Design, Synthesis and Evaluation of Novel Tyrosine-Based DNA Gyrase B Inhibitors

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Summary

The discovery and synthesis of new tyrosine-based inhibitors of DNA gyrase B (GyrB), that target its ATPase subunit, is reported. 24 compounds were synthesized and evaluated for activity against DNA gyrase and DNA topoisomerase IV. Antibacterial properties of selected GyrB inhibitors have been demonstrated by their activity against *S. aureus* and *E. faecalis* in the low micromolar range. The most promising compounds, **8a** and **13e,** inhibited *E coli* and *S. aureus* GyrB with IC_{50} values of 40 and 30 μM. The same compound also inhibited the growth of *S. aureus* and *E. faecalis* with minimal inhibitory concentrations (MIC_{90}) of 14 and 28 μg mL^{-1}, respectively.

Keywords: GyrB, antibacterial, topoisomerase, pyrroleamide, tyrosine

Introduction

DNA gyrase and topoisomerase IV (topo IV) are clinically validated targets that catalyze reactions involving the transient break of both strands of DNA in an ATPase-dependent manner.[1,2] Negatively supercoiled DNA is crucial for cell processes including DNA replication, transcription, and recombination, making both enzymes essential for bacterial survival. DNA gyrase is primarily responsible for initiation of DNA replication, elongation and introduction of negative supercoils in front of the replication fork thereby relieving torsional strain during replication. Topo IV is primarily involved in the control of DNA supercoiling and in the decatenation of daughter chromosomes after DNA replication. [3]

DNA gyrase exists as a heterotetrameric complex similar to that of topo IV, being composed of two pairs of GyrA and GyrB subunits homologous to two pairs of ParC and ParE subunits of topo IV, respectively. Fluoroquinolones are currently the only clinically relevant inhibitors of the two enzymes.[4] They bind at the interfaces between the GyrA subunits in gyrase and ParC subunits in topoisomerase IV, stabilizing the covalent enzyme-DNA complexes and leading to the termination of replication cycle and cell death.[5] Despite the clinical and commercial success of fluoroquinolones, their use nowadays is limited due to some serious side effects and, especially, to the emergence of fluoroquinolone-resistant bacteria.[6,7] Surprisingly, ATPase activity of bacterial DNA gyrase that resides in the GyrB/ParE subunit remains so far pharmacologically underexploited, even though it is effectively targeted by aminocoumarin natural products such as clorobiocin and novobiocin (Figure 1), the former being introduced into the clinic in the 1960s.[8,9] In the last ten years, interest in the design of novel small molecular inhibitors of gyrase ATP subunit has intensified, partially because of the elucidation of many crystal structures by X-ray crystallography. A wide variety of new low molecular weight ligands that compete with the binding of ATP to GyrB have been synthesized and shown to exhibit antibacterial activity, however none have yet progressed into the clinic.[10,11] Antibacterial compounds that target the ATP-binding site of GyrB have a great potential because of the latter's essentiality and presence only in bacterial cells. In addition, it has been demonstrated that ATP competitive inhibitors are likely to avoid cross-resistance with fluoroquinolones, making them interesting candidates for the fight against fluoroquinolone resistant pathogens.[5,12]

Figure 1. Representative examples of oroidin and GyrB inhibitors.

Pyrrolecarboxamide-containing compounds such as oroidin[13] (Figure 1) are widely present in sponge-derived marine natural products, many of them exhibiting diverse biological activities, for example modulatory activity on voltage-gated sodium channels[14,15] and biofilm formation.[16,17] In addition, ester isosteres of pyrrolecarboxamide are present in a class of naturally occurring antibiotics clorobiocin and coumermycin A1, produced by *Streptomyces* strains, which inhibit bacterial type IIA topoisomerases. The strategy of incorporating substituted pyrroleamides was applied in the design of low-molecular weight inhibitors of GyrB in fragment-based NMR screening by AstraZeneca.[18] The continuation of their research gave a series of potent pyrroleamide derivatives;AZD5099 progressed to phase 1 human clinical trial but was withdrawn in 2012 due to safety and efficacy reasons (Figure 1).[18-21] Other potent GyrB inhibitors with the 1*H*-pyrrole-2-carboxamide moiety, such as *N*-phenyl-4,5-dibromopyrroleamides, *N*-phenylindolamides[22-24] and 4,5,6,7-tetrahydrobenzo[1,2‑d]thiazoles,[25] were reported by our research group recently. However, none of them displayed any significant antibacterial activity.

We describe here the discovery of two series of compounds, containing the 1*H*-pyrrole-2-carboxamido-tyrosine moiety, which inhibit the ATPase activity of DNA-gyrase B and have antibacterial activity. The general strategy for their design is depicted in Figure 2. We postulated that it might be possible to obtain an inhibitor of gyrase ATPase subunit using only two diversification points at a pyrroleamide fragment and a substituted aromatic amine fragment linked to a central amino acid moiety. Importantly, because of its synthetic simplicity, this amino acid linker would provide access to various structure activity relationship (SAR) and optimization opportunities (Figure 2). According to our model and on the basis of inhibitors from the literature,[12] the pyrroleamide was thought to be involved as both H-bond acceptor and H-bond donor in hydrogen bonds to an active-site Asp73 and a tightly bound water molecule in the active site of the enzyme. This binding pattern mimics the binding of the adenine of ATP as determined by X-ray crystallography.[26]

Next, we introduced various aromatic fragments R^3 that could extend outside the ATP binding pocket and form additional cation-π interactions with the highly conserved Arg136 residue localized at the entry of the ATP-binding pocket and/or could be involved in hydrogen bonding interactions with Arg76 in the proximity of the first arginine residue. This region of the enzyme differs from that in its mammalian homologues as well as from other ATPases of the GHKL protein family. In addition, binding of an inhibitor to this region has an effect that differs importantly from that caused by ATP binding, enabling selectivity makes an important difference compared to ATP-binding and enables selectivity of designed inhibitors towards a myriad of ATP utilizing enzymes. These predicted interactions of both moieties are conserved across known GyrB inhibitors.[27]

Lastly, the bulky and hydrophobic tyrosine was applied as an amino acid linker which could extend into a more open and hydrophobic region of the ATP ribose ring binding pocket and may be involved in hydrogen bonding interactions between the phenolic OH group and one of the a two arginines (Arg76 and Arg136) in the binding site.

Figure 2. Structure and modifications of the designed inhibitors.

Our preliminary docking studies suggested 1*H*-pyrrolecarboxamide to be crucial for binding into the adenine binding pocket. The role of substituents on pyrrole functionality in binding is, on the one hand, to occupy the hydrophobic pocket of the enzyme and, on the other hand, to increase the acidity of the pyrrole NH group and thus strengthen the interaction with Asp73. It was therefore expected that varians of the 1*H*-pyrrole ring substituents would have great influence on the GyrB inhibitory activity. The preparation of the first series of designed inhibitors thus started with optimization of the 1*H*-pyrrolecarboxamide fragment, while keeping the aminophenyl moiety fixed. Furthermore, other heterocycles, such as indole and indazole, were also installed in this position instead of the pyrrole to increase binding energy by occupying a larger space of the adenine binding pocket.

Results and discussion

Preparation of the first series is presented in Scheme 1. Esters **8a-f** were synthesized in four steps, starting from *L*-Tyr (**4**), which was protected with the *tert*-butoxycarbonyl (Boc) group.[28] The *N*-protected derivative **5** was then coupled to methyl 3-aminobenzoate, using DCC and HOBt[29] as activating agents at 0°C, to form **6** which was further deprotected with a solution of HCl gas in THF to form the salt **7**that precipitated from the reaction mixture. Compound **7** then reacted with the corresponding 2-trichloroacetyl- 1*H*-pyrrole derivatives, using Na_2CO_3 as a base in DMF at 40°C, to give **8a**, **8c** and **8d**. However, the reaction with 4,5-dichloro-2-trichloroacetyl-1*H*-pyrrole did not proceed well and a mixture of products that were hard to purify was obtained. The 4,5-dichloro-1*H*-pyrrole-2-carboxylic acid was therefore used, successfully, in the coupling reaction with **7** using EDC, HOBt and NMM in DMF to give **8b**. The same coupling procedure, using indole-2-carboxylic and indazole-3-carboxylic acid, was also successfully used in the preparation of **8e** and **8f**, respectively. Hydrolysis of **8a-f** with 2M LiOH in H_2O/THF led to the corresponding acids **9a-f**.

Six esters **8a-f** and their corresponding carboxylic acids **9a-f** were evaluated on the *E.coli* gyrase inhibition assay. The results are presented in Table 1 as IC_{50} values. Of 12 compounds tested only dibromo and dichloro derivatives **8a**, **9a** and **9b** displayed activity against *E. coli* gyrase with IC_{50} values of 40, 16 and 24 μM, respectively. In general, the dibromopyrrole gave a better inhibition than a dichloropyrrole which is most apparent when active and inactive esters **8a** and **8b** are compared. The difference, however, is not so pronounced when the two acidic counterparts, **9a** and **9b,** are compared, only a small difference in activity,that falls within the limits of experimental error, is observed. The nitro derivatives **8c**,**d** and **9c**,**d**, which were prepared with the aim of improving the interaction with Asp73 by increasing the acidity of the pyrrole NH group, were completely inactive at concentrations below 250 μM. This indicates the importance of hydrophobic interactions between the pyrrole ring and the hydrophobic binding pocket. Furthermore, the results indicate that the bulkier indole and indazole groups were unable to optimally occupy the hydrophobic binding pocket, thus making the interactions of compounds **8e**,**f** and **9e**,**f** with Asp73 less strong. In addition, compounds **9a** and **9b,** with a free carboxylic acid group, were more potent than esters **8a** and **8b**. Both of

these findings are in agreement with those from our previous study.[22] Importantly, measurements of K_d with the surface plasmon resonance assay (SPR) on G24 protein, which is the smallest fragment of the N terminal part of the B subunit still able to bind the ATP molecule,[28] gave results for **8a** (K_d = 46 μM) similar to the IC_{50} value (40 μM) from our screening test, which indicates the specificity of binding into the active site of the enzyme.

Having defined 4,5-dibromopyrrolecarboxamide as a promising moiety which enables strong binding into the adenine binding pocket, variations in aminobenzoic part of molecule **9a** were investigated. Compounds with different aliphatic and aromatic functionalities in place of aminobenzoic acid were prepared in four steps according to Scheme 2. First attempts to prepare **11** from Tyr-OMe hydrochloride (**10**) and 4,5-dibromo-2-trichloroacetyl-1*H*-pyrrole were not successful. Compound **11** was therefore prepared from 4,5-dibromo-1*H*-pyrrole-2-carboxylic acid using EDC and HOBt as activating agents. The ester **11** was further hydrolyzed to carboxylic acid **12** which was readily coupled with various aliphatic amines under mild reaction conditions using EDC and HOBt at 0°C to give esters **13a-d** and **13h**. On the other hand, aromatic amines, being much less nucleophilic, required heating at 60°C to obtain **13e-g** as racemic mixtures in much lower yields than those for their aliphatic counterparts **13a-d** and **13h**. The esters **13a-e** were hydrolyzed with LiOH to obtain carboxylic acids **14a-e**.

Scheme 1. Reagents and conditions: (i) Boc_2O, dioxane/water 1/1, 0°C, 24h, 90%; (ii) methyl 3-aminobenzoate, DCC, HOBt, CH_2Cl_2/DMF 1/1, 0°C to r.t., 24h, 56%; (iii) $HCl_{(g)}$, THF, 0°C, 20 min, 98%; (iv) for **8a**,**8 c**, **8d**: $RCOCCl_3$, Na_2CO_3, DMF, 40°C, 24h, 20-74%; (v) 4,5-dichloro-2-trichloroacetyl-1*H*-pyrrole (for **8b**), indole-2-carboxylic acid (for **8e**), indazole-3-carboxylic acid (for **8f**), EDC, HOBt, NMM, DMF, 0 °C to r.t., 24h, 39–50%; (vi) 2M LiOH, H_2O/THF=1/1, r.t., 2.5h, 95%.

Table 1. Inhibitory activities against *E. coli* and *S. aureus* DNA gyrase.

Table 2. Antibacterial activities.

According to our model (Figure 2), the benzoate moiety should bind in the proximity of both arginines. Different types of interactions between inhibitors and the two arginines (Arg76 and Arg136) have been described.[12] The majority of published compounds have an aromatic ring at this position which forms cation-π interactions with at least one of two arginines and/or a functional group which forms proton donor-acceptor type of interaction with one of arginines. To evaluate the importance of π-stacking interactions in our compounds and to potentially improve the activity, in addition to benzoate, phenol and phenyl groups in position R different aliphatic esters **13a-c**, carboxylic acids **14a-c** and amide **13h** were used. In addition, two dicarboxylic acid esters **13b,13c** were prepared to obtain interactions with both arginines. However, the results of *E. coli* gyrase inhibition assays, presented in Table 1, were disappointing. With the exception of aspartate derivative **13b** and amide **13h** all compounds of this type showed IC_{50} values far above 50 μM, which confirmed the importance of π-stacking interactions for inhibitory activity. Surprisingly, it was demonstrated that the presence of a -COOH group on the aromatic ring has a detrimental effect on the activity and indicated that these compounds do not form proton donor-acceptor interaction of the carboxyl group with one of two arginines. Indeed, when a COOH group was replaced with a OH group (compound **13f**), or was absent (compound **13g**), the activity improved to 13 μM and 1.9 μM, respectively. Even when the -COOH group was positioned next to a -OH group (compounds **13e** and **14e),** the inhibitory activity on *E. coli* gyrase was still lower than those for **13f** and **13g**. These results indicate that the interactions of the phenylcarboxylic moiety are mostly of a cation-π stacking nature. Lastly, the ester **13d** and carboxylic acid **14d** in which a methylene group was inserted between the phenyl ring and the amino group inhibited the *E.coli* enzyme with 0.97 μM and 0.62 μM, respectively.

Compounds which were active against *E. coli* gyrase were then selected for testing against gyrase from *S. aureus*. Only **13f** displayed a significant inhibitory activity, with an IC_{50} value of 28 μM.

Compounds **8a**, **13f**, **14d** and **14e**, which were all active against *E. coli* gyrase, were then tested against *E. coli* and *S. aureus* topoisomerase IV but were found to be inactive at concentrations below 100 μM. A dual targeting intracellular inhibitor of both enzymes GyrB and ParE would be expected to show lower frequency of resistance and larger antibacterial potency.[30] Given the apparent similarity between GyrB and ParE, particularly in the amino acids making crucial interactions with current inhibitors, the selectivity for GyrB may be surprising. On the other hand, our results are in agreement with those of Eakin *et al*[18] who also observed similar selectivity of pyrroleamide inhibitors. It

appears that subtle conformational changes or dynamic differences between the two enzymes could result in the observed differences in activities.

The antimicrobial activities of all active compounds with *E. coli* IC_{50} activity equal to or below 50 μM were evaluated against two Gram-positive (*S. aureus* ATCC 25923, *E. faecalis* ATCC 292122) and three Gram-negative (*E. coli* ATCC 25922, *ΔtolC*, *ΔimpA*) strains. Three promising compounds, **8a**, **13e** and **13f,** were identified, all of which possessed modest antibacterial activity against both *S. aureus* and *E. faecalis* (Table 1). Compounds **8a** and **13e**, which were the most potent, inhibited *S aureus* and *E. faecalis* with MIC_{90} values of 14 and 28 μg mL^{-1}. However, when the activities of both **8a** and **13e** against *S. aureus* are compared to their IC_{50} activities there is no clear correlation between enzymatic and antibacterial activity. Several literature examples[19,21,31-37] indicate, with some notable exceptions,[18,38] that nanomolar inhibition of GyrB is required for antibacterial activities comparable to our results. The discrepancy in correlation between enzymatic and antibacterial activity could indicate that their antibacterial properties are not just a consequence of GyrB inhibitory activity but most probably also of additional mechanism. Furthermore, the higher antibacterial activities of ester **8a** and **13e** compared to their acidic counterparts **9a** and **14e** could be attributed to insufficient penetration of compounds **9a** and **14e** through the bacterial cell wall due to their acidic nature.[39] These results are in accordance with the recently reported correlation on *S. pneumoniae* between activity (pMIC) of compounds and their pKa.[40] However, none of the compounds evaluated possessed any inhibitory activity against wild-type *E.coli* at concentrations below 250 μM. Additional evaluation against *ΔtolC* and *ΔimpA E. coli* strains suggests that some of them (**13d**, **13e** and **13f)** are efflux pump substrates and **13e** and **13f** do not possess the physicochemical properties necessary for cell wall penetration in *E. coli*.

Scheme 2. Reagents and conditions: (i)4,5-dibromopyrrole-2-carboxylic acid, EDC, HOBt, NMM, DMF, 0 °C to r.t., 16h, 79%; (ii) LiOH, H_2O/THF = 1/1, r.t., 1.5h, 95%; (iii) for **13a-d**, **13h**: R^1NH_2, EDC, HOBt, NMM, DMF, 0 °C to r.t., 16h, 45-75%, (iv) for **13e-g**: Ar-NH_2, EDC, HOBt, DMAP, NMM, DMF, 0 °C, 20 min to 60 °C, 16h, 14-53%; (v) 2M LiOH, H_2O/THF, 25 °C, 24h, 95%.

Conclusion

In conclusion, the design, synthesis and biological evaluation of a new class of tyrosine-based diamides as potential DNA gyrase B inhibitors is reported. The compounds displayed sub-micromolar to low micromolar IC_{50} values against *E. coli* gyrase. In addition, three compounds with antibacterial activity were discovered. The most promising compounds, **8a** and **13e**, inhibited the growth of *S. aureus* and *E. faecalis* with minimal inhibitory concentrations (MIC_{90}) of 14 and 28 μg mL^{-1}. All three

compounds still have antibacterial activities much lower than that of ciprofloxacin, but provide a good rationale for further optimization of this class of compounds towards more potent DNA gyrase and topoisomerase IV inhibitors with even better antibacterial activity.

Chemistry

General

Chemicals were obtained from Acros, Aldrich Chemical Co., and Fluka and used without further purification. Analytical TLC was performed on silica gel Merck 60 F_{254} plates (0.25 mm), using visualization with UV light and ninhydrin. Column chromatography was carried out on silica gel 60 (particle size 240–400 mesh). HPLC analyses were performed on an Agilent Technologies 1100 instrument with a G1365B UV-VIS detector, a G1316A thermostat and a G1313A autosampler using a Phenomenex Luna 5 µm C18column (4.6 × 150 mm or 4.6 × 250 mm) and flow rate of 1.0 mL/min. The eluent consisted of trifluoroacetic acid (0.1% in water) as solvent A and methanol or acetonitrile as solvent B. Melting points were determined on a Reichert hot stage microscope and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded at 400 and 100 MHz, respectively, on a Bruker AVANCE III 400 spectrometer in DMSO-d_6, $CDCl_3$ or MeOH-d_4 solution, with TMS as the internal standard. IR spectra were recorded on a Perkin-Elmer Spectrum BX FT-IR spectrometer or Thermo Nicolet Nexus 470 ESP FT-IR spectrometer. Mass spectra were obtained using a VGAnalytical Autospec Q mass spectrometer. Optical rotations were measured on a Perkin-Elmer 241 MC polarimeter. The reported values for specific rotation are average values of 10 successive measurements using an integration time of 5 s. The purity of the tested compounds was established by HPLC using Agilent EclipsePlus C18 column 4.6 mm x 150 mm, flow rate: 1.0 mL/min; injection volume 5 µl: Optical purity of the tested compounds was established by HPLC using. Kromasil 3-Cellucoat column; 150x4.6 mm, flow rate: 1.0 mL/min; injection volume 5 µl.

Some NMR spectra as well as the InChI codes of the investigated compounds are provided as Supporting information.

*Boc-L-tyrosine (**5**)*[41]

L-tyrosine (8.80 g, 48.6 mmol) and NaOH (3.89 g, 97.2 mmol) were dissolved in a mixture of 1,4-dioxane (100 mL) and water (100 mL). The solution was cooled to 0°C prior to the addition of Boc_2O (10.64 g, 48.6 mmol). After 48 h concentrated hydrochloric acid was added until pH = 1 was reached, then the resulting solution was extracted with EtOAc (3x 100 mL). Combined organic fractions were washed with brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure to yield white crystals. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 1.40 (s, 9H, Boc-CH_3), 2.98 (dd, J = 14.0, 6.0 Hz, 1H, benzyl-

CH_2), 3.02 (dd, *J* = 14.0, 6.0 Hz, 1H, benzyl-CH_2), 4.53 (ddd, *J* = 8.4, 6.0, 6.0 Hz, 1H, Tyr-α-CH, 5.10 (d, *J* = 8.4 Hz, 1H, NH), 6.69 (d, *J* = 8.2 Hz, 2H, ArH), 6.95 (d, *J* = 8.2 Hz, ArH), 7.6 – 9.2 (bs, 2H, OH and COOH) ppm.

*(S)-methyl 3-(2-((tert-butoxycarbonyl)amino)-3-(4-hydroxyphenyl)propanamido) benzoate (**6**)*

Boc-*L*-tyrosine (5.03 g, 17.9 mmol), methyl 3-aminobenzoate[42] (5.39 g, 35.8 mmol) and 1-hydroxybenzotriazole were dissolved in a mixture of 15 ml of dichloromethane and 15 ml of DMF and cooled to 0°C prior to the addition of *N,N'*-dicyclohexylcarbodiimide (5.54 g, 26.9 mmol). The resulting solution was allowed to warm to room temperature during 24 h. The solvents were evaporated under reduced pressure and the residue dissolved in EtOAc (100 mL). The solution was washed with 10% water solution of citric acid (2x50 mL), saturated water solution of $NaHCO_3$ (2x50 mL) and brine (50 mL). Organic phase was dried over Na_2SO_4, filtered and concentrated under reduced pressure. Crude product was purified by flash column chromatography (n-hexane : EtOAc = 1 : 1) to yield pale pink powder. Yield 56%, mp 187°C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.33 (s, 9H, Boc-CH_3), 2.73 (dd, *J* = 13.8, 9.7 Hz, 1H, benzyl-CH_2), 2.87 (dd, *J* = 13.8, 4.8 Hz, 1H, benzyl-CH_2), 3.86 (s, 3H, Me-ester-CH_3), 4.22 (ddd, *J* = 9.7, 7.9, 4.8 Hz, 1H, Tyr-α-CH), 6.65 (d, *J* = 8.5, 2H, ArH), 7.09 (d, *J* =7.9 Hz, 1H, Boc-NH), 7.10 (d, *J* =8.5, 2H, ArH), 7.46 (t, *J* = 7.9, 1H, ArH), 7.64 (dt, *J* = 7.9, 1.7 Hz, 1H, ArH), 7.85 (dd, *J* = 7.9, 1.7 Hz, 1H, ArH), 8.25 (dd, 1.7, 1.7 Hz, 1H, ArH), 9.19 (s, 1H, phenol-OH), 10.23 (s, 1H, Ar-amide-NH) ppm. ^{13}CNMR (DMSO-d_6, 100 MHz): δ 28.19, 36.58, 52.24, 57.07, 78.12, 114.85, 119.72, 123.70, 123.85, 127.87, 129.28, 130.08, 130.16, 139.36, 155.43, 155.83, 166.08, 171.36 ppm. MS (ESI): *m/z* 413.2 ([MH]$^-$, 100). HRMS (ESI) for $C_{22}H_{25}N_2O_6$: calculated, 413.1718; found, 413.1721. $[\alpha]_D^{20}$ = -49.1 (*c* 1.00, MeOH). RP-HPLC purity: 98.5%.

*(S)-3-(4-hydroxyphenyl)-1-((3-(methoxycarbonyl)phenyl)amino)-1-oxopropan-2-aminium chloride (**7**)*

Compound **6** (1.63 g, 3.92 mmol) was dissolved in THF (40 mL) and bubbled for 20 min with anhydrous HCl gas on ice bath. After the addition of diethylether (100 mL) white precipitate was formed, which was filtered, dried in vacuum for 1 h and stored in desiccator. Yield 98%, mp: 140 – 145°C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 3.01 (dd, *J* = 13.8, 7.3 Hz, 1H, benzyl-CH_2), 3.12 (dd, *J* = 13.8, 6.5 Hz, 1H, benzyl-CH_2), 3.86 (s, 3H, Me-ester-CH_3), 4.2 (bs, 1H, Tyr-α-CH), 6.70 (d, *J* = 8.5, 2H, ArH), 7.10 (d, *J* = 8.5, 2H, ArH), 7.50 (dd, *J* = 8.1, 7.8 Hz, 1H, ArH), 7.69 (ddd, *J* = 7.8, 1.6, 1.0 Hz, 1H, ArH), 7.85 (ddd, *J* = 8.1, 2.1, 1.0 Hz, 1H, ArH), 8.26 (dd, *J* = 2.1, 1.8 Hz, 1H, ArH), 8.4 (bs, 3H, NH_3), 9.41 (s, 1H), 11.14 (s, 1H) ppm. ^{13}C NMR (DMSO-d_6, 100 MHz): δ 36.19, 52.32, 54.56, 115.36, 120.00, 124.02, 124.66, 129.48, 130.22, 130.52, 138.46, 138.51, 156.58, 165.94, 167.24 ppm. MS (ESI): *m/z* 315.1 ([MH]$^+$, 100). HRMS (ESI) for $C_{17}H_{19}N_2O_4$: calculated, 315.1339; found, 315.1339. $[\alpha]_D^{20}$ = -35.6 (*c* 0.60, MeOH). RP-HPLC purity 97.4%

*General procedure for the preparation of methyl benzoates **8a, c** and **d.***

Compound **7** (1 eq.) and Na_2CO_3 (1.1 eq.) were dissolved in DMF (1mL/mmol) under Ar atmosphere and heated to 40°C. A solution of 2-trichloroacetyl-1*H*-pyrrole derivative in DMF (1mL/mmol) was subsequently added dropwise during 30 min and stirred for 18h at 45°C. The reaction mixture was then concentrated, dissolved in EtOAc and washed with 10% solution of citric acid, 1 M NaOH and brine successively. Organic phase was dried over Na_2SO_4, filtered and concentrated.

*(S)-methyl 3-(2-(4,5-dibromo-1H-pyrrole-2-carboxamido)-3-(4-hydroxyphenyl)propanamido)benzoate (**8a**)*

Compound **8a** was prepared from **7** and 2,2,2-trichloro-1-(4,5-dibromo-1H-pyrrol-2-yl)ethan-1-one and recrystallized from *n*-hexane/EtOAc mixture to give the title compound as brownish crystals. Yield 20%. mp 126 – 132°C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.88 (dd, J = 13.8, 10.0 Hz, 1H, benzyl-CH_2), 3.01 (dd, J = 13.8, 4.9 Hz, 1H, benzyl-CH_2), 3.85 (s, 3H, Me-ester-CH_3), 4.68 (ddd, J = 10.0, 8.1, 4.9 Hz, 1H, Tyr-α-CH), 6.64 (d, J = 8.5 Hz, 2H, phenol-2,4-H), 7.07 (d, J = 2.7 Hz, 1H, ArH), 7.14 (d, J = 8.5 Hz, 2H, ArH), 7.47 (dd, J = 8.1, 7.9 Hz, 1H, ArH), 7.65 (ddd, J = 7.9, 1.6, 1.0 Hz, 1H, ArH), 7.86 (ddd, J = 8.1, 2.1, 1.0 Hz, 1H, ArH), 8.26 (dd, J = 2.1, 1.6 Hz, 1H, ArH), 8.37 (d, J = 8.1 Hz, 1H, amide-NH), 9.19 (s, 1H, Ar-amide-NH), 10.39 (s, 1H, phenol-OH), 12.64 (d, J = 2.7, 1H, pyrrole-NH) ppm. ^{13}C NMR (DMSO-d_6, 100 MHz): δ 37.23, 41.89, 52.11, 100.23, 102.44, 105.24, 114.93, 115.04, 124.99, 126.94, 127.16, 127.33, 128.11, 129.16, 130.27, 144.77, 156.03, 157.32, 166.10, 170.70 ppm. MS (ESI): *m/z* 562.0 ([MH]$^-$, 51), 563.9 ([MH+2]$^-$,100), 566.0 ([MH-2]$^-$, 49). HRMS (ESI) for $C_{22}H_{18}N_3O_5Br_2$: calculated, 561.9619; found, 561.9599. $[\alpha]_D^{20}$ = -93.1 (c 0.36, MeOH). RP-HPLC purity: 96%. ee = 92%.

*(**S**)-methyl 3-(3-(4-hydroxyphenyl)-2-(5-nitro-1H-pyrrole-2-carboxamido)propanamido)benzoate (**8c**)*

Compound **8c** was prepared from **7** and 2,2,2-trichloro-1-(5-nitro-1H-pyrrol-2-yl)ethan-1-one to yield orange powder. Yield 60%, mp 124°C, ^{1}H NMR (MeOH-d_4, 400 MHz): δ 3.04 (dd, J = 13.8, 8.2 Hz, 1H, benzyl-CH_2), 3.17 (dd, J = 13.8, 7.1 Hz, 1H, benzyl-CH_2), 3.90 (Me-ester-CH_3), 4.83 (dd, J = 8.2, 7.1 Hz, 1H, Tyr-α-CH), 6.68 (d, J = 8.5 Hz, 2H, ArH), 6.90 (d J = 4.3 Hz, 1H, ArCH), 7.07 (d, J = 4.3 Hz, 1H, ArCH), 7.12 (d, J = 8.5 Hz, 2H, ArH), 7.41 (dd, J = 8.2, 7.7 Hz, 1H, ArH), 7.73 (ddd, J = 8.2, 2.3, 1.1 Hz, 1H, ArH), 7.75 (ddd, J = 7.7, 1.7, 1.1 Hz, 1H, ArH), 8.19 (dd, J = 2.3, 1.7 Hz, 1H, ArH) ppm. ^{13}C-NMR (DMSO-d_6, 100MHz): δ 36.74, 52.22, 55.66, 110. 92, 113.15, 114.83, 114.98, 119.89, 123.83, 124.09, 127.41, 129.30, 130.07, 138.61, 139.10, 155.89, 166.01, 170.36 ppm. IR (ATR): ν 3281, 3143, 2952, 1702, 1637, 1556, 1514, 1443, 1290, 1236, 1204, 1106, 808, 754, 681 cm^{-1}. MS (ESI): *m/z* 451.1 ([MH]$^-$, 100). HRMS (ESI) for $C_{22}H_{19}N_4O_7$: calculated, 451.1259; found, 451.1250. $[\alpha]_D^{20}$ = -22.5 (c 0.42, MeOH). RP-HPLC purity: 96%. ee = 74%

*(**S**)-methyl 3-(3-(4-hydroxyphenyl)-2-(4-nitro-1H-pyrrole-2-carboxamido) propanamido)benzoate (**8d**)*

Compound **8d** was prepared from **7** and 2,2,2-trichloro-1-(4-nitro-1H-pyrrol-2-yl)ethan-1-one to yield orange powder. Yield 40%. mp 140°C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.91 (dd, J = 13.8, 10.2 Hz, 1H, benzyl-CH_2), 3.04 (dd, 13.8, 5.0 Hz, 1H, benzyl-CH_2), 3.85 (s, 3H, Me-ester-CH_3), 4.73 (ddd, J = 10.2, 8.1, 5.0, 1H, Tyr-α-CH), 6.65 (d, J = 8.4 Hz, 2H, ArH), 7.17 (d, J = 8.4 Hz, 2H, ArH), 7.47 (dd, J = 8.0, 8.0 Hz, 1H, ArH), 7.62 (d, J = 1.6 Hz, 1H, ArCH), 7.65 (ddd, J = 8.0, 1.9, 1.3 Hz, 1H, ArH), 7.88 (ddd, J = 8.0, 1.9, 1.2 Hz, 1H, ArH), 7.91 (d, J = 1.6 Hz, 1H, ArCH), 8.27 (dd, J = 1.9, 1.9 Hz, 1H, ArH), 8.74 (d, J = 8.1, 1H, amide-NH), 9.2 (bs, 1H, OH), 10.43 (s, 1H, Ar-amide-NH), 12.4 (bs, 1H, pyrrole-NH) ppm. ^{13}C NMR (DMSO-d_6, 100 MHz): δ 36.45, 52.25, 55.81, 106.07, 114.95, 119.89, 122.88, 123.84, 124.04, 126.49, 127.84, 129.31, 130.12, 136.33, 136.33, 139.23, 155.87, 159.44, 166.06, 170.69, 172.16 ppm. MS (ESI): *m/z* 451.1 ([MH]$^-$, 100). HRMS (ESI) for $C_{22}H_{19}N_4O_7$: calculated, 451.1259; found, 451.1249. $[\alpha]_D^{20}$ = -42.1 (*c* 0.50, MeOH). RP-HPLC purity: 96%. ee = 98%.

*General procedure for the preparation of benzoates **8b**,**e**,**f**, tyrosinate **11** and 4,5-dibromo-1H-pyrrolecarboxamides **13a-d**.*

1*H*-pyrrole-2-carboxilic acid derivative (1 eq.) or **12** (1 eq.), compound **7** (1 eq.) or **10** or corresponding amine, 1-hydroxybenzotriazole (1 eq) and *N*-methylmorpholine (2 eq.) were dissolved in DMF (1mL/mmol) and cooled to 0°C prior to the addition of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (1 eq.). The resulting solution was allowed to warm to room temperature during 24 h. The reaction mixture was then concentrated, dissolved in EtOAc and washed with 10% water solution of citric acid, 1M NaOH and brine successively. Organic phase was dried over Na_2SO_4, filtered and concentrated.

*(S)-methyl 3-(2-(4,5-dichloro-1H-pyrrole-2-carboxamido)-3-(4-hydroxyphenyl)propanamido) benzoate (**8b**)*

Compound **8b** was prepared from **7** and 2,2,2-trichloro-1-(4,5-dichloro-1H-pyrrol-2-yl)ethan-1-one. Crude product was purified by flash column chromatography, mobile phase: *n*-hexane :EtOAc = 2 : 1 to 1 : 1. Brownish crystals. Yield 45%. mp: 125–127°C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.88 (dd, J = 13.9, 9.9 Hz, 1H, benzyl-CH_2), 3.01 (d, J = 13.9, 1H, benzyl-CH_2), 3.85 (s, 3H, Me-ester-CH_3), 4.7 (m, 1H, Tyr-α-CH), 6.64 (d, J = 7.8 Hz, 2H, ArH), 7.04 (d, J = 1.3 Hz, 1H, ArCH), 7.14 (d, J = 7.8 Hz, 2H, ArH), 7.47 (t, J = 8.1 Hz, 1H ArH), 7.65 (d, J = 8.1 Hz, 1H, ArH), 7.86 (d, J = 8.1 Hz, 1H, ArH), 8.26 (s, 1H, ArH), 8.40 (d, J = 7.5 Hz, 1H, amide-NH), 9.19 (s, 1H, Ar-amide-NH), 10.40 (s, 1H, phenol-OH), 12.68 (d, J = 1.3, 1H, pyrrole-NH) ppm. IR (ATR): ν 3273, 2952, 1705, 1682, 1621, 1514, 1434, 1299, 1239, 1201, 1173, 752 cm^{-1}. MS (ESI): *m/z* = 476.1 ([MH]$^+$, 100), 478.1 ([MH+2]$^+$,

64), 480.1 ([MH+4]$^+$, 10). HRMS (ESI) for $C_{22}H_{20}N_3O_5Cl_2$: calculated, 476.0775; found, 476.0779. $[\alpha]_D^{20}$ = +32.3 (*c* 1.00, MeOH). RP-HPLC purity: 97%. ee = 98%.

*(S)-methyl 3-(3-(4-hydroxyphenyl)-2-(1H-indole-2-carboxamido)propanamido)benzoate (**8e**)*

Compound **8e** was prepared from **7** and 1*H*-indole-2-carboxylic acid. The crude product was recrystallized from EtOAc/n-hexane mixture to afford pale yellow crystals. Yield: 39%. mp: 127 – 130°C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.99 (dd, *J* = 13.7, 9.8 Hz, 1H, benzyl-CH_2), 3.07 (dd, *J* = 13.7, 5.1 Hz, 1H, benzyl-CH_2), 3.87 (s, 3H, Me-ester-CH_3), 4.79 (ddd, *J* = 9.8, 7.9, 5.1 Hz, 1H, Tyr-α-CH), 6.65 (d, *J* = 8.5 Hz, 2H, ArH), 7.04 (ddd, *J* = 8.0, 6.9, 0.8 Hz, 1H, ArH), 7.18 (ddd, *J* = 8.3, 6.9, 1.1 Hz, 1H, ArH), 7.20 (d, *J* = 8.5 Hz, 2H, ArH), 7.27 (dd, *J* = 2.2, 0.9 Hz, 1H, ArH), 7.41 (dd, *J* = 8.3, 0.8 Hz, 1H, ArH), 7.49 (dd, *J* = 8.0, 8.0 Hz, 1H, ArH), 7.65 (dd, *J* = 8.0, 0.9 Hz, 1H, ArH), 7.67 (ddd, *J* = 8.0, 1.6, 1.2 Hz, 1H, ArH), 7.90 (ddd, *J* = 8.0, 2.2, 1.2 Hz, 1H, ArH), 8.29 (dd, *J* = 2.2, 1.6 Hz, 1H, ArH), 8.72 (d, *J* = 7.9 Hz, 1H, amide-NH), 9.18 (s, 1H, Ar-amide-NH), 10.46 (s, 1H, phenol-OH), 11.53 (d, *J* = 2.2 Hz, 1H, indole-NH) ppm. IR (ATR): ν 3283, 2951, 1705, 1624, 1593, 1540, 1514, 1488, 1439, 1300, 1231, 1202, 1173, 1107, 812, 746 cm^{-1}. MS (ESI): *m/z* 456.1([MH]$^-$, 100). HRMS (ESI) for $C_{26}H_{22}N_3O_5$: calculated, 456.1565; found, 456.1555. $[\alpha]_D^{20}$ = +34.2 (*c* 1.00, MeOH). RP-HPLC purity: 96%. ee = 42%

*(S)-methyl 3-(3-(4-hydroxyphenyl)-2-(1H-indazole-3-carboxamido)propanamido) benzoate (**8f**)*

Compound **8f** was prepared from **7** and 1*H*-indazole-3-carboxylic acid to yield white crystals. mp: 123 – 124°C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 3.08 (dd, *J* = 15.4, 6.3, 1H, benzil-CH_2), 3.11 (dd, *J* = 15.4, 6.3, 1H, benzyl-CH_2), 3.85 (s, 3H, Me-ester-CH_3), 4.86 (td, *J* = 8.0, 6.3 Hz, 1H, Tyr-α-CH), 6.64 (d, *J* = 8.5 Hz, 2H, ArH), 7.11 (d, *J* = 8.5 Hz, 2H, ArH), 7.23 (ddd, *J* = 8.2, 7.0, 0.7 Hz, 1H, ArH), 7.41 (ddd, *J* = 8.4, 7.0, 1.0 Hz, 1H, ArH), 7.48 (dd, *J* = 7.9, 7.9 Hz, 1H, ArH), 7.62 (ddd, *J* = 8.4, 1.0, 1.0 Hz), 1H, ArH), 7.66 (ddd, *J* = 7.9, 1.8, 1.0 Hz, 1H, ArH), 7.87 (ddd, *J* = 7.9, 2.2, 1.0 Hz, 1H, ArH), 8.11 (dd, *J* = 8.2, 0.7 Hz, 1H, ArH), 8.20 (d, *J* = 8.0 Hz, 1H, amide-NH), 8.27 (dd, *J* = 2.2, 1.8 Hz, 1H, ArH), 9.21 (s, 1H, Ar-amide-NH), 10.44 (s, 1H, phenol-OH), 13.66 (s, 1H, indazole-NH). IR (ATR): ν 3267, 1702, 1639, 1613, 1534, 1514, 1439, 1292, 1234, 1170, 750 cm^{-1}. MS (ESI): *m/z* 459.2, ([MH]$^+$, 100). HRMS (ESI) for $C_{25}H_{23}N_4O_5$: calculated, 459.1663; found, 459.1664. $[\alpha]_D^{20}$ = -111.1 (*c* 0.42, MeOH). RP-HPLC purity: 97%.

*Methyl (4,5-dibromo-1H-pyrrole-2-carbonyl)-L-tyrosinate (**11**)*

Compound **11**[43][2] was prepared from **10**[41] and 4,5-dibromo-1*H*-pyrrole-2-carboxylic acid. The crude material was passed through a short pad of silica gel to obtain white solid. Yield 79 %. mp 94 – 100°C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.88 (dd, *J* = 13.9, 9.9 Hz, 1H, benzyl-CH_2), 2.99 (dd, *J* =

13.9, 5.3 Hz, 1H, benzyl-CH_2), 3.61 (s, 3H, CH3), 4.51 (ddd, *J* = 9.9, 8.0, 5.4 Hz, 1H, Tyr-α-CH), 6.64 (d, *J* = 8.5 Hz, 2H, ArH), 7.01 (d, *J* = 2.4 Hz, 1H, ArH), 7.03 (d, *J* = 8.5, 2H, ArH), 8.43 (d, *J* = 8.0 Hz, 1H, amide-NH), 9.21 (s, 1H, OH), 12.68 (d, *J* = 2.4 Hz, 1H, pyrrole-NH) ppm. ^{13}C-NMR (DMSO-d_6, 100 MHz): δ ^{13}C NMR (DMSO-d_6, 100 MHz): δ 35.73, 53.98, 97.87, 104.92, 113.20, 115.00, 127.63, 127.95, 129.98, 155.87, 158.75, 173.27 ppm. IR (ATR): ν 3272, 2952, 1724, 1316, 1557, 1512, 1435, 1209, 1173 cm^{-1}MS (ESI): *m/z* 546.9 ([MH+2]$^+$, 100), 444.9 ([MH]$^+$, 40.4), 448.9 ([MH+4]$^+$, 48.6). HRMS (ESI) for $C_{15}H_{15}N_2O_4Br_2$: calculated, 444.9393; found, 444.9396. $[\alpha]_D^{20}$ = -49.1 (*c* 1.00, MeOH). RP-HPLC purity: 95%.

*Methyl 1-((4,5-dibromo-1H-pyrrole-2-carbonyl)-L-tyrosyl)piperidine-4-carboxylate (**13a**)*

Compound **13b** was prepared from **12** and methyl piperidine-4-carboxylate. Mobile phase for flash column chromatography: DCM : MeOH = 40 : 1. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 0.95 – 1.1 (m, 1H), 1.20 – 1.50 (m, 2H), 1.65 – 1.85 (m, 2H), 2.65 – 2.96 (m, 4H), 3.02 – 3.13 (m, 1H), 3.58 (s, 3H, CH_3), 3.60 (s, 3H, CH_3'), 3.80 – 3.95 (m, 1H), 4.10 – 4.24 (m, 1H), 4.89 – 4.99 (m, 1H, Tyr-α-CH, Tyr-α-CH'), 6.61 (d, *J* = 8.4 Hz, 2H, ArOH, ArOH'), 7.03 (d, *J* = 8.4 Hz, 2H, ArH), 7.04 (d, *J* = 8.4 Hz, 2H, ArH'), 7.06 (d, *J* = 1.9 Hz, 1H, ArH), 7.07 (d, *J* = 1.9 Hz, 1H, ArH'), 8.40 (d, *J* = 8.1 Hz, 1H, amide-NH), 8.43 (d, *J* = 8.1 Hz, 1H, amide-NH'), 9.16 (s, 1H, phenol-OH), 9.17 (phenol-OH'), 12.63 (d, *J* = 1.9 Hz, 1H, pyrrole-NH), 12.66 (d, *J* = 1.9 Hz, 1H, pyrrole-NH') ppm. IR (ATR): ν 3154, 2951, 1724, 1610, 1544. 1513, 1448, 1378, 1206, 1172, 1037 cm^{-1}. MS (ESI): *m/z* 556.0 ([MH+2]$^-$, 100), 554.0 ([MH]$^-$, 51.4), 558.0 ([MH+4]$^-$, 48.6). HRMS (ESI) for $C_{21}H_{22}N_3O_5Br_2$: calculated, 553.9932; found, 553.9920. $[\alpha]_D^{20}$ = -10.1 (*c* 1.00, MeOH). RP-HPLC purity: 98%.

*Dimethyl (4,5-dibromo-1H-pyrrole-2-carbonyl)-L-tyrosyl-L-aspartate (**13b**)*

Compound **13c** was prepared from **12** and dimethyl *L*-aspartate. Mobile phase for flash column chromatography: DCM : MeOH = 40 : 1 to 20 : 1. Yield: 75 %, off-white solid. mp: 98 – 101°C. ^{1}H-NMR (DMSO-d_6, 400 MHz): δ 2.72 (dd, *J* = 16.4, 7.1 Hz, 1H, Asp-β-CH_2), 2.73 (dd, *J* = 13.8, 10.7 Hz, 1H, benzyl-CH_2), 2.83 (dd, *J* = 16.4, 6.2 Hz, 1H Asp-β-CH_2), 2.95 (dd, *J* = 13.8, 3.8 Hz, 1H, benzyl-CH_2), 3.60 (s, 3H, CH_3), 3.61 (s, 3H, CH_3), 4.57 (ddd, *J* = 10.7, 8.6, 3.8 Hz, 1H, Tyr-α-CH), 4.66 (ddd, *J* = 7.9, 6.2, 7.1 Hz, 1H, Asp-α-CH), 6.61 (d, *J* = 8.5 Hz, 2H, ArH), 7.01 (d, *J* = 2.7 Hz, 1H, ArH), 7.08 (d, *J* = 8.5 Hz, 2H, ArH), 8.20 (d, *J* = 8.6 Hz, 1H, Asp-NH), 8.58 (d, *J* = 7.9 Hz, 1H, Tyr-NH), 9.15 (s, 1H, OH), 12.62 (d, *J* = 2.7 Hz, 1H, pyrrole-NH) ppm. IR (ATR): ν 3279, 2952, 1732, 1629, 1543, 1504, 1436, 1372, 1217, 1172 cm^{-1}. MS (ESI): *m/z* 574.0 ([MH+2]$^-$, 100), 572.0 ([MH]$^-$, 51.4), 576.0 ([MH+4]$^-$, 48.6). HRMS for $C_{20}H_{20}N_3O_7Br_2$: calculated, 571.9673; found, 571.9662.

*Dimethyl (4,5-dibromo-1H-pyrrole-2-carbonyl)-L-tyrosyl-L-glutamate (**13c**)*

Compound **13d** was prepared from **12** and dimethyl *L*-aspartate. Mobile phase for flash column chromatography: DCM : MeOH = 20 : 1. Yield: 80 %, off-white solid. mp: 96 – 98°C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.82 (ddt, *J* = 13.7, 9.2, 7.5 Hz, 1H, Glu-β-CH_2), 1.98 (dtd, *J* = 13.7, 7.5, 5.5 Hz, 1H, Glu-β-CH_2), 2.30 (t, *J* = 7.5 Hz, 2H, Glu-γ-CH_2), 2.75 (dd, *J* = 13.8, 10.4 Hz, 1H, benzyl-CH_2), 2.91 (dd, *J* = 13.8, 4.5 Hz, 1H, benzyl-CH_2), 3.58 (s, 3H, CH_3), 3.62 (s, 3H, CH_3), 4.30 (ddd, *J* = 9.2, 7.8, 5.5 Hz, 1H, Glu-α-CH), 4.63 (ddd, *J* = 10.4, 8.6, 4.5 Hz, 1H, Tyr-α-CH), 6.61 (d, *J* = 8.4 Hz, 2H, ArH), 7.03 (d, *J* = 2.8 Hz, 1H ArH), 7.08 (d, *J* = 8.4Hz, 2H, ArH), 8.19 (d, *J* = 8.6 Hz, 1H, Tyr-NH), 8.54 (d, *J* = 7.8 Hz, Glu-NH), 9.14 (s, 1H, OH), 12.63 (d, *J* = 2.8 Hz, 1H, pyrrole-NH). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 26.14, 29.43, 36.99, 50.97, 51.40, 51.97, 54.30, 97.82, 104.66, 113.33, 114.82, 127.69, 127.95, 130.04, 155.76, 158.48, 171.62, 171.97, 172.56. IR (ATR): ν 3288, 2952, 1725, 1630, 1557, 1513, 1436, 1415, 1326, 1209, 1172, 973, 825, 756 cm^{-1}. MS (ESI): *m/z* 590.0 ($[MH+2]^+$, 100), 588.0 ($[MH]^+$, 51.4), 592.0 ($[MH+4]^+$, 48.6). HRMS (ESI) for $C_{21}H_{24}N_3O_7Br_2$: calculated, 587.9976; found, 587.9976. $[\alpha]_D^{20}$ = -36.7 (*c* 1.00, MeOH). RP-HPLC purity: 96%. ee = 90%

*Methyl (S)-4-((2-(4,5-dibromo-1H-pyrrole-2-carboxamido)-3-(4-hydroxyphenyl)propanamido)methyl)benzoate (**13d**)*

Compound **13d** was prepared from **12** and 4-(methoxycarbonyl)phenyl)methanaminium chloride. Mobile phase for flash column chromatography: DCM : MeOH = 40:1 to 20:1. Yield: 45%, white crystals. mp: 135°C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.81 (dd, *J* = 13.8, 9.9 Hz, 1H, Tyr-benzyl-CH_2), 2.98 (dd, *J* = 13.8, 5.1 Hz, 1H, Tyr-benzyl-CH_2), 3.84 (s, 3H, CH_3), 4.32 (dd, *J* = 16.4, 6.1 Hz, 1H, NH-benzyl-CH_2), 4.38 (dd, *J* = 16.4, 6.1 Hz, 1H, NH-benzil-C$\underline{H_2}$), 4.58 (ddd, *J* = 9.9, 8.4, 5.1 Hz, 1H, Tyr-α-CH), 6.63 (d, *J* = 8.5 Hz, 2H, ArH), 7.04 (d, *J* = 2.6 Hz, 1H, ArH), 7.08 (d, *J* = 8.5 Hz, 2H, ArH), 7.29 (d, *J* = 8.3 Hz, 2H, ArH), 7.88 (d, *J* = 8.3 Hz, 2H, benzoate-3,5-H), 8.27 (d, *J* = 8.4 Hz, 1H, amide-NH(at pyrrole), 8.65 (t, *J* = 6.1 Hz, 1H, amide-NH at aminomethylbenzoate), 9.19 (s, 1H, phenol-OH), 12.64 (d, *J* = 2.6 Hz, pyrrole-NH) ppm. ^{13}C NMR (DMSO-d_6, 100 MHz): δ 36.68, 41.86, 52.11, 54.91, 97.85, 104.711, 113.41, 114.93, 127.17, 127.76, 128.04, 128.11, 129.16, 130.09, 145.08, 155.81, 158.68, 166.12, 171.53 ppm. IR (ATR): ν 3282, 2950, 1702, 1613, 1557, 1513, 1434, 1415, 1281, 1224, 1178, 1107, 1019, 972, 832, 752, 705 cm^{-1}. MS (ESI): *m/z* 578.0 ($[MH+2]^-$, 100), 576.0 ($[MH]^-$, 51.4), 580.0 ($[MH+4]^-$, 48.6). HRMS (ESI) for $C_{23}H_{20}N_3O_5Br_2$: calculated, 575.9775; found, 575.9770. $[\alpha]_D^{20}$ = -8.96 (*c* 1.00, MeOH). RP-HPLC purity: 100%. ee = 91%

*General procedure for the preparation of the target compounds **9a-f**, **12** and **14a-e***

Compound **8a-f**, **12** or **14a-e** (1 eq.) was dissolved in a mixture of THF (1 mL/mmol) and LiOH solution (5 eq., 1M solution), and stirred overnight at room temperature. EtOAc and 1M NaOH were then added, layers were separated and organic phase washed again with 1M NaOH. To the combined water phases concentrated hydrochloric acid was added until pH = 1 was reached. The compound was then extracted to EtOAc, combined organic phases were washed with brine, dried over Na_2SO_4, filtered and concentrated. The crude residue is purified by flash column chromatography or crystallized.

*(S)-3-(2-(4,5-dibromo-1H-pyrrole-2-carboxamido)-3-(4-hydroxyphenyl) propanamido)benzoic acid (**9a**)*

Compound **9a** was prepared from **8a** and the oily residue obtained was purified by flash column chromatography, mobile phase: *n*-hexane:EtOAc:AcOH = 1:1:0.1 to yield brown crystals. Yield 95%. mp: 127 – 132°C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.88 (dd, *J* = 13.7 Hz, 10.3 Hz, 1H, benzyl-H), 3.01 (dd, *J* = 13.7, 4.5 Hz, 1H, benzyl-H), 4.69 (ddd, *J* = 10.3, 8.0, 4.5 Hz, 1H, Tyr-α-CH), 6.64 (d, *J* = 8.4 Hz, 2H, ArH), 7.07 (s, 1H, ArH), 7.14 (d, *J* = 8.4 Hz, 2H, ArH), 7.43 (dd, *J* = 7.9, 7.9 Hz, 1H, ArH), 7.63 (d, *J* = 7.9 Hz, 1H, ArH), 7.84 (d, *J* = 7.9 Hz, 1H, ArH), 8.22 (s, 1H, ArH), 8.37 (d, *J* = 8.0 Hz, 1H, amide-NH), 9.19 (s, 1H, Ar-amide-NH), 10.36 (s, 1H, phenol-OH), 12.64 (s, 1H, pyrrole-NH), 13.0 (bs, 1H, COOH) ppm. IR (ATR): ν 3144, 2359, 1683, 1612, 1554, 1513, 1437, 1392, 1301, 1232, 1106, 974, 809, 752 cm^{-1}. MS (ESI): *m/z* 548.0 ([MH]$^-$, 51), 550.0 ([MH+2]$^-$, 100), 552.0 ([MH+4]$^-$, 49). HRMS for $C_{21}H_{16}N_3O_5Br_2$: calculated, 547.9462; found, 547.9469. $[\alpha]_D^{20}$ = +27.0 (*c* 1.00, MeOH). RP-HPLC purity: 95%. ee = 90%.

*(S)-3-(2-(4,5-dichloro-1H-pyrrole-2-carboxamido)-3-(4-hydroxyphenyl)propan amido)benzoic acid (**9b**)*

Compound **9b** was prepared from **8b** to yield brown crystals. mp: 127–132°C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.89 (dd, *J* = 13.8, 10.1 Hz, 1H, benzyl-H), 3.02 (dd, 13.8, 4.8 Hz, 1H, benzyl-H), 4.70 (ddd, *J* = 10.1, 7.9, 4.8 Hz, 1H, Tyr-α-CH), 6.65 (d, *J* = 8.4 Hz, 2H, ArH), 7.05 (s, 1H, ArH), 7.15 (d *J* = 8.4 Hz, 2H, ArH), 7.44 (dd, *J* = 8.0, 7.8 Hz, 1H, ArH), 7.64 (ddd, *J* = 7.8, 1.6, 1.1 Hz, 1H, ArH), 7.85 (ddd, *J* = 8.0, 2.3, 1.0 Hz, 1H, ArH), 8.23 (dd, *J* = 2.3, 1.6 Hz, 1H, ArH), 8.41 (d *J* = 7.9 Hz, 1H, amide-NH), 9.20 (s, 1H, Ar-amide-NH), 10.37 (s, 1H, phenol-OH), 12.70 (s, 1H, pyrrole-NH), 12.9 (bs, COOH) ppm. IR (ATR): ν 3270, 1677, 1612, 1599, 1560, 1513, 1436, 1229, 1104, 1019, 812, 754 cm^{-1}. MS (ESI): *m/z* 460.1 ([MH]$^-$, 100), 462.1 ([MH+2]$^-$, 64), 464.1 ([MH+4]$^-$, 10). HRMS for $C_{21}H_{16}N_3O_5Cl_2$: calculated, 460.0472; found, 460.0472. $[\alpha]_D^{20}$ = +29.2 (*c* 1.00, MeOH). ee= 97%

*(S)-3-(3-(4-hydroxyphenyl)-2-(5-nitro-1H-pyrrole-2-carboxamido)propanamido) benzoic acid (**9c**)*

Compound **9c** was prepared from **8c** to yield brown crystals. Yield 96%. mp 125 – 134°C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.91 (m, 1H, benzyl-CH_2), 3.07 (m, 1H, benzyl-CH_2), 4.80 (m, 1H, Tyr-α-CH), 6.66 (d, *J* = 8.1 Hz, 2H, ArH), 6.93 (s, 1H, ArH), 7.15 (m, 3H, ArH in pyrrole CH), 7.65 (dd, *J* = 7.6, 7.6 Hz, 1H, ArH), 7.65 (d, *J* = 7.6 Hz, 1H, ArH), 7.85 (d, *J* = 7.6 Hz, 1H, ArH), 8.24 (s, 1H), 8.86 (d, *J* = 6.7 Hz, pyrrole-CONH), 9.23 (s, 1H), 10.45 (s, 1H, phenol-OH), 12.6-13.3 (bs, 1H, COOH), 13.53 (s, 1H, pyrrole-NH) ppm. IR (ATR): ν 3276, 3128, 1696, 1638, 1592, 1556, 1514, 1446, 1367, 1288, 1231, 1174, 1106, 1046, 807, 754 cm^{-1}. MS (ESI): *m/z* 437.1 ([MH]$^-$, 100). HRMS (ESI) for $C_{21}H_{17}N_4O_7$: calculated, 437.1103; found, 437.1094. $[\alpha]_D^{20}$ = +11.7 (*c* 1.00, MeOH). RP-HPLC purity:97%. ee = 74%

*(S)-3-(3-(4-hydroxyphenyl)-2-(4-nitro-1H-pyrrole-2-carboxamido)propanamido) benzoic acid (**9d**)*

Compound **9d** was prepared from **8d** to yield brown crystals. Yield 95%. mp: 163–165°C. ^{1}H NMR (MeOH-d_4, 400 MHz): δ 3.06 (dd, *J* = 13.7, 8.3 Hz, 1H, benzyl-H), 3.20 (dd, *J* = 13.7, 7.1 Hz, 1H, benzyl-H), 4.87 (dd, *J* = 8.3, 7.1 Hz, 1H, Tyr-α-CH), 6.71 (d, *J* = 8.5 Hz, 2H, ArH), 6.91 (d, *J* = 4.3 Hz, 1H, ArH), 7.07 (d, *J* = 4.3 Hz, 1H, ArH), 7.15 (d, *J* = 8.5 Hz, 2H, ArH), 7.42 (dd, *J* = 8.0, 8.0 Hz, 1H, ArH), 7.73 – 7.77 (m, 2H, ArH), 8.17 (t, *J* = 2.0 Hz, ArH) ppm. ^{13}C NMR (DMSO-d_6, 100 MHz): δ 38.51, 57.61, 111.37, 113.42, 116.41, 122.76, 125.89, 126.69, 128.84, 130.04, 130.66, 131.51, 132.74, 139.78, 140.68, 157.53, 161.43, 169.57, 172.43 ppm. IR (ATR): ν 3267, 3137, 1693, 1671, 1611, 1593, 1531, 1514, 1446, 1414, 1370, 1312, 1259, 1205, 1174, 1105, 889, 839, 811, 749 cm^{-1}. MS (ESI): *m/z* 437.1 ([MH]$^-$, 100). HRMS (ESI) for $C_{21}H_{17}N_4O_7$: calculated, 437.1103; found, 437.1093. $[\alpha]_D^{20}$ = +49.7 (*c* 1.00, MeOH). RP-HPLC purity: 95%. ee = 98%.

*(S)-3-(3-(4-hydroxyphenyl)-2-(1H-indole-2-carboxamido)propanamido)benzoic acid (**9e**)*

Compound **9e** was prepared from compound **8e**. The crude product was purified by crystallization from the mixture of *n*-hexane and EtOAc to get white crystals. Yield 97%. mp: 146 – 149°C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.97 (dd, *J* = 13.6, 10.0 Hz, 1H, benzyl-CH_2), 3.06 (dd, *J* = 13.6, 4.9 Hz, 1H, benzyl-CH_2), 4.78 (ddd, *J* = 10.0, 8.2, 4.9 Hz, 1H, Tyr-α-CH), 6.64 (d, *J* = 8.4 Hz, ArH), 7.03 (ddd, *J* = 8.0, 7.0, 0.9 Hz, 1H, indole-5-H), 7.17 (ddd, *J* = 8.3, 7.0, 1.1 Hz, 1H, indole-6-H), 7.19 (d, *J* = 8.4 Hz, 2H, ArH), 7.25 (dd, *J* = 2.1, 0.8 Hz, 1H, indole-3-H), 7.40 (dd, *J* = 8.3, 0.9 Hz, 1H, ArH), 7.44 (dd, *J* = 8.0, 8.0 Hz), 7.61-7.65 (m, 2H, ArH), 7.85 (ddd, *J* = 8.0, 1.9, 1.0 Hz, 1H, ArH), 8.24 (dd, *J* = 1.9, 1.9 Hz, 1H, ArH), 8.70 (d, *J* = 8.2 Hz, 1H, pyrrole-CONH), 9.23 (s, 1H, Ar-amide-NH), 10.41 (s, 1H, phenol-OH), 11.5 (d, *J* = 2.1 Hz, 1H, pyrrole-NH), 16.5 (bs, 1H, COOH) ppm. IR (ATR): ν 3236, 1707, 1687, 1635, 1618, 1540, 1514, 1485, 1373, 1300, 1251, 1198, 1168, 1115, 1047, 809, 757, 679,

649 cm^{-1}. MS (ESI): *m/z* 442.1 ($[MH]^-$, 100). HRMS (ESI) for $C_{25}H_{20}N_3O_5$: calculated, 442.1408; found, 442.1400. $[\alpha]_D^{20}$ = +29.1 (*c* 1.00, MeOH). RP-HPLC purity: 99%. ee =42%.

*3-(3-(4-hydroxyphenyl)-2-(1H-indazole-3-carboxamido)propanamido)benzoic acid (**9f**)*

Compound **9f** was prepared from compound **8f**. The crude material was purified using flash column chromatography and mobile phase *n*-hexane : EtOAc : AcOH = 1:5:0.05 to yield white crystals. Yield 95%. mp: 125–130°C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 3.06-3.11 (m, 2H, benzyl-CH_2), 4.85 (m, 1H, Tyr-α-CH), 6.63 (d, *J* = 8.5 Hz, 2H, ArH), 7.11 (d, *J* = 8.5 Hz, 2H, ArH), 7.23 (ddd, *J* = 8.1, 7.0, 0.7 Hz, 1H, ArH), 7.41 (ddd, *J* = 8.3, 7.0, 1.1 Hz, 1H, ArH), 7.44 (dd, *J* = 7.9, 7.9 Hz, 1H, ArH), 7.62 (ddd, *J* = 8.3, 0.7, 0.7 Hz, 1H, ArH), 7.63 (ddd, *J* = 7.9, 1.7, 1.0 Hz, 1H, ArH), 7.84 (ddd, *J* = 7.9, 2.1, 1.0 Hz, 1H, ArH), 8.10 (d, *J* = 8.1 Hz, 1H), 8.18 (d, *J* = 8.1 Hz, 1H), 8.22 (dd, *J* = 2.1, 1.7 Hz, 1H, ArH). IR (ATR): ν 3261, 1642, 1613, 1593, 1534, 1514, 1444, 1234, 1169, 749 cm^{-1}. MS (ESI) *m/z* = 445.1 ($[MH]^+$, 100). HRMS (ESI) for $C_{24}H_{21}N_4O_5$: calculated, 445.1506; found, 445.1499. RP-HPLC purity: 96%. ee = 0%

*4,5-dibromo-1H-pyrrole-2-carbonyl-L-tyrosine (**12**)*

Compound **12** was prepared from **11** to yield brownish crystals. Yield 95%. mp: 113 – 117°C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.85 (dd, J = 13.8, 10.3 Hz, 1H, benzyl-CH_2), 3.03 (dd, *J* = 13.8, 4.3 Hz, 1H, benzyl-CH_2), 4.47 (ddd, *J* = 10.3, 8.3, 4.3 Hz, 1H, Tyr-α-CH), 6.64 (d, *J*=8.4 Hz, 2H, ArH), 7.02 (s, 1H, ArH), 7.06 (d, *J* = 8.4 Hz, 2H, ArH), 8.3 (d, *J* = 8.3 Hz, 1H, amide-NH), 9.20 (s, 1H, phenol-OH), 12.67 (s, 1H, pyrrole-NH), 12.7 (bs, 1H, COOH) ppm. ^{13}C NMR (DMSO-d_6, 100 MHz): δ 35.71, 53.96, 97.85, 104.90, 113.18, 114.99, 127.60, 127.93, 129.95, 155.85, 158.72, 173.25 ppm. IR (ATR): ν 3174, 2924, 1716, 1612, 1557, 1413, 1212 cm^{-1}. MS (ESI) *m/z* = 432.9 ($[MH+2]^+$, 100), 430.9 ($[MH]^+$, 40.4), 434.9 ($[MH+4]^+$, 48.6). HRMS (ESI) for $C_{14}H_{13}N_2O_4Br_2$: calculated, 430.9237; found, 430.9240. $[\alpha]_D^{20}$ = -37.5 (*c* 1.00, MeOH). RP-HPLC purity: 96%.

*1-((4,5-dibromo-1H-pyrrole-2-carbonyl)-L-tyrosyl)piperidine-4-carboxylic acid (**14a**)*

Compound **14a** was prepared from compound **13a**. After neutralization of water phase, white precipitate forms, which is filtered and dried *in vacuo* for 1 h. mp: 158 – 161°C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.10 – 1.50 (m, 2H), 1.68 – 1.84 (m, 2H), 2.60 – 3.16 (m, 5H), 3.80 – 3.94 (m, 1H), 4.07 – 4.29 (m, 1H), 4.96 (m, 1H, Tyr-α-CH), 6.63 (d, *J* = 8.4 Hz, 2H, ArH), 7.06 (m, 3H, ArH), 8.41 (d, *J* = 8.3 Hz, amide-NH), 8.45 (d, *J* = 8.3 Hz, amide-NH'), 9.18 (s, 1H, phenol-OH), 9.19 (s, 1H, phenol-OH'), 12.25 (s, 1H, COOH), 12.65 (s, 1H, pyrrole-NH), 12.68 (s, 1H, pyrrole-NH') ppm. IR (ATR): ν 3150, 2957, 1706, 1609, 1546, 1448, 1378, 1208, 1173 cm^{-1}.MS (ESI): *m/z* 542.0 ($[MH+2]^-$, 100),

540.0 ([MH]$^-$, 51.4), 544.0 ([MH+4]$^-$, 48.6). HRMS (ESI) for $C_{20}H_{20}N_3O_5Br_2$: calculated, 539.9775; found, 539.9764. $[\alpha]_D^{20}$ = 97.6 (*c* 0.21, DMSO). RP-HPLC purity: 95%.

*(4,5-dibromo-1H-pyrrole-2-carbonyl)-L-tyrosyl-L-aspartic acid (**14b**)*

Compound **14b** was prepared from compound **13b**. After neutralization of water phase, white precipitate forms, which is filtered and dried *in vacuo* for 1 h. mp: 230 – 233 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.6 (dd, *J* = 16.8, 6.9 Hz, 1H, Asp-β-CH_2), 2.7 (m, 2H, Asp-β-CH_2 and benzyl-CH_2), 2.98 (dd, *J* = 14.0, 3.4 Hz, benzyl-CH_2), 4.6 (m, 2H, Tyr-α-CH in Asp-α-CH), 6.60 (d, *J* = 8.5 Hz, 2H, ArH), 7.00 (d, *J* = 2.7 Hz, 1H, ArH), 7.08 (d, *J* = 8.5 Hz, 2H, ArH), 8.19 (d, *J* = 8.7 Hz, 1H, Glu-NH), 8.43 (d, *J* = 8.0 Hz, Tyr-NH), 9.14 (s, 1H, OH), 12.1 – 12.6 (bs, 2H, COOH), 12.61 (d, *J* = 2.7 Hz, 1H, pyrrole-NH) ppm. ^{13}C NMR (DMSO-d_6, 100 MHz): δ 38.08, 38.31, 51.32, 56.42, 100.10, 106.49, 115.04, 116.33, 128.52, 129.51, 131.48, 157.29, 161.38, 173.43, 175.41, 175.50 ppm. IR (ATR): ν 3404, 3288, 3066, 2969, 1718, 1690, 1674, 1625, 1542, 1505, 1279 cm^{-1}. MS (ESI): *m/z* 545.9 ([MH+2]$^-$, 100), 543.9 ([MH]$^-$, 51.4), 547.9 ([MH+4]$^-$, 48.6). HRMS (ESI) for $C_{18}H_{16}N_3O_7Br_2$: calculated, 543.9360; found, 543.9343. RP-HPLC purity: diastereomers: 84.4% and 13.9% .

*(4,5-dibromo-1H-pyrrole-2-carbonyl)-L-tyrosyl-L-glutamic acid (**14c**)*

Compound **14c** was prepared from compound **13c**. After neutralization of water phase, white precipitate forms, which is filtered and dried *in vacuo* for 1 h. mp: 132 – 135°C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.77 (dtd, *J* = 13.9, 7.6, 8.1 Hz, 1H, Glu-β-CH_2), 1.97 (dtd, *J* 13.9, 7.6, 5.2 Hz, 1H, Glu-β-CH_2), 2.23 (t, *J* = 7.6 Hz, 2H, Glu-γ-CH), 2.74 (dd, *J* = 13.8, 10.8 Hz, 1H, benzyl-CH_2), 2.92 (dd, *J* = 13.8, 4.2 Hz, 1H, benzyl-CH_2), 4.24 (ddd, *J* = 8.7, 8.1, 5.2 Hz, 1H, Glu-α-CH), 4.65 (ddd, 10.8, 8.0, 4.2 Hz, 1H, Tyr-α-CH), 6.60 (d, *J* = 8.4 Hz, 2H, ArH), 7.02 (d, *J* = 2.6 Hz, 1H, ArH), 7.09 (d, *J* = 8.4 Hz, 2H, ArH), 8.17 (d, *J* = 8.7 Hz, 1H, Glu-NH), 8.4 (d, *J* = 8.0 Hz, 1H, Tyr-NH), 9.14 (s, 1H, OH), 12.0-12.6 (bs, 2H, COOH), 12.62 (d, *J* = 2.6 Hz, pyrrole-NH) ppm. ^{13}C NMR (DMSO-d_6, 100 MHz): δ 26.48, 29.89, 37.09, 51.08, 54.33, 97.79, 104.61, 113.28, 114.81, 127.73, 128.10, 130.06, 155.71, 158.45, 171.53, 173.13, 173.69 ppm. IR (ATR): ν 3420, 3356, 3295, 3105, 2962, 1725, 1660, 1555, 1515, 1609, 1224, 1167, 1113, 984, 896, 814 cm^{-1}. MS (ESI): *m/z* 559.9 ([MH+2]$^-$, 100), 558.0 ([MH]$^-$, 51.4), 561.9 ([MH+4]$^-$, 48.6). HRMS (ESI) for $C_{19}H_{18}N_3O_7Br_2$: calculated, 557.9517; found, 557.9500. $[\alpha]_D^{20}$ = -27.6 (*c* 1.00, MeOH). RP-HPLC purity: 98%.

*(S)-4-((2-(4,5-dibromo-1H-pyrrole-2-carboxamido)-3-(4-hydroxyphenyl)propanamido)methyl)benzoic acid (**14d**)*

Compound **14d** was prepared from compound **13d**. After neutralization of water phase, white precipitate forms, which is filtered and dried *in vacuo* for 1 h. mp: 232 – 235°C. ^{1}H NMR (DMSO-d_6,

400 MHz): δ 2.82 (dd, *J* = 13.7, 10.1 Hz, 1H, Tyr-benzyl-CH_2), 2.99 (dd, *J* = 13.7, 5.2 Hz, 1H, benzyl-CH_2), 4.33 (dd, *J* = 16.1, 5.9 Hz, 1H, NH-benzyl-C$\underline{H_2}$), 4.38 (dd, *J* = 16.1, 5.9 Hz, 1H, NH-benzyl-CH_2), 4.6 (ddd, *J* = 10.1, 8.2, 5.2 Hz, 1H, Tyr-α-CH), 6.64 (d, *J* = 8.5 Hz, 2H, ArH), 7.05 (s, 1H, ArH), 7.09 (d, *J* = 8.5 Hz, 2H, ArH), 7.27 (d, *J* = 8.3 Hz, 2H, ArH), 7.87 (d, *J* = 8.3 Hz, ArH), 8.28 (d, *J* = 8.2 Hz, 1H, amide-NH at pyrrole), 8.65 (t, *J* = 5.9 Hz, 1H, amide-NH at aminomethylbenzoate), 9.20 (s, 1H, OH), 12.66 (s, 1H, pyrrole-NH), 12.9 (bs, 1H, COOH) ppm. ^{13}C NMR (DMSO-d_6, 100 MHz): δ 36.70, 41.88, 54.91, 97.87, 104.72, 113.41, 114.97, 127.00, 127.76, 128.11, 129.17, 129.30, 130.08, 144.55, 155.81, 158.67, 167.20, 171.49 ppm. IR (ATR): ν 3293, 3115, 2947, 1700, 1626, 1590, 1553, 1513, 1449, 1405, 1382, 1243, 1172, 751 cm^{-1}. MS (ESI): *m/z* 564.0 ([MH+2]$^-$, 100), 562.0 ([MH]$^-$, 51.4), 566.0 ([MH+4]$^-$, 48.6). HRMS (ESI) for $C_{22}H_{18}N_3O_5Br_2$: calculated, 561.9619; found, 561.9613. $[\alpha]_D^{20}$ = -8.3 (*c* 1.00, MeOH). RP-HPLC purity: 98%. ee = 93%

*(S)-5-(2-(4,5-dibromo-1H-pyrrole-2-carboxamido)-3-(4-hydroxyphenyl)propanamido)-2-hydroxybenzoic acid (**14e**)*

Compound **14e** was prepared from compound **13e**. After neutralization of water phase, white precipitate forms, which is filtered and dried *in vacuo* for 1 h. mp: 246 – 250°C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.87 (dd, *J* = 13.8, 9.9 Hz, 1H, benzyl-CH_2), 3.00 (dd, *J* = 13.8, 4.8 Hz, 1H, benzyl-CH_2), 4.65 (ddd, *J* = 9.9, 8.2, 4.8 Hz, 1H, Tyr-α-CH), 6.64 (d, *J* = 8.4 Hz, 2H, ArH), 6.93 (d, *J* = 9.0 Hz, 1H, ArH), 7.07 (d, *J* = 2.7 Hz, 1H, ArH), 7.13 (d, *J* = 8.4 Hz, 2H, ArH), 7.68 (dd, *J* = 9.0, 2,7 Hz, 1H, ArH), 8.09 (d, *J* = 2.7 Hz, 1H, ArH), 8.35 (d, *J* = 8.2 Hz, 1H, amide-NH), 9.19 (s, 1H, Ar-amide NH), 10.13 (s, 1H, Tyr-OH), 11.0 (bs, 1H, salicyl-OH), 12.64 (d, *J* = 2.7 Hz, 1H, pyrrole-NH), 14.0 (bs, 1H, COOH) ppm. ^{13}C NMR (DMSO-d_6, 100 MHz): δ 36.65, 55.51, 97.88, 104.82, 112.43, 113.47, 114.92, 117.22, 120.76, 127.65, 127.65, 130.11, 130.58, 155.82, 157.18, 158.77, 170.15, 171.68 ppm. IR (ATR): ν 3173, 3122, 3073, 2957, 1662, 1595, 1550, 1450, 1284, 1226, 1172, 834, 673 cm^{-1}. MS (ESI): *m/z* 565.9 ([MH+2]$^-$, 100), 563.9 ([MH]$^-$, 51.4), 567.9 ([MH+4]$^-$, 48.6), [M-H]$^-$. HRMS (ESI) for $C_{21}H_{16}N_3O_6Br_2$: calculated, 563.9411; found, 563.9406. $[\alpha]_D^{20}$ = +12.9 (*c* 1.00, MeOH). RP-HPLC purity: 96%.

*General procedure for the synthesis of **13e-h**.*

Compound **12** (1 eq.), aromatic amine (1.1 eq.), 1-hydrohybenzotriazole (1 eq.), DMAP (1 eq.) and *N*-methylmorpholine (4 eq.) were dissolved in DMF (10 mL/mmol) and cooled to 0°C prior to addition of EDC (1.1 eq.). The reaction mixture was stirred for 15 min at 0°C and overnight at 60°C, concentrated and the residue dissolved in EtOAc. The resulting solution was then washed with 10% water solution of citric acid, concentrated solution of $NaHCO_3$ and brine successively. Organic phase was dried over Na_2SO_4, filtered and concentrated. The crude residue was purified by flash column chromatography

*Methyl 5-(2-(4,5-dibromo-1H-pyrrole-2-carboxamido)-3-(4-hydroxyphenyl)propanamido)-2-hydroxybenzoate (**13e**)*

Compound **13e** was prepared from **12** and methyl 5-aminosalicylate. Mobile phase: DCM : MeOH = 40:1 to 20:1. Recrystalization from a mixture of DCM and MeOH. Pale yellow crystals. Yield 53%. mp: 217 – 220°C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.86 (dd, J = 13.8, 9.8 Hz, 1H, benzyl-CH_2), 2.99 (dd, J = 13.8, 5.1 Hz, 1H, benzyl-CH_2), 3.89 (s, 3H, CH_3), 4.63 (ddd, J = 9.8, 8.2, 5.1 Hz, 1H, Tyr-α-CH), 6.63 (d, J = 8.5 Hz, 2H, ArH), 6.95 (d, J = 9.0 Hz, 1H, ArH), 7.06 (d, J = 2.8 Hz, 1H, ArH), 7.12 (d, J = 8.5 Hz, 2H, ArH), 7.66 (dd, J = 9.0, 2.7 Hz, 1H, ArH), 8.09 (d, J = 2.7, 1H, ArH), 8.33 (d, J = 8.2, 1H, amide-NH), 9.18 (s, 1H, Ar-amide-NH), 10.13 (s, 1H, salicyl-OH), 10.27 (s, 1H, Tyr-OH), 12.63 (d, J = 2.8, 1H, pyrrole-NH) ppm. ^{13}C NMR (DMSO-d_6, 100 MHz): δ 36.59, 52.52, 55.48, 97.86, 104.81, 112.47, 113.44, 114.89, 117.57, 120.45, 127.57, 127.61, 130.07, 130.72, 155.79, 156.03, 158.75, 168.92, 170.13 ppm. IR (ATR): ν 3381, 3259, 3149, 1669, 1627, 1560, 1512, 1492, 1199, 1167, 1083, 973, 826, 793 cm^{-1}. MS (ESI): *m/z* 580.0 ([MH+2]$^-$, 100), 578.0 ([MH]$^-$, 51.4), 582.0 ([MH+4]$^-$, 48.6). HRMS (ESI) for $C_{22}H_{18}N_3O_6Br_2$: calculated, 577.9568; found, 577.9562. RP-HPLC purity: 97%. ee = 0%

*4,5-dibromo-N-(3-(4-hydroxyphenyl)-1-((3-hydroxyphenyl)amino)-1-oxopropan-2-yl)-1H-pyrrole-2-carboxamide (**13f**)*

Compound **13g** was prepared from **12** and 3-aminophenol. The crude product after extraction was dissolved in MeOH and the yellow impurity filtered off. Mother liquor was concentrated and purified by flash column chromatography, mobile phase: DCM:MeOH = 20:1. Yield: 14%. White crystals. Mp: 145 – 147°C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.84 (dd, J = 13.8, 10.3 Hz, 1H, benzyl-CH_2), 2.96 (dd, J = 13.8, 4.6 Hz, 1H, benzyl-CH_2), 4.69 (ddd, J = 10.3, 8.4, 4.6 Hz, 1H, Tyr-α-CH), 6.45 (ddd, J = 8.0, 2.3, 0.9 Hz, 1H, ArH), 6.63 (d, J = 8.5, 2H, ArH), 6.96 (ddd, J = 8.0, 2.1, 0.9 Hz, 1H, ArH), 7.06 (d, J = 2.6 Hz, 1H, ArH), 7.07 (dd, J = 8.0, 8.0 Hz, 1H, ArH), 7.13 (d, J = 8.5 Hz, 2H, ArH), 7.17 (dd, J = 2.3, 2.1 Hz, 1H, ArH), 8.33 (d, J = 8.2 Hz, 1H, amide-NH), 9.18 (s, 1H, OH), 9.39 (s, 1H, OH), 10.04 (s, 1H), 12.63 (d, J = 2.6 Hz, 1H, pyrrole-NH) ppm. ^{13}C NMR (DMSO-d_6, 100 MHz): δ 38.59, 57.20, 100.12, 106.47, 108.76, 112.50, 115.15, 116.29, 128.38, 128.95, 130.51, 131.44, 140.39, 157.33, 158.87, 161.29, 172.20 ppm. MS (ESI): *m/z* = 521.9 ([MH+2]$^-$, 100), 519.9 ([MH]$^-$, 51.4), 523.9 ([MH+4]$^-$, 48.6). HRMS (ESI) for $C_{20}H_{16}N_3O_4Br_2$: calculated, 519.9513; found, 519.9508. RP-HPLC purity: 96%. ee= 0%

*4,5-dibromo-N-(3-(4-hydroxyphenyl)-1-oxo-1-(phenylamino)propan-2-yl)-1H-pyrrole-2-carboxamide (**13g**)*

Compound **13h** was prepared from **12** and aniline. The crude product after extraction was dissolved in MeOH and the yellow impurity filtered off. Mother liquor was concentrated and purified by flash column chromatography, mobile phase: DCM:MeOH = 40:1. Yield: 14%, pale yellow solid. mp: 130°C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.86 (dd, J = 13.8, 10.2 Hz, 1H, benzyl-CH_2), 2.98 (dd, J = 13.8, 4.5 Hz, 1H, benzyl-CH_2), 4.71 (ddd, J = 10.2, 8.2, 4.5 Hz, 1H, Tyr-α-CH), 6.63 (d, J = 8.5 Hz, 2H, ArH), 7.04 (ddd, J = 7.4, 1.2, 1.2 Hz, 1H, ArH), 7.06 (d, J = 2.6 Hz, 1H, ArH), 7.13 (d, J = 8.5 Hz, 2H, ArH), 7.30 (dd, J = 8.6, 7.4 Hz, 2H, ArH), 7.59 (dd, J = 8.6, 1.2 Hz, 2H, ArH), 8.35 (d, J = 8.2Hz, amide-NH), 9.17 (s, 1H, phenol-OH), 10.16 (s, 1H, Ar-amide-NH), 12.63 (d, J = 2.6, 1H, pyrrole-NH) ppm. ^{13}C NMR (DMSO-d_6, 100 MHz): δ 29.02, 47.67, 90.56, 96.92, 105.60, 106.72, 112.12, 115.98, 118.82, 119.39, 120.23, 121.88, 129.74, 147.80, 151.76, 162.74 ppm. IR (ATR): ν 3265, 1596, 1497, 1443, 1414, 1391, 1311, 1229, 1171, 751 cm^{-1}. MS (ESI): *m/z* 506.0 ([MH+2]$^-$, 100), 504.0 ([MH]$^-$, 51.4), 508 ([MH+4]$^-$, 48.6). HRMS (ESI) for $C_{20}H_{16}N_3O_3Br_2$: calculated, 503.9564; found, 503.9558. RP-HPLC purity: 95%. ee = 0%

*1-((4,5-dibromo-1H-pyrrole-2-carbonyl)-L-tyrosyl)piperidine-4-carboxamide (**13h**)*

Compound **13a** was prepared from **12** and isonipecotamide. The oily residue was purified by flash column chromatography, mobile phase: DCM : MeOH = 9: 1 to yield white crystals. Yield 47%. mp: 168 – 172°C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.21 – 1.44 (m, 2H), 1.54 – 1.72 (m, 2H), 2.21 – 2.32 (m, 1H), 2.70 – 2.90 (m, 3H), 2.98 – 3.09 (m, 1H), 3.88 – 3.98 (m, 1H), 4.20 – 4.37 (m, 1H), 4.91 – 4.99 (m, 1H, Tyr-α-CH, Tyr-α-CH'), 6.61 (d, J = 8.3 Hz, 2H, ArH, ArH'), 6.77 (s, 1H, amide-NH_2), 6.80 (s, 1H, amide-NH_2'), 7.04 (d, J = 8.3 Hz, 2H, ArH, ArH'), 7.07 (d, J = 2.7 Hz, 1H, ArH, ArH'), 7.23 (s, 1H, amide-NH_2), 7.24 (amide-NH_2'), 8.36 (d, J = 8.2 Hz, amide-NH), 8.43 (d, J = 8.2 Hz, amide-NH'), 9.16 (s, 1H, phenol-OH), 9.17 (s, 1H, phenol-OH'), 12.61 (d, J = 2.7 Hz, 1H, pyrrole-NH), 12.67 (d, J = 2.7 Hz, pyrrole-NH') ppm. IR (ATR): ν 3186, 1609, 1544, 1511, 1447, 1378, 1213 cm^{-1}. MS (ESI): *m/z* 541.0 ([MH+2]$^-$, 100), 539.0 ([MH]$^-$, 51.4), 543.0 ([MH+4]$^-$, 48.6). HRMS (ESI) for $C_{20}H_{21}N_4O_4Br_2$: calculated, 538.9935; found, 538.9945. $[\alpha]_D^{20}$ = -6.85 (*c* 1.00, DMF). RP-HPLC purity: 99%

Biology assay methods

E. coli and S. aureus DNA Gyrase ATPase assay

In vitro inhibitory activity screening and determination of IC_{50} values on *E. coli* and *S. aureus* Gyrase was performed on black streptavidin-coated 96-well microtiter plates (Thermo Scientific Pierce) as

previously reported protocol.[22,25] The plate was first rehydrated with the wash buffer supplied (20 mM Tris-HCl (pH 7.6), 137 mM NaCl, 0.01% (w/v) BSA, 0.05% (v/v) Tween 20). Biotinylated oligonucelotide was immobilized onto the wells. The excess of oligonucleotide was then washed off and the enzyme assay carried out in the wells. The final reaction volume of 30 µL in buffer (35 mM Tris HCl, pH 7.5; 24 mM KCl; 4 mM $MgCl_2$; 2 mM DTT; 1.8 mM spermidine; 1 mM ATP; 6.5% (w/v) glycerol; 0.1 mg/mL albumin) contained 1.5 U of DNA gyrase from *E. coli* or *S.aureus*, 0.75 µg of relaxed pNO1 plasmid, and 3 µL of inhibitors solution in 10% DMSO and 0.008% Tween 20. Reactions were incubated for 30 min at 37°C and, after addition of the TF buffer (50 mM NaOAc pH 5.0, 50 mM NaCl and 50 mM MgCl2), which terminated the enzymatic reaction, for another 30 min at room temperature to allow triplex formation (biotin−oligonucleotide− plasmid). The unbound plasmid was then washed off using TF buffer, and a solution of SybrGOLD stain in T10 buffer (10 mM Tris × HCl, pH 8.0, and 1 mM EDTA) was added. After mixing, the fluorescence (excitation, 485 nm; emission, 535 nm) was read using a BioTek's Synergy H4 microplate reader. Preliminary screening was performed at inhibitor concentrations of 100 and 10 µM. For the most potent compounds, IC_{50} was determined with seven concentrations of the inhibitors. IC_{50} values were calculated using GraphPad Prism software and represent the concentration of inhibitor where the residual activity of the enzyme is 50% in three independent measurements; the final result is given as their average value. Novobiocin (IC_{50} = 0.17 µM (lit.0.08 µM44) for *E. coli* DNA gyrase and IC_{50} = 0.041 µM (lit. 0.01 µM44) for *S. aureus* DNA gyrase) were used as a positive control.

***E. coli* and *S. aureus* Topoisomerase IV ATPase assay**

In vitro inhibitory activity screening and determination of IC_{50} values on *E. coli* and S. aureus topoisomerase IV was performed on the black streptavidin-coated 96-well microtiter plates (Thermo Scientific Pierce) as previously reported protocol.[22,25] The plate was first rehydrated with the supplied wash buffer (20 mM Tris-HCl (pH 7.6), 137 mM NaCl, 0.01% (w/v) BSA, 0.05% v/v) Tween 20), and biotinylated oligonucelotide was immobilized onto the wells. The excess of oligonucleotide was then washed off, and the enzyme assay carried out in the wells. The final reaction volume of 30 µL in buffer (40 mM HEPES KOH (pH 7.6), 100 mM potassium glutamate, 10 mM magnesium acetate, 10 mM DTT, 1 mM ATP, 0.05 mg/mL albumin) contained 1.5 *U of topoisomerase IV from E. coli or S. aureus*, 0.75 µg of supercoiled pNO1 plasmid, and 3 µL of inhibitors solution in 10% DMSO and 0.008% Tween 20. Reactions were incubated for 30 min at 37 °C, and after addition of the TF buffer (50 mM NaOAc, pH 5.0, 50 mM NaCl and 50 mM $MgCl_2$), which terminated the enzymatic reaction, for another 30 min at room temperature to allow triplex formation (biotin−oligonucleotide−plasmid). The unbound plasmid was then washed off using TF buffer and the solution of SybrGOLD stain in T10 buffer (10 mM Tris-HCl pH 8.0 and 1 mM EDTA) was added. After mixing, fluorescence (excitation, 485 nm; emission, 535 nm) was read using a

BioTek's Synergy H4 microplate reader. Preliminary screening was performed at inhibitor concentrations of 100 and 10 μM. For most potent compounds, IC50 was determined with seven concentrations of the inhibitors. IC_{50} values were calculated using GraphPad Prism software and represent the concentration of inhibitor where the residual activity of the enzyme is 50% in three independent measurements; the final result is given as their average value. Novobiocin (IC50 = 11 μM (lit. 10 μM44) for *E. coli* topoisomerase IV and IC50 = 27 μM (lit. 20 μM44) for *S. aureus* topoisomerase IV) was used as a positive control. *Esherichia coli* DNA Gyrase ATPase Assay. Compounds were diluted in DMSO and water to give 2 mM concentration in 50% DMSO, then serially diluted in 50% DMSO/water. Then 10 μL was added to a final assay volume of 100 μL, giving a final DMSO concentration in the assays of 5% (v/v). Compounds were tested between either 200 and 0.005 μM or 25 and 0.0001 μM. Controls also contained a final concentration of 5% DMSO. E. coli gyrase ATPase activity (Inspiralis) was measured in a linked assay which follows the hydrolysis of ATP via the conversion of NADH to NAD+. E. coli DNA gyrase (10 μL of 500 nM) was incubated at 25 °C in a final volume of 100 μL containing 1× assay buffer (50 mM Tris·HCl (pH 7.5), 1 mM EDTA, 5 mM $MgCl_2$, 5 mM DTT, 10% (w/v) glycerol)), 800 μM phosphoenolpyruvate, 400 μM NADH, 1.5 μL of phosphokinase/lactate dehydrogenase (PK/LDH) enzyme mix, and 0.36 μM linear pBR322 plus or minus inhibitors. The order of addition to the wells was buffer/water/DNA mix, then compounds, and then the enzyme. This was equilibrated and the A340 measured for 10 min at 25 °C. Reactions were then initiated by the addition of ATP (Mg2+) to 2 mM and the decrease in A340 measured over time. Raw data were collected as a change in OD340 with time (values quoted as milli OD340/min) and converted to A % of the 100% control (enzyme in the absence of inhibitors) after subtraction of the background (no enzyme) rate. These were analyzed using SigmaPlot Version 12.5 (2014).

Determination of Antibacterial Activity.

Antimicrobial assays were performed by the broth microdilution method following the guidelines of the Clinical and Laboratory Standards Institute (CLSI; Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically, Approved Standards − Ninth Edition; M07-A9, Vol. 32, No. 2). Primarily the following CLSI recommended quality control strains for susceptibility testing were used in the antibacterial assays: *Enterococcus faecalis* (Gram positive, ATCC 29212), *Staphylococcus aureus* (Gram positive, ATCC 25923), *Escherichia coli* (Gram negative, ATCC 25922), and *Pseudomonas aeruginosa* (Gram negative, ATCC 27853). In addition, *E. coli* tolC::Tn10, efflux deficient strain lacking functional tolC gene, and *E. coli* imp4213::Tn10 strain with altered permeability of the outer membrane, were used. Primary screening of compounds against the ATCC strains was initially carried out at a final concentration of 50 μM (n = 3). Compounds that showed >50% inhibition in the primary screen were tested further at several concentrations to confirm

the activity and to determine MIC values. The compounds were further assayed against the E. coli strains in 10 2-fold dilutions starting from 256 μg/mL concentration. Azithromycin (USP, cat. 1046056), ciprofloxacin (USP, cat. 1134313 or ICN Biomedicals, Inc.) and Meropenem (USP, cat. 1392454) were used as standard antibiotics.

Surface Plasmon Resonance (SPR) Measurements.

Surface plasmon resonance (SPR) measurements for compounds were performed on a BiacoreX machine using CM5 sensor chip (Biacore, GE Healthcare) as previously reported protocol.[22,25] The system was primed twice with running buffer (10 mM Hepes, pH 7.4, 150 mM NaCl, 3 mM EDTA, 0.005% surfactant P20). The G24 protein was immobilized on the second flow cell of a sensor chip CM5 using the standard amino coupling method. Briefly, the carboxymethylated dextran layer was activated with a 7 min pulse of 1-ethyl-3-(3-(dimethylamino)propyl)-carbodiimide (EDC) and N-hydroxysuccinimide (NHS) mixed in a 1:1 ratio. Protein was diluted to the final concentration of 50 μg/mL in 10 mM sodium acetate (pH = 4.5) and injected in two short pulses to reach the final immobilization level around 17400 response units. Finally, the rest of the surface was deactivated with a 7 min injection of ethanolamine. The first flow cell was activated with EDC/NHS and deactivated with ethanolamine and served as a reference cell for subtraction of nonspecific binding. Analytes were prepared as DMSO 100× stock solutions and were diluted with a running buffer prior to the injection. They were injected at a flow rate of 30 μL/min for 90 s, and dissociation was monitored for an additional 120 s. Because the dissociation of analytes from the ligand was rapid, no regeneration protocol was needed. For the titration of analytes, the 1% of the DMSO was added to the running buffer in order to diminish the difference in refractive index between the samples and running buffer. Selected compounds 3, 24, 25, 32, 35, 36, and 43 were tested at at least eight different concentrations (depending on their solubility) in three parallel titrations. The sensorgrams were analyzed using BiaEval software (Biacore, GE Healthcare). Equilibrium binding responses were determined from the binding levels 5 s before the stop of the injection. Kd values were determined by fitting of the data to a 1:1 steady state binding model as described in results.

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Conflict of Interest

The authors have declared no conflicts of interest.

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Graphical abstract

Design, Synthesis and Evaluation of Tyrosine-Based DNA Gyrase B Inhibitors with Antibacterial Activity Against Gram-Positive Bacteria

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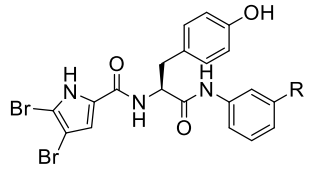


IC_{50} (*E. coli* GyrB) = 13 μM
IC_{50} (*S. aureus* GyrB) = 28 μM
MIC_{90} (*S. aureus*) = 38 μg mL^{-1}
MIC_{90} (*E. faecalis*) = 38 μg mL^{-1}

Cmp.	*R*	**IC_{50} (μM) GyrB**		**MIC_{90} (μg mL^{-1})**	
		E. coli	*S. aureus*	*S. aureus*	*E. faecalis*
8a	COOMe	40	>250	14	28
13e	OH	13	28	38	38

A series of novel tyrosine-based inhibitors of DNA gyrase B (GyrB), were designed, synthesized and evaluated against DNA gyrase and DNA topoisomerase IV. Compounds **8a** and **13e,** which inhibited *E coli* and *S. aureus* GyrB and displayed inhibitory activity against the growth of *S. aureus* and *E. faecalis* were identified as promising compounds for further structural optimization.

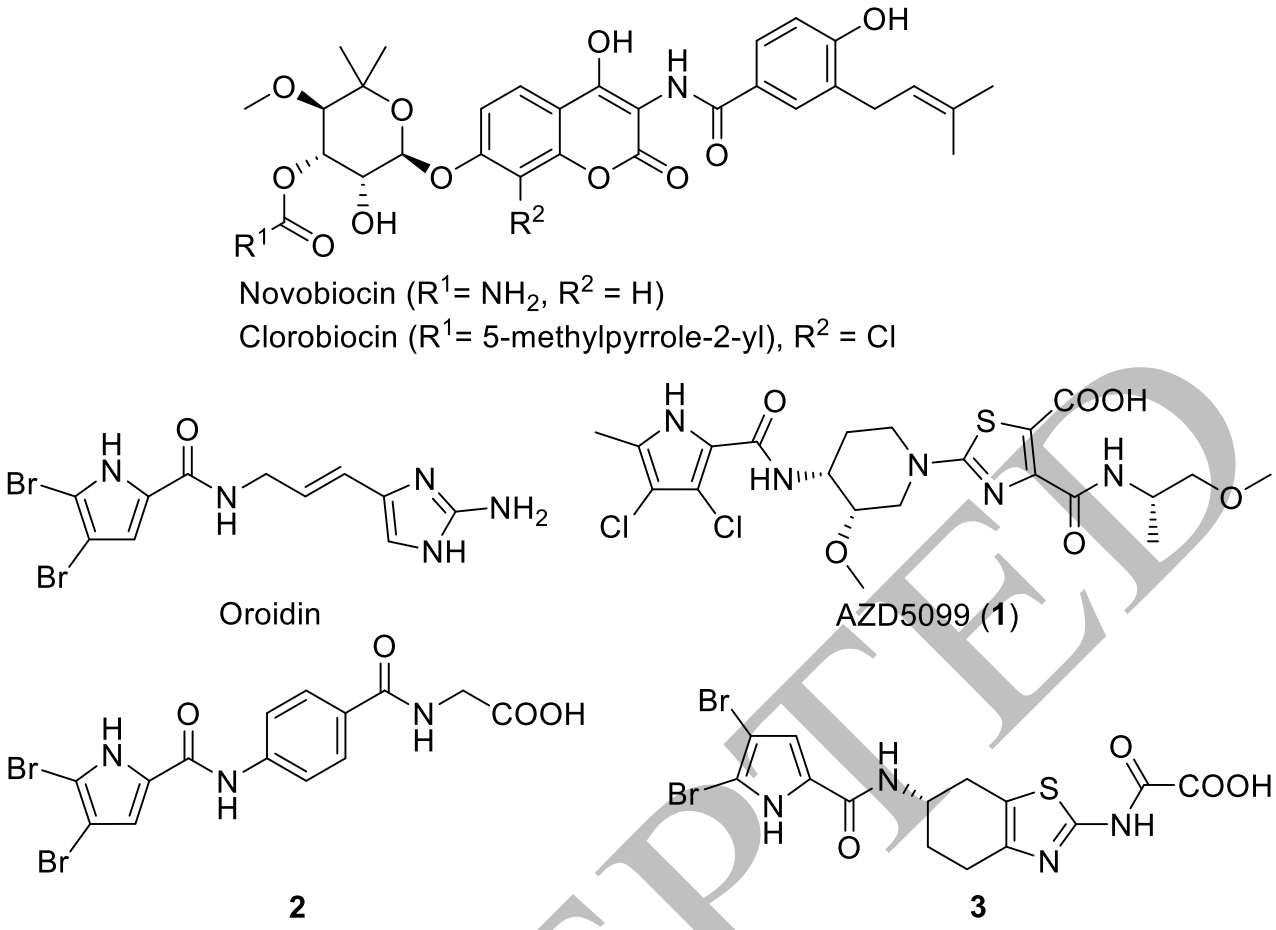


Figure 1. Representative examples of oroidin and GyrB inhibitors.

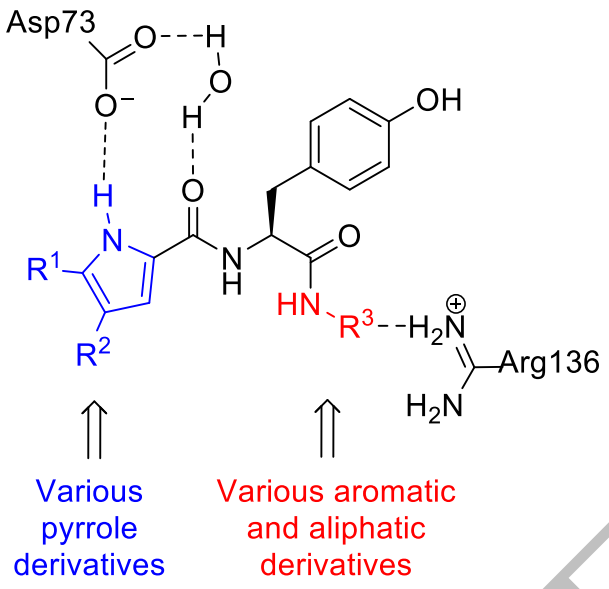
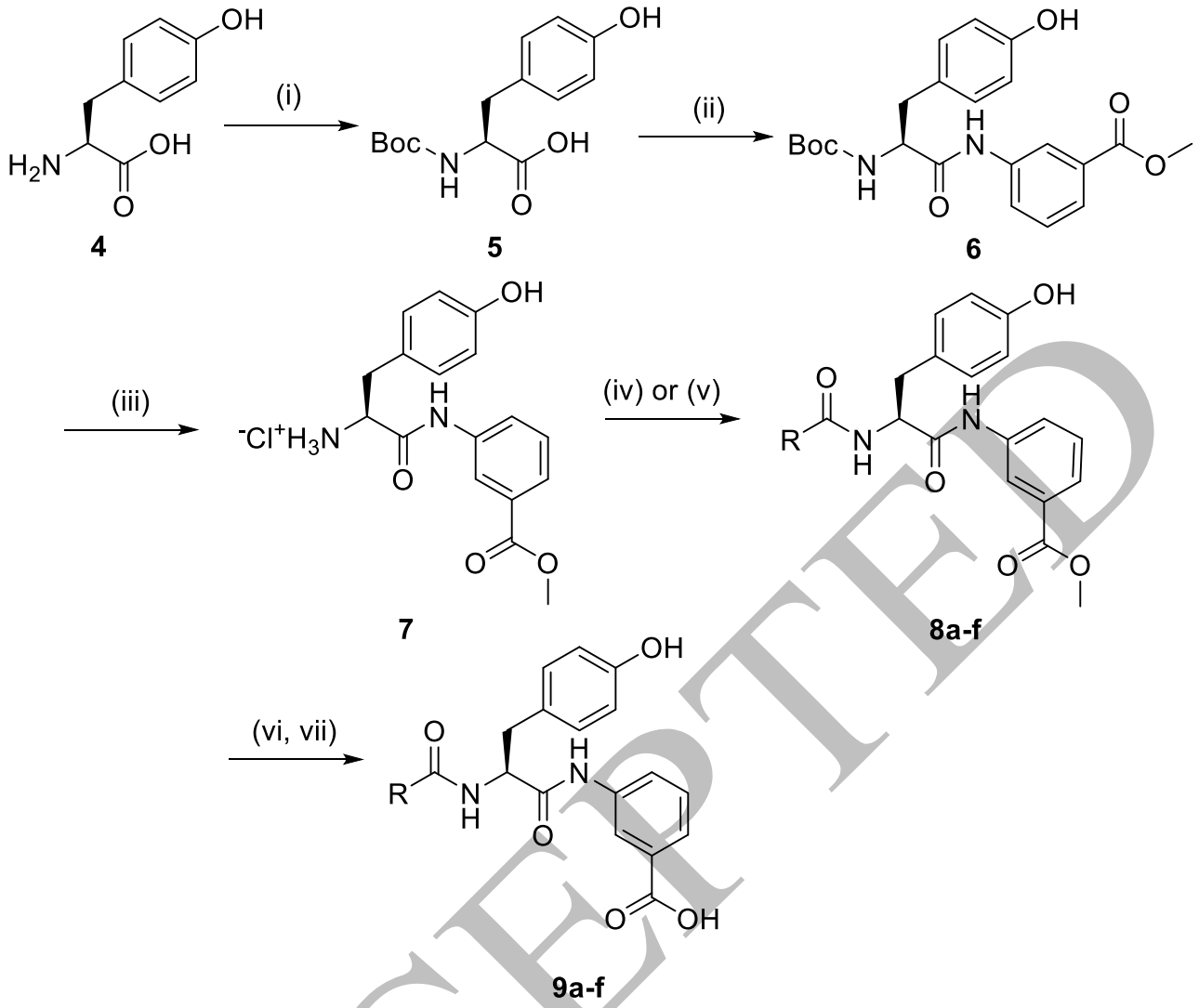
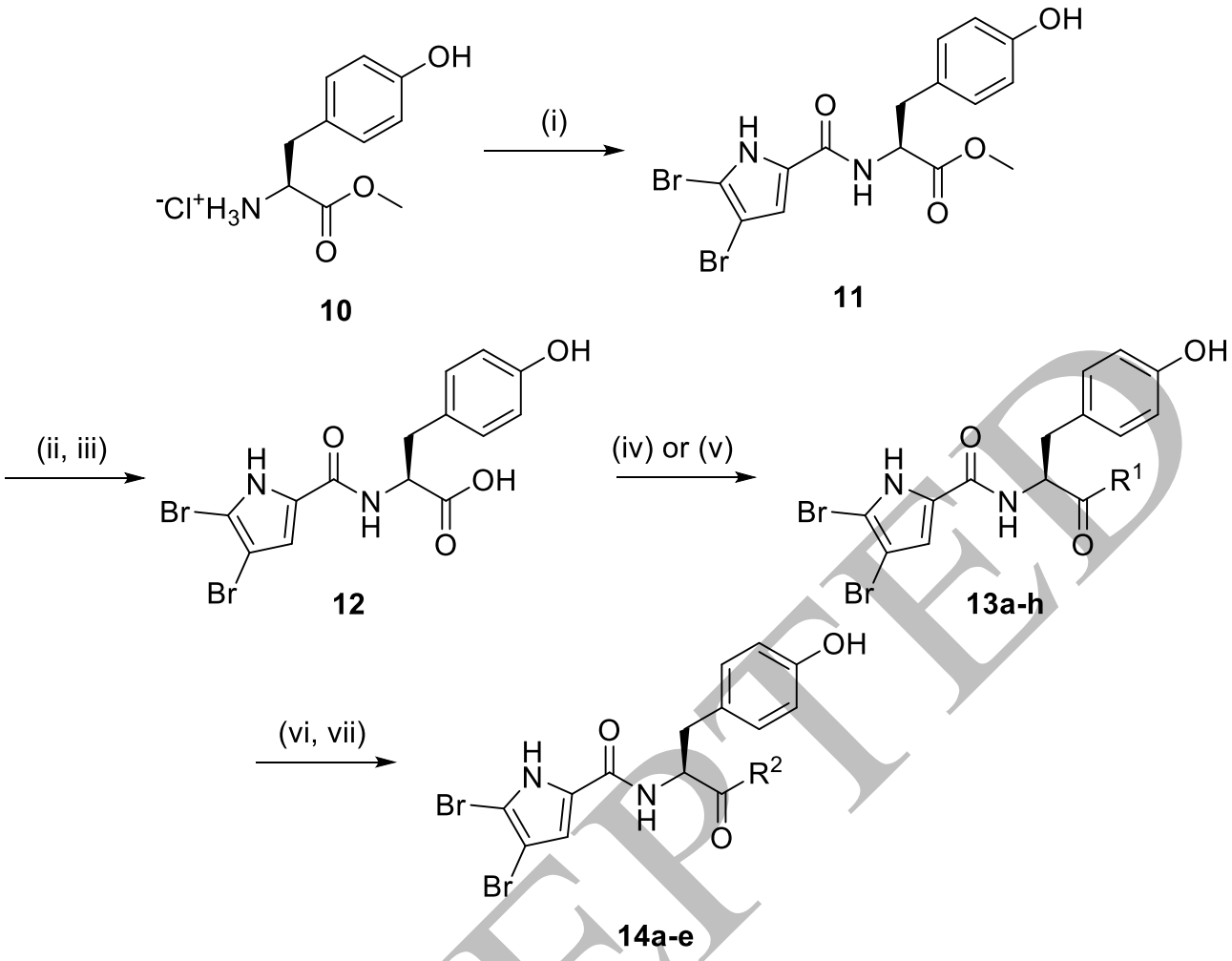


Figure 2. Structure and modifications of the designed inhibitors.



Scheme 1. Reagents and conditions: (i) Boc_2O, dioxane/water 1/1, 0°C, 24h, 90%; (ii) methyl 3-aminobenzoate, DCC, HOBt, CH_2Cl_2/DMF 1/1, 0°C to r.t., 24h, 56%; (iii) $HCl_{(g)}$, THF, 0°C, 20 min, 98%; (iv) for **8a**,**8 c**, **8d**: $RCOCCl_3$, Na_2CO_3, DMF, 40°C, 24h, 20-74%; (v) 4,5-dichloro-2-trichloroacetyl-1*H*-pyrrole (for **8b**), indole-2-carboxylic acid (for **8e**), indazole-3-carboxylic acid (for **8f**), EDC, HOBt, NMM, DMF, 0 °C to r.t., 24h, 39–50%; (vi) 2M LiOH, H_2O/THF=1/1, r.t., 2.5h, 95%, (vii) HCl.



Scheme 2. Reagents and conditions: (i) 4,5-dibromopyrrole-2-carboxylic acid, EDC, HOBt, NMM, DMF, 0 °C to r.t., 16h, 79%; (ii) LiOH, H_2O/THF = 1/1, r.t., 1.5h, 95%, (iii) HCl; (iv) for **13a-d**, **13h**: R^1NH_2, EDC, HOBt, NMM, DMF, 0 °C to r.t., 16h, 45-75%, (v) for **13e-g**: Ar-NH_2, EDC, HOBt, DMAP, NMM, DMF, 0 °C, 20 min to 60 °C, 16h, 14-53%; (vi) 1. 2M LiOH, H_2O/THF, 25 °C, 24h, 95%, (vii) H^+.