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-\textit{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 \textit{Escherichia coli} displaying improved inhibition of \textit{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 \textit{E. coli} and \textit{S. aureus}. The crystal structure of the 2-((2-(4,5-dibromo-1\textit{H}-pyrrole-2-carboxamido)benzothiazol-6-yl)amino)-2-oxoacetic acid (24) in complex with \textit{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.\textsuperscript{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 \textit{Escherichia coli}\textsuperscript{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,\textsuperscript{4} topoisomerase IV, a bacterial enzyme with a similar structure, function, and inhibitor susceptibility to DNA gyrase was identified.\textsuperscript{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. The GyrA and ParC subunits are involved in DNA transit, while the GyrB and ParE subunits contain an ATP-binding domain. 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. 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. 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.

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.\(^\text{18}\)

**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\(^\text{18,19,20}\) 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 approaches\(^2\) and exploiting opportunities offered to medicinal chemistry by aromatic heterocycles,\(^2\) benzimidazole ureas,\(^2\) bithiazoles,\(^2\) thiazolopyridines,\(^2\) pyrrolopyrimidines,\(^2\) imidazopyridines,\(^2\) benzothiazoles\(^2\) (Figure 2) and other structural types\(^3\) have been studied as dual inhibitors of GyrB and ParE subunits of bacterial gyrase and topoisomerase IV.\(^4,5\)

**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]thiazole\(^6\) and N-phenyl-4,5-dibromopyrrolamide\(^7\) 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-1H-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.\(^6,7\) 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 - \(\pi\) 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.\textsuperscript{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.\textsuperscript{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 \textit{S. aureus} DNA gyrase and \textit{E. coli} and \textit{S. aureus} topoisomerase IV because these three enzymes possess a smaller hydrophobic pocket compared to that of \textit{E. coli} DNA gyrase.\textsuperscript{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 \textit{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.

\textbf{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<sub>ligand 24</sub> in green, C<sub>ligand 13</sub> in orange, C<sub>GyrB</sub> 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-1H-pyrrole-2-carbonyl chloride or 4,5-dichloro-1H-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

![Scheme 1](image)

Reagents 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-1H-pyrrole-2-carboxylic acid (for 8, 9 and 10) or 4,5-dichloro-1H-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-1H-pyrrol-2-yl)ethan-1-one or 2,2,2-trichloro-1-(4,5-dichloro-1H-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**

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\[ \text{O}_2N\text{S} \quad a \quad \text{H}_2N\text{S} \quad b \quad H_2N\text{S} \quad c \quad H_2N\text{S} \]
\[ 1 \quad 15 \quad 16 \quad X=\text{Br} \quad 17 \quad X=\text{Cl} \]
```

18 X=Br, n=0, R=Et
19 X=Br, n=1, R=Me
20 X=Br, n=2, R=Me
21 X=Cl, n=0, R=Et
22 X=Cl, n=1, R=Me
23 X=Cl, n=2, R=Me
24 X=Br, n=0
25 X=Br, n=1
26 X=Br, n=2
27 X=Cl, n=0
28 X=Cl, n=1
29 X=Cl, n=2

*Reagents and conditions: (a) H₂ (2.1 bar), 10% Pd/C, EtOH, rt, 6 h; (b) 2,2,2-trichloro-1-(4,5-dibromo-1H-pyrrol-2-yl)ethan-1-one (for 16) or 2,2,2-trichloro-1-(4,5-dichloro-1H-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).

<table>
<thead>
<tr>
<th>compound</th>
<th>X</th>
<th>n</th>
<th>R</th>
<th>DNA gyrase IC₅₀ [µM]</th>
<th>topoisomerase IV IC₅₀ [µM]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td><em>E. coli</em></td>
<td><em>S. aureus</em></td>
</tr>
<tr>
<td>novobiocin</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.17</td>
<td>0.040</td>
</tr>
<tr>
<td>8</td>
<td>Br</td>
<td>0</td>
<td>Et</td>
<td>0.081 ± 0.05</td>
<td>&gt; 100</td>
</tr>
<tr>
<td>9</td>
<td>Br</td>
<td>1</td>
<td>Me</td>
<td>0.24 ± 0.02</td>
<td>&gt; 100</td>
</tr>
<tr>
<td>10</td>
<td>Br</td>
<td>2</td>
<td>Me</td>
<td>0.87 ± 0.21</td>
<td>&gt; 100</td>
</tr>
<tr>
<td>11</td>
<td>Cl</td>
<td>0</td>
<td>Et</td>
<td>1.8 ± 0.4</td>
<td>&gt; 100</td>
</tr>
<tr>
<td>12</td>
<td>Cl</td>
<td>1</td>
<td>Me</td>
<td>7.9 ± 2.1</td>
<td>&gt; 100</td>
</tr>
<tr>
<td>13</td>
<td>Br</td>
<td>0</td>
<td>H</td>
<td>0.038 ± 0.001</td>
<td>&gt; 100</td>
</tr>
<tr>
<td>14</td>
<td>Br</td>
<td>2</td>
<td>H</td>
<td>0.057 ± 0.018</td>
<td>&gt; 100</td>
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</tbody>
</table>

dichloro-1H-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 μM. The inhibition of *S. aureus* DNA gyrase was observed only for the dibromopyrrole carboxylic acid 25 (IC$_{50}$ = 5.6 μM) and dichloropyrrole carboxylic acids 27-29, with IC$_{50}$ values in the range of 0.51 to 94 μM. 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 μM) and 28 in the dichloro series (IC$_{50}$ = 8.5 μM) against *S. aureus* gyrase. The nanomolar activity of the dichloro oxalyl derivative 27 (IC$_{50}$ = 0.51 μM) in comparison to dibromo analogue 24 (IC$_{50}$ >100 μM), as well as to malonyl analogue 28 (IC$_{50}$ = 8.5 μM) and succinyl analogue 29 (IC$_{50}$ = 94 μM) 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).

<table>
<thead>
<tr>
<th>compound</th>
<th>X</th>
<th>n</th>
<th>R</th>
<th>DNA gyrase IC₅₀ [μM]</th>
<th>topoisomerase IV IC₅₀ [μM]</th>
</tr>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>E. coli</td>
<td>S. aureus</td>
</tr>
<tr>
<td>novobiocin</td>
<td></td>
<td></td>
<td>-</td>
<td>0.17</td>
<td>0.040</td>
</tr>
<tr>
<td>16</td>
<td>Br</td>
<td>-</td>
<td>-</td>
<td>2.0 ± 0.2</td>
<td>&gt; 100</td>
</tr>
<tr>
<td>17</td>
<td>Cl</td>
<td>-</td>
<td>-</td>
<td>&gt; 100</td>
<td>&gt; 100</td>
</tr>
<tr>
<td>18</td>
<td>Br</td>
<td>0</td>
<td>Et</td>
<td>29 ± 6</td>
<td>&gt; 100</td>
</tr>
<tr>
<td>19</td>
<td>Br</td>
<td>1</td>
<td>Me</td>
<td>2.0 ± 0.4</td>
<td>&gt; 100</td>
</tr>
<tr>
<td>20</td>
<td>Br</td>
<td>2</td>
<td>Me</td>
<td>7.0 ± 0.9</td>
<td>&gt; 100</td>
</tr>
<tr>
<td>21</td>
<td>Cl</td>
<td>0</td>
<td>Et</td>
<td>&gt; 100</td>
<td>&gt; 100</td>
</tr>
<tr>
<td>22</td>
<td>Cl</td>
<td>1</td>
<td>Me</td>
<td>0.22 ± 0.02</td>
<td>&gt; 100</td>
</tr>
<tr>
<td>23</td>
<td>Cl</td>
<td>2</td>
<td>Me</td>
<td>35 ± 12</td>
<td>&gt; 100</td>
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<tr>
<td>24</td>
<td>Br</td>
<td>0</td>
<td>H</td>
<td>0.058 ± 0.031</td>
<td>&gt; 100</td>
</tr>
<tr>
<td>25</td>
<td>Br</td>
<td>1</td>
<td>H</td>
<td>0.14 ± 0.06</td>
<td>5.6 ± 2.6</td>
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<tr>
<td>26</td>
<td>Br</td>
<td>2</td>
<td>H</td>
<td>0.033 ± 0.025</td>
<td>&gt; 100</td>
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<tr>
<td>27</td>
<td>Cl</td>
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<td>H</td>
<td>0.087 ± 0.001</td>
<td>0.51 ± 0.23</td>
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<tr>
<td>28</td>
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<td>H</td>
<td>0.12 ± 0.02</td>
<td>8.5 ± 1</td>
</tr>
<tr>
<td>29</td>
<td>Cl</td>
<td>2</td>
<td>H</td>
<td>0.43 ± 0.11</td>
<td>94 ± 25</td>
</tr>
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</table>

**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 IC$_{50}$ 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 (IC$_{50}$ (*E. coli*) = 11 µM; IC$_{50}$ (*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 ΔtolC 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 µg/mL) in ΔtolC, than in the wild type E. coli strain that contained efflux pump (MIC > 256 µg/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. 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. 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*\textsubscript{ligand} in *green* for model 1 and in *yellow* for model 2, *C*\textsubscript{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-\textit{d}]thiazole-based DNA gyrase B inhibitors\textsuperscript{29} 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-1H-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. $^1$H NMR and $^{13}$C spectra were recorded at 400 and 100 MHz, respectively, on a Bruker AVANCE III spectrometer in 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 × 150 mm or 4.6 mm × 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 °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 × 10 mL saturated NaHCO$_3$, 3 × 10 mL 10% citric acid, 3 × 10 mL water and 3 × 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, DMSO-d₆) δ 1.34 (t, 3H, J = 7.1 Hz, CH₃CH₂), 4.34 (q, 2H, J = 7.1 Hz, CH₃CH₂), 7.97 (d, 1H, J = 8.9 Hz, Ar-H4), 8.31 (dd, 1H, J = 8.9 Hz, Ar-H5), 9.12 (dd, 1H, J = 2.4 Hz), 13.64 (s br, 1H, CONH) ppm; ¹³C NMR (100 MHz, DMSO-d₆) 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 C₁₁H₈N₃O₅S ([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, DMSO-d₆) δ 3.68 (s, 3H, OCH₃), 3.82 (s, 2H, COCH₂CO), 7.93 (d, 1H, J = 8.9 Hz, Ar-H4), 8.30 (dd, 1H, J = 8.9 Hz, Ar-H5), 9.09 (d, 1H, J = 2.4 Hz, Ar-H7), 13.19 (s br, 1H, CONH) ppm; ¹³C NMR (100 MHz, DMSO-d₆) 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 C₁₁H₁₀N₃O₅S ([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, 1046, 997, 954, 908, 829 cm⁻¹; ¹H NMR (400 MHz, DMSO-d₆) δ  2.70 (m, 2H, COCH₂), 2.83 (m, 2H, COCH₂), 3.62 (s, 3H, OCH₃), 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; ¹³C NMR (100 MHz, DMSO-d₆) 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 C₁₂H₁₂N₃O₃S ([M+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, 1258, 1160, 1112, 1058, 1011, 964, 907, 865, 812, 802 cm⁻¹; ¹H NMR (400 MHz, DMSO-d₆) δ 1.31 (t, 3H, J = 7.1 Hz, CH₂CH₃), 4.31 (q, 2H, J = 7.1 Hz, CH₂CH₃), 5.34 (s br, 2H, NH₂), 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); ¹³C NMR (100 MHz, DMSO-d₆): 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 C₁₁H₁₂N₃O₃S ([M+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, DMSO-d₆) δ 3.62 (s, 2H, COCH₂CO), 3.67 (s, 3H, OCH₃), 5.24 (s br, 2H, NH₂), 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; ¹³C NMR (100 MHz, DMSO-d₆) δ 42.10, 52.14, 103.97, 114.49, 120.99, 132.92, 139.43, 145.91, 152.87, 164.41, 167.51 ppm; HRMS (ESI⁺) m/z for C₁₁H₁₂N₃O₃S ([M+H]⁺): calcd. 266.0599, found 266.0606.

**Methyl 4-(6-aminobenzo[d]thiazol-2-yl)amino)-4-oxo-4-butanenoate (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, 1456, 1472, 14737, 1487, 1311, 1271, 1232, 1151, 1081, 1060, 999, 983, 915, 898 cm⁻¹; ¹H NMR (400 MHz, DMSO-d₆) δ 2.64 (m, 2H, COCH₂), 2.72 (m, 2H, COCH₂), 3.61 (s, 3H, OCH₃), 5.16 (s br, 2H, NH₂), 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; ¹³C NMR (100 MHz, DMSO-d₆) 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 C₁₂H₁₄N₃O₃S ([M+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, 1252, 1187, 1163, 1107, 1042, 1014, 984, 974, 961, 869, 824, 810 cm⁻¹; ¹H NMR (400 MHz, DMSO-d₆) δ 1.33 (t, 3H, J = 7.1 Hz, CH₂C₃H₇), 4.33 (q, 2H, J = 7.1 Hz, CH₂CH₃), 7.26 (d, 1H, J = 2.7 Hz, pyrrole-H3), 7.72 (dd, 1H, J = 8.8 Hz, J = 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), 10.07 (s, 1H, NHCO), 12.96 (s br, 1H, NHCOCO), 13.17 (s br, 1H, pyrrole-NH) ppm; ¹³C NMR (100 MHz, DMSO-d₆) 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: tᵣ = 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, DMSO-d₆) δ 3.68 (s, 5H, OCH₃, COCH₂CO), 7.26 (d, 1H, ¹J = 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, NHCOCH₂), 12.94 (d, 1H, ³J = 2.7 Hz, pyrrole-NH) ppm; ¹³C NMR (100 MHz, DMSO-d₆): 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 C_{16}H_{11}Br₂N₄O₄S ([M-H]⁻): calcd. 512.8868, found 512.8879; HPLC: tᵣ = 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, DMSO-d₆): δ 2.67 (m, 2H, CH₂CO), 2.78 (m, 2H, CH₂CO), 3.62 (s, 3H, OCH₃), 144.72, 156.92, 157.23, 170.92, 172.56 ppm; HRMS (ESI) m/z for C_{17}H_{13}Br₂N₄O₄S ([M-H]⁻): calcd. 526.9024, found 526.9035; HPLC: tᵣ = 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) v 3410, 3173, 2986, 1752, 1705, 1654, 1608, 1579, 1545, 1521, 1465, 1412, 1339, 1285, 1231, 1188, 1146, 1015, 961, 868, 812, 747 cm\(^{-1}\); \(^1\)H NMR (400 MHz, DMSO-\(d_6\)): \(\delta\) 1.33 (t, 3H, \(J = 7.1\) Hz, \(\text{CH}_2\text{CH}_3\)), 4.33 (q, 2H, \(J = 7.1\) Hz, \(\text{CH}_2\text{CH}_3\)), 7.23 (s, 1H, pyrrole-3), 7.72 (dd, 1H, \(J = 8.8\) Hz, \(J_4 = 2.0\) Hz, \(\text{Ar-H}_5\)), 7.79 (d, 1H, \(J = 8.8\) Hz, \(\text{Ar-H}_4\)), 8.44 (d, 1H, \(J = 2.0\) Hz, \(\text{Ar-H}_7\)), 10.08 (s, 1H, NHCO), 13.01 (s br, 1H, NHCO\(\text{CH}_2\)), 13.18 (s br, 1H, pyrrole-NH) ppm; \(^{13}\)C NMR (100 MHz, DMSO-\(d_6\)) \(\delta\) 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, 159.0 ppm (2 carbonyl signals not seen due to broadening). HRMS (ESI) \(m/z\) for C\(_{16}\)H\(_{11}\)Cl\(_2\)N\(_4\)O\(_4\)S ([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) v 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\(^{-1}\); \(^1\)H NMR (400 MHz, DMSO-\(d_6\)): \(\delta\) 3.68 (s, 5H, \(\text{CH}_2\text{OCH}_3\)), 7.22 (d, 1H, \(J = 2.8\) Hz, pyrrole-H3), 7.68 (dd, 1H, \(J = 8.8\) Hz, \(J_4 = 2.0\) Hz, \(\text{Ar-H}_5\)), 7.74 (d, 1H, \(J = 8.8\) Hz, \(\text{Ar-H}_4\)), 8.38 (d, 1H, \(J = 2.0\) Hz, \(\text{Ar-H}_7\)), 10.04 (s, 1H, NHCO), 12.54 (s br, 1H, NHCO\(\text{CH}_2\)), 12.98 (d, 1H, \(J = 2.8\) Hz, pyrrole-NH) ppm; \(^{13}\)C NMR (100 MHz, DMSO-\(d_6\)) \(\delta\) 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
C_{16}H_{11}Cl_{2}N_{4}O_{4}S ([M-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$^{-1}$; $^1$H NMR (400 MHz, DMSO-$d_6$): δ 7.27 (d, 1H, $^3J = 2.8$ 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, 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 C_{14}H_{17}Br_{2}N_{4}O_{4}S ([M-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$^{-1}$; $^1$H NMR (400 MHz, DMSO-$d_6$) δ 2.59 (m, 2H, CH$_2$CO), 2.73 (m,
2H, CH$_2$CO), 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, NHCOCH$_2$. COOH), 12.93 (d, 1H, $^4J$ = 2.8 Hz, pyrrole-NH) ppm; $^{13}$C NMR (100 MHz, DMSO-d$_6$) $\delta$ 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 C$_{16}$H$_{11}$Br$_2$N$_4$O$_4$S ([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). $^1$H NMR (400 MHz, DMSO-d$_6$) $\delta$ 4.79 (s br, 2H, 6-NH$_2$), 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-NH$_2$), 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 Na$_2$CO$_3$ (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-1H-pyrrol-2-yl)ethan-1-one (1.60 g, 4.32 mmol) or 2,2,2-trichloro-1-(4,5-dichloro-1H-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-1H-pyrrole-2-carboxamide (16).** Prepared according to general procedure E from 15 and 2,2,2-trichloro-1-(4,5-dibromo-1H-pyrro-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-d₆): 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-d₆): 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: tᵣ = 17.34 min (100 % at 254 nm).

**N-(6-Aminobenzo[d]thiazol-2-yl)-4,5-dichloro-1H-pyrrole-2-carboxamide (17).** Prepared according to general procedure E from 15 and 2,2,2-trichloro-1-(4,5-dichloro-1H-pyrro-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-d₆): 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-d₆): δ 104.00, 108.90, 112.58, 114.46, 115.49, 120.59, 126.03, 133.01, 139.44, 145.82, 153.35, 156.99 ppm; HRMS (ESI) m/z for C₁₂H₇Cl₂N₄OS ([M-H]⁻): calcd 324.9718, found 324.9709; HPLC: tᵣ = 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-d₆ + 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, ³J = 8.8 Hz, Ar-H₄), 7.77 (dd, 1H, ³J = 8.8 Hz, ⁴J = 2.0 Hz, Ar-H₅), 8.41 (d, 1H, ⁴J = 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-d₆): 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: tᵣ = 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) v 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\(^{-1}\);

\(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) 3.52 (s, 2H, COCH\(_2\)CO), 3.67 (s, 3H, OCH\(_3\)), 7.50 (s br, 1H, pyrrole-H3), 7.53 (dd, 1H, \(^3\)J = 8.7 Hz, \(^4\)J = 1.8 Hz, Ar-H5), 7.70 (d, 1H, \(^3\)J = 8.7 Hz, Ar-H4), 8.31 (d, 1H, \(^4\)J = 1.8 Hz, Ar-H7), 10.43 (s, 1H, NH), 12.57 (s br, 1H, NH), 13.24 (s br, 1H, NH) ppm; \(^13\)C NMR (100 MHz, DMSO-\(d_6\)): \(\delta\) 43.42, 51.97, 99.00, 108.50, 111.48, 115.93, 118.51, 120.38, 125.67, 132.11, 134.81, 144.81, 157.01, 157.44, 168.13 ppm; HRMS (ESI\(^+\)) m/z for C\(_{16}\)H\(_{11}\)Br\(_2\)N\(_4\)O\(_4\)S ([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) v 3364, 1718, 1660, 1612, 1578, 1533, 1470, 1436, 1408, 1382, 1316, 1276, 1205, 1170 cm\(^{-1}\); \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) 28.46, 30.79, 51.36, 99.00, 108.46, 111.14, 115.93, 118.39, 120.36, 125.69, 135.32, 144.43, 157.09, 157.44, 169.74, 172.86 ppm 2.59-2.68 (m, 4H, COCH\(_2\)CH\(_2\)CO), 3.61 (s, 3H, OCH\(_3\)), 7.51-7.54 (m, 2H, pyrrole-H3, Ar-H5), 7.69 (d, 1H, \(^3\)J = 8.1 Hz, Ar-H4), 8.32 (d, 1H, \(^4\)J = 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 NMR (100 MHz, DMSO-\(d_6\)) \(\delta\) HRMS (ESI\(^+\)) m/z for C\(_{17}\)H\(_{15}\)Br\(_2\)N\(_4\)O\(_4\)S ([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, 1306, 1279, 1230, 1191, 1115, 1064, 1016, 956, 882, 852 cm$^{-1}$; $^1$H NMR (400 MHz, DMSO-d$_6$ + TFA) δ 1.33 (t, 3H, J = 7.1 Hz, CH$_3$CH$_3$), 4.32 (q, 2H, J = 7.1 Hz, CH$_2$CH$_3$), 7.49 (s, 1H, pyrrole-H3), 7.73 (d, 1H, $^3$J = 8.7 Hz, Ar-H4), 7.78 (dd, 1H, $^3$J = 8.7 Hz, $^4$J = 2.0 Hz, Ar-H5), 8.41 (d, 1H, $^4$J = 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, 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 C$_{16}$H$_{11}$Cl$_2$N$_4$O$_4$S ([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$^{-1}$; $^1$H NMR (400 MHz, DMSO-d$_6$) δ 3.56 (s, 2H, COCH$_2$CO), 3.66 (s, 3H, OCH$_3$), 7.46 (s br, 1H, pyrrole-H3), 7.58 (dd, 1H, $^3$J = 8.7 Hz, $^4$J = 1.9 Hz, Ar-H5), 7.70 (d, 1H, $^3$J = 8.7 Hz, Ar-H4), 8.33 (d, 1H, $^4$J = 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, 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 C$_{16}$H$_{11}$Cl$_2$N$_4$O$_4$S ([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, DMSO-d₆) δ 2.64 (m, 4H, CH₂CH₂CO), 3.61 (s, 3H, OCH₃), 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, CONHCH₂), 12.57 (s br, 1H, NH), 13.30 (s br, 1H, NH) ppm; ¹³C NMR (100 MHz, DMSO-d₆): 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 C₁₇H₁₃Cl₂N₄O₄S ([M-H]⁻): calcd 439.0035, found 439.0040; HPLC: tᵣ = 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, DMSO-d₆) 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; ¹³C NMR (100 MHz, DMSO-d₆) 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 C₁₄H₁₃Br₂N₄O₄S ([M-H]⁻): calcd 484.8555, found 484.8543; HPLC: tᵣ = 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,
H NMR (400 MHz, DMSO-\textit{d}_6) \delta 3.42 (s, 2H, COCH\textsubscript{2}CO), 7.50 (d, 1H, \textit{J} = 2.3 Hz, pyrrole-H3), 7.53 (dd, 1H, \textit{J} = 8.7 Hz, \textit{J} = 1.6 Hz, Ar-H5), 7.70 (d, 1H, \textit{J} = 8.7 Hz, Ar-H4), 8.32 (d, 1H, \textit{J} = 1.6 Hz, Ar-H7), 10.33 (s, 1H, NHCOCH\textsubscript{2}), 12.60 (s br, 2H, NH, COOH), 13.23 (s br, 1H, NH) ppm; \textsuperscript{13}C NMR (100 MHz, DMSO-\textit{d}_6): \delta 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) \textit{m/z} for C\textsubscript{15}H\textsubscript{9}Br\textsubscript{2}N\textsubscript{4}O\textsubscript{4}S ([M-H]): calcd 498.8711, found 498.8714; HPLC: \textit{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) \nu 3259, 3123, 2994, 2512, 1879, 1651, 1608, 1557, 1528, 1468, 1408, 1388, 1323, 1297, 1259, 1221, 1178, 1105, 1082 cm\textsuperscript{-1}; \textsuperscript{1}H NMR (400 MHz, DMSO-\textit{d}_6) \delta 2.55-2.60 (m, 4H, COCH\textsubscript{2}CH\textsubscript{2}CO), 7.50 (s, 1H, pyrrole-H3), 7.53 (dd, 1H, \textit{J} = 8.7 Hz, \textit{J} = 2.0 Hz, Ar-H5), 7.69 (d, 1H, \textit{J} = 8.7 Hz, Ar-H4), 8.32 (d, 1H, \textit{J} = 2.0 Hz, Ar-H7), 10.16 (s, 1H, NHCOCH\textsubscript{2}), 12.16 (s br, 1H, NH/COOH), 12.56 (s br, 1H, NH/COOH), 13.23 (s br, 1H, NH) ppm; \textsuperscript{13}C NMR (100 MHz, DMSO-\textit{d}_6) \delta 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) \textit{m/z} for C\textsubscript{16}H\textsubscript{11}Br\textsubscript{2}N\textsubscript{4}O\textsubscript{4}S ([M-H]): calcd 512.8868, found 512.8854; HPLC: \textit{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) \nu 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; \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) 7.48 (s, 1H, pyrrole-H3), 7.73 (d, 1H, \(^3\)J = 8.8 Hz, Ar-H4), 7.79 (dd, 1H, \(^3\)J = 8.8 Hz, \(^4\)J = 1.8 Hz, Ar-H5), 8.43 (d, 1H, \(^4\)J = 1.8 Hz, Ar-H7), 10.86 (s, 1H, NHCO), 12.64 (s br, 1H, NH), 13.32 (s br, 1H, NH) ppm, signal for COOH not observed; \(^13\)C NMR (100 MHz, DMSO-\(d_6\)) \(\delta\) 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 C\(_{14}\)H\(_7\)Cl\(_2\)N\(_4\)O\(_4\)S ([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 °C. IR (ATR) \(\nu\) 3110, 1679, 1613, 1545, 1468, 1417, 1392, 1373, 1322, 1256, 1173, 1143, 1021, 991, 939, 867, 805, 703 cm⁻¹; \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) 3.40 (s, 2H, COCH\(_2\)CO), 7.48 (d, 1H, \(^4\)J = 2.3 Hz, pyrrole-H3), 7.54 (dd, 1H, \(^3\)J = 8.7 Hz, \(^4\)J = 1.7 Hz, Ar-H5), 7.70 (d, 1H, \(^3\)J = 8.7 Hz, Ar-H4), 8.32 (d, 1H, \(^4\)J = 1.7 Hz, Ar-H7), 10.33 (s, 1H, NHCOCH\(_2\)CO), 12.60 (s br, 2H, NH, COOH), 13.31 (s br, 1H, NH) ppm; \(^13\)C NMR (100 MHz, DMSO-\(d_6\)): \(\delta\) 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 C\(_{15}\)H\(_9\)Cl\(_2\)N\(_4\)O\(_4\)S ([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 °C; IR (ATR) \(\nu\) 3254, 2988, 2362, 1655, 1609, 1552, 1529, 1469, 1420, 1397, 1331, 1302, 1260, 1231, 1184, 1057, 1023, 981, 872, 841, 820, 742 cm⁻¹; \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) 2.60 (m, 4H, CH\(_2\)CH\(_2\)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, CONHCH$_2$), 11.80-12.80 (s br, 2H, COOH, NH), 13.30 (s br, 1H, NH) ppm; $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 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 C$_{16}$H$_{11}$Cl$_2$N$_4$O$_4$S ([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 IC$_{50}$ 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 MgCl$_2$, 2 mM DTT, 1.8 mM spermidine, 1mM ATP, 6.5 % (w/v) glycerol, 0.001 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 3μL 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 MgCl$_2$], 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 ×) in T10 buffer [10 mM Tris × HCl (pH 8.0) and 1 mM EDTA] was added, mixed and the fluorescence ($\lambda_{ex} = 485$ nm; $\lambda_{em} = 535$ 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, IC$_{50}$ values were
determined with seven concentrations of the inhibitors. GraphPad Prism program was used for calculating IC$_{50}$ value which represents the concentration of inhibitor where the activity of the enzyme is reduced by 50%. IC$_{50}$ values were determined in three independent measurements, and their average value is given as a result. As a positive control novobiocin (Sigma-Aldrich) [IC$_{50}$ = 0.17 μM (lit. 0.08 μM)$^{31,32}$ for E. coli gyrase and IC$_{50}$ = 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 IC$_{50}$ 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 MgCl$_2$], 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 ($\lambda_{ex}$ = 485 nm; $\lambda_{em}$ = 535 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, IC\textsubscript{50} values were determined with seven concentrations of the inhibitors. GraphPad Prism program was used for calculating IC\textsubscript{50} value, which represents the concentration of inhibitor where the activity of the enzyme is reduced by 50\%. The IC\textsubscript{50} values were determined in three independent measurements and their average value is given as a result. As a positive control novobiocin [IC\textsubscript{50} = 11 μM (lit. 10 μM)\textsuperscript{31,32} for \textit{E. coli} topoisomerase IV and IC\textsubscript{50} = 27 μM (lit. 20 μM)\textsuperscript{31,32} for \textit{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: \textit{Enterococcus faecalis} (Gram positive, ATCC 29212), \textit{Staphylococcus aureus} (Gram positive, ATCC 25923), \textit{Escherichia coli} (Gram negative, ATCC 25922) and \textit{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, Martinsread, 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.

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>X-ray source</td>
<td>PXI/X06SA (SLS³)</td>
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<tr>
<td>Wavelength [Å]</td>
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<tr>
<td>Detector</td>
<td>PILATUS 6M</td>
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<tr>
<td>Temperature [K]</td>
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</tr>
<tr>
<td>Space group</td>
<td>C 2</td>
</tr>
</tbody>
</table>
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)
$R_{\text{sym}}$ [%] 7.6 (44.6)
$R_{\text{meas}}$ [%] 9.5 (56.9)
Mean(I)/sd$^b$ 11.17 (3.14)

$^a$SWISS LIGHT SOURCE (SLS, Villingen, Switzerland). $^b$Calculated from independent reflections.

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

<table>
<thead>
<tr>
<th>Property</th>
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<tbody>
<tr>
<td>Resolution [Å]</td>
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<tr>
<td>Number of reflections (working/test)</td>
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<tr>
<td>$R_{\text{cryst}}$ [%]</td>
<td>25.8</td>
</tr>
<tr>
<td>$R_{\text{free}}$ [%] $^b$</td>
<td>28.9</td>
</tr>
<tr>
<td>Total number of atoms:</td>
<td></td>
</tr>
<tr>
<td>Protein</td>
<td>2807</td>
</tr>
<tr>
<td>Water</td>
<td>6</td>
</tr>
<tr>
<td>Ligand</td>
<td>30</td>
</tr>
<tr>
<td>Iodide</td>
<td>1</td>
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</table>
Deviation from ideal geometry c

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
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</thead>
<tbody>
<tr>
<td>Bond lengths [Å]</td>
<td>0.007</td>
</tr>
<tr>
<td>Bond angles [°]</td>
<td>1.11</td>
</tr>
<tr>
<td>Bonded B’s [Å²] d</td>
<td>1.3</td>
</tr>
</tbody>
</table>

Ramachandran plot e

<table>
<thead>
<tr>
<th>Region</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Most favored regions [%]</td>
<td>89.9</td>
</tr>
<tr>
<td>Additional allowed regions [%]</td>
<td>10.1</td>
</tr>
<tr>
<td>Generously allowed regions [%]</td>
<td>0.0</td>
</tr>
<tr>
<td>Disallowed regions [%]</td>
<td>0.0</td>
</tr>
</tbody>
</table>

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. Their geometries were optimized using MMFF94 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. Molecular docking of the final molecules was performed using FlexX, as available in LeadIT, 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).\textsuperscript{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,\textsuperscript{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.\textsuperscript{38}

**Screening against PAINS.** To evaluate a library of the synthesized compounds against PAINS,\textsuperscript{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/1d1d0424ba15da08cde7e18e9a27d55b1a6cb797/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


(38) BioSolve IT (GmbH), LeadIT version 2.1.3.


$\textit{E. coli} \text{ DNA gyrase}
\text{IC}_{50} = 58 \text{ nM}$