.28 ppm; HRMS (ESI $^-$) m/z for $\text{C}_{15}\text{H}_9\text{Br}_2\text{N}_4\text{O}_4\text{S}$ ($[\text{M-H}]^-$): calcd 498.8711, found 498.8714; HPLC: $t_r = 19.74$ min (95.9 % at 254 nm).

4-((2-(4,5-dibromo-1H-pyrrole-2-carboxamido)benzo[d]thiazol-6-yl)amino)-4-oxobutanoic acid (26). Prepared by alkaline hydrolysis of methyl ester **20** (0.025 g, 0.047 mmol) according to general procedure D; whitish crystals, yield 0.019 g (78 %); mp 315 °C (decomposition); IR (ATR) ν 3259, 3123, 2994, 2512, 1879, 1651, 1608, 1557, 1528, 1468, 1408, 1388, 1323, 1297, 1259, 1221, 1178, 1105, 1082 cm^{-1} ; ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 2.55-2.60 (m, 4H, $\text{COCH}_2\text{CH}_2\text{CO}$), 7.50 (s, 1H, pyrrole-H3), 7.53 (dd, 1H, $^3J = 8.7$ Hz, $^4J = 2.0$ Hz, Ar-H5), 7.69 (d, 1H, $J = 8.7$ Hz, Ar-H4), 8.32 (d, 1H, $^4J = 2.0$ Hz, Ar-H7), 10.16 (s, 1H, NHCOCH_2), 12.16 (s br, 1H, NH/COOH), 12.56 (s br, 1H, NH/COOH), 13.23 (s br, 1H, NH) ppm; ^{13}C NMR (100 MHz, $\text{DMSO-}d_6$) δ 28.78, 30.98, 98.95, 108.49, 111.08, 115.91, 118.36, 120.21, 125.79, 132.00, 135.40, 144.22, 157.14, 157.16, 170.01, 173.83 ppm; HRMS (ESI $^-$) m/z for $\text{C}_{16}\text{H}_{11}\text{Br}_2\text{N}_4\text{O}_4\text{S}$ ($[\text{M-H}]^-$): calcd 512.8868, found 512.8854; HPLC: $t_r = 19.96$ min (96.3 % at 254 nm).

2-((2-(4,5-Dichloro-1H-pyrrole-2-carboxamido)benzo[d]thiazol-6-yl)amino)-2-oxoacetic acid (27). Prepared by alkaline hydrolysis of ethyl ester **21** (0.085 g, 0.20 mmol) according to general procedure D; grey crystals, yield 0.045 g (57 %); mp. 320-322 °C; IR (ATR) ν 3369, 2971, 2900, 2361, 1691, 1609, 1560, 1521, 1458, 1420, 1383, 1334, 1310, 1262, 1184, 1138, 1109, 1056,

1026, 987, 869, 810, 728 cm^{-1} ; ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 7.48 (s, 1H, pyrrole-H3), 7.73 (d, 1H, $^3J = 8.8$ Hz, Ar-H4), 7.79 (dd, 1H, $^3J = 8.8$ Hz, $^4J = 1.8$ Hz, Ar-H5), 8.43 (d, 1H, $^4J = 1.8$ Hz, Ar-H7), 10.86 (s, 1H, NHCOCO), 12.64 (s br, 1H, NH), 13.32 (s br, 1H, NH) ppm, signal for COOH not observed; ^{13}C NMR (100 MHz, $\text{DMSO-}d_6$) δ 109.07, 112.74, 113.11, 118.32, 119.63, 120.20, 122.40, 131.83, 133.73, 145.22, 157.13, 157.35, 157.91, 162.16 ppm; HRMS (ESI $^-$) m/z for $\text{C}_{14}\text{H}_7\text{Cl}_2\text{N}_4\text{O}_4\text{S}$ ($[\text{M-H}]^-$): calcd 396.9565, found 396.9569; HPLC: $t_r = 20.05$ min (96.7 % at 254 nm).

3-((2-(4,5-Dichloro-1H-pyrrole-2-carboxamido)benzo[d]thiazol-6-yl)amino)-3-oxopropanoic acid (28). Prepared from compound **22** (0.100 g, 0.234 mmol) according to general procedure D; brownish crystals, yield 0.045 g (46 %); mp. 240-243 $^\circ\text{C}$. IR (ATR) ν 3110, 1679, 1613, 1545, 1468, 1417, 1392, 1373, 1322, 1256, 1173, 1143, 1021, 991, 939, 867, 805, 703 cm^{-1} ; ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 3.40 (s, 2H, COCH_2CO), 7.48 (d, 1H, $^4J = 2.3$ Hz, pyrrole-H3), 7.54 (dd, 1H, $^3J = 8.7$ Hz, $^4J = 1.7$ Hz, Ar-H5), 7.70 (d, 1H, $^3J = 8.7$ Hz, Ar-H4), 8.32 (d, 1H, $^4J = 1.7$ Hz, Ar-H7), 10.33 (s, 1H, NHCOCH_2), 12.60 (s br, 2H, NH, COOH), 13.31 (s br, 1H, NH) ppm; ^{13}C NMR (100 MHz, $\text{DMSO-}d_6$): δ 43.93, 109.05, 111.38, 113.05, 118.24, 118.48, 120.28, 122.45, 132.03, 135.01, 144.50, 157.33, 157.46, 164.48, 169.28 ppm; HRMS (ESI $^-$) m/z for $\text{C}_{15}\text{H}_9\text{Cl}_2\text{N}_4\text{O}_4\text{S}$ ($[\text{M-H}]^-$): calcd 410.9722, found 410.9712; HPLC: $t_r = 19.04$ min (97.5 % at 254 nm).

4-((2-(4,5-Dichloro-1H-pyrrole-2-carboxamido)benzo[d]thiazol-6-yl)amino)-4-oxobutanoic acid (29). Prepared from compound **23** (0.050 g, 0.11 mmol) according to general procedure D; grey crystals, yield 0.025 g (52 %); mp 298-301 $^\circ\text{C}$; IR (ATR) ν 3254, 2988, 2362, 1655, 1609, 1552, 1529, 1469, 1420, 1397, 1331, 1302, 1260, 1231, 1184, 1057, 1023, 981, 872, 841, 820, 742 cm^{-1} ; ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 2.60 (m, 4H, $\text{CH}_2\text{CH}_2\text{CO}$), 7.48 (s br, 1H, pyrrole-

H3), 7.54 (dd, 1H, $^3J = 8.7$ Hz, $^4J = 1.8$ Hz, Ar-H5), 7.69 (d, 1H, $J = 8.7$ Hz, Ar-H4), 8.32 (d, 1H, $^4J = 1.8$ Hz, Ar-H7), 10.18 (s, 1H, CONHCH₂), 11.80-12.80 (s br, 2H, COOH, NH), 13.30 (s br, 1H, NH) ppm; ^{13}C NMR (100 MHz, DMSO-*d*₆) δ 28.78, 30.97, 109.04, 111.09, 113.02, 118.19, 118.38, 120.21, 122.47, 131.97, 135.44, 144.12, 157.13, 157.15, 170.02, 173.82 ppm; HRMS (ESI⁺) m/z for C₁₆H₁₁Cl₂N₄O₄S ([M-H]⁺): calcd 424.9878, found 424.9873; HPLC: t_r = 19.32 min (97.5 % at 254 nm).

Evaluation of inhibitory activities on *E. coli* and *S. aureus* DNA gyrase. Commercially available assay (Inspiralis) for the determination of IC₅₀ values (Inspiralis) was used on streptavidin-coated black 96-well microtiter plates (Thermo Scientific Pierce). Wash buffer [137 mM NaCl, 20 mM Tris-HCl (pH 7.6), 0.01 % (w/v) BSA, 0.05 % (v/v) Tween 20] was used to rehydrate the plates and then biotinylated oligonucleotide was immobilized onto the plates. Wash buffer was used to remove the non-bound oligonucleotide. The assay was carried out with the final volume of 30 μL in buffer [35 mM Tris-HCl (pH 7.5), 24 mM KCl, 4 mM MgCl₂, 2 mM DTT, 1.8 mM spermidine, 1mM ATP, 6.5 % (w/v) glycerol, 0.0001 g/mL albumin] containing 1.5 U of gyrase from *E. coli* or *S. aureus*, 0.75 μg of relaxed pNO1 plasmid, and inhibitor (in 3 μL of 10 % DMSO and 0.008 % Tween 20 solution). After 30 min incubation at 37 °C the enzymatic reaction was terminated with the addition of the TF buffer [50 mM NaOAc (pH 5.0), 50 mM NaCl, and 50 mM MgCl₂], and left for another 30 min at room temperature to allow the formation of biotin-oligonucleotide-plasmid triplex and then TF buffer was used to wash off the unbound plasmid. 200 μL of SybrGOLD stain (diluted 1000 $\times$) in T10 buffer [10 mM Tris $\times$ HCl (pH 8.0) and 1 mM EDTA] was added, mixed and the fluorescence ($\lambda_{\text{ex}} = 485$ nm; $\lambda_{\text{em}} = 535$ nm) was measured with BioTek's Synergy H4 microplate reader. Inhibitor concentrations of 100 and 10 μM were used for initial screening. For selected compounds, IC₅₀ values were

determined with seven concentrations of the inhibitors. GraphPad Prism program was used for calculating IC₅₀ value which represents the concentration of inhibitor where the activity of the enzyme is reduced by 50%. IC₅₀ values were determined in three independent measurements, and their average value is given as a result. As a positive control novobiocin (Sigma-Aldrich) [IC₅₀ = 0.17 µM (lit. 0.08 µM)^{31, 32} for *E. coli* gyrase and IC₅₀ = 0.041 µM (lit. 0.01 µM)^{31, 32} for *S. aureus* gyrase] was used.

Evaluation of inhibitory activities on *E. coli* and *S. aureus* topoisomerase IV.

Commercially available assay (Inspiralis) for the determination of IC₅₀ values was used on streptavidin-coated black 96-well microtiter plates (Thermo Scientific Pierce). Wash buffer [137 mM NaCl, 20 mM Tris-HCl (pH 7.6), 0.01 % (w/v) BSA, 0.05 % (v/v) Tween 20] was used to rehydrate the plates and then biotinylated oligonucleotide was immobilized on the plates. Wash buffer was used to remove the non-bound oligonucleotide. The assay was carried out with the final volume of 30 µL, using buffer [40 mM HEPES (pH 7.6), 100 mM potassium glutamate, 10 mM magnesium acetate, 10 mM DTT, 1mM ATP, 0.00005 g/mL albumin] containing 1.5 U of topoisomerase IV from *E.coli* or *S. aureus*, 0.75 µg of supercoiled pNO1 plasmid and inhibitor (in 3µL of 10 % DMSO and 0.008 % Tween 20 solution). The mixture was incubated for 30 min at 37 °C, then the enzymatic reaction was terminated with the addition of the TF buffer [50 mM NaCl, 50 mM NaOAc (pH 5.0), and 50 mM MgCl₂], and left for another 30 min at room temperature to allow the formation of biotin-oligonucleotide-plasmid triplex, whereupon TF buffer was used to wash off the unbound plasmid. 200 µL of SybrGOLD stain (diluted 1000 ×) in T10 buffer [10 mM Tris × HCl (pH 8.0) and 1 mM EDTA] was added, mixed and the fluorescence ($\lambda_{\text{ex}} = 485 \text{ nm}$; $\lambda_{\text{em}} = 535 \text{ nm}$) was measured with a BioTek's Synergy H4 microplate reader. Inhibitor concentrations of 100 and 10 µM were used for initial screenin. For

selected compounds, IC₅₀ values were determined with seven concentrations of the inhibitors. GraphPad Prism program was used for calculating IC₅₀ value, which represents the concentration of inhibitor where the activity of the enzyme is reduced by 50%. The IC₅₀ values were determined in three independent measurements and their average value is given as a result. As a positive control novobiocin [IC₅₀ = 11 µM (lit. 10 µM)^{31,32} for *E. coli* topoisomerase IV and IC₅₀ = 27 µM (lit. 20 µM)^{31,32} for *S. aureus* topoisomerase IV] was used.

Determination of antibacterial activity. Antimicrobial assays were performed by the broth microdilution method following the Clinical Laboratory Standards Institute (CLSI; Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically, Approved Standards-Ninth Edition; M07-A9, Vol. 32, No. 2) guidelines. In the antibacterial assays the following CLSI recommended quality control strains for susceptibility testing were used: *Enterococcus faecalis* (Gram positive, ATCC 29212), *Staphylococcus aureus* (Gram positive, ATCC 25923), *Escherichia coli* (Gram negative, ATCC 25922) and *Pseudomonas aeruginosa* (Gram negative, ATCC 27853). A final compounds' concentration of 50 µM ($n = 3$) was used for determination of their antimicrobial activity against the ATCC strains.

Crystallography. Protein production, crystallization, preparation of the inhibitor-protein complexes, data collection and processing, structure modeling and refinement were performed by Proteros Biostructures GmbH, Martinsried, Germany.

Protein production. The expression of GyrB was performed according to previously established protocols using a suitable previously established construct that allowed production of homogenous protein in preparative amounts. The protein was purified using affinity and gel

filtration chromatography steps that yielded homogenous protein with a purity better than 95% as judged from Coomassie stained SDS-PAGE.

Crystallization. The purified protein was used in crystallization trials employing both a standard screen (ca. 1200 different conditions) as well as crystallization conditions identified in literature. Conditions initially obtained have been optimized using standard strategies, systematically varying critical parameters influencing crystallization, such as protein concentration, temperature, drop ratio, and others. These conditions were also refined by systematical variation of pH and precipitant concentrations.

Data Collection and Processing. A cryo-protocol was established using PROTEROS Standard Protocols. Crystals were flash-frozen and measured at the temperature of 100 K. The X-ray diffraction data were collected from crystals of complex of the ligand **24** with GyrB at the Swiss Light Source (Villigen, Switzerland) using cryogenic conditions. The crystals belonged to space group C2. The programs XDS and XSCALE were used for data processing. Tables 3 and 4 summarize the data collection and refinement statistics.

Table 3. Data collection and processing statistics for compound **24**.

X-ray source	PXI/X06SA (SLS ^a)
Wavelength [Å]	1.00000
Detector	PILATUS 6M
Temperature [K]	100
Space group	C 2

Cell: a; b; c; [Å]	116.29; 49.58; 70.21
α ; β ; γ ; [°]	90.0; 93.5; 90.0
Resolution [Å]	2.83 (3.08-2.83)
Unique reflections	9437 (2091)
Multiplicity	2.5 (2.4)
Completeness [%]	96.7 (96.5)
R _{sym} [%]	7.6 (44.6)
R _{meas} [%]	9.5 (56.9)
Mean(I)/sd ^b	11.17 (3.14)

^aSWISS LIGHT SOURCE (SLS, Villingen, Switzerland). ^bCalculated from independent reflections.

Table 4. Refinement statistics for compound **24**.^a

Resolution [Å]	70.08-2.83
Number of reflections (working/test)	8772 / 665
R _{cryst} [%]	25.8
R _{free} [%] ^b	28.9
Total number of atoms:	
Protein	2807
Water	6
Ligand	30
Iodide	1

Deviation from ideal geometry ^c

Bond lengths [Å]	0.007
Bond angles [°]	1.11
Bonded B's [Å ²] ^d	1.3

Ramachandran plot ^e

Most favored regions [%]	89.9
Additional allowed regions [%]	10.1
Generously allowed regions [%]	0.0
Disallowed regions [%]	0.0

^aValues were defined in REFMAC5, without sigma cutoff. ^bTest-set contains 6.8% of measured reflections. ^cRoot mean square deviations from geometric target values. ^dCalculation performed with MOLEMAN. ^eCalculation done with PROCHECK..

Molecular modeling.

Ligand and protein preparation. Three-dimensional models of the final compounds were built in ChemBio3D Ultra 13.0.³³ Their geometries were optimized using MMFF94³⁴ force field, partial atomic charges were added and energy minimized until the gradient value was below 0.001 kcal/(mol Å). Further refinement with GAMESS interface in ChemBio3D Ultra 13.0 using the semi-empirical PM3 method, QA optimization algorithm and Gasteiger-Hückel charges for all atoms for 100 steps was performed.³⁴ Molecular docking of the final molecules was performed using FlexX,^{35, 36} as available in LeadIT,³⁷ running on four octal core AMD Opteron CPU processors, 16 GB RAM, two 750 GB hard drives, under 64-bit Scientific Linux 6.0. Receptor Wizard in LeadIT was used for protein preparation. The binding site was defined as

amino acid residues within a radius of 7 Å around an X-ray crystal structure ligand (PDB entry: 4DUH).²² Correct tautomers and protonation states were assigned and hydrogen atoms were added to the binding site residues using the Receptor Wizard. The ligand and water molecules, except HOH614, were deleted from the X-ray structure of the complex.

Ligand docking. The FlexX hybrid algorithm (enthalpy and entropy driven ligand binding), as available in LeadIT,³⁷ 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. Docking binding modes 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.³⁸

Screening against PAINS. To evaluate a library of the synthesized compounds against PAINS,⁴⁰ all tested compounds were screened against the PAINS filter using Python script filter_pains.py, downloaded from GitHub (https://github.com/Team-SKI/snippets/blob/1d1d0424ba15da08cdc7e18c9a27d55b1a6cb797/Python/filter_pains.py). All compounds passed the PAINS filter.

ASSOCIATED CONTENT

Supporting Information

Description of structure modeling and refinement (PDF). The Supporting Information is available free of charge on the ACS Publications website at DOI:

AUTHOR INFORMATION

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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ABBREVIATIONS

DMF, *N,N*-dimethylformamide; TFA, trifluoroacetic acid; GyrA, DNA gyrase subunit A; GyrB, DNA gyrase subunit B; ParC, topoisomerase IV subunit C; ParE, topoisomerase IV subunit E; RA, residual activity; SAR, structure-activity relationship; SPR, surface plasmon resonance.

REFERENCES

- (1) Silver, S. L. Challenges of antibacterial discovery. *Clin. Microb. Rev.* **2011**, *24*, 79-109.
- (2) Davies, J.; Davies, D. Origins and evolution of antibiotic resistance. *Microb. Mol. Biol. Rev.* **2010**, *74*, 417-433.
- (3) Gellert, M.; Mizuuchi, K.; O'Dea M. H.; Nash, H.A. DNA gyrase: an enzyme that introduces superhelical turns into DNA. *Proc. Natl. Acad. Sci. U.S.A.* **1976**, *73*, 3872-3876.
- (4) Wigley, D. B.; Davies, G. J.; Dodson, E. J.; Maxwell, A.; Dodson, G. Crystal structure of an N-terminal fragment of the DNA-gyrase B protein. *Nature* **1991**, *351*, 624-629.
- (5) Kato, J.; Nishimura, Y.; Imamura, R.; Niki, H.; Hiraga, S.; Suzuki, J. New topoisomerase essential for chromosome segregation in *E. coli*. *Cell* **1990**, *63*, 393-404.
- (6) Kato, J.; Suzuki, H.; Ikeda, H. Purification and characterization of DNA topoisomerase IV in *Escherichia coli*. *J. Biol. Chem.*, **1992**, *267*, 25676-25684.
- (7) Collin, F.; Karkare, S.; Maxwell, A. Exploiting bacterial DNA gyrase as a drug target: current state and perspectives, *Appl. Microb. Biotechnol.* **2011**, *92*, 479-497.
- (8) Champoux, J.J. DNA topoisomerases: structure, function and mechanism. *Annu. Rev. Biochem.*, **2001**, *70*, 369-413.
- (9) Tomašič, T.; Mašič, L. P. Prospects for developing new antibacterials targeting bacterial type IIA topoisomerases. *Curr. Med. Chem.* **2014**, *14*, 130-151.
- (10) Naeem, A.; Badshah, S. L.; Muska, M.; Ahmad, N.; Khan, K. *Molecules* **2016**, *21*, 268; doi: 10.3390/molecules21040268. The current case of quinolones: synthetic approaches and antibacterial activity.

- (11) Cheng, G.; Hao, H.; Dai, M.; Liu, Z.; Yu, S.; Meng, X. Antibacterial action of quinolones: From target to network. *Eur. J. Med. Chem.* **2013**, *66*, 555-562.
- (12) Perez, J.; Lupala, C. S.; Gomez_Gutierrez, P. Designing type II topoisomerase inhibitors: a molecular modeling approach. *Curr. Top. Med. Chem.* **2014**, *14*, 40-50.
- (13) Mayer, C.; Janin, Y. L. Non-quinolone inhibitors of bacterial type IIA topoisomerases: A feat of bioisosterism. *Chem. Rev.* **2014**, *114*, 2313_2342.
- (14) Azam, A. M.; Thathan, J.; Jubie, S. Dual targeting DNA gyrase (GyrB) and topoisomerase IV (ParE) inhibitors: A review. *Bioorg. Chem.* **2015**, *62*, 41-63.
- (15) (a) Cui, S. F.; Addla, D.; Zhou, C. He. Novel 3-aminothiazolquinolones: design, synthesis, bioactive evaluation, SARs, and preliminary antibacterial mechanism. *J. Med. Chem.* **2016**, *59*, 4488-4510. (b) Zhang, D.; Addla, D.; Ponmani, J.; Wang, A.; Xie, D.; Wang, Y. N.; Zhang, S. L.; Geng, R. X.; C, G. X.; Li, S.; Zhou, C. H. Discovery of membrane active benzimidazole quinolones-based topoisomerase inhibitors as potential DNA-binding antimicrobial agents, *Eur. J. Med. Chem.* **2016**, *111*, 160–182.
- (16) Zidar, N.; Tomašič, T.; Macut, H.; Sirc, A.; Brvar, M.; Montalvão, S.; Tammela, P.; Ilaš, J.; Kikelj, D. New *N*-phenyl-4,5-dibromopyrrolamides as ATPase inhibitors of DNA gyrase. *Eur. J. Med. Chem.* **2016**, *117*, 197-211.
- (17) . O'Dowd, H.; Shannon, D. E.; Chandupatla, K. R.; Dixit, V.; Engrakul, J. J.; Ye, Z.; Jones, S. M.; O'Brien, C. F.; Nicolau, D. P.; Tessier, P. R.; Crandon, J. L.; Song, B.; Macikenas, D.; Hanzelka, B. L.; Le Tiran, A.; Bennani, Y. L.; Charifson, P. S.; Grillot, A. -L. Discovery and characterization of a water-soluble prodrug of a dual inhibitor of bacterial DNA gyrase and topoisomerase IV. *ACS Med. Chem. Lett.* **2015**, *6*, 822-826.

- (18) Bisacchi, G. S.; Manchester, J.I. A new-class antibacterial-almost. Lessons in drug discovery and development: A critical analysis of more than 50 years of effort toward ATPase inhibitors of DNA gyrase and topoisomerase IV. *ACS Infect. Dis.* **2014**, *1*, 4-41.
- (19) Lewis, R. J.; Singh, O. M.; Smith, C. V.; Skarzynski, T.; Maxwell, A.; Wonacott, A. J.; Wigley, D. B. The nature of inhibition of DNA gyrase by the coumarins and the cyclothialidines revealed by X-ray crystallography. *EMBO J.* **1996**, *15*, 1412-1420.
- (20) Tsai, F. T. F.; Singh, O. M. P.; Skarzynski, T.; Wonacott, A. J.; Weston, S.; Tucker, A.; Pauptit, R. A.; Breeze, A. L.; Poyser, J. P.; O'Brien, R.; Ladbury, J. E.; Wigley, D. B. The high-resolution crystal structure of a 24-kDa gyrase B fragment from *E. coli* complexed with one of the most potent coumarin inhibitors, clorobiocin. *Proteins: Struct., Funct., Bioinf.* **1997**, *28*, 41-52.
- (21) *Scaffold hopping in medicinal chemistry*; Brown, N., Ed.; Wiley-VCH Verlag GmbH & Co. KGaA: Weinheim, 2014.
- (22) *Heterocyclic chemistry in drug discovery*; (Li, J. J., Ed.), John Wiley and Sons: Hoboken, 2013.
- (23) Charifson, P. S.; Grillot, A. L.; Grossman, T. H.; Parsons, J. D.; Badia, M.; Bellon, S.; Deininger, D. D.; Drumm, J. E.; Gross, C. H.; LeTiran, A.; Liao, Y. S.; Mani, N.; Nicolau, D. P.; Perola, E.; Ronkin, S.; Shannon, D.; Swenson, L. L.; Tang, Q.; Tessier, P. R.; Tian, S. K.; Trudeau, M.; Wang, T. S.; Wei, Y. Y.; Zhang, H.; Stamos, D. Novel dual targeting benzimidazole urea inhibitors of DNA gyrase and topoisomerase IV possessing potent antibacterial activity: intelligent design and evolution through the judicious use of structure-guided design and structure-activity relationship. *J. Med. Chem.* **2008**, *51*, 5243-5263.

- (24) Brvar, M.; Perdih, A.; Renko, M.; Anderluh, G.; Turk, D.; Šolmajer, T. Structure-based discovery of substituted 4,5'-bithiazoles as novel DNA gyrase inhibitors, *J. Med. Chem.* **2012**, *55*, 6413-6426.
- (25) Ghorpade, S. R.; Kale, M. G.; McKinney, D. C.; Peer, M. S. H.; Raichurkar, Int. Pat. Appl. WO 2009147431 A1, **2009**, PCT/GB2009/050609, June 2009.
- (26) Tari, L. W.; Trzoss, M.; Bensen, D. C.; Li, X. M.; Chen, Z. Y.; Lam, T. Zhang, J. H.; Creighton, C. J.; Cunningham, M. L.; Kwan, B.; Stigham, M.; Shaw, K. J.; Lightstone, F. C.; Wong, S. E.; Ngueyen, T. B.; Nix, J.; Finn, J. Pyrrolopyrimidine inhibitors of DNA gyrase B (GyrB) and topoisomerase IV (ParE). Part I: structure guided discovery and optimization of dual targeting agents with potent, broad-spectrum enzymatic activity. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 1529-1536.
- (27) Starr, J. T.; Sciotti, R. J.; Hanna, D. L.; Huband, M. D.; Mullins, L. M.; Cai, H.; Gage, J. W.; Lockard, M.; Rauckhorst, M. R.; Owen, R. M.; Lall, M. S.; Tomilo, M.; Chen, H. F.; McCurdy, S. P.; Barbachyn, M. R. 5-(2-Pyrimidinyl)-imidazo[1,2-*a*]pyridines are antibacterial agents targeting the ATPase domains of DNA gyrase and topoisomerase IV. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 5302-5306.
- (28) Haydon, D. J.; Czaplewski, L. G.; Palmer, N. J.; Mitchell, D. R.; Atherall, J. F.; Steele, C. R.; Ladduwahetty, T., Int. Pat. Appl- WO2007146093 A1, December 2007. Application No. PCT/GB2007/002314, June 2007.
- (29) Axford, L. C.; Agarwal, P. K.; Anderson, K. H.; Andrau, L. N.; Atherall, J.; Barker, S.; Bennett, J. M.; Blair, M.; Collins, I.; Czaplewski, L. G.; Davies, D. T.; Gannon, C. T.; Kumar, D.; Lancett, P.; Logan, A.; Lunnis, C. J.; Mitchell, D. R.; Offermann, D. A.; Palmer, J. T.; Palmer, N.; Pitt, G. R. W.; Pommier, S.; Price, D.; Rao, B. N.; Saxena, R.; Shukla, T.; Singh, A. K.;

Singh, M.; Srivastava, A.; Steele, C.; Stokes, N. R.; Thomaides-Brears H.; Tyndall, E. M.; Watson, D.; Haydon, D. J. Design, synthesis and biological evaluation of α -substituted isonipecotic acid benzothiazole analogues as potent bacterial type II topoisomerase inhibitors. *Bioorg. Med. Chem. Lett.* **2013**, 23, 6598-6603.

(30) Tomašič, T.; Katsamakos, S.; Hodnik, Ž.; Ilaš, J.; Brvar, M.; Šolmajer, T.; Montalvao, S.; Tammela, P.; Banjanac, M.; Ergović, G.; Anderluh, M.; Mašič, L.P.; Kikelj, D., Discovery of 4,5,6,7-Tetrahydrobenzo[1,2-*d*]thiazoles as novel DNA gyrase inhibitors targeting the ATP-binding site. *J. Med. Chem.* **2015**, 58, 5501-5521.

(31) Zidar, N.; Macut, H.; Tomašič, T.; Brvar, M.; Montalvao, S.; Tammela, P.; Šolmajer, T.; Mašič, L.P.; Ilaš, J.; Kikelj, D., *N*-Phenyl-4,5-dibromopyrrolamides and *N*-Phenylindolamides as ATP competitive DNA gyrase B inhibitors: Design, synthesis and evaluation. *J. Med. Chem.* **2015**, 58, 6179-6194.

(32) Alt, S.; Mitchenall, L. A.; Maxwell, A.; Heide, L. Inhibition of DNA gyrase and DNA topoisomerase IV of *Staphylococcus aureus* and *Escherichia coli* by aminocoumarin antibiotics. *J. Antimicrob. Chemother.* **2011**, 66, 2061-2069.

(33) Kampranis, S. C.; Gormley, N. A.; Tranter, R.; Orphanides, G.; Maxwell, A. Probing the binding of coumarins and cyclothiadienes to DNA gyrase. *Biochemistry* **1999**, 38, 1967-1976.

(34) GAMESS interface, ChemBio3D Ultra 13.0, ChemBioOffice Ultra 13.0, CambridgeSoft.

(35) Halgren, T. A. Merck molecular force field .1. Basis, form, scope, parameterization, and performance of MMFF94. *J. Comput. Chem.* **1996**, 17, 490-519.

(36) Rarey, M.; Kramer, B.; Lengauer, T.; Klebe, G. A fast flexible docking method using an incremental construction algorithm. *J. Mol. Biol.* **1996**, 261, 470-489.

- (37) Rarey, M.; Wefing, S.; Lengauer, T. Placement of medium-sized molecular fragments into active sites of proteins. *J. Comput. Aid. Mol. Des.* **1996**, *10*, 41-54.
- (38) BioSolve IT (GmbH), LeadIT version 2.1.3.
- (39) PyMOL, Delano Scientific LLC, San Francisco, CA, <http://pymol.sourceforge.net> (May 15, 2016).
- (40) Baell, J. B.; Holloway, G. A. New substructure filters for removal of pan assay interference compounds (PAINS) from screening libraries and for their exclusion in bioassays. *J. Med. Chem.* **2010**, *53*, 2719-2740.

Table of Contents Graphic

E. coli DNA gyrase
 $IC_{50} = 58 \text{ nM}$