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New dual ATP-competitive inhibitors of bacterial DNA gyrase and topoisomerase IV active against ESKAPE pathogens

Martina Durcik [a], Ákos Nyerges [b, 1], Žiga Skok [a], Darja Gramec Skledar [a], Jurij Trontelj [a], Nace Zidar [a], Janez Ilaš [a], Anamarija Zega [a], Cristina D. Cruz [c], Päivi Tammela [c], Martin Welin [d], Yengo R. Kimbung [d], Dorota Focht [d], Ondřej Benek [e], Tamás Révész [b], Gábor Draskovits [b], Petra Éva Szili [b], Lejla Daruka [b], Csaba Pál [b], Danijel Kikelj [a], Lucija Peterlin Mašič [a, **], Tihomir Tomašič [a, *]

[a] University of Ljubljana, Faculty of Pharmacy, Aškerčeva cesta 7, 1000, Ljubljana, Slovenia
[b] Synthetic and Systems Biology Unit, Institute of Biochemistry, Biological Research Centre, Szeged, H-6726, Hungary
[c] Drug Research Program, Division of Pharmaceutical Biosciences, Faculty of Pharmacy, University of Helsinki, P.O. Box 56 (Viikinkaari 5 E), 00014, Helsinki, Finland
[d] SARomics Biostructures, Medicon Village, Lund, Sweden
[e] University of Hradec Kralove, Faculty of Science, Department of Chemistry, Rokitanskeho 62, 500 03, Hradec Kralove, Czech Republic

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ABSTRACT

The rise in multidrug-resistant bacteria defines the need for identification of new antibacterial agents that are less prone to resistance acquisition. Compounds that simultaneously inhibit multiple bacterial targets are more likely to suppress the evolution of target-based resistance than monotargeting compounds. The structurally similar ATP binding sites of DNA gyrase and topoisomerase IV offer an opportunity to accomplish this goal. Here we present the design and structure-activity relationship analysis of balanced, low nanomolar inhibitors of bacterial DNA gyrase and topoisomerase IV that show potent antibacterial activities against the ESKAPE pathogens. For inhibitor **31c**, a crystal structure in complex with *Staphylococcus aureus* DNA gyrase B was obtained that confirms the mode of action of these compounds. The best inhibitor, **31h**, does not show any *in vitro* cytotoxicity and has excellent potency against Gram-positive (MICs: range, 0.0078–0.0625 μg/mL) and Gram-negative pathogens (MICs: range, 1–2 μg/mL). Furthermore, **31h** inhibits GyrB mutants that can develop resistance to other drugs. Based on these data, we expect that structural derivatives of **31h** will represent a step toward clinically efficacious multitargeting antimicrobials that are not impacted by existing antimicrobial resistance.

1. Introduction

The discovery of antibiotics led to tremendous improvements in the success rates of treating bacterial infections, and the consequent reduced mortality. This big step forward was also seen for other medical areas, as safe organ transplants and cancer and heart disease surgery then became possible [1,2]. Not surprisingly, the use of each antibiotic was soon followed by the development of resistance in the target bacterial species [1]. With inappropriate use, and indeed major overuse mainly in treating livestock, the problem of antibacterial resistance escalated [3]. One of the major threats both in hospitals and in the community are the 'ESKAPE' pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacter* species) [2,4]. Most of these pathogens are included in the World Health Organization priority list of pathogens for which new therapies are urgently needed [5].

At the same time, the pharmaceutical industry has been withdrawing from antibacterial research and development, due to the high costs of the research needed and the low potential return as a result of the development of bacterial resistance [6–8]. On a

** Corresponding author.
* Corresponding author.
E-mail addresses: Lucija.PeterlinMasic@ffa.uni-lj.si (L.P. Mašič), Tihomir.Tomasic@ffa.uni-lj.si (T. Tomašič).
[1] Present address: Department of Genetics, Harvard Medical School, Boston, MA 02215, USA.

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Abbreviations

ATCC	American Type Culture Collection
DCM	dichloromethane
DMF	N,N-dimethylformamide
DMSO	dimethylsulfoxide
GHKL	gyrase Hsp90 histidine kinase and MutL
GyrA	DNA gyrase subunit A
GyrB	DNA gyrase subunit B
EDC	N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide
HepG2	human hepatocellular carcinoma cell line
HOBt	hydroxybenzotriazole
hTopoIIα	human DNA topoisomerase IIα
LDH	lactate dehydrogenase
MCF-7	breast cancer cell line
MH	Mueller–Hinton
MRSA	methicillin-resistant Staphylococcus aureus
NMM	N-methylmorpholine
NMP	N-methyl-2-pyrrolidone;
ParC	topoisomerase IV subunit C
ParE	topoisomerase IV subunit E
PAβN	phenylalanine-arginine β-naphthylamide
TFA	trifluoroacetic acid;
THF	tetrahydrofuran;
Topo IV	topoisomerase IV
VISA	vancomycin-intermediate S. aureus

positive note, initiatives have been promoted to boost research and development into new antibacterials, such as the Innovative Medicines Initiative of 'New Drugs for Bad Bugs' programme, the 'Combating Antibiotic-Resistant Bacteria Biopharmaceutical Accelerator', the 'Joint Programming Initiative on Antimicrobial Resistance', and others [9,10].

The bacterial enzymes DNA gyrase and topoisomerase IV (TopoIV) are validated targets for the development of new antibacterial agents. Both are heterotetrameric enzymes that catalyze changes in DNA topology during DNA replication, recombination, and transcription [11]. DNA gyrase consists of two GyrA and two GyrB subunits, while its paralog TopoIV consists of two ParC and two ParE subunits. The GyrA and ParC subunits are responsible for cleavage and reunion of the DNA and are the targets of the therapeutically used fluoroquinolones [12]. However, resistance against broad-spectrum fluoroquinolones is rising rapidly and serious side-effects have emerged that led to the EMA recommendation in 2018 to restrict, and even suspend, the use of some fluoroquinolone antibiotics [13,14]. The role of the GyrB and ParE subunits is to provide the energy necessary for the DNA ligation process through ATP hydrolysis [11,15]. The most well-known inhibitors of GyrB/ParE are aminocoumarins, with their representative novobiocin, which was used in the clinic primarily to treat penicillin-resistant *S. aureus* infections, although it was later withdrawn from therapy due to the development of target-based resistance and toxicity [15].

In the last years, we have discovered and optimized several new structural classes of pyrrolamide-based GyrB and ParE inhibitors [16–22]. Recently, we also developed inhibitors **I** and **II** (Figs. 1 and 2) as balanced dual-targeting inhibitors of *S. aureus* GyrB and ParE. These inhibitors interact with multiple evolutionarily conserved amino acids in the ATP binding pockets of both enzymes. **I** and **II** are highly potent against a broad panel of multidrug-resistant *S. aureus* clinical strains and resistance mutations against these compounds are particularly rare [23]. **I** and **II** bypass the existing and clinically widespread resistance mechanisms, including those that reduce the efficacy of other DNA gyrase and TopoIV inhibitors. Additionally, *de novo* resistance mutations against **I** and **II** are rare and have a limited impact on resistance levels. Furthermore, **I** and **II** inhibit Gram-negative pathogens *in vitro*, although at higher concentrations than Gram-positive pathogens [23].

In the present study, we present the structure-based design of **I** and **II** analogs and a comprehensive structure-activity relationship study, with the aim to develop a class of optimized ATP-competitive dual-targeting inhibitors with broad-spectrum activities against the emerging ESKAPE pathogens. The crystal structure of a representative inhibitor **31c** in complex with *S. aureus* GyrB showed the detailed interaction pattern at the molecular level. The selected inhibitor of this series, **31h**, showed potent low nanomolar dual inhibition of *E. coli* and *S. aureus* DNA gyrase and TopoIV, selectivity against human DNA topoisomerase IIα (hTopoIIα), broad spectrum antibacterial activity including against GyrB mutants and clinical isolates, and no *in vitro* cytotoxicity. In the *in vitro* metabolic study using an S9 fraction, we also identified the main metabolites of **31h**, with no reactive metabolites detected.

2. Results and discussion

2.1. Structure-based design

Recently, we carried out the rational design and evaluation of two new benzothiazole-6-carboxylic acid-based inhibitors **I** and **II** that showed low target-based resistance potential (Figs. 1 and 2) [23]. The crystal structure of inhibitor **II** in complex with *S. aureus* GyrB (Supplementary information, Fig. S1) revealed that the pyrrolamide moiety forms crucial hydrogen bonds with Asp81 of *S. aureus* GyrB (Asp73 in *E. coli*) and the conserved water molecule, along with additional hydrophobic interactions. The benzothiazole core of **II** is important for cation-π interactions with Arg84 (Arg76 in *E. coli*). Hydrophobic interactions with Pro87 (Pro79 in *E. coli*) are formed with the benzothiazole core and the benzyloxy group of **II**, while the carboxyl group forms a salt bridge with Arg144 (Arg136 in *E. coli*) [23]. This crystal structure and the promising microbiological data for inhibitors **I** and **II** served as the basis for hit-to-lead optimization of the benzothiazole class of GyrB inhibitors, with the aim to obtain new balanced dual GyrB and ParE inhibitors with broad spectrum activities against the ESKAPE pathogens, and with low target-based resistance potential. The latter was pursued by structure-based design of dual GyrB/ParE inhibitors that form potent interactions with amino-acid residues that are essential for the enzymatic function (e.g., Asp73, Arg76, Pro79 in *E. coli* GyrB), and therefore do not mutate and are conserved within the bacterial strains [23,24]. The second aim of the present study was to define the *in vitro* selectivity, cytotoxicity, genotoxicity, and metabolism of the representative new inhibitor. This design of the optimized analogs of **I** and **II** is presented in Figs. 1 and 2, respectively.

Based on compound **I**, we synthesized four new series of inhibitors with different substitution patterns around the benzothiazole scaffold (Fig. 1, type 1–4 compounds). For the type 1 compounds, 4,5-dichloro-, 4,5-dibromo-, and 4-bromo-3-chloro-5-methyl-1*H*-pyrrole were introduced into the molecules. However, better results overall were obtained for inhibitor **I** than for type 1 compounds, therefore 3,4-dichloro-5-methyl-1*H*-pyrrole was used as the pyrrole moiety in compounds of types 2–4. In the type 2 series, the carboxylic acid was replaced with its bioisosteres or with hydrogen-bond-acceptor moieties, which can form hydrogen

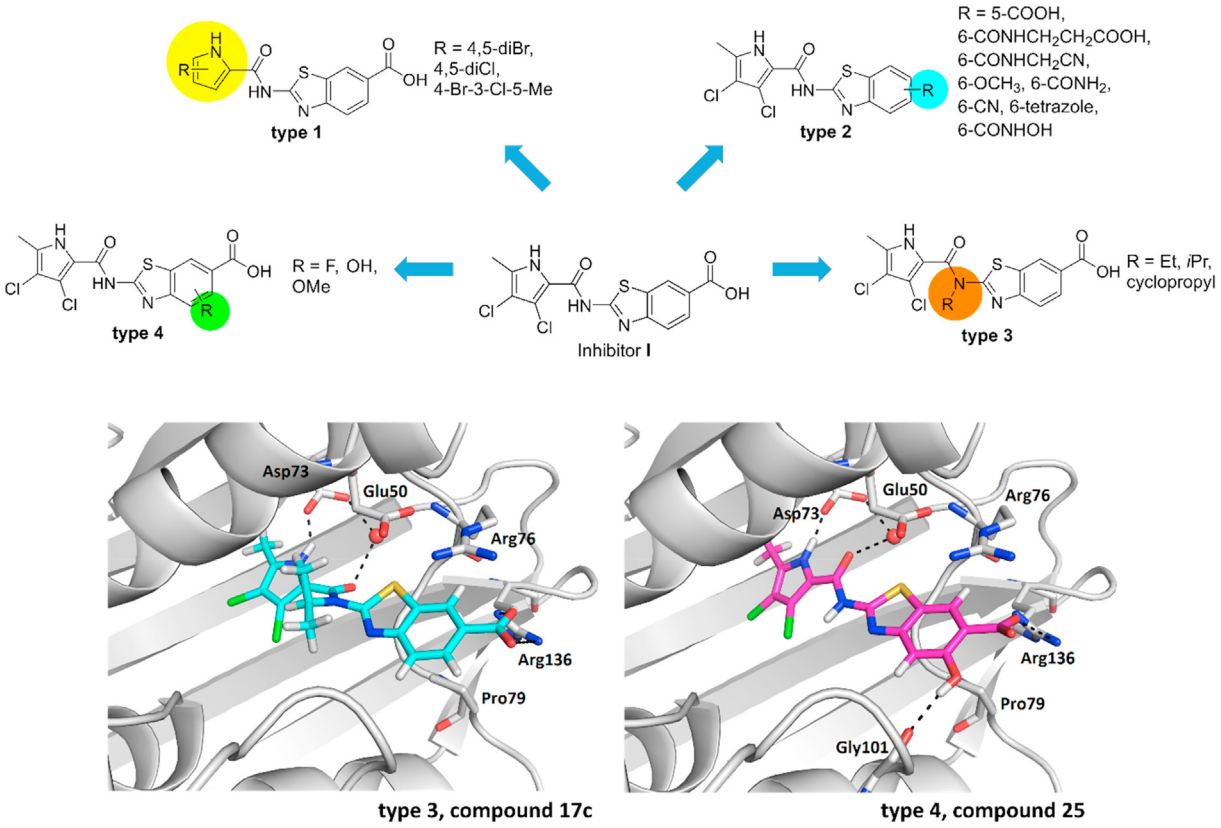


Fig. 1. Design of type 1–4 compounds based on inhibitor **I** [23] and the predicted docking pose for type 3 and 4 compounds **17c** and **25** in the ATP-binding site of *E. coli* DNA gyrase B (gray cartoon; PDB code: 4DUH). For clarity, only selected amino-acid residues are shown as sticks. Hydrogen bonds are presented as black dashed lines. Conserved water molecule is presented as a red sphere.

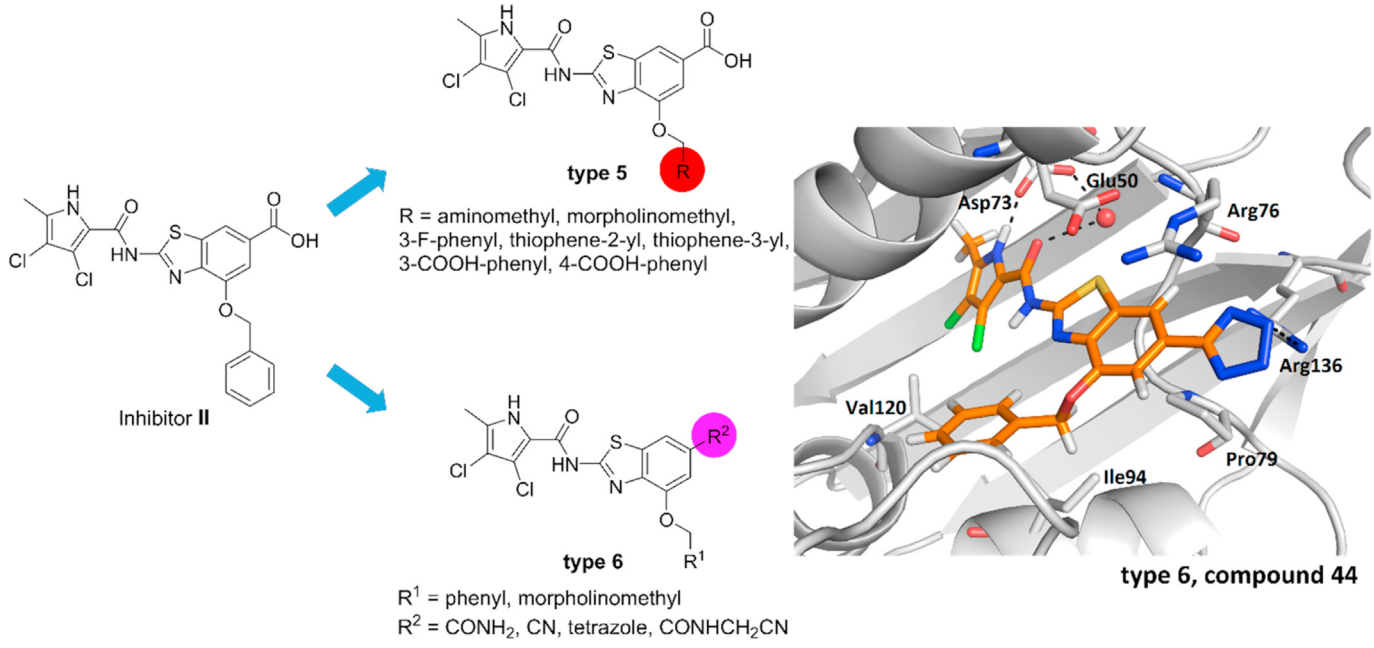


Fig. 2. Design of type 5 and 6 compounds based on inhibitor **II** [23] and the predicted docking pose for type 6 compound **44** in the ATP-binding site of *E. coli* DNA gyrase B (gray cartoon; PDB code: 4DUH). For clarity, only selected amino-acid residues are shown as sticks. Hydrogen bonds are presented as black dashed lines. Conserved water molecule is presented as a red sphere.

bonds or ionic interactions with Arg136 (*E. coli* numbering). The aim of this modification was to reduce the acidic character of compound **I**, to improve bacterial cell-wall penetration. To make inhibitors less planar than **I**, type 3 compounds were prepared that were derivatized at the amide nitrogen with aliphatic groups (Fig. 1, compound **17c**). In the type 4 compounds, we attached fluoro,

methoxy, or hydroxyl groups to positions 4 or 5 of the bicycle, which can form additional interactions in the binding site according to the predictions from the docking analysis (Fig. 1, compound **25**).

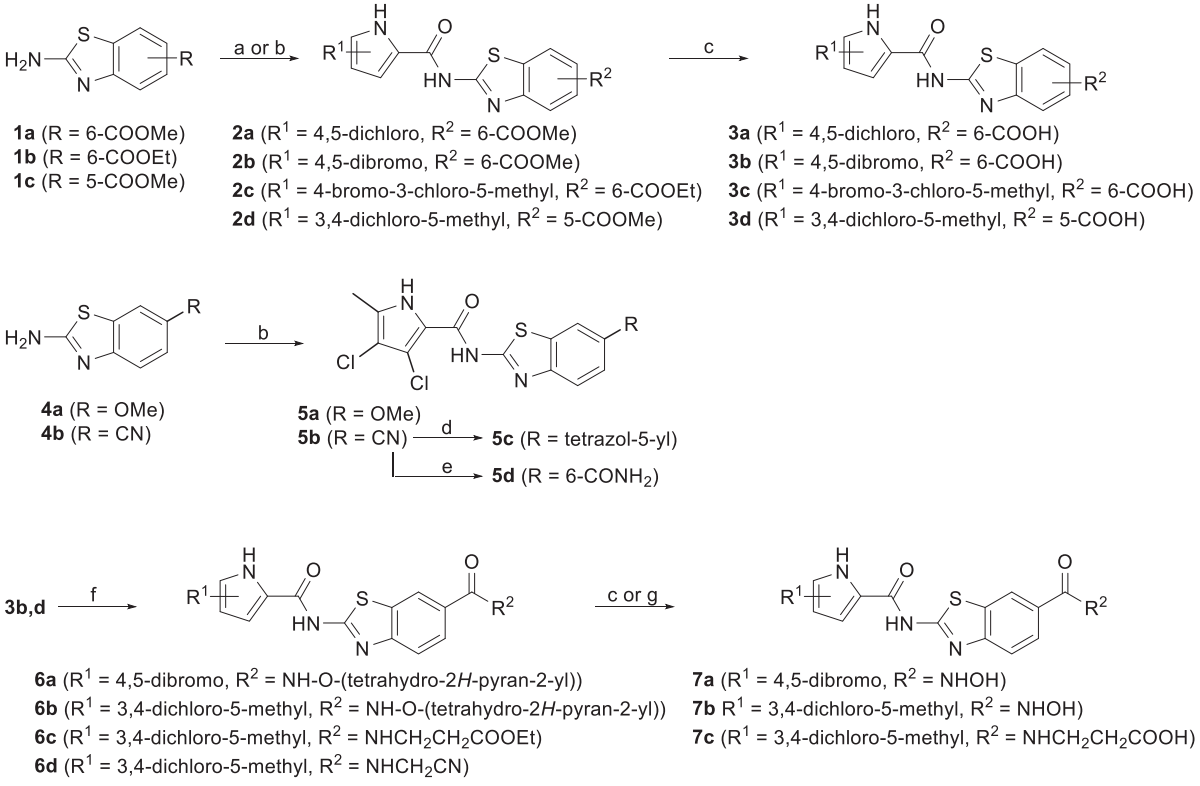
For the optimization of compound **II**, we changed the part with the phenyl substituent to gain additional interactions with the target in the lipophilic floor of GyrB and ParE (Fig. 2, type 5, 6 compounds). In the type 5 compounds, the phenyl ring was replaced with a variety of aromatic and aliphatic substituents with hydrophobic, acidic, and basic properties. In the type 6 compounds, we replaced the carboxylic group with its bioisotere tetrazole (Fig. 2, compound **44**), and with cyano or (substituted) carboxamide groups (Fig. 2).

2.2. Chemistry

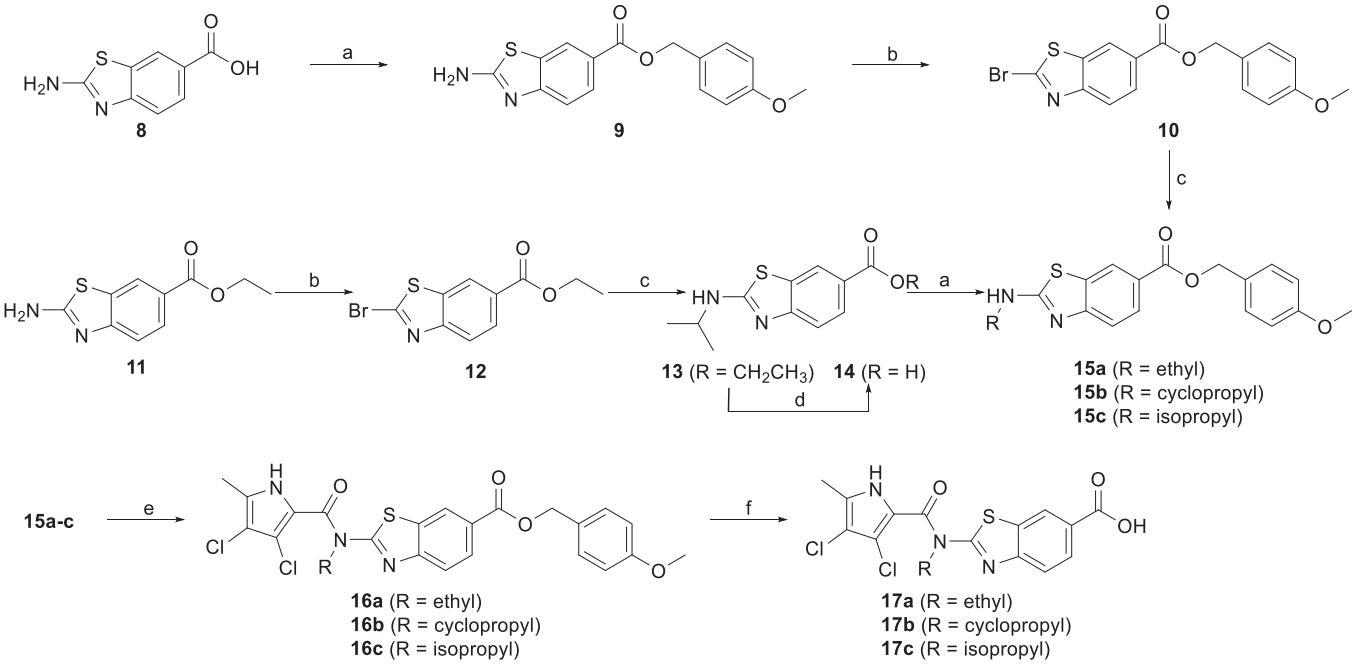
The synthesis of the type 1 and type 2 compounds is shown in Scheme 1. First, a set of commercially available 2-aminobenzo [*d*] thiazoles **1a-c** were coupled to pyrrole derivatives, to obtain **2a-d**. Esters **2a-d** were subjected to alkaline hydrolysis, to obtain final compounds **3a-d**. Commercially available 2-aminobenzo[*d*]thiazoles **4a** and **4b** were coupled to 3,4-dichloro-5-methyl-1*H*-pyrrole-2-carboxylic acid, to obtain final compounds **5a** and **5b**, and compound **5b** was additionally cyclized to its tetrazole analog **5c** using sodium azide and ammonium chloride. Compound **5b** was converted also to its amide analog **5d** using KOH in *N*-methyl-2-pyrrolidone. Compounds **6a-d** were prepared by coupling *O*-(tetrahydro-2*H*-pyran-2-yl)hydroxylamine (for **6a, 6b**), β-alanine ethyl ester (for **6c**), or *N*-cyanomethylamine (for **6d**) to the carboxylic acids **3b-d**. The tetrahydro-2*H*-pyran-2-yl protecting group of **6a** and **6b** was removed with trifluoroacetic acid, to obtain the final hydroxamic acids **7a** and **7b**, and the ethyl ester of **6c** was hydrolyzed under alkaline conditions to obtain **7c**.

Scheme 2 presents the synthesis of the type 3 compounds, with substituents at the amide nitrogen. To prepare *N*-alkyl derivatives, first the carboxyl group of 2-aminobenzo[*d*]thiazole-6-carboxylic acid (**8**) was protected as *para*-methoxybenzyl ester **9**. The use of this protecting group was crucial for the successful preparation of **17a-c**, as alkaline hydrolysis of ethyl esters also resulted in cleavage of the alkylated amide moiety. The amino group of **9** was replaced with bromine using *tert*-butyl nitrite and copper (II) bromide, and the bromide **10** obtained was substituted with either ethylamine (for **15a**) or cyclopropylamine (for **15b**). In parallel, ethyl 2-aminobenzo[*d*]thiazole-6-carboxylate (**11**) was also converted into bromine derivative **12**, which was substituted with isopropylamine to obtain compound **13**. Ethyl ester of **13** was hydrolyzed to obtain **14**, and replaced with a *para*-methoxybenzyl group to obtain compound **15c**. Intermediates **15a-c** were reacted with 3,4-dichloro-5-methyl-1*H*-pyrrole-2-carbonyl chloride to obtain compounds **16a-c**. In the final step, *para*-methoxybenzyl ester was cleaved with acidolysis, to yield **17a-c**.

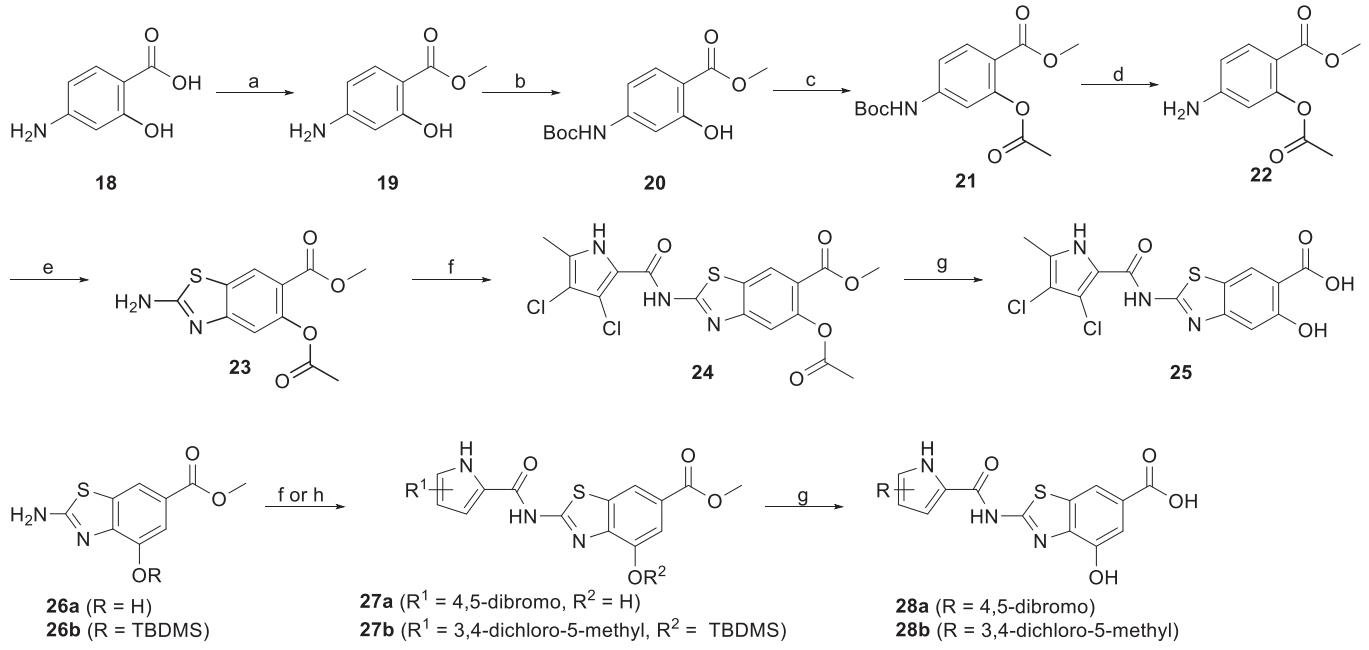
Scheme 3 shows the synthesis of type 4 compounds **25**, **28a**, and **28b** with hydroxy substituents on positions 4 or 5 of the benzothiazole core. First, methyl ester **19** was formed from the 4-aminosalicylic acid (**18**) using Fischer esterification, after which the amino group was protected as *tert*-butyl carbamate, to obtain **20**. Then the hydroxyl group was protected with acetyl chloride, to provide compound **21**. The Boc protecting group was removed, to get **22**, and then cyclization was performed using Br_2 and KSCN in acetic acid, to obtain the benzothiazole intermediate **23**. Compound



Scheme 1. Reagents and conditions: (a) 2,2,2-trichloro-1-(4,5-dichloro-1*H*-pyrrol-2-yl)ethan-1-one (for **2a**) or 2,2,2-trichloro-1-(4,5-dibromo-1*H*-pyrrol-2-yl)ethan-1-one (for **2b**), Na_2CO_3, DMF, 80 °C, 15 h; (b) *i:* 4-bromo-3-chloro-5-methyl-1*H*-pyrrole-2-carboxylic acid (for **2c**) or 3,4-dichloro-5-methyl-1*H*-pyrrol-2-carboxylic acid (for **2d** and **5a**, **5b**), $(COCl)_2$, anhydrous DCM, 20 °C, 15 h, *ii:* **1a-c**, toluene, 130 °C, 15 h; (c) 2 M NaOH, 1,4-dioxane or MeOH, 80 °C (for **3a-d**) or 20 °C (for **7c**), 15 h; (d) NaN_3, NH_4Cl, DMF, 100 °C, 15 h; (e) KOH, NMP, 115 °C, 48 h; (f) *O*-(tetrahydro-2*H*-pyran-2-yl)hydroxylamine (for **6a**, **6b**) or β-alanine ethyl ester (for **6c**), or 2-aminoacetonitrile hydrochloride (for **6d**), EDC, HOBt, NMM, DMF, 20 °C, 15 h; (g) CF_3COOH, DCM, 20 °C, 2 h (for **7a**, **7b**).



Scheme 2. Reagents and conditions: (a) 4-methoxybenzyl chloride, K_2CO_3, anhydrous DMF, 20 °C, 15 h; (b) $CuBr_2$, *tert*-butyl nitrite, CH_3CN 0 °C–20 °C, 15 h; (c) ethylamine (for **15a**), cyclopropylamine (for **15b**), or isopropylamine (for **13**), THF, 20 °C, 15 h; (d) 2 M NaOH, 1,4-dioxane, 20 °C, 15 h; (e) *i:* 3,4-dichloro-5-methyl-1*H*-pyrrol-2-carboxylic acid, $(COCl)_2$, anhydrous DCM, 20 °C, 15 h, *ii:* **15a-c**, toluene, 130 °C, 15 h; (f) 1 M HCl in CH_3COOH, CH_3COOH, 20 °C, 15 h.



Scheme 3. Reagents and conditions: (a) H_2SO_4, MeOH, 65 °C, 15 h; (b) di-*tert*-butyl dicarbonate, 70 °C, 48 h; (c) acetic anhydride, pyridine, CH_3CN, 70 °C, 48 h; (d) 2 M HCl in diethyl ether, 20 °C, 15 h; (e) KSCN, Br_2, CH_3COOH, 10 °C, then 20 °C, 15 h, saturated aq. $NaHCO_3$ solution; (f) *i:* 3,4-dichloro-5-methyl-1*H*-pyrrol-2-carboxylic acid, $(COCl)_2$, anhydrous DCM, 20 °C, 15 h, *ii:* 23 (for 24) or 26b (for 27b), toluene, 130 °C, 15 h; (g) 4 M NaOH (for 25) or 1 M NaOH (for 28a, 28b), 1,4-dioxane or methanol, 80 °C (for 25) or 40 °C (for 28a, 28b); (h) 26a, 2,2,2-trichloro-1-(4,5-dibromo-1*H*-pyrrol-2-yl)ethan-1-one, Na_2CO_3, DMF, 80 °C, 15 h (for 27a).

23 was then coupled to 3,4-dichloro-5-methyl-1*H*-pyrrole-2-carbonyl chloride, and finally, compound **24** obtained was subjected to alkaline hydrolysis, to provide target compound **25**. To synthesize 4-hydroxy substituted **28a** and **28b**, first **26a** and **26b** were prepared according to previously described procedures [25]. Compounds **26a** and **26b** were then coupled with the corresponding pyrrole derivative, to obtain compounds **27a** and **27b**, which were subjected to alkaline hydrolysis to obtain **28a** and **28b**. In the same reaction step, the *tert*-butyldimethylsilyl protecting group was removed from **27b**.

The combined synthetic steps for the preparation of type 4 and 5 compounds with substituents on position 4 of the benzothiazole

core are shown in Scheme 4. Various 4-substituted 2-aminobenzo[*d*]thiazoles (**29a-i**) were synthesized according to described procedures [25]. Compounds **29a-i** were coupled to 3,4-dichloro-5-methyl-1*H*-pyrrole-2-carbonyl chloride, to obtain methyl esters **30a-i**, which were then converted to the final carboxylic acid analogs (**31a-i**) under alkaline hydrolysis conditions. Finally, compound **32** was prepared by Boc deprotection of **31d** using acidolysis.

Scheme 5 presents the synthesis of type 6 compounds with the carboxyl group at position 6 replaced. Compound **33** was synthesized with EDC and HOBt-promoted coupling between **31c** and 2-aminoacetonitrile hydrochloride. Compound **37** was prepared by first hydrolyzing benzothiazole **34** to its carboxylic acid derivative **35**, which was coupled to 2-aminoacetonitrile hydrochloride. Compound **36** obtained was then reacted with 3,4-dichloro-5-methyl-1*H*-pyrrole-2-carbonyl chloride to obtain final product **37**. To obtain tetrazole (**44**) and amide (**47**) analogs of compound **II**, methyl 3-(benzyloxy)-4-nitrobenzoate (**38**) was converted to amide **39** using saturated NH_3 solution in methanol. Compound **39** was then used in two different pathways. Reaction with triphenylphosphine oxide, triethylamine, and oxalyl chloride afforded the cyano analog **40**. The nitro group of **40** was reduced to obtain amine **41** using tin (II) chloride, and then cyclization was performed with Br_2 and KSCN in acetic acid, to obtain the benzothiazole **42**. Compound **43** was prepared by coupling **42** with 3,4-dichloro-5-methyl-1*H*-pyrrole-2-carbonyl chloride, and then the tetrazole ring was formed from the cyano group using ammonium chloride and sodium azide in DMF, to obtain final compound **44**. Also, compound **39** was reduced to **45** with tin (II) chloride. Cyclization of **45** to the benzothiazole intermediate **46**, and the subsequent coupling of **46** with 3,4-dichloro-5-methyl-1*H*-pyrrole-2-carbonyl chloride were performed, to obtain the final amide analog **47**.

2.3. In vitro enzyme inhibition and antibacterial activity

The new compounds were examined for their inhibitory activities against DNA gyrase from *E. coli* and *S. aureus* in supercoiling assays, and against TopoIV from *E. coli* and *S. aureus* in relaxation assays (Table 1, types 1–4; Table 2, types 5, 6). Compounds showed potent inhibition of *E. coli* DNA gyrase, with several with IC_{50} values < 10 nM. Several of the new compounds showed potent dual *S. aureus* DNA gyrase and TopoIV inhibition; e.g., **3c**, **25**, **31c**, and **31e-i** had IC_{50} values < 100 nM against both of these target enzymes. In particular, type 5 compounds **31c** and **31e-i** showed potent inhibition of *S. aureus* and *E. coli* DNA gyrase and *S. aureus* TopoIV (IC_{50} < 100 nM), but weaker *E. coli* TopoIV inhibition (IC_{50} < 400 nM). Compounds with IC_{50} values < 500 nM against DNA gyrase from *E. coli* were tested for antibacterial activity against *E. faecalis* and the ESKAPE pathogens *S. aureus*, *K. pneumoniae*, *A. baumannii*, *P. aeruginosa*, and *E. coli* (Table 3 and Supplementary Information, Table S1).

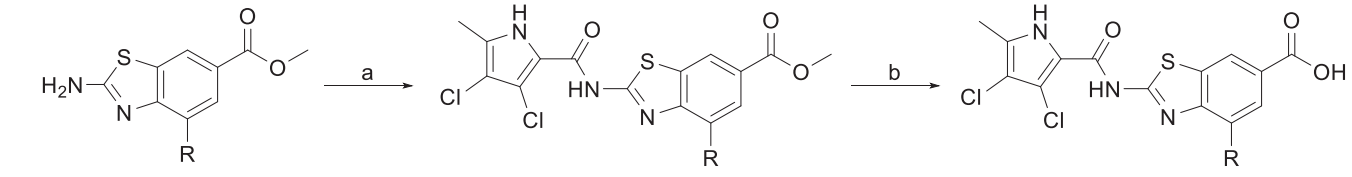
Bacterial strains that were used: *S. aureus* ATCC 29213, *S. aureus* (MRSA) ATCC 43300, *E. faecalis* ATCC 29212, *A. baumannii* ATCC 17978, *P. aeruginosa* ATCC 27853, *K. pneumoniae* ATCC 10031, and *E. coli* ATCC 25922.

Measurements were performed according to the Clinical and Laboratory Standards Institute guidelines, with three independent measurements.

Type 1 compounds **3a-c** with 4,5-dichloro-, 4,5-dibromo-, and 4-bromo-3-chloro-5-methylpyrroles, respectively, are potent inhibitors of *E. coli* DNA gyrase (Table 1, IC_{50} < 20 nM). Among these, **3c** with 4-bromo-3-chloro-5-methylpyrrole displayed balanced low nanomolar dual inhibition of *S. aureus* DNA gyrase and TopoIV (Table 1, IC_{50} 24 nM, 61 nM, respectively), as comparable to inhibitor **I**. This also resulted in broad-spectrum activity for **3c** (Table 3, MICs: 0.0625 μg/mL against *E. faecalis*; 2 μg/mL against *K. pneumoniae*).

Moving the carboxyl group to position 5 of the benzothiazole core (type 2 compound **3e**) or replacing the carboxyl group on position 6 with hydrogen bond acceptors (type 2 compounds **5a**, **5b**) led to loss of enzyme inhibition, and consequently of the antibacterial activities. This appears to be because **5a** and **5b** cannot form ionic interactions with Arg136, which are crucial for potent activity. Compounds **7a** and **7b** with a hydroxamic acid group as a less acidic bioisostere of the carboxyl group were weaker inhibitors of DNA gyrase and TopoIV. On the other hand, compounds **5c**, **5d**, **6d**, and **7c** with the carboxylic acid bioisostere tetrazole or carboxamide, all retained potent activities against DNA gyrase from *E. coli* and *S. aureus* (Table 1), which for compounds **5c** and **7c** translated in activities against *E. faecalis*, with MICs of 1 μg/mL and 2 μg/mL, respectively.

Type 3 compounds with substituents at the amide nitrogen were prepared with the aim to reduce the high level of planarity in the structure. Additionally, with substituents at this position, there was the possibility to form additional interactions with the



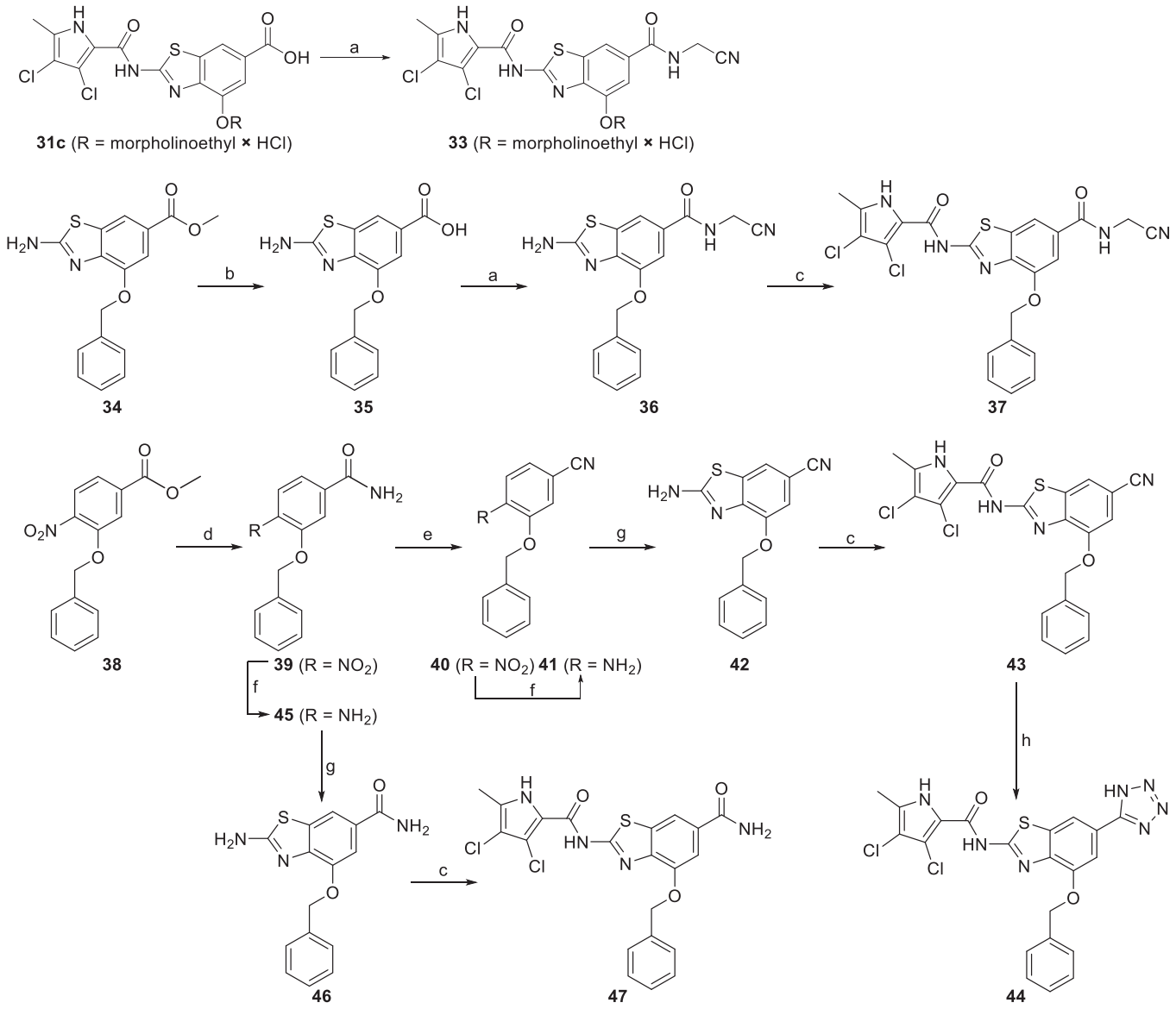
29a (R = F)
29b (R = OCH_3)
29c (R = O-(2-morpholinoethyl))
29d (R = OCH_2CH_2NHBoc)
29e (R = O-CH_2-(3-$COOCH_3$)Ph)
29f (R = O-CH_2-(4-$COOCH_3$)Ph)
29g (R = O-CH_2-(thiophen-2-yl))
29h (R = O-CH_2-(thiophen-3-yl))
29i (R = O-CH_2-(3-F)Ph)

30a (R = F)
30b (R = OCH_3)
30c (R = O-(2-morpholinoethyl))
30d (R = OCH_2CH_2NHBoc)
30e (R = O-CH_2-(3-$COOCH_3$)Ph)
30f (R = O-CH_2-(4-$COOCH_3$)Ph)
30g (R = O-CH_2-(thiophen-2-yl))
30h (R = O-CH_2-(thiophen-3-yl))
30i (R = O-CH_2-(3-F)Ph)

31a (R = F)
31b (R = OCH_3)
31c (R = O-(2-morpholinoethyl))
31d (R = OCH_2CH_2NHBoc)
31e (R = O-CH_2-(3-$COOCH_3$)Ph)
31f (R = O-CH_2-(4-$COOCH_3$)Ph)
31g (R = O-CH_2-(thiophen-2-yl))
31h (R = O-CH_2-(thiophen-3-yl))
31i (R = O-CH_2-(3-F)Ph)

32 (R = $OCH_2CH_2NH_2$ × HCl)

Scheme 4. Reagents and conditions: (a) *i:* 3,4-dichloro-5-methyl-1*H*-pyrrol-2-carboxylic acid, $(COCl)_2$, anhydrous DCM, 20 °C, 15 h, *ii:* **29a-i** (for **30a-i**), toluene, 130 °C, 15 h; (b) 1 M NaOH, 1,4-dioxane or MeOH, 40 °C, 15–72 h; (c) 4 M HCl in 1,4-dioxane, 1,4-dioxane, 3 h, 20 °C.



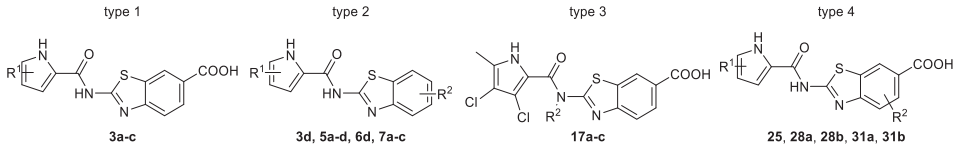
Scheme 5. Reagents and conditions: (a) 2-aminoacetonitrile hydrochloride, EDC, HOBt, NMM, DMF, 20 °C, 15 h; (b) 1 M NaOH, MeOH, 80 °C, 15 h; (c) *i:* 3,4-dichloro-5-methyl-1*H*-pyrrol-2-carboxylic acid, $(COCl)_2$, anhydrous DCM, 20 °C, 15 h, *ii:* **36**, **42** or **46**, toluene, 130 °C, 15 h; (d) saturated NH_3 solution in MeOH, 65 °C, 15 h; (e) PPh_3O, Et_3N, $(COCl)_2$, anhydrous CH_3CN, 20 °C, 30 min; (f) $SnCl_2$, MeOH/EtOAc, 55 °C, 15 h; (g) KSCN, Br_2, CH_3COOH, 10 °C, then 20 °C, 15 h, 25% NH_3 aq. solution; (h) NaN_3, NH_4Cl, DMF, 125 °C, 15 h.

lipophilic floor of the enzyme. The difference in activities between compounds **17a-c** showed that increasing the size of the substituents from ethyl to cyclopropyl to isopropyl led to weaker activities (Table 1). Compounds **17a** and **17b** showed potent antibacterial activities against Gram-positive methicillin-resistant *S. aureus* (MRSA) and *E. faecalis*, with MICs of 0.125 μg/mL to 8 μg/mL. For **17a**, moderate activity was also observed against Gram-negative *A. baumannii* and *K. pneumoniae* (Table 3, MICs of 16 μg/mL).

Type 4 and 5 inhibitors were the most potent compounds in this class. Adding small substituents, such as hydroxyl, methoxy, or fluoro (i.e., type 4 compounds), or larger substituents (i.e., type 5 compounds) to position 4 resulted in strong inhibition of DNA gyrase. Except for dibromopyrrole analog **28a**, all of these compounds showed IC_{50} < 10 nM for *E. coli* DNA gyrase and <61 nM for *S. aureus* DNA gyrase (Tables 1 and 2).

A co-crystal structure was obtained for type 5 compound **31c** in complex with the 24 kDa fragment of *S. aureus* GyrB at a resolution of 1.7 Å (Fig. 3, PDB code 6TTG). This confirmed the predicted binding mode of these inhibitors in the ATP binding site of *S. aureus* DNA gyrase. One hydrogen bond was formed between the pyrrole NH group and Asp81 (Asp73 in *E. coli*), and a second hydrogen bond was formed between the amide carbonyl oxygen and the conserved water molecule, which was coordinated by Asp81, Gly85, and Thr173. The amide carbonyl oxygen also formed a direct hydrogen bond with Thr173. In the lipophilic pocket of the enzyme, 3,4-dichloro-5-methylpyrrole formed hydrophobic interactions with Ile51, Val79, Gly85, Ile86, Pro87, Ile102, Leu103, Thr173, and Ile175. The benzothiazole moiety formed a π-cation interaction with the Arg84 side chain (Arg76 in *E. coli*), while the aromatic carboxylate formed two additional hydrogen bonds with Arg144 (Arg136 in *E. coli*). As predicted, a 2-morpholinoethoxy substituent at position 4 of the benzothiazole core was oriented toward the lipophilic floor, although due to the missing loop in the crystal structure (amino

Table 1
Inhibitory activities of type 1–4 compounds against *E. coli* and *S. aureus* DNA gyrase and topoisomerase IV (TopoIV).



Cpd.	R^1	R^2	IC_{50} (nM)[a]			
			E. coli		*S. aureus*	
			DNA gyrase	**TopoIV**	**DNA gyrase**	**TopoIV**
Type 1 inhibitors						
3a	4,5-diCl	_	19 ± 1	280 ± 20	500 ± 280	>1000
3b	4,5-diBr	_	12 ± 7	>1000	>1000	300 ± 150
3c	3-Cl-4-Br-5-Me	_	<10	220 ± 90	24 ± 13	61 ± 25
Type 2 inhibitors						
3d	3,4-diCl-5-Me	5-COOH	830 ± 150	>1000	>1000	>1000
5a	3,4-diCl-5-Me	6-OMe	>1000	>1000	>1000	>1000
5b	3,4-diCl-5-Me	6-CN	>1000	>1000	>1000	>1000
5c	3,4-diCl-5-Me	6-(Tetrazol-5-yl)	<10	660 ± 180	25 ± 12	270 ± 140
5d	3,4-diCl-5-Me	6-$CONH_2$	11 ± 2	490 ± 80	59 ± 28	320 ± 160
6d	3,4-diCl-5-Me	6-$CONHCH_2CN$	16 ± 4	>1000	310 ± 100	>1000
7a	4,5-diBr	6-CONHOH	89 ± 20	>1000	>1000	>1000
7b	3,4-diCl-5-Me	6-CONHOH	280 ± 10	>1000	720 ± 310	>1000
7c	3,4-diCl-5-Me	6-$CONHCH_2$ CH_2COOH	<10	210 ± 20	71 ± 12	40 ± 11
Type 3 inhibitors						
17a	_	Ethyl	<10	>1000	32 ± 15	230 ± 80
17b	_	Cyclopropyl	24 ± 1	>1000	>1000	>1000
17c	_	Isopropyl	320 ± 170	>1000	>1000	>1000
Type 4 inhibitors						
25	3,4-diCl-5-Me	5-OH	<10	95 ± 4	61 ± 4	46 ± 10
28a	4,5-diBr	4-OH	46 ± 33	180 ± 40	170 ± 70	240 ± 110
28b	3,4-diCl-5-Me	4-OH	<10	130 ± 10	22 ± 6	120 ± 60
31a	3,4-diCl-5-Me	4-F	<10	200 ± 10	17 ± 0	240 ± 40
31b	3,4-diCl-5-Me	4-OMe	<10	160 ± 20	44 ± 12	390 ± 60
I			13 ± 0	500 ± 280	72 ± 20	66 ± 22
II			<10	350 ± 50	16 ± 5	68 ± 31
Novobiocin			170 ± 20	11000 ± 2000	34 ± 7	27000 ± 7000

[a] IC_{50}, concentration (mean ± SD of three independent experiments) that inhibits enzyme activity by 50%.

acids 105–127), no interactions with the enzyme were observed, except from a hydrogen bond with a crystal water molecule. Compound **31c** therefore targets amino acids Asp81, Arg84, and Pro87 in *S. aureus* GyrB, which have been shown to be essential for the enzymatic activity of GyrB in *E. coli* [24], and to be fully conserved across over 1000 phylogenetically diverse bacterial genomes [23].

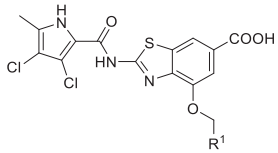
Type 5 compounds with larger and mostly aromatic substituents on position 4 of the benzothiazole core were the best balanced dual-acting compounds, as seen for **31e**, **31f**, and **31i** with IC_{50} values of 13 nM, 12 nM, and <10 nM against *S. aureus* TopoIV (Table 2). Type 4 compound **31a**, with a fluoro substituent on position 4, showed very good inhibition of Gram-positive bacteria and Gram-negative *K. pneumoniae* (Table 3), although these activities were not improved over those of the inhibitor **I**. The addition of polar substituents to the type 4 and 5 compounds (i.e., hydroxyl: **25**, **28a**, **28b**; morpholinoethoxy: **31c**; aminoethoxy: **32**) had negative impacts on their antibacterial activities (Supplementary Information, Table S1). Although the recently reported rules for compound accumulation in Gram-negative bacteria, known as the 'eNTRy rules' [26], indicate that primary amines are important for drug entry, compound **32** was devoid of antibacterial activity. Similarly, other compounds with polar groups lacked antibacterial activities despite their excellent on-target inhibition (i.e., **31e**, **31f**, **32**, **33**). These inhibitors have greater topological polar surface areas in comparison with other compounds (Supplementary Information, Table S2), which might be why they cannot penetrate bacterial cell walls, and consequently why they lack antibacterial activities. On the other hand, replacing the phenyl ring of inhibitor **II** with its bioisosteric replacement thiophene ring resulted in the two best compounds of the series, **31g** and **31h**, which showed exceptional activities against Gram-positive and Gram-negative bacteria (Table 3).

Type 6 compounds with an aliphatic nitrile group in the side chain on position 6 (**33**, **37**) or carboxyl group bioisosteres on position 6 (**44**, **47**), were potent inhibitors of *E. coli* DNA gyrase (IC_{50} < 11 nM), while the aromatic nitrile **43** was devoid of activity, which is in line with the observations for **5b**. Tetrazole **44** also showed potent *S. aureus* DNA gyrase inhibition and good TopoIV inhibition (Table 2). The only notable antibacterial activity against Gram-positive bacteria was seen for **37** and **44** (Table 3).

The structure–activity relationship of the new series of inhibitors is summarized in Supplementary Information Fig. S2 while the predicted docking pose of type 5 inhibitor **31h** and the interactions that it forms in the binding site are shown in Fig. 4. The strongest activity of type 5 compounds can be attributed to the substituents at position 4, which form additional interactions with the lipophilic floor of the binding site, as observed for the co-crystal structure of *S. aureus* GyrB in complex with **31c** (Fig. 3) and by the docking binding mode of **31h** (Fig. 4). Furthermore, we superimposed the crystal structures of *E. coli* GyrB (PDB entry: 1KZN) and ParE (PDB entry: 1S14) with those of *S. aureus* GyrB (PDB entry: 6TTG) and ParE (PDB entry: 4URN) (Fig. S4) to better understand the difference in the observed inhibition of DNA gyrase and

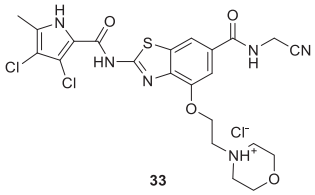
Table 2
Inhibitory activities of type 5 and 6 compounds against *E. coli* and *S. aureus* DNA gyrase and topoisomerase IV (TopoIV).

type 5

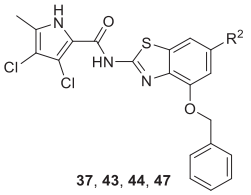


31c, **31e-i**, **32**

type 6



33



37, **43**, **44**, **47**

Cpd.	R^1	R^2	IC_{50} (nM)[a]			
			E. coli		*S. aureus*	
			DNA gyrase	**TopoIV**	**DNA gyrase**	**TopoIV**
Type 5 inhibitors						
31c		–	<10	220 ± 140	17 ± 6	66 ± 3
31e		–	<10	170 ± 10	<10	13 ± 0
31f		–	<10	260 ± 70	<10	12 ± 5
31g		–	<10	300 ± 80	<10	88 ± 38
31h		–	<10	340 ± 70	<10	84 ± 38
31i		–	<10	54 ± 28	<10	<10
32		–	<10	>1000	48 ± 25	160 ± 60
Type 6 inhibitors						
33		$CONHCH_2CN$	<10	>1000	100 ± 40	520 ± 370
37	–	$CONHCH_2CN$	<10	>1000	180 ± 80	>1000
43	–	CN	>1000	>1000	>1000	>1000
44	–	Tetrazol-5-yl	<10	680 ± 250	26 ± 7	89 ± 17
47	–	$CONH_2$	11 ± 2	>1000	>1000	>1000
I			13 ± 0	500 ± 280	72 ± 20	66 ± 22
II			<10	350 ± 50	16 ± 5	68 ± 31
Novobiocin			170 ± 20	11000 ± 2000	34 ± 7	27000 ± 7000

topoisomerase IV. The most noticeable differences in binding sites are observed in the pyrrole binding pocket (Fig. S4). However, the unresolved loop in the lipophilic floor surrounding the substituent at position 4 of the benzothiazole core may also contribute significantly to the observed difference in IC_{50} values against the four enzymes.

2.4. Microbiological profiling of compound 31h

Overall, **31h** was selected as the best compound of this class of inhibitors, as in addition to its potent inhibition of DNA gyrase and TopoIV, it had the best antibacterial activity profile against all of the ESKAPE pathogens. The MICs of **31h** against the Gram-negative bacteria *A. baumannii*, *P. aeruginosa*, *K. pneumoniae*, and *E. coli* were 1, 2, 2, and 4 μg/mL, respectively. For the Gram-positive bacteria, the MICs were even better, as 0.0156 μg/mL for *E. faecalis* and the compound was exceptionally active also against the MRSA strain (Table 3, MIC 0.0625 μg/mL). Additionally, **31h** was tested on the ESKAPE pathogens *E. faecium* (ATCC 700221) and vancomycin-intermediate *S. aureus* (VISA; ATCC 700699), where it showed MICs of 0.0078 μg/mL and 0.0313 μg/mL, respectively. Therefore, **31h** was further explored in terms of its microbiological profile. We examined how certain GyrB mutations (Arg144, Thr173 and Ile175) in MRSA and VISA strains influence the antibacterial activities of **31h**. As **31h** also forms important interactions among others also with these three amino acids of GyrB, their mutations might lead to decreased susceptibility to inhibitor **31h**. However, **31h** also showed very potent activities here, with MICs against the MRSA mutants of 0.125–0.25 μg/mL, and against the VISA mutants of 0.0625 μg/mL (Table 4). These mutants thus confer very low levels of resistance to **31h**.

To determine whether compound **31h** undergoes efflux in

Table 3
Minimum inhibitory concentrations (MICs) for the DNA gyrase and/or topoisomerase IV inhibitors with activities against the indicated Gram-positive and Gram-negative bacterial strains.

Cpd.	MIC (μg/mL) [a]						
	Gram positive			Gram-negative			
	S. aureus	*S. aureus* (MRSA)[b]	*E. faecalis*	*A. baumannii*	*P. aeruginosa*	*K. pneumoniae*	*E. coli*
3c	0.125	2	0.0625	4	8	2	>64
5c	16	16	1	>64	>64	16	>64
7b	64	32	0.5	>64	>64	>64	>64
7c	32	8	2	>64	>64	8	>64
17a	0.125	8	0.125	16	>64	16	32
17b	2	2	0.25	>64	>64	64	>64
25	16	4	1	>64	>64	8	>64
28b	16	4	2	>64	>64	4	>64
31a	**0.5**	**0.0625**	**0.0625**	**8**	**64**	**4**	**16**
31b	2	0.5	0.25	16	64	2	64
31g	**0.065**	**0.5**	**0.0163**	**1**	**4**	**4**	**8**
31h	**0.25**	**0.0625**	**0.0156**	**1**	**2**	**2**	**4**
31i	0.0625	0.0625	0.001	2	64	>64	>64
37	4	4	16	>64	>64	>64	>64
44	4	4	0.125	>64	>64	>64	>64
I	1	0.125	0.125	4	8	1	4
II	0.5	0.0625	<0.0313	2	2	4	16

[a] MIC, minimum inhibitory concentration.
[b] MRSA, methicillin-resistant *S. aureus*.

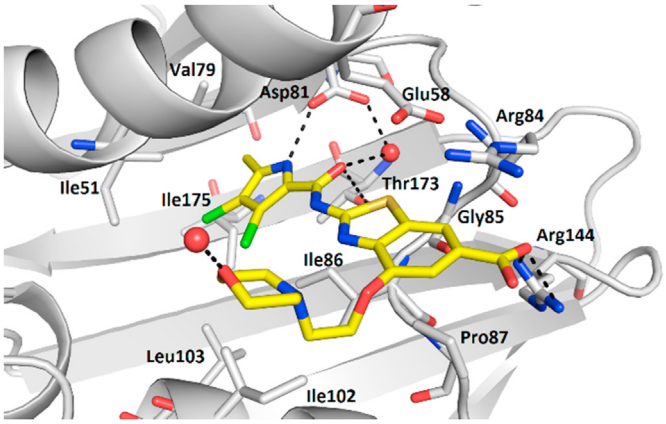


Fig. 3. Co-crystal structure of *S. aureus* DNA gyrase B (gray cartoon; PDB entry: 6TTG) in complex with inhibitor **31c** (yellow sticks). For clarity, only amino-acid residues that interact with **31c** are shown as sticks. Water molecules are presented as red spheres and hydrogen bonds are shown as dashed black lines.

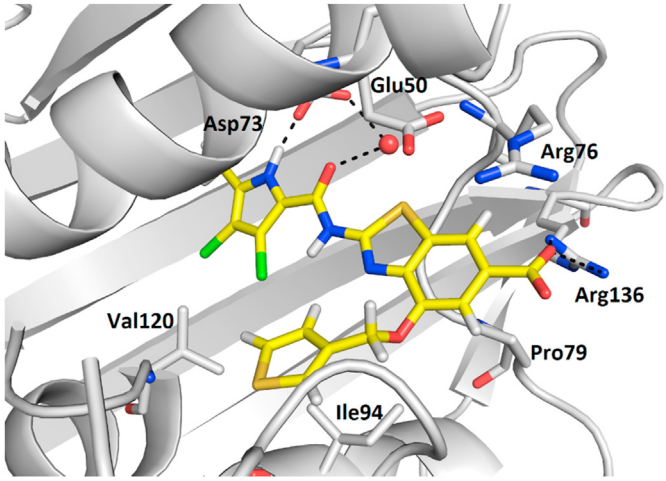


Fig. 4. The predicted docking pose of type 5 inhibitor **31h** (yellow sticks) that shows the interactions that are important for activity against the *E. coli* DNA gyrase B (gray cartoon; PDB code 4DUH) ATP-binding site. For clarity, only selected amino-acid residues are shown as sticks. Hydrogen bonds are presented as black dashed lines. The detailed interaction pattern is shown in Supplementary Information Fig. S3.

Gram-negative bacteria, it was tested against the highly resistant *E. coli* MG1655 strain without and with the efflux pump substrate phenylalanine-arginine β-naphthylamide (PAβN). Without PAβN, **31h** showed no activity, whereas with PAβN, **31h** inhibited this *E. coli* strain with a very good MIC of 0.125 μg/mL (Table 4). This confirmed that compound **31h** is a substrate for the efflux pumps in the *E. coli* MG1655 strain. To determine the importance of the interaction between the terminal carboxylate of **31h** and Arg136 in *E. coli*, inhibitor **31h** was tested on the *E. coli* MG1655 strain with the R136C mutation in the absence and presence of PAβN and the activity of **31h** was only 8-fold weaker (Table 4). Although interaction with Arg136 is important for bacterial inhibition, a very good MIC of 2 μg/mL was obtained against the mutated strain, which showed that the interactions formed between **31h** and the rest of the GyrB active site are strong enough to provide good antibacterial activity. These promising data are in line with our design strategy, where compounds form strong interactions with Asp81, Arg84, and Pro87 (*S. aureus* GyrB numbering), which are essential for the enzymatic function of GyrB.

Additionally, **31h** was tested against Gram-negative clinical isolates, where it showed good activity against *P. aeruginosa*, *E. coli*, and ciprofloxacin resistant *A. baumannii* and *E. coli* clinical strains, with MICs from 0.5 μg/mL to 16 μg/mL (Table 5).

2.5. In vitro selectivity and toxicity evaluation of compound 31h

Human DNA TopoII catalyzes the introduction of topological changes into the DNA molecule. Its ATP-binding domain belongs to the GHKL ATPase family, and it is similar to those of DNA gyrase and TopoIV [27]. To determine the selectivity of **31h** for these bacterial enzymes, we evaluated its inhibition of hTopoIIα in the DNA relaxation assay. Compound **31h** had an IC_{50} of 45.0 μM against hTopoIIα (Supplementary Information, Fig. S5). **31h** is thus over 450-fold selective for DNA gyrase from *E. coli* or *S. aureus*, and 13-fold and 54-fold selective for TopoIV from *E. coli* and *S. aureus*, respectively.

Table 4
Minimum inhibitory concentrations (MICs) for **31h** against wild-type methicillin-resistant *S. aureus* (MRSA), vancomycin-intermediate *S. aureus* (VISA), and *E. coli*, and their GyrB mutants.

Bacteria	Strain	MIC (μg/mL)[a]	Fold-change (mutant vs. wild type)
S. aureus (MRSA)	Wild-type	0.0625	-
	GyrB R144I	0.25	4-fold
	GyrB T173A	0.125	2-fold
S. aureus (VISA)	Wild-type	0.0313	-
	GyrB R144I	0.0625	2-fold
	GyrB I175T	0.0625	2-fold
E. coli MG1655	Wild-type	>64	_
	GyrB R136C	>64	_
E. coli MG1655	Wild type	0.125	_
(+50 μg/mL PAβN[b])	GyrB R136C	2	8-fold

[a] MIC, minimum inhibitory concentration.
[b] PAβN, phenylalanine-arginine β-naphthylamide (efflux pump substrate).

Table 5
MICs for **31h** against Gram-negative clinical isolates.

Bacteria	Strain	MIC (μg/mL)[a]
A. baumannii CIPr[b]	ATCC BAA-1605	0.5
E. coli	20204	1
E. coli CIPr	31995	2
E. coli CIPr	31859	2
P. aeruginosa	19488	16

[a] MIC, minimum inhibitory concentration.
[b] CIPr, ciprofloxacin-resistant clinical isolate.

Compound **31h** was tested for *in vitro* cytotoxicity using the lactate dehydrogenase assay in liver cancer HepG2 cells and breast cancer MCF-7 cells. Here, **31h** showed no cytotoxicity up to 100 μM (Supplementary Information, Fig. S6). The genetic toxicity of **31h** was also evaluated in a standard micronucleus test on Chinese hamster ovary K1 cells, without and with a rat liver S9 fraction (Supplementary Information, Table S3). In the absence of the S9 fraction, **31h** showed no genetic toxicity up to 16 μM, which was 495-fold, 123-fold, and 247-fold higher than the MICs of **31h** against *E. faecalis,* MRSA, and VISA, respectively. Conversely, in the presence of the S9 fraction, compound **31h** showed no genotoxicity up to 62 μM, which was 1917-fold, 478-fold, and 957-fold higher than the MICs against *E. faecalis*, MRSA, and VISA. To identify the main *in vitro* metabolites of **31h**, *in vitro* metabolic transformation studies were performed using the rat liver S9 fraction. Here, we identified three main metabolites shown in Scheme 6: a metabolite hydroxylated on the methylpyrrole moiety; a metabolite hydroxylated on the benzothiazole ring; and a direct glucuronide conjugate (as either an acylglucuronide or an *N*-glucuronide) (Supplementary Information, Figs. S7–S10).

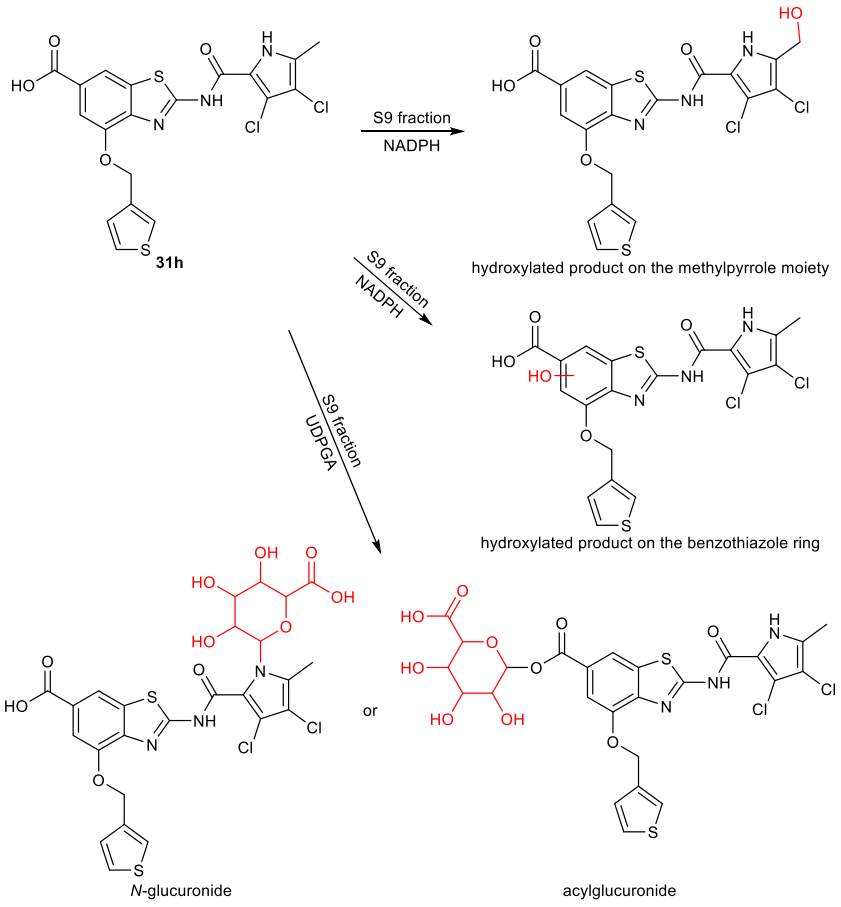
3. Conclusions

In conclusion, we have designed and synthesized new multi-targeting benzothiazole-based DNA gyrase and TopoIV inhibitors. The crystal structure of inhibitor **31c** in complex with *S. aureus* DNA gyrase resolved to a resolution of 1.7 Å confirmed the binding mode of these types of inhibitors in the ATP binding site. Compound **31h** showed potent low nanomolar dual inhibition of GyrB and ParE from *S. aureus* and *E. coli*, and excellent broad-spectrum antibacterial activity against resistant pathogens belonging to ESKAPE group, including MRSA, VISA, *K. pneumoniae*, *A. baumannii*, and *P. aeruginosa*. Compound **31h** also showed potency against clinical *A. baumannii*, *E. coli*, and *P. aeruginosa* isolates. Furthermore, **31h** showed good selectivity for DNA gyrase and TopoIV over hTopoII, no cytotoxicity in MCF-7 and HepG2 cells, no *in vitro* genotoxicity at concentrations up to 16 μM, and no formation of reactive metabolites. Further microbiological evaluation against mutated strains revealed promising results. Thus, analogs of this class represent new and much-needed scaffolds for the further development of new antibacterials for treatment of resistant Gram-positive and Gram-negative infections.

4. Experimental section

4.1. General chemistry information

Chemicals were obtained from Acros Organics (Geel, Belgium), Sigma-Aldrich (St. Louis, MO, USA), and Apollo Scientific (Stockport, UK), and were used without further purification. Analytical TLC was performed on silica gel Merck 60 F_{254} plates (0.25 mm), using visualization with UV light and spray reagents. Column chromatography was carried out on silica gel 60 (particle size, 240–400 mesh). Analytical reversed-phase HPLC analyses were performed on a 1260 Infinity II LC system (Agilent Technologies Inc., Santa Clara, CA, USA) for method A or a Dionex Ultimate 3000 binary rapid separation LC system (Thermo Scientific, Thermo Fisher Scientific, Waltham, MA, USA) for methods B and C. Method A: A Waters XBridge C18 column was used (3.5 μm, 4.6 mm × 150 mm), with flow rate of 1.5 mL/min and sample injection volume of 10 μL. The mobile phase consisted of acetonitrile (solvent A) and 0.1% formic acid in 1% acetonitrile in ultrapure water (solvent B). The gradient (defined for solvent A) was: 0–1.0 min, 25%; 1.0–6.0 min, 25%–98%; 6.0–6.5 min, 98%; 6.5–7.5 min, 98%–25%; 7.5–10.5 min, 25%. Method B: An Agilent Extend-C18 column was used (3.5 μm, 4.6 × 150 mm), with flow rate of 1.0 mL/min and sample injection volume of 10 μL. The mobile phase consisted of acetonitrile (solvent A) and 0.1% trifluoroacetic acid (TFA) in ultrapure water (solvent B). The gradient (defined for solvent A) was: 0–16 min, 30–90%; 16–20 min, 90%; 20–21 min, 90-30%. Method C: A Waters Acquity UPLC CSH C18 column was used (1.7 μm, 2.1 × 50 mm), with flow rate of 0.4 mL/min and sample injection volume of 1–4 μL. The mobile phase consisted of acetonitrile (solvent A) and 0.1% trifluoroacetic acid (TFA) in ultrapure water (solvent B). The gradient (defined for solvent A) was: 0–8 min, 30–90%; 8–10 min, 90%; 10–11 min, 90-30%. ^{1}H and ^{13}C NMR spectra were recorded at 400 and 100 MHz, respectively, on a Bruker AVANCE III 400 spectrometer (Bruker Corporation, Billerica, MA, USA) in DMSO-d_6 or $CDCl_3$ solutions, with TMS as the internal standard. Mass spectra were obtained using Exactive Plus Orbitrap mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) or an ADVION expression CMS^{L} mass spectrometer (Advion Inc., Ithaca, USA).



Scheme 6. Identified metabolic transformation products of **31h**.

4.2. Synthetic procedures and analytical data

General procedure A. Synthesis of compounds 2a, 2b, and 27a (with 2a as an example). To a solution of methyl 2-aminobenzo[*d*]thiazole-6-carboxylate (**1a**, 201 mg, 0.964 mmol) in *N*,*N*-dimethylformamide, Na_2CO_3 (102 mg, 0.964 mmol) and 2,2,2-trichloro-1-(4,5-dichloro-1*H*-pyrrole-2-yl)-ethan-1-one (295 mg, 1.06 mmol) were added, and the reaction mixture was stirred at 80 °C overnight. The reaction mixture was cooled to room temperature, 10% citric acid aqueous solution was added, and the mixture was cooled in an ice bath. The precipitate that formed was filtered off and dried to give 2a (240 mg) as an off-white solid.

Methyl 2-(4,5-dichloro-1*H*-pyrrole-2-carboxamido)benzo[*d*]thiazole-6-carboxylate (2a). Yield: 240 mg (70.0%); off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.89 (s, 3H), 7.54 (s, 1H), 7.84 (d, J = 8.5 Hz, 1H), 8.04 (dd, J = 8.5, 1.7 Hz, 1H), 8.68 (d, J = 1.7 Hz, 1H), 12.87 (s, 1H), 13.37 (s, 1H). HRMS (ESI$^-$) *m/z* for $C_{14}H_8Cl_2N_3O_3S$ ([M − H]$^-$): calculated 367.9660, found 367.9663.

Methyl 2-(4,5-dibromo-1*H*-pyrrole-2-carboxamido)benzo[*d*]thiazole-6-carboxylate (2b). Synthesized according to general procedure A. Yield: 91.6% (1.01 g); light brown solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.89 (s, 3H), 7.55 (d, J = 8.5 Hz, 1H), 7.81 (s, 1H), 8.04 (dd, J = 8.5, 1.8 Hz, 1H), 8.68 (d, J = 1.8 Hz, 1H), 12.84 (s, 1H), 13.30 (s, 1H). HRMS (ESI$^-$) *m/z* for $C_{14}H_8Br_2N_3O_3S$ ([M − H]$^-$): calculated 455.8653, found 455.8663.

General procedure B. Synthesis of compounds 2c, 2d, 5a, 5b, 16a-c, 24, 27b, 30a-i, 37, 43, and 47 (with 2c as an example). To a suspension of 4-bromo-3-chloro-5-methyl-1*H*-pyrrole-2-carboxylic acid (101 mg, 0.42 mmol) in anhydrous dichloromethane (10 mL) oxalyl chloride (0.181 mL, 2.11 mmol) was added dropwise and the solution stirred at room temperature under an argon atmosphere overnight. The solvent was evaporated under reduced pressure, ethyl 2-aminobenzo [*d*]thiazole-6-carboxylate (**1b**, 94 mg, 0.42 mmol) and toluene (20 mL) were added, and the suspension was stirred at 130 °C overnight. The precipitate in the reaction mixture was filtered off, resuspended in 1 M HCl (100 mL), sonicated and filtered off. The crude product was dispersed in methanol (100 mL), heated, filtered off and dried, to obtain **2c** (121 mg) as a gray solid.

Ethyl 2-(4-bromo-3-chloro-5-methyl-1*H*-pyrrole-2-carboxamido)benzo[*d*]thiazole-6-carboxylate (2c). Synthesized according to general procedure B using ethyl 2-aminobenzo [*d*] thiazole-6-carboxylate (**1b**, 0.094 mg, 0.42 mmol). Yield: 121 mg (64.6%); gray solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.35 (t, J = 7.1 Hz, 3H), 2.28 (s, 3H), 4.35 (q, J = 7.1 Hz, 2H), 7.83 (s, 1H), 8.03 (dd, J = 8.5, 1.8 Hz, 1H), 8.66 (s, 1H), 11.95 (s, 1H), 12.43 (s, 1H). HRMS (ESI$^+$) *m/z* for $C_{14}H_9Cl_2N_4O_2S$ ([M+H]$^+$): calculated 441.9622, found 441.9619.

Methyl 2-(3,4-dichloro-5-methyl-1*H*-pyrrole-2-carboxamido)benzo[*d*]thiazole-5-carboxylate (2d). Synthesized according to general procedure B using methyl 2-aminobenzo [*d*] thiazole-5-carboxylate (**1c**, 250 mg, 1.20 mmol). The crude product was dispersed in acetone (100 mL), sonicated, filtered off and dried. Yield: 210 mg (45.5%); off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.24 (s, 3H), 3.93 (s, 3H), 7.81 (d, J = 8.8 Hz, 1H), 8.09 (dd, J = 8.9, 2.5 Hz, 1H), 8.54 (d, J = 2.5 Hz, 1H), 9.92 (s, 1H), 12.20 (s, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 10.92, 53.26, 108.85, 111.83, 112.32, 119.13, 122.35, 122.45, 125.79, 126.38, 128.34, 128.61, 138.86, 157.52, 165.94. HRMS (ESI$^-$) *m/z* for $C_{15}H_{10}Cl_2N_3O_3S$ ([M − H]$^-$):

calculated 381.9825, found 381.9830.

General procedure C. Synthesis of compounds 3a-d, 7c, 14, 25, 28a, 28b, 31a-f, and 35 (with 3b as an example). Methyl 2-(4,5-dibromo-1*H*-pyrrole-2-carboxamido)benzo [*d*]thiazole-6-carboxylate (**2b**, 0.800 g, 1.74 mmol) was suspended in 1,4-dioxane (30 mL), 2 M NaOH (4.35 mL, 8.70 mmol) was added, and the reaction mixture was stirred at 80 °C overnight. The solvent was removed under reduced pressure, the residue was acidified with 1 M HCl to pH 1, and the precipitate obtained was filtered off and dried to give **3b** (565 mg) as a brown solid.

2-(4,5-Dichloro-1*H*-pyrrole-2-carboxamido)benzo [*d*]thiazole-6-carboxylic acid (3a). Synthesized according to general procedure C using methyl 2-(4,5-dichloro-1*H*-pyrrole-2-carboxamido)benzo [*d*]thiazole-6-carboxylate (**2a**, 240 mg, 0.65 mmol). Yield: 205 mg (88.7%); brown solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.53 (d, J = 2.1 Hz, 1H), 7.82 (d, J = 8.4 Hz, 1H), 8.02 (dd, J = 8.5, 1.7 Hz, 1H), 8.64 (d, J = 1.7 Hz, 1H), 12.86 (s, 1H), 13.37 (s, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 109.71, 114.13, 119.05, 120.34, 122.80, 124.27, 126.28, 127.84, 132.12, 152.17, 158.06, 161.97, 167.51. HRMS (ESI$^-$) m/z for $C_{13}H_6Cl_2N_3O_3S$ ([M − H]$^-$): calculated 353.9498, found 353.9507. HPLC: t_r 5.80 min (92.9% at 254 nm), method A.

2-(4,5-Dibromo-1*H*-pyrrole-2-carboxamido)benzo[*d*]thiazole-6-carboxylic acid (3b). Yield: 565 mg (68.5%); brown solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.55 (d, J = 2.7 Hz, 1H), 7.82 (d, J = 8.5 Hz, 1H), 8.02 (dd, J = 8.5, 1.7 Hz, 1H), 8.64 (d, J = 1.4 Hz, 1H), 12.83 (s, 1H), 13.31 (s, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 99.10, 110.11, 116.74, 120.14, 124.11, 126.45, 127.03, 127.77, 132.13, 152.26, 158.46, 162.21, 167.70. HRMS (ESI$^-$) m/z for $C_{13}H_6Br_2N_3O_3S$ ([M − H]$^-$): calculated 441.8497, found 441.8501. HPLC: t_r 5.89 min (96.1% at 254 nm), method A.

2-(4-Bromo-3-chloro-5-methyl-1*H*-pyrrole-2-carboxamido) benzo[*d*]thiazole-6-carboxylic acid (3c). Synthesized according to general procedure C using ethyl 2-(4-bromo-3-chloro-5-methyl-1*H*-pyrrole-2-carboxamido)benzo [*d*]thiazole-6-carboxylate (**2c**, 100 mg, 0.23 mmol). Yield: 80 mg (85.4%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.28 (d, J = 11.0 Hz, 3H), 7.74 (d, J = 8.2 Hz, 1H), 7.99 (d, J = 8.6 Hz, 1H), 8.56 (s, 1H), 12.53 (s, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 12.60, 98.29, 117.47, 119.30, 119.53, 124.17, 126.62, 127.87, 131.76, 132.08, 151.17, 158.65, 162.81, 167.86. HRMS (ESI$^+$) m/z for $C_{14}H_{10}BrClN_3O_3S$ ([M+H]$^+$): calculated 413.9309, found 413.9321. HPLC: t_r 6.44 min (95.5% at 254 nm), method A.

2-(3,4-Dichloro-5-methyl-1*H*-pyrrole-2-carboxamido)benzo [*d*]thiazole-5-carboxylic acid (3d). Synthesized according to general procedure C using methyl 2-(3,4-dichloro-5-methyl-1*H*-pyrrole-2-carboxamido)benzo [*d*]thiazole-5-carboxylate (**2e**, 100 mg, 0.260 mmol) at room temperature. The product was isolated by cooling the reaction mixture to room temperature, then it was acidified using ion exchange resin (Amberlite IR120; pH<5) and stirred at room temperature for 5 min. DMF was added to dissolve the precipitate that formed. The resin was filtered off and the filtrate was evaporated to dryness. The residue was dispersed in acetone, sonicated and filtered off. The product was washed with acetonitrile and diethyl ether and dried. Yield: 64 mg (66.4%); off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.25 (s, 3H), 7.79 (d, J = 8.8 Hz, 1H), 8.09 (dd, J = 8.8, 2.3 Hz, 1H), 8.50 (d, J = 2.2 Hz, 1H), 9.88 (s, 1H), 12.21 (s, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 11.30, 109.23, 112.62, 112.71, 119.61, 123.04, 125.79, 127.87, 128.26, 128.93, 139.11, 157.90, 167.94. HRMS (ESI$^-$) m/z for $C_{14}H_8Cl_2N_3O_3S$ ([M − H]$^-$): calculated 367.9669, found 367.9661. HPLC: t_r 6.61 min (96.1% at 254 nm), method A.

3,4-Dichloro-*N*-(6-methoxybenzo[*d*]thiazol-2-yl)-5-methyl-1*H*-pyrrole-2-carboxamide (5a). Synthesized according to general procedure B using 6-methoxybenzo [*d*]thiazol-2-amine (**4a**, 110 mg, 0.515 mmol). The crude product was dispersed in methanol, sonicated, filtered off, washed with methanol and dried. Yield: 74 mg (40%); brown solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.26 (s, 3H), 3.82 (s, 3H), 7.05 (dd, J = 2.6, 8.8 Hz, 1H), 7.58 (d, J = 2.6 Hz, 1H), 7.64 (d, J = 8.8 Hz, 1H), 11.57 (s, 1H), 12.30 (s, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 10.89, 55.55, 104.80, 109.53, 114.44, 114.89, 117.49, 120.32, 128.11, 128.80, 129.55, 132.38, 141.39, 156.11. HRMS (ESI$^+$) m/z for $C_{14}H_{12}Cl_2N_3O_2S$ ([M+H]$^+$): calculated 356.0022, found 356.0020. HPLC: t_r 7.19 min (98.0% at 254 nm), method A.

3,4-Dichloro-*N*-(6-cyanobenzo[*d*]thiazol-2-yl)-5-methyl-1*H*-pyrrole-2-carboxamide (5b). Synthesized according to general procedure B using 2-aminobenzo [*d*]thiazole-6-carbonitrile (**4b**, 400 mg, 2.28 mmol). The crude product was dispersed in acetonitrile, sonicated, filtered off, and dried. Yield: 310 mg (82%); gray solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.27 (s, 3H), 7.78−8.00 (m, 2H), 8.59 (s, 1H), 12.06 (s, 1H), 12.39 (s, 1H). HRMS (ESI$^+$) m/z for $C_{14}H_9Cl_2N_4OS$ ([M+H]$^+$): calculated 350.9869, found 350.9859. HPLC: t_r 7.06 min (98.0% at 254 nm), method A.

***N*-(6-(1*H*-Tetrazol-5-yl)benzo[*d*]thiazol-2-yl)-3,4-dichloro-5-methyl-1*H*-pyrrole-2-carboxamide (5c).** A solution of 3,4-dichloro-*N*-(6-cyanobenzo [*d*]thiazol-2-yl)-5-methyl-1*H*-pyrrole-2-carboxamide (**5b**, 100 mg, 0.285 mmol), ammonium chloride (152 mg, 2.85 mmol), and sodium azide (185 mg, 2.85 mmol) in DMF (5 mL) was heated to 100 °C for 15 h. The mixture was cooled down to room temperature, water (7 mL) and ethyl acetate (7 mL) were added, and the mixture was acidified with 1 M HCl to pH 3−4. The precipitate was filtered off, washed with ethyl acetate, and dried. The crude product was dispersed in acetone (5 mL), sonicated, filtered off, and dried. Yield: 67 mg (59.7%); brown solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.29 (s, 3H), 7.95 (s, 1H), 8.08−8.18 (m, 1H), 8.70 (s, 1H), 12.00 (s, 1H), 12.41 (s, 1H), the signal for the tetrazole NH not seen. HRMS (ESI$^+$) m/z for $C_{14}H_{10}Cl_2N_7OS$ ([M+H]$^+$): calculated 394.0039, found 394.0039. HPLC: t_r 6.13 min (95.3% at 254 nm), method A.

2-(3,4-Dichloro-5-methyl-1*H*-pyrrole-2-carboxamido)benzo [*d*]thiazole-6-carboxamide (5d). 3,4-dichloro-*N*-(6-cyanobenzo [*d*]thiazol-2-yl)-5-methyl-1*H*-pyrrole-2-carboxamide (**5b**, 100 mg, 0.28 mmol) was dissolved in *N*-methyl-2-pyrrolidone (10 mL), pulverized KOH (320 mg, 5.96 mmol) was added, and the reaction mixture was stirred at 100 °C overnight. Additional 10 equivalents of KOH (160 mg, 2.8 mmol) were added and the reaction mixture was stirred at 115 °C overnight. The reaction mixture was cooled to room temperature, diluted with water, and acidified with 2 M HCl. The resulting precipitate was filtered off and purified with flash column chromatography using hexane/ethyl acetate/THF (1:1:1) → ethyl acetate/THF (1:1) as eluent. Yield: 10 mg, (10.4%); white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.27 (s, 3H), 7.40 (s, 1H), 7.76 (d, J = 8.5 Hz, 1H), 7.96 (d, J = 8.1 Hz, 1H), 8.04 (s, 1H), 8.49 (s, 1H), 11.99 (s, 1H), 12.52 (s, 1H). HRMS (ESI$^-$) m/z for $C_{14}H_9Cl_2N_4O_2S$ ([M − H]$^-$): calculated 366.9829, found 366.9822. HPLC: t_r 5.70 min (95.3% at 254 nm), method A.

General procedure D. Synthesis of compounds 6a-d, 33, and 36 (with 6a as an example). A solution of 2-(4,5-dibromo-1*H*-pyrrole-2-carboxamido)benzo [*d*]thiazole-6-carboxylic acid (**3b**, 100 mg, 0.225 mmol) in *N*,*N*-dimethylformamide (3 mL) was cooled to 0 °C and then EDC (42 mg, 0.27 mmol) and HOBt (40 mg, 0.295 mmol) were added. The pH was adjusted to 8 with *N*-methylmorpholine, and the reaction mixture was stirred for 20 min at 0 °C. Then *O*-(tetrahydro-2*H*-pyran-2-yl)hydroxylamine (26 mg, 0.225 mmol) was added, and reaction mixture was stirred overnight at room temperature. The solvent was evaporated *in vacuo* and the residue dissolved in ethyl acetate (30 mL), and washed successively with 1% citric acid (15 mL), saturated aqueous $NaHCO_3$ solution (15 mL), and brine (30 mL). The organic phase was dried over Na_2SO_4 and filtered, and the solvent evaporated off

under reduced pressure.

2-(4,5-Dibromo-1*H*-pyrrole-2-carboxamido)-*N*-((tetrahydro-2*H*-pyran-2-yl)oxy)benzo[*d*]thiazole-6-carboxamide (6a). Crude product was purified using flash column chromatography using dichloromethane/methanol (20:1) as eluent. Yield: 8.7 mg (7.0%); beige solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.57 (s, 3H), 1.74 (s, 3H), 3.50−3.59 (m, 1H), 4.01−4.15 (m, 1H), 5.03 (s, 1H), 7.55 (d, J = 2.7 Hz, 1H), 7.79−7.89 (m, 2H), 8.42 (d, J = 1.6 Hz, 1H), 11.69 (s, 1H), 12.79 (s, 1H), 13.28 (s, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 18.78, 25.22, 28.38, 61.81, 97.68, 101.41, 116.00, 119.85, 121.45, 123.41, 124.67, 125.61, 127.14, 132.14, 151.79, 159.66, 161.96, 164.71. HRMS (ESI$^+$) m/z for $C_{18}H_{17}Br_2N_4O_4S$ ([M+H]$^+$): calculated 542.9332, found 542.9334.

2-(3,4-Dichloro-5-methyl-1*H*-pyrrole-2-carboxamido)-*N*-((tetrahydro-2*H*-pyran-2-yl)oxy)benzo[*d*]thiazole-6-carboxamide (6b). Synthesized according to general procedure D using 2-(3,4-dichloro-5-methyl-1*H*-pyrrole-2-carboxamido)benzo [*d*]thiazole-6-carboxylic acid (**3d**, 0.150 g, 0.41 mmol). Yield: 70 mg (36.8%); off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.56 (s, 3H), 1.74 (s, 3H), 2.24 (d, J = 2.2 Hz, 3H), 3.50−3.58 (m, 1H), 4.03−4.13 (m, 1H), 5.01 (s, 1H), 7.63 (d, J = 9.5 Hz, 1H), 7.72−7.80 (m, 1H), 8.26 (d, J = 8.6 Hz, 1H), 11.61 (s, 1H). HRMS (ESI$^+$) m/z for $C_{14}H_9Cl_2N_4O_2S$ ([M+H]$^+$): calculated 469.0499, found 469.0495.

Ethyl 3-(2-(3,4-dichloro-5-methyl-1*H*-pyrrole-2-carboxamido)benzo[*d*]thiazole-6-carboxamido)propanoate (6c). Synthesized according to general procedure D using 3,4-dichloro-5-methyl-1*H*-pyrrole-2-carboxamido)benzo [*d*]thiazole-6-carboxylic acid (**3d**, 80 mg, 0.22 mmol). Yield: 47 mg (45.5%); light brown solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.19 (t, J = 7.0 Hz, 3H), 2.28 (s, 3H), 2.61 (t, J = 6.9 Hz, 2H), 3.53 (q, J = 5.7 Hz, 2H), 4.09 (q, J = 7.2 Hz, 2H), 7.79 (s, 1H), 7.93 (d, J = 8.5 Hz, 1H), 8.46 (s, 1H), 8.63 (t, J = 4.6 Hz, 1H), 11.92 (s, 1H), 12.34 (s, 1H). HRMS (ESI$^+$) m/z for $C_{19}H_{19}Cl_2N_4O_4S$ ([M+H]$^+$): calculated 469.0499, found 469.0497.

***N*-(Cyanomethyl)-2-(3,4-dichloro-5-methyl-1*H*-pyrrole-2-carboxamido)benzo[*d*]thiazole-6-carboxamide (6d).** Synthesized according to general procedure D using 3,4-dichloro-5-methyl-1*H*-pyrrole-2-carboxamido)benzo [*d*]thiazole-6-carboxylic acid (**3d**, 60 mg, 0.162 mmol). Yield: 45 mg (68.0%); gray solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.28 (s, 3H), 4.35 (d, J = 5.4 Hz, 2H), 7.83 (s, 1H), 7.95 (dd, J = 8.5, 1.8 Hz, 1H), 8.52 (s, 1H), 9.27 (t, J = 5.5 Hz, 1H), 11.97 (s, 1H), 12.34 (s, 1H). HRMS (ESI$^+$) m/z for $C_{16}H_{12}Cl_2N_5O_2S$ ([M+H]$^+$): calculated 408.0083, found 408.0082. HPLC: t_r 6.14 min (95.2% at 254 nm), method A.

General procedure E. Synthesis of compounds 7a, 7b (with 7a as an example). A solution of 2-(4,5-dibromo-1*H*-pyrrole-2-carboxamido)-*N*-((tetrahydro-2*H*-pyran-2-yl)oxy)benzo [*d*]thiazole-6-carboxamide (**6a**, 8.7 mg, 0.016 mmol) in dichloromethane (5 mL) and CF_3COOH (0.012 mL, 0.161 mmol) was stirred at room temperature for 2 h. The precipitate was filtered off, washed with dichloromethane, and dried to give **7a** (1.7 mg) as a white solid.

2-(4,5-Dibromo-1*H*-pyrrole-2-carboxamido)-*N*-hydroxybenzo[*d*]thiazole-6-carboxamide (7a). Yield: 1.7 mg (23%); white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.55 (s, 1H), 7.76−7.90 (m, 2H), 8.40 (s, 1H), 11.27 (s, 1H), 12.78 (s, 1H), 13.28 (s, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 99.62, 109.34, 116.80, 120.38, 121.37, 125.53, 126.05, 128.51, 132.03, 151.07, 157.72, 160.81, 164.57. HRMS (ESI$^+$) m/z for $C_{13}H_9Br_2N_4O_3S$ ([M+H]$^+$): calculated 458.87566, found 458.87604. HPLC: t_r 5.12 min (96.1% at 254 nm), method A.

2-(3,4-Dichloro-5-methyl-1*H*-pyrrole-2-carboxamido)-*N*-hydroxybenzo[*d*]thiazole-6-carboxamide (7b). Synthesized according to general procedure E using 2-(3,4-dichloro-5-methyl-1*H*-pyrrole-2-carboxamido)-*N*-((tetrahydro-2*H*-pyran-2-yl)oxy)benzo [*d*]thiazole-6-carboxamide (**6b**, 0.070 g, 0.15 mmol). Yield: 23 mg (40.0%); brown solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.28 (s, 3H), 7.78 (d, J = 8.6 Hz, 1H), 7.85 (dd, J = 8.4, 1.7 Hz, 1H), 8.39 (d, J = 1.6 Hz, 1H), 11.27 (s, 1H), 11.92 (s, 1H), 12.40 (s, 1H). HRMS (ESI$^-$) m/z for $C_{14}H_9Cl_2N_4O_3S$ ([M − H]$^-$): calculated 382.97669, found 382.97842. HPLC: t_r 5.46 min (97.4% at 254 nm), method A.

3-(2-(3,4-Dichloro-5-methyl-1*H*-pyrrole-2-carboxamido) benzo[*d*]thiazole-6-carboxamido)propanoic acid (7c). Synthesized according to general procedure C using ethyl 3-(2-(3,4-dichloro-5-methyl-1*H*-pyrrole-2-carboxamido)benzo [*d*]thiazole-6-carboxamido)propanoate (**6c**, 33 mg, 0.070 mmol) and 1 M NaOH, and the reaction mixture was stirred at room temperature, not at 80 °C. Yield: 30 mg (95.7%); brown solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.28 (s, 3H), 2.55 (t, J = 7.5 Hz, 2H), 3.49 (q, J = 7.0, Hz, 2H), 7.78 (d, J = 8.4 Hz, 1H), 7.93 (dd, J = 8.5, 1.8 Hz, 1H), 8.47 (d, J = 1.2 Hz, 1H), 8.61 (t, J = 5.5 Hz, 1H), 12.43 (s, 1H). HRMS (ESI$^-$) m/z for $C_{17}H_{13}Cl_2N_4O_4S$ ([M − H]$^-$): calculated 439.0040, found 439.0043. HPLC: t_r 5.72 min (90.7% at 254 nm), method A.

General procedure F. Synthesis of compounds 9 and 15c (with 9 as an example). To a solution of 2-aminobenzo [*d*]thiazole-6-carboxylic acid (1.50 g, 7.7 mmol) in dry DMF (10 mL), potassium carbonate (1.60 g, 11.58 mmol) and 4-methoxybenzyl chloride (1.45 mL, 9.24 mmol) were added, and the mixture was stirred at room temperature for 14 h. The solvent was removed under reduced pressure, and to the residue, ethyl acetate (30 mL) and water (30 mL) were added. The organic phase was dried over Na_2SO_4 and filtered, and the solvent evaporated under reduced pressure, to give **9** (219 mg) as a white solid.

4-Methoxybenzyl 2-aminobenzo[*d*]thiazole-6-carboxylate (9). Yield: 219 mg (9.0%); white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.76 (s, 3H), 5.26 (s, 2H), 6.93−6.99 (m, 2H), 7.40−7.48 (m, 3H), 7.88 (dd, J = 8.5, 1.8 Hz, 1H), 8.37 (d, J = 1.8 Hz, 1H), 8.53 (s, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 55.59, 66.19, 114.35, 117.58, 122.20, 123.07, 127.62, 128.74, 130.42, 131.67, 157.47, 159.65, 166.01, 170.32. MS (ESI) m/z = 314.5 ([M+H]$^+$).

General procedure G. Synthesis of compounds 10 and 12 (with 12 as an example). To a solution of ethyl 2-aminobenzo [*d*] thiazole-6-carboxylate (**11**, 8.0 g, 36.0 mmol) and $CuBr_2$ (16.07 g, 72.0 mmol) in acetonitrile (200 mL), *tert*-butyl nitrite (7.42 mL, 72.0 mmol) was added in an ice bath. The reaction mixture was stirred at room temperature for 14 h. The solvent was removed under reduced pressure, and to the residue, ethyl acetate (200 mL) and NH_4Cl solution (200 mL) were added. The organic phase was washed with brine (100 mL) and NH_4Cl solution (100 mL), dried over Na_2SO_4, and filtered, and the solvent was evaporated under reduced pressure. Compound **12** (8.0 g) was obtained as a pale brown solid.

4-Methoxybenzyl 2-bromobenzo[*d*]thiazole-6-carboxylate (10). Synthesized according to general procedure G using 4-methoxybenzyl 2-aminobenzo [*d*]thiazole-6-carboxylate (**9**, 0.300 g, 0.95 mmol). The reaction mixture was stirred at room temperature for only 2 h. Yield: 0.354 g (98.1%); light brown solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.77 (s, 3H), 5.32 (s, 2H), 6.98 (d, J = 8.7 Hz, 2H), 7.45 (d, J = 8.7 Hz, 2H), 88.07−8.08 (m, 2H), 8.80 (s, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 55.61, 66.97, 114.39, 122.74, 124.68, 127.31, 127.96, 128.27, 130.62, 137.72, 144.76, 155.21, 159.79, 165.53. MS (ESI) m/z = 398.8 ([M + Na−2H]$^-$).

Ethyl 2-bromobenzo[*d*]thiazole-6-carboxylate (12) [28]. Yield: 8.0 g (78%); pale brown solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.36 (t, J = 7.1 Hz, 3H), 4.37 (q, J = 7.1 Hz, 2H), 8.10 (s, 2H), 8.82 (s, 1H).

General procedure H. Synthesis of compounds 13, 15a, and 15b (with 13 as an example). A solution of ethyl 2-bromobenzo [*d*] thiazole-6-carboxylate (**12**, 1.00 g, 3.49 mmol) and isopropylamine (2.86 mL, 34.9 mmol) in THF (70 mL) was stirred at room temperature for 14 h. The solvent was removed under reduced pressure. The crude product was dissolved in ethyl acetate (100 mL) and

washed successively with 1% citric acid (2 × 50 mL), saturated $NaHCO_3$ solution (50 mL), and brine (50 mL). The organic phase was dried over Na_2SO_4 and filtered, and the solvent was removed under reduced pressure, to give **13** (0.915 g) as a yellow oil.

Ethyl 2-(isopropylamino)benzo[*d*]thiazole-6-carboxylate (13). Yield: 0.915 g (99.1%), yellow oil. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.22 (d, J = 6.5 Hz, 6H), 1.32 (t, J = 7.1 Hz, 3H), 3.96–4.11 (m, 1H), 4.28 (q, J = 7.1 Hz, 2H), 7.41 (d, J = 8.4 Hz, 1H), 7.81 (dd, J = 8.4, 1.8 Hz, 1H), 8.28 (d, J = 1.6 Hz, 1H), 8.35 (d, J = 7.3 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 14.72, 22.72, 46.57, 60.81, 117.69, 122.34, 122.90, 127.57, 130.88, 157.27, 166.09, 168.54. MS (ESI) m/z = 265.0 ([M+H]$^+$).

2-(Isopropylamino)benzo[*d*]thiazole-6-carboxylic acid (14). Synthesized according to general procedure C using ethyl 2-(isopropylamino)benzo [*d*]thiazole-6-carboxylate (**13**, 0.940 g, 3.56 mmol), with pH was adjusted to 3. Yield: 0.749 g (89.1%); gray solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.22 (d, J = 6.6 Hz, 6H), 4.02 (q, J = 6.6 Hz, 1H), 7.39 (d, J = 8.1 Hz, 1H), 7.79 (d, J = 8.3 Hz, 1H), 8.13–8.45 (m, 2H), 12.62 (s, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 22.73, 46.53, 117.62, 123.10, 123.33, 127.80, 130.71, 156.98, 167.67, 168.30. MS (ESI) m/z = 237.1 ([M+H]$^+$).

4-Methoxybenzyl 2-(ethylamino)benzo[*d*]thiazole-6-carboxylate (15a). Synthesized according to general procedure H using 4-methoxybenzyl 2-bromobenzo [*d*]thiazole-6-carboxylate (**11**, 0.230 g, 0.61 mmol). Yield: 0.203 g (97.5%); white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.21 (t, J = 7.0 Hz, 3H), 3.41 (q, J = 7.0 Hz, 2H), 3.76 (s, 3H), 5.25 (s, 2H), 6.92–7.01 (m, 2H), 7.42 (dt, J = 8.5, 2.3 Hz, 3H), 7.82 (dd, J = 8.5, 1.8 Hz, 1H), 8.30 (d, J = 1.8 Hz, 1H), 8.42 (t, J = 5.3 Hz, 1H). MS (ESI) m/z = 343.1 ([M+H]$^+$).

4-Methoxybenzyl 2-(cyclopropylamino)benzo[*d*]thiazole-6-carboxylate (15b). Synthesized according to general procedure H using 4-methoxybenzyl 2-bromobenzo [*d*]thiazole-6-carboxylate (**11**, 0.279 g, 0.74 mmol). Yield: 0.211 g (80.7%); white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.55–0.64 (m, 2H), 0.79 (td, J = 6.9, 4.7 Hz, 2H), 2.73 (s, 1H), 3.76 (s, 3H), 5.26 (s, 2H), 6.93–7.00 (m, 2H), 7.39–7.51 (m, 3H), 7.82–7.88 (m, 1H), 8.38 (d, J = 1.8 Hz, 1H), 8.77 (s, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 7.32, 26.71, 55.59, 66.22, 114.36, 118.03, 122.16, 123.28, 127.70, 128.73, 130.43, 131.12, 133.51, 157.27, 159.66, 166.02. MS (ESI) m/z = 355.0 ([M+H]$^+$).

4-Methoxybenzyl 2-(isopropylamino)benzo[*d*]thiazole-6-carboxylate (15c). Synthesized according to general procedure F using 2-(isopropylamino)benzo [*d*]thiazole-6-carboxylic acid (**14**, 0.568 g, 2.40 mmol). The crude product was recrystallized from ethyl acetate. Yield: 0.806 g (94.1%), white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.22 (d, J = 6.5 Hz, 6H), 3.76 (s, 3H), 4.02 (q, J = 6.7 Hz, 1H), 5.25 (s, 2H), 6.92–7.02 (m, 2H), 7.31–7.50 (m, 3H), 7.82 (dd, J = 8.4,1.8 Hz, 1H), 8.29 (d, J = 1.8 Hz, 1H), 8.36 (d, J = 7.3 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 22.67, 46.73, 55.57, 66.21, 114.34, 117.55, 122.31, 123.11, 127.77, 128.71, 128.96, 130.41, 156.60, 159.65, 165.95, 168.56. MS (ESI) m/z = 355.1 ([M − H]$^-$).

4-Methoxybenzyl 2-(3,4-dichloro-*N*-ethyl-5-methyl-1*H*-pyrrole-2-carboxamido)benzo[*d*]thiazole-6-carboxylate (16a). Synthesized according to general procedure B using 4-methoxybenzyl 2-(ethylamino)benzo [*d*]thiazole-6-carboxylate (**15a**, 0.180 g, 0.53 mmol). Yield: 0.078 g (28.6%); white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.22 (t, J = 7.0 Hz, 3H), 2.25 (s, 3H), 3.76 (s, 3H), 4.40 (q, J = 7.0Hz, 2H), 5.30 (s, 2H), 6.97–7.02 (m, 2H), 7.45 (d, J = 8.7 Hz, 2H), 7.88–7.95 (m, 1H), 8.03 (dd, J = 8.5, 1.8 Hz, 1H), 8.68 (dd, J = 1.7, 0.4 Hz, 1H), 12.50 (s, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 11.43, 14.52, 45.79, 55.61, 66.62, 109.09, 113.10, 114.38, 118.17, 121.42, 124.30, 125.67, 127.67, 128.51, 130.33, 130.50, 132.90, 152.32, 159.72, 162.29, 163.66, 165.81. MS (ESI) m/z = 516.0 ([M − H]$^-$).

4-Methoxybenzyl 2-(3,4-dichloro-*N*-cyclopropyl-5-methyl-1*H*-pyrrole-2-carboxamido)benzo[*d*]thiazole-6-carboxylate (16b). Synthesized according to general procedure B using 4-methoxybenzyl 2-(cyclopropylamino)benzo [*d*]thiazole-6-carboxylate (**15b**, 0.201 g, 0.57 mmol). Yield: 0.092 g (30.6%); white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.59–0.66 (m, 2H), 1.04 (q, J = 6.9 Hz, 2H), 2.26 (s, 3H), 3.68 (tt, J = 7.3, 3.9 Hz, 1H), 3.77 (s, 3H), 5.31 (s, 2H), 6.95–7.01 (m, 2H), 7.43–7.47 (m, 2H), 7.87–7.93 (m, 1H), 8.02 (dt, J = 8.6, 2.1 Hz, 1H), 8.66–8.69 (m, 1H), 12.31 (s, 1H). MS (ESI) m/z = 530.0 ([M+H]$^+$).

4-Methoxybenzyl 2-(3,4-dichloro-*N*-isopropyl-5-methyl-1*H*-pyrrole-2-carboxamido)benzo[*d*]thiazole-6-carboxylate (16c). Synthesized according to general procedure B using 4-methoxybenzyl 2-(isopropylamino)benzo [*d*]thiazole-6-carboxylate (**15c**, 0.150 g, 0.42 mmol). The crude product was purified by preparative TLC using dichloromethane/methanol (20:1) as eluent. Yield: 0.031 g (13.8%); white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.56 (s, 6H), 2.30 (s, 3H), 3.82 (s, 3H), 5.03 (p, J = 6.8 Hz, 1H), 5.31 (s, 2H), 6.90–6.95 (m, 2H), 7.37–7.43 (m, 2H), 7.81 (d, J = 8.5 Hz, 1H), 8.10 (dd, J = 8.6, 1.7 Hz, 1H), 8.40 (d, J = 1.6 Hz, 1H), 9.09 (s, 1H).

General procedure I. Synthesis of compounds 17a-c (with 17a as an example). To a suspension of 4-methoxybenzyl 2-(3,4-dichloro-*N*-ethyl-5-methyl-1*H*-pyrrole-2-carboxamido)benzo [*d*] thiazole-6-carboxylate (63 mg, 0.12 mmol) in glacial acetic acid (5 mL), 1 M HCl in acetic acid (1.2 mmol) was added, and the reaction mixture was stirred at room temperature overnight. The precipitate was filtered off, washed with diethyl ether, and dried *in vacuo* to obtain **17a** (13 mg) as a white solid.

2-(3,4-Dichloro-*N*-ethyl-5-methyl-1*H*-pyrrole-2-carboxamido)benzo[*d*]thiazole-6-carboxylic acid (17a). Yield: 13 mg (26.9%); white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.23 (t, J = 6.9 Hz, 3H), 2.26 (s, 3H), 4.41 (q, J = 6.9 Hz, 2H), 7.90 (d, J = 8.5 Hz, 1H), 8.02 (dd, J = 8.5, 1.6 Hz, 1H), 8.63 (d, J = 1.5 Hz, 1H), 12.52 (s, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 10.86, 13.96, 45.20, 108.46, 112.44, 117.65, 120.67, 123.68, 126.26, 127.26, 129.69, 132.13, 151.42, 161.70, 162.66, 166.88. HRMS (ESI$^+$) m/z for $C_{16}H_{14}Cl_2N_3O_3S$ ([M+H]$^+$): calculated 398.0127, found 398.0125. HPLC: t_r 3.813 min (97.4% at 254 nm), method C.

2-(3,4-Dichloro-*N*-cyclopropyl-5-methyl-1*H*-pyrrole-2-carboxamido)benzo[*d*]thiazole-6-carboxylic acid (17b). Synthesized according to general procedure I using 4-methoxybenzyl 2-(3,4-dichloro-*N*-cyclopropyl-5-methyl-1*H*-pyrrole-2-carboxamido)benzo [*d*]thiazole-6-carboxylate (50 mg, 0.094 mmol). Yield: 10 mg (25.9%); white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.63 (s, 2H), 1.04 (d, J = 6.9 Hz, 2H), 2.27 (s, 3H), 7.89 (d, J = 8.5 Hz, 1H), 8.00 (dd, J = 8.6, 1.8 Hz, 1H), 8.62 (d, J = 1.8 Hz, 1H), 12.32 (s, 1H). ^{13}C NMR (101 MHz, DMSO- d_6) δ 10.95, 11.44, 32.53, 109.50, 114.65, 119.20, 121.17, 124.14, 126.52, 127.71, 130.32, 132.64, 152.49, 162.34, 165.03, 167.51. HRMS (ESI$^+$) m/z for $C_{17}H_{14}Cl_2N_3O_3S$ ([M+H]$^+$): calculated 410.0127, found 410.0124. HPLC: t_r 3.820 min (92.5% at 254 nm), method C.

2-(3,4-Dichloro-*N*-isopropyl-5-methyl-1*H*-pyrrole-2-carboxamido) benzo[*d*]thiazole-6-carboxylic acid (17c). Synthesized according to general procedure I using 4-methoxybenzyl 2-(3,4-dichloro-*N*-isopropyl-5-methyl-1*H*-pyrrole-2-carboxamido) benzo [*d*]thiazole-6-carboxylate (29 mg, 0.055 mmol). Yield: 18 mg (80.2%); white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.51 (d, J = 6.2 Hz, 6H), 2.23 (s, 3H), 4.96 (s, 1H), 7.83 (d, J = 8.2 Hz, 1H), 7.97 (d, J = 7.0 Hz, 1H), 8.53 (s, 1H), 12.53 (s, 1H). HRMS (ESI$^+$) m/z for $C_{17}H_{16}Cl_2N_3O_3S$ ([M+H]$^+$): calculated 412.0284, found 412.0279. HPLC: t_r 3.873 min (98.0% at 254 nm), method C.

Methyl 4-amino-2-hydroxybenzoate (19) [29]. To a solution of 4-aminosalicylic acid (**18**, 7.50 g, 49.0 mmol) in methanol (70 mL), H_2SO_4 (4 mL, 75 mmol) was added, and the solution was stirred at 65 °C for 24 h. The solvent was removed *in vacuo*, and the residue dissolved in ethyl acetate (100 mL) and neutralized with saturated aqueous $NaHCO_3$ solution (100 mL). The phases were separated, the

water phase was extracted with ethyl acetate (3 × 75 mL), and the combined organic phases were washed with brine (3 × 75 mL), dried over Na_2SO_4, and filtered, and the solvent removed *in vacuo*. Yield: 7.26 g (88.6%); brown solid; mp: 99–104 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.90 (s, 3H), 4.12 (s, 2H), 6.12–6.20 (m, 2H), 7.64 (dd, J = 8.0, 0.9 Hz, 1H), 10.97 (s, 1H).

Methyl 4-((*tert*-butoxycarbonyl)amino)-2-hydroxybenzoate (20) [30]. To a solution of methyl 4-amino-2-hydroxybenzoate (9.57 g, 57.3 mmol), di-*tert*-butyl dicarbonate (13.8 g, 63.0 mmol) was added, and the mixture was stirred at 70 °C for 48 h. The solvent was removed under reduced pressure, to the residue ethyl acetate (100 mL) and water were added, and the phases were separated. The organic phase was washed with 1 M HCl (3 × 40 mL) and brine (3 × 40 mL), dried over Na_2SO_4, and filtered, and the solvent was removed *in vacuo*. The crude product was purified with flash column chromatography using ethyl acetate/hexane (1:7) as eluent. Yield: 6.52 g (43%); white crystals. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.54 (s, 9H), 3.94 (s, 3H), 6.62 (s, 1H), 6.95 (dd, J = 8.7, 2.2 Hz, 1H), 7.01 (d, J = 2.1 Hz, 1H), 7.76 (d, J = 8.7 Hz, 1H), 10,86 (s, 1H).

Methyl 2-acetoxy-4-((*tert*-butoxycarbonyl)amino)benzoate (21) [31]. To a solution of methyl 4-((*tert*-butoxycarbonyl)amino)-2-hydroxybenzoate (1.28 g, 4.8 mmol) in acetonitrile (15 mL), pyridine (0.694 mL, 8.6 mmol) and acetic anhydride (0.869 mL, 8.6 mmol) were added, and the mixture was stirred at 70 °C for 48 h. The solvent was removed under reduced pressure, and the residue was dissolved in ethyl acetate (40 mL). The organic phase was washed with 1 M HCl (3 × 20 mL) and brine (3 × 20 mL), dried over Na_2SO_4, and filtered, and the solvent was removed *in vacuo*. To the crude product, hexane was added, with the resulting suspension sonicated, and the solid was filtered off, washed with hexane, and dried. Yield: 1.24 g (84%); white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.54 (s, 9H), 2.36 (s, 3H), 3.86 (s, 3H), 6.74 (s, 1H) 7.14 (dd, J = 8.7, 1.7 Hz, 1H), 7.40 (d, J = 1.7 Hz, 1H), 7.97 (d, J = 8.7 Hz, 1H).

Methyl 2-acetoxy-4-aminobenzoate (22) [31]. To methyl 2-acetoxy-4-((*tert*-butoxycarbonyl)amino)benzoate (1.24 g, 4.01 mmol), 2 M HCl in diethyl ether (35 mL) was added, and the mixture was stirred at room temperature overnight. The product precipitated as a white solid and was filtered off, washed with ether, and dried. The filtrate contained the remaining starting material, so the solvent was removed under reduced pressure, to the residue, 2 M HCl in diethyl ether (5 mL) was added, and the mixture was stirred at room temperature overnight. The white precipitate was filtered off, washed with ether, and dried. The combined product in the form of a hydrochloride salt (0.911 g) was dissolved in ethyl acetate (100 mL), and the organic phase was washed with saturated aqueous $NaHCO_3$ solution (50 mL) and brine (50 mL), dried over Na_2SO_4, and filtered, and the solvent was removed *in vacuo*. Yield: 0.760 g (86%); light brown solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.23 (s, 3H), 3.68 (s, 3H), 6.20 (br s, 2H), 6.22 (d, J = 2.2 Hz, 1H), 6.46 (dd, J = 8.7, 2.2 Hz, 1H), 7.65 (d, J = 8.7 Hz, 1H).

General procedure J. Synthesis of compounds 23, 42, and 46 (with 23 as an example). A solution of methyl 2-acetoxy-4-aminobenzoate (0.716 g, 3.43 mmol) and KSCN (1.33 g, 13.7 mmol) in acetic acid (12 mL) was stirred at room temperature for 20 min. It was then cooled to 10 °C, bromine (0.351 mL, 6.85 mmol) in acetic acid (3 mL) was added, and the mixture was stirred at room temperature overnight. The suspension was cooled in an ice bath, and neutralized with saturated aqueous $NaHCO_3$ solution (500 mL), with the resulting precipitate filtered off and dried at 60 °C for 1.5 h. The solid was purified by adding diethyl ether (50 mL), with the suspension obtained sonicated, and the solid filtered off and washed with diethyl ether. To the crude product, methanol (100 mL) was added, the mixture was heated to reflux, and the undissolved solid was filtered off. Methanol was removed under reduced pressure, to obtain **23** (205 mg) as a yellow solid.

Methyl 5-acetoxy-2-aminobenzo[*d*]thiazole-6-carboxylate (23). Yield: 205 mg (23%); yellow solid.^{1}H NMR (400 MHz, DMSO-d_6) δ 2.28 (s, 3H), 3.78 (s, 3H), 7.09 (s, 1H), 8.08 (s, 2H), 8.29 (s, 1H). MS (ESI) m/z = 289.0 ([M+Na]$^+$).

Methyl 5-acetoxy-2-(3,4-dichloro-5-methyl-1*H*-pyrrole-2-carboxamido)benzo[*d*]thiazole-6-carboxylate (24). Synthesized according to general procedure B using methyl 5-acetoxy-2-aminobenzo [*d*]thiazole-6-carboxylate (**23**, 100 mg, 0.376 mmol). The crude product was dispersed in diethyl ether (20 mL), sonicated, filtered off, and dried. Yield: 56 mg (33%); brown solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.28 (s, 3H), 2.32 (s, 3H), 3.84 (s, 3H), 7.60 (s, 1H), 8.67 (s, 1H), 12.06 (s, 1H), 12.36 (s, 1H).

2-(3,4-Dichloro-5-methyl-1*H*-pyrrole-2-carboxamido)-5-hydroxybenzo[*d*]thiazole-6-carboxylic acid (25). Synthesized according to general procedure C using methyl 5-acetoxy-2-(3,4-dichloro-5-methyl-1*H*-pyrrole-2-carboxamido)benzo [*d*]thiazole-6-carboxylate (**24**, 39 mg, 0.088 mmol) and 4 M NaOH (0.221 mL, 0.88 mmol). The crude product was dispersed in diethyl ether (10 mL), sonicated, filtered off, and dried. Yield: 22.3 mg (66%); brown solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.27 (s, 3H), 6.94 (s, 1H), 8.22 (s, 1H), 12.00 (s, 1H), 12.31 (s, 1H). HRMS (ESI$^-$) m/z for $C_{14}H_9Cl_2N_3O_4S$ ([M − H]$^-$): calculated 383.9618, found 383.9621. HPLC: t_r 8.890 min (99.1% at 254 nm), method B.

Methyl 2-(4,5-dibromo-1*H*-pyrrole-2-carboxamido)-4-hydroxybenzo[*d*]thiazole-6-carboxylate (27a). Synthesized according to general procedure A using methyl 2-amino-4-hydroxybenzo [*d*]thiazole-6-carboxylate (**26a**, 248 mg, 0.67 mmol). Yield: 167 mg (31.8%); off-brown solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.86 (s, 3H), 7.47 (d, J = 1.6 Hz, 1H), 7.56 (d, J = 2.5 Hz, 1H), 8.10 (d, J = 1.5 Hz, 1H), 10.32 (s, 1H), 12.90 (s, 1H), 13.26 (s, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 52.58, 99.59, 109.23, 111.98, 114.59, 116.73, 125.91, 126.08, 133.78, 150.03, 157.67, 159.74, 166.58, 171.75. HRMS (ESI$^+$) m/z for $C_{14}H_{10}Br_2N_3O_4S$ ([M+H]$^+$): calculated 473.8753, found 473.8749.

Methyl 4-((*tert*-butyldimethylsilyl)oxy)-2-(3,4-dichloro-5-methyl-1*H*-pyrrole-2-carboxamido)benzo[*d*]thiazole-6-carboxylate (27b). Synthesized according to general procedure B using methyl 2-amino-4-((*tert*-butyldimethylsilyl)oxy)benzo [*d*] thiazole-6-carboxylate (**26b**, 87 mg, 0.26 mmol). Yield: 70 mg (52.9%); white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.10 (s, 3H), 3.87 (s, 3H), 7.41 (s, 1H), 8.26 (s, 1H), 11.84 (s, 1H), 12.46 (s, 1H). HRMS (ESI$^+$) m/z for $C_{21}H_{26}Cl_2N_3O_4SSi$ ([M+H]$^+$): calculated 514.0785, found 514.0783.

2-(4,5-Dibromo-1*H*-pyrrole-2-carboxamido)-4-hydroxybenzo[*d*]thiazole-6-carboxylic acid (28a). Synthesized according to general procedure C using methyl 2-(4,5-dibromo-1*H*-pyrrole-2-carboxamido)-4-hydroxybenzo [*d*]thiazole-6-carboxylate (100 mg, 0.21 mmol). Yield: 83 mg (85.5%); black solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.48 (d, J = 1.6 Hz, 1H), 7.56 (d, J = 2.8 Hz, 1H), 8.04 (d, J = 1.5 Hz, 1H), 10.31 (s, 1H), 12.87 (s, 1H), 13.27 (d, J = 2.8 Hz, 1H). HRMS (ESI$^-$) m/z for $C_{13}H_6Br_2N_3O_4S$ ([M − H]$^-$): calculated 457.8451, found 457.8458. HPLC: t_r 5.173 min (98.8% at 254 nm), method B.

2-(3,4-Dichloro-5-methyl-1*H*-pyrrole-2-carboxamido)-4-hydroxybenzo[*d*]thiazole-6-carboxylic acid (28b). Synthesized according to general procedure C using methyl 4-((*tert*-butyldimethylsilyl)oxy)-2-(3,4-dichloro-5-methyl-1*H*-pyrrole-2-carboxamido)benzo [*d*]thiazole-6-carboxylate (**27b**, 70 mg, 0.21 mmol). An additional 500 μL of 2 M NaOH was added, and the reaction mixture was stirred at 80 °C for a further 15 h. Yield: 7.8 mg (14.8%); brown solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.27 (s, 3H), 7.44 (d, J = 1.6 Hz, 1H), 8.05 (d, J = 1.5 Hz, 1H), 10.25 (s, 1H), 11.93 (s,

1H), 12.36 (s, 1H), 12.80 (s, 1H). HRMS (ESI$^-$) *m/z* for $C_{14}H_8Cl_2N_3O_4S$ ([M − H]$^-$): calculated 383.9618, found 383.9622. HPLC: t_r 6.310 min (96.2% at 254 nm), method B.

Methyl 2-(3,4-dichloro-5-methyl-1*H*-pyrrole-2-carboxamido)-4-fluorobenzo[*d*]thiazole-6-carboxylate (30a). Synthesized according to general procedure B using methyl 2-amino-4-fluorobenzo [*d*]thiazole-6-carboxylate (**29a**, 350 mg, 1.55 mmol). Yield: 242 mg (38.9%); brown solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.28 (s, 3H), 3.90 (s, 3H), 7.78 (dd, J = 11.2, 1.5 Hz, 1H), 8.55 (d, J = 1.5 Hz, 1H), 12.30 (s, 1H), 12.37 (s, 1H). HRMS (ESI$^+$) *m/z* for $C_{15}H_{11}FCl_2N_3O_3S$ ([M+H]$^+$): calculated 401.9877, found 401.9853.

Methyl 2-(3,4-dichloro-5-methyl-1*H*-pyrrole-2-carboxamido)-4-methoxybenzo[*d*]thiazole-6-carboxylate (30b). Synthesized according to general procedure B using methyl 2-amino-4-methoxybenzo [*d*]thiazole-6-carboxylate (**29b**, 368 mg, 1.55 mmol). Yield: 345 mg (53.9%); brown solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.28 (s, 3H), 3.90 (s, 3H), 4.00 (s, 3H), 7.50 (d, J = 1.4 Hz, 1H), 8.29 (d, J = 1.2 Hz, 1H), 12.15 (s, 1H), 12.29 (s, 1H). HRMS (ESI$^+$) *m/z* for $C_{16}H_{14}Cl_2N_3O_4S$ ([M+H]$^+$): calculated 414.0077, found 414.0073.

4-(2-((2-(3,4-Dichloro-5-methyl-1*H*-pyrrole-2-carboxamido)-6-(methoxycarbonyl)benzo[*d*]thiazol-4-yl)oxy) ethyl)morpholin-4-ium chloride (30c). Synthesized according to general procedure B using methyl 2-amino-4-(2-morpholinoethoxy)benzo [*d*]thiazole-6-carboxylate (**29c**, 261 mg, 0.773 mmol). Yield: 70 mg (16.5%); beige solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.28 (s, 3H), 3.20−3.30 (m, 2H), 3.65 (s, 4H), 3.79 (t, 2H), 3.90 (s, 3H), 4.04 (d, J = 12.5 Hz, 2H), 4.69 (s, 2H), 7.60 (d, J = 1.4 Hz, 1H), 8.36 (s, 1H), 10.68 (s, 1H), 12.12 (s, 1H), 12.68 (s, 1H). HRMS (ESI$^+$) *m/z* for $C_{21}H_{23}Cl_2N_4O_5S$ ([M+H]$^+$): calculated 513.0761, found 513.0751.

Methyl 4-(2-((*tert*-butoxycarbonyl)amino)ethoxy)-2-(3,4-dichloro-5-methyl-1*H*-pyrrole-2-carboxamido)benzo[*d*]thiazole-6-carboxylate (30d). Synthesized according to general procedure B using methyl 2-amino-4-(2-((*tert*-butoxycarbonyl)amino) ethoxy)benzo [*d*]thiazole-6-carboxylate (**29d**, 200 mg, 0.544 mmol). Yield: 38 mg (12.8%); beige solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.40 (s, 9H), 2.28 (s, 3H), 3.41 (m, 2H, signal is overlapping with the signal for water),3.89 (s, 3H), 4.22 (t, J = 5.8 Hz, 2H), 7.06 (t, J = 4.8 Hz, 1H), 7.50 (d, J = 1.4 Hz, 1H), 8.29 (s, 1H), 12.26 (s, 2H). HRMS (ESI$^+$) *m/z* for $C_{22}H_{24}Cl_2N_4O_6SNa$ ([M+Na]$^+$): calculated 565.0686, found 565.0682.

Methyl 2-(3,4-dichloro-5-methyl-1*H*-pyrrole-2-carboxamido)-4-((3-(methoxycarbonyl)benzyl)oxy)benzo[*d*] thiazole-6-carboxylate (30e). Synthesized according to general procedure B using methyl 2-amino-4-((3-(methoxycarbonyl) benzyl)oxy)benzo [*d*]thiazole-6-carboxylate (**29e**, 77 mg, 0.207 mmol). Yield: 73 mg (64.4%); light gray solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.27 (s, 3H), 3.88 (s, 3H), 3.89 (s, 3H), 5.43 (s, 2H), 7.61 (s, 1H), 7.63−7.65 (m, 1H), 7.83 (s, 1H), 7.98 (dt, J = 7.7, 1.5 Hz, 1H), 8.14−8.17 (m, 1H), 8.32 (d, J = 0.9 Hz, 1H), 12.21 (s, 1H), 12.25 (s, 1H). HRMS (ESI$^-$) *m/z* for $C_{24}H_{18}Cl_2N_3O_6S$ ([M − H]$^-$): calculated 546.0299, found 546.0300.

Methyl 2-(3,4-dichloro-5-methyl-1*H*-pyrrole-2-carboxamido)-4-((4-(methoxycarbonyl)benzyl)oxy)benzo[*d*] thiazole-6-carboxylate (30f). Synthesized according to general procedure B using methyl 2-amino-4-((4-(methoxycarbonyl) benzyl)oxy)benzo [*d*]thiazole-6-carboxylate (**29f**, 68 mg, 0.185 mmol). Yield: 72 mg (70.9%); light gray solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.26 (s, 3H), 3.87 (s, 3H), 3.88 (s, 3H), 5.44 (s, 2H), 7.61 (d, J = 1.3 Hz, 1H), 7.69 (d, J = 8.2 Hz, 2H), 8.03 (d, J = 8.3 Hz, 2H), 8.31 (d, J = 0.9 Hz, 1H), 12.19 (s, 1H), 12.27 (s, 1H). HRMS (ESI$^-$) *m/z* for $C_{24}H_{18}Cl_2N_3O_6S$ ([M − H]$^-$): calculated 546.0299, found 546.0302.

Methyl 2-(3,4-dichloro-5-methyl-1*H*-pyrrole-2-carboxamido)-4-(thiophen-2-ylmethoxy)benzo[*d*]thiazole-6-carboxylate (30g). Synthesized according to general procedure B using methyl 2-amino-4-(thiophen-2-ylmethoxy)benzo [*d*]thiazole-6-carboxylate (**29g**, 120 mg, 0.375 mmol). Yield: 122 mg (65.6%); light gray solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.26 (s, 3H), 3.89 (s, 3H), 5.53 (s, 2H), 7.08 (dd, J = 5.1, 3.4 Hz, 1H), 7.28 (dd, J = 3.5, 1.2 Hz, 1H), 7.60 (dd, J = 5.1, 1.3 Hz, 1H), 7.65 (d, J = 1.5 Hz, 1H), 8.31 (d, J = 1.4 Hz, 1H), 12.21−12.23 (m, 2H). HRMS (ESI$^+$) *m/z* for $C_{20}H_{16}Cl_2N_3O_4S$ ([M+H]$^+$): calculated 495.9954, found 495.9946.

Methyl 2-(3,4-dichloro-5-methyl-1*H*-pyrrole-2-carboxamido)-4-(thiophen-3-ylmethoxy)benzo[*d*]thiazole-6-carboxylate (30h). Synthesized according to general procedure B using methyl 2-amino-4-(thiophen-3-ylmethoxy)benzo [*d*]thiazole-6-carboxylate (**29h**, 93 mg, 0.290 mmol). Yield: 63 mg (43.7%); light gray solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.26 (s, 3H), 3.89 (s, 3H), 5.32 (s, 2H), 7.26 (dd, J = 4.9, 1.3 Hz, 1H), 7.58−7.64 (m, 2H), 7.64−7.70 (m, 1H), 8.30 (d, J = 1.4 Hz, 1H), 12.20 (s, 1H), 12.22 (s, 1H). HRMS (ESI$^-$) *m/z* for $C_{20}H_{14}Cl_2N_3O_4S$ ([M − H]$^-$): calculated 493.9808, found 493.9810.

Methyl 2-(3,4-dichloro-5-methyl-1*H*-pyrrole-2-carboxamido)-4-((3-fluorobenzyl)oxy)benzo[*d*]thiazole-6-carboxylate (30i). Synthesized according to general procedure B using methyl 2-amino-4-((3-fluorobenzyl)oxy)benzo [*d*]thiazole-6-carboxylate (**29i**, 110 mg, 0.331 mmol). Yield: 132 mg (71.7%); light gray solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.27 (s, 3H), 3.89 (s, 3H), 5.36 (s, 2H), 7.17−7.27 (m, 1H), 7.39−7.41 (m, 2H), 7.45−7.53 (m, 1H), 7.61 (s, 1H), 8.31 (s, 1H), 12.19 (s, 1H), 12.26 (s, 1H). HRMS (ESI$^+$) *m/z* for $C_{22}H_{17}FCl_2N_3O_4S$ ([M+H]$^+$): calculated 508.0295, found 508.0293.

2-(3,4-Dichloro-5-methyl-1*H*-pyrrole-2-carboxamido)-4-fluorobenzo[*d*]thiazole-6-carboxylic acid (31a). Synthesized according to general procedure C using methyl 2-(3,4-dichloro-5-methyl-1*H*-pyrrole-2-carboxamido)-4-fluorobenzo [*d*]thiazole-6-carboxylate (**30a**, 100 mg, 0.249 mmol). The crude product was dispersed in acetonitrile, sonicated, and heated, and the precipitate was filtered off and dried. Yield: 43 mg (44.5%); brown solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.29 (s, 3H), 7.75 (dd, J = 11.2, 1.5 Hz, 1H), 8.52 (d, J = 1.4 Hz, 1H), 12.26 (s, 1H), 12.34 (s, 1H), 13.23 (br s, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 11.04, 110.04, 112.37, 112.57, 115.79, 116.67, 120.08, 120.11, 126.81, 130.35, 134.56, 156.67, 161.63, 166.20. HRMS (ESI$^-$) *m/z* for $C_{14}H_7Cl_2FN_3O_3S$ ([M − H]$^-$): calculated 385.9580, found 385.9564. HPLC: t_r 8.983 min (95.1% at 254 nm), method B.

2-(3,4-Dichloro-5-methyl-1*H*-pyrrole-2-carboxamido)-4-methoxybenzo[*d*]thiazole-6-carboxylic acid (31b). Synthesized according to general procedure C using methyl 2-(3,4-dichloro-5-methyl-1*H*-pyrrole-2-carboxamido)-4-methoxybenzo [*d*]thiazole-6-carboxylate (**30b**, 162 mg, 0.391 mmol). Yield: 120 mg (76.7%); brown solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.28 (s, 3H), 3.98 (s, 3H), 7.50 (d, J = 1.4 Hz, 1H), 8.24 (d, J = 1.4 Hz, 1H), 12.02 (br s, 1H), 12.41 (s, 1H), 12.66 (br s, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 11.01, 55.77, 107.78, 109.87, 115.41, 116.07, 116.91, 126.79, 130.03, 132.65, 141.53, 151.03, 156.69, 159.52, 167.07. HRMS (ESI$^-$) *m/z* for $C_{15}H_{10}Cl_2N_3O_4S$ ([M − H]$^-$): calculated 397.9764, found 397.9779. HPLC: t_r 7.867 min (96.7% at 254 nm), method B.

4-(2-((6-Carboxy-2-(3,4-dichloro-5-methyl-1*H*-pyrrole-2-carboxamido)benzo[*d*]thiazol-4-yl)oxy)ethyl)morpholin-4-ium chloride (31c). Synthesized according to general procedure C using 4-(2-((2-(3,4-dichloro-5-methyl-1*H*-pyrrole-2-carboxamido)-6-(methoxycarbonyl)benzo [*d*]thiazol-4-yl)oxy)ethyl)morpholin-4-ium chloride (**30c**, 70 mg, 0.127 mmol). Yield: 54 mg (78.7%); brownish-red solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.29 (s, 3H), 3.32 (m, 2H), 3.66 (m, 4H), 3.84 (t, J = 11.9 Hz, 2H), 4.06 (m, 2H), 4.71

(s, 2H), 7.58 (d, J = 1.4 Hz, 1H), 8.30 (d, J = 1.3 Hz, 1H), 11.13 (s, 1H), 12.20 (br s, 1H), 12.86 (s, 1H). HRMS (ESI$^-$) m/z for $C_{20}H_{19}Cl_2N_4O_5S$ ([M − H]$^-$): calculated 497.0448, found 497.0458. HPLC: t_r 5.030 min (98.0% at 254 nm), method B.

4-(2-((*tert*-Butoxycarbonyl)amino)ethoxy)-2-(3,4-dichloro-5-methyl-1*H*-pyrrole-2-carboxamido)benzo[*d*]thiazole-6-carboxylic acid (31d). Synthesized according to general procedure C using methyl 4-(2-((*tert*-butoxycarbonyl)amino)ethoxy)-2-(3,4-dichloro-5-methyl-1*H*-pyrrole-2-carboxamido)benzo [*d*]thiazole-6-carboxylate (**30d**, 35 mg, 0.064 mmol). Yield: 7.6 mg (22.3); brownish-red solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.39 (s, 9H), 2.28 (s, 3H), 3.39 (m, 2H, signal is overlapping with the signal for water), 4.10 (t, J = 6.0 Hz, 2H), 6.96−7.05 (m, 1H), 7.34 (d, J = 1.4 Hz, 1H), 7.92 (d, J = 1.5 Hz, 1H), 12.70 (s, 1H), 12.80 (s, 1H), 12.97 (s, 1H). HRMS (ESI$^-$) m/z for $C_{21}H_{21}Cl_2N_4O_6S$ ([M − H]$^-$): calculated 527.0564, found 527.0565.

4-((3-Carboxybenzyl)oxy)-2-(3,4-dichloro-5-methyl-1*H*-pyrrole-2-carboxamido)benzo[*d*]thiazole-6-carboxylic acid (31e). To a suspension of methyl 2-(3,4-dichloro-5-methyl-1*H*-pyrrole-2-carboxamido)-4-((3-(methoxycarbonyl)benzyl)oxy)benzo [*d*]thiazole-6-carboxylate (60 mg, 0.109 mmol) in 1,4-dioxane, 2 M NaOH (274 μL, 0.547 mmol) was added, and the suspension stirred at 60 °C overnight. Then, 5 equivalents 2 M NaOH were added, and the reaction mixture was stirred at 75 °C for 7 days. The reaction was followed using HPLC-MS, and 5 equivalents of fresh 2 M NaOH were added, and the reaction mixture was stirred at 75 °C for 7 days. After the reaction was finished, the solvent was evaporated under reduced pressure, the residue was acidified with 1 M HCl to pH 1, and the precipitate obtained was filtered off. The crude product was suspended in methanol, heated, and filtered off. Yield: 33 mg (58.5%); brown solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.26 (s, 3H), 5.41 (s, 2H), 7.58 (t, J = 7.7 Hz, 1H), 7.63 (d, J = 1.4 Hz, 1H), 7.80 (dt, J = 7.8, 1.4 Hz, 1H), 7.96 (dt, J = 7.8, 1.4 Hz, 1H), 8.12 (d, J = 1.8 Hz, 1H), 8.27 (d, J = 1.4 Hz, 1H), 12.19 (br s, 1H), 12.25 (s, 1H), 13.06 (br s, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 11.00, 69.49, 109.05, 109.93, 115.59, 116.38, 116.81, 126.69, 128.83, 128.86, 129.00, 130.03, 130.92, 132.58, 132.82, 137.17, 141.65, 149.92, 156.65, 159.72, 167.04, 167.17. HRMS (ESI$^+$) m/z for $C_{22}H_{16}Cl_2N_3O_6S$ ([M+H]$^+$): calculated 520.0131, found 520.0146. HPLC: t_r 8.323 min (95.8% at 254 nm), method B.

4-((4-Carboxybenzyl)oxy)-2-(3,4-dichloro-5-methyl-1*H*-pyrrole-2-carboxamido)benzo[*d*]thiazole-6-carboxylic acid (31f). Synthesized according to general procedure C using methyl 2-(3,4-dichloro-5-methyl-1*H*-pyrrole-2-carboxamido)-4-((4-(methoxycarbonyl)benzyl)oxy)benzo [*d*]thiazole-6-carboxylate (**30f**, 63 mg, 0.115 mmol). Yield: 57 mg (95.5%); brown crystals. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.27 (s, 3H), 5.43 (s, 2H), 7.61 (d, J = 1.4 Hz, 1H), 7.66 (d, J = 8.4 Hz, 2H), 8.01 (d, J = 8.4 Hz, 2H), 8.27 (d, J = 1.3 Hz, 1H), 12.17 (br s, 1H), 12.28 (s, 1H), 13.02 (br s, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 11.03, 69.44, 109.19, 109.94, 115.63, 116.47, 116.84, 126.70, 127.45, 127.84, 129.48, 130.00, 130.34, 132.87, 141.63, 149.86, 156.68, 159.75, 167.01, 167.09. HRMS (ESI$^-$) m/z for $C_{22}H_{14}Cl_2N_3O_6S$ ([M − H]$^-$): calculated 517.9986, found 517.9989. HPLC: t_r 8.380 min (95.4% at 254 nm), method B.

General procedure K. Synthesis of compounds 31g-i (with 31h as an example). To a suspension of methyl 2-(3,4-dichloro-5-methyl-1*H*-pyrrole-2-carboxamido)-4-(thiophen-3-ylmethoxy) benzo [*d*]thiazole-6-carboxylate (**30h**, 45 mg, 0.091 mmol) in methanol (15 mL), 1 M NaOH (0.455 mL, 0.455 mmol) was added, and the reaction mixture was stirred at 40 °C overnight. An additional 0.455 mL 1 M NaOH was added, and the reaction mixture was stirred at 40 °C for 3 days. The solvent was evaporated *in vacuo*, the residue was acidified with 1 M HCl to pH 1, and the precipitate formed was filtered off. The crude product was suspended in methanol, heated, and filtered to get **31h** (30 mg) as a light gray solid.

2-(3,4-Dichloro-5-methyl-1*H*-pyrrole-2-carboxamido)-4-(thiophen-2-ylmethoxy)benzo[*d*]thiazole-6-carboxylic acid (31g). Synthesized according to general procedure K using methyl 2-(3,4-dichloro-5-methyl-1*H*-pyrrole-2-carboxamido)-4-(thiophen-2-ylmethoxy)benzo [*d*]thiazole-6-carboxylate (**30g**, 110 mg, 0.344 mmol). The crude product was purified with flash column chromatography using dichloromethane/methanol (9:1) as eluent. Yield: 50 mg (46.7%); beige solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.26 (s, 3H), 5.52 (s, 2H), 7.08 (dd, J = 5.1, 3.4 Hz, 1H), 7.28 (dd, J = 3.4, 1.2 Hz, 1H), 7.60 (dd, J = 5.1, 1.2 Hz, 1H), 7.64 (d, J = 1.5 Hz, 1H), 8.26 (d, J = 1.4 Hz, 1H), 12.17 (s, 1H), 12.23 (s, 1H), 12.98 (s, 1H). HRMS (ESI$^+$) m/z for $C_{19}H_{14}Cl_2N_3O_4S_2$ ([M+H]$^+$): calculated 481.9797, found 481.9792. HPLC: t_r 4.503 min (95.4% at 254 nm), method C.

2-(3,4-Dichloro-5-methyl-1*H*-pyrrole-2-carboxamido)-4-(thiophen-3-ylmethoxy)benzo[*d*]thiazole-6-carboxylic acid (31h). Yield: 30 mg (68.3%); light gray solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.26 (s, 3H), 5.30 (s, 2H), 7.25 (dd, J = 4.9, 1.3 Hz, 1H), 7.57−7.63 (m, 2H), 7.63−7.68 (m, 1H), 8.25 (d, J = 1.4 Hz, 1H), 12.17 (s, 1H), 12.22 (s, 1H), 13.00 (s, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 11.53, 65.84, 109.50, 110.41, 116.06, 116.76, 117.37, 125.36, 127.13, 127.23, 128.62, 130.47, 133.28, 137.94, 142.24, 150.52, 157.08, 160.07, 167.54. HRMS (ESI$^-$) m/z for $C_{19}H_{14}Cl_2N_3O_4S_2$ ([M+H]$^+$): calculated 481.9797, found 481.9794. HPLC: t_r 4.527 min (95.1% at 254 nm), method C.

2-(3,4-Dichloro-5-methyl-1*H*-pyrrole-2-carboxamido)-4-((3-fluorobenzyl)oxy)benzo[*d*]thiazole-6-carboxylic acid (31i). Synthesized according to general procedure K using methyl 2-(3,4-dichloro-5-methyl-1*H*-pyrrole-2-carboxamido)-4-((3-fluorobenzyl)oxy)benzo [*d*]thiazole-6-carboxylate (**30i**, 58 mg, 0.114 mmol) in methanol (15 mL), with the reaction mixture first stirred for 4 days. An additional 2 equivalents of 1 M NaOH (228 μL, 0.228 mmol) were added, and the reaction mixture was stirred for a further 1 day. The crude product was purified by suspension in methanol, heating, and filtering off. Yield: 36 mg (64.3%); brown crystals. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.27 (s, 3H), 5.35 (s, 2H), 7.18−7.26 (m, 1H), 7.38−7.40 (m, 2H), 7.46−7.53 (m, 1H), 7.61 (d, J = 1.2 Hz, 1H), 8.27 (d, J = 1.1 Hz, 1H), 12.25 (s, 1H), 12.16 (s, 1H), 13.01 (br s, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 11.02, 69.15, 109.04, 109.93, 114.59, 114.71, 114.81, 114.92, 115.60, 116.44, 116.85, 124.03, 124.05, 126.68, 129.97, 130.45, 130.53, 132.83, 139.48, 139.55, 149.84, 156.65, 159.75, 160.90, 163.32, 167.01. HRMS (ESI$^-$) m/z for $C_{21}H_{13}Cl_2FN_3O_4S$ ([M − H]$^-$): calculated 491.9993, found 491.9997. HPLC: t_r 11.797 min (99.0% at 254 nm), method B.

2-((6-Carboxy-2-(3,4-dichloro-5-methyl-1*H*-pyrrole-2-carboxamido)benzo[*d*]thiazol-4-yl)oxy)ethan-1-aminium chloride (32). To a suspension of 4-(2-((*tert*-butoxycarbonyl)amino) ethoxy)-2-(3,4-dichloro-5-methyl-1*H*-pyrrole-2-carboxamido) benzo [*d*]thiazole-6-carboxylic acid (**31d**, 7.6 mg, 0.014 mmol) in 1,4-dioxane (1 mL), 4 M HCl in 1,4-dioxane (1 mL) was added, and the reaction mixture was stirred at room temperature for 5 h. The precipitate in the reaction mixture was filtered off, washed with methanol, and dried. Yield: 3 mg (44.9%); brownish-red solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.28 (s, 3H), 3.30 (m, 2H, signal is overlapping with the signal for water), 4.45 (t, J = 5.0 Hz, 2H), 7.59 (d, J = 1.1 Hz, 1H), 8.10 (s, 3H), 8.31 (s, 1H), 12.07 (br s, 1H), 12.50 (s, 1H), 13.08 (br s, 1H). HRMS (ESI$^+$) m/z for $C_{16}H_{15}Cl_2N_4O_4S$ ([M+H]$^+$): calculated 429.0186, found 429.0187. HPLC: t_r 3.733 min (95.1% at 254 nm), method B.

4-(2-((6-((Cyanomethyl)carbamoyl)-2-(3,4-dichloro-5-methyl-1*H*-pyrrole-2-carboxamido)benzo[*d*]thiazol-4-yl)oxy) ethyl)morpholin-4-ium chloride (33). Synthesized according to general procedure D using 4-(2-((6-carboxy-2-(3,4-dichloro-5-methyl-1*H*-pyrrole-2-carboxamido)benzo [*d*]thiazol-4-yl)oxy) ethyl)morpholin-4-ium chloride (**31c**, 19 mg, 0.035 mmol). Here,

1 M HCl was used in the isolation process instead of 1% citric acid. After addition of organic and water phases, the precipitate formed was filtered off and dried. Yield: 14 mg (68.0%); beige solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.29 (s, 3H), 3.25−3.34 (m, 2H), 3.62−3.73 (m, 4H), 3.76−3.88 (m, 2H), 4.01−4.10 (m, 2H), 4.36 (d, J = 5.4 Hz, 2H), 4.71 (t, 2H), 7.62 (d, J = 1.5 Hz, 1H), 8.21 (d, J = 0.5 Hz, 1H), 9.40 (t, J = 5.5 Hz, 1H), 10.93 (s, 1H), 12.20 (s, 1H), 12.78 (s, 1H). HRMS (ESI$^+$) m/z for $C_{22}H_{23}Cl_2N_6O_4S$ ([M+H]$^+$): calculated 537.0873, found 537.0861. HPLC: t_r 4.313 min (96.9% at 254 nm), method B.

2-Amino-4-(benzyloxy)benzo[*d*]thiazole-6-carboxylic acid (35). Synthesized according to general procedure C using methyl 2-amino-4-(benzyloxy)benzo [*d*]thiazole-6-carboxylate (**34**, 400 mg, 1.27 mmol). After addition of 1 M HCl, the water phase was cooled to 0 °C for 3 h, and the white precipitate formed was filtered off. Yield: 340 mg (89.0%); white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 5.23 (s, 2H), 7.31−7.43 (m, 3H), 7.45 (d, J = 1.5 Hz, 1H), 7.47−7.51 (m, 2H), 7.88 (s, 2H), 7.93 (d, J = 1.5 Hz, 1H), 12.68 (s, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 70.43, 110.73, 116.43, 124.05, 128.30, 128.33, 128.83, 132.03, 137.54, 146.53, 148.37, 167.64, 168.93. MS (ESI) m/z = 299.1 ([M − H]$^-$).

2-Amino-4-(benzyloxy)-*N*-(cyanomethyl)benzo[*d*]thiazole-6-carboxamide (36). Synthesized according to general procedure D using 2-amino-4-(benzyloxy)benzo [*d*]thiazole-6-carboxylic acid (**35**, 330 mg, 1.10 mmol). Yield: 93 mg (27%); white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.32 (d, J = 5.4 Hz, 2H), 5.24 (s, 2H), 7.33−7.38 (m, 1H), 7.39−7.45 (m, 2H), 7.47 (d, J = 1.6 Hz, 1H), 7.48−7.52 (m, 2H), 7.83 (s, 2H), 7.86 (d, J = 1.6 Hz, 1H), 9.08 (t, J = 5.5 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 28.26, 70.63, 109.30, 114.15, 118.28, 126.27, 128.38, 128.51, 128.85, 132.26, 137.48, 145.97, 148.54, 166.75, 168.29. MS (ESI) m/z = 337.1 ([M − H]$^-$).

4-(Benzyloxy)-*N*-(cyanomethyl)-2-(3,4-dichloro-5-methyl-1*H*-pyrrole-2-carboxamido)benzo[*d*]thiazole-6-carboxamide (37). Synthesized according to general procedure B using 2-amino-4-(benzyloxy)-*N*-(cyanomethyl)benzo [*d*]thiazole-6-carboxamide (**36**, 77 mg, 0.228 mmol). Yield: 45 mg (38.5%); brown solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.26 (s, 3H), 4.37 (d, J = 5.4 Hz, 2H), 5.32 (s, 2H), 7.36−7.48 (m, 3H), 7.51−7.59 (m, 2H), 7.63 (d, J = 1.3 Hz, 1H), 8.15 (d, J = 1.3 Hz, 1H), 9.28 (t, J = 5.5 Hz, 1H), 12.18 (br s, 1H), 12.22 (s, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 11.55, 28.35, 70.71, 108.17, 110.43, 114.35, 116.17, 117.25, 118.20, 128.67, 128.89, 128.96, 129.50, 130.44, 133.33, 136.98, 141.56, 150.81, 156.92, 159.38, 166.72. HRMS (ESI$^-$) m/z for $C_{23}H_{16}Cl_2N_5O_3S$ ([M − H]$^-$): calculated 512.0356, found 512.0361. HPLC: t_r 11.253 min (95.2% at 254 nm), method B.

3-(Benzyloxy)-4-nitrobenzamide (39). A solution of methyl 3-(benzyloxy)-4-nitrobenzoate (1.19 g, 4.15 mmol) in saturated methanol solution of ammonia was stirred in a pressure tube at 65 °C overnight. The precipitate formed was filtered off. The mother liquid was evaporated under reduced pressure, the residue crystallized from acetonitrile, and the crystals combined with the filtered precipitate. Yield: 1.01 g (89.4%); pale yellow crystals. ^{1}H NMR (400 MHz, DMSO-d_6) δ 5.37 (s, 2H), 7.33−7.49 (m, 5H), 7.59 (dd, J = 8.4, 1.6 Hz, 1H), 7.73 (s, 1H), 7.87 (d, J = 1.5 Hz, 1H), 7.98 (d, J = 8.4 Hz, 1H), 8.24 (s, 1H). MS (ESI) m/z = 272.9 ([M+H]$^+$).

3-(Benzyloxy)-4-nitrobenzonitrile (40). To a solution of 3-(benzyloxy)-4-nitrobenzamide (700 mg, 2.57 mmol) in dry acetonitrile (10 mL), triphenylphosphine oxide (7.15 mg, 0.026 mmol), triethylamine (1.08 mL, 7.71 mmol), and oxalyl chloride (441 μL, 5.14 mmol) were added, and the reaction mixture stirred at room temperature for 30 min. The triethylammonium chloride that was formed was filtered off, and the mother liquid was evaporated. The crude product was crystallized from methanol. Yield: 650 mg (99.4%); off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 5.38 (s, 2H), 7.35−7.47 (m, 5H), 7.66 (dd, J = 8.3, 1.6 Hz, 1H), 8.07−8.11 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 71.66, 116.44, 117.74, 120.10, 125.52, 126.15, 128.12, 128.84, 129.08, 135.75, 142.96, 150.93. MS (ESI) m/z = 253.1 ([M − H]$^-$).

General procedure L. Synthesis of compounds 41 and 45 (with 41 as an example). 3-(Benzyloxy)-4-nitrobenzonitrile (643 mg, 2.53 mmol) was dissolved in methanol/ethyl acetate (1:3, 40 mL), tin (II) chloride (1.7 g, 8.86 mmol) was added, and the reaction mixture was stirred at 55 °C overnight. The solvent was evaporated *in vacuo*, the residue was neutralized with saturated aqueous $NaHCO_3$ solution and extracted with ethyl acetate (3 × 100 mL). The combined organic phases were washed with brine (100 mL) and filtered, and the solvent was evaporated under reduced pressure, to obtain **41** (321 mg) as a white solid.

4-Amino-3-(benzyloxy)benzonitrile (41) [32]. Yield: 321 mg (56.6%); white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 5.16 (s, 2H), 5.82 (s, 2H), 6.69 (d, J = 8.2 Hz), 7.12 (dd, J = 8.2, 1.8 Hz, 1H), 7.22 (d, J = 1.7 Hz, 1H), 7.31−7.36 (m, 1H), 7.37−7.43 (m, 2H), 7.47−7.53 (m, 2H).

2-Amino-4-(benzyloxy)benzo[*d*]thiazole-6-carbonitrile (42). Synthesized according to general procedure J using 4-amino-3-(benzyloxy)benzonitrile (**41**, 315 mg, 1.31 mmol). The reaction mixture was neutralized with 25% aqueous NH_3 solution instead of $NaHCO_3$, and the product was extracted with ethyl acetate (2 × 50 mL). The combined organic phases were washed with brine (20 mL), dried over Na_2SO_4, and filtered, and the solvent was evaporated under reduced pressure. The crude product was suspended in methanol and filtered off. Yield: 126 mg (34.0%); yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 5.22 (s, 2H), 7.32−7.38 (m, 2H), 7.38−7.44 (m, 2H), 7.45−7.50 (m, 2H), 7.84 (d, J = 1.4 Hz, 1H), 8.02 (s, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 70.69, 102.78, 112.77, 119.27, 120.14, 128.15, 128.49, 128.88, 132.78, 137.12, 147.03, 148.77, 169.44. MS (ESI) m/z = 280.1 ([M − H]$^-$).

***N*-(4-(Benzyloxy)-6-cyanobenzo[*d*]thiazol-2-yl)-3,4-dichloro-5-methyl-1*H*-pyrrole-2-carboxamide (43).** Synthesized according to general procedure B using 2-amino-4-(benzyloxy) benzo [*d*]thiazole-6-carbonitrile (**42**, 120 mg, 0.427 mmol). Yield: 126 mg (64.6%); gray solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.26 (s, 3H), 5.32 (s, 2H), 7.37−7.49 (m, 3H), 7.53−7.54 (m, 2H), 7.57 (d, J = 1.1 Hz, 1H), 8.21 (d, J = 1.0 Hz, 1H), 12.25 (s, 1H), 12.29 (br s, 1H). HRMS (ESI$^-$) m/z for $C_{21}H_{13}Cl_2N_4O_2S$ ([M − H]$^-$): calculated 455.0142, found 455.0147. HPLC: t_r 14.237 min (97.6% at 254 nm), method B.

***N*-(4-(Benzyloxy)-6-(1*H*-tetrazol-5-yl)benzo[*d*]thiazol-2-yl)-3,4-dichloro-5-methyl-1*H*-pyrrole-2-carboxamide (44).** To a solution of *N*-(4-(benzyloxy)-6-cyanobenzo [*d*]thiazol-2-yl)-3,4-dichloro-5-methyl-1*H*-pyrrole-2-carboxamide (100 mg, 0.219 mmol) in *N,N*-dimethylformamide (5 mL), ammonium chloride (117 mg, 2.19 mmol) and sodium azide (142 mg, 2.19 mmol) were added, and the reaction mixture was stirred at 125 °C overnight. Ethyl acetate (10 mL) and 1 M HCl (5 mL) were added, and the precipitate formed (the starting compound) was filtered off. The phases of the mother liquid were separated, the organic phase was washed with brine (5 mL), dried over Na_2SO_4, and filtered, and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography using dichloromethane/methanol/acetic acid (30:1:0.1) as eluent. Yield: 4 mg (3.7%); beige solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.26 (s, 3H), 5.36 (s, 2H), 7.37−7.48 (m, 3H), 7.57−7.59 (m, 2H), 7.78 (d, J = 1.5 Hz, 1H), 8.29 (d, J = 1.4 Hz, 1H), 12.18 (br s, 1H), 12.22 (s, 1H), signal for tetrazole NH is not seen. HRMS (ESI$^-$) m/z for $C_{21}H_{14}Cl_2N_7O_2S$ ([M − H]$^-$): calculated 498.0312, found 498.0319. HPLC: t_r 11.120 min (95.1% at 254 nm), method B.

4-Amino-3-(benzyloxy)benzamide (45). Synthesized according to general procedure L using 3-(benzyloxy)-4-nitrobenzamide (300 mg, 1.102 mmol). Yield: 260 mg (97.4%); yellow oil. ^{1}H NMR (400 MHz, DMSO-d_6) δ 5.14 (s, 2H), 5.29 (s, 2H), 6.63 (d, J = 8.2 Hz,

1H), 6.92 (s, 1H), 7.28–7.36 (m, 3H), 7.36–7.44 (m, 2H), 7.45 (d, $J = 1.8$ Hz, 1H), 7.48–7.55 (m, 2H), 7.60 (s, 1H). MS (ESI) $m/z = 243.0$ ($[M+H]^+$).

2-Amino-4-(benzyloxy)benzo[*d*]thiazole-6-carboxamide (46). Synthesized according to general procedure J using 4-amino-3-(benzyloxy)benzamide (**45**, 255 mg, 1.053 mmol). The reaction mixture was neutralized with 25% aqueous NH_3 solution instead of $NaHCO_3$. The crude product was purified only by resuspension in methanol, heating to reflux, and filtering off the undissolved solid. The methanol was evaporated, to obtain the product **46**. Yield: 307 mg (97.0%); orange solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 5.22 (s, 2H), 7.23 (s, 1H), 7.32–7.37 (m, 1H), 7.38–7.44 (m, 2H), 7.45–7.51 (m, 3H), 7.74 (s, 2H), 7.83 (d, $J = 1.5$ Hz, 1H), 7.86 (s, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 70.52, 109.68, 113.99, 127.92, 128.01, 128.33, 128.83, 131.98, 137.64, 145.34, 148.43, 167.81, 167.99. MS (ESI) $m/z = 300.1$ ($[M+H]^+$).

4-(Benzyloxy)-2-(3,4-dichloro-5-methyl-1*H*-pyrrole-2-carboxamido)benzo[*d*]thiazole-6-carboxamide (47). Synthesized according to general procedure B using 2-amino-4-(benzyloxy) benzo [*d*]thiazole-6-carboxamide (**46**, 190 mg, 0.635 mmol). The crude product was purified with flash column chromatography using dichloromethane/methanol (20:1) as eluent. Yield: 20 mg (6.6%); light gray solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.26 (s, 3H), 5.30 (s, 2H), 7.36–7.47 (m, 4H), 7.51–7.57 (m, 2H), 7.64 (d, $J = 1.3$ Hz, 1H), 8.04 (s, 1H), 8.13 (s, 1H), 12.12 (s, 1H), 12.22 (s, 1H). HRMS (ESI^-) m/z for $C_{21}H_{15}Cl_2N_4O_3S$ ($[M - H]^-$): calculated 473.0247, found 473.0255. HPLC: t_r 9.740 min (95.0% at 254 nm), method B.

4.3. Determination of inhibitory activities on E. coli and S. aureus DNA gyrase and topoisomerase IV

The assay for the determination of the IC_{50} values was performed according to previously reported procedures [33]. IC_{50} values were determined using seven concentrations of the inhibitors, with GraphPad Prism 6.0 software used to calculate the values. IC_{50} values were determined in three independent measurements, and their means are given as the final result.

4.4. Determination of inhibitory activities on human DNA topoisomerase IIα

Inhibitory activities were determined in an assay from Inspiralis using streptavidin-coated 96-well microtiter plates (Thermo Scientific Pierce). First, the plates were rehydrated with buffer (20 mM Tris/HCl, 0.01% [w/v] bovine serum albumin, 0.05% [v/v] Tween 20, 137 mM NaCl, pH 7.6), and then the biotinylated oligonucleotide was immobilized. After washing off the unbound oligonucleotide, the enzyme assays were performed. The reaction volume was 30 μL in buffer (50 mM Tris/HCl, 10 mM $MgCl_2$, 125 mM NaCl, 5 mM dithiothreitol, 0.1 μg/mL albumin, 1 mM ATP, pH 7.5) that contained 1.5 U human DNA topoisomerase II, 0.75 μg supercoiled pNO1 plasmid, and 3 μL inhibitor solution in 10% dimethylsulfoxide (DMSO) containing 0.008% Tween 20. Reaction solutions were incubated at 37 °C for 30 min. TF buffer (50 mM NaOAc, 50 mM NaCl, 50 mM $MgCl_2$, pH 5.0) was then added to terminate the enzymatic reaction. After additional incubation for 30 min at room temperature, during which the biotin–oligonucleotide–plasmid triplex was formed, the unbound plasmid was washed off using TF buffer, and Diamond Dye in T10 buffer (10 mM Tris/HCl, 1 mm EDTA, pH 8.0) was added. The fluorescence was measured using a microplate reader (Synergy™ 4 Hybrid; BioTek, VT, USA; excitation: 485 nm; emission: 537 nm). The initial screening was done at 100 μM or 10μM concentrations of inhibitors. For the most active inhibitors, the IC_{50} was determined using seven concentrations of each compound. The GraphPad Prism 6.0 software was used to calculate the IC_{50} values. The data are reported as the means of three independent measurements. Etoposide was used as the positive control (IC_{50} 71 μM).

4.5. Determination of antibacterial activity

Clinical microbiology control strains of A. baumannii (ATCC 17978, ATCC19606), ciprofloxacin-resistant *A. baumannii* (ATCC BAA-1605), *E. faecalis* (ATCC 29212), *E. faecium* (ATCC 700221), *E. coli* (ATCC 25922), *K. pneumoniae* (ATCC 700603, ATCC 10031), *P. aeruginosa* (ATCC 27853), *S. aureus* (ATCC 25913), methicillin-resistant *S. aureus* (ATCC 43300) and vancomycin-intermediate *S. aureus* (ATCC 700699) were obtained from American Type Culture Collection (ATCC) via Microbiologics Inc. (St. Cloud, MN, USA). *E. coli* MG1655 originated from the laboratory collection of Dr. Csaba Pal. *E. coli* (20204), ciprofloxacin-resistant *E. coli* (31995, 31859), and *P. aeruginosa* (19488) clinical strains were obtained from the strain collection of the University of Szeged (Hungary). The GyrB mutant strains of methicillin-resistant *S. aureus* (ATCC 43300) and vancomycin-intermediate *S. aureus* (ATCC 700699) were generated according to the published protocol [23]. The GyrB mutant strain of *E. coli* MG1655 was constructed using pORTMAGE [34] recombineering (Addgene plasmid # 120418; http://n2t.net/addgene:120418; RRID:Addgene_120418) according to the published protocol [35].

Mueller Hinton Agar or cation-adjusted Mueller Hinton II broth (MHBII) were used for growth of the bacteria under standard laboratory conditions, for antimicrobial susceptibility tests, and for selection of resistant variants. To prepare the MHBII broth, 22 g MHBII powder (containing 3 g beef extract, 17.5 g acid hydrolysate of casein, 1.5 g starch; Becton Dickinson and Co.) was dissolved in 1 L water. MHBII agar was prepared by the addition of 14 g agar (Bacto; Molar Chemicals) to 1 L broth.

Minimum inhibitory concentrations (MICs) were determined using a standard serial broth microdilution technique, according to the Clinical and Laboratory Standards Institute guidelines [36]. For details on MIC determination see Supplementary Information.

4.6. Molecular docking

Three-dimensional models of the designed DNA gyrase B and topoisomerase IV inhibitors were built in Chem3D 18.0 (PerkinElmer Inc., Massachusetts, USA). The geometries and charges of the ligands were optimized using the MM2 force field, and partial atomic charges were assigned. The energy was minimized until the gradient was <0.001 kcal/(mol × Å). Molecular docking calculations were performed using Schrödinger Release 2019–1 (Schrödinger, LLC, New York, NY, USA, 2019). The crystal structure of *E. coli* DNA gyrase B in complex with bithiazole inhibitor (PDB entry: 4DUH) was retrieved from the Protein Data Bank. The protein was then prepared using Protein Preparation Wizard with the default settings. The receptor grid was calculated for the ligand-binding site, and the designed compounds were docked using the Glide XP protocol, as implemented in Schrödinger Release 2019–1 (Glide, Schrödinger, LLC, New York, NY, USA, 2019).

4.7. Crystallography

Protein production, crystallization, preparation of the inhibitor–protein complexes, data collection and processing, and structure modeling and refinement were performed by SARomics Biostructures, Medicon Village, Lund, Sweden. For details on crystallography see Supplementary Information.

4.8. In vitro cytotoxicity and genotoxicity measurements

The cytotoxicity of compound **31h** in HepG2 and MCF-7 cells was determined using the lactate dehydrogenase (LDH) assay (Thermo Fisher Scientific, Waltham, MA, USA). Briefly, the HepG2 and MCF-7 cells were cultured in Eagle's minimum essential medium (Gibco, Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 2 mM L-glutamine (Sigma-Aldrich, St. Louis, MO, USA), 100 U/mL penicillin (Sigma-Aldrich, St. Louis, MO, USA), 100 μg/mL streptomycin (Sigma-Aldrich, St. Louis, MO, USA), and 10% fetal bovine serum (Gibco, Thermo Fisher Scientific, Waltham, MA, USA), at 37 °C and under 5% CO_2. The LDH assays for compound **31h** were performed according to manufacturer instructions using cytotoxicity assay kits (CyQUANT™ LDH; Thermo Fisher Scientific, Waltham, MA, USA). The genetic toxicity analysis of compound **31h** was performed in an *in vitro* micronucleus test, according to the published protocol [37], at Eurofins Panlabs (St. Charles, MO, US). For details on cytotoxicity and genotoxicity assays see Supplementary Information.

4.9. In vitro metabolism assay

The *in vitro* metabolism study was performed using the rat hepatic S9 fraction in phosphate buffer, pH 7.4, supplemented with $MgCl_2$, NADPH, GSH, UDPGA, PAPS and alamethicin as co-factors/additives. The following were purchased from Sigma-Aldrich (St. Louis, MO, USA): pooled S9 from liver; β-nicotinamide adenine dinucleotide phosphate (NADPH, reduced tetra (cyclohexylammonium salt; CAS 100929-71-3); L-glutathione (GSH, 98%; CAS 70-18-8); uridine diphosphate glucuronic acid (UDPGA; ammonium salt; 98%–100%; CAS 43195-60-4); adenosine 3′-phosphate 5′-phosphosulfate (PAPS, lithium salt hydrate; CAS 109434-21-1); alamethicin (CAS 27061-78-5); sodium phosphate monobasic, anhydrous (>98%; CAS 7558-80-7); and magnesium chloride (98%; CAS 7786-30-3). Details on metabolism assay can be found in Supplementary Information.

Data availability

The co-crystal structure of *S. aureus* DNA gyrase subunit B in complex with **31c** has been deposited as PDB entry 6TTG.

Author contributions

L.P.M. and T.T. conceived the study. M.D. and O.B. carried out the synthesis. T.T. planned and carried out docking experiments. Ž.S. carried out enzyme activity testing. A.N., C.D.C., P.T., T.R., G.D., P.S., L.D., and C.P. planned and carried out microbiology testing. M.W., Y.R.K., and D.F. carried out crystallography. M.D. and D.G.S. carried out cytotoxicity testing. M.D., D.G.S., and J.T. carried out metabolism assay. M.D., A.N., N.Z., J.I., A.Z., C.P., D.K., L.P.M., and T.T. contributed to the interpretation of the results. M.D. wrote the manuscript with input from all authors. All authors have given approval to the final version of the manuscript.

Conflict of Interest Statement

The authors declare conflict of interest. A PCT patent application (New class of DNA gyrase and/or topoisomerase IV inhibitors with activity against gram-positive and gram-negative bacteria: PCT/EP2019/073412073412073412), has been filed by T. Tomašič, N. Zidar, M. Durcik, J. Ilaš, A. Zega, C. Durante Cruz, P. Tammela, C. Pál, A. Nyerges, D. Kikelj, L. Peterlin Mašič.

Declaration of Competing Interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejmech.2021.113200.

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