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New *N*-phenylpyrrolamide inhibitors of DNA gyrase with improved antibacterial activity†

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This study presents the discovery of a new series of *N*-phenylpyrrolamide inhibitors of bacterial DNA gyrase with improved antibacterial activity. The most potent inhibitors had low nanomolar IC₅₀ values against *Escherichia coli* DNA gyrase (IC₅₀; 2–20 nM) and *E. coli* topoisomerase IV (22i, IC₅₀ = 143 nM). Importantly, none of the compounds showed activity against human DNA topoisomerase II α , indicating selectivity for bacterial targets. Among the tested compounds, **22e** emerged as the most effective against Gram-positive bacteria with minimum inhibitory concentration (MIC) values of 0.25 $\mu\text{g mL}^{-1}$ against *Staphylococcus aureus* ATCC 29213 and MRSA, and 0.125 $\mu\text{g mL}^{-1}$ against *Enterococcus faecalis* ATCC 29212. For Gram-negative bacteria, compounds **23b** and **23c** showed the greatest efficacy with MIC values ranging from 4 to 32 $\mu\text{g mL}^{-1}$ against *E. coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Acinetobacter baumannii* ATCC 17978 and *A. baumannii* ATCC 19606. Notably, compound **23b** showed promising activity against the clinically relevant Gram-negative pathogen *Klebsiella pneumoniae* ATCC 10031, with an MIC of 0.0625 $\mu\text{g mL}^{-1}$. Furthermore, compounds **23a** and **23c** exhibited significantly lower susceptibility to resistance development compared to novobiocin in *S. aureus* ATCC 29213 and *K. pneumoniae* ATCC 10031. Overall, the most promising compounds of this series showed excellent on-target potency, marking a significant improvement over previous *N*-phenylpyrrolamide inhibitors.

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1. Introduction

Antimicrobial resistance (AMR) is among the top ten global health threats of the 21st century.¹ Antibiotic-resistant bacteria are projected to cause at least 10 million deaths annually by 2050.² The World Health Organization (WHO) identifies six bacterial species as critical or high priority: the Gram-negative *Escherichia coli*, *Klebsiella pneumoniae*, *Acinetobacter baumannii* and *Pseudomonas aeruginosa*, and the Gram-positive *Staphylococcus aureus* and *Streptococcus pneumoniae*.³ Among these, methicillin-resistant *Staphylococcus aureus* (MRSA) accounted

for the most AMR-related deaths in 2019,³ and the incidence of community-acquired MRSA infections is continuing to rise.^{4,5}

DNA gyrase is an essential enzyme for bacterial survival as it plays an important role in DNA replication, transcription, and recombination by facilitating the cleavage and rejoining of DNA strands.⁶ Topoisomerase IV, structurally and functional similar enzyme to DNA gyrase, is vital for bacterial cell division. Both enzymes form tetrameric complexes necessary for DNA binding, cleavage and transport: DNA gyrase consists of two GyrA and two GyrB subunits, while topoisomerase IV comprises two ParC and two ParE subunits.^{6,7} The cleavage and rejoining of DNA require the hydrolysis of an ATP molecule. The ATP-binding site is located on the GyrB subunit of DNA gyrase and the ParE subunit of topoisomerase IV. Due to their structural similarities, these enzymes offer exceptional opportunities for dual targeting with new antibacterial compounds, potentially reducing the likelihood of bacteria developing resistance.⁸

The most well-known inhibitors of DNA gyrase and topoisomerase IV are fluoroquinolones, which act by binding to the GyrA and/or ParC subunit and stabilizing the enzyme-DNA complex.^{9,10} Fluoroquinolones are among the most common and effective antibacterial agents, but side effects and

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increasing bacterial resistance limit their use.^{11,12} Currently, no GyrB inhibitors that work through an ATP-competitive mechanism are available in clinical practice.¹³ Although several classes of ATP-competitive inhibitors targeting DNA gyrase and/or topoisomerase IV have been identified in recent decades, including pyridylureas,¹⁴ pyrimidinoindoles,¹⁵ benzimidazoleureas,¹⁶ benzothiazoles,^{17–22} pyrazolopyridones,²³ and pyrrolamides,²⁴ none have been approved for clinical use. Two ATP-competitive DNA gyrase inhibitors are currently in clinical trials: fobrepodacin or SPR720 for non-tuberculous mycobacterial infections²⁵ and DS-2969b for *Clostridium difficile* infections.²⁶ Further research is needed to develop GyrB/ParE inhibitors with enhanced antibacterial activity, reduced toxicity and favourable ADME (absorption, distribution, metabolism, and excretion) properties.

We have recently discovered a new class of DNA gyrase inhibitors, the *N*-phenylpyrrolamides (Fig. 1).^{27–32} Building on the first X-ray crystal structure of an *N*-phenylpyrrolamide inhibitor **A** bound to the active site of GyrB (Fig. 1),²⁷ we developed several classes of optimised inhibitors. By substituting the 4,5-dibromopyrrole ring in compound **A** with a 3,4-dichloro-5-methyl-substituted pyrrole (Fig. 1, compound **B**), we achieved a twofold increase in activity against *E. coli* DNA gyrase, improving an IC₅₀ value from 450 nM to 280 nM.²⁸ Further enhancement was achieved by introducing an isopropoxy group on the central phenyl ring (Fig. 1, compound **C**), resulting in an IC₅₀ of 47 nM against *E. coli* DNA gyrase, along with weak activity against Gram-positive *E. faecalis*.²⁸ The antibacterial activity was further improved by replacing the carboxyl group with an isosteric group on the right-hand side of the molecule (Fig. 1, compound **D**).²⁹ Structural optimisation at the three key positions marked in Fig. 1 (compound **D**) proved critical for the potent on-target and antibacterial activity of this

class of DNA gyrase inhibitors. In this study, we present the synthesis and evaluation of a new series of *N*-phenylpyrrolamide DNA gyrase inhibitors, featuring a basic substituent at the central benzene core, which exhibit improved antibacterial activity. For selected promising inhibitors, we performed an in-depth evaluation, including determination of selectivity, frequency of resistance and thermodynamic solubility.

2. Results and discussion

2.1. Structure-based design

We have designed a series of new *N*-phenylpyrrolamide DNA gyrase inhibitors by systematically varying the substituents R¹, R², R³ and R⁴ (Fig. 2). In most of these new compounds, we used the 3,4-dichloro-5-methyl-substituted pyrrole as R¹, as this has been shown to be optimal in our previous inhibitor series.^{28,29,32} To investigate the effects of different substituents on the pyrrole ring, we also synthesised a small set of 4,5-dibromopyrrole analogues. The NH group of the amide bond was left unsubstituted in most compounds (R² = H, Fig. 2), although we also prepared two compounds with a methyl or benzyl substituent at this position. In this way, we aimed to disrupt the planarity of the central core and thus improve the physicochemical properties of the compounds. As R³ (Fig. 2), we introduced various nitrogen-containing heterocycles, including piperazine, 3-aminopyrrolidine, 3-aminopiperidine, piperidin-4-amine, morpholine, and 3-aminomethylpiperidine. These groups were chosen to (i) promote additional interactions with the lipophilic floor of the enzyme, (ii) enable additional hydrogen bonding through the NH group of the ring, and/or (iii) increase antibacterial activity, as the presence of a positive charge has been associated with enhanced accumulation in *E. coli*.^{33–35} At the right end of

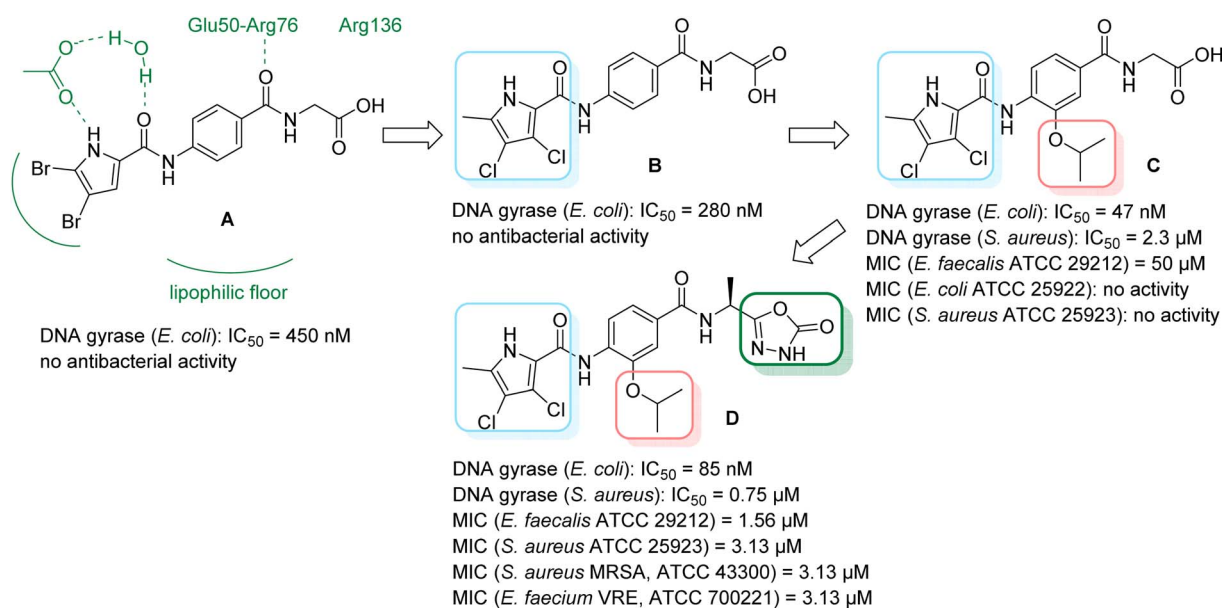


Fig. 1 A brief summary of the optimisation pathway for *N*-phenylpyrrolamide inhibitors of DNA gyrase. Starting from compound **A**,²⁷ the on-target and antibacterial activities increased through compounds **B** and **C**,²⁸ to compound **D**.²⁹



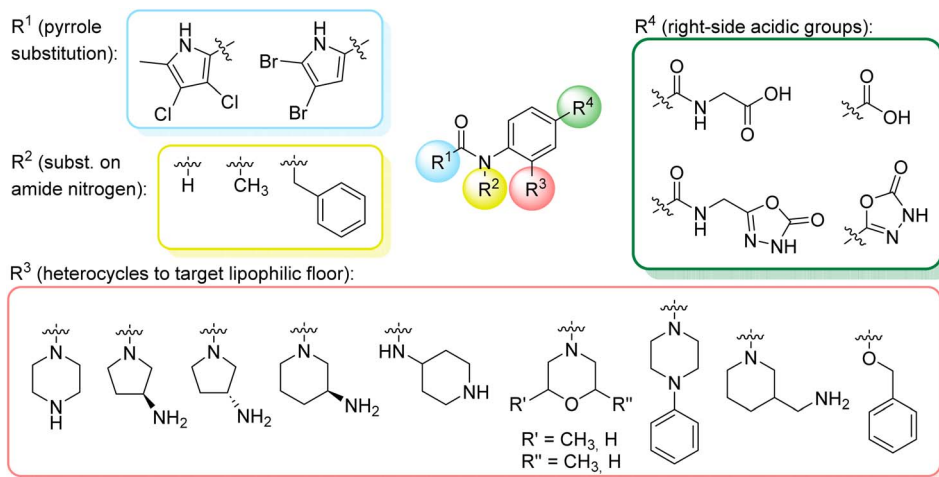


Fig. 2 Optimisation plan of *N*-phenylpyrrolamide inhibitors of DNA gyrase.

the molecules (R^4 , Fig. 2), we introduced either a carboxyl group or an isosteric 5-oxo-1,3,4-oxadiazole group, both capable of interacting with the positively charged Arg136 residue or forming cation- π interactions with the Glu50-Arg76 salt bridge. In some compounds, these groups were directly attached to the central phenyl ring, while in others they were connected *via* an additional three-atom linker to modulate the spatial arrangement and binding interactions.

2.2. Chemistry

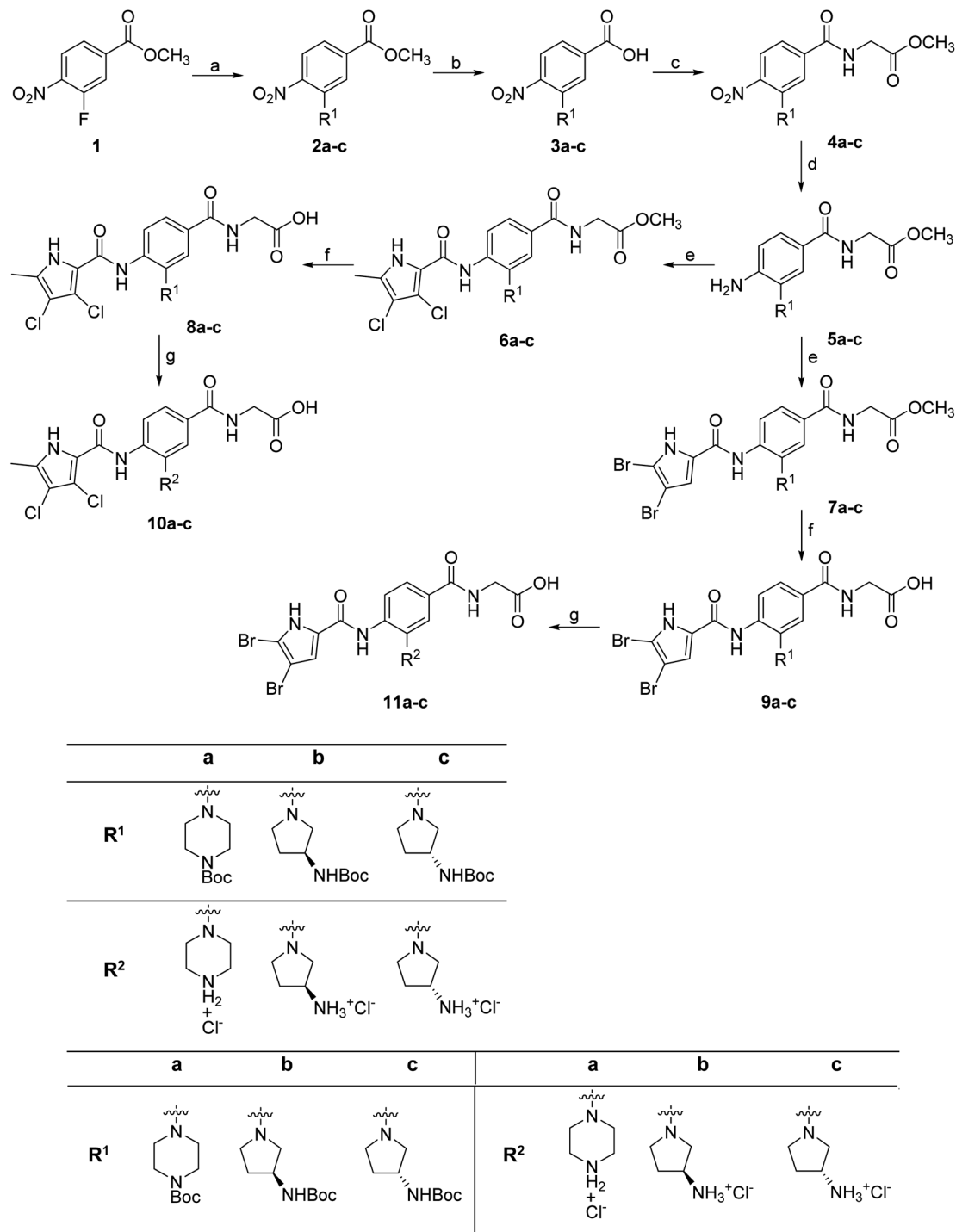
The synthesis of compounds **8–11** is shown in Scheme 1. In the first step, the fluorine in the methyl 3-fluoro-4-nitrobenzoate (**1**) was substituted with either Boc-piperazine or Boc-3-aminopyrrolidine as nucleophile. Next, the methyl ester groups of **2a–c** were hydrolysed with sodium hydroxide solution, and the glycine methyl ester was attached to the free carboxyl groups of **3a–c** *via* TBTU-promoted coupling. The nitro groups of **4a–c** were then reduced to the amino groups by hydrogenation with Pd/C as catalyst. Either 3,4-dichloro-5-methyl-1*H*-pyrrole-2-carboxylic acid (for the synthesis of **6a–c**) or 4,5-dibromo-1*H*-pyrrole-2-carboxylic acid (for the synthesis of **7a–c**) were attached to the amino groups of **5a–c** *via* the amide bond by first activating the carboxyl group with oxalyl chloride to form the acid chloride. The final compounds were obtained by first hydrolysing the methyl ester groups of **6a–c** and **7a–c** to the carboxyl groups (**8a–c** and **9a–c**), followed by the removal of the Boc protecting groups with hydrochloric acid in 1,4-dioxane, resulting in compounds **10a–c** and **11a–c**. For compound **10a**, an analogue (**14**) was also synthesised, in which the terminal carboxyl group on the right-hand side of the molecule was replaced with a 5-oxo-1,3,4-oxadiazole group. The synthesis of this compound is shown in Scheme 2. In the first step, the methyl ester group of **6c** was converted to the hydrazide group by reaction with hydrazine monohydrate in a mixture of MeOH and THF under reflux to give **12**. The hydrazide group was then

cyclised to the 5-oxo-1,3,4-oxadiazole ring using 1,1'-carbonyldiimidazole (CDI) as a reagent. To obtain the final compound **14**, the Boc protecting group was removed by acidolysis with hydrochloric acid in 1,4-dioxane.

To prepare compounds **17–20**, we used the procedure shown in Scheme 3. Starting from 3-fluoro-4-nitrobenzoic acid methyl ester (**1**), we prepared a series of analogues (**2a–b**, **15c–i**) by introducing an amine-containing heterocycle through nucleophilic substitution of fluorine at the central benzene core. After reduction of the nitro groups to the amino groups to form **16a–i**, the 3,4-dichloro-5-methyl-1*H*-pyrrole-2-carboxylic acid was attached *via* the amide bond by first activating the carboxyl group with oxalyl chloride or thionyl chloride to give **17a–i**. Compounds **18**, **19** and **20** were prepared from **17a–i** by hydrolysing the methyl ester groups and removing the Boc protecting groups by acidolysis with hydrochloric acid. Compounds **17a–g** were further converted into analogues in which the 5-oxo-1,3,4-oxadiazole group was introduced in place of the carboxyl group on the right-hand side (Scheme 4). First, the methyl ester group of **17a–g** was converted to a hydrazide group using hydrazine monohydrate as reagent (**21a–g**), which was followed by a reaction with CDI to give compounds **22a–g**. The final step involved the acidolytic cleavage of the Boc protecting group in **22a–d**, resulting in the desired compounds **23a–d**.

The synthesis of the morpholine derivative **27a** with the 4,5-dibromopyrrole group on the left-hand side, and the synthesis of the 3,4-dichloro-5-methylpyrrole analogue **27b** with the methyl group on the amide nitrogen, are shown in Scheme 5. To introduce the methyl group on the amino group of **16e**, a reductive amination reaction was employed. First, the methylenimine intermediate was formed using paraformaldehyde as a reagent, which was then reduced with sodium borohydride to give the methylamino compound **24**. In the next step, 3,4-dichloro-5-methyl-1*H*-pyrrole-2-carboxylic acid or 4,5-dibromo-1*H*-pyrrole-2-carboxylic acid was coupled with the amino groups of **16e** and **24** to obtain the amides **25a–b**. After hydrazinolysis





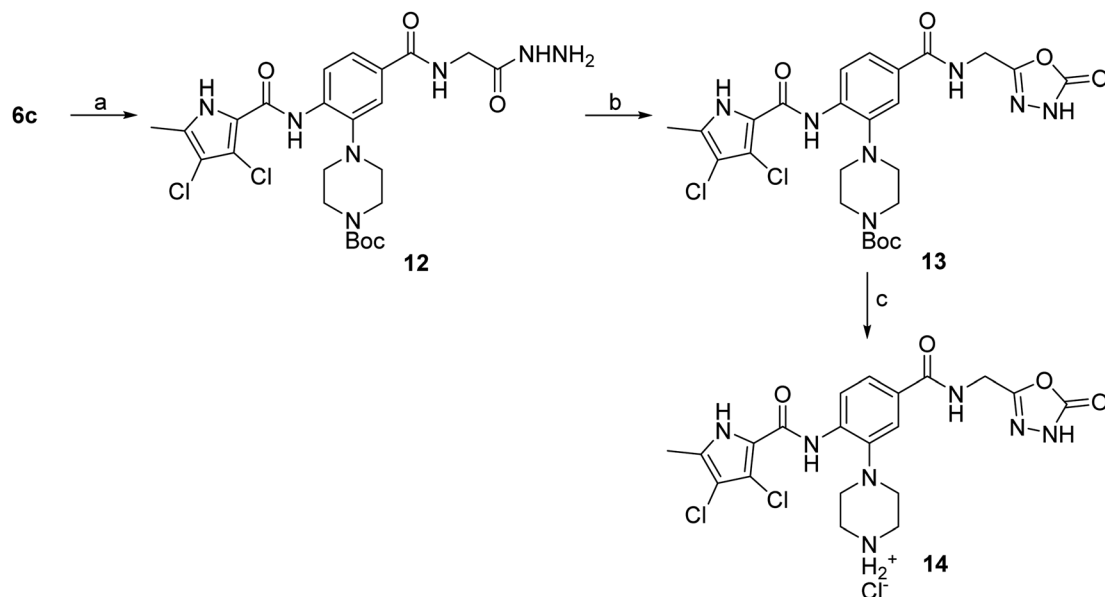
Scheme 1 Reagents and conditions: (a) corresponding amine, K_2CO_3 , DMF, 70 °C, 15 h; (b) 1 M NaOH, MeOH or MeOH and THF, rt, 15 h; (c) glycine methyl ester hydrochloride, TBTU, NMM, DMF, rt, 20 h; (d) H_2 , Pd/C, MeOH, rt, 4–5 h; (e) i: 3,4-dichloro-5-methylpyrrole-2-carboxylic acid or 4,5-dibromo-1H-pyrrole-2-carboxylic acid, oxalyl chloride, anhydrous CH_2Cl_2 , rt, 15 h, then ii: **5a–c**, anhydrous pyridine, anhydrous CH_2Cl_2 , rt, 15 h; (f) 1 M NaOH, MeOH or MeOH and THF, rt, 15 h; (g) 4 M HCl in 1,4-dioxane, THF (for some compounds), rt, 1–2 h.

of the methyl ester groups of **26a–b** and subsequent cyclization to 5-oxo-1,3,4-oxadiazole using CDI as reagent, the final compounds **27a–b** were obtained.

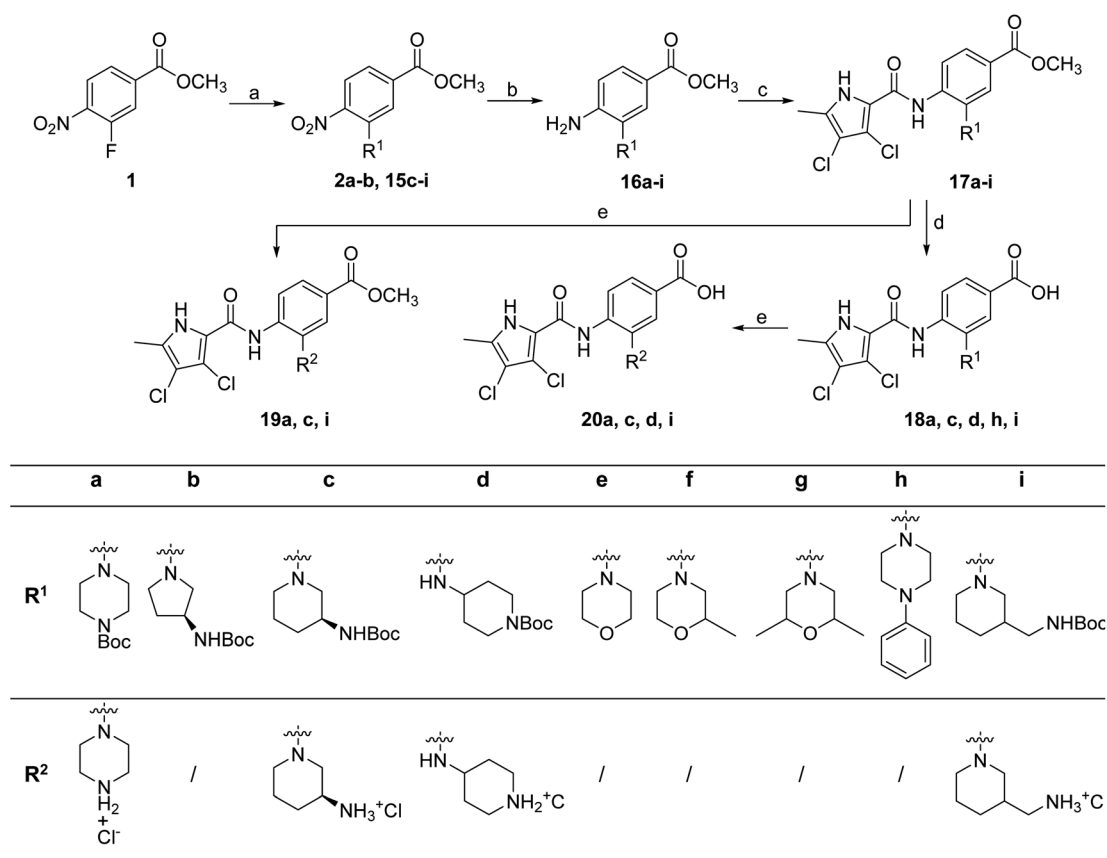
To prepare compound **33**, which features a benzyl group on the amide nitrogen, the procedure shown in Scheme 6 was

followed. After reduction of the nitro group of 4-nitrobenzoic acid methyl ester (**28**) by catalytic hydrogenation, the benzyl substituent was added in two steps. First, the benzylimine was formed in the reaction between **29** and benzaldehyde, and then the imine group was reduced with sodium borohydride to give



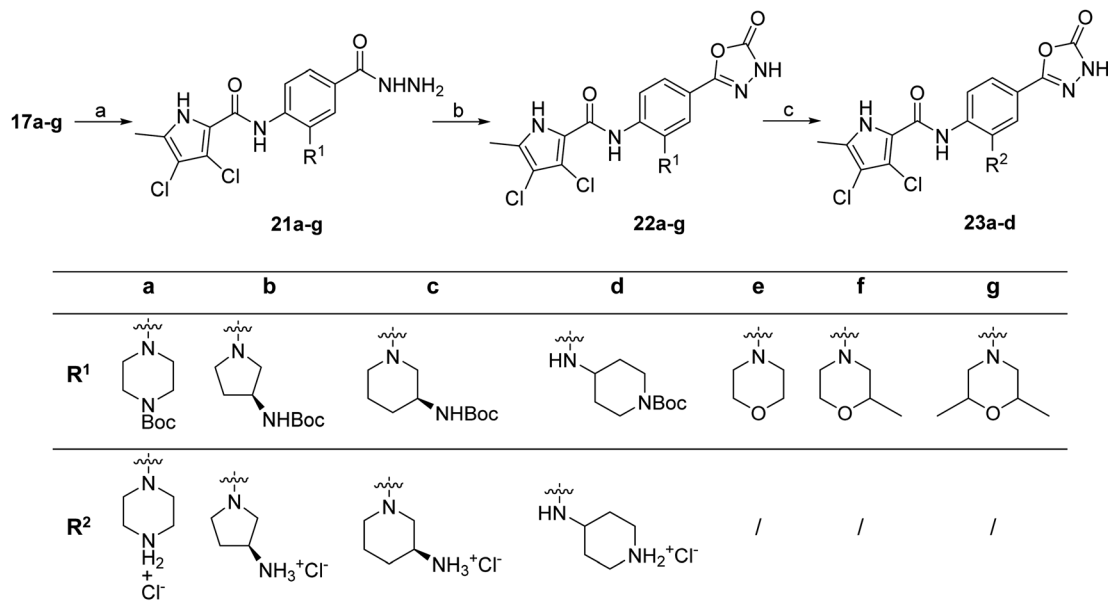


Scheme 2 Reagents and conditions: (a) hydrazine monohydrate, MeOH/THF (1 : 1), reflux, 20 h; (b) CDI, 1,4-dioxane/DMF (3 : 1), 101 °C, 20 h; (c) 4 M HCl in 1,4-dioxane, rt, 2 h.



Scheme 3 Reagents and conditions: (a) corresponding amine, K₂CO₃, DMF, 70 °C, 15 h; (b) H₂, Pd/C, MeOH or MeOH and THF, rt, 1–5 h; (c) i: 3,4-dichloro-5-methylpyrrole-2-carboxylic acid, oxalyl chloride, anhydrous CH₂Cl₂, rt, 15 h (or thionyl chloride, 75 °C, 1 h, for the synthesis of 17e), then ii: 16a–i, anhydrous pyridine, anhydrous CH₂Cl₂, rt, 15 h (or toluene, 130 °C, 15 h, for the synthesis of 17e); (d) 1 M NaOH, MeOH or MeOH and THF, rt (or 60 °C for 18d and 18h), 15 h; (e) 1 M HCl in acetic acid or 4 M HCl in 1,4-dioxane, THF or CH₂Cl₂ (for some compounds), rt, 5–15 h.

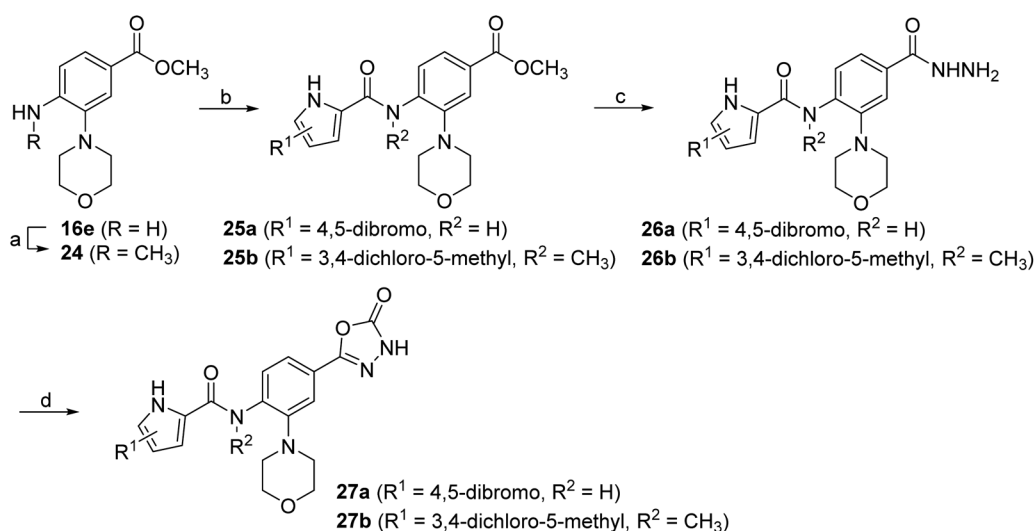




Scheme 4 Reagents and conditions: (a) hydrazine monohydrate, MeOH, THF, high-pressure tube, 120 °C, 15–48 h; (b) CDI, 1,4-dioxane, DMF, 101 °C, 15–24 h; (c) 4 M HCl in 1,4-dioxane, DMF (for some compounds), rt, 3–15 h.

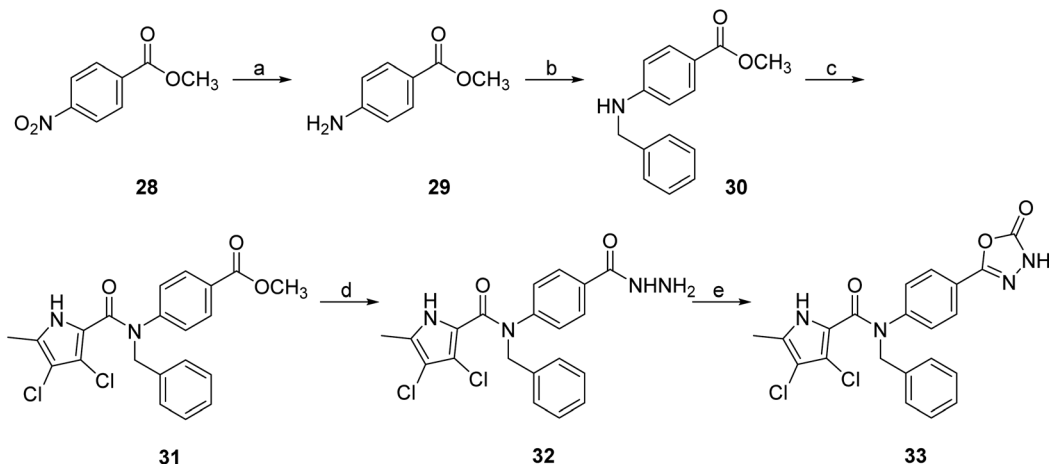
30. Next, the 3,4-dichloro-5-methyl-1*H*-pyrrole-2-carboxylic acid was coupled with the amino group of **30** *via* an amide bond to give **31**. To obtain the final compound **33**, the methyl ester group of **31** was first converted to the hydrazide group to give **32** in the reaction with hydrazine monohydrate. This was followed by cyclisation with CDI to form the 5-oxo-1,3,4-oxadiazole group. An analogue of **33** with the benzyloxy substituent attached to the benzene core (**39**) was prepared according to the procedure shown in Scheme 7. In the first step, the benzyl group was attached to the phenolic hydroxyl group of **34** using benzyl

bromide as reagent and potassium carbonate as base. Subsequently, compound **35** was reacted with hydrazine monohydrate to form hydrazide **36**, which was then converted to the 5-oxo-1,3,4-oxadiazole group with CDI. The nitro group of **37** was then reduced to the amino group, and finally the 4-dichloro-5-methyl-1*H*-pyrrole-2-carboxylic acid was attached to **38** *via* an amide bond. This was achieved by first activating the carboxyl group with oxalyl chloride to give the final compound **39**.



Scheme 5 Reagents and conditions: (a) i: paraformaldehyde, NaOMe, MeOH, 40 °C, 15 h, then ii: NaBH₄, 40 °C, 20 h; (b) i: 3,4-dichloro-5-methylpyrrole-2-carboxylic acid or 4,5-dibromo-1*H*-pyrrole-2-carboxylic acid, oxalyl chloride, anhydrous CH₂Cl₂, rt, 15 h, then ii: **16e** or **24**, anhydrous pyridine, anhydrous CH₂Cl₂, rt, 15 h; (c) hydrazine monohydrate, MeOH, THF, high-pressure tube, 120 °C, 15 h; (d) CDI, 1,4-dioxane, DMF, 101 °C, 15 h.





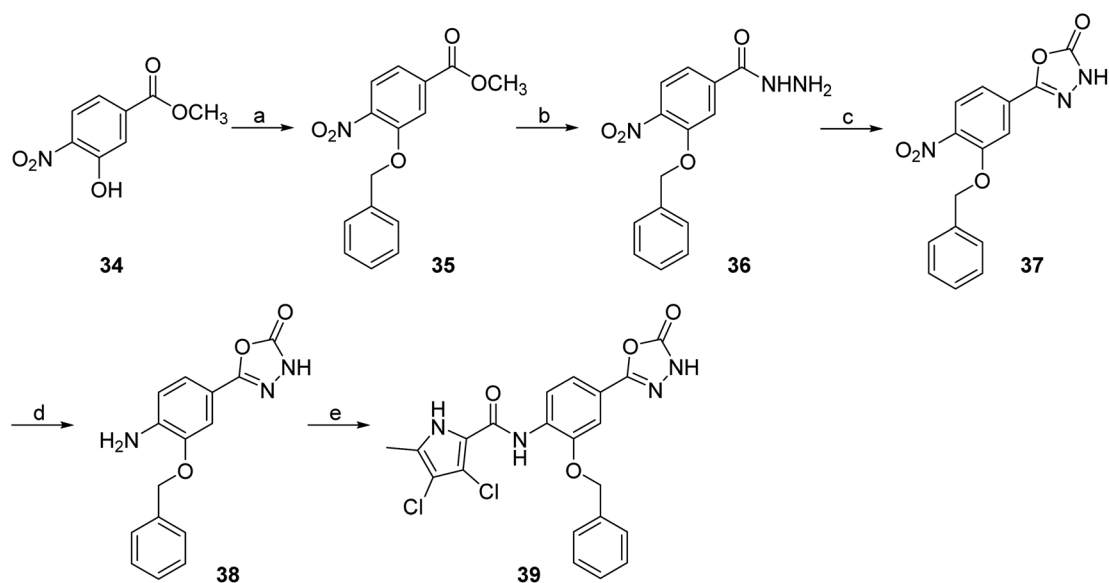
Scheme 6 Reagents and conditions: (a) H₂, Pd/C, MeOH, THF, rt, 3 h; (b) i: benzaldehyde, Na₂SO₄, CH₂Cl₂, reflux, 15 h, ii: NaBH₄, MeOH, rt, 15 h; (c) i: 3,4-dichloro-5-methylpyrrole-2-carboxylic acid, oxalyl chloride, anhydrous CH₂Cl₂, rt, 15 h, then ii: **30**, anhydrous pyridine, anhydrous CH₂Cl₂, rt, 15 h; (d) hydrazine monohydrate, MeOH, THF, high-pressure tube, 100 °C, 2 d; (e) CDI, 1,4-dioxane, 101 °C, 15 h.

2.3. Biological evaluation

2.3.1 Inhibition of DNA gyrase, topoisomerase IV and human DNA topoisomerase II α . The compounds were tested for their inhibitory activity against DNA gyrase and topoisomerase IV from *E. coli* and *S. aureus* in supercoiling and relaxation plate assays, respectively. The selectivity was determined against a structurally similar enzyme, human DNA topoisomerase II α . Enzyme inhibition data are shown in Tables 1 and 2, either as residual activities (RA) at a given concentration or as half-maximal inhibitory concentrations (IC₅₀ values).

Table 1 shows the enzyme inhibitory activities of compounds **6–14**, which feature carboxyl or 5-oxo-1,3,4-oxadiazole groups connected to the central phenyl ring through a three-atom

linker. The comparison of compound **8a** (IC₅₀ = 84.1 nM) with its methyl ester analogue **6a** (IC₅₀ = 4080 nM) highlights the critical role of the terminal acidic group for the potent activity on *E. coli* gyrase. Furthermore, Table 1 shows that compounds containing the 3,4-dichloro-5-methyl-substituted pyrrole ring on the left-hand side of the molecules (**8a–c**, **10a–c**) exhibit, on average, 10- to 50-fold greater potency on *E. coli* gyrase than those with the 4,5-dibromopyrrole ring (**9a–c**, **11a–c**). A similar difference in activity is observed when the compounds with the Boc protecting group at the nitrogen-containing heterocycle (**8a–c**, **9a–c**, **13**) are compared with the Boc-deprotected analogues (**10a–c**, **11a–c**, **14**). This difference may be attributed to the free NH group in the Boc-deprotected



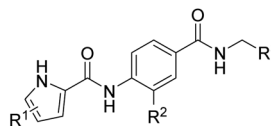
Scheme 7 Reagents and conditions: (a) K₂CO₃, benzyl bromide, CH₃CN, rt, 60 °C, 3 h; (b) hydrazine monohydrate, MeOH, THF, 65 °C, 15 h; (c) CDI, 1,4-dioxane, 101 °C, 15 h; (d) Fe, glacial acetic acid, rt, 1.5 h; (e) i: 3,4-dichloro-5-methylpyrrole-2-carboxylic acid, oxalyl chloride, anhydrous CH₂Cl₂, rt, 15 h, then ii: **38**, anhydrous pyridine, anhydrous CH₂Cl₂, rt, 15 h.

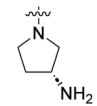
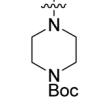
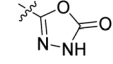
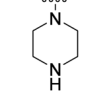
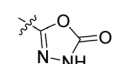
Table 1 Inhibitory activities of compounds **6–14** against DNA gyrase and topoisomerase IV from *E. coli* and *S. aureus*, and human DNA topoisomerase II α

Comp.	R ¹	R ²	R ³	IC ₅₀ ^a (nM) or RA ^b (%)				
				<i>E. coli</i> gyrase	<i>S. aureus</i> gyrase	<i>E. coli</i> topo IV	<i>S. aureus</i> topo IV	Human topo II α
6a	3,4-Dichloro-5-methyl		COOCH ₃	4080 nM	n.d. ^c	n.d.	n.d.	n.d.
8a	3,4-Dichloro-5-methyl		COOH	84.1 nM	37% (1 μ M)	93% (1 μ M)	22% (1 μ M)	n.d.
8b	3,4-Dichloro-5-methyl		COOH	196 nM	n.d.	n.d.	n.d.	n.d.
8c	3,4-Dichloro-5-methyl		COOH	162 nM	n.d.	n.d.	n.d.	n.d.
9a	4,5-Dibromo		COOH	362 nM	n.d.	n.d.	n.d.	n.d.
9b	4,5-Dibromo		COOH	4110 nM	n.d.	n.d.	n.d.	n.d.
9c	4,5-Dibromo		COOH	5390 nM	n.d.	n.d.	n.d.	n.d.
10a	3,4-Dichloro-5-methyl		COOH	40.3 nM	621 nM	93% (1 μ M)	4190 nM	78% (10 μ M)
10b	3,4-Dichloro-5-methyl		COOH	19.3 nM	112 nM	958 nM	2560 nM	82% (10 μ M)
10c	3,4-Dichloro-5-methyl		COOH	21.3 nM	1240 nM	2370 nM	2030 nM	112% (40 μ M)
11a	4,5-Dibromo		COOH	841 nM	n.d.	97% (10 μ M)	73% (10 μ M)	100% (40 μ M)
11b	4,5-Dibromo		COOH	235 nM	46% (10 μ M)	84% (10 μ M)	91% (10 μ M)	n.d.



Table 1 (Contd.)



Comp.	R ¹	R ²	R ³	IC ₅₀ ^a (nM) or RA ^b (%)				
				<i>E. coli</i> gyrase	<i>S. aureus</i> gyrase	<i>E. coli</i> topo IV	<i>S. aureus</i> topo IV	Human topo II α
11c	4,5-Dibromo		COOH	996 nM	78% (10 μ M)	75% (10 μ M)	n.d.	n.d.
13	3,4-Dichloro-5-methyl			483 nM	n.d.	n.d.	n.d.	n.d.
14	3,4-Dichloro-5-methyl			22.4 nM	37% (1 μ M)	2970 nM	2700 nM	111% (10 μ M)

^a Concentration of compound that inhibits the enzyme activity by 50%. ^b Residual activity of the enzyme at 1 μ M or 10 μ M concentration of the compound. ^c Not determined. Compounds **10a–c**, **11a–c** and **14** were prepared as hydrochloride salts.

compounds, which potentially forms additional interactions with the enzyme, or to steric hindrance caused by the Boc group. An important observation from Table 1 is that compound **10a** (IC₅₀ = 40.3 nM) with the terminal carboxyl group has about two times lower potency than its analogue **14** (IC₅₀ = 22.4 nM) with the 5-oxo-1,3,4-oxadiazole ring as the carboxyl isostere. This difference likely arises from stronger interactions between the 5-oxo-1,3,4-oxadiazole ring and the positively charged Arg136 residue in the enzyme's binding site. Among the compounds shown in Table 1, **10b**, **10c** and **14** exhibit the highest potency against *E. coli* DNA gyrase with IC₅₀ values around 20 nM. Compound **10b** also proves to be the most potent inhibitor of *S. aureus* DNA gyrase, with an IC₅₀ value of 112 nM. However, the inhibitory effects of these compounds on topoisomerase IV in both *E. coli* and *S. aureus* are generally less potent, with IC₅₀ values in the low micromolar range. Importantly, all compounds tested exhibit exceptional selectivity for human DNA topoisomerase II α .

Table 2 shows the enzyme inhibitory activities of compounds **18–39**, which differ from **6–14** by having the carboxyl or 5-oxo-1,3,4-oxadiazole groups directly attached to the central benzene ring. Consistent with the results in Table 1, the compound with a 3,4-dichloro-5-methyl-substituted pyrrole ring (**22e**, IC₅₀ = 82.2 nM) exhibits approximately five times greater potency than its counterpart with the 4,5-dibromopyrrole ring (**27b**, IC₅₀ = 433 nM). On the other hand, although compound **20i** (IC₅₀ = 6.34 nM), which contains a free carboxyl group, is about twice as potent as its methyl ester analogue **19i** (IC₅₀ = 14.2 nM), the activities of the methyl esters are slightly weaker in similar pairs, **20c** (IC₅₀ = 134 nM) and **19c** (IC₅₀ = 120 nM),

and **20a** (RA = 67% at 1 μ M) and **19a** (IC₅₀ = 100 nM). As observed in Table 1, compounds with the Boc-protecting group on the nitrogen-containing heterocycle (**18c**, **18i**, **22a**, **22c**) generally exhibit lower efficacy against *E. coli* DNA gyrase compared to their Boc-deprotected analogues (**20c**, **20i**, **23a**, **23c**). Moreover, compounds with an additional heterocyclic basic centre, such as the piperazine derivative **23a** (IC₅₀ = 17.9 nM), the 3-aminopyrrolidine derivative **23b** (IC₅₀ = 2.80 nM), the 3-aminopiperidine derivative **23c** (IC₅₀ = 27.2 nM), and the piperidin-4-amine derivative **23d** (IC₅₀ = 4.40 nM), demonstrate slightly greater potency than those with a morpholine ring (**22e–g**, IC₅₀ = 53.5–109 nM). This increased potency may be attributed to additional interactions formed by the NH group in the heterocyclic rings. Docking studies of compounds **20i** and **23d** with the active site of GyrB (Fig. 3) support this hypothesis, revealing potential ionic interactions between the amino groups of these compounds and the Asp49 residue.

Compound **39** (IC₅₀ = 886 nM), featuring a benzyloxy substituent, exhibits lower activity compared to other compounds in the series. This reduced activity may be due to the bulkiness of the benzyloxy group or its inability to form additional polar interactions with the enzyme. Similarly, compounds **27a** and **33** with a methyl or benzyl substituent on the amide nitrogen (R², Fig. 2) show weak enzyme inhibitory activities, suggesting that the free amide NH group is crucial for effective binding. Overall, the compounds listed in Table 2 (**18–39**), in which the carboxyl or 5-oxo-1,3,4-oxadiazole groups are directly attached to the central phenyl ring, exhibit slightly greater potency than those listed in Table 1 (**6–14**), which include an additional three-atom linker. The most effective DNA

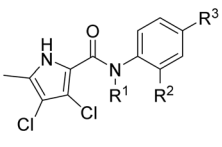
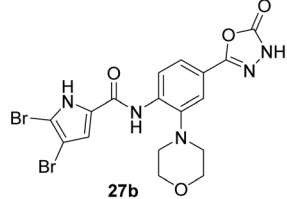
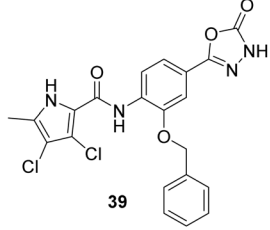
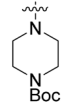

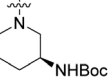





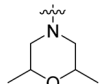

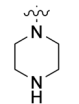

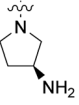

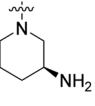

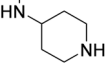






Table 2 Inhibitory activities of compounds 18–39 against DNA gyrase and topoisomerase IV from *E. coli* and *S. aureus*, and human DNA topoisomerase II α

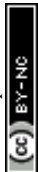
Comp.	R ¹	R ²	R ³	IC ₅₀ ^a (μM) or RA ^b (%)				
				<i>E. coli</i> gyrase	<i>S. aureus</i> gyrase	<i>E. coli</i> topo IV	<i>S. aureus</i> topo IV	Human topo II α
			18-23, 27a, 33					
			27b					
			39					
18a	H		COOH	159 nM	54% (1 μM)	99% (1 μM)	21% (1 μM)	n.d. ^c
18c	H		COOH	67% (1 μM)	n.d.	n.d.	n.d.	n.d.
18h	H		COOH	199 nM	n.d.	85% (10 μM)	n.d.	n.d.
18i	H		COOH	89% (0.1 μM)	n.d.	n.d.	n.d.	128% (100 μM)
19a	H		COOCH ₃	100 nM	45% (1 μM)	102% (1 μM)	95% (1 μM)	n.d.
19c	H		COOCH ₃	120 nM	27% (1 μM)	79% (1 μM)	n.d.	n.d.
19i	H		COOCH ₃	14.2 nM	n.d.	533 nM	n.d.	n.d.
20a	H		COOH	67% (1 μM)	n.d.	106% (1 μM)	92% (1 μM)	116% (40 μM)
20c	H		COOH	134 nM	n.d.	78% (1 μM)	n.d.	n.d.
20d	H		COOH	532 nM	n.d.	89% (10 μM)	n.d.	n.d.
20i	H		COOH	6.34 nM	28% (0.1 μM)	143 nM	0% (1 μM)	28% (100 μM)



Table 2 (Contd.)

Comp.	R ¹	R ²	R ³	IC ₅₀ ^a (μM) or RA ^b (%)				
				<i>E. coli</i> gyrase	<i>S. aureus</i> gyrase	<i>E. coli</i> topo IV	<i>S. aureus</i> topo IV	Human topo IIα
								
								
								
18-23, 27a, 33								
27b								
39								
22a	H			59.0 nM	n.d.	104% (1 μM)	n.d.	100% (100 μM)
22c	H			90% (0.1 μM)	n.d.	n.d.	n.d.	n.d.
22e	H			82.2 nM	n.d.	1230 nM	n.d.	93% (100 μM)
22f	H			53.5 nM	n.d.	103% (1 μM)	n.d.	98% (100 μM)
22g	H			109 nM	n.d.	105% (1 μM)	n.d.	97% (100 μM)
23a	H			17.9 nM	n.d.	71% (1 μM)	n.d.	86% (100 μM)
23b	H			2.80 nM	39% (0.1 μM)	105% (1 μM)	99% (0.1 μM)	86300 nM
23c	H			27.2 nM	41% (0.1 μM)	448 nM	406 nM	90% (50 μM)
23d	H			4.40 nM	n.d.	1257 nM	n.d.	99% (10 μM)
27a	CH ₃			98% (10 μM)	n.d.	103% (1 μM)	n.d.	n.d.
27b	—	—	—	433 nM	n.d.	103% (1 μM)	n.d.	98% (100 μM)
33	Bzl	H		81% (1 μM)	n.d.	n.d.	n.d.	92% (100 μM)
39	—	—	—	886 nM	n.d.	101% (1 μM)	n.d.	104% (100 μM)

^a Concentration of compound that inhibits the enzyme activity by 50%. ^b Residual activity of the enzyme at 1 μM or 10 μM concentration of the compound. ^c Not determined. Compounds **19a**, **19c**, **19i**, **20a**, **20c-d**, **20i** and **23a-d** were prepared as hydrochloride salts.



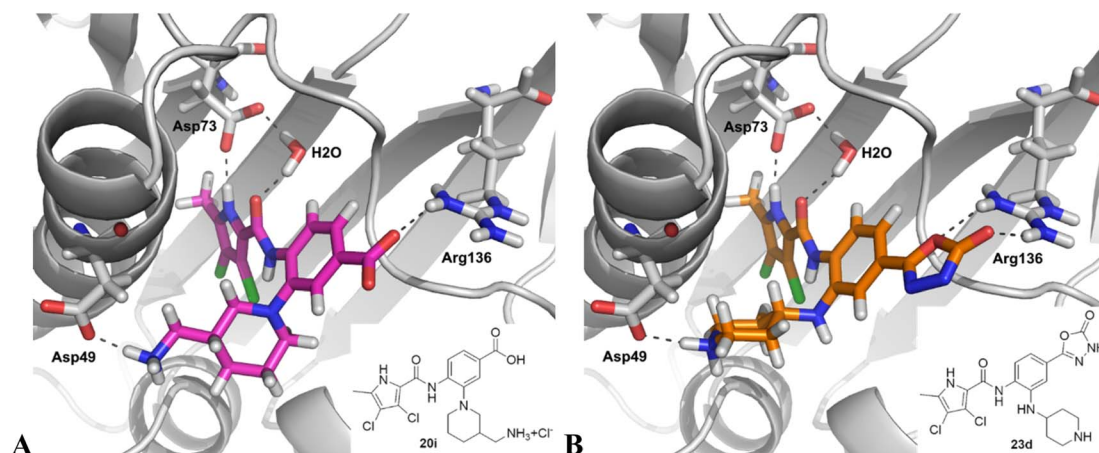


Fig. 3 Binding modes of compounds **20i** (A) and **23d** (B) in the *E. coli* GyrB (grey) (PDB code: 4ZVI),²⁷ as predicted by docking with Glide. The ligand and the key amino acid side chains are shown as stick models coloured according to the chemical atom type. The predicted H-bonds are shown as grey dotted lines. The figure was created with PyMOL.³⁶

gyrase inhibitors in this series are compound **20i** with a carboxyl group and the 5-oxo-1,3,4-oxadiazoles **23a–d**. Compound **20i** is also the most effective against topoisomerase IV, with a promising IC_{50} value of 143 nM against *E. coli* topoisomerase IV. Additionally, compound **23c** shows significant activity against topoisomerase IV with IC_{50} values of 448 nM and 406 nM for *E. coli* and *S. aureus* topoisomerase IV, respectively. Importantly, none of the compounds are active on human DNA topoisomerase II α . Compound **23b** was further evaluated in a supercoiling assay against DNA gyrase from *A. baumannii* and *P. aeruginosa*. The IC_{50} values were 15.3 nM for *A. baumannii* DNA gyrase and 3.63 nM for *P. aeruginosa* DNA gyrase (Table 2S, ESI†).

2.3.2 Antibacterial activity

2.3.2.1. Activity against selected Gram-positive and Gram-negative bacteria. The most potent inhibitors were tested

against a range of Gram-positive (*S. aureus* ATCC 29213, *S. aureus* ATCC 43300 – MRSA, *S. aureus* ATCC 700699 – VISA, *E. faecalis* ATCC 29212 and *E. faecium* ATCC 35667) and Gram-negative (*E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *A. baumannii* ATCC 17978, *A. baumannii* ATCC 19606, *K. pneumoniae* ATCC 10031, *K. pneumoniae* ATCC 700603, *E. spp. cloacae* ATCC 13047 and *K. aerogenes* ATCC 13408) bacterial strains using the broth microdilution method according to the Clinical and Laboratory Standards Institute guidelines.³⁷ The antibacterial activities, expressed as minimum inhibitory concentrations (MICs), are shown in Tables 1S (ESI),† 3, 4 and Fig. 1S (ESI).† The dose–response curves for the selected most promising compounds are shown in Fig. 4.

Unfortunately, none of the compounds shown in Table 1S† exhibit significant antibacterial activity at concentrations below $32 \mu\text{g mL}^{-1}$, with the exception of compound **13**. This

Table 3 Minimum inhibitory concentrations (MICs) of compounds **18a–20d** against selected Gram-positive and Gram-negative bacteria

Species	MIC ^a ($\mu\text{g mL}^{-1}$)									
	18a	18c	18h	18i	19a	19c	19i	20a	20c	20d
Gram-positive bacteria										
<i>S. aureus</i> ATCC 29213	4	16	>64	32	>32	8	8	>32	>32	>64
<i>S. aureus</i> ATCC 43300 (MRSA)	n.d. ^b	n.d.	>64	n.d.	n.d.	n.d.	2	>64	>64	>64
<i>S. aureus</i> ATCC 700699 (VISA)	n.d.	n.d.	>64	n.d.	n.d.	n.d.	1	>64	>64	>64
<i>E. faecalis</i> ATCC 29212	n.d.	n.d.	>64	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	>64
<i>E. faecium</i> ATCC 35667	16	>32	n.d.	>32	>32	8	1	>32	>32	n.d.
Gram-negative bacteria										
<i>E. coli</i> ATCC 25922	>32	>32	>64	>32	>32	>32	4	>32	>32	>64
<i>P. aeruginosa</i> ATCC 27853	>32	>32	>64	>32	>32	>32	>32	>32	>32	>64
<i>A. baumannii</i> ATCC 17978	n.d.	n.d.	>64	n.d.	n.d.	n.d.	8	>64	>64	>64
<i>A. baumannii</i> ATCC 19606	>32	>32	n.d.	>32	16	16	8	>32	>32	n.d.
<i>K. pneumoniae</i> ATCC 10031	n.d.	n.d.	>64	n.d.	n.d.	4	8	>64	>64	>64
<i>K. pneumoniae</i> ATCC 700603	>32	>32	n.d.	>32	>32	>32	>32	>32	>32	n.d.
<i>E. spp. cloacae</i> ATCC 13047	n.d.	n.d.	>64	n.d.	n.d.	n.d.	>64	>64	>64	>64
<i>K. aerogenes</i> ATCC 13408	>32	>32	n.d.	>32	>32	>32	>32	>32	>32	n.d.

^a Minimum inhibitory concentration. ^b Not determined.



Table 4 Minimum inhibitory concentrations (MICs) of compounds 20i–39 against selected Gram-positive and Gram-negative bacteria

Species	MIC ^a (μg mL ⁻¹)								
	20i	22c	22e	22f	23a	23b	23c	23d	39
Gram-positive bacteria									
<i>S. aureus</i> ATCC 29213	32	32	0.25	>64	2	1	2	>64	>32
<i>S. aureus</i> ATCC 43300 (MRSA)	n.d. ^b	n.d.	0.25	>64	4	2	2	>64	>32
<i>S. aureus</i> ATCC 700699 (VISA)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	>64	>32
<i>E. faecalis</i> ATCC 29212	n.d.	n.d.	0.125	1	0.5	1	2	>64	>32
<i>E. faecium</i> ATCC 35667	32	16	n.d.	n.d.	n.d.	n.d.	2	n.d.	n.d.
Gram-negative bacteria									
<i>E. coli</i> ATCC 25922	>32	>32	>64	>64	>64	8	16	>64	>32
<i>P. aeruginosa</i> ATCC 27853	>32	>32	>64	>64	>64	32	16	>64	>32
<i>A. baumannii</i> ATCC 17978	n.d.	n.d.	>64	>64	>64	8	16	>64	>32
<i>A. baumannii</i> ATCC 19606	>32	>32	n.d.	n.d.	n.d.	n.d.	4	n.d.	n.d.
<i>K. pneumoniae</i> ATCC 10031	n.d.	n.d.	>64	>64	0.25	0.0625	0.25	2	>32
<i>K. pneumoniae</i> ATCC 700603	>32	>32	n.d.	n.d.	n.d.	>32	>32	n.d.	n.d.
<i>E. spp. cloacae</i> ATCC 13047	n.d.	n.d.	>64	>64	>64	32	>64	>64	>32
<i>K. aerogenes</i> ATCC 13408	>32	>32	n.d.	n.d.	n.d.	>32	>32	n.d.	n.d.

^a Minimum inhibitory concentration. ^b Not determined.

compound demonstrates promising activity, showing a potency of 1 μg mL⁻¹ against *S. aureus* ATCC 29213 and 32 μg mL⁻¹ against *E. faecium* (ATCC 35667). However, the antibacterial activity of compounds shown in Tables 3 and 4 is more promising. In general, compounds with 5-oxo-1,3,4-oxadiazole group (22c, 22e, 22f, 23a–d and 39) exhibit stronger antibacterial activity than compounds with free carboxyl group (18a, 18c, 18h, 18i, 20a, 20c, 20d and 20i). The 5-oxo-1,3,4-oxadiazole group can interact with Arg136 similarly as the carboxyl group, but due to its higher pK_a value, the compounds are likely to have better permeability through bacterial membranes. Among the carboxyl derivatives, 18h, 20a, 20c and 20d show no activity, while 18a, 18c, 18i and 20i show weak inhibition of growth of Gram-positive *S. aureus* ATCC 29213 (MIC ~ 4–32 μg mL⁻¹), and 18a and 20i also of *E. faecium* ATCC 35667 (MIC ~ 16–32 μg mL⁻¹). None of these compounds are active against Gram-negative strains up to 32 μg mL⁻¹. Interestingly, three active carboxyl derivatives (18a, 18c, 18i) possess the Boc protecting group at the heterocycle and are more potent than their Boc-deprotected analogues 20a, 20c, and 20i, despite having weaker on-target activity. This suggests that while the Boc group may hinder binding to the enzyme, it potentially enhances accumulation in Gram-positive bacteria by facilitating cellular entry or reducing efflux. Furthermore, methyl esters 19a, 19c and 19i show higher antibacterial activity than their carboxyl analogues 20a, 20c and 20i, although their on-target activity is lower. This is likely due to their increased lipophilicity, which improves penetration through the bacterial cell wall and membrane. Among the entire series, the 5-oxo-1,3,4-oxadiazole derivatives 22e, 22f and 23a–d show the most promising antibacterial activity (Table 4), particularly against Gram-positive bacteria. The MIC values against *S. aureus* ATCC 29213, *S. aureus* ATCC 43300 (MRSA), *E. faecalis* ATCC 29212, and *E. faecium* ATCC 35667 fall within the low micromolar range.

Notably, the morpholine derivative 22e exhibits the highest potency, with MIC values of 0.25 μg mL⁻¹ against *S. aureus* ATCC 29213 and MRSA, and 0.125 μg mL⁻¹ against *E. faecalis* ATCC 29212. Interestingly, compounds 23a–d with an additional basic centre in the heterocycle bound to the R³ position (Fig. 1), are the only ones showing activity against Gram-negative bacteria. This aligns with recent findings that the presence of a positive charge can enhance accumulation in Gram-negative bacteria.^{33–35} Compounds 23b and 23c demonstrate activity against *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *A. baumannii* ATCC 17978, and *A. baumannii* ATCC 19606 with MIC values from 4 to 32 μg mL⁻¹. The most notable result is the very high potency of compounds 23b–d against the clinically relevant Gram-negative strain *K. pneumoniae* ATCC 10031, with MIC values below 1 μg mL⁻¹. The most potent compound against *K. pneumoniae* ATCC 10031 is 23b with an MIC of 0.0625 μg mL⁻¹. However, none of the tested compounds exhibit activity against the extended-spectrum β-lactamase (ESBL)-producing strain *K. pneumoniae* ATCC 700603.

2.3.2.2. Activity against fluoroquinolone-resistant strains. Four compounds with the most promising antibacterial activities (22e and 23a–c) were further evaluated against fluoroquinolone-resistant clinical isolates of *S. aureus* and *K. pneumoniae* (Table 5). Two different *S. aureus* and four different *K. pneumoniae* isolates were used, originating from patient samples from multiple hospitals in Hungary. The fluoroquinolone-resistant phenotype of the clinical isolates was previously confirmed in-house. The results presented in Table 5 show that while the compounds were inactive against fluoroquinolone-resistant strains of *K. pneumoniae*, they exhibited similar activity against both fluoroquinolone-sensitive and fluoroquinolone-resistant strains of *S. aureus*. This underscores their potential for the treatment of high-priority MRSA infections.



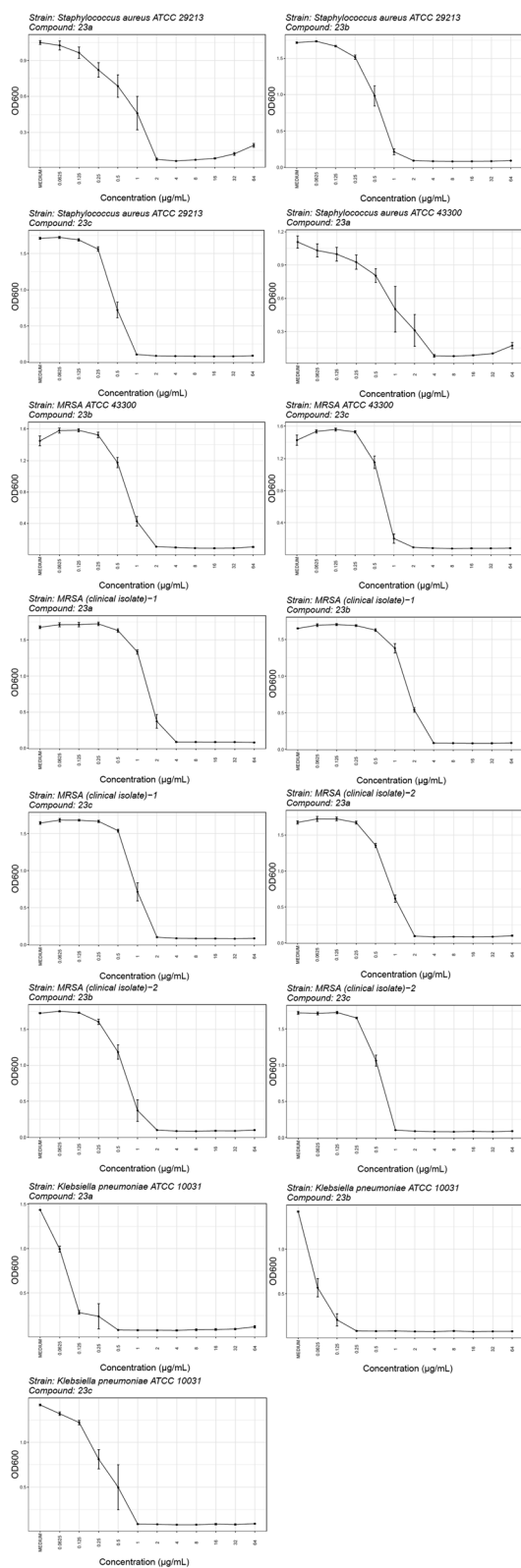


Fig. 4 Dose–response curves for compounds 23a–c against the selected Gram-positive (*S. aureus* ATCC 29213, *S. aureus* ATCC 43300 – MRSA, MRSA clinical isolate-1, MRSA clinical isolate-2) and Gram-negative strains (*K. pneumoniae* ATCC 10031).

Table 5 Minimal inhibitory concentrations (MICs) of compounds 22e and 23a–c against fluoroquinolone-resistant clinical isolates of *S. aureus* and *K. pneumoniae*

Species	MIC ^c ($\mu\text{g mL}^{-1}$)					
	22e	23a	23b	23c	Novo. ^a	Zoli. ^b
<i>S. aureus</i> ATCC 29213	0.25	2	1	2	0.25	1
<i>S. aureus</i> ATCC 43300 (MRSA)	0.25	4	2	2	0.5	0.125
MRSA clinical isolate-1	0.5	4	4	4	0.25	0.125
MRSA clinical isolate-2	0.5	2	2	2	0.25	<0.0625
<i>K. pneumoniae</i> ATCC 10031	>64	0.25	0.0625	0.25	0.5	0.25
<i>K. pneumoniae</i> NCTC 13440	>64	>64	>64	>64	>64	64
<i>K. pneumoniae</i> SE K13	>64	>64	8	32	>64	32
<i>K. pneumoniae</i> SE K14	>64	>64	>64	>64	>64	>64
<i>K. pneumoniae</i> K9–K25	>64	>64	>64	>64	>64	>64

^a Novobiocin. ^b Zoliflodacin. ^c Minimum inhibitory concentration. Fluoroquinolone-sensitive control strains are shown in bold. Each measurement was performed in at least four replicates.

2.3.2.3. Frequency of resistance. The spontaneous frequency of resistance was determined for compounds 23a and 23c in the strains of *S. aureus* ATCC 29213 and *K. pneumoniae* ATCC 10031 (Fig. 5 and Table 3S[†]). The results show that both compounds are significantly less resistance-prone compared to novobiocin in both bacterial strains. The frequency of resistance was generally lower in *S. aureus* than in *K. pneumoniae*. Notably, no resistant colonies of *S. aureus* were detected for compound 23c when selected up to 4-fold MIC. In addition, we determined the MIC values of colonies isolated from the frequency of resistance plates after selection to assess the resistance level of single mutational events (Table 4S[†]). The change in MIC values compared to the ancestor is only 4-fold for 23c in *S. aureus*.

2.3.3 Thermodynamic solubility. The thermodynamic solubility of selected compounds was evaluated by preparing saturated solutions in phosphate-buffered saline (PBS) with a pH of 7.4, followed by quantification using ultra-performance liquid chromatography (UPLC) (Table 6). Most compounds demonstrated moderate water solubility, ranging from 10 to 100 mg L⁻¹. Notably, compound 10a with a 3,4-dichloro-5-methyl-substituted pyrrole ring was more soluble than its analogue 11a with the 4,5-dibromopyrrole ring. Presence of the 5-oxo-1,3,4-oxadiazole groups on the central phenyl ring (23a) significantly reduced the solubility to less than 1 mg L⁻¹. Conversely, the introduction of the primary amino group in the substituent at R³ (23c) notably enhanced water solubility.



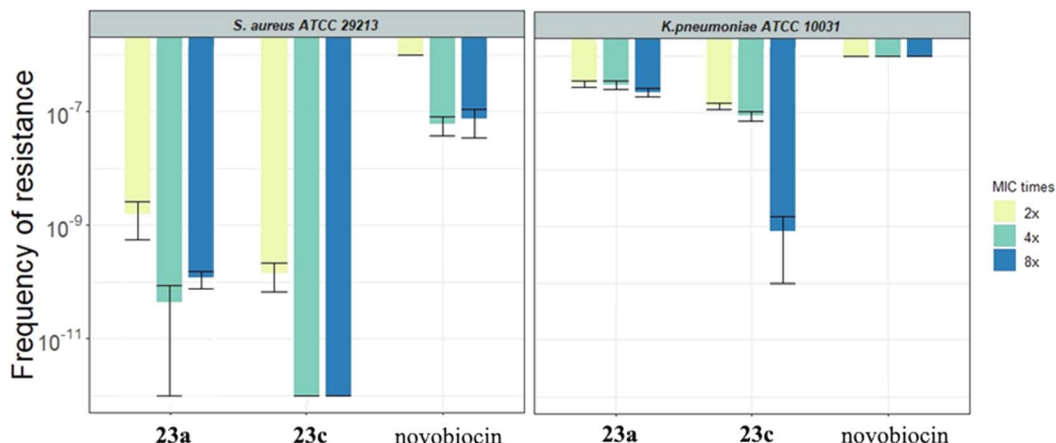


Fig. 5 Spontaneous frequency of resistance to 23a, 23c and novobiocin in *S. aureus* ATCC 29213 (left panel) and *K. pneumoniae* ATCC 10031 (right panel). The bars represent the average of 3 independent biological replicates. The error bars indicate the 95% confidence interval. Values at 10^{-6} indicate that the plates contained an uncountable abundance of colonies (therefore the frequency of resistance is above the limit of detection), while 10^{-12} indicate that no colonies were observed under the experimental conditions (the frequency of resistance is below the limit of detection).

Table 6 Thermodynamic solubility of selected compounds

Comp.	Structure	Therm. Solubility ^a [mg L ⁻¹]	Comp.	Structure	Therm. Solubility [mg L ⁻¹]
10a		98	23a		<1
11a		27	23c		31
20c		24	22f		<1

^a Thermodynamic solubility.

3. Conclusions

We have designed and synthesised a new series of *N*-phenylpyrrolamide inhibitors of DNA gyrase with improved antibacterial activity compared to earlier compounds of this structural

class. Among these, the most potent inhibitors of *E. coli* DNA gyrase were compounds **10b**, **10c**, and **20i** with a carboxyl group on the right-hand side (IC_{50} ; 6.3–21 nM) and **14** and **23a–d** with a 5-oxo-1,3,4-oxadiazole group at this position (IC_{50} ; 2.8–27 nM). Compound **21i** also showed a promising IC_{50} value of 143 nM



against a structurally similar enzyme, *E. coli* topoisomerase IV. Importantly, none of the compounds showed activity against human DNA topoisomerase II α , indicating selectivity for bacterial targets. The most effective compound against Gram-positive bacteria was the morpholine derivative **22e** with MIC values of 0.25 $\mu\text{g mL}^{-1}$ against *S. aureus* ATCC 29213 and MRSA, and 0.125 $\mu\text{g mL}^{-1}$ against *E. faecalis* ATCC 29212. Compounds **23b** and **23c** were most effective against Gram-negative *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *A. baumannii* ATCC 17978, and *A. baumannii* ATCC 19606 with MIC values from 4 to 32 $\mu\text{g mL}^{-1}$. The most effective compound against the clinically relevant Gram-negative *K. pneumoniae* ATCC 10031 was **23b** with a MIC of 0.0625 $\mu\text{g mL}^{-1}$. Importantly, compounds **22e** and **23a–c** retained similar levels of activity against both fluoroquinolone-sensitive and fluoroquinolone-resistant clinical isolates of MRSA, highlighting their potential for the treatment of high-priority MRSA infections. Moreover, compounds **23a** and **23c** demonstrated markedly lower susceptibility to resistance development in *S. aureus* ATCC 29213 and *K. pneumoniae* ATCC 10031 compared to novobiocin. Overall, the most promising compounds in the series showed excellent on-target activity and improved antibacterial activity compared to previous series of *N*-phenylpyrrolamides.

4. Experimental

4.1. Chemistry – general information

Chemicals were purchased from Sigma-Aldrich (St. Louis, USA), Acros Organics (Geel, Belgium), TCI (Tokyo, Japan) and Apollo Scientific (Stockport, UK). Thin layer chromatography (TLC) was performed using Merck 60 F254 silica gel plates (0.25 mm) and visualized with UV light and spray reagents. Silica gel 60 (particle size 240–400 mesh) was used for column chromatography. Infrared (IR) spectra were recorded using a Thermo Nicolet Nexus 470 ESP FT-IR spectrometer (Thermo Fisher Scientific, Waltham, USA). HPLC analyses were performed using a Dionex Ultimate 3000 binary rapid separation LC system (Thermo Scientific, Thermo Fisher Scientific, Waltham, MA, USA). Method A (for compounds **18h**, **20d**, and **23d**): Agilent Eclipse Plus C18 column (5 μm , 4.6 \times 150 mm) or Agilent Extend-C18 column (3.5 μm , 4.6 \times 150 mm), flow rate of 1.0 mL min $^{-1}$ 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%. The compounds were detected at 254 and 280 nm. Method B (for compounds **22a**, **22e**, **22f**, **22g**, **23a**, **27a**, and **27b**): Waters Acquity UPLC HSS C18 column (1.8 μm , 2.1 \times 50 mm), flow rate of 0.4 mL min $^{-1}$ and sample injection volume of 1.75 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: 0–10 min, 10–90%; 10–11 min, 90%. The compounds were detected at 254 and 280 nm. Melting points were determined using a Reichert hot stage microscope. Proton (^1H), carbon-13 (^{13}C) and DEPT nuclear magnetic resonance (NMR) spectra were recorded at frequencies of 400 and 100 MHz, respectively. The recordings were

performed with a Bruker AVANCE III 400 spectrometer (Bruker Corporation, Billerica, USA) in solutions of CDCl_3 or DMSO-d_6 , with TMS as internal standard. Mass spectra were obtained using a VG Analytical Autospec Q mass spectrometer (Fisons, VG Analytical, Manchester, UK). The purity of the analysed key compounds was determined to be $\geq 95\%$.

4.2. Synthetic procedures and analytical data

4.2.1 General procedure A

4.2.1.1 Synthesis of tert-butyl 4-(5-(methoxycarbonyl)-2-nitrophenyl)piperazine-1-carboxylate (2a). To a suspension of methyl 3-fluoro-4-nitrobenzoate (**1**, 5.00 g, 25.0 mmol) and potassium carbonate (4.16 g, 30.0 mmol) in DMF (100 mL), piperazine N1-Boc protected (4.68 g, 25.0 mmol) was added. The mixture was heated at 70 $^\circ\text{C}$ for 15 h. The solvent was evaporated under reduced pressure. EtOAc (150 mL) and H_2O (75 mL) were added and the compound was extracted into the organic layer. The organic phase was washed with water (50 mL) and brine (2 \times 50 mL), dried over Na_2SO_4 , filtered and the solvent was removed under reduced pressure to give **2a** as a yellow solid. Yield 7.56 g (83%); yellow solid; mp 88–90 $^\circ\text{C}$. ^1H NMR (400 MHz, DMSO-d_6): δ 7.96 (d, 1H, J = 8.4 Hz, ArH), 7.80 (d, 1H, J = 1.6 Hz, ArH), 7.68 (dd, 1H, J = 8.4, 1.7 Hz, ArH), 3.90 (s, 3H, CH_3), 3.40–3.50 (m, 4H, 2 \times CH_2), 2.98–3.08 (m, 4H, 2 \times CH_2), 1.43 (s, 9H, *t*Bu) ppm. ^{13}C NMR (100 MHz, DMSO-d_6): δ 164.8, 153.8, 145.4, 144.9, 133.8, 125.9, 122.6, 122.3, 79.1, 52.8, 50.8, 42.8, 28.0 ppm.

4.2.1.2 3-(4-(tert-Butoxycarbonyl)piperazin-1-yl)-4-nitrobenzoic acid (3a). To the solution of **2a** (200 mg, 0.55 mmol) in methanol (10 mL), 1 M NaOH (0.81 mL, 0.82 mmol) was added and the reaction mixture was stirred for 15 h at rt. 1 M HCl was added dropwise to pH 7 and the solvent was removed under reduced pressure. To the crude residue were added 1 M HCl to pH 4, water (20 mL) and ethyl acetate (25 mL). The phases were separated and organic phase was washed with water (20 mL) and brine (20 mL), dried over Na_2SO_4 , filtered and evaporated to give 171 mg of the product as orange crystals. Yield 171 mg (89%); orange crystals; mp 170–175 $^\circ\text{C}$. ^1H NMR (400 MHz, DMSO-d_6): δ 7.75–7.73 (m, 2H, ArH-2, ArH-5), 7.58 (dd, 1H, J = 6.2, 1.4 Hz, ArH-6), 3.41–3.47 (m, 4H, 2 \times CH_2), 2.93–2.95 (m, 4H, 2 \times CH_2), 1.42 (s, 9H, *t*Bu) ppm. ^{13}C NMR (100 MHz, DMSO-d_6): δ 166.6, 153.8, 153.6, 144.7, 144.4, 125.2, 122.9, 122.1, 79.1, 51.1, 42.8, 28.0 ppm. IR (ATR): ν 2976, 2931, 1686, 1570, 1419, 1365, 1231, 1160, 1126, 966, 749 cm^{-1} . MS (ESI) m/z = 350.13 ($[\text{M} - \text{H}]^-$).

4.2.1.3 tert-Butyl 4-(5-((2-methoxy-2-oxoethyl)carbamoyl)-2-nitrophenyl)piperazine-1-carboxylate (4a). To the solution of **3a** (0.900 g, 2.4 mmol) and TBTU (1.01 g, 3.1 mmol) in DMF (20 mL) NMM (0.53 mL, 4.8 mmol) was added and the solution was stirred for 30 min at rt. Glycine methyl ester hydrochloride (333 mg, 2.7 mmol) was added and reaction mixture was stirred at rt for 20 h. The solvent was removed under reduced pressure, the residue was dissolved in ethyl acetate (20 mL) and washed with water (2 \times 20 mL), saturated NaHCO_3 solution (2 \times 20 mL) and brine (20 mL), then organic phase was dried over Na_2SO_4 , filtered and evaporated. The crude product was purified with



flash column chromatography using ethyl acetate/petroleum ether 1 : 1 as mobile phase to give **4a** as orange oil (0.852 g). Yield 0.852 g (84%); orange oil. $^1\text{H NMR}$ (400 MHz, DMSO- d_6): δ 9.22 (t, 1H, $J = 5.8$ Hz, NH), 7.94 (d, 1H, $J = 8.5$ Hz, ArH-5), 7.76 (d, 1H, $J = 1.7$ Hz, ArH-2), 7.59 (dd, 1H, $J = 8.5, 1.7$ Hz, ArH-6), 4.05 (d, 2H, $J = 5.8$ Hz, CH_2), 3.67 (s, 3H, CH_3), 3.43–3.48 (m, 4H, $2 \times \text{CH}_2$, overlapped with the signal of water), 3.00–3.03 (m, 4H, $2 \times \text{CH}_2$), 1.42 (s, 9H, *t*Bu) ppm. $^{13}\text{C NMR}$ (100 MHz, DMSO- d_6): δ 170.1, 165.0, 153.8, 144.9, 144.4, 137.8, 125.7, 121.1, 120.8, 79.1, 51.8, 51.0, 43.7, 41.3, 28.0 ppm. IR (ATR): ν 3324, 2978, 1750, 1691, 1668, 1521, 1418, 1365, 1229, 1209, 1661, 1000, 834, 744 cm^{-1} . MS (ESI) $m/z = 445.01$ ($[\text{M} + \text{Na}]^+$).

4.2.1.4 tert-Butyl 4-(2-amino-5-((2-methoxy-2-oxoethyl)carbamoyl)phenyl)piperazine-1-carboxylate (5a). The solution of **4a** (2.51 g, 5.9 mmol) in methanol (90 mL) was stirred under argon for 15 min. Pd/C (0.500 g) was added, the solution was saturated with hydrogen and the reaction mixture was stirred for 4 h under hydrogen atmosphere. The catalyst was filtered off and the solvent was evaporated. The crude product was purified with flash column chromatography using ethyl acetate/petroleum ether 2 : 1 as mobile phase to obtain 2.08 g of **5a** as white crystals. Yield 2.08 g (90%); white crystals; mp 80–84 °C. $^1\text{H NMR}$ (400 MHz, DMSO- d_6): δ 8.59 (t, 1H, $J = 5.8$ Hz, NH), 7.53 (d, 1H, $J = 1.9$ Hz, ArH-6), 7.48 (dd, 1H, $J = 8.4, 1.9$ Hz, ArH-4), 6.74 (d, 1H, $J = 8.4$ Hz, ArH-3), 4.58–6.07 (br s, 2H, NH_2), 3.99 (d, 2H, $J = 5.8$ Hz, CH_2), 3.69 (s, 3H, CH_3), 3.51–3.63 (m, 4H, $2 \times \text{CH}_2$, overlapped with the signal of water), 2.75–2.85 (m, 4H, $2 \times \text{CH}_2$), 1.49 (s, 9H, *t*Bu) ppm. $^{13}\text{C NMR}$ (100 MHz, DMSO- d_6): δ 170.8, 166.5, 153.9, 145.8, 136.7, 124.5, 121.0, 119.1, 113.1, 78.8, 51.6, 50.6, 43.2, 41.1, 28.1 ppm. IR (ATR): ν 3479, 3367, 3308, 2935, 1743, 1693, 1610, 1504, 1415, 1365, 1248, 1205, 1163, 1119, 768 cm^{-1} . MS (ESI) $m/z = 415.07$ ($[\text{M} + \text{Na}]^+$).

4.2.2 General procedure B

4.2.2.1 4-(2-(3,4-Dichloro-5-methyl-1H-pyrrole-2-carboxamido)-5-((2-methoxy-2-oxoethyl)carbamoyl)phenyl)piperazine-1-carboxylate (6a). To a solution of 3,4-dichloro-5-methyl-1H-pyrrole-2-carboxylic acid (0.178 g, 0.92 mmol) in anhydrous dichloromethane (10 mL), oxalyl chloride (0.31 mL, 3.7 mmol) was added dropwise and the solution was stirred at rt for 15 h under argon atmosphere. The solvent was evaporated under reduced pressure. Fresh anhydrous dichloromethane (5 mL), **5a** (0.300 g, 0.76 mmol) and pyridine (2 mL) were added and the reaction mixture was stirred under argon at rt for 15 h. Solvent was removed under reduced pressure, the residue was dissolved in ethyl acetate (15 mL) and washed with H_2O (10 mL), HCl 1 M solution (15 mL) and brine (2×15 mL). During the extraction the product precipitated and was filtered off. The crude product was triturated with water and the undissolved solid was filtered off. The product was then triturated with diethyl ether and the undissolved solid was filtered off to give **6a** as a grey solid. Yield 246 mg (57%); grey solid; mp 194–197 °C. $^1\text{H NMR}$ (400 MHz, DMSO- d_6): δ 12.43 (s, 1H, NH), 9.79 (s, 1H, NH), 8.93 (t, 1H, $J = 5.7$ Hz, NH), 8.48 (d, 1H, $J = 8.4$ Hz, ArH-3), 7.91 (d, 1H, $J = 1.9$ Hz, ArH-6), 7.75 (dd, 1H, $J = 8.6, 1.9$ Hz, ArH-4), 4.02 (d, 2H, $J = 5.7$ Hz, CH_2), 3.67 (s, 3H, CH_3), 3.50–3.57 (m, 4H, $2 \times \text{CH}_2$), 2.82–2.85 (m, 4H, $2 \times \text{CH}_2$), 2.24 (s, 3H, CH_3), 1.44 (s, 9H, *t*Bu) ppm. $^{13}\text{C NMR}$ (100 MHz, DMSO- d_6): δ 170.5,

165.6, 156.5, 153.7, 141.0, 136.7, 129.8, 128.4, 125.3, 121.0, 118.8, 118.6, 109.7, 108.6, 79.1, 52.1, 51.7, 41.2, 28.0, 10.8 ppm. One peak not seen. IR (ATR): ν 3299, 2978, 1757, 1672, 1647, 1621, 1510, 1410, 1367, 1250, 1201, 1170, 1122, 947, 761 cm^{-1} . MS (ESI) $m/z = 566$ ($[\text{M} - \text{H}]^-$). HRMS for $\text{C}_{25}\text{H}_{30}\text{Cl}_2\text{N}_5\text{O}_6$: calculated 566.1573, found 566.1572. HPLC: Agilent Eclipse Plus C18 column (5 μm , 4.6×150 mm); mobile phase: 30–90% of acetonitrile in TFA (0.1%) in 16 min, 90% acetonitrile to 20 min; flow rate 1.0 mL min^{-1} ; injection volume: 10 μL ; t_{R} : 14.083 min (96.1% at 280 nm).

4.2.2.2 tert-Butyl 4-(2-(4,5-dibromo-1H-pyrrole-2-carboxamido)-5-((2-methoxy-2-oxoethyl)carbamoyl)phenyl)piperazine-1-carboxylate (7a). Synthesised according to General procedure B from **5a** (0.600 g, 1.5 mmol), 4,5-dibromo-1H-pyrrole-2-carboxylic acid (493 mg, 1.8 mmol) and oxalyl chloride (0.629 mL, 7.3 mmol). The precipitate that was formed during the extraction was filtered off to obtain crude product **1**. The two phases of the mother liquor were separated and organic phase was washed with water (20 mL), saturated NaHCO_3 solution (2×20 mL) and brine (2×20 mL), dried over Na_2SO_4 , filtered and the solvent evaporated to obtain crude product **2**. The combined crude products were triturated with diethyl ether and the undissolved solid was filtered off and dried to give **7a** (801 mg) as a grey solid. Yield 801 mg (83%); grey solid; mp 140–143 °C. $^1\text{H NMR}$ (400 MHz, DMSO- d_6): δ 13.08 (d, 1H, $J = 2.7$ Hz, NH), 9.19 (s, 1H, NH), 8.93 (t, 1H, $J = 5.7$ Hz, NH), 8.10 (d, 1H, $J = 8.4$ Hz, ArH-3), 7.75 (d, 1H, $J = 1.9$ Hz, ArH-6), 7.68 (dd, 1H, $J = 8.4, 1.9$ Hz, ArH-4), 7.17 (d, 1H, $J = 2.7$ Hz, ArH), 4.01 (d, 2H, $J = 5.7$ Hz, CH_2), 3.67 (s, 3H, CH_3), 3.52–3.57 (m, 4H, $2 \times \text{CH}_2$), 2.81–2.83 (m, 4H, $2 \times \text{CH}_2$), 1.43 (s, 9H, *t*Bu) ppm. $^{13}\text{C NMR}$ (100 MHz, DMSO- d_6): δ 170.5, 165.9, 156.9, 153.8, 142.7, 135.1, 129.2, 127.5, 123.9, 121.1, 119.8, 113.5, 106.8, 98.7, 79.0, 51.7, 51.4, 41.2, 28.0 ppm. One peak not seen. IR (ATR): ν 3201, 2950, 1755, 1736, 1645, 1549, 1516, 1430, 1250, 1204, 1168, 937, 759, 680 cm^{-1} . MS (ESI) $m/z = 640.0$ ($[\text{M} - \text{H}]^-$). HRMS for $\text{C}_{24}\text{H}_{28}\text{Br}_2\text{N}_5\text{O}_6$: calculated 640.0406, found 640.0403.

4.2.2.3 (3-(4-(tert-Butoxycarbonyl)piperazin-1-yl)-4-(3,4-dichloro-5-methyl-1H-pyrrole-2-carboxamido)benzoyl)glycine (8a). To the solution of **6a** (0.160 g, 0.28 mmol) in methanol (10 mL) 1 M NaOH (0.84 mL, 0.84 mmol) was added and the mixture was stirred at rt for 15 h. The mixture was neutralized with 1 M HCl and methanol was removed under reduced pressure. The pH was adjusted to 4 with 1 M HCl, ethyl acetate was added and the resulting precipitate was filtered off and dried to obtain **8a** as a grey solid. Yield 138 mg (89%); grey solid; mp >300 °C. $^1\text{H NMR}$ (400 MHz, DMSO- d_6): δ 12.63 (br s, 1H, COOH), 12.43 (s, 1H, NH), 9.79 (s, 1H, NH), 8.81 (t, 1H, $J = 5.7$ Hz, NH), 8.48 (d, 1H, $J = 8.6$ Hz, ArH-5), 7.91 (d, 1H, $J = 1.9$ Hz, ArH-2), 7.75 (dd, 1H, $J = 8.6, 1.9$ Hz, ArH-6), 3.93 (d, 2H, $J = 5.7$ Hz, CH_2), 3.50–3.58 (m, 4H, $2 \times \text{CH}_2$), 2.82–2.85 (m, 4H, $2 \times \text{CH}_2$), 2.24 (s, 3H, CH_3), 1.44 (s, 9H, *t*Bu) ppm. $^{13}\text{C NMR}$ (100 MHz, DMSO- d_6): δ 171.4, 165.5, 156.5, 153.7, 140.9, 136.6, 129.8, 128.6, 125.3, 121.0, 118.8, 118.5, 109.7, 108.6, 79.1, 52.1, 41.1, 28.0, 10.8 ppm. One peak not seen. DEPT 45 NMR (100 MHz, DMSO- d_6) δ 125.8, 121.5, 119.1, 52.6, 41.7, 28.5, 11.2 ppm. One peak not seen. DEPT 135 NMR (100 MHz, DMSO- d_6) δ 125.8, 121.5, 119.0, 52.6 (negative), 41.7 (negative), 28.5, 11.2 ppm. One peak not seen. IR



(ATR): ν 3369, 3287, 3108, 2973, 1748, 1632, 1505, 1481, 1409, 1366, 1253, 1170, 1138, 1083, 759, 621 cm^{-1} . MS (ESI) m/z = 552.0 ($[\text{M} - \text{H}]^-$). HRMS for $\text{C}_{24}\text{H}_{28}\text{Cl}_2\text{N}_5\text{O}_6$: calculated 552.1417, found 552.1411. HPLC: Agilent Eclipse Plus C18 column (5 μm , 4.6 \times 150 mm); mobile phase: 30–90% of acetonitrile in TFA (0.1%) in 16 min, 90% acetonitrile to 20 min; flow rate 1.0 mL min^{-1} ; injection volume: 10 μL ; t_{R} : 12.420 min (98.1% at 280 nm).

4.2.2.4 (S)-3-(3-((tert-Butoxycarbonyl)amino)pyrrolidin-1-yl)-4-(3,4-dichloro-5-methyl-1H-pyrrole-2-carboxamido)benzoyl glycine (8b). To the solution of **6b** (155 mg, 0.27 mmol) in a mixture of methanol (10 mL) and tetrahydrofuran (2 mL) 1 M NaOH (1.09 mL, 1.09 mmol) was added and the mixture was stirred at rt for 15 h. To the mixture 1 M HCl was added to reach pH 7 and methanol was removed under reduced pressure. 1 M HCl was added to the aqueous residue to reach pH 4, after which ethyl acetate (15 mL) was added. The undissolved precipitate was filtered off and dried to give **8b** (81 mg). The mother liquor was poured into a separating funnel and the two phases were separated. The organic phase was washed with brine (2 \times 10 mL), dried with Na_2SO_4 , filtered and evaporated. Diethyl ether was added to the residue, the resulting suspension was sonicated and the precipitate filtered off and dried (26 mg). The pure products were combined to give **8b** (107 mg) as brown crystals. Yield 107 mg (71%); brown crystals; mp 210–215 $^\circ\text{C}$. ^1H NMR (400 MHz, DMSO-d_6): δ 12.57 (br s, 1H, COOH), 12.38 (s, 1H, NH), 9.50 (s, 1H, NH), 8.82 (t, 1H, J = 5.8 Hz, NH), 8.27 (d, 1H, J = 8.5 Hz, Ar-H-5), 7.76 (d, 1H, J = 1.8 Hz, Ar-H-2), 7.63 (dd, 1H, J = 8.5, 1.8 Hz, Ar-H-6), 7.19 (d, 1H, J = 6.5 Hz, NH), 4.07–4.14 (m, 1H, CH), 3.92 (d, 2H, J = 5.8 Hz, CH_2), 3.27–3.33 (m, 1H, CH, overlapping with the signal for water), 3.14–3.19 (m, 1H, CH), 3.00–3.06 (m, 1H, CH), 2.89–2.94 (m, 1H, CH), 2.15–2.24 (m, 4H, CH, CH_3), 1.81–1.89 (m, 1H, CH), 1.39 (s, 9H, *t*Bu) ppm. ^{13}C NMR (100 MHz, DMSO-d_6): δ 171.4, 165.9, 156.6, 155.2, 139.8, 135.3, 129.5, 129.2, 123.1, 119.7, 119.2, 118.8, 110.1, 108.6, 77.8, 57.5, 50.9, 49.8, 41.2, 31.3, 28.2, 10.8 ppm. IR (ATR): ν 3311, 3177, 2976, 1710, 1694, 1634, 1585, 1506, 1331, 1169, 860, 765 cm^{-1} . $[\alpha]_{\text{D}}^{25}$ –12.0 (*c* 0.125, MeOH). MS (ESI) m/z = 552.0 ($[\text{M} - \text{H}]^-$). HRMS for $\text{C}_{24}\text{H}_{28}\text{Cl}_2\text{N}_5\text{O}_6$: calculated 552.1417, found 552.1412. HPLC: Agilent Eclipse Plus C18 column (5 μm , 4.6 \times 150 mm); mobile phase: 30–90% of acetonitrile in TFA (0.1%) in 16 min, 90% acetonitrile to 20 min; flow rate 1.0 mL min^{-1} ; injection volume: 10 μL ; t_{R} : 11.366 min (97.0% at 280 nm).

4.2.2.5 (R)-3-(3-((tert-Butoxycarbonyl)amino)pyrrolidin-1-yl)-4-(3,4-dichloro-5-methyl-1H-pyrrole-2-carboxamido)benzoyl glycine (8c). To the solution of **6c** (200 mg, 0.35 mmol) in a mixture of methanol (8 mL) and tetrahydrofuran (3 mL) 1 M NaOH (1.41 mL, 1.4 mmol) was added and the mixture was stirred at rt for 15 h. Water (10 mL) was added to the mixture and the pH was adjusted to 4 with 1 M HCl, after which the obtained precipitate was filtered off and dried to give **8c** (91 mg) as a grey solid. Yield 91 mg (47%); grey solid; mp 210–214 $^\circ\text{C}$. ^1H NMR (400 MHz, DMSO-d_6): δ 12.57 (br s, 1H, COOH), 12.38 (s, 1H, NH), 9.50 (s, 1H, NH), 8.81 (t, 1H, J = 5.8 Hz, NH), 8.27 (d, 1H, J = 8.5 Hz, ArH-5), 7.76 (d, 1H, J = 1.8 Hz, ArH-2), 7.63 (dd, 1H, J = 8.5, 1.8 Hz, ArH-6), 7.19 (d, 1H, J = 6.5 Hz, NH), 4.07–

4.14 (m, 1H, CH), 3.92 (d, 2H, J = 5.8 Hz, CH_2), 3.27–3.33 (m, 1H, CH, overlapping with the signal for water), 3.14–3.19 (m, 1H, CH), 3.00–3.06 (m, 1H, CH), 2.89–2.94 (m, 1H, CH), 2.15–2.24 (m, 4H, CH, CH_3), 1.81–1.89 (m, 1H, CH), 1.39 (s, 9H, *t*Bu) ppm. ^{13}C NMR (100 MHz, DMSO-d_6): δ 171.4, 166.1, 156.6, 155.4, 139.9, 135.1, 129.6, 129.3, 122.9, 120.0, 119.0, 118.7, 110.2, 108.7, 78.0, 57.4, 50.7, 49.7, 41.5, 31.2, 28.1, 10.7 ppm. IR (ATR): ν 3440, 3292, 2977, 2931, 2840, 1733, 1687, 1636, 1506, 1403, 1244, 1063, 1041, 764, 605 cm^{-1} . $[\alpha]_{\text{D}}^{25}$ +11.8 (*c* 0.187, MeOH). MS (ESI) m/z = 552.1 ($[\text{M} - \text{H}]^-$). HRMS for $\text{C}_{24}\text{H}_{28}\text{Cl}_2\text{N}_5\text{O}_6$: calculated 552.1417, found 552.1414. HPLC: Agilent Eclipse Plus C18 column (5 μm , 4.6 \times 150 mm); mobile phase: 30–90% of acetonitrile in TFA (0.1%) in 16 min, 90% acetonitrile to 20 min; flow rate 1.0 mL min^{-1} ; injection volume: 10 μL ; t_{R} : 11.366 min (96.9% at 280 nm).

4.2.2.6 (3-(4-(tert-Butoxycarbonyl)piperazin-1-yl)-4-(4,5-dibromo-1H-pyrrole-2-carboxamido)benzoyl)glycine (9a). To the solution of **7a** (200 mg, 0.32 mmol) in methanol (10 mL) 1 M NaOH (1.24 mL, 1.2 mmol) was added and the mixture was stirred at rt for 15 h. The mixture was neutralized with 1 M HCl and the methanol was removed under reduced pressure. Water (10 mL) was added, the pH was adjusted to 4 with 1 M HCl, ethyl acetate (20 mL) was added, and the organic phase was washed with water (10 mL) and brine (2 \times 10 mL), dried over Na_2SO_4 , filtered and the solvent removed. To the residue diethyl ether was added, the resulting suspension was sonicated and the precipitate was filtered off to give **9a** (115 mg) as a grey solid. Yield 115 mg (59%); grey solid; mp >300 $^\circ\text{C}$. ^1H NMR (400 MHz, DMSO-d_6): δ 13.08 (s, 1H, NH), 12.59 (br s, 1H, COOH), 9.20 (s, 1H, NH), 8.81 (t, 1H, J = 5.8 Hz, NH), 8.10 (d, 1H, J = 8.5 Hz, ArH-5), 7.76 (d, 1H, J = 1.9 Hz, ArH-2), 7.68 (dd, 1H, J = 8.5, 1.9 Hz, ArH-6), 7.17 (d, 1H, J = 2.8 Hz, ArH), 3.93 (d, 2H, J = 5.8 Hz, CH_2), 3.51–3.57 (m, 4H, 2 \times CH_2), 2.81–2.83 (m, 4H, 2 \times CH_2), 1.43 (s, 9H, *t*Bu) ppm. ^{13}C NMR (100 MHz, DMSO-d_6): δ 171.4, 165.7, 156.9, 153.8, 142.6, 135.0, 129.4, 127.6, 123.9, 121.0, 119.8, 113.5, 106.8, 98.7, 79.0, 51.4, 41.2, 28.0 ppm. One peak not seen. IR (ATR): ν 3101, 3053, 2972, 1745, 1630, 1505, 1406, 1368, 1172, 1140, 830, 756, 631 cm^{-1} . MS (ESI) m/z = 626.0 ($[\text{M} - \text{H}]^-$). HRMS for $\text{C}_{23}\text{H}_{26}\text{Br}_2\text{N}_5\text{O}_6$: calculated 626.0250, found 626.0255. HPLC: Agilent Eclipse Plus C18 column (5 μm , 4.6 \times 150 mm); mobile phase: 30–90% of acetonitrile in TFA (0.1%) in 16 min, 90% acetonitrile to 20 min; flow rate 1.0 mL min^{-1} ; injection volume: 10 μL ; t_{R} : 11.169 min (95.8% at 280 nm).

4.2.2.7 (S)-3-(3-((tert-Butoxycarbonyl)amino)pyrrolidin-1-yl)-4-(4,5-dibromo-1H-pyrrole-2-carboxamido)benzoyl)glycine (9b). To the solution of **7b** (250 mg, 0.38 mmol) in methanol (10 mL) 1 M NaOH (1.52 mL, 1.52 mmol) was added and the mixture was stirred at rt for 15 h. The mixture was neutralized with 1 M HCl and methanol was removed under reduced pressure. The pH was adjusted to 4 with 1 M HCl, ethyl acetate was added, and the organic phase was washed with water (10 mL) and brine (2 \times 10 mL), dried over Na_2SO_4 , filtered and the solvent removed. To the residue diethyl ether was added, the resulting suspension was sonicated and the precipitate was filtered off to give **9b** (188 mg) as a grey solid. Yield 188 mg (77%); grey solid; mp 219–223 $^\circ\text{C}$. ^1H NMR (400 MHz, DMSO-d_6): δ 12.89 (s, 1H, NH), 12.47 (br s,



1H, COOH), 9.52 (s, 1H, NH), 8.80 (t, 1H, $J = 5.8$ Hz, NH), 7.29–7.35 (m, 3H, 3 × ArH), 7.13–7.20 (m, 1H, ArH), 4.01–4.06 (m, 1H, CH), 3.91 (d, 2H, $J = 5.8$ Hz, CH₂), 3.31–3.43 (m, 2H, 2 × CH, overlapping with the signal for water), 3.21–3.27 (m, 1H, CH), 3.04–3.08 (m, 1H, CH), 2.03–2.11 (m, 1H, CH), 1.75–1.84 (m, 1H, CH), 1.37 (s, 9H, *t*Bu) ppm. ¹³C NMR (100 MHz, DMSO-*d*₆): δ 171.4, 166.3, 157.6, 155.2, 144.4, 131.7, 128.3, 128.0, 127.8, 117.5, 114.5, 113.5, 105.7, 98.2, 77.8, 55.9, 49.8, 48.3, 41.2, 30.5, 28.2 ppm. IR (ATR): ν 3376, 3276, 3104, 1735, 1692, 1666, 1628, 1509, 1399, 1383, 1236, 1153, 976, 767 cm⁻¹. [α]_D²⁵ –37.3 (c 0.126, MeOH). MS (ESI) $m/z = 626.0$ ([M – H]⁻). HRMS for C₂₃H₂₆Br₂N₅O₆: calculated 626.0250, found 626.0239. HPLC: Agilent Eclipse Plus C18 column (5 μm, 4.6 × 150 mm); mobile phase: 30–90% of acetonitrile in TFA (0.1%) in 16 min, 90% acetonitrile to 20 min; flow rate 1.0 mL min⁻¹; injection volume: 10 μL; t_R : 9.325 min (98.6% at 280 nm).

4.2.2.8 (R)-3-(3-((tert-Butoxycarbonyl)amino)pyrrolidin-1-yl)-4-(4,5-dibromo-1H-pyrrole-2-carboxamido)benzoyl)glycine (9c). To the solution of **7c** (300 mg, 0.47 mmol) in a mixture of methanol (10 mL) and tetrahydrofuran (1 mL) 1 M NaOH (1.86 mL, 1.8 mmol) was added and the mixture was stirred at rt for 15 h. The mixture was neutralized with 1 M HCl and methanol was removed under reduced pressure. Water (10 mL) was added and the pH was adjusted to 4 with 1 M HCl. The precipitate was filtered off and dried to obtain **9c** (234 mg) as a grey solid. Yield 234 mg (80%); grey solid; mp 218–223 °C. ¹H NMR (400 MHz, DMSO-*d*₆): δ 12.89 (s, 1H, NH), 12.47 (br s, 1H, COOH), 9.52 (s, 1H, NH), 8.80 (t, 1H, $J = 5.8$ Hz, NH), 7.29–7.35 (m, 3H, 3 × ArH), 7.13–7.20 (m, 2H, NH, ArH), 4.01–4.06 (m, 1H, CH), 3.91 (d, 2H, $J = 5.8$ Hz, CH₂), 3.39–3.44 (m, 2H, 2 × CH, overlapping with the signal for water), 3.20–3.26 (m, 1H, CH), 3.04–3.07 (m, 1H, CH), 2.03–2.10 (m, 1H, CH), 1.75–1.84 (m, 1H, CH), 1.37 (s, 9H, *t*Bu) ppm. ¹³C NMR (100 MHz, DMSO-*d*₆): δ 171.4, 166.3, 157.6, 155.2, 144.3, 135.1, 129.6, 128.0, 127.8, 117.5, 114.5, 113.5, 105.8, 98.2, 77.8, 55.9, 49.8, 48.3, 41.2, 30.5, 28.2 ppm. Signal for one aromatic carbon not seen. IR (ATR): ν 3376, 3276, 3104, 1735, 1692, 1666, 1628, 1509, 1399, 1383, 1236, 1153, 976, 767 cm⁻¹. [α]_D²⁵ +35.3 (c 0.133, MeOH). MS (ESI) $m/z = 626.0$ ([M – H]⁻). HRMS for C₂₃H₂₆Br₂N₅O₆: calculated 626.0250, found 626.0252. HPLC: Agilent Eclipse Plus C18 column (5 μm, 4.6 × 150 mm); mobile phase: 30–90% of acetonitrile in TFA (0.1%) in 16 min, 90% acetonitrile to 20 min; flow rate 1.0 mL min⁻¹; injection volume: 10 μL; t_R : 9.325 min (95.1% at 280 nm).

4.2.2.9 4-(5-((Carboxymethyl)carbamoyl)-2-(3,4-dichloro-5-methyl-1H-pyrrole-2-carboxamido)phenyl)piperazin-1-ium chloride (10a). Compound **8a** (50 mg, 0.09 mmol) was suspended in 4 M HCl in 1,4-dioxane (5 mL) and THF (2 mL), and the mixture was stirred at rt for 1 h. The solvents were evaporated, to the solid residue diethyl ether was added, the resulting suspension was sonicated and the solid was filtered off to give **10a** (39 mg) as a grey solid. Yield 39 mg (89%); grey solid; mp 262–265 °C. ¹H NMR (400 MHz, DMSO-*d*₆): δ 12.59 (br s, 1H, COOH), 12.49 (s, 1H, NH), 9.59 (s, 1H, NH), 9.23 (br s, 2H, NH₂⁺), 8.96 (t, 1H, $J = 5.7$ Hz, NH), 8.45 (d, 1H, $J = 8.6$ Hz, ArH-3), 7.83 (d, 1H, $J = 1.8$ Hz, ArH-6), 7.79 (dd, 1H, $J = 8.6, 1.8$ Hz, ArH-4), 3.93 (d, 2H, $J = 5.7$ Hz, CH₂), 3.24–3.31 (m, 4H, 2 × CH₂), 3.09–3.12 (m, 4H, 2 × CH₂), 2.24 (s, 3H, CH₃) ppm. ¹³C NMR (100 MHz, DMSO-*d*₆):

δ 171.4, 165.5, 156.6, 140.3, 136.4, 129.7, 128.9, 125.3, 120.7, 118.9, 118.8, 110.0, 108.6, 48.9, 43.4, 41.2, 10.8 ppm. DEPT 45 NMR (100 MHz, DMSO-*d*₆) δ 125.7, 121.2, 119.4, 49.5, 44.0, 41.7, 11.2 ppm. DEPT 135 NMR (100 MHz, DMSO-*d*₆) δ 125.7, 121.2, 119.4, 49.5 (negative), 44.0 (negative), 41.7 (negative), 11.2 ppm. IR (ATR): ν 3402, 3307, 3240, 2834, 1734, 1645, 1588, 1504, 1412, 1211, 1040, 920, 763 cm⁻¹. MS (ESI) $m/z = 452.0$ ([M – H]⁻). HRMS for C₁₉H₂₀Cl₂N₅O₄: calculated 452.0892, found 452.0891. HPLC: Agilent Eclipse Plus C18 column (5 μm, 4.6 × 150 mm); mobile phase: 20–40% of acetonitrile in phosphate buffer (pH = 6.8) in 16 min, 40% acetonitrile to 20 min; flow rate 1.0 mL min⁻¹; injection volume: 10 μL; t_R : 9.087 min (99.6% at 280 nm).

4.2.2.10 (S)-1-(5-((Carboxymethyl)carbamoyl)-2-(3,4-dichloro-5-methyl-1H-pyrrole-2-carboxamido)phenyl)pyrrolidin-3-aminium chloride (10b). The solution of **8b** (40 mg, 0.072 mmol) in a mixture of 4 M HCl in 1,4-dioxane (1 mL) and THF (1 mL) was stirred at rt for 2 h. The solvent was removed, to the residue diethyl ether was added, the resulting suspension was sonicated and the undissolved solid was filtered off and dried to give **10b** (34 mg) as a beige solid. Yield 24 mg (97%); beige solid; mp 216–220 °C. ¹H NMR (400 MHz, DMSO-*d*₆): δ 12.6 (s, 1H, NH), 9.47 (s, 1H, NH), 8.92 (t, 1H, $J = 5.8$ Hz, NH), 8.43 (s, 3H, NH₃⁺), 8.17 (d, 1H, $J = 8.5$ Hz, ArH-3), 7.79 (d, 1H, $J = 1.8$ Hz, ArH-6), 7.65 (dd, 1H, $J = 8.5, 1.8$ Hz, ArH-4), 3.92 (d, 2H, $J = 5.8$ Hz, CH₂), 3.66–3.71 (m, 1H, CH, overlapping with the signal for water), 3.47–3.52 (m, 1H, CH, overlapping with the signal for water), 3.36–3.42 (m, 1H, CH), 3.08–3.12 (m, 1H, CH), 2.95–3.00 (m, 1H, CH), 2.24–2.34 (m, 4H, CH, CH₃), 1.96–2.06 (m, 1H, CH) ppm. Signal for COOH not seen. ¹³C NMR (100 MHz, DMSO-*d*₆): δ 171.4, 165.8, 156.7, 139.3, 134.8, 129.5, 129.1, 122.9, 121.0, 119.2, 118.7, 111.2, 108.7, 55.1, 50.3, 49.0, 41.2, 29.6, 10.8 ppm. DEPT 45 NMR (100 MHz, DMSO-*d*₆) δ 175.7, 173.2, 123.4, 121.4, 119.8, 55.6, 50.8, 49.5, 41.7, 30.1, 11.2 ppm. DEPT 135 NMR (100 MHz, DMSO-*d*₆) δ 123.4, 121.4, 119.8, 55.6 (negative), 50.8, 49.5 (negative), 41.7, 30.1 (negative), 11.2 (negative) ppm. IR (ATR): ν 3365, 3263, 2937, 1729, 1640, 1509, 1507, 1410, 1317, 1259, 1222, 1041, 762 cm⁻¹. [α]_D²⁵ –11.9 (c 0.117, MeOH). MS (ESI) $m/z = 452.0$ ([M – H]⁻). HRMS for C₁₉H₂₀Cl₂N₅O₄: calculated 452.0892, found 452.0881. HPLC: Agilent Eclipse Plus C18 column (5 μm, 4.6 × 150 mm); mobile phase: 20–40% of acetonitrile in phosphate buffer (pH = 6.8) in 16 min, 40% acetonitrile to 20 min; flow rate 1.0 mL min⁻¹; injection volume: 10 μL; t_R : 9.252 min (99.3% at 280 nm).

4.2.2.11 (R)-1-(5-((Carboxymethyl)carbamoyl)-2-(3,4-dichloro-5-methyl-1H-pyrrole-2-carboxamido)phenyl)pyrrolidin-3-aminium chloride (10c). The solution of **8c** (35 mg, 0.063 mmol) in 4 M HCl in 1,4-dioxane (5 mL) was stirred at rt for 2 h. The solvent was removed, to the residue diethyl ether was added, the resulting suspension was sonicated and the undissolved solid was filtered off and dried to give **10c** (22 mg) as a grey solid. Yield 22 mg (71%); grey solid; mp 217–221 °C. ¹H NMR (400 MHz, DMSO-*d*₆): δ 12.6 (s, 1H, NH), 9.47 (s, 1H, NH), 8.92 (t, 1H, $J = 5.8$ Hz, NH), 8.43 (s, 3H, NH₃⁺), 8.17 (d, 1H, $J = 8.5$ Hz, ArH-3), 7.79 (d, 1H, $J = 1.8$ Hz, ArH-6), 7.65 (dd, 1H, $J = 8.5, 1.8$ Hz, ArH-4), 3.92 (d, 2H, $J = 5.8$ Hz, CH₂), 3.66–3.71 (m, 1H, CH, overlapping with the signal for water), 3.47–3.52 (m, 1H, CH,



overlapping with the signal for water), 3.36–3.42 (m, 1H, CH), 3.08–3.12 (m, 1H, CH), 2.95–3.00 (m, 1H, CH), 2.24–2.34 (m, 4H, CH, CH₃), 1.96–2.06 (m, 1H, CH) ppm. Signal for COOH not seen. ¹³C NMR (100 MHz, DMSO-d₆): δ 171.4, 165.8, 156.7, 139.3, 134.8, 129.5, 129.1, 122.9, 121.0, 119.2, 118.7, 111.2, 108.7, 55.1, 50.3, 49.0, 41.2, 29.6, 10.8 ppm. IR (ATR): ν 3348, 3235, 3017, 2922, 1733, 1636, 1602, 1509, 1410, 1317, 1042, 764, 607 cm⁻¹. [α]_D²⁵ +11.0 (c 0.100, MeOH). MS (ESI) *m/z* = 452.1 ([M – H]⁻). HRMS for C₁₉H₂₀Cl₂N₅O₄: calculated 452.0892, found 452.0898. HPLC: Agilent Eclipse Plus C18 column (5 μm, 4.6 × 150 mm); mobile phase: 20–40% of acetonitrile in phosphate buffer (pH = 6.8) in 16 min, 40% acetonitrile to 20 min; flow rate 1.0 mL min⁻¹; injection volume: 10 μL; *t*_R: 9.252 min (99.4% at 280 nm).

4.2.2.12 4-(5-((Carboxymethyl)carbamoyl)-2-(4,5-dibromo-1H-pyrrole-2-carboxamido)phenyl)piperazin-1-ium chloride (11a). Compound **9a** (50 mg, 0.079 mmol) was dissolved in 4 M HCl in 1,4-dioxane (4 mL) and THF (1 mL), and the solution was stirred at rt for 2 h. The solvents were evaporated, to the solid residue diethyl ether was added, the resulting suspension was sonicated and the solid was filtered off to give **11a** (45 mg) as a beige solid. Yield 45 mg (100%); beige solid; mp 225–228 °C. ¹H NMR (400 MHz, DMSO-d₆): δ 13.19 (d, 1H, *J* = 2.8 Hz, NH), 12.52 (br s, 1H, COOH), 9.24 (br s, 2H, NH₂⁺), 9.19 (s, 1H, NH), 8.95 (t, 1H, *J* = 5.8 Hz, NH), 8.06 (d, 1H, *J* = 9.0 Hz, ArH-3), 7.69–7.72 (m, 2H, ArH-4,6), 7.29 (d, 1H, *J* = 2.8 Hz, ArH), 3.93 (d, 2H, *J* = 5.8 Hz, CH₂), 3.27–3.36 (m, 4H, 2 × CH₂), 3.08–3.10 (m, 4H, 2 × CH₂) ppm. ¹³C NMR (100 MHz, DMSO-d₆): δ 171.4, 165.7, 157.0, 142.2, 134.9, 129.7, 127.5, 123.9, 122.0, 119.5, 114.0, 106.7, 98.6, 48.2, 43.0, 41.2 ppm. DEPT 45 NMR (100 MHz, DMSO-d₆) δ 124.4, 122.5, 120.1, 114.5, 48.7, 43.6, 41.7 ppm. DEPT 135 NMR (100 MHz, DMSO-d₆) δ 124.4, 122.4, 120.1, 114.5, 48.7 (negative), 43.6 (negative), 41.7 (negative) ppm. IR (ATR): ν 3326, 3199, 2798, 2719, 1730, 1655, 1507, 1406, 1305, 1178, 950, 746 cm⁻¹. MS (ESI) *m/z* = 526.0 ([M – H]⁻). HRMS for C₁₈H₁₈Br₂N₅O₄: calculated 525.9726, found 525.9727. HPLC: Agilent Eclipse Plus C18 column (5 μm, 4.6 × 150 mm); mobile phase: 20–40% of acetonitrile in phosphate buffer (pH = 6.8) in 16 min, 40% acetonitrile to 20 min; flow rate 1.0 mL min⁻¹; injection volume: 10 μL; *t*_R: 6.884 min (98.4% at 280 nm).

4.2.2.13 (S)-1-(5-((Carboxymethyl)carbamoyl)-2-(4,5-dibromo-1H-pyrrole-2-carboxamido)phenyl)pyrrolidin-3-aminium chloride (11b). Suspension of **9b** (70 mg, 0.11 mmol) in 4 M HCl in 1,4-dioxane (5 mL) was stirred at rt for 2 h. The precipitate was filtered off and washed with diethyl ether and dried to give **11b** (48 mg) as a grey solid. Yield 48 mg (76%); grey solid; mp 209–212 °C. ¹H NMR (400 MHz, DMSO-d₆): δ 13.01 (d, 1H, *J* = 2.4 Hz, NH), 12.38 (br s, 1H, COOH), 9.61 (s, 1H, NH), 8.84 (t, 1H, *J* = 5.8 Hz, NH), 8.22 (s, 3H, NH₃⁺), 7.52–7.50 (m, 1H, ArH), 7.43–7.41 (m, 2H, 2 × ArH), 7.36 (d, 1H, *J* = 2.4 Hz, ArH), 3.91 (d, 2H, *J* = 5.8 Hz, CH₂), 3.88 (s, 1H, CH, overlapping with the signal for water), 3.49–3.53 (m, 2H, 2 × CH), 3.20–3.23 (m, 1H, CH), 3.01–3.07 (m, 1H, CH), 2.20–2.29 (m, 1H, CH), 1.94–2.04 (m, 1H, CH) ppm. ¹³C NMR (100 MHz, DMSO-d₆): δ 171.4, 166.2, 157.4, 142.6, 131.1, 129.7, 127.8, 127.0, 119.0, 115.9, 114.4, 105.8, 98.4, 53.6, 49.4, 47.7, 41.2, 29.1 ppm. IR (ATR): ν 3374, 3188, 2943, 1725, 1630, 1507, 1411, 1389, 1328, 1221, 1180, 974, 868,

755 cm⁻¹. [α]_D²⁵ –11.1 (c 0.189, MeOH). MS (ESI) *m/z* = 526.0 ([M – H]⁻). HRMS for C₁₈H₁₈Br₂N₅O₄: calculated 525.9726, found 525.9726. HPLC: Agilent Eclipse Plus C18 column (5 μm, 4.6 × 150 mm); mobile phase: 20–40% of acetonitrile in phosphate buffer (pH = 6.8) in 16 min, 40% acetonitrile to 20 min; flow rate 1.0 mL min⁻¹; injection volume: 10 μL; *t*_R: 5.908 min (95.2% at 280 nm).

4.2.2.14 (R)-1-(5-((Carboxymethyl)carbamoyl)-2-(4,5-dibromo-1H-pyrrole-2-carboxamido)phenyl)pyrrolidin-3-aminium chloride (11c). The solution of **9c** (70 mg, 0.11 mmol) in a mixture of 1,4-dioxane (10 mL) and 4 M HCl in 1,4-dioxane (5 mL) was stirred at rt for 2 h. The solvent was removed under reduced pressure, to the residue diethyl ether was added, the resulting suspension was sonicated, the precipitate was filtered off, washed with diethyl ether and dried to give **11c** (62 mg) as a grey solid. Yield 62 mg (98%); grey solid; mp 209–214 °C. ¹H NMR (400 MHz, DMSO-d₆): δ 13.01 (d, 1H, *J* = 2.4 Hz, NH), 12.38 (br s, 1H, COOH), 9.61 (s, 1H, NH), 8.84 (t, 1H, *J* = 5.8 Hz, NH), 8.22 (s, 3H, NH₃⁺), 7.52–7.50 (m, 1H, ArH), 7.43–7.41 (m, 2H, 2 × ArH), 7.36 (d, 1H, *J* = 2.4 Hz, ArH), 3.74–3.94 (m, 3H, CH, CH₂ overlapping with the signal for water), 3.49–3.53 (m, 2H, 2 × CH), 3.20–3.23 (m, 1H, CH), 3.01–3.07 (m, 1H, CH), 2.20–2.29 (m, 1H, CH), 1.94–2.04 (m, 1H, CH) ppm. ¹³C NMR (100 MHz, DMSO-d₆): δ 171.7, 167.6, 157.9, 143.0, 131.1, 129.2, 127.3, 127.2, 119.0, 115.8, 114.0, 106.4, 98.8, 53.4, 49.4, 47.9, 41.2, 28.9 ppm. DEPT 45 NMR (100 MHz, DMSO-d₆) δ 127.3, 119.6, 116.5, 114.8, 54.2, 49.9, 48.3, 41.7, 29.6 ppm. DEPT 135 NMR (100 MHz, DMSO-d₆) δ 127.3, 119.6, 116.5, 114.8, 54.2 (negative), 49.9, 48.3 (negative), 41.7 (negative), 29.6 (negative) ppm. IR (ATR): ν 3418, 3313, 2954, 2875, 1725, 1632, 1506, 1411, 1388, 1211, 1180, 974, 757 cm⁻¹. [α]_D²⁵ +10.8 (c 0.120, MeOH). MS (ESI) *m/z* = 526.0 ([M – H]⁻). HRMS for C₁₈H₁₈Br₂N₅O₄: calculated 525.9726, found 525.9727. HPLC: Agilent Eclipse Plus C18 column (5 μm, 4.6 × 150 mm); mobile phase: 20–40% of acetonitrile in phosphate buffer (pH = 6.8) in 16 min, 40% acetonitrile to 20 min; flow rate 1.0 mL min⁻¹; injection volume: 10 μL; *t*_R: 5.908 min (95.1% at 280 nm).

4.2.2.15 tert-Butyl 4-(2-(3,4-dichloro-5-methyl-1H-pyrrole-2-carboxamido)-5-((2-hydrazineyl-2-oxoethyl)carbamoyl)phenyl)piperazine-1-carboxylate (12). To the solution of compound **6c** (0.589 g, 1.0 mmol) in a mixture of methanol (10 mL) and THF (10 mL) hydrazine hydrate solution (80%, 0.50 mL, 10.2 mmol) was added and the mixture was stirred under reflux for 20 h. The solvent was removed under reduced pressure, to the residue ethanol was added and the resulting suspension was sonicated, the undissolved solid was filtered off and dried to give **12** (430 mg) as a white solid. Yield 430 mg (76%); white solid; mp 156–160 °C. ¹H NMR (400 MHz, DMSO-d₆): δ 12.43 (s, 1H, NH), 9.79 (s, 1H, NH), 9.23 (br s, 1H, NH), 8.71 (t, 1H, *J* = 5.8 Hz, NH), 8.46 (d, 1H, *J* = 8.6 Hz, ArH-3), 7.92 (d, 1H, *J* = 1.8 Hz, ArH-6), 7.75 (dd, 1H, *J* = 8.6, 1.8 Hz, ArH-4), 4.67 (br s, 2H, NH₂), 3.84 (d, 2H, *J* = 5.8 Hz, CH₂), 3.49–3.57 (m, 4H, 2 × CH₂), 2.82–2.85 (m, 4H, 2 × CH₂), 2.24 (s, 3H, CH₃), 1.44 (s, 9H, *t*Bu) ppm. ¹³C NMR (100 MHz, DMSO-d₆): δ 168.4, 165.6, 156.5, 153.7, 140.8, 136.4, 129.8, 128.9, 125.4, 121.2, 118.8, 118.4, 109.7, 108.6, 79.1, 52.1, 41.3, 28.0, 10.8 ppm. Signal for one aliphatic carbon not seen. IR (ATR): ν 3263, 1690, 1641, 1509, 1410, 1262, 1245, 1168, 1132,



1040, 713 cm^{-1} . MS (ESI) $m/z = 566.0$ ($[\text{M} - \text{H}]^-$). HRMS for $\text{C}_{24}\text{H}_{30}\text{Cl}_2\text{N}_7\text{O}_5$: calculated 566.1685, found 566.1682. HPLC: Agilent Eclipse Plus C18 column (5 μm , 4.6×150 mm); mobile phase: 30–90% of acetonitrile in TFA (0.1%) in 16 min, 90% acetonitrile to 20 min; flow rate 1.0 mL min^{-1} ; injection volume: 10 μL ; t_{R} : 10.065 min (96.3% at 280 nm).

4.2.2.16 tert-Butyl 4-(2-(3,4-dichloro-5-methyl-1H-pyrrole-2-carboxamido)-5-(((5-oxo-4,5-dihydro-1,3,4-oxadiazol-2-yl)methyl)carbamoyl)phenyl)piperazine-1-carboxylate (13). The solution of **12** (392 mg, 0.69 mmol) and CDI (224 mg, 1.38 mmol) in a mixture of 1,4-dioxane (15 mL) and DMF (5 mL) was stirred at 101 $^{\circ}\text{C}$ for 20 h. The solvent was removed and the residue was purified with flash column chromatography using dichloromethane/methanol (10 : 1) as eluent to give **13** (70 mg) as a white solid. Yield 70 mg (17%); white solid; mp 147–151 $^{\circ}\text{C}$. $^1\text{H NMR}$ (400 MHz, DMSO-d_6): δ 12.43 (s, 1H, NH), 12.29 (s, 1H, NH), 9.79 (s, 1H, NH), 9.03 (t, 1H, $J = 5.8$ Hz, NH), 8.47 (d, 1H, $J = 8.6$ Hz, ArH-3), 7.90 (d, 1H, $J = 1.9$ Hz, ArH-6), 7.76 (dd, 1H, $J = 8.6$, 1.9 Hz, ArH-4), 4.39 (d, 2H, $J = 5.6$ Hz, CH_2), 3.45 (4H, $2 \times \text{CH}_2$, overlapping with the signal for water), 2.81–2.85 (m, 4H, $2 \times \text{CH}_2$), 2.24 (s, 3H, CH_3), 1.43 (s, 9H, *t*Bu) ppm. $^{13}\text{C NMR}$ (100 MHz, DMSO-d_6): δ 165.5, 156.5, 154.9, 154.5, 153.7, 141.0, 136.8, 129.9, 128.1, 125.4, 121.0, 118.8, 118.6, 109.8, 108.6, 79.1, 52.0, 30.8, 28.0, 10.7 ppm. Signal for one aliphatic carbon not seen. IR (ATR): ν 3262, 2979, 2935, 1776, 1644, 1506, 1411, 1247, 1165, 1130, 1091, 1041, 762 cm^{-1} . MS (ESI) $m/z = 592.0$ ($[\text{M} - \text{H}]^-$). HRMS for $\text{C}_{25}\text{H}_{28}\text{Cl}_2\text{N}_7\text{O}_6$: calculated 592.1478, found 592.1474. HPLC: Agilent Eclipse Plus C18 column (5 μm , 4.6×150 mm); mobile phase: 30–90% of acetonitrile in TFA (0.1%) in 16 min, 90% acetonitrile to 20 min; flow rate 1.0 mL min^{-1} ; injection volume: 10 μL ; t_{R} : 12.838 min (97.1% at 280 nm).

4.2.2.17 4-(2-(3,4-Dichloro-5-methyl-1H-pyrrole-2-carboxamido)-5-(((5-oxo-4,5-dihydro-1,3,4-oxadiazol-2-yl)methyl)carbamoyl)phenyl)piperazin-1-ium chloride (14). The solution of **13** (65 mg, 0.12 mmol) in a mixture of 1,4-dioxane (10 mL) and 4 M HCl in 1,4-dioxane (6 mL) was stirred for 2 h at rt. The precipitate was filtered off and dried to give **14** (46 mg) as a grey solid. Yield 46 mg (79%); grey solid; mp 258–262 $^{\circ}\text{C}$. $^1\text{H NMR}$ (400 MHz, DMSO-d_6): δ 12.46 (s, 1H, NH), 12.34 (s, 1H, NH), 9.60 (s, 1H, NH), 9.18 (t, 1H, $J = 5.6$ Hz, NH), 9.02 (br s, 2H, NH_2^+), 8.46 (d, 1H, $J = 8.6$ Hz, ArH-3), 7.84 (d, 1H, $J = 1.9$ Hz, ArH-6), 7.78 (dd, 1H, $J = 8.6$, 1.8 Hz, ArH-4), 4.39 (d, 2H, $J = 5.6$ Hz, CH_2), 3.24–3.31 (m, 4H, $2 \times \text{CH}_2$), 3.06–3.12 (m, 4H, $2 \times \text{CH}_2$), 2.24 (s, 3H, CH_3) ppm. $^{13}\text{C NMR}$ (100 MHz, DMSO-d_6): δ 165.7, 156.6, 154.9, 154.4, 140.3, 136.6, 129.9, 128.3, 125.4, 120.9, 118.9, 118.8, 110.0, 108.7, 48.8, 43.5, 35.1, 10.7 ppm. DEPT 45 NMR (100 MHz, DMSO-d_6) δ 125.9, 121.3, 119.4, 49.3, 43.9, 35.6, 11.2 ppm. DEPT 135 NMR (100 MHz, DMSO-d_6) δ 125.9, 121.3, 119.4, 49.3 (negative), 43.9 (negative), 35.6 (negative) ppm. IR (ATR): ν 3245, 2956, 2797, 1773, 1639, 1507, 1460, 1309, 1258, 922, 842, 730 cm^{-1} . MS (ESI) $m/z = 492.1$ ($[\text{M} - \text{H}]^-$). HRMS for $\text{C}_{20}\text{H}_{20}\text{Cl}_2\text{N}_7\text{O}_4$: calculated 492.0954, found 492.0959. HPLC: Agilent Eclipse Plus C18 column (5 μm , 4.6×150 mm); mobile phase: 20–40% of acetonitrile in phosphate buffer (pH = 6.8) in 16 min, 40% acetonitrile to 20 min; flow rate 1.0 mL min^{-1} ; injection volume: 10 μL ; t_{R} : 13.668 min (96.7% at 280 nm).

4.2.2.18 Methyl (S)-3-(3-((tert-butoxycarbonyl)amino)piperidin-1-yl)-4-nitrobenzoate (15c). Synthesised according to General procedure A from **1** (1.07 g, 5.39 mmol), (3S)-3-amino-piperidine 3-Boc protected (1.00 g, 5.35 mmol) and K_2CO_3 (0.894 g, 6.47 mmol). Yield 87% (1.78 g); orange solid; mp 120–124 $^{\circ}\text{C}$. $^1\text{H NMR}$ (400 MHz, CDCl_3): δ 7.83 (d, 1H, $J = 1.5$ Hz, Ar-H-2), 7.77 (d, 1H, $J = 8.4$ Hz, Ar-H-5), 7.71 (dd, 1H, $J = 8.4$, 1.5 Hz, ArH-6), 5.05–5.15 (m, 1H, NH), 3.97 (s, 3H, CH_3), 3.88–3.97 (m, 1H, CH), 3.26–3.30 (m, 1H, CH), 2.92–3.12 (m, 3H, CH, CH_2), 1.84–1.93 (m, 1H, CH), 1.64–1.80 (m, 3H, CH, CH_2), 1.48 (s, 9H, *t*Bu) ppm. IR (ATR): ν 3444, 3122, 3067, 2966, 1727, 1616, 1598, 1521, 1487, 1438, 1421, 1358, 1282, 1227, 1188, 1156, 1111, 1080, 987, 917, 905, 851, 840, 799, 772, 738, 686 cm^{-1} .

4.2.2.19 tert-Butyl 4-(2-amino-5-(methoxycarbonyl)phenyl)piperazine-1-carboxylate (16a). To the solution of compound **2a** (5.44 g, 16.0 mmol) in a mixture of methanol (200 mL) and tetrahydrofuran (90 mL) under an argon atmosphere Pd-C (1.00 g) was added, the mixture was saturated with hydrogen and stirred under a hydrogen atmosphere at rt for 4 h. The catalyst was filtered off and the solvent was removed under reduced pressure to obtain **16a** (4.87 g) as a white solid. Yield 90% (4.87 g); white solid; mp 135–137 $^{\circ}\text{C}$. $^1\text{H NMR}$ (400 MHz, DMSO-d_6): δ 7.49 (dd, 1H, $J = 8.4$, 1.9 Hz, ArH), 7.44 (d, 1H, $J = 1.9$ Hz, ArH), 6.70 (d, 1H, $J = 8.4$ Hz, ArH), 5.73 (br s, 2H, NH_2), 3.74 (s, 3H, CH_3), 3.44–3.59 (m, 4H, $2 \times \text{CH}_2$), 2.68–2.84 (m, 4H, $2 \times \text{CH}_2$), 1.43 (s, 9H, *t*Bu) ppm. $^{13}\text{C NMR}$ (100 MHz, DMSO-d_6): δ 166.3, 153.9, 147.6, 136.7, 126.8, 120.6, 116.4, 113.1, 78.8, 51.2, 50.4, 43.2, 28.0 ppm. IR (ATR): ν 3412, 3319, 2977, 2811, 1685, 1614, 1579, 1510, 1477, 1440, 1417, 1362, 1323, 1302, 1261, 1248, 1218, 1157, 1133, 1107, 1066, 1052, 1039, 993, 945, 930, 908, 870, 843, 829, 811, 769, 649, 624 cm^{-1} . MS (ESI) $m/z = 336.2$ ($[\text{M} + \text{H}]^+$). HRMS for $\text{C}_{17}\text{H}_{26}\text{O}_3\text{N}_4$: calculated 336.1923, found 336.1924.

4.2.2.20 tert-Butyl 4-(2-(3,4-dichloro-5-methyl-1H-pyrrole-2-carboxamido)-5-(methoxycarbonyl)phenyl)piperazine-1-carboxylate (17a). Synthesised according to General procedure B from 3,4-dichloro-5-methyl-1H-pyrrole-2-carboxylic acid (0.850 g, 4.38 mmol) and **16a** (1.62 g, 4.82 mmol). During extraction, the product precipitated and was filtered off. The crude product was successively triturated with methanol and tetrahydrofuran, and the undissolved solid was filtered off and dried to give **17a** (0.589 g) as a white solid. Yield 25% (0.589 g); white solid; mp 132–136 $^{\circ}\text{C}$. $^1\text{H NMR}$ (400 MHz, DMSO-d_6): δ 12.45 (s, 1H, NH), 9.79 (s, 1H, NH), 8.51 (d, 1H, $J = 8.6$ Hz, ArH), 7.78–7.89 (m, 2H, $2 \times$ ArH), 3.84 (s, 3H, CH_3), 3.52 (br s, 4H, $2 \times \text{CH}_2$), 2.78–2.87 (m, 4H, $2 \times \text{CH}_2$), 2.24 (s, 3H, CH_3), 1.44 (s, 9H, *t*Bu) ppm. IR (ATR): ν 3252, 2975, 2892, 2364, 1722, 1686, 1639, 1593, 1524, 1488, 1455, 1410, 1378, 1365, 1332, 1273, 1258, 1245, 1218, 1194, 1164, 1131, 1116, 1098, 1079, 1041, 998, 980, 939, 894, 860, 844, 818, 748, 740, 711, 664, 650, 632, 622, 611 cm^{-1} . MS (ESI) $m/z = 511.2$ ($[\text{M} + \text{H}]^+$). HRMS for $\text{C}_{23}\text{H}_{29}\text{N}_4\text{O}_5\text{Cl}_2$: calculated 511.1515, found 511.1524. HPLC (0–16 min, 30–90% ACN in 0.1% TFA, 16–20 min, 90% ACN in 0.1% TFA, Agilent Eclipse Plus C18: 5 μm , 4.6×150 mm): t_{R} 16.803 min (98.3% at 280 nm).

4.2.2.21 3-(4-(tert-Butoxycarbonyl)piperazin-1-yl)-4-(3,4-dichloro-5-methyl-1H-pyrrole-2-carboxamido)benzoic acid (18a).



To a solution of compound **17a** (201 mg, 0.391 mmol) in a mixture of methanol (10 mL) and tetrahydrofuran (7 mL) 1 M NaOH (1.56 mL, 1.56 mmol) was added and the mixture was stirred at rt for 15 h. The solvent was removed under reduced pressure, to the residue water (10 mL) was added and the mixture was acidified to pH 4 with 1 M HCl. The water phase was extracted with ethyl acetate (20 mL), the organic phase was washed with brine (10 mL), dried over Na₂SO₄ and the solvent was removed under reduced pressure. The crude product was triturated with diethyl ether and the undissolved solid was filtered off and dried to obtain **18a** as a pale pink solid (70 mg). Yield 35% (70 mg); pale pink solid; mp 260–262 °C. ¹H NMR (400 MHz, DMSO-*d*₆): δ 12.87 (s, 1H, COOH), 12.45 (s, 1H, NH), 9.78 (s, 1H, NH), 8.49 (d, 1H, *J* = 8.6 Hz, ArH), 7.77–7.88 (m, 2H, 2 × ArH), 3.42–3.63 (m, 4H, 2 × CH₂), 2.75–2.91 (m, 4H, 2 × CH₂), 2.24 (s, 3H, CH₃), 1.44 (s, 9H, *t*Bu) ppm. ¹³C NMR (100 MHz, DMSO-*d*₆): δ 166.7, 156.6, 153.8, 141.1, 137.8, 129.9, 127.3, 125.7, 122.6, 118.7, 118.7, 109.8, 108.6, 79.1, 51.9, 28.0, 10.8 ppm. One peak not seen. IR (ATR): ν 3261, 2980, 2363, 1713, 1644, 1609, 1594, 1523, 1489, 1456, 1429, 1410, 1376, 1366, 1338, 1280, 1248, 1211, 1199, 1169, 1137, 1105, 1088, 1042, 1005, 956, 897, 865, 833, 800, 765, 724, 700, 646, 629, 619, 609 cm⁻¹. MS (ESI) *m/z* = 495.1 ([M – H]⁻). HRMS for C₂₂H₂₅N₄O₅Cl₂: calculated 495.1202, found 495.1206. HPLC (0–16 min, 30–90% ACN in 0.1% TFA, 16–20 min, 90% ACN in 0.1% TFA, Agilent Eclipse Plus C18: 5 μm, 4.6 × 150 mm): *t*_R 14.030 min (96.3% at 280 nm).

4.2.2.22 (S)-3-(3-((tert-Butoxycarbonyl)amino)piperidin-1-yl)-4-(3,4-dichloro-5-methyl-1H-pyrrole-2-carboxamido)benzoic acid (18c). To a solution of compound **17c** (203 mg, 0.391 mmol) in a mixture of methanol (5 mL) and tetrahydrofuran (4 mL) 1 M NaOH (3.13 mL, 3.13 mmol) was added and the mixture was stirred at rt for 15 h. The solvent was removed under reduced pressure, to the residue water (10 mL) was added and the mixture was acidified to pH 4 with 1 M HCl. The water phase was extracted with ethyl acetate (20 mL), the organic phase was washed with brine (10 mL), dried over Na₂SO₄ and the solvent was removed under reduced pressure to give **18c** (101 mg) as a pale-yellow solid. Yield 50% (101 mg); pale yellow solid; mp 231–233 °C. ¹H NMR (400 MHz, DMSO-*d*₆): δ 12.89 (br s, 1H, COOH), 12.46 (s, 1H, NH), 9.83 (s, 1H, NH), 8.49 (d, 1H, *J* = 8.5 Hz, ArH-5), 7.75–7.91 (m, 2H, 2 × ArH), 6.80–6.99 (m, 1H, NHBoc), 3.55–3.67 (m, 1H, CH), 2.91–3.03 (m, 1H, CH), 2.78–2.88 (m, 1H, CH), 2.53–2.62 (m, 2H, CH₂), 2.25 (s, 3H, CH₃), 1.75–1.96 (m, 2H, CH₂), 1.60–1.74 (m, 1H, CH), 1.36 (s, 9H, *t*Bu), 1.23–1.30 (m, 1H, CH) ppm. IR (ATR): ν 3326, 2959, 2362, 2163, 2032, 2001, 1969, 1717, 1686, 1639, 1588, 1519, 1490, 1437, 1405, 1365, 1318, 1289, 1262, 1173, 1122, 1107, 1082, 1068, 1038, 1026, 997, 960, 928, 877, 864, 839, 805, 765, 731, 676, 648, 625, 608 cm⁻¹. [α]_D²⁰ = +0.44° (c 0.297, THF). MS (ESI) *m/z* = 511.2 ([M + H]⁺). HRMS for C₂₃H₂₉N₄O₅Cl₂: calculated 511.1515, found 511.1512. HPLC (0–16 min, 30–90% ACN in 0.1% TFA, 16–20 min, 90% ACN in 0.1% TFA, Agilent Eclipse Plus C18: 5 μm, 4.6 × 150 mm): *t*_R 13.706 min (97.7% at 280 nm).

4.2.2.23 3-((1-(tert-Butoxycarbonyl)piperidin-4-yl)amino)-4-(3,4-dichloro-5-methyl-1H-pyrrole-2-carboxamido)benzoic acid (18d). To a solution of **17d** (40 mg, 0.076 mmol) in methanol,

1 M NaOH (0.761 mL, 0.761 mmol) was added and the reaction mixture was stirred at 60 °C for 15 h. The solvent was evaporated *in vacuo* and the residue was neutralised with 1 M HCl to pH 7. The resulting precipitate was filtered off and dried to obtain **18d** (30 mg) as a light brown solid. Yield 77% (30 mg); light brown solid. ¹H NMR (400 MHz, DMSO-*d*₆): δ 12.60 (br s, 1H, COOH), 12.30 (s, 1H, NH), 8.93 (s, 1H, NH), 7.80 (d, 1H, *J* = 8.3 Hz, ArH), 7.33–7.43 (m, 2H, 2 × ArH), 5.07 (d, 1H, *J* = 6.9 Hz, NH), 3.86 (d, 2H, *J* = 13.6 Hz, CH₂), 3.48 (d, 1H, *J* = 9.7 Hz, CH), 2.97 (s, 2H, CH₂), 2.24 (s, 3H, CH₃), 1.88 (d, 2H, *J* = 12.7 Hz, CH₂), 1.40 (s, 9H, *t*Bu), 1.25–1.38 (m, 2H, CH₂) ppm.

4.2.2.24 4-(3,4-Dichloro-5-methyl-1H-pyrrole-2-carboxamido)-3-(4-phenylpiperazin-1-yl)benzoic acid (18h). To a solution of **17h** (30 mg, 0.062 mmol) in methanol, 1 M NaOH (0.616 mL, 0.616 mmol) was added and the reaction mixture was stirred at 50 °C for 15 h. The solvent was evaporated *in vacuo* and the residue was neutralised with 1 M HCl to pH 7. The resulting precipitate was filtered off to afford **17h** (28.8 mg) as a light brown solid. Yield 99% (28.8 mg); light brown solid; mp >300 °C. ¹H NMR (400 MHz, DMSO-*d*₆): δ 12.90 (s, 1H, COOH), 12.47 (s, 1H, NH), 9.87 (s, 1H, NH), 8.51 (d, 1H, *J* = 8.8 Hz, ArH), 7.91 (s, 1H, ArH), 7.84 (s, 1H, ArH), 7.27 (s, 2H, 2 × ArH), 7.03 (s, 2H, 2 × ArH), 6.84 (s, 1H, ArH), 3.33 (4H, overlapped with the signal for water, 2 × CH₂), 3.04 (s, 4H, 2 × CH₂), 2.24 (s, 3H, CH₃) ppm. IR (ATR): ν 3257, 3176, 3132, 3073, 2961, 2883, 2825, 1749, 1680, 1632, 1586, 1557, 1518, 1492, 1438, 1409, 1375, 1289, 1271, 1254, 1234, 1154, 1134, 1091, 1044, 966, 922, 883, 767 cm⁻¹. HRMS (ESI⁺) *m/z* for C₂₃H₂₃Cl₂N₄O₃ ([M + H]⁺): calculated 473.1142, found 473.1138. HPLC (0–16 min, 30–90% ACN in 0.1% TFA, 16–20 min, 90% ACN in 0.1% TFA, Agilent Extend-C18 column: 3.5 μm, 4.6 × 150 mm): *t*_R 15.437 min (95.50% at 254 nm).

4.2.2.25 3-(3-((tert-Butoxycarbonyl)amino)methyl)piperidin-1-yl)-4-(3,4-dichloro-5-methyl-1H-pyrrole-2-carboxamido)benzoic acid (18i). To a solution of compound **17i** (180 mg, 0.33 mmol) in a mixture of methanol (10 mL) and tetrahydrofuran (10 mL) 1 M NaOH (1.32 mL, 1.32 mmol) was added and the mixture was stirred at rt for 15 h. The solvent was removed under reduced pressure, EtOAc (10 mL) and 1 M HCl (10 mL) were added to the residue. The phases were separated, the organic phase was washed with brine (2 × 10 mL), dried over Na₂SO₄, filtered and the solvent was removed under reduced pressure to give **18i** (160 mg) as a white solid. Yield 92% (160 mg); white solid; mp 221–224 °C. ¹H NMR (400 MHz, DMSO-*d*₆): δ 12.86 (br s, 1H, COOH), 12.44 (s, 1H, NH), 9.86 (s, 1H, NHBoc), 8.49 (d, 1H, *J* = 8.4 Hz, Ar-H-5), 7.86 (d, 1H, *J* = 1.8 Hz, Ar-H-2), 7.78 (dd, 1H, *J* = 8.4, 1.8 Hz, Ar-H-6), 6.90 (t, 1H, *J* = 5.8 Hz, NHBoc), 2.81–2.98 (m, 3H, 3 × CH), 2.69–2.80 (m, 1H, CH), 2.59–2.69 (m, 1H, CH), 2.35–2.40 (m, 1H, CH), 2.24 (s, 3H, CH₃), 1.83–1.93 (m, 1H, CH), 1.72–1.82 (m, 2H, 2 × CH), 1.59–1.71 (m, 1H, CH), 1.31 (s, 9H, *t*Bu), 0.99–1.09 (m, 1H, CH) ppm. ¹³C NMR (100 MHz, DMSO-*d*₆): δ 166.9, 156.6, 155.6, 142.3, 137.8, 129.9, 126.9, 125.7, 122.6, 118.7, 118.3, 109.9, 108.6, 77.4, 57.1, 53.3, 43.6, 37.3, 28.1, 27.6, 25.4, 10.7 ppm. IR (ATR): ν 3271, 2959, 2808, 1709, 1675, 1641, 1603, 1519, 1487, 1435, 1300, 1254, 1166, 1091, 728, 594 cm⁻¹. MS (ESI) *m/z* = 525.17 ([M + H]⁺). HRMS for C₂₄H₃₁Cl₂N₄O₅: calculated 525.1666, found 525.1662. HPLC: Agilent Zorbax 80 Å Extend-C18 (3.5 μm, 4.6 × 150 mm); mobile phase: 5%



acetonitrile in 0.1% TFA to 8 min, 5–95% of acetonitrile from 8 to 15 min, 95% acetonitrile from 15 to 16 min, 95–5% of acetonitrile from 16 to 18 min, 5% of acetonitrile from 18 to 21 min; flow rate 1.0 mL min⁻¹; injection volume: 10 μL; *t*_R: 13.587 min (99.4% at 254 nm).

4.2.2.26 *4-(2-(3,4-Dichloro-5-methyl-1H-pyrrole-2-carboxamido)-5-(methoxycarbonyl)phenyl)piperazin-1-ium chloride (19a)*. Compound **17a** (102 mg, 0.200 mmol) was dissolved in 1 M HCl solution in acetic acid (5 mL) and the mixture was stirred at rt for 5 h. The solvent was removed under reduced pressure, the solid residue was successively triturated with diethyl ether and water, and the undissolved solid was filtered off and dried to give **19a** (71 mg) as a white solid. Yield 35% (71 mg); white solid; mp 244–248 °C. ¹H NMR (400 MHz, DMSO-*d*₆): δ 12.51 (s, 1H, NH), 9.67 (s, 1H, NH), 9.07 (s, 2H, NH₂⁺), 8.53 (d, 1H, *J* = 8.6 Hz, ArH), 7.87 (dd, 1H, *J* = 8.6, 1.9 Hz, ArH), 7.82 (d, 1H, *J* = 1.9 Hz, ArH), 3.86 (s, 3H, COOCH₃), 3.22–3.31 (m, 4H, 2 × CH₂), 3.05–3.14 (m, 4H, 2 × CH₂), 2.25 (s, 3H, CH₃) ppm. ¹³C NMR (100 MHz, DMSO-*d*₆): δ 165.5, 156.7, 140.4, 138.2, 130.0, 127.6, 124.5, 122.3, 119.1, 118.7, 110.2, 108.7, 52.1, 48.7, 43.5, 10.8 ppm. DEPT 45 NMR (100 MHz, DMSO-*d*₆) δ 128.1, 122.7, 119.6, 52.6, 49.3, 43.9, 11.2 ppm. DEPT 135 NMR (100 MHz, DMSO-*d*₆) δ 128.1, 122.7, 119.6, 52.6, 49.3 (negative), 43.9 (negative), 11.2 ppm. IR (ATR): ν 3357, 3291, 3162, 3124, 2954, 2853, 2773, 2720, 2607, 2451, 2363, 1767, 1710, 1632, 1615, 1592, 1573, 1525, 1491, 1446, 1405, 1375, 1355, 1324, 1313, 1284, 1272, 1254, 1225, 1192, 1132, 1120, 1104, 1091, 1043, 1017, 1001, 983, 962, 947, 894, 859, 817, 761, 730, 711, 655, 639, 615 cm⁻¹. MS (ESI) *m/z* = 411.1 ([M - H]⁻). HRMS for C₁₈H₂₁N₄O₃Cl₂: calculated 411.0991, found 411.0998. HPLC (0–16 min, 30–90% ACN in 0.1% TFA, 16–20 min, 90% ACN in 0.1% TFA, Agilent Eclipse Plus C18: 5 μm, 4.6 × 150 mm): *t*_R 6.367 min (100% at 280 nm).

4.2.2.27 *(S)-1-(2-(3,4-Dichloro-5-methyl-1H-pyrrole-2-carboxamido)-5-(methoxycarbonyl)phenyl)piperidin-3-aminium chloride (19c)*. Compound **17c** (102 mg, 0.209 mmol) was dissolved in 1 M HCl solution in acetic acid (5 mL) and the mixture was stirred at rt for 15 h. The solvent was removed under reduced pressure, the solid residue was triturated with diethyl ether, and the undissolved solid was filtered off and dried to give **19c** (79 mg) as a brown solid. Yield 74% (79 mg); brown solid; mp 201–203 °C. ¹H NMR (400 MHz, DMSO-*d*₆): δ 12.55 (s, 1H, NH), 9.64 (s, 1H, NH), 8.47 (d, *J* = 8.5 Hz, ArH), 8.16 (br s, 3H, NH₃⁺), 7.79–7.90 (m, 2H, 2 × ArH), 3.86 (s, 3H, CH₃), 3.26–3.36 (m, 1H, CH), 3.14–3.23 (m, 1H, CH), 2.86–2.94 (m, 1H, CH), 2.65–2.79 (m, 2H, CH₂), 2.25 (s, 3H, CH₃), 2.05–2.16 (m, 1H, CH), 1.85–1.92 (m, 1H, CH), 1.63–1.79 (m, 1H, CH), 1.43–1.58 (m, 1H, CH) ppm. ¹³C NMR (100 MHz, DMSO-*d*₆): δ 165.6, 156.6, 141.3, 137.8, 129.9, 127.0, 124.5, 122.1, 119.3, 118.6, 110.3, 108.7, 54.7, 52.3, 52.1, 47.5, 27.5, 23.6, 10.7 ppm. IR (ATR): ν 2949, 2153, 2013, 1987, 1970, 1712, 1636, 1589, 1515, 1489, 1438, 1414, 1376, 1318, 1287, 1257, 1204, 1105, 1041, 994, 941, 873, 844, 765, 731, 657, 608 cm⁻¹. [α]_D²⁰ = -0.72° (*c* 0.337, THF). MS (ESI) *m/z* = 425.1 ([M + H]⁺). HRMS for C₁₉H₂₃N₄O₃Cl₂: calculated 425.1147, found 425.1145. HPLC (0–16 min, 30–90% ACN in 0.1% TFA, 16–20 min, 90% ACN in 0.1% TFA, Agilent

Eclipse Plus C18: 5 μm, 4.6 × 150 mm): *t*_R 7.370 min (98.9% at 280 nm).

4.2.2.28 *(1-(2-(3,4-Dichloro-5-methyl-1H-pyrrole-2-carboxamido)-5-(methoxycarbonyl)phenyl)piperidin-3-yl)methanaminium chloride (19i)*. Compound **17i** (100 mg, 0.851 mmol) was dissolved in 4 M HCl solution in dioxane (10 mL) and the mixture was stirred for 4 h. After completion of the reaction, the solvent was removed under reduced pressure and the obtained solid was washed with diethyl ether (2 × 5 mL) to give **19i** (70 mg) as a beige solid. Yield 79% (70 mg); beige solid; mp 194–198 °C. ¹H NMR (400 MHz, DMSO-*d*₆): δ 12.53 (s, 1H, NH), 9.93 (s, 1H, NH), 8.58 (d, *J* = 8.4 Hz, Ar-H-3), 8.02 (br s, 3H, NH₃⁺), 7.94 (d, *J* = 1.8 Hz, Ar-H-6), 7.89 (dd, *J* = 8.4, 1.8 Hz, Ar-H-4), 3.91 (s, 3H, COOCH₃), 3.08–3.14 (m, 1H, CH), 2.89–2.98 (m, 1H, CH), 2.76–2.87 (m, 2H, 2 × CH), 2.58–2.68 (m, 2H, 2 × CH), 2.30 (s, 3H, CH₃), 2.07–2.19 (m, 1H, CH), 1.90–2.00 (m, 1H, CH), 1.80–1.89 (m, 1H, CH), 1.68–1.79 (m, 1H, CH), 1.16–1.25 (m, 1H, CH) ppm. ¹³C NMR (100 MHz, DMSO-*d*₆): δ 165.7, 156.6, 142.1, 138.4, 130.1, 127.1, 124.3, 122.7, 118.7, 118.5, 110.0, 108.7, 55.5, 53.5, 52.1, 41.8, 34.9, 27.1, 25.0, 10.8 ppm. DEPT 45 NMR (100 MHz, DMSO-*d*₆) δ 127.6, 123.2, 119.0, 56.1, 54.0, 52.5, 42.3, 35.4, 27.6, 25.5, 11.3 ppm. DEPT 135 NMR (100 MHz, DMSO-*d*₆) δ 127.6, 123.2, 119.0, 56.1 (negative), 54.0 (negative), 52.5, 42.3 (negative), 35.4, 27.6 (negative), 25.5 (negative), 11.3 ppm. IR (ATR): ν 2948, 1717, 1640, 1590, 1517, 1488, 1414, 1289, 1257, 1199, 1121, 1036, 1007, 764 cm⁻¹. MS (ESI) *m/z* = 439.13 ([M + H]⁺). HRMS for C₂₀H₂₅Cl₂N₄O₃: calculated 439.1293, found 439.1298. HPLC: Agilent Zorbax 80 Å Extend-C18 (3.5 μm, 4.6 × 150 mm); mobile phase: 5% acetonitrile in 0.1% TFA to 8 min, 5–95% of acetonitrile from 8 to 15 min, 95% acetonitrile from 15 to 16 min, 95–5% of acetonitrile from 16 to 18 min, 5% of acetonitrile from 18 to 21 min; flow rate 1.0 mL min⁻¹; injection volume: 10 μL; *t*_R: 9.373 min (95.6% at 254 nm).

4.2.2.29 *4-(5-Carboxy-2-(3,4-dichloro-5-methyl-1H-pyrrole-2-carboxamido)phenyl)piperazin-1-ium chloride (20a)*. Compound **18a** (50 mg, 0.100 mmol) was dissolved in a mixture of 1 M HCl solution in acetic acid (5 mL), tetrahydrofuran (2 mL) and dichloromethane (4 mL) and the mixture was stirred at rt for 5 h. The solvent was removed under reduced pressure, the solid residue was successively triturated with diethyl ether and water, and the undissolved solid was filtered off and dried to give **20a** (16 mg) as a pale pink solid. Yield 32% (16 mg); pale pink solid; mp 258–261 °C. ¹H NMR (400 MHz, DMSO-*d*₆): δ 12.50 (s, 1H, NH), 9.68 (s, 1H, NH), 8.50 (d, *J* = 8.4 Hz, ArH), 7.76–7.92 (m, 2H, 2 × ArH), 3.22–3.29 (m, 4H, 2 × CH₂), 3.03–3.11 (m, 4H, 2 × CH₂), 2.25 (s, 3H, CH₃) ppm. Signal for NH₂⁺ is overlapping with the signal for water. Signal for COOH proton not seen. ¹³C NMR (100 MHz, DMSO-*d*₆): δ 183.4, 178.3, 166.7, 156.6, 140.4, 137.8, 129.9, 127.7, 122.4, 118.8, 110.1, 108.7, 49.0, 43.6, 10.8 ppm. IR (ATR): ν 3293, 3005, 2822, 2470, 2363, 1684, 1651, 1588, 1529, 1488, 1457, 1406, 1374, 1316, 1285, 1229, 1192, 1142, 1120, 1089, 1047, 963, 923, 885, 849, 787, 771, 726, 704, 647, 634, 606 cm⁻¹. MS (ESI) *m/z* = 395.1 ([M - H]⁻). HRMS for C₁₇H₁₇N₄O₃Cl₂: calculated 395.0678, found 395.0670. HPLC (0–16 min, 30–90% ACN in 0.1% TFA, 16–20 min, 90% ACN in 0.1%



TFA, Agilent Eclipse Plus C18: 5 μm , 4.6 \times 150 mm): t_{R} 4.769 min (98.1% at 280 nm).

4.2.2.30 (*S*)-1-(5-Carboxy-2-(3,4-dichloro-5-methyl-1H-pyrrole-2-carboxamido)phenyl)piperidin-3-aminium chloride (**20c**). Compound **18c** (70 mg, 0.14 mmol) was dissolved in 1 M HCl solution in acetic acid (5 mL) and the mixture was stirred at rt for 15 h. The solvent was removed under reduced pressure, the solid residue was triturated with diethyl ether, and the undissolved solid was filtered off and dried to give **20c** (64 mg) as a pale-yellow solid. Yield 91% (64 mg); pale yellow solid; mp 240–244 $^{\circ}\text{C}$. ^1H NMR (400 MHz, DMSO- d_6): δ 12.54 (br s, 1H, NH), 9.63 (s, 1H, NH), 8.45 (d, $J = 9.1$ Hz, ArH), 7.77–7.85 (m, 2H, 2 \times ArH), 3.28–3.33 (m, 1H, CH), 3.14–3.21 (m, 1H, CH), 2.85–2.93 (m, 1H, CH), 2.64–2.78 (m, 2H, CH $_2$), 2.25 (s, 3H, CH $_3$), 2.04–2.14 (m, 1H, CH), 1.84–1.92 (m, 1H, CH), 1.63–1.76 (m, 1H, CH), 1.44–1.58 (m, 1H, CH) ppm. Signals for COOH and NH $_3^+$ protons not seen. ^{13}C NMR (100 MHz, DMSO- d_6): δ 166.7, 156.6, 141.2, 137.4, 129.8, 127.1, 125.8, 122.2, 119.2, 118.7, 110.3, 108.7, 54.8, 52.3, 47.6, 27.5, 23.6, 10.7 ppm. IR (ATR): ν 2926, 2154, 2050, 1992, 1682, 1634, 1589, 1518, 1490, 1406, 1322, 1249, 1201, 1125, 1107, 1090, 1043, 1017, 960, 942, 836, 766, 743, 728, 649, 608 cm^{-1} . $[\alpha]_{\text{D}}^{20} = -0.55^{\circ}$ (c 0.264, THF). MS (ESI) $m/z = 411.1$ ($[\text{M} + \text{H}]^+$). HRMS for C $_{18}$ H $_{21}$ N $_4$ O $_3$ Cl $_2$: calculated 411.0991, found 411.1002. HPLC (0–16 min, 30–90% ACN in 0.1% TFA, 16–20 min, 90% ACN in 0.1% TFA, Agilent Eclipse Plus C18: 5 μm , 4.6 \times 150 mm): t_{R} 5.947 min (99.7% at 280 nm).

4.2.2.31 4-((5-Carboxy-2-(3,4-dichloro-5-methyl-1H-pyrrole-2-carboxamido)phenyl)amino)piperidin-1-ium chloride (**20d**). To a suspension of **18d** (30 mg, 0.059 mmol) in 1,4-dioxane (1 mL), 4 M HCl in 1,4-dioxane (4 mL) was added and the reaction mixture was stirred at rt for 4 h. The resulting precipitate was filtered off, washed with diethyl ether and dried. Yield 94% (24.7 mg); pale yellow solid; mp 260–265 $^{\circ}\text{C}$. ^1H NMR (400 MHz, DMSO- d_6): δ 12.52 (s, 2H, NH, COOH), 9.14 (s, 1H, NH), 8.79 (s, 1H, NH), 8.65 (s, 1H, NH), 7.83 (d, 1H, $J = 8.1$ Hz, ArH), 7.35–7.44 (m, 2H, 2 \times ArH), 3.64 (1H, overlapped with the signal for water, CH), 3.25–3.35 (m, 3H, CH, CH $_2$), 3.05 (q, 2H, $J = 10.6$, 11.3 Hz, CH $_2$), 2.25 (s, 3H, CH $_3$), 2.05 (d, 2H, $J = 13.9$ Hz, CH $_2$), 1.60–1.75 (m, 2H, CH $_2$) ppm. Signal for one NH proton not seen. IR (ATR): ν 3117, 2955, 2786, 2323, 1722, 1657, 1620, 1581, 1519, 1500, 1471, 1402, 1371, 1335, 1311, 1282, 1218, 1179, 1135, 1117, 1090, 1076, 1055, 1031, 992, 965, 933, 901, 872, 846, 803, 763, 748, 725, 638, 606 cm^{-1} . HRMS (ESI $^+$) m/z for C $_{18}$ H $_{21}$ Cl $_2$ N $_4$ O $_3$ ($[\text{M} + \text{H}]^+$): calculated 411.0985, found 411.0980. HPLC (0–16 min, 30–90% ACN in 0.1% TFA, 16–20 min, 90% ACN in 0.1% TFA, Agilent Extend-C18 column: 3.5 μm , 4.6 \times 150 mm): t_{R} 12.480 min (95.11% at 254 nm).

4.2.2.32 (1-(5-Carboxy-2-(3,4-dichloro-5-methyl-1H-pyrrole-2-carboxamido)phenyl)piperidin-3-yl)methanaminium chloride (**20i**). Compound **18i** (59 mg, 0.11 mmol) was dissolved in 4 M HCl solution in 1,4-dioxane (8 mL). The mixture was stirred for 12 h. The solvent was removed under reduced pressure and the solid was washed with diethyl ether (2 \times 5 mL) to give **20i** (49 mg) as a white solid. Yield 96% (49 mg); white solid; mp 234–238 $^{\circ}\text{C}$. ^1H NMR (400 MHz, DMSO- d_6): δ 12.48 (s, 1H, NH), 9.87 (s, 1H, NH), 8.50 (d, $J = 8.4$ Hz, Ar-H-3), 7.97 (br s, 3H, NH $_3^+$), 7.88 (d, $J = 1.8$ Hz, Ar-H-6), 7.81 (dd, $J = 8.4$, 1.8 Hz, Ar-H-4), 3.03–3.10 (m,

1H, CH), 2.83–2.92 (m, 1H, CH), 2.71–2.80 (m, 2H, 2 \times CH), 2.54–2.63 (m, 3H, $J = 9.0$ Hz, 3 \times CH), 2.25 (s, 3H, CH $_3$), 2.02–2.13 (m, 1H, CH), 1.85–1.95 (m, 1H, CH), 1.75–1.83 (m, 1H, CH), 1.63–1.73 (m, 1H, CH), 1.09–1.19 (m, 1H, CH) ppm. ^{13}C NMR (100 MHz, DMSO- d_6): δ 166.7, 156.6, 142.0, 137.9, 129.9, 127.1, 125.6, 122.8, 118.7, 118.4, 110.0, 108.6, 55.6, 53.6, 41.8, 34.8, 27.2, 25.0, 10.8 ppm. IR (ATR): ν 3446, 3070, 2888, 1722, 1635, 1605, 1521, 1422, 1378, 1219, 1201, 1140, 1041, 837, 814, 752, 730, 660, 546 cm^{-1} . MS (ESI) $m/z = 425.11$ ($[\text{M} + \text{H}]^+$). HRMS for C $_{19}$ H $_{23}$ Cl $_2$ N $_4$ O $_3$: calculated 425.1142, found 425.1137. HPLC: Agilent Zorbax 80 \AA Extend-C18 (3.5 μm , 4.6 \times 150 mm); mobile phase: 5% acetonitrile in 0.1% TFA to 8 min, 5–95% of acetonitrile from 8 to 15 min, 95% acetonitrile from 15 to 16 min, 95–5% of acetonitrile from 16 to 18 min, 5% of acetonitrile from 18 to 21 min; flow rate 1.0 mL min $^{-1}$; injection volume: 10 μL ; t_{R} : 8.557 min (99.2% at 254 nm).

4.2.2.33 *tert*-Butyl 4-(2-(3,4-dichloro-5-methyl-1H-pyrrole-2-carboxamido)-5-(hydrazinecarbonyl)phenyl)piperazine-1-carboxylate (**21a**). To the solution of **17a** (0.71 g, 1.38 mmol) in a mixture of MeOH (20 mL) and THF (10 mL) in a high-pressure tube, hydrazine hydrate (64%, 4.71 mL, 96.8 mmol) was added. The tube was sealed and the reaction mixture was stirred at 120 $^{\circ}\text{C}$ for 15 h. The tube was cooled to rt and the precipitate was filtered off and dried to give **21a** (0.579 g) as a white solid. Yield 82% (0.579 g); white solid; mp 277–280 $^{\circ}\text{C}$. ^1H NMR (400 MHz, DMSO- d_6): δ 12.42 (s, 1H, NH), 9.74 (s, 1H, NH), 9.71 (s, 1H, NH), 8.43 (d, $J = 8.6$ Hz, ArH), 7.84 (s, 1H, ArH), 7.70 (d, $J = 8.6$ Hz, ArH), 4.47 (s, 2H, NH $_2$), 3.57–3.47 (m, 4H, 2 \times CH $_2$), 2.85–2.77 (m, 4H, 2 \times CH $_2$), 2.24 (s, 3H, CH $_3$), 1.44 (s, 9H, *t*Bu) ppm.

4.2.2.34 *tert*-Butyl 4-(2-(3,4-dichloro-5-methyl-1H-pyrrole-2-carboxamido)-5-(5-oxo-4,5-dihydro-1,3,4-oxadiazol-2-yl)phenyl)piperazine-1-carboxylate (**22a**). To a solution of compound **21a** (0.500 g, 0.980 mmol) in a mixture of DMF (30 mL) and 1,4-dioxane (15 mL) 1,1'-carbonyldiimidazole (0.640 g, 3.93 mmol) was added and the reaction mixture was stirred at 101 $^{\circ}\text{C}$ for 15 h. The solvent was removed under reduced pressure, the crude product was successively triturated with acetonitrile, water and THF, the undissolved solid was filtered off and dried. The crude product was crystallized from DMF and dried to give **22a** (226 mg) as a white solid. Yield 43% (226 mg); white solid; mp 260–263 $^{\circ}\text{C}$. ^1H NMR (400 MHz, DMSO- d_6): δ 12.50 (s, 1H, NH), 12.45 (s, 1H, NH), 9.73 (s, 1H, NH), 8.53 (d, $J = 8.6$ Hz, ArH), 7.69 (s, 1H, ArH), 7.64 (d, $J = 8.5$ Hz, ArH), 3.52 (s, 4H, 2 \times CH $_2$), 2.88–2.80 (m, 4H, 2 \times CH $_2$), 2.24 (s, 3H, CH $_3$), 1.44 (s, 9H, *t*Bu) ppm. ^{13}C NMR (100 MHz, DMSO- d_6): δ 157.0, 154.9, 154.2, 154.0, 153.9, 142.3, 136.9, 130.4, 123.5, 120.1, 119.5, 119.2, 110.3, 109.1, 79.6, 52.2, 44.3, 28.5, 11.2 ppm. Peaks of two aromatic carbons overlapping. HRMS for C $_{23}$ H $_{27}$ O $_5$ N $_6$ Cl $_2$ ($[\text{M} + \text{H}]^+$): calculated 537.14099, found 537.14145. HPLC (0–10 min, 10–90% ACN in 0.1% TFA, 10–11 min, 90% ACN in 0.1% TFA, Waters Acquity UPLC HSS C18 column: 1.8 μm , 2.1 \times 50 mm): t_{R} 6.560 min (97.45% at 254 nm, 96.79% at 280 nm).

4.2.2.35 *tert*-Butyl (*S*)-1-(2-(3,4-dichloro-5-methyl-1H-pyrrole-2-carboxamido)-5-(5-oxo-4,5-dihydro-1,3,4-oxadiazol-2-yl)phenyl)pyrrolidin-3-yl)carbamate (**22b**). To a solution of compound **21b** (100 mg, 0.192 mmol) in a mixture of DMF (2.5 mL) and 1,4-



dioxane (5 mL) 1,1'-carbonyldiimidazole (94 mg, 0.576 mmol) was added and the reaction mixture was stirred at 101 °C for 15 h. The solvent was removed under reduced pressure, the crude product was successively triturated with acetonitrile and methanol, the undissolved solid was filtered off and dried to give **22b** (24 mg) as a pale-yellow solid. Yield 23% (24 mg); pale yellow solid; mp 260–263 °C. ¹H NMR (400 MHz, DMSO-d₆): δ 12.40 (br s, 2H, 2 × NH), 9.50 (s, 1H, NH), 8.32 (d, *J* = 8.6 Hz, ArH), 7.60 (s, 1H, ArH), 7.53 (d, *J* = 8.6 Hz, ArH), 7.20 (d, 1H, NHBoc), 4.15–4.07 (m, 1H, CH), 3.33–3.24 (m, 1H, CH), 3.24–3.17 (m, 1H, CH), 3.12–3.04 (m, 1H, CH), 2.96–2.91 (m, 1H, CH), 2.25 (s, 3H, CH₃), 2.22–2.16 (m, 1H, CH), 1.90–1.80 (m, 1H, CH), 1.40 (s, 9H, *t*Bu) ppm. IR (ATR): ν 3342, 2980, 2850, 2361, 1721, 1681, 1643, 1620, 1602, 1512, 1491, 1416, 1366, 1329, 1275, 1216, 1171, 1118, 1086, 1037, 1003, 958, 897, 833, 761, 731, 657, 625, 608, 571 cm⁻¹. [α]_D²⁰ = 0.035 (c 0.255, DMF). HRMS for C₂₃H₂₇Cl₂N₆O₅: calculated 537.1414, found 537.1416.

4.2.2.36 tert-Butyl (S)-1-(2-(3,4-dichloro-5-methyl-1H-pyrrole-2-carboxamido)-5-(5-oxo-4,5-dihydro-1,3,4-oxadiazol-2-yl)phenyl)piperidin-3-yl)carbamate (22c). To a solution of compound **21c** (240 mg, 0.449 mmol) in a mixture of DMF (5 mL) and 1,4-dioxane (10 mL) 1,1'-carbonyldiimidazole (219 mg, 1.35 mmol) was added and the reaction mixture was stirred at 101 °C for 15 h. The solvent was removed under reduced pressure, the crude product was triturated with a mixture of acetonitrile and methanol (20 mL, 1 : 1), the undissolved solid was filtered off and dried to give **22c** (202 mg) as a brown solid. Yield 84% (202 mg); brown solid; mp 260–263 °C. ¹H NMR (400 MHz, DMSO-d₆): δ 12.55 (s, 1H, NH), 12.44 (s, 1H, NH), 9.76 (s, 1H, NH), 8.53 (d, *J* = 8.6 Hz, 1H, ArH), 7.64–7.62 (m, 2H, 2 × ArH), 6.89 (d, *J* = 7.5 Hz, 1H, NHBoc), 3.70–3.54 (m, 1H, CH), 3.05–2.91 (m, 1H, CH), 2.91–2.77 (m, 1H, CH), 2.70–2.54 (m, 2H, CH₂), 2.24 (s, 3H, CH₃), 1.99–1.75 (m, 2H, CH₂), 1.74–1.58 (m, 1H, CH), 1.27–1.17 (m, 1H, CH), 1.36 (s, 9H, *t*Bu) ppm. ¹³C NMR (100 MHz, DMSO-d₆): δ 156.6, 154.8, 154.4, 153.5, 142.2, 136.5, 129.9, 122.8, 119.4, 118.9, 118.7, 118.7, 108.6, 77.7, 57.4, 52.9, 47.6, 29.3, 28.2, 24.4, 10.8 ppm. IR (ATR): ν 3317, 2963, 2360, 1778, 1687, 1634, 1582, 1514, 1491, 1403, 1362, 1326, 1251, 1170, 1125, 1072, 1034, 945, 913, 827, 747 cm⁻¹. [α]_D²⁰ = -0.73° (c 0.318, THF). MS (ESI) *m/z* = 549.1 ([M - H]⁻). HRMS for C₂₄H₂₇N₆O₅Cl₂: calculated 549.1420, found 549.1436. HPLC (0–16 min, 30–90% ACN in 0.1% TFA, 16–20 min, 90% ACN in 0.1% TFA, Agilent Eclipse Plus C18: 5 μm, 4.6 × 150 mm): *t*_R 11.960 min (95.1% at 280 nm).

4.2.2.37 tert-Butyl 4-((2-(3,4-dichloro-5-methyl-1H-pyrrole-2-carboxamido)-5-(5-oxo-4,5-dihydro-1,3,4-oxadiazol-2-yl)phenyl)amino)piperidine-1-carboxylate (22d). To a suspension of **21d** (60 mg, 0.114 mmol) in a mixture of 1,4-dioxane and DMF (2 : 1, 15 mL), 1,1'-carbonyldiimidazole (55.6 mg, 0.343 mmol) was added and the reaction mixture was stirred at 101 °C for 24 h. The solvent was evaporated *in vacuo*, the residue was suspended in acetonitrile, sonicated, filtered off and dried. Yield 56% (35 mg); yellow solid; mp 168–171 °C. ¹H NMR (400 MHz, DMSO-d₆): δ 12.49 (s, 1H, NH), 12.26 (s, 1H, NH), 8.84 (s, 1H, NH), 7.78–7.74 (m, 1H, ArH), 7.19–7.14 (m, 2H, 2 × ArH), 5.19 (d, 1H, *J* = 7.1 Hz, NH), 3.92–3.79 (m, 2H, CH₂), 3.53 (s, 1H, CH), 2.93 (m, 2H, CH₂), 2.23 (s, 3H, CH₃), 1.89 (d, 2H, *J* = 9.7 Hz, CH₂), 1.40 (s,

9H, *t*Bu), 1.37–1.25 (m, 2H, CH₂) ppm. IR (ATR): ν 3347, 3328, 3265, 3122, 2974, 2931, 2852, 1767, 1660, 1590, 1533, 1496, 1478, 1417, 1366, 1322, 1241, 1212, 1175, 1143, 1093, 1074, 1043, 939, 860, 823, 734 cm⁻¹. HRMS for C₂₄H₂₉Cl₂N₆O₅: calculated 551.15710, found 551.15704.

4.2.2.38 3,4-Dichloro-5-methyl-N-(2-morpholino-4-(5-oxo-4,5-dihydro-1,3,4-oxadiazol-2-yl)phenyl)-1H-pyrrole-2-carboxamide (22e). To a solution of compound **21e** (400 mg, 0.970 mmol) in a mixture of DMF (20 mL) and 1,4-dioxane (20 mL) 1,1'-carbonyldiimidazole (310 mg, 1.94 mmol) was added and the reaction mixture was stirred at 101 °C for 15 h. The solvent was removed under reduced pressure, the crude product was successively triturated with acetonitrile, water and MeOH, and the undissolved solid was filtered off and dried. The crude product was crystallized from DMF and dried to obtain **22e** (42.9 mg) as a white solid. Yield 11% (42.9 mg); white solid; mp 304–305 °C. ¹H NMR (400 MHz, DMSO-d₆): δ 12.54 (s, 1H, NH), 12.44 (s, 1H, NH), 9.75 (s, 1H, NH), 8.54 (d, 1H, *J* = 8.6 Hz, ArH), 7.70 (d, 1H, *J* = 2.0 Hz, ArH), 7.64 (dd, 1H, *J* = 8.5, 1.9 Hz, ArH), 3.72–2.84 (m, 4H, 2 × CH₂), 2.84–2.95 (m, 4H, 2 × CH₂), 2.24 (s, 3H, CH₃) ppm. ¹³C NMR (100 MHz, DMSO-d₆): δ 157.0, 154.9, 154.0, 153.9, 142.4, 137.0, 130.4, 123.4, 120.1, 119.6, 119.2, 118.9, 109.1, 110.3, 67.0, 52.6, 11.2 ppm. HRMS for C₁₈H₁₈O₄N₅Cl₂ ([M + H]⁺): calculated 438.07237, found 438.07304. HPLC (0–10 min, 10–90% ACN in 0.1% TFA, 10–11 min, 90% ACN in 0.1% TFA, Waters Acquity UPLC HSS C18 column: 1.8 μm, 2.1 × 50 mm): *t*_R 5.333 min (97.38% at 254 nm, 97.45% at 280 nm).

4.2.2.39 3,4-Dichloro-5-methyl-N-(2-(2-methylmorpholino)-4-(5-oxo-4,5-dihydro-1,3,4-oxadiazol-2-yl)phenyl)-1H-pyrrole-2-carboxamide (22f). To a solution of compound **21f** (310 mg, 0.703 mmol) in a mixture of DMF (20 mL) and 1,4-dioxane (10 mL) 1,1'-carbonyldiimidazole (456 mg, 2.81 mmol) was added and the reaction mixture was stirred at 101 °C for 15 h. The solvent was removed under reduced pressure, the solid residue was successively triturated with acetonitrile, water and THF, and the undissolved solid was filtered off and dried. The crude product was crystallized from DMF and dried to give **22f** (169 mg) as a white solid. Yield 53% (169 mg); white solid; mp 302–303 °C. ¹H NMR (400 MHz, DMSO-d₆): δ 12.56 (s, 1H, NH), 12.44 (s, 1H, NH), 9.75 (s, 1H, NH), 8.55 (d, 1H, *J* = 8.6 Hz, ArH), 7.68 (d, 1H, *J* = 2.0 Hz, ArH), 7.64 (dd, 1H, *J* = 8.6, 2.0 Hz, ArH), 3.88–3.93 (m, 1H, CH), 3.69–3.81 (m, 2H, 2 × CH), 2.78–2.94 (m, 3H, 3 × CH), 2.55–2.61 (m, 1H, CH), 2.24 (s, 3H, CH₃), 1.12 (d, 3H, *J* = 6.2 Hz, CH₃) ppm. ¹³C NMR (100 MHz, DMSO-d₆): δ 157.0, 154.9, 154.0, 153.9, 142.2, 137.1, 130.4, 123.4, 120.0, 119.5, 119.2, 119.0, 109.1, 110.3, 72.1, 66.7, 58.6, 52.1, 19.3, 11.2 ppm. DEPT 45 NMR (100 MHz, DMSO-d₆) δ 123.4, 120.0, 119.0, 72.2, 66.7, 58.6, 52.1, 19.3, 11.2 ppm. DEPT 135 NMR (100 MHz, DMSO-d₆) δ 123.4, 120.0, 119.0, 72.2, 66.7 (negative), 58.6 (negative), 52.1 (negative), 19.3, 11.2 ppm. HRMS for C₁₉H₁₈O₄N₅Cl₂ ([M - H]⁻): calculated 450.07428, found 450.07413. HPLC (0–10 min, 10–90% ACN in 0.1% TFA, 10–11 min, 90% ACN in 0.1% TFA, Waters Acquity UPLC HSS C18 column: 1.8 μm, 2.1 × 50 mm): *t*_R 5.703 min (95.50% at 254 nm, 95.08% at 280 nm).

4.2.2.40 3,4-Dichloro-N-(2-(2,6-dimethylmorpholino)-4-(5-oxo-4,5-dihydro-1,3,4-oxadiazol-2-yl)phenyl)-5-methyl-1H-pyrrole-2-



carboxamide (**22g**). To the solution of compound **21g** (217 mg, 0.492 mmol) in a mixture of DMF (8 mL) and 1,4-dioxane (15 mL) 1,1'-carbonyldiimidazole (319 mg, 1.97 mmol) was added and the reaction mixture was stirred at 101 °C for 15 h. The solvent was removed under reduced pressure, the solid residue was successively triturated with acetonitrile, MeOH and THF, and the undissolved solid was filtered off and dried. The crude product was crystallized from DMF and dried to obtain **22g** (112 mg) as a white solid. Yield 49% (112 mg); white solid; mp 301–303 °C. ¹H NMR (400 MHz, DMSO-*d*₆): δ 12.57 (s, 1H, NH), 12.45 (s, 1H, NH), 9.79 (s, 1H, NH), 8.57 (d, 1H, *J* = 8.6 Hz, ArH), 7.61–7.71 (m, 2H, 2 × ArH), 3.77–3.86 (m, 2H, 2 × CH), 2.86–2.93 (m, 2H, 2 × CH), 2.43–2.48 (m, 2H, 2 × CH), 2.24 (s, 3H, CH₃), 1.12 (d, 6H, *J* = 6.1 Hz, 2 × CH₃) ppm. ¹³C NMR (100 MHz, DMSO-*d*₆): δ 157.0, 154.9, 154.0, 153.9, 141.9, 137.1, 130.4, 123.5, 119.9, 119.5, 119.3, 119.1, 109.1, 110.2, 72.0, 58.1, 19.3, 11.2 ppm. HRMS for C₂₀H₂₂O₄N₅Cl₂ ([M + H]⁺): calculated 466.10399, found 466.10434. HPLC (0–10 min, 10–90% ACN in 0.1% TFA, 10–11 min, 90% ACN in 0.1% TFA, Waters Acquity UPLC HSS C18 column: 1.8 μm, 2.1 × 50 mm): *t*_R 6.077 min (98.33% at 254 nm, 98.27% at 280 nm).

4.2.2.41 *4-(2-(3,4-Dichloro-5-methyl-1H-pyrrole-2-carboxamido)-5-(5-oxo-4,5-dihydro-1,3,4-oxadiazol-2-yl)phenyl)piperazin-1-ium chloride* (**23a**). To a solution of **22a** (60.0 mg, 0.110 mmol) in DMF (6 mL) 4 M HCl in 1,4-dioxane (6 mL) was added and the reaction mixture was stirred at rt for 15 h. The solvent was removed under reduced pressure and the solid residue was triturated with acetonitrile, the undissolved solid was filtered off and dried to give **23a** (47.4 mg) as a white solid. Yield 91% (47.4 mg); white solid; mp 287–289 °C. ¹H NMR (400 MHz, DMSO-*d*₆): δ 12.63 (s, 1H, NH), 12.51 (s, 1H, NH), 9.60 (s, 1H, NH), 9.17 (s, 2H, NH₂⁺), 8.53 (dd, 1H, *J* = 8.7, 1.3 Hz, ArH), 7.68 (d, 1H, *J* = 8.5 Hz, ArH), 7.62 (s, 1H, ArH), 3.23–3.31 (m, 4H, 2 × CH₂), 3.08–3.15 (m, 4H, 2 × CH₂), 2.25 (s, 3H, CH₃) ppm. ¹³C NMR (100 MHz, DMSO-*d*₆): δ 157.1, 154.9, 154.0, 153.8, 141.5, 136.9, 130.4, 123.9, 120.4, 119.6, 119.2, 118.8, 110.7, 109.2, 49.2, 43.9, 11.2 ppm. HRMS for C₁₈H₁₉O₃N₆Cl₂ ([M + H]⁺): calculated 437.08880, found 437.08902. HPLC (0–10 min, 10–90% ACN in 0.1% TFA, 10–11 min, 90% ACN in 0.1% TFA, Waters Acquity UPLC HSS C18 column: 1.8 μm, 2.1 × 50 mm): *t*_R 3.107 min (96.56% at 254 nm, 95.85% at 280 nm).

4.2.2.42 *(S)-1-(2-(3,4-Dichloro-5-methyl-1H-pyrrole-2-carboxamido)-5-(5-oxo-4,5-dihydro-1,3,4-oxadiazol-2-yl)phenyl)pyrrolidin-3-aminium chloride* (**23b**). To a solution of compound **22b** (24 mg, 0.045 mmol) in DMF (1 mL) 4 M HCl in 1,4-dioxane (3 mL) was added and the reaction mixture was stirred at rt for 15 h. The solvent was removed under reduced pressure and the solid residue was successively triturated with diethyl ether and acetonitrile, the undissolved solid was filtered off and dried to give **23b** (13 mg) as an off-white solid. Yield 60% (13 mg); off-white solid; mp > 230 °C. ¹H NMR (400 MHz, DMSO-*d*₆): δ 12.63 (s, 1H, NH), 12.58 (s, 1H, NH), 9.50 (s, 1H, NH), 8.32 (br s, 3H, NH₃⁺), 8.25 (d, 1H, *J* = 8.5 Hz, ArH), 7.61 (d, 1H, *J* = 1.9 Hz, ArH), 7.55 (dd, 1H, *J* = 8.5, 1.9 Hz, ArH), 3.87–3.97 (s, 1H, CH), 3.50–3.57 (m, 1H, CH), 3.37–3.45 (m, 1H, CH), 3.02–3.14 (m, 2H, 2 × CH), 2.26–2.32 (m, 1H, CH), 1.98–2.06 (m, 1H, CH),

2.25 (s, 3H, CH₃) ppm. IR (ATR): ν 2969, 2360, 2341, 2187, 1994, 1768, 1637, 1584, 1519, 1490, 1360, 1311, 1257, 1179, 1091, 1040, 958, 927, 832, 707 cm⁻¹. [α]_D²⁵ 1.25 (c 0.271, DMF). HRMS for C₁₈H₁₉Cl₂N₆O₃: calculated 437.0890, found 437.0890. HPLC (0–16 min, 30–90% ACN in 0.1% TFA, 16–20 min, 90% ACN in 0.1% TFA, Agilent Eclipse Plus C18: 5 μm, 4.6 × 150 mm): *t*_R 3.523 min (96.05% at 254 nm).

4.2.2.43 *(S)-1-(2-(3,4-Dichloro-5-methyl-1H-pyrrole-2-carboxamido)-5-(5-oxo-4,5-dihydro-1,3,4-oxadiazol-2-yl)phenyl)piperidin-3-aminium chloride* (**23c**). To a solution of compound **22c** (133 mg, 0.241 mmol) in DMF (2 mL) 4 M HCl in 1,4-dioxane (6 mL) was added and the reaction mixture was stirred at rt for 15 h. The solvent was removed under reduced pressure and the solid residue was successively triturated with diethyl ether and acetonitrile, the undissolved solid was filtered off and dried to give **23c** (71 mg) as an off-white solid. Yield 60% (71 mg); off-white solid; mp > 230 °C. ¹H NMR (400 MHz, DMSO-*d*₆): δ 12.64 (s, 1H, NH), 12.57 (s, 1H, NH), 9.58 (s, 1H, NH), 8.46 (d, *J* = 8.4 Hz, 1H, ArH), 8.27 (br s, 3H, NH₃⁺), 7.63–7.66 (m, 2H, 2 × ArH), 3.26–3.40 (s, 1H, CH), 3.16–3.25 (m, 1H, CH), 2.83–2.94 (m, 1H, CH), 2.65–2.81 (m, 2H, CH₂), 2.25 (s, 3H, CH₃), 2.05–2.13 (m, 1H, CH), 1.83–1.93 (m, 1H, CH), 1.62–1.77 (m, 1H, CH), 1.44–1.62 (m, 1H, CH) ppm. ¹³C NMR (100 MHz, DMSO-*d*₆): δ 156.6, 154.4, 153.4, 141.9, 136.0, 129.8, 122.8, 120.3, 119.2, 118.7, 118.2, 110.3, 108.7, 54.7, 52.1, 47.5, 27.5, 23.6, 10.8 ppm. IR (ATR): ν 3100, 2361, 1766, 1650, 1613, 1511, 1488, 1408, 1373, 1333, 1255, 1204, 1125, 1086, 1039, 945, 919, 836, 749, 708 cm⁻¹. MS (ESI) *m/z* = 449.1 ([M – H]⁻). HRMS for C₁₉H₁₉N₆O₃Cl₂: calculated 449.0896, found 449.0890. HPLC (0–16 min, 30–90% ACN in 0.1% TFA, 16–20 min, 90% ACN in 0.1% TFA, Agilent Eclipse Plus C18: 5 μm, 4.6 × 150 mm): *t*_R 4.633 min (95.8% at 280 nm).

4.2.2.44 *4-((2-(3,4-Dichloro-5-methyl-1H-pyrrole-2-carboxamido)-5-(5-oxo-4,5-dihydro-1,3,4-oxadiazol-2-yl)phenyl)amino)piperidin-1-ium chloride* (**23d**). To a suspension of **22d** (25 mg, 0.045 mmol) in 1,4-dioxane (7 mL), 4 M HCl in 1,4-dioxane (3 mL) was added and the reaction mixture was stirred at rt for 3 h. The resulting precipitate was filtered off, washed with diethyl ether and dried to give **23d** as a pale-yellow solid. Yield 100% (22 mg); pale yellow solid; mp 212–217 °C. ¹H NMR (400 MHz, DMSO-*d*₆): δ 12.55 (s, 1H, NH), 12.53 (s, 1H, NH), 9.10 (s, 1H, NH), 8.72 (s, 2H, NH₂⁺), 7.79 (d, *J* = 8.2 Hz, ArH), 7.17 (m, 2H, 2 × ArH), 3.67–3.72 (m, 1H, CH), 3.25–3.35 (m, 2H, CH₂), 3.01–3.11 (m, 2H, CH₂), 2.24 (s, 3H, CH₃), 2.06 (dd, 2H, *J* = 13.2, 2.8 Hz, CH₂), 1.65–1.72 (m, 2H, CH₂) ppm. ¹³C NMR (100 MHz, DMSO-*d*₆): δ 167.9, 157.6, 138.7, 130.9, 128.9, 127.7, 123.4, 120.3, 119.6, 115.3, 112.1, 109.1, 47.3, 42.1, 28.6, 11.2 ppm. HRMS (ESI⁺) *m/z* for C₁₉H₂₁Cl₂N₆O₃ ([M + H]⁺): calculated 451.1047, found 451.1040. HPLC (0–16 min, 30–90% ACN in 0.1% TFA, 16–20 min, 90% ACN in 0.1% TFA, Agilent Extend-C18 column: 3.5 μm, 4.6 × 150 mm): *t*_R 12.587 min (92.82% at 254 nm).

4.2.2.45 *Methyl 4-(methylamino)-3-morpholinobenzoate* (**24**). To a solution of methyl 4-amino-3-morpholinobenzoate (**16e**, 1.50 g, 6.35 mmol) and sodium methoxide (0.48 g, 8.89 mmol) in MeOH (35 mL) paraformaldehyde (0.48 g, 15.9 mmol) was added in one portion. The reaction mixture was heated to 40 °C



and stirred for 15 h. Solid NaBH₄ (0.48 g, 12.7 mmol) was added to the reaction mixture and stirred at 40 °C for 5 h. Additional 2.00 equivalents of solid NaBH₄ (0.48 g, 12.7 mmol) was added and the mixture was stirred at 40 °C for 15 h. The reaction mixture was quenched with 80 mL of saturated aqueous NaHCO₃ and then diluted with 80 mL of ethyl acetate. The phases were separated and the aqueous layer was washed with ethyl acetate (3 × 45 mL). The organic layers were combined, washed with brine (2 × 50 mL) and dried over Na₂SO₄. The solvent was removed under reduced pressure and the crude product was purified by column chromatography using ethyl acetate/hexane (2 : 3) as eluent. The crude product was purified by washing the solid with ethyl acetate to give **24** (413 mg) as a white solid. Yield 26% (413 mg); white solid; mp 125–126 °C. ¹H NMR (400 MHz, DMSO-*d*₆): δ 7.63 (d, *J* = 8.5 Hz, 1H, ArH), 7.47 (s, 1H, ArH), 6.56 (d, *J* = 8.4 Hz, 1H, ArH), 5.86 (q, *J* = 4.8 Hz, 1H, NH), 3.73–3.80 (m, 7H, 2 × CH₂, COOCH₃), 2.81 (d, *J* = 4.9 Hz, 3H, NCH₃), 2.72–2.79 (m, 4H, 2 × CH₂) ppm.

4.2.2.46 Methyl 4-(4,5-dibromo-1H-pyrrole-2-carboxamido)-3-morpholinobenzoate (25a). To a solution of 4,5-dibromo-1H-pyrrole-2-carboxylic acid (0.500 g, 1.86 mmol) in anhydrous dichloromethane (20 mL) oxalyl chloride (0.80 mL, 9.30 mmol) was added and the mixture was stirred for 15 h at room temperature under an argon atmosphere. The solvent was evaporated under reduced pressure, anhydrous dichloromethane (15 mL), **16e** (293 mg, 1.24 mmol) and pyridine (1.5 mL) were added, and the reaction mixture was stirred for 15 h under argon atmosphere at room temperature. The product was filtered off and dried to give **25a** as a pale brown solid. Yield 84% (0.507 g); pale brown solid; mp 270–274 °C. ¹H NMR (400 MHz, DMSO-*d*₆): δ 13.13 (s, 1H, NH), 9.28 (s, 1H, CONH), 8.15 (d, *J* = 8.3 Hz, 1H, ArH), 7.71–7.80 (m, 2H, 2 × ArH), 7.15 (s, 1H, ArH), 3.85 (s, 3H, COOCH₃), 3.75–3.82 (m, 4H, 2 × CH₂), 2.82–2.90 (m, 4H, 2 × CH₂) ppm.

4.2.2.47 4,5-Dibromo-N-(4-(hydrazinecarbonyl)-2-morpholino-phenyl)-1H-pyrrole-2-carboxamide (26a). To a solution of **25a** (485 mg, 1.00 mmol) in MeOH (15 mL) and THF (10 mL) in a pressure tube hydrazine monohydrate (3.40 mL, 69.8 mmol) was added and the reaction mixture was stirred at 120 °C for 15 h. The precipitate was filtered off and dried. The solvent of the mother liquor was evaporated under reduced pressure, MeOH (1 mL) was added to the crude residue, the undissolved solid was filtered off and combined with the precipitate from the first filtration. Yield 54% (263 mg); pale brown solid; mp 258–261 °C. ¹H NMR (400 MHz, DMSO-*d*₆): δ 13.08 (s, 1H, NH), 9.75 (s, 1H, NH), 9.22 (s, 1H, NH), 8.01 (d, *J* = 8.3 Hz, 1H, ArH), 7.68 (s, 1H, ArH), 7.61 (d, *J* = 8.5 Hz, 1H, ArH), 7.13 (s, 1H, ArH), 4.40–4.62 (m, 2H, NH₂), 3.72–3.85 (m, 4H, 2 × CH₂), 2.81–2.90 (m, 4H, 2 × CH₂) ppm.

4.2.2.48 4,5-Dibromo-N-(2-morpholino-4-(5-oxo-4,5-dihydro-1,3,4-oxadiazol-2-yl)phenyl)-1H-pyrrole-2-carboxamide (27a). To a solution of **26a** (248 mg, 0.510 mmol) in 1,4-dioxane (10 mL) and DMF (20 mL) 1,1'-carbonyldiimidazole (336 mg, 2.07 mmol) was added, and the reaction mixture was stirred at 101 °C for 15 h. The solvent was evaporated under reduced pressure and the solid was washed successively with acetonitrile, water, and diethyl ether. The crude product was recrystallized from DMF

and washed with THF to give **27a** as a pale-yellow solid. Yield 51% (133 mg); pale yellow solid; mp >300 °C. ¹H NMR (400 MHz, DMSO-*d*₆): δ 12.52 (br s, 2H, 2 × NH), 9.35 (s, 1H, NH), 8.17 (d, *J* = 8.3 Hz, 1H, ArH), 7.51–7.62 (m, 2H, 2 × ArH), 7.11 (s, 1H, ArH), 3.77–3.86 (m, 4H, 2 × CH₂), 2.83–2.93 (m, 4H, 2 × CH₂) ppm. ¹³C NMR (100 MHz, DMSO-*d*₆): δ 157.4, 154.9, 154.1, 144.2, 135.4, 128.1, 123.0, 122.0, 120.4, 117.5, 114.1, 107.4, 99.1, 66.9, 52.0 ppm. HRMS for C₁₇H₁₆O₄N₅Br₂ [M + H]⁺: calculated 511.95626, found 511.95636. HPLC (0–10 min, 10–90% ACN in 0.1% TFA, 10–11 min, 90% ACN in 0.1% TFA, Waters Acquity UPLC HSS C18 column: 1.8 μm, 2.1 × 50 mm): *t*_R 4.900 min (95.01% at 254, 95.00% at 280 nm).

4.2.2.49 3,4-Dichloro-N,5-dimethyl-N-(2-morpholino-4-(5-oxo-4,5-dihydro-1,3,4-oxadiazol-2-yl)phenyl)-1H-pyrrole-2-carboxamide (27b). To a solution of **26b** (63.9 mg, 0.150 mmol) in 1,4-dioxane (3 mL) and DMF (5 mL) 1,1'-carbonyldiimidazole (73.0 g, 0.450 mmol) was added, and the reaction mixture was stirred at 101 °C for 15 h. The solvent was evaporated under reduced pressure and the solid was washed successively with acetonitrile, water, 1,4-dioxane, and methanol to give **27b** as a pale-yellow solid. Yield 15% (10 mg); pale yellow solid; mp 257–259 °C. ¹H NMR (400 MHz, DMSO-*d*₆): δ 12.52 (s, 1H, NH), 11.89 (s, 1H, NH), 7.49 (s, 2H, 2 × ArH), 7.32 (s, 1H, ArH), 3.68 (s, 4H, 2 × CH₂), 3.37 (s, 3H, NCH₃), 2.71–3.00 (m, 4H, 2 × CH₂), 2.13 (s, 3H, CH₃) ppm. ¹³C NMR (100 MHz, DMSO-*d*₆): δ 162.4, 154.9, 153.8, 148.4, 140.6, 130.6, 127.6, 123.3, 121.4, 120.9, 117.1, 109.0, 107.2, 66.0, 51.5, 37.5, 11.1 ppm. HRMS for C₁₉H₁₈O₄N₅Cl₂ [M – H][–]: calculated 450.07394, found 450.07413. HPLC (0–10 min, 10–90% ACN in 0.1% TFA, 10–11 min, 90% ACN in 0.1% TFA, Waters Acquity UPLC HSS C18 column: 1.8 μm, 2.1 × 50 mm): *t*_R 4.243 min (99.49% at 254, 99.82% at 280 nm).

4.2.2.50 Methyl 4-aminobenzoate (29). The solution of **28** (3.77 g, 20.8 mmol) in a mixture of MeOH and THF (100 mL) was stirred under argon for 15 min. Pd/C (0.913 g) was added, the solution was saturated with hydrogen and the reaction mixture was stirred for 3 h under hydrogen atmosphere. The catalyst was filtered off and the solvent was evaporated to give **29** (2.62 g) as a pale brown solid. Yield 2.62 g (83%); pale brown solid; mp 90–93 °C. ¹H NMR (400 MHz, DMSO-*d*₆): δ 7.64 (d, 2H, *J* = 8.8 Hz, 2 × ArH), 6.57 (d, 2H, *J* = 8.8 Hz, 2 × ArH), 5.85 (s, 2H, NH₂), 3.73 (s, 3H, CH₃) ppm. IR (ATR): ν 3407, 3330, 3226, 3044, 2988, 2944, 2845, 1681, 1635, 1596, 1574, 1513, 1433, 1313, 1283, 1199, 1175, 1117, 1078, 973, 851, 842, 767, 697, 638 cm^{–1}.

4.2.2.51 Methyl 4-(benzylamino)benzoate (30). To the solution of **29** (2.07 g, 13.7 mmol) in CH₂Cl₂ (70 mL) benzaldehyde (3.9 mL, 38.9 mmol) was added and the mixture was stirred at rt for 10 min, Na₂SO₄ (7.76 g, 54.6 mmol) was then added and the mixture was stirred at reflux for 15 h. The mixture was cooled to rt, filtered and the solvent was removed under reduced pressure. The residue was dissolved in MeOH and cooled on an ice bath, NaBH₄ (1.03 g, 27.3 mmol, 2 eq.) was then added in small portions and the reaction mixture was stirred at rt for 15 h. A few mL of water were added and the solvent was removed under reduced pressure. The residue was dissolved in EtOAc (160 mL) and water (80 mL), the phases were separated and the organic phase was washed with brine (2 × 80 mL), dried over Na₂SO₄,



filtered and the solvent evaporated. The crude product was purified with flash column chromatography using dichloromethane as the eluent. The fractions containing the product were combined, the solvent was removed under reduced pressure, the solid residue was triturated with hexane and the undissolved solid was filtered off and dried to give **30** (0.959 g) as a white solid. Yield 0.959 g (29%); white solid; mp 107–109 °C. ¹H NMR (400 MHz, DMSO-d₆): δ 7.66 (d, 2H, *J* = 8.8 Hz, 2 × ArH), 7.29–7.39 (m, 4H, 4 × ArH), 7.20–7.28 (m, 1H, ArH), 7.14 (t, 1H, *J* = 6.0 Hz, NH), 6.61 (d, 2H, *J* = 8.8 Hz, 2 × ArH), 4.34 (d, 2H, *J* = 6.0 Hz, CH₂), 3.72 (s, 3H, CH₃) ppm. IR (ATR): ν 3416, 3027, 3005, 2952, 1686, 1598, 1569, 1529, 1494, 1456, 1432, 1419, 1344, 1312, 1275, 1236, 1186, 1172, 1111, 1064, 1027, 1003, 962, 909, 834, 767, 738, 700, 644, 618 cm⁻¹.

4.2.2.52 Methyl 4-(*N*-benzyl-3,4-dichloro-5-methyl-1*H*-pyrrole-2-carboxamido)benzoate (31). To a suspension of 3,4-dichloro-5-methyl-1*H*-pyrrole-2-carboxylic acid (0.775 g, 4.00 mmol) in anhydrous dichloromethane (55 mL), oxalyl chloride (1.39 mL, 16.0 mmol) was added dropwise and the reaction mixture was stirred at rt under an argon atmosphere for 15 h. The solvent was evaporated under reduced pressure, **30** (0.959 g, 3.97 mmol), anhydrous pyridine (8 mL) and anhydrous CH₂Cl₂ (30 mL) were added and the reaction mixture was stirred at rt under an argon atmosphere overnight. The solvent was removed *in vacuo*, and to the residue EtOAc (55 mL) and water (45 mL) were added. The phases were separated, the organic phase was washed with 1 M HCl (35 mL), saturated NaHCO₃ solution (35 mL) and brine (2 × 20 mL), dried over Na₂SO₄, filtered and the solvent removed *in vacuo*. The crude product was purified with flash column chromatography using EtOAc/hexane (1 : 7 to 1 : 3) as eluent to give **31** (0.925 g) as a brown solid. Yield 0.925 g (56%); brown solid; mp 135–140 °C. ¹H NMR (400 MHz, DMSO-d₆): δ 12.19 (s, 1H, NH), 7.79 (d, *J* = 8.7 Hz, 2H, 2 × ArH), 7.19–7.31 (m, 5H, 5 × ArH), 7.19 (d, *J* = 8.7 Hz, 2H, 2 × ArH), 5.16 (s, 2H, CH₂), 3.79 (s, 3H, COOCH₃), 2.15 (s, 3H, pyrrole-CH₃) ppm. IR (ATR): ν 3265, 3140, 3034, 2950, 1709, 1626, 1600, 1575, 1509, 1483, 1456, 1436, 1412, 1376, 1356, 1314, 1280, 1217, 1180, 1117, 1100, 1067, 1016, 977, 861, 764, 749, 733, 701, 669, 640, 613 cm⁻¹. HRMS for C₂₁H₁₉O₃N₂Cl₂ ([M + H]⁺): calculated 417.07672, found 417.07615.

4.2.2.53 *N*-Benzyl-3,4-dichloro-*N*-(4-(hydrazinecarbonyl)phenyl)-5-methyl-1*H*-pyrrole-2-carboxamide (32). To the solution of **31** (308 mg, 0.738 mmol) in a mixture of MeOH (10 mL) and THF (6 mL) in a high-pressure tube hydrazine hydrate (64%, 1.80 mL, 36.9 mmol) was added. The tube was sealed and the reaction mixture was stirred at 100 °C for 2 d. The tube was cooled to rt, the solvent was removed under reduced pressure and to the residue water was added to obtain a white suspension. The solid was filtered off and dried. The crude product was triturated with acetonitrile and the undissolved solid was filtered off and dried to give the first portion of **32** (145 mg). The filtrate was concentrated under reduced pressure and the residue was purified with flash column chromatography using CH₂Cl₂/MeOH (50 : 1 to 25 : 1) as eluent to obtain the second part of **32** (125 mg). Both fractions of the product were combined. Yield 88% (270 mg); white solid; mp 144–147 °C. ¹H NMR (400 MHz, DMSO-d₆): δ 12.07 (s, 1H, pyrrole-NH), 9.70 (s,

1H, NH), 7.66 (d, *J* = 8.7 Hz, 2H, 2 × ArH), 7.20–7.32 (m, 5H, 5 × ArH), 7.12 (d, 2H, *J* = 8.6 Hz, 2 × ArH), 5.14 (s, 2H, CH₂), 4.47 (s, 2H, NH₂), 2.14 (s, 3H, pyrrole-CH₃) ppm. ¹³C NMR (101 MHz, DMSO-d₆): δ 165.4, 161.9, 145.3, 137.7, 130.6, 128.8, 128.2, 128.1, 128.1, 127.8, 127.6, 126.0, 120.6, 110.2, 107.7, 52.8, 11.3 ppm. IR (ATR): ν 3313, 3236, 2986, 2856, 1655, 1599, 1572, 1540, 1487, 1454, 1426, 1379, 1329, 1286, 1228, 1202, 1103, 1069, 1029, 978, 861, 697, 625 cm⁻¹. HRMS for C₂₀H₁₉O₂N₄Cl₂ ([M + H]⁺): calculated 417.08796, found 417.08769.

4.2.2.54 *N*-Benzyl-3,4-dichloro-5-methyl-*N*-(4-(5-oxo-4,5-dihydro-1,3,4-oxadiazol-2-yl)phenyl)-1*H*-pyrrole-2-carboxamide (33). The solution of **32** (245 mg, 0.590 mmol) and CDI (352 mg, 2.17 mmol) in 1,4-dioxane (10 mL) was stirred for 15 h at 101 °C. The solvent was removed under reduced pressure and the residue was purified with flash column chromatography starting with dichloromethane and proceeding with dichloromethane/methanol (50 : 1) as eluent to give **33** (43 mg) as a pale-yellow solid. Yield 43 mg (17%); pale yellow solid; mp 202–204 °C. ¹H NMR (400 MHz, DMSO-d₆): δ 12.54 (s, 1H, NH), 12.13 (s, 1H, NH), 7.64 (d, *J* = 8.6 Hz, 2H, 2 × ArH), 7.19–7.32 (m, 7H, 7 × ArH), 5.15 (s, 2H, CH₂), 2.14 (s, 3H, pyrrole-CH₃) ppm. ¹³C NMR (101 MHz, DMSO-d₆): δ 161.8, 154.9, 153.7, 145.6, 137.6, 128.9, 128.5, 128.1, 127.7, 126.9, 126.1, 121.4, 120.4, 110.3, 107.8, 52.8, 11.3 ppm. IR (ATR): ν 3202, 2840, 1769, 1597, 1578, 1510, 1481, 1427, 1407, 1381, 1351, 1275, 1209, 1182, 1075, 1031, 955, 925, 841, 750, 696, 667, 645, 613 cm⁻¹. HRMS for C₂₁H₁₇O₃N₄Cl₂ ([M + H]⁺): calculated 443.06722, found 443.06693. HPLC: Waters Acquity UPLC BEH C18 (1.7 μm, 2.1 × 50 mm); mobile phase: 10–90% of acetonitrile in TFA (0.1%) in 10 min; flow rate 0.4 mL min⁻¹; injection volume: 1.75 μL; *t*_R: 5.163 min (95.23% at 254 nm, 95.38% at 280 nm).

4.2.2.55 Methyl 3-(benzyloxy)-4-nitrobenzoate (35). To a stirred suspension of methyl 3-hydroxy-4-nitrobenzoate (**34**, 500 mg, 2.53 mmol) and potassium carbonate (699 mg, 5.06 mmol) in acetonitrile (10 mL) benzyl bromide (0.30 mL, 2.53 mmol) was added and the mixture was stirred at 60 °C for 3 h. The solvent was removed under reduced pressure and to the residue ethyl acetate (20 mL) and water (20 mL) were added, and separated. The organic phase was washed with brine (2 × 20 mL), dried over Na₂SO₄, filtered and the solvent removed under reduced pressure to give **35** (620 mg) as a yellow solid. Yield 85% (620 mg); yellow solid; mp 90–93 °C. ¹H NMR (400 MHz, DMSO-d₆): δ 8.03 (d, *J* = 8.4 Hz, Ar-H), 7.90 (d, *J* = 1.2 Hz, Ar-H), 7.70 (dd, *J* = 8.4, 1.2 Hz, Ar-H), 7.35–7.48 (m, 5H, 5 × Ar-H), 5.41 (s, 2H, CH₂), 3.92 (s, 3H, COOCH₃) ppm. ¹³C NMR (100 MHz, DMSO-d₆): δ 165.2, 150.9, 142.9, 136.1, 134.7, 129.0, 128.7, 127.9, 125.7, 122.0, 116.2, 71.2, 53.4 ppm. IR (ATR): ν cm⁻¹. MS (ESI) *m/z* = 310.1 ([M + Na]⁺).

4.2.2.56 3-(Benzyloxy)-4-nitrobenzohydrazide (36). To a solution of **35** (4.60 g, 16.3 mmol) in MeOH (100 mL) and THF (100 mL), hydrazine monohydrate 80% solution (7.95 mL, 163 mmol) was added. The reaction mixture was stirred at 65 °C for 15 h and the solvent was removed under reduced pressure. The residue was suspended in ethanol and the flask was placed in the refrigerator for 1 h. The precipitate was filtered off, suspended in water, the suspension was sonicated, the solid filtered off and dried to give 1.44 g of **36**. The ethanol from the



mother liquor of the previous filtration was removed and the residue was purified with flash column chromatography (dichloromethane/methanol 10/1) to give 2.09 g of **36**. Yield 77% (3.54 g). $^1\text{H NMR}$ (400 MHz, DMSO-d_6): δ 10.06 (s, 1H, NH), 7.97 (d, $J = 8.4$ Hz, ArH), 7.83 (d, $J = 1.5$ Hz, ArH), 7.54 (dd, $J = 8.4$, 1.6 Hz, ArH), 7.40–7.48 (m, 4H, 4 \times ArH), 7.34–7.38 (m, 1H, ArH), 5.37 (s, 2H, CH_2), 4.63 (s, 2H, NH_2) ppm. MS (ESI) $m/z = 285.9$ ($[\text{M} - \text{H}]^-$).

4.2.2.57 5-(3-(Benzyloxy)-4-nitrophenyl)-1,3,4-oxadiazol-2(3H)-one (37). To a solution of compound **30** (3.50 g, 12.2 mmol) in 1,4-dioxane (175 mL) 1,1'-carbonyldiimidazole (2.96 g, 18.3 mmol) was added and the reaction mixture was stirred at 101 °C for 15 h. The solvent was removed under reduced pressure, the residue was suspended in methanol, the suspension was sonicated, heated and the solid filtered off to give 3.22 g of **37**. The mother liquor was evaporated and purified with flash column chromatography (dichloromethane/methanol 30/1). The purified product was combined with the product after filtration. Yield 98% (3.74 g). $^1\text{H NMR}$ (400 MHz, DMSO-d_6): δ 12.91 (s, 1H, NH), 8.06 (d, $J = 8.4$ Hz, ArH), 7.73 (d, $J = 1.6$ Hz, ArH), 7.54 (dd, $J = 8.4$, 1.6 Hz, ArH), 7.33–7.50 (m, 5H, 5 \times ArH), 5.43 (s, 2H, CH_2) ppm. MS (ESI) $m/z = 312.0$ ($[\text{M} - \text{H}]^-$).

4.2.2.58 5-(4-Amino-3-(benzyloxy)phenyl)-1,3,4-oxadiazol-2(3H)-one (38). Compound **37** (523 mg, 1.67 mmol) was suspended in acetic acid (25 mL), iron (932 mg, 16.7 mmol) was added and the reaction mixture was stirred at rt for 90 min. Water was added and the iron was filtered through Celite. The flask was placed on an ice bath for 1 h upon which the product in the mother liquor crystallized. The product was filtered off and dried. Yield 57% (269 mg). $^1\text{H NMR}$ (400 MHz, DMSO-d_6): δ 12.22 (s, 1H, NH), 7.51 (d, $J = 7.0$ Hz, 2 \times Ar-H), 7.40 (s, 2H, 2 \times Ar-H), 7.33 (s, 1H, ArH), 7.21 (d, $J = 1.8$ Hz, ArH), 7.16 (dd, $J = 8.2$, 1.8 Hz, ArH), 6.73 (d, $J = 8.2$ Hz, ArH), 5.54 (s, 2H, NH_2), 5.18 (s, 2H, CH_2) ppm. MS (ESI) $m/z = 283.9$ ($[\text{M} + \text{H}]^+$).

4.2.2.59 N-(2-(Benzyloxy)-4-(5-oxo-4,5-dihydro-1,3,4-oxadiazol-2-yl)phenyl)-3,4-dichloro-5-methyl-1H-pyrrole-2-carboxamide (39). Synthesised according to General procedure B from 3,4-dichloro-5-methyl-1H-pyrrole-2-carboxylic acid (0.123 g, 0.71 mmol) and **38** (0.150 g, 0.53 mmol). During extraction, the product precipitated and was filtered off. The crude product was successively triturated with acetonitrile, methanol and diethyl ether and the undissolved solid was filtered off. The crude product was purified by crystallization from DMF to give **39** (0.113 g) as a white solid. Yield 47% (0.113 g); white solid; mp 293–295 °C. $^1\text{H NMR}$ (400 MHz, DMSO-d_6): δ 12.59 (s, 1H, NH), 12.42 (s, 1H, NH), 9.20 (s, 1H, NH), 8.56 (d, $J = 8.5$ Hz, ArH), 7.51–7.62 (m, 3H, 3 \times Ar-H), 7.42–7.49 (m, 4H, 4 \times Ar-H), 5.30 (s, 2H, CH_2), 2.20 (s, 3H, CH_3) ppm. HRMS for $\text{C}_{21}\text{H}_{15}\text{O}_4\text{N}_4\text{Cl}_2$: calculated 457.04758, found 457.04776. HPLC (30–90% ACN in 0.1% TFA in 10 min, UPLC): t_R 5.430 min (98.87% at 254 nm, 98.66% at 280 nm).

4.3. Determination of inhibitory activities on DNA gyrase and topoisomerase IV

For the determination of inhibitory activities against DNA gyrase and topoisomerase IV from *E. coli*, *S. aureus*, *A.*

baumannii and *P. aeruginosa*, assay kits from Inspiralis were used according to the previously described procedures.²⁸ Seven different inhibitor concentrations were used to determine the IC_{50} values, which were subsequently calculated using GraphPad Prism 6.0 software. For the most important inhibitors, IC_{50} values were determined based on three independent measurements, and the final results reported are mean values.

4.4. Determination of inhibitory activity on human DNA topoisomerase II α

Assay kits from Inspiralis were used for the experiments according to established protocols.³⁸ Seven different inhibitor concentrations were used to determine the IC_{50} values, which were subsequently calculated using GraphPad Prism 6.0 software. For the most important inhibitors, IC_{50} values were determined based on three independent measurements, and the final results reported are mean values.

4.5. Determination of antibacterial activity

Clinical control strains of *S. aureus* ATCC 29213, *S. aureus* ATCC 43300 (MRSA), *S. aureus* ATCC 700699 (VISA), *E. faecalis* ATCC 29212, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *A. baumannii* ATCC 17978, *K. pneumoniae* ATCC 10031 and *E. spp. cloacae* ATCC 13047 were obtained from the University of Szeged (Hungary). Clinical control strains of *E. faecium* ATCC 35667, *A. baumannii* ATCC 19606, *K. pneumoniae* ATCC 700603, and *K. aerogenes* ATCC 13408 were obtained from Microbiology Inc. (St. Cloud, Minnesota, USA). The antimicrobial assays were performed using the broth microdilution method in 96-well plates. The procedure complied with the Clinical and Laboratory Standards Institute guidelines³⁷ and was performed following established methodologies.³¹ For selected compounds, the MIC values were determined by dose–response experiments (reported values are from at least two independent experiments, each with three replicates per concentration).

4.6. Determination of frequency of resistance

To assess the spontaneous frequency of resistance, approximately 1010 cells from stationary-phase MHBII broth cultures of *S. aureus* ATCC 29213 and *K. pneumoniae* ATCC 10031 (supplied from Microbiology) were plated on antibiotic-containing plates according to a standard protocol.³⁹ Prior to plating, bacteria were cultured overnight in MHBII medium at 37 °C with shaking at 250 rpm, harvested by centrifugation and washed once in an equal volume of PBS to remove all by-products of bacterial metabolism from the media. After washing, the bacterial suspension was concentrated to approximately 1012 CFU mL^{-1} and from this concentrated cell suspension (1 mL) approximately 1010 cells were plated onto each MHBII agarose plate. Agarose was used instead of agar to reduce drug adsorption and improve assay performance. Petri dishes (145 mm) were filled with 40 mL of MHBII agarose medium containing the selective antibiotic at concentrations of 2 \times , 4 \times and 8 \times MIC of the respective antibiotic. Each experiment was performed in at least 3 biological replicates. The plates were incubated at 37 °C for 72 hours. Simultaneously, the



total CFU was determined by plating appropriate dilutions onto antibiotic-free MHBII agar plates. The resistance frequency for each strain was calculated by dividing the number of colonies formed after 72 hours of incubation at 37 °C by the initial viable cell count.

4.7. Molecular modelling

Molecular docking calculations were performed using Schrödinger Release 2023–3 (Schrödinger, LLC, New York, NY, USA, 2023). The crystal structure of *E. coli* DNA gyrase B in complex with a 4,5-dibromopyrrolamide-based inhibitor (PDB entry: 4ZVI)²⁷ was prepared using the Protein Preparation Wizard with the default settings. The receptor grid was calculated for the inhibitor-binding site and the designed compounds were docked using the Glide XP protocol as implemented in Schrödinger Release 2023–3 (Glide, Schrödinger, LLC, New York, NY, USA, 2023). The highest scored docking conformation was used for analysis and presentation.

4.8. Thermodynamic solubility assay

Thermodynamic water solubility was evaluated by preparing saturated solutions of selected compounds in phosphate buffered saline (PBS) with a pH of 7.4 and subsequent quantification by UPLC analysis. First, excess amounts of the compounds (1–2 mg) were weighed into Eppendorf tubes and 500 µL PBS was added to each. The resulting suspensions were shaken at 100 rpm and 25 °C. After 24 hours, the suspensions were centrifuged at 18000 rpm, and the supernatant was diluted fivefold with water/acetonitrile (1/1) to prepare the samples for UPLC analyses. In addition, 10 mM DMSO stocks of the compound were diluted in water/acetonitrile (1/1) to prepare samples for calibration curves (seven concentration points for each compound). Samples were then analysed using the Thermo Scientific Dionex Ultimate 3000 Binary Rapid Separation liquid chromatography system (Thermo Fisher Scientific, USA) equipped with a Waters C18 Acquity UPLC HSS column (1.8 µm, 2.1 × 50 mm). The mobile phase consisted of 0.1% TFA in ultrapure water (A) and acetonitrile (B), with the gradient expressed as % of B: 0–1 min 10%, 1–4 min 10–90%, 4–5 min 90%. The flow rate was set to 0.4 mL min⁻¹ with an injection volume of 10 µL. Data processing was performed using Chromeleon CDS software (Thermo Fisher Scientific, USA), and solubilities were calculated using Excel (Microsoft, USA).

Data availability

The data supporting this article have been included as part of the ESI.† The data include minimum inhibitory concentrations of compounds 6–14, inhibitory activities of 23b against DNA gyrase from *A. baumannii* and *P. aeruginosa*, spontaneous frequency of resistance table, minimum inhibitory concentrations of 23a and 23c adapted lines, synthetic procedures and analytical data, and ¹H and ¹³C NMR spectra of the representative compounds.

Author contributions

All authors conceived the project and designed the experiments. A. E. C., F. F., M. P., P. P., D. B. T., Ž. S., M. D., M. S., J. D., C. D. C., P. É. S., L. D. performed the experiments. All authors analysed the data and contributed to manuscript preparation and revision.

Conflicts of interest

The authors declare no competing financial interest.

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