Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry 12 (2004) 5115–5131
Bioorganic & Medicinal Chemistry

Combinatorial synthesis of substituted 3-(2-indolyl)piperidines and 2-phenyl indoles as inhibitors of ZipA–FtsZ interaction

Lee D. Jennings,[a,*] Kenneth W. Foreman,[e] Thomas S. Rush, III,[e] Desiree H. H. Tsao,[e] Lidia Mosyak,[e] Scott L. Kincaid,[a] Mohani N. Sukhdeo,[a] Alan G. Sutherland,[a] Weidong Ding,[b] Cynthia Hess Kenny,[b] Chantel L. Sabus,[c] Hanlan Liu,[c] Elizabeth G. Dushin,[d] Soraya L. Moghazeh,[d] Pornpen Labthavikul,[d] Peter J. Petersen,[d] Margareta Tuckman[d] and Alexey V. Ruzin[d]

[a]*Wyeth Research, Department of Medicinal Chemistry, 401 N. Middletown Road, Pearl River, NY 10965, USA*
[b]*Wyeth Research, Department of Biophysics/Enzymology, 401 N. Middletown Road, Pearl River, NY 10965, USA*
[c]*Wyeth Research, Department of Chemical Technology, 401 N. Middletown Road, Pearl River, NY 10965, USA*
[d]*Wyeth Research, Department of Infectious Disease Discovery Research, 401 N. Middletown Road, Pearl River, NY 10965, USA*
[e]*Wyeth Research, Departments of Computational Chemistry and Structural Biology, 35 Cambridge Park Dr., Cambridge, MA 02140, USA*

Received 10 May 2004; revised 13 July 2004; accepted 15 July 2004
Available online 7 August 2004

Abstract—The ZipA–FtsZ protein–protein interaction is a potential target for antibacterial therapy. The design and parallel synthesis of a combinatorial library of small molecules, which target the FtsZ binding area on ZipA are described. Compounds were demonstrated to bind to the FtsZ binding domain of ZipA by HSQC NMR and to inhibit cell division in a cell elongation assay.

1. Introduction

The prokaryotic tubulin analog FtsZ is an essential protein involved in the process of cell division in both Gram-negative and Gram-positive bacteria. Cytoplasmic FtsZ becomes localized in the medial division site of the septal ring at an early stage in the division cycle and remains associated with the leading edge of the ingrowing septum during cytokinesis.[1] Impairment of septal ring assembly and consequent failure of the thermosensitive *ftsZ84* mutant of *Escherichia coli* to divide above the restrictive temperature demonstrates the importance of FtsZ in cell division.[2] FtsZ is nearly universally present in Gram-positive and Gram-negative bacteria and has also been identified in plants and moss.[3]

A search for proteins that bind FtsZ in *E. coli* led to the discovery of a previously unknown membrane-anchored protein called ZipA.[4] ZipA is localized at the site of cell division at a very early stage of the division cycle and tethers the FtsZ protofilaments to the membrane during invagination of the septum. The structure of the C-terminus of ZipA and its interaction with FtsZ has been elucidated by X-ray crystallography.[5] The binding of FtsZ protein to the membrane bound ZipA is necessary for septum formation in *E. coli*, as demonstrated by the formation of nonseptate filaments after the overexpression or depletion of ZipA.[4] Sequence analysis indicates that ZipA homologues are present in *Haemophilus influenzae*, *Salmonella typhimurium*, and *Pseudomonas aeruginosa*[4,6] and a study of the *H. influenzae* genome using high-density transposon mutagenesis identified genes encoding both ZipA and FtsZ as essential for growth and viability.[7] However, proteins with significant homology to ZipA have not been found in Gram-positive bacteria and also appear to be absent in the Gram-negative bacteria *Helicobacter pylori*, *Borrelia burgdorferi*, and *Treponema pallidum*, indicating that

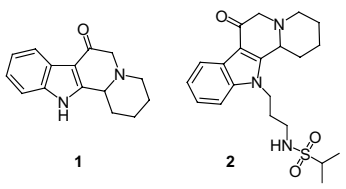
Keywords: ZipA; FtsZ; Bacterial cell division; Protein–protein interaction; Antiinfectives.
* Corresponding author at present address: Chemical and Screening Sciences, Wyeth Research, 401 N. Middletown Road, Pearl River, NY 10965, USA. Tel.: +1-845-602-3618; fax: +1-845-602-3045; e-mail: jenninl@wyeth.com

0968-0896/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2004.07.031

ZipA is conserved in only a subset of Gram-negative genomes.[8]

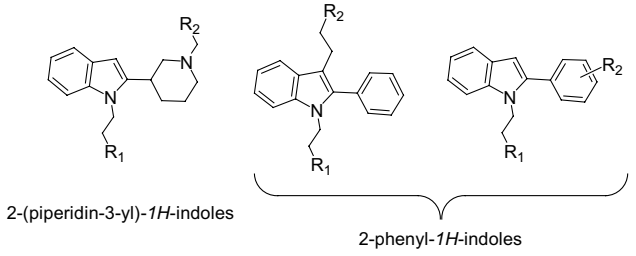
Illnesses caused by respiratory pathogens, in particular *H. influenzae*, *Streptococcus pneumoniae*, and *Moraxella catarrhalis*, are major global public health problems.[9] These pathogens are the causative agents of a variety of life threatening infections and disease states such as bacteremia, chronic bronchitis, Gram-negative bacillary bacteremia, epiglottitis, endocarditis, bacterial meningitis, pneumonia, and sepsis. The appearance of strains of respiratory pathogens resistant to the antibiotics commonly used to eradicate them is becoming more frequent and is a cause of growing public and professional concern.[10] Because ZipA is required for cell division in several Gram-negative bacilli, inhibition of the ZipA–FtsZ interaction represents a promising new target for antibacterial action. We elected to pursue small molecule inhibitors of the binding of FtsZ by identified bacterial ZipA's while at the same time searching for ZipA analogues in Gram-positive pathogens.

The complex between the C-terminal FtsZ binding domain of ZipA ($ZipA_{185–328}$) and the C-terminal fragment of FtsZ ($FtsZ_{367–383}$) has been elucidated both by X-ray crystallography and by NMR,[5,11] facilitating the discovery effort. In this complex, the C-terminal FtsZ peptide forms an α-helix and fills a solvent-exposed hydrophobic cavity on the surface of ZipA. The α-helix conformation of the $FtsZ_{367–383}$ peptide directs only six amino acid sidechains toward important interaction with ZipA. Furthermore, alanine mutagenesis of the $FtsZ_{367–383}$ peptide showed that just three peptide sidechains, contiguous in space in the X-ray structure, account for the bulk of the binding to $ZipA_{185–328}$. These results suggest that a small molecule might interfere with this interface. The location at which molecules bind to the C-terminal domain on ZipA can be determined without resort to X-ray crystallography using 2-D HSQC NMR.[12] Recently, we have reported the discovery of a drug-like small molecule inhibitor of ZipA–FtsZ interaction, 1,2,3,4,12,12b-hexahydro-indolo[2,3-*a*]quinolizin-7-one (**1**; IC_{50} = 1170 μM).[13,14] Derivatization of **1** on the indole nitrogen atom afforded interesting compounds, exemplified by **2**, which we were able to co-crystallize with ZipA but were not significantly more potent than **1**. Here we describe the application of combinatorial synthesis and structure based design to aid our efforts in finding potent inhibitors of the ZipA–FtsZ interaction.

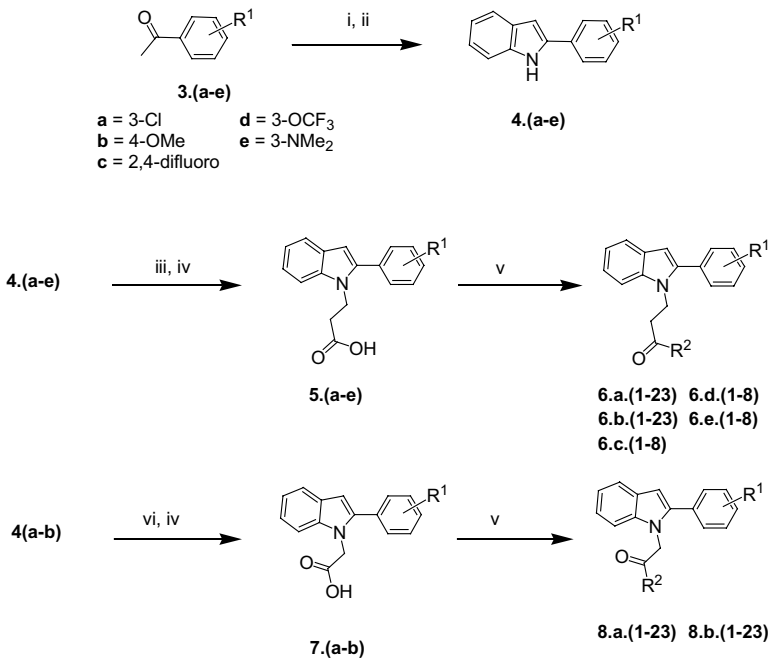


2. Chemistry

The interaction between ZipA and FtsZ is mainly the product of complementary hydrophobic contacts.[5] Binding of FtsZ by ZipA is rather strong, with a K_D estimated to be 0.2 μM.[15] Molecules **1** and **2** were found by X-ray crystallography to occupy one of the same shallow hydrophobic depressions on the surface of ZipA used to bind FtsZ. Inspection of the crystal structures also showed that these small molecule ligands could make hydrogen bonds through bound water molecules to backbone residues in the binding pocket. Our design strategy was to (i) continue to make hydrophobic contact with the FtsZ binding cavity using a lipophilic aromatic scaffold, (ii) include substituents with hydrogen bond donating groups, which would anchor the molecule to the binding surface in a manner of our own design, and (iii) introduce substituents with ionizable groups at their termini to improve the solubility of the molecules. To satisfy our design criteria, the molecules would need to have two or more substituent groups. Because the indoloquinolizinone scaffold provides only one site for synthetic diversification, we decided to modify or replace the indoloquinolizinone scaffold. Deletion of the C ring and repositioning or deletion of the nitrogen atom in the piperidine ring offered the more easily accessible 2-(piperidin-3-yl)-1*H*-indoles and 2-phenyl-1*H*-indoles. These scaffolds were anticipated to cover the hydrophobic pocket in the FtsZ binding domain of ZipA and provide greater synthetic flexibility. Substituents on the piperidine ring or on the indole nucleus would potentially be oriented in the correct direction for making hydrogen bonds to the tightly bound water molecule previously mentioned or be able to cover nearby hydrophobic areas.



The desired target compounds were obtained by way of the following synthetic routes. Phenyl hydrazine was condensed and cyclized with selected acetophenones **3**(**a**–**e**) to afford the 2-phenyl-1*H*-indoles **4**(**a**–**e**) (Scheme 1). Compounds **4**(**a**–**e**) were elaborated to the desired 1-substituted 2-phenyl-1*H*-indoles using standard methods. Amines used in making the final products were selected using the following experimental design process.[16] Molecular property descriptors [molecular weight (mw), (unaligned) dipole moment, *c*log*P* (both Hansch and Leo fragment method), *S*logP (both Ghose and Crippen atom based methods), globularity, Van der Waals volume, and Van der Waals surface area] were calculated for a large set of commercially available amines. Examination of the resulting data set using multivariate data analysis tool SIMCA-P[17] revealed that of these properties, mw, *c*log*P*, *S*log*P*, volume and surface area were highly correlated. Amines were selected using a D-optimal design of the three-dimensional property space made up of the *c*log*P*, dipole moment, and globularity



Scheme 1. Synthesis of 2-phenyl-1*H*-indoles. Reagents and conditions: (i) phenyl hydrazine, EtOH; (ii) polyphosphoric acid, 130 °C; (iii) methyl acrylate, potassium *tert*-butoxide, toluene; (iv) NaOH, MeOH; (v) amine R^2 [see Table 1], EDCI, HOBt, *i*-Pr_2EtN; (vi) methyl bromoacetate, NaH, DMF.

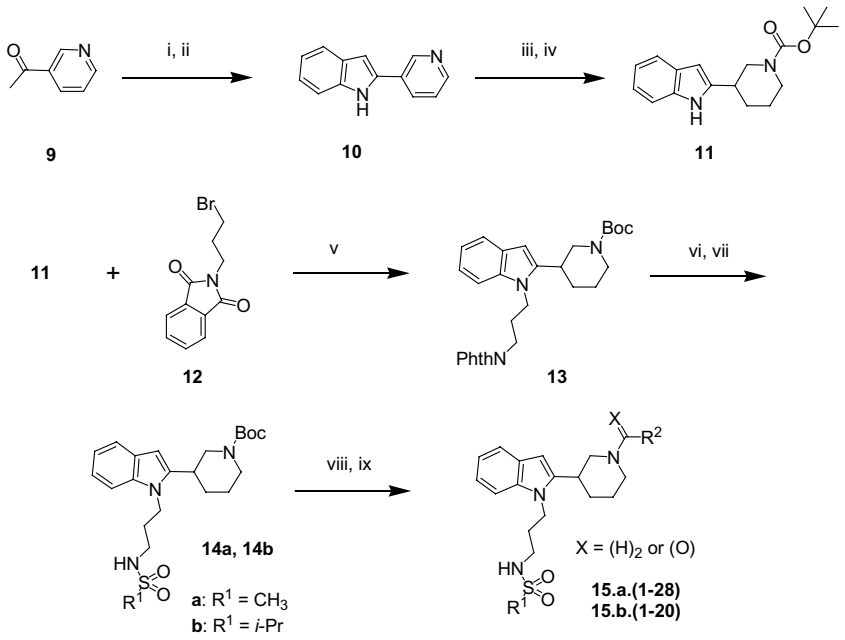
to arrive at a design set having 23 members. This gave a set of amines that was optimally distributed in lipophilicity, polarity, and deviation from sphericity. Intermediates **5.a**, 5**b**, **7.a**, and **7.b**, synthesized from 3-chlorobenzophenone (**3.a**) and 4-methoxyacetophenone (**3.b**), were coupled with the entire set of amines to afford a library of 92 compounds. Intermediates **5.**(**c**–**e**) and **7.**(**c**–**e**) were coupled with a subset of this larger building block set to give a more narrowly focused library of 24 compounds.

Novel 2-(piperidin-3-yl)-1*H*-indoles **15.a.**(**1**–**28**) and **15.b.**(**1**–**20**) were prepared as shown in Scheme 2. Condensation and cyclization of phenyl hydrazine with 3-acetylpyridine (**9**) gave indole **10**. The pyridine ring of **10** was selectively reduced by hydrogenation over platinum and subsequently protected with di(*tert*-butyl)dicarbonate to give **11**. Compound **11** was alkylated with *N*-(3-bromopropyl)phthalimide (**12**) to give **13**, which was deprotected and sulfonylated with either methyl or isopropyl sulfonyl chloride to give **14.a** and **14.b**. Intermediates **14.a** and **14.b** were deprotected with TFA and either alkylated or acylated in parallel on the piperidinyl nitrogen to give *N*-(3-indol-1-yl-propyl)-alkylsulfonamides **15.a.**(**1**–**28**) and **15.b.**(**1**–**20**). Alkyl bromides (R^2CH_2Br) and acid chlorides (R^2COCl) were selected from sets of commercially available reagents and from simple substituted benzyl bromides known in the literature. 1-(3-Aminopropyl)-1*H*-indoles **16.a.**(**1**–**17**) and 3-(indol-1-yl) propionamides **19.a.**(**1**–**20**), **19.b.**(**1**–**21**), **19.c.**(**1**–**21**), and **19.d.**(**1**–**22**) were synthesized by similar routes from intermediate **13** and **11** (Schemes 3 and 4) using the same sets of alkyl bromides and acid chlorides used in the synthesis of alkylsulfonamides **15.a.**(**1**–**28**) and **15.b.**(**1**–**20**). The four amines chosen for use in the synthesis of propionamides **19.a.**(**1**–**20**), **19.b.**(**1**–**21**), **19.c.**(**1**–**21**), and **19.d.**(**1**–**22**) (Scheme 4) were a subset of the 23 diverse amines used in the synthesis of aryl indoles **6.a.**(**1**–**23**) and **6.b.**(**1**–**23**) and were selected based on the observed effectiveness of **6.b.19** (vide infra) in inhibiting binding of FtsZ by ZipA in our fluorescence binding assay.

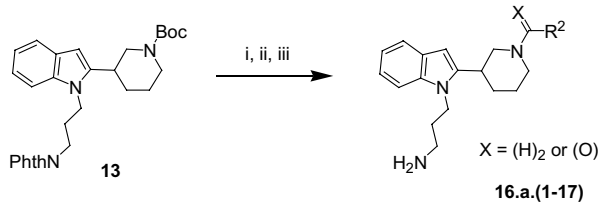
Synthesis of the desired 1,3-disubstituted-2-phenyl indoles **26.a.**(**1**–**4**) began with 4-benzoylbutyric acid **20** (Scheme 5). Fisher indole synthesis with phenyl hydrazine using the procedure of Hutchins and Chapman[18] gave the propionic acid intermediate **21**. Functional group interconversion, protection, alkylation on nitrogen, and Boc-protection on nitrogen gave intermediate **23**. Compound **23** was sequentially deprotected and functionalized to give indoles **26.a.**(**1**–**4**). The amines used in the synthesis of **26.a.**(**1**–**4**) were the same as those used in the synthesis of the 3-(indol-1-yl)-propionamides (Scheme 4). 3-(3-Aminopropyl) substituted indoles **28.a.**(**1**–**21**) were also synthesized from intermediate **23** (Scheme 6). In this case, a new properties-based experimental design was done on a large set of commercially available amines to select a different diverse set of amines for use in the synthesis of **28.a.**(**1**–**21**).

3. Results

New compounds were evaluated for their effect on the binding of FtsZ peptide to ZipA using $ZipA_{185–328}$ and an analog of the C-terminal FtsZ peptide in a fluorescence polarization competition assay.[19] In an initial assay, percent inhibition was measured at three concentrations: 1, 0.5, and 0.125 mM. IC_{50}'s were



Scheme 2. Synthesis of substituted 2-(piperidin-3-yl)-1*H*-indoles. Reagents and conditions: (i) phenyl hydrazine, EtOH; (ii) polyphosphoric acid, 130 °C; (iii) H_2, Pt/C, AcOH; (iv) di(*tert*-butyl)dicarbonate; (v) NaH, DMF, cat. $(n\text{-butyl})_4N^+I^-$; (vi) $H_2NNH_2{\cdot}H_2O$, EtOH; (vii) R^1SO_2Cl, NEt_3, CH_2Cl_2; (viii) trifluoroacetic acid, CH_2Cl_2; (ix) either R^2CH_2Br, Na_2CO_3, acetone, cat. $(n\text{-butyl})_4N^+I^-$, or R^2COCl, Na_2CO_3, CH_2Cl_2.



Scheme 3. Synthesis of substituted 1-(3-aminopropyl)-1*H*-indoles. Reagents and conditions: (i) trifluoroacetic acid, CH_2Cl_2; (ii) either R^2CH_2Br, Na_2CO_3, acetone, cat. $(n\text{-butyl})_4N^+I^-$, or R^2COCl, Na_2CO_3, CH_2Cl_2; (iii) $H_2NNH_2{\cdot}H_2O$, EtOH.

determined for selected compounds by serial dilution of the test compound with 500 nM ZipA and 160 nM fluorescein-FtsZ peptide. Percent inhibition scores and IC_{50}'s for only the most potent members of each analog set are shown.

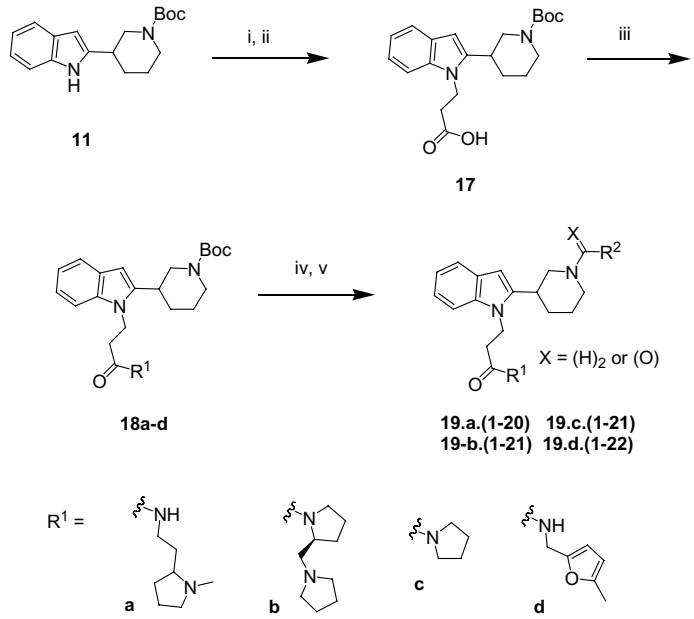
2-Phenyl-1*H*-indoles alkylated on nitrogen were synthesized and assayed first (Table 1). No definitive SAR trends were discernable as few of the compounds inhibited FtsZ binding to a significant extent at the tested concentrations. However, compounds having sidechains containing tertiary amines, for example **6.a.6**, **6.a.7**, **6.a.19**, and particularly **6.b.19**, were represented disproportionately among the compounds with significant percent inhibition scores at 0.5 mM concentration. This information was used for selecting substituent groups and designing targets in subsequent iterations.

No hits were found in the library of *N*-(3-indol-1-yl-propyl)-alkylsulfonamides [**15.a.(1–28)** and **15.b.(1–20)**] (Table 2). However, using the information gained from screening the 2-phenyl-1*H*-indoles, we modified the synthesis to afford a library of indoles having the basic 3-aminopropyl substituent group [**16.a.(1–17)**]. This series contained one of the most active indole ZipA inhibitors in this study, **16.a.4**.

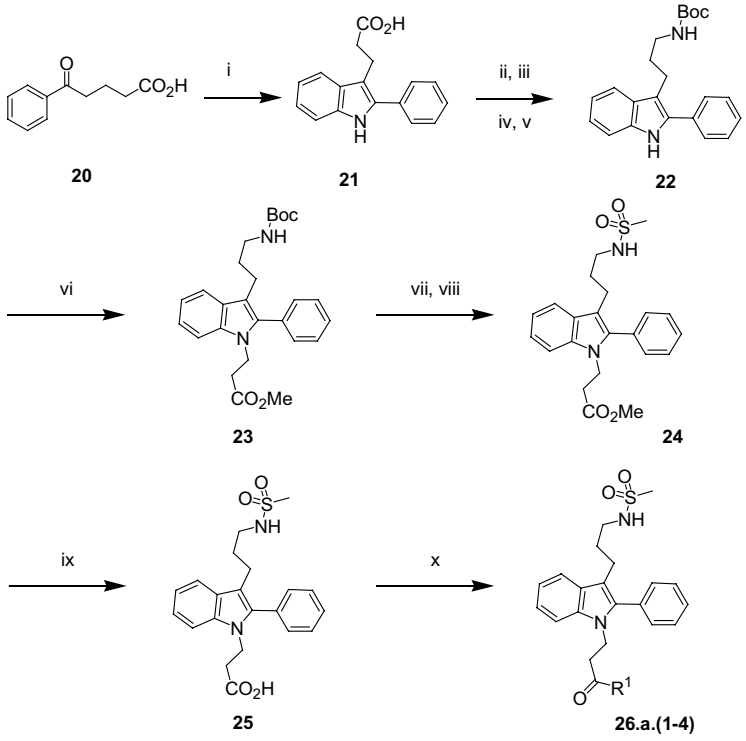
Screening of the library of 3-(indol-1-yl)-propionamides [**19.(a–d).(1–22)**] in the fluorescence polarization assay also showed that the majority of the compounds did not inhibit FtsZ binding to a significant extent at the tested concentrations, with only 15% of the members having percent inhibition greater than 25% at 0.5 mM. Compound **19.b.15** stood out from the others in the initial screen (Table 3) and its activity (IC_{50} = 271 μM) was confirmed by a dose response curve. Again, SAR trends were uncertain, but ZipA–FtsZ inhibition was generally highest with molecules having the basic tertiary amine (*S*)-(+)-1-(2-pyrrolidin-1-ylmethyl)pyrrolidine at R^1 and a lipophilic R^2 group.

2-Phenyl indoles differentially substituted on the 1- and 3-positions were designed to cover the hydrophobic FtsZ binding area, display a sidechain of appropriate length capable of making hydrogen bonds to the bound water molecule, and display a sidechain with a water solubilizing group. Only two compounds showed greater than 25% inhibition at 0.5 mM (Table 4). IC_{50}'s for these were not determined.

In order to verify that compounds were interacting with *E. coli* ZipA in the FtsZ binding region, selected compounds were evaluated for binding to $ZipA_{185–328}$ by a 2-D HSQC NMR experiment using 50 μM ^{15}N-labeled $ZipA_{185–328}$ and 500 μM compound in an aqueous solution containing 10% DMSO. Compound **19.b.15** was



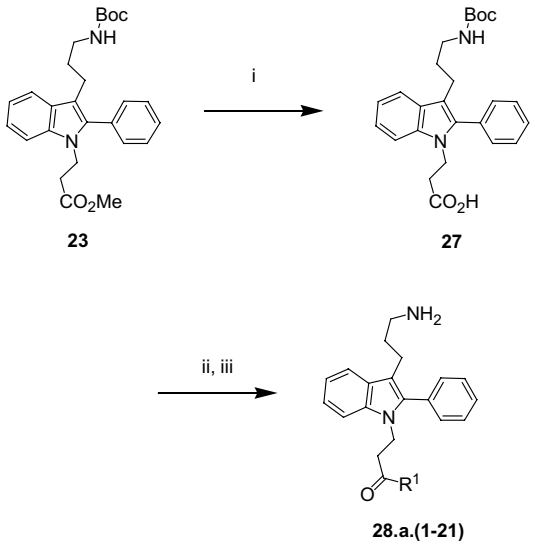
Scheme 4. Synthesis of substituted 3-(indol-1-yl)-propionamides. Reagents and conditions: (i) methyl acrylate, potassium *tert*-butoxide, toluene; (ii) NaOH, MeOH; (iii) amine **a**, **b**, **c**, or **d**, EDCI, HOBt, *i*-Pr_2EtN; (iv) trifluoroacetic acid, CH_2Cl_2; (v) either R^2CH_2Br, Na_2CO_3, acetone, cat. $(n\text{-butyl})_4N^+I^-$ or R^2COCl, Na_2CO_3, CH_2Cl_2.



Scheme 5. Synthesis of 1,3-disubstituted-2-phenyl indoles. Reagents and conditions: (i) phenyl hydrazine, $ZnCl_2$, AcOH; (ii) $SOCl_2$, CH_2Cl_2; (iii) NH_4OH, CH_2Cl_2; (iv) $LiAlH_4$, THF; (v) di(*tert*-butyl)dicarbonate; (vi) methyl acrylate, KO*t*-Bu, toluene, 150 °C, microwave irradiation; (vii) 20% TFA, CH_2Cl_2; (viii) CH_3SO_2Cl, NEt_3, CH_2Cl_2; (ix) NaOH, MeOH; (x) amine **a**, **b**, **c**, or **d**, EDCI, HOBt, *i*-Pr_2EtN.

tested and showed significant perturbation of the nearby protein residues in the FtsZ binding region, indicating that it was binding to ZipA at this site.

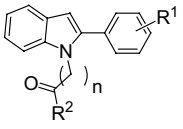
Selected compounds were evaluated for activity as inhibitors of bacterial cell growth, both to correlate in vitro and in vivo activities and to assure that no potential leads were missed. Compounds were tested for activity against bacterial cell growth in two ways. First, compounds were examined by microscopy to see whether or not they caused cell elongation at sublethal concentrations in *Bacillus subtilis* (a Gram-positive bacteria)



Scheme 6. Synthesis of 3-(3-aminopropyl) indoles. Reagents and conditions: (i) NaOH, MeOH; (ii) primary or secondary amine, EDCI, HOBt, *i*-Pr_2EtN; (iii) 20% TFA, CH_2Cl_2.

and in two strains of *E. coli* 390 (inner membrane permeable mutant) and CH4 (a ZipA gene knock-out complemented with ZipA supplied on a thermosensitive plasmid).[4] Nalidixic acid, which acts by a ZipA-independent mechanism, was used as a positive control for cell elongation. Mean values were obtained from light scattering histograms (10,000 total counts/sample, forward light scatter, FACSort flow cytometer, BD Biosciences, San Jose, CA) and the percent change in mean values compared to sample not treated with compound was calculated. Because we have found that percent change values generally reflect the extent of cell elongation, these values were used as an indirect measure of change in cell length. Cytotoxic compounds that caused the formation of cell debris were distinguished from compounds causing cell elongation in this assay visually by both microscopy and histogram shape. Results obtained from testing of **16.a.4** and **19.b.15** in the cell elongation assay (Table 5) are consistent with cell division inhibition at the indicated concentrations in *B. subtilis* and *E. coli* CH4. **16.a.4** was tested at too high a concentration against *E. coli* 390, causing debris for-

Table 1. Inhibition of fluorescein-FtsZ binding to ZipA (185–328) by 2-phenyl-1*H*-indoles



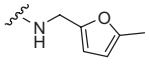
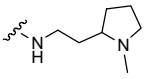
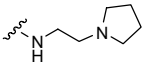
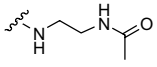
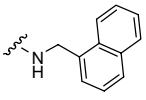
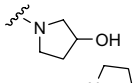
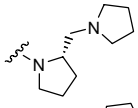
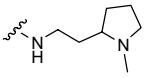
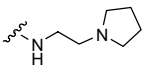
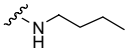
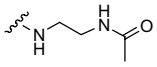
Structure ID	R^1	*n*	R^2	Inhibition at 0.5 mM concn (%)	IC_{50} (μM)
6.a.1	3-Cl	2		23.9	2528
6.a.6	3-Cl	2		21.6	>2000
6.a.7	3-Cl	2		20.4	1627
6.a.12	3-Cl	2		17.8	—
6.a.13	3-Cl	2		19.5	3290
6.a.16	3-Cl	2		22.2	>2000
6.a.19	3-Cl	2		22	3226
6.b.6	4-MeO	2		18.2	—
6.b.7	4-MeO	2		19.3	—
6.b.9	4-MeO	2		18.3	—
6.b.12	4-MeO	2		19.9	2468

Table 1 (*continued*)

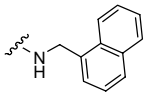
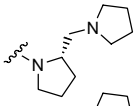
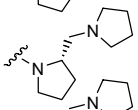
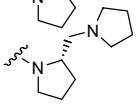
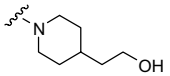
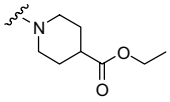
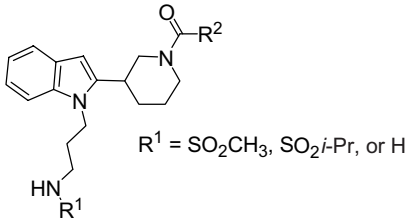
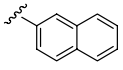
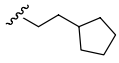
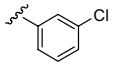
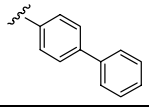
Structure ID	R^1	*n*	R^2	Inhibition at 0.5 mM concn (%)	IC_{50} (μM)
6.b.13	4-MeO	2		17.9	—
6.b.19	4-MeO	2		44.3	—
6.d.5	3-CF_3O	2		17.9	—
8.a.19	3-Cl	1		19	—
8.a.22	3-Cl	1		23.7	>2000
8.a.23	3-Cl	1		19.9	—

Table 2. Inhibition of fluorescein-FtsZ Binding to ZipA (185–328) by *N*-(3-indol-1-yl-propyl)-alkylsulfonamides and 1-(3-aminopropyl)-1*H*-indoles



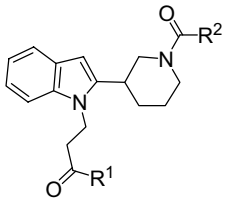
Structure ID	R^1	R^2	Inhibition at 0.5 mM concn (%)	IC_{50} (μM)
15.a.19	SO_2CH_3		93.1	>2000
15.b.17	SO_2i-Pr		94.9	>2000
15.b.18	SO_2i-Pr		40.8	>2000
16.a.3	H		23.7	
16.a.4	H		26.8	296

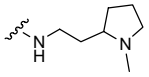
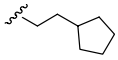
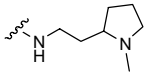
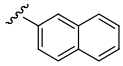
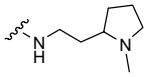
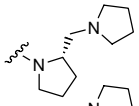
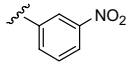
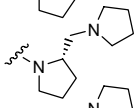
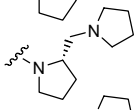
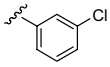
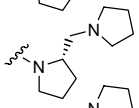
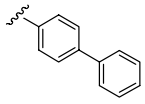
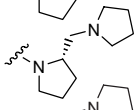
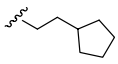
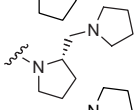
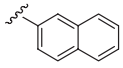
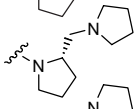
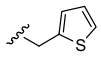
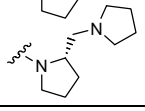
mation (observed by both microscopy and abnormally shaped histogram) and **19.b.15** had no effect on cell division at the tested concentration. Second, an MIC was determined by the microbroth dilution method. Bacterial species in the panel included *S. aureus* (ATCC 29213), *B. subtilis*, *S. pneumoniae*, *H. influenzae* (ATCC 49247), *M. catarrhalis*, and *E. coli*. Results for the most potent inhibitors of FtsZ binding are collected in Table 6. The MIC values are probably consistent with the potency of inhibition of ZipA–FtsZ interaction measured using the fluorescence polarization assay. None of the compounds tested inhibited the growth of *E. coli* (wt) at 200 μM.

4. Conclusion

In several species of Gram-negative bacteria, ZipA, a membrane-anchored protein, must interact with the key cell division protein FtsZ for septum formation and subsequent cell division to take place. The widespread phylogenic distribution of FtsZ, and the identification of ZipA in multiple Gram-negative organisms, supports the idea that inhibition of ZipA–FtsZ is a promising molecular target. Nevertheless, ZipA features several challenges beyond those typically encountered in enzyme targets. Crystal structures reveal a relatively flat and featureless hydrophobic surface that serves as

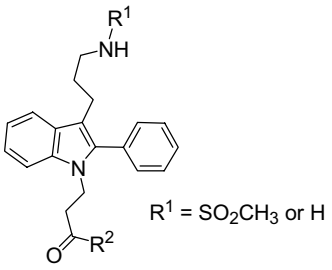
Table 3. Inhibition of fluorescein-FtsZ binding to ZipA (185-328) by substituted 3-(indol-1-yl) propionamides

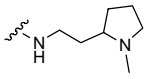
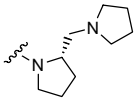
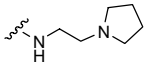
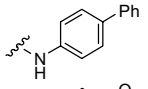
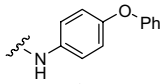
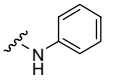
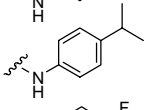
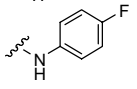
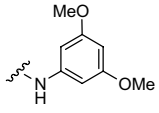


Structure ID	R^1	R^2	Inhibition at 0.5 mM concn (%)	IC_{50} (μM)
19.a.17			29.9	733
19.a.18			29.3	808
19.a.20			33	—
19.b.12			28.2	—
19.b.13			25.8	1070
19.b.14			33.3	
19.b.15			78.1	271
19.b.18			37.7	802
19.b.19			44.3	546
19.b.20			27.7	
19.b.21			34.7	1099

ZipA's interface with FtsZ. These surfaces contrast strikingly with typical enzyme active sites that are deeply invaginated, with a combination of hydrophilic and hydrophobic groups lining the surface. Designing molecules with sufficient binding strength is therefore a challenge, since the maximal affinity of each atom must be quite small.[20] Hence, our challenge was to find optimal arrangements of atoms. The need to maintain a favorable pharmaceutical profile, solubility, specificity, and cell penetration still applied, complicating our efforts. Our best compound, **16.a.4**, (IC_{50} = 296 μM, K_D = 105 μM) did not meet our program goal of an inhibitor of ZipA–FtsZ with a $K_D \sim 10$ μM and in vitro microbial growth inhibition at $\leqslant$16 μg/mL. However, it was demonstrated by NMR that compounds from this series bind to ZipA at the FtsZ binding site and, moreover, cell-based assays indicated that small molecule inhibitors of ZipA–FtsZ could indeed inhibit cell division in both *B. subtilis* and *E. coli*. Cell division inhibitory activity observed against *B. subtilis* might be considered as a hint for existence of ZipA analog(s) in Gram-positive bacteria. Alternatively, such an activity might indicate that these compounds are not specific inhibitors of ZipA–FtsZ interaction in vivo and are acting on other molecular targets. Further investigation is necessary to explore these hypotheses. Although the compounds collected in Table 6 are relatively large (mw 437–589), all were experimentally determined to be at least moderately soluble in water at pH 7.4 (19–50 μg/mL) and highly permeable in an artificial membrane permeability assay (1.3–7.0 Pe $\times 10^{-6}$ cm/sec).[21] The marginally better MIC's observed for the inhibitors tested against the inner membrane permeable *E. coli* mutant (Table 6, column 6) relative to the wild type (MIC > 200 μM) suggest that the weak in vitro activity observed in the antimicrobial susceptibility testing might be due to

Table 4. Inhibition of fluorescein-FtsZ binding to ZipA (185–328) by 1,3-disubstituted-2-phenyl indoles



Structure ID	R^1	R^2	Inhibition at 0.5 mM concn (%)
26.a.1	SO_2CH_3		15.2
26.a.2	SO_2CH_3		17
26.a.3	SO_2CH_3		16.5
28.a.15	H		15.2
28.a.16	H		61.4
28.a.17	H		14.4
28.a.19	H		62.7
28.a.20	H		18
28.a.21	H		17.5

impermeability of the inhibitors but this is not reconcilable with the results from the membrane permeability assay.

Due to the difficult nature of the target site, we hoped that better starting scaffolds could be found. High throughput screening of our compound collection using the fluorescence polarization assay, and similarity searching using the hits that were found by HTS, afforded additional leads. We will report on the discovery and optimization of these leads in another communication.

5. Experimental

NMR data were recorded in $CDCl_3$ on either a 300 or 400 MHz Bruker Avance DRX spectrometer equipped with a *z*-gradient QNP probe. Typical ^1H NMR spectra were acquired with 64 K data points over a spectral width of 8250 Hz. 256 scans were acquired for each spectrum.

Table 5. Effect of **16.a.4** and **19.b.15** on cell division in cell elongation assay

Structure ID	Concn (μg/mL)	%Change in mean[a]	Microscopy[b]
B. subtilis, 168			
16.a.4	12.5 (μM)	127	20% elong
19.b.15	12.5 (μM)	44	10% fil; 50% elong
Nalidixic acid	5	273	100% elong
E. coli, 390			
16.a.4	200 (μM)	72	Debris
19.b.15	25 (μM)	−18	5% elong
Nalidixic acid	5	332	100% elong
E. coli, CH4			
16.a.4	6.25 (μM)	49	20% elong
19.b.15	100 (μM)	39	20% elong
Nalidixic acid	5	301	100% elong

[a] Percent change of histogram mean = $(\text{mean}_x - \text{mean}_c)/\text{mean}_c \times 100\%$, where mean_x represents compound-treated cells and mean_c represents untreated cells.

[b] Visual estimate of the percentage of elongated cells out of total cell population observed in microscope field. 'fil' Stands for 'filamented' and 'elong' stands for 'elongated'.

High resolution mass spectra (HRMS) were obtained using a Bruker (Billerica, MA) APEXII FTICR mass spectrometer equipped with an actively shielded 9.4 T superconducting magnet (Magnex Scientific Ltd, UK), and an external Bruker APOLLO ESI source. The sample was prepared in acetonitrile and flow injected into the mass spectrometer with carrier solvent consisting of 1:1 (v:v) water–acetonitrile (0.25% formic acid) at a flow rate of 50 μL/min. The mass spectrum was externally calibrated using HP tuning mix.

High performance liquid chromatography/mass spectrometry (LC–MS) was performed on a Hewlett–Packard 1100 MSD with a 254 nm DAD detector coupled with a API-ES detector, scanning a mass range between 150 and 700 amu using either a Xterra C_{18} column (inner dimensions 30 mm × 2.1 mm, 5 μm particle size) at 50 °C and a two solvent system of 0.02% aqueous formic acid and 0.02% formic acid in acetonitrile, flowing at a rate of 1.0 mL/min, (Condition A), or Keystone Aquasil column (inner dimensions 50 mm × 2 mm, 5 μm particle size) at 40 °C and a two solvent system of 10 mM aqueous NH_4OAc and acetonitrile, flowing at a rate of 0.8 mL/min (condition B). Preparative high pressure liquid chromatography with automatic fraction collection (RP-HPLC) was performed on a Gilson Semi-Preparative HPLC system with UNIPOINT Software v. 1.71, using a Phenomenex C_{18} Luna column (inner dimensions 21.2 mm × 100 mm, 5 μm particle size) and a two solvent system of 0.05% aqueous NH_4OH and acetonitrile containing 0.05% NH_4OH flowing at 22.5 mL/min. All reactions requiring anhydrous conditions and/or an inert atmosphere were performed under positive nitrogen atmosphere. All solvents and reagents were used as received from commercial suppliers.

Table 6. Antimicrobial susceptibility testing of selected compounds against Gram-positive and Gram-negative bacteria

	S. aureus (+)	*B. subtilis* (+)	*S. pneumoniae* (+)	*H. influenzae* (−)	*M. catarrhalis* (−)	*E. coli* (imp) (−)
Structure						
	MIC (μM)					
16.a.4	25	25	12.5	100	12.5	50
19.a.17	200	200	200	>200	200	200
19.a.18	100	200	100	>200	100	200
19.a.20	200	200	>200	>200	>200	200
19.b.15	100	100	50	>200	50	200
19.b.18	200	100	100	>200	100	200
19.b.19	200	100	100	>200	50	200
26.a.16	50	25	50	100	25	50
26.a.19	50	25	50	100	50	50
	MIC (μg/mL)					
16.a.4	16	16	8	64	16	32
Piperacillin	4	—	2	<0.12	<0.12	<0.12

5.1. Biological methods

The fluorescence polarization competition assay[19] was performed as described in the text, as was the cell elongation assay. Determination of antibacterial activity by the microbroth dilution method was performed following the commonly accepted procedure as described previously.[13,22]

5.2. Physiochemical methods

Parallel artificial membrane permeation assay (PAMPA) was performed as described in the literature.[21] UV absorptions of compounds in the acceptor wells of a UV transparent plate were measured with a SPECTRAMax® 190 microplate spectrophotometer (Molecular Device Corporation, Sunnyvale, CA, USA) at absorption wavelengths between 190 and 500 nm. All the PAMPA experiments were performed on the PSR4p robotic instrument (pION INC., Woburn, MA, USA). PSR4p Command Software (Version 1.6) was used to control the instrument and process the data.

5.3. 2-(3-Chloro-phenyl)-1*H*-indole (4a)

2-(4-Methoxyphenyl)-1*H*-indole (**4b**), 2-(2,4-difluorophenyl)-1*H*-indole (**4c**), 2-(3-trifluoromethoxyphenyl)-1*H*-indole (**4d**), and 2-(3-dimethylaminophenyl)-1*H*-indole (**4e**) were prepared via Fisher indole synthesis following the method reported by Slatt and Bergman for the synthesis of 2-(4-methoxyphenyl)-1*H*-indole.[23]

5.4. 3-[2-(3-Chloro-phenyl)-indol-1-yl]-propionic acid methyl ester

KO*t*-Bu (0.96 g, 7.85 mmol) and tetra-*n*-butyl ammonium iodide (1.32 g, 3.57 mmol) was added to a solution of 2-(3-chloro-phenyl)-1*H*-indole (**4a**) (3.0 g, 13.18 mmol) in 120 mL toluene. The reaction mixture was stirred at room temperature for 40 min upon which time methyl acrylate (3.4 g, 39.53 mmol) was added. The reaction was stirred at room temperature overnight, concentrated, and taken up in 150 mL EtOAc. The solution was washed with brine and dried over K_2CO_3, and the solvent was evaporated to afford 4.38 g (>100%) of the crude product, which was used in the next step without further purification.

5.5. 3-[2-(3-Chloro-phenyl)-indol-1-yl]-propionic acid (5a)

Aqueous NaOH (6.98 mL 10 N) was added to a solution of 3-[2-(3-chloro-phenyl)-indol-1-yl]-propionic acid methyl ester (4.38 g) in 120 mL MeOH. The reaction was stirred at room temperature overnight. The reaction mixture was acidified by addition of 6 N aq HCl and then partitioned between EtOAc and water. The organic phase was dried over K_2CO_3 and concentrated to afford 4.51 g (>100%) of **5a**, which was used in the next step without any purification.

5.6. [2-(3-Chloro-phenyl)-indol-1-yl]-acetic acid (7a)

2-(3-Chloro-phenyl)-1*H*-indole (**4a**) (2.5 g, 10.98 mmol) and tetra-*n*-butyl ammonium iodide (0.25 g, 1.0 mmol) was added to a slurry of NaH (0.63 g) in 109 mL anhydrous DMF. The reaction mixture was stirred at room temperature for 30 min upon which time the reaction was cooled to 0 °C in ice and methyl bromoacetate (1.25 g, 13.18 mmol) was added. The reaction was stirred at room temperature overnight, poured into 600 mL water, and extracted with EtOAc. The organic phase was washed with brine, dried over K_2CO_3, and evaporated to afford 3.74 g of the methyl ester. The ester was hydrolyzed by the same procedure used to prepare 3-[2-(3-chloro-phenyl)-indol-1-yl]-propionic acid (**5a**) to give 3.36 g (>100%) of the title compound.

5.7. General procedure for the preparation of compounds 6.(a–e).(1–23) and 8.(a–b).(1–23)

Indole propionic acid **5**(**a**–**e**) or indole acetic acid **7**(**a**–**b**) (0.05 g, approximately 0.167 mmol depending on MW), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI; 48 mg, 0.251 mmol), 1-hydroxybenzotriazole (HOBt; 34 mg, 0.251 mmol), and diisopropylethyl amine (DIEA; 65 mg, 0.5 mmol) were combined in 2.5 mL DMF and shaken for 15–20 min using an orbital shaker. Amine (0.192 mmol) was added and the reaction was left to shake overnight. PS-Car-

bonate resin (0.661 g, 3 equiv) (Argonaut Technologies, Foster City, CA) was added and the reaction was shaken for a further 5 h. Scavenging of excess amine was confirmed by TLC. The reactions were filtered and concentrated and the crude products were redissolved in either DMSO or MeOH and purified by RP-HPLC.

5.7.1. 3-[2-(3-Chloro-phenyl)-indol-1-yl]-*N*-(5-methyl-furan-2-ylmethyl)-propionamide (6.a.1). (21%); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.22 (s, 3H), 2.45 (t, J = 7.2 Hz, 2H), 4.21 (d, J = 5.6 Hz, 2H), 4.54 (t, J = 7.2 Hz, 2H), 5.65 (br s, 1H), 5.85 (d, J = 2 Hz, 1H), 5.99 (d, J = 2 Hz, 1H), 6.53 (s, 1H), 7.10–7.19 (m, 1H), 7.20–7.30 (m, 1H), 7.31–7.51 (m, 5H), 7.59–7.64 (m, 1H). Anal. Calcd for $C_{23}H_{22}ClN_2O_2^{1+}$: 393.13644. Found 393.13579 ($[M+H]^{1+}$, Δ = −0.65 mmu, ESI-FTMS).

5.7.2. 3-[2-(3-Chloro-phenyl)-indol-1-yl]-*N*-[2-(1-methyl-pyrrolidin-2-yl)-ethyl]-propionamide (6.a.6). (24%); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.40–1.50 (m, 2H), 1.53–1.70 (m, 3H), 1.78–1.88 (m, 1H), 2.07–2.15 (m, 1H), 2.19 (s, 3H), 2.42 (t, J = 7.6 Hz, 2H), 2.95–3.10 (m, 2H), 3.25–3.35 (m, 1H), 4.51 (t, J = 7.6 Hz, 2H), 6.88 (br s, 1H), 7.13 (t, J = 7.4 Hz, 1H), 7.25 (t, J = 7.4 Hz, 1H), 7.35–7.50 (m, 5H), 7.61 (d, J = 8.4 Hz, 1H). Anal. Calcd for $C_{24}H_{29}ClN_3O^{1+}$: 410.19937. Found 410.20007 ($[M+H]^{1+}$, Δ = 0.7 mmu, ESI-FTMS).

5.7.3. 3-[2-(3-Chloro-phenyl)-indol-1-yl]-*N*-(2-pyrrolidin-1-yl-ethyl)-propionamide (6.a.7). (23%); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.72–1.78 (m, 4H), 2.35–2.50 (m, 4H), 2.55–2.75 (m, 4H), 3.19 (q, J = 5.6 Hz, 2H), 4.53 (t, J = 6 Hz, 2H), 6.44–6.50 (br s, 1H), 6.53 (s, 1H), 7.13 (t, J = 7.4 Hz, 1H), 7.24 (t, J = 7.4 Hz, 1H), 7.35–7.42 (m, 3H), 7.45–7.50 (m, 2H), 7.60 (d, J = 8 Hz, 1H). Anal. Calcd for $C_{23}H_{27}ClN_3O^{1+}$: 396.18372. Found 396.18455 ($[M+H]^{1+}$, Δ = 0.83 mmu, ESI-FTMS).

5.7.4. 3-[2-(3-Chloro-phenyl)-indol-1-yl]-*N*-naphthalen-1-ylmethyl-propionamide (6.a.13). (28%); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.44 (t, J = 7.2 Hz, 2H), 4.57 (t, J = 7.6 Hz, 2H), 4.69 (d, J = 5.2 Hz, 2H), 5.63–5.71 (br s, 1H), 6.52 (s, 1H), 7.14 (t, J = 7.4 Hz, 1H), 7.22–7.29 (m, 2H), 7.32–7.49 (m, 4H), 7.43–7.40 (m, 4H), 7.60 (d, J = 8 Hz, 1H), 7.77–7.82 (m, 2H), 7.82–7.87 (m, 1H). Anal. Calcd for $C_{28}H_{24}ClN_2O^{1+}$: 439.15717. Found 439.15677 ($[M+H]^{1+}$, Δ = −0.66 mmu, ESI-FTMS).

5.7.5. 3-[2-(3-Chloro-phenyl)-indol-1-yl]-1-(3-hydroxy-pyrrolidin-1-yl)-propan-1-one (6.a.16). (12%); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.67–1.90 (m, 2H), 2.43–2.53 (m, 2H), 3.0–3.11 (m, 1H), 3.15–3.23 (m, 1H), 3.35–3.47 (m, 2H), 3.48–3.55 (m, 1H), 4.28–4.37 (m, 1H), 4.55–4.62 (m, 2H), 6.56 (s, 1H), 7.10–7.17 (m, 1H), 7.23–7.29 (m, 2H), 7.35–7.48 (m, 3H), 7.49 (s, 1H), 7.62 (d, J = 7.6 Hz, 1H). Anal. Calcd for $C_{21}H_{22}ClN_2O_2^{1+}$: 369.13644. Found 369.13603 ($[M+H]^{1+}$, Δ = −0.41 mmu, ESI-FTMS).

5.7.6. 3-[2-(3-Chloro-phenyl)-indol-1-yl]-1-(2-pyrrolidin-1-ylmethyl-pyrrolidin-1-yl)-propan-1-one (6.a.19). (22%); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.60–2.05 (m, 8H), 2.18–2.26 (m, 1H), 2.18–2.23 (m, 1H), 2.47 (t, J = 5.4 Hz, 2H), 2.63–2.73 (m, 1H), 2.90–2.98 (m, 1H), 3.10–3.15 (m, 1H), 3.25–3.30 (m, 1H), 3.31 (t, J = 5.4 Hz, 1H), 4.13 (br s, 1H), 4.56 (t, J = 5.4 Hz, 2H), 4.60–4.70 (m, 1H), 6.56 (d, J = 3.2 Hz, 1H), 7.14 (t, J = 6.4 Hz, 1H), 7.21–7.29 (m, 2H), 7.35–7.50 (m, 4H), 7.62 (d, J = 7.6 Hz, 1H). Anal. Calcd for $C_{26}H_{31}ClN_3O^{1+}$: 436.21502. Found 436.21363 ($[M+H]^{1+}$, Δ = −1.39 mmu, ESI-FTMS).

5.7.7. *N*-(2-Acetylamino-ethyl)-3-(2-phenyl-indol-1-yl)-propionamide (6.b.12). (17%); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.86 (s, 3H), 2.39 (t, J = 6.8 Hz, 2H), 3.02–3.08 (m, 2H), 3.10–3.16 (m, 2H), 3.88 (s, 3H), 4.53 (t, J = 6.8 Hz, 2H), 5.48 (br s, 1H), 5.7 (br s, 1H), 6.50 (s, 1H), 7.01 (d, J = 8.8 Hz, 2H), 7.13 (t, J = 5.4 Hz, 1H), 7.22 (t, J = 5.4 Hz, 1H), 7.42 (d, J = 8.8 Hz, 2H), 7.46 (d, J = 7.6 Hz, 1H), 7.60 (d, J = 7.6 Hz, 1H). Anal. Calcd for $C_{22}H_{26}N_3O_3^{1+}$: 380.19687. Found 380.19634 ($[M+H]^{1+}$,Δ = −0.53 mmu, ESI-FTMS).

5.7.8. 2-[2-(3-Chloro-phenyl)-indol-1-yl]-1-(2-pyrrolidin-1-ylmethyl-pyrrolidin-1-yl)-ethanone (8.a.19). (24%), A mixture of *cis* and *trans* isomers inseparable by chromatography; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.6–1.8 (m, 6H), 1.85–2.10 (m, 6H), 2.40–2.55 (m, 2H), 2.55–2.70 (m, 2H), 3.28–3.43 (m, 1H), 3.45–3.55 (m, 0.5H), 3.63–3.70 (m, 0.5H), 3.95 (q, J = 6 Hz, 0.5H), 4.29–4.37 (m, 0.5H), 4.74 (s, 1H), 4.78 (d, J = 18 Hz, 0.5H), 5.2 (d, J = 18 Hz, 0.5H), 6.6 (d, J = 5.6 Hz, 1H), 7.10–7.16 (m, 1H), 7.19–7.23 (m, 2H), 7.33–7.47 (m, 3H), 7.5 (d, J = 13.2 Hz, 1H), 7.63 (d, J = 7.6 Hz, 1H). Anal. Calcd for $C_{25}H_{29}ClN_3O^{1+}$: 422.19937. Found 422.1982 ($[M+H]^{1+}$, Δ = −1.17 mmu, ESI-FTMS).

5.7.9. 2-[2-(3-Chloro-phenyl)-indol-1-yl]-1-[4-(2-hydroxy-ethyl)-piperidin-1-yl]-ethanone (8.a.22). (20%); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.94–1.06 (m, 1H), 1.10–1.21 (m, 1H), 1.50 (q, J = 6.4 Hz, 2H), 1.55–1.80 (m, 5H), 2.59–2.64 (m, 1H), 3.03 (t, J = 12 Hz, 1H), 3.65 (t, J = 6.4 Hz, 2H), 4.59 (d, J = 13.2 Hz, 1H), 4.83 (d, J = 2 Hz, 2H), 6.62 (s, 1H), 7.10–7.16 (m, 1H), 7.21–7.23 (m, 2H), 7.36–7.40 (m, 3H), 7.45 (d, J = 1.6 Hz, 1H), 7.63 (d, J = 7.6 Hz, 1H). Anal. Calcd for $C_{23}H_{26}ClN_2O_2^{1+}$: 397.16774. Found 397.16743 ($[M+H]^{1+}$, Δ = −0.31 mmu, ESI-FTMS).

5.7.10. 1-{2-[2-(3-Chloro-phenyl)-indol-1-yl]-acetyl}-piperidine-4-carboxylic acid ethyl ester (8.a.23). (25%); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.27 (t, J = 7.2 Hz, 3H), 1.50–1.70 (m, 2H), 1.83–2.0 (m, 2H), 2.50–2.60 (m, 1H), 2.90–3.0 (m, 1H), 3.10–3.20 (m, 1H), 3.70 (d, J = 13.6 Hz, 1H), 4.17 (q, J = 7.2 Hz, 2H), 4.36 (d, J = 13.6 Hz, 1H), 4.83 (s, 2H), 6.62 (s, 1H), 7.14 (t, J = 6.8 Hz, 1H), 7.19–7.23 (m, 2H), 7.37 (s, 3H), 7.45 (s, 1H), 7.63 (d, J = 8 Hz, 1H). Anal. Calcd for $C_{24}H_{26}ClN_2O_3^{1+}$: 425.16265. Found 425.16157 ($[M+H]^{1+}$, Δ = −1.08 mmu, ESI-FTMS).

5.8. *N*-Phenyl-N′-(1-pyridin-3-yl-ethylidene)-hydrazine

3-Acetylpyridine (31.1 mL, 277.1 mmol) was added to a solution of phenyl hydrazine (27.3 mL, 277.4 mmol) in ethanol (45 mL). The reaction mixture was refluxed with

stirring for 45 min. After cooling to room temperature, the solid was filtered and washed. The solid was dried to yield a yellow solid of *N*-phenyl-*N*′-(1-pyridin-3-yl-ethylidene)-hydrazine (52.2 g, 247 mmol, 89%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.27 (s, 3H), 6.88–6.96 (m, 1H), 7.17–7.33 (m, 4H), 7.46 (s, 1H), 8.12 (dd, J = 8.1, 1.9 Hz, 1H), 8.53 (dd, J = 4.7, 1.4 Hz, 1H), 8.99 (d, J = 2.1 Hz, 1H).

5.9. 2-Pyridin-3-yl-1*H*-indole (10)

The hydrazine obtained from the previous step (9.1 g, 43.12 mmol) was mixed with polyphosphoric acid (PPA) (30 mL) to form a paste. The reaction mixture was heated with manual stirring to 180 °C. The mixture was then removed from heat and cooled to 100 °C before pouring slowly into water (600 mL). At this point, the yellowish solid was filtered and washed. The solid was suspended in 1 N NaOH (600 mL) and the mixture was extracted four times with ethyl acetate. The organic extracts were evaporated to give the indole (7.26 g, 37.4 mmol, 86%) as a yellow solid. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.88 (s, 1H), 7.13–7.25 (m, 2H), 7.30–7.40 (m, 2H), 7.65 (d, J = 7.8 Hz, 1H), 7.95 (d, J = 7.0 Hz, 1H), 8.53 (dd, J = 4.8 Hz, 1.5 Hz 1H), 8.97 (d, J = 1.7 Hz 1H), 9.10 (s, 1H).

5.10. 2-Piperidin-3-yl-1*H*-indole

Acetic acid (210 mL) and 2-pyridin-3-yl-1*H*-indole (6.14 g, 31.61 mmol) were added to 5% wt. Palladium on carbon (3.00 g, 1.847 mmol) in a dry par bottle. The slurry was hydrogenated at 50 psi for 3–4 days in a Parr hydrogenation apparatus. The slurry was filtered through fluted filter paper to remove the catalyst and the filtrate was concentrated to give an oil. The oil was then redissolved in ethyl acetate and 10 N aqueous NaOH was added until the pH of the solution was about 12. The alkaline solution was extracted twice with 600 mL of ethyl acetate. The combined organic extracts were washed once with 800 mL water, once with 800 mL saturated aqueous NaCl and dried over K_2CO_3. The solvent was evaporated to yield 2-piperidin-3-yl-1*H*-indole (4.63 g, 23.1 mmol, 73%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.55–1.62 (m, 2H), 1.70–1.83 (m, 2H), 2.0–2.14 (m, 2H), 2.85–3.0 (m, 2H), 3.25 (d, J = 9.1 Hz, 1H), 6.23 (s, 1H), 7.03–7.14 (m, 2H), 7.30 (d, J = 7.8 Hz, 1H), 7.54 (d, J = 7.5 Hz, 1H), 8.51 (s, 1H); *m*/*z* 201.1 [M+H].

5.11. 3-(1*H*-Indol-2-yl)-piperidine-1-carboxylic acid *tert*-butyl ester (11)

Di-*tert*-butyl dicarbonate (20.7 mL, 20.64 mmol) was added to a solution of 2-piperidin-3-yl-1*H*-indole (6.2 g, 30.96 mmol) in anhydrous THF (30 mL) at 0 °C. The reaction mixture was stirred overnight at room temperature. The mixture was then poured into 400 mL of water and aqueous layer was extracted 3 × 250 mL ethyl acetate. The combined organic extracts were washed with 1 × 300 mL water, 1 × 300 mL saturated aqueous NaCl, dried over K_2CO_3, and evaporated. The residue was purified on silica gel using 0–20% ethyl acetate/hexane to yield 2.42 g (8.06 mmol, 26%) of the ester **11**. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.48 (s, 9H), 1.6–1.8 (m, 2H), 2.10–2.35 (m, 2H), 2.85–3.35 (m, 4H), 3.43–3.80 (m, 1H), 6.28 (s, 1H), 7.04–7.15 (m, 2H), 7.32 (d, J = 7.3 Hz, 1H), 7.54 (d, J = 7.3 Hz, 1H), 9.10 (s, 1H); *m*/*z* 301.1 [M+H].

5.12. 3-{1-[3-(1,3-Dioxo-1,3-dihydro-isoindol-2-yl)-propyl]-1*H*-indol-2-yl}-piperidine-1-carboxylic acid *tert*-butyl ester (13)

3-(1*H*-Indol-2-yl)-piperidine-1-carboxylic acid *tert*-butyl ester (21.1 g, 72.9 mmol) was added to a stirred suspension of NaH (4.4 g, 109.4 mmol) in 110 mL anhydrous DMF at 0 °C. After stirring for 10 min, tetra-*n*-butyl ammonium iodide (6.7 g, 18.2 mmol) and *N*-(3-bromopropyl)phthalimide (25.4 g, 94.8 mmol) (**12**) were added. The reaction was stirred at room temperature overnight. The mixture was then poured into 300 mL of water and the aqueous layer was extracted three times with 150 mL ethyl acetate. The combined organic extracts were washed 1 × 100 mL water, 1 × 100 mL saturated aqueous NaCl, dried over K_2CO_3 and evaporated. The residue was purified on silica gel using 0–25% ethyl acetate/hexane to yield 18.62 g (38.2 mmol, 52%) of **13**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.45 (s, 9H), 1.45–1.8 (m, 4H), 2.11–2.22 (m, 3H), 2.7–2.9 (m, 3H), 3.73–3.82 (m, 2H), 4.24 (t, J = 7.7 Hz, 2H), 6.27 (s, 1H), 7.05 (d, J = 7.7 Hz, 1H), 7.14 (d, J = 7.7 Hz, 1H), 7.28 (s, 1H), 7.52 (d, J = 7.7 Hz, 1H), 7.71–7.74 (m, 2H), 7.83–7.86 (m, 2H); *m*/*z* 488.1 [M+H].

5.13. 3-[1-(3-Amino-propyl)-1*H*-indol-2-yl]-piperidine-1-carboxylic acid *tert*-butyl ester

Hydrazine (0.46 g, 14.3 mmol) was added to a solution of 4.65 g (9.5 mmol) ester **13** in 15 mL *n*-butanol. The reaction was heated to 75 °C for 18 h. The solvent was evaporated. The residue was partitioned between ethyl acetate and water. The layers were separated and the aqueous layer extracted three times with ethyl acetate. The organic layers were combined and washed one time with saturated aqueous NaCl, and dried over K_2CO_3. The solvent was evaporated to yield 3-[1-(3-amino-propyl)-1*H*-indol-2-yl]-piperidine-1-carboxylic acid *tert*-butyl ester, 3.84 g (10.7 mmol, >100%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.46 (s, 9H), 1.66–1.8 (m, 3H), 2.04–2.15 (m, 3H), 2.67–2.87 (m, 5H), 4.10–4.27 (m, 4H), 5.0 (br s, 2H), 6.27 (s, 1H), 7.03–7.17 (m, 2H), 7.32 (d, J = 8.1 Hz, 1H), 7.54 (d, J = 7.7 Hz, 1H); *m*/*z* 358.2 [M+H].

5.14. 3-[1-(3-Methanesulfonylamino-propyl)-1*H*-indol-2-yl]-piperidine-1-carboxylic acid *tert*-butyl ester (14a)

Triethylamine (5.9 mL, 42.5 mmol) was added to a suspension of 3-[1-(3-amino-propyl)-1*H*-indol-2-yl]-piperidine-1-carboxylic acid *tert*-butyl ester (3.8 g, 10.6 mmol) in dichloromethane (50 mL). Methanesulfonyl chloride (1.65 mL, 21.2 mmol) was then added slowly to the mixture. The reaction was stirred overnight and was poured into 100 mL of water. The layers were separated and the organic layer washed one time with 100 mL water and twice with 100 mL aqueous $NaHCO_3$, dried over K_2CO_3 and evaporated to yield 3-[1-(3-methanesulfonyl-

amino-propyl)-1*H*-indol-2-yl]-piperidine-1-carboxylic acid *tert*-butyl ester (4.6 g, 10.5 mmol, 100%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.81–1.27 (m, 1H), 1.51 (s, 9H), 1.55–1.70 (m, 2H), 1.73–1.89 (m, 2H), 1.99–2.09 (m, 1H), 2.1–2.21 (m, 1H), 2.22–2.41 (m, 1H), 2.46–2.62 (m, 1H), 2.79–3.0 (m, 4H), 3.14–3.43 (m, 2H), 4.12–4.45 (m, 4H), 6.02 (s, 1H), 6.31 (s, 1H), 7.09 (t, J = 7.7 Hz, 1H), 7.18 (td, J = 8.0, 1.0 Hz, 1H), 7.30 (d, J = 8.2 Hz, 1H), 7.56 (d, J = 7.8 Hz, 1H); *m/z* 436.1 [M+H].

5.15. 3-[1-(3-*iso*-Propylsulfonylamino-propyl)-1*H*-indol-2-yl]-piperidine-1-carboxylic acid *tert*-butyl ester (14b)

Compound **14b** was prepared by an analogous procedure from 3-[1-(3-amino-propyl)-1*H*-indol-2-yl]-piperidine-1-carboxylic acid *tert*-butyl ester and isopropylsulfonyl chloride.

5.16. *N*-[3-(2-Piperidin-3-yl-indol-1-yl)-propyl]-methanesulfonamide

Trifluoroacetic acid (25 mL, 325 mmol) was added to a suspension of **14a** (4.6 g, 10.6 mmol) in dichloromethane (25 mL) at 0 °C. The reaction was stirred at 0 °C for 0.5 h. The reaction was evaporated and the residue was partitioned between dichloromethane and water. The layers were separated and the aqueous layer extracted once with CH_2Cl_2. Organic layers were combined and dried over K_2CO_3. The solvent was evaporated to yield the title compound (3.6 g, 10.6 mmol, 100%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.72–2.13 (m, 8H), 2.57–2.76 (m, 2H), 2.88 (s, 3H), 3.07–3.28 (m, 4H), 3.35–3.43 (m, 1H), 4.26 (t, J = 7.4 Hz, 2H), 6.30 (s, 1H), 7.08 (t, J = 7.1 Hz, 1H), 7.17 (t, J = 8.0 Hz, 1H), 7.27 (d, J = 7.7 Hz, 1H), 7.55 (d, J = 7.7 Hz, 1H).

5.17. General procedure for the preparation of compounds 15.a.(1–28)

(*Condition A*) Sodium carbonate (20.67 mg, 0.2 mmol), tetrabutylammonium iodide (5.54 mg, 0.02 mmol), and 0.2 mmol of the requisite alkyl halide was added to a solution of *N*-[3-(2-piperidin-3-yl-indol-1-yl)-propyl]-methanesulfonamide (0.15 mmol) in acetone (2 mL). The reaction was stirred at 50 °C for 18 h. The solvents were evaporated. The residue was dissolved in 1.5 mL DMSO and purified by RP-HPLC. (*Condition B*) To a solution of *N*-[3-(2-piperidin-3-yl-indol-1-yl)-propyl]-methanesulfonamide (0.15 mmol) in dichloromethane (2 mL) was added sodium carbonate (63.6 mg, 0.6 mmol), and 0.3 mmol of the requisite acid chloride. The reaction was stirred at 50 °C for 18 h. The solvents were evaporated and the residue was dissolved in 1.5 mL DMSO and purified by RP-HPLC.

Compounds **15.b.(1–20)** were prepared by an analogous procedure using 3-[1-(3-*iso*-propylsulfonylamino-propyl)-1*H*-indol-2-yl]-piperidine-1-carboxylic acid *tert*-butyl ester (**14b**).

5.17.1. *N*-(3-{2-[1-(Naphthalene-2-carbonyl)-piperidin-3-yl]-indol-1-yl}-propyl)-methane sulfonamide (15.a.19). (28%); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.59–1.72 (m, 1H), 1.72–1.81 (m, 1H), 2.02–2.15 (m, 1H), 2.22 (d, J = 14.9 Hz, 1H), 2.25–2.38 (m, 1H), 2.75 (t, J = 14 Hz, 2H), 2.99 (s, 3H), 3.11–3.30 (m, 3H), 3.31–3.42 (m, 1H), 3.98 (d, J = 14 Hz, 1H), 4.30–4.40 (m, 1H), 4.50–4.60 (m, 1H), 4.83 (d, J = 14 Hz, 1H), 6.39 (s, 1H), 6.52 (br s, 1H), 7.10 (t, J = 7.9 Hz, 1H), 7.20 (t, J = 7.9 Hz, 1H), 7.36 (d, J = 8 Hz, 1H), 7.54–7.63 (m, 4H), 7.89–7.94 (m, 3H), 8.10 (s, 1H). Anal. Calcd for $C_{28}H_{32}N_3O_3S^{1+}$: 490.21589. Found 490.21458 ($[M+H]^{1+}$, Δ = −1.31 mmu, ESI-FTMS).

5.17.2. Propane-2-sulfonic acid (3-{2-[1-(3-methyl-butyryl)-piperidin-3-yl]-indol-1-yl}-propyl)-amide (15.b.17). (17%); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.96 (d, J = 4.8 Hz, 3H), 1.00 (d, J = 4.8 Hz, 3H), 1.37–1.40 (m, 6H), 1.68–1.80 (m, 1H), 1.85–2.0 (m, 2H), 2.10–2.45 (m, 5H), 2.90–3.02 (m, 1H), 3.09–3.40 (m, 4H), 4.0 (d, J = 14 Hz, 1H), 4.20–4.32 (m, 1H), 4.38–4.48 (m, 1H), 4.77 (d, J = 14 Hz, 1H), 6.22 (d, J = 7.4 Hz, 1H), 6.30 (s, 1H), 7.07 (t, J = 7.9 Hz, 1H), 7.17 (t, J = 7.9 Hz, 1H), 7.30 (d, J = 8 Hz, 1H), 7.55 (d, J = 8 Hz, 1H). Anal. Calcd for $C_{24}H_{38}N_3O_3S^{1+}$: 448.26284. Found 448.26155 ($[M+H]^{1+}$, Δ = −1.29 mmu, ESI-FTMS).

5.17.3. Propane-2-sulfonic acid (3-{2-[1-(3-cyclopentyl-propionyl)-piperidin-3-yl]-indol-1-yl}-propyl)-amide (15.b.18). (14%); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.06–1.18 (m, 2H), 1.37–1.40 (m, 6H), 1.50–1.80 (m, 10H), 1.83–2.0 (m, 3H), 2.18–2.30 (m, 2H), 2.30–2.40 (m, 3H), 2.90–3.05 (m, 1H), 3.10–3.40 (m, 4H), 3.98 (d, J = 14 Hz, 1H), 4.20–4.30 (m, 1H), 4.40–4.50 (m, 1H), 4.75 (d, J = 14 Hz, 1H), 6.40 (d, J = 9 Hz, 1H), 6.30 (s, 1H), 7.07 (t, J = 7.9 Hz, 1H), 7.17 (t, J = 7.9 Hz, 1H), 7.30 (d, J = 8 Hz, 1H), 7.55 (d, J = 8 Hz, 1H). Anal. Calcd for $C_{27}H_{42}N_3O_3S^{1+}$: 488.29414. Found 488.29299 ($[M+H]^{1+}$, Δ = −1.15 mmu, ESI-FTMS).

5.18. 2-[3-(2-Piperidin-3-yl-indol-1-yl)-propyl]-isoindole-1,3-dione

Trifluoroacetic acid (25 mL, 325 mmol) was added to a solution of **13** (4.65 g, 9.5 mmol) in dichloromethane (25 mL) at 0 °C. The reaction was stirred at 0 °C for 0.5 h. The reaction was concentrated and the residue was partitioned between dichloromethane and water. The layers were separated and the aqueous layer extracted once with dichloromethane. The organic layers were combined and dried over K_2CO_3. The solvent was evaporated to yield the title compound. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.57–1.81 (m, 3H), 2.04–2.21 (m, 3H), 2.34 (s, 1H), 2.64–2.76 (m, 2H), 2.82–2.89 (m, 1H), 3.12 (d, J = 12.1 Hz, 1H), 3.29 (d, J = 11.6 Hz, 1H), 3.78 (t, J = 7.1 Hz, 2H), 4.20 (d, J = 7.7 Hz, 1H), 6.26 (s, 1H), 7.06 (t, J = 1.0 Hz, 1H), 7.12 (t, J = 7.1 Hz, 1H), 7.25 (d, J = 9.1 Hz, 2H), 7.51 (d, J = 7.6 Hz, 1H), 7.70–7.73 (m, 2H), 7.82–7.85 (m, 2H).

5.19. General procedure for the preparation of compounds 16.a.(1–17)

(*Condition A*) Sodium carbonate (80.55 mg, 0.76 mmol) and 0.38 mmol of the requisite acid chloride was added

to a solution of 2-[3-(2-piperidin-3-yl-indol-1-yl)-propyl]-isoindole-1,3-dione (73.62 mg, 0.19 mmol) in dichloromethane (0.75 mL). The reaction was stirred at 50 °C for 18 h and the solution was concentrated. The crude phthalimide was dissolved in n-butanol (1.5 mL) and 9 μL (0.29 mmol) hydrazine was then added. The reaction was heated to 75 °C for 18 h. The solvent was evaporated and the residue was dissolved in 1.5 mL DMSO and purified by RP-HPLC. (*Condition B*) To a solution of 2-[3-(2-piperidin-3-yl-indol-1-yl)-propyl]-isoindole-1,3-dione (73.62 mg, 0.19 mmol) in dichloromethane (2 mL) was added sodium carbonate (63.6 mg, 0.6 mmol), and 0.3 mmol of the requisite acid chloride. The reaction was stirred at 50 °C for 18 h and the solution was concentrated. The crude phthalimide was dissolved in *n*-butanol (1.5 mL) and 9 μL (0.29 mmol) hydrazine was then added. The reaction was heated to 75 °C for 18 h. The solvent was evaporated and the residue was dissolved in 1.5 mL DMSO and purified by RP-HPLC.

5.19.1. {3-[1-(3-Amino-propyl)-1*H*-indol-2-yl]-piperidin-1-yl}-biphenyl-4-yl-methanone (16.a.4). (29%); ^{1}H NMR ($CDCl_3$–CF_3CO_2D 300 MHz) δ 1.60–1.78 (m, 1H), 1.80–2.05 (m, 3H), 2.06–1.37 (m, 2H), 2.74 (t, J = 12 Hz, 1H), 2.95–3.17 (m, 2H), 3.24 (t, J = 12 Hz, 1H), 3.83–3.87 (m, 1H), 3.95 (d, J = 14 Hz, 1H), 4.20–4.40 (m, 2H), 4.75 (d, J = 14 Hz, 1H), 6.36 (s, 1H), 7.07–7.25 (m, 3H), 7.35–7.50 (m, 5H), 7.54–7.62 (m, 5H), 7.72–7.9 (br s, 2H). Anal. Calcd for $C_{29}H_{33}N_3O^{1+}$: 438.25399. Found 438.25405 ([M+H]$^{1+}$, Δ = 0.06 mmu, ESI-FTMS).

5.20. 3-[1-(2-methoxycarbonyl-ethyl)-1*H*-indol-2-yl]-piperidine-1-carboxylic acid *tert*-butyl ester

Potassium *tert*-butoxide (3.29 mL, 26.94 mmol) and a catalytic amount of tetra-*n*-butylammonium iodide was added to a solution **11** (13.49 g, 44.91 mmol) in 150 mL of toluene. The mixture was stirred at room temperature for 20 min, at which time methyl acrylate (12.13 mL, 134.72 mmol) was added and the reaction was stirred at room temperature over three nights. The reaction mixture was then heated to 65 °C for 4 h, upon which time the reaction mixture was concentrated and the residue redissolved in ethyl acetate. The organic layer was washed with water and aqueous NaCl, dried over K_2CO_3, and concentrated to give the title compound (14.76 g, 38.2 mmol, 85%) as a brown oil which solidified overnight when stored under vacuum: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.45–1.52 (m, 2H), 1.52 (s, 9H), 1.60–1.73 (m, 3H), 1.73–1.86 (m, 1H), 2.10–2.19 (m, 1H), 2.72–2.90 (m, 3H), 3.68 (s, 3H), 4.10–4.33 (m, 1H), 4.42–4.57 (m, 2H), 6.29 (s, 1H), 7.06 (t, J = 7 Hz, 1H), 7.18 (t, J = 7 Hz, 1H), 7.32 (d, J = 8 Hz, 1H), 7.54 (d, J = 8 Hz, 1H), *m*/*z* 387 [M+H].

5.21. 3-[1-(2-Carboxyethyl)-1*H*-indol-2-yl]-piperidine-1-carboxylic acid *tert*-butyl ester (17)

Aqueous NaOH (10 N, 19.10 mL) was added to a solution of 3-[1-(2-methoxycarbonyl-ethyl)-1*H*-indol-2-yl]-piperidine-1-carboxylic acid *tert*-butyl ester (14.76 g, 38.19 mmol) in 300 mL methanol. The solution was stirred for 4–5 h at room temperature. The reaction mixture was acidified to pH $\sim$ 2 with 6 N HCl and partitioned between ethyl acetate and water. The organic layer was washed with 1.5 L of water, 400 mL aqueous NaCl, dried over K_2CO_3, and concentrated to give **17** (14.9 g, quantitative): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.35 (s, 9H), 1.39–1.50 (m, 3H), 1.50–1.72 (m, 2H), 1.95–2.02 (m, 1H), 2.41–2.52 (m, 1H), 2.55–2.72 (m, 1H), 2.72–2.89 (m, 1H), 3.91–4.02 (m, 1H), 4.02–4.18 (m, 1H), 4.18–4.33 (m, 2H), 6.135 (s, 1H), 6.94–7.03 (m, 2H), 7.26 (d, J = 7.1 Hz, 1H), 7.46 (d, J = 7.1 Hz, 1H); *m*/*z* 373 [M+H].

5.22. 3-(1-{2-[3-(1-Methyl-pyrrolidin-2-yl)-propyl-carbamoyl]-ethyl}-1*H*-indol-2-yl)-piperidine-1-carboxylic acid *tert*-butyl ester (18a)

1-Ethyl-3-(3-dimethylamino-ethyl)carbodiimide (EDCI; 1.77 g, 9.22 mmol), hydroxybenzotriazole (HOBT; 1.25 g, 9.22 mmol), and diisopropylethylamine (3.21 mL, 18.44 mmol) was added to a solution of **17** (2.29 g, 6.15 mmol) in 55 mL of DMF. The solution was stirred for approximately 20–25 min at room temperature, upon which time 2-(2-aminoethyl)-1-methyl-pyrrolidine (0.90 g, 7.07 mmol) was added. The reaction was stirred overnight at room temperature. The reaction mixture was poured into 600 mL of water and extracted three times with ethyl acetate (100 mL). The combined organic extracts were washed twice with 300 mL water, 1 × 300 mL aqueous NaCl, dried over K_2CO_3, and concentrated to give the title compound which was dried under vacuum overnight (2.85 g, 96%): LC/MS Data (Condition A, molecular ion and retention time): *m*/*z* 483 [M+H], 2.00 min.

Compounds **18b**, **18c**, and **18d** were prepared by an analogous procedure from acid **17** using (*S*)-(+)-1-(2-pyrrolidinylmethyl)pyrrolidine, pyrrolidine, and 5-methylfurfuryl-amine.

5.23. 3-{1-[3-Oxo-3-(2-pyrrolidin-1-ylmethyl-pyrrolidin-1-yl)-propyl]-1*H*-indol-2-yl}-piperidine-1-carboxylic acid *tert*-butyl ester (18b)

(2.74 g, 88%): LC/MS Data (Condition A, molecular ion and retention time): *m*/*z* 509 [M+H], 2.29 min.

5.24. 3-[1-(3-Oxo-3-pyrrolidin-1-yl-propyl)-1*H*-indol-2-yl]-piperidine-1-carboxylic acid *tert*-butyl ester (18c)

(2.68 g, quantitative yield): LC/MS Data (Condition A, molecular ion and retention time): *m*/*z* 426 [M+H], 3.19 min.

5.25. 3-(1-{2-[(5-Methyl-furan-2-ylmethyl)-carbamoyl]-ethyl}-1*H*-indol-2-yl)-piperidine-1-carboxylic acid *tert*-butyl ester (18d)

(2.87 g, quantitative yield): LC/MS Data (Condition A, molecular ion and retention time): *m*/*z* 466 [M+H], 3.39 min.

Compounds **19.a.(1–20)**, **19.b.(1–21)**, **19.c.(1–21)**, and **19.d.(1–22)** were prepared from **18a–d** by a procedure

analogous to that used for the preparation of compounds **15.a.(1–28)**.

5.26. 3-{2-[1-(3-Cyclopentyl-propionyl)-piperidin-3-yl]-indol-1-yl}-*N*-[2-(1-methyl-pyrrolidin-2-yl)-ethyl]-propionamide (19.a.17)

(18%); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.06–1.18 (m, 2H), 1.40–2.23 (m, 21H), 2.30–2.90 (m, 7H), 3.0–3.05 (m, 1H), 3.10–3.20 (m, 2H), 3.25–3.30 (m, 1H), 3.35–3.40 (m, 1H), 3.98 (d, J = 14 Hz, 1H), 4.20–4.34 (m, 1H), 4.37–4.58 (m, 1H), 4.58–4.70 (m, 1H), 4.78 (d, J = 14 Hz, 1H), 6.29 (s, 1H), 7.07 (t, J = 7.9 Hz, 1H), 7.18 (t, J = 7.9 Hz, 1H), 7.38–7.50 (m, 2H), 7.52 (d, J = 7.6 Hz, 1H). Anal. Calcd for $C_{31}H_{47}N_4O_2^{1+}$: 507.36936. Found 507.36816 ($[M+H]^{1+}$, Δ = −1.2 mmu, ESI-FTMS).

5.27. 3-{2-[1-(Biphenyl-4-carbonyl)-piperidin-3-yl]-indol-1-yl}-1-(2-pyrrolidin-1-ylmethyl-pyrrolidin-1-yl)-propan-1-one (19.b.15)

(14%); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.78–0.95 (m, 2H), 1.2–1.45 (m, 2H), 1.50–2.00 (m, 6H), 2.05–2.18 (m, 4H), 2.20–2.33 (m, 1H), 2.80–3.10 (m, 4H), 3.20–3.35 (m, 2H), 3.40–3.53 (m, 1H), 3.65–3.80 (m, 2H), 3.85–4.05 (m, 2H), 4.07–4.17 (m, 1H), 4.20–4.27 (m, 1H), 4.30–4.40 (m, 1H), 4.50–4.67 (m, 2H), 4.83–4.93 (m, 1H), 7.10–7.35 (m, 4H), 7.38–7.55 (m, 4H), 7.55–7.65 (m, 3H), 7.65–7.75 (m, 2H). Anal. Calcd for $C_{38}H_{45}N_4O_2^{1+}$: 589.35371. Found 589.3534 ($[M+H]^{1+}$, Δ = −0.31 mmu, ESI-FTMS).

5.28. 1-(2-Pyrrolidin-1-ylmethyl-pyrrolidin-1-yl)-3-{2-[1-(2-thiophen-2-yl-acetyl)-piperidin-3-yl]-indol-1-yl}-propan-1-one (19.b.20)

(7%); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.55–2.0 (m, 10H), 2.05–2.80 (m, 9H), 2.80–3.05 (m, 2H), 3.08–3.25 (m, 3H), 3.25–3.40 (m, 2H), 3.95–4.05 (m, 1H), 4.15–4.25 (m, 1H), 4.38–4.80 (m, 4H), 6.27 (s, 1H), 6.85–7.0 (m, 2H), 7.05–7.23 (m, 3H), 7.26–7.38 (m, 1H), 7.5–7.55 (m, 1H), 7.65–7.75 (m, 1H). Anal. Calcd for $C_{31}H_{41}N_4O_2S^{1+}$: 533.29448. Found 533.29306 ($[M+H]^{1+}$, Δ = −0.42 mmu, ESI-FTMS).

5.29. 3-(2-Phenyl-1*H*-indol-3-yl)-propionic acid (21)

Phenyl hydrazine (1.54 g, 14.28 mmol) and $ZnCl_2$ (3.78 g, 26.99 mmol)was added to a solution of 4-benzoylbutyric acid (2.74 g, 14.28 mmol) in 33 mL acetic acid. The reaction was heated to 70 °C for 4 h and allowed to cool. The $ZnCl_2$ was removed by filtration and the reaction solution was concentrated. The residue was dissolved in EtOAc and washed four times with water and four times with brine and dried and concentrated to afford 3.84 g (>100% yield) of the title compound as an orange solid: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.70–2.80 (m, 2H), 3.21–3.30 (m, 2H), 7.12–7.25 (m, 2H), 7.33–7.41 (m, 2H), 7.42–7.58 (m, 4H), 7.65 (d, J = 7.5 Hz, 1H), 8.05 (s, 1H), 10.5 (br s, 1H); *m*/*z* 266 [M+H].

5.30. 3-(2-Phenyl-1*H*-indol-3-yl)-propionamide

Thionyl chloride (0.44 mL, 6.036 mmol) was added dropwise to a solution of **21** (0.62 g, 2.33 mmol) in 20 mL CH_2Cl_2, cooled to 0 °C in ice. The reaction mixture was stirred at 0 °C for 45 min and allowed to stir at room temperature for 2 h. The reaction solution was recooled to 0 °C and 30 mL aqueous ammonium hydroxide was added slowly. The reaction was again stirred at 0 °C for 45 min and allowed to stir at room temperature for 1 h. The reaction was poured into water, extracted with CH_2Cl_2, washed with water and brine, dried, and concentrated to afford the title compound (0.51 g, 1.92 mmol, 82.5 yield) as a yellow solid: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.70–2.80 (m, 2H), 3.21–3.30 (m, 2H), 5.25 (br s, 2H), 7.10–7.25 (m, 2H), 7.33–7.40 (m, 2H), 7.40–7.58 (m, 4H), 7.65 (d, J = 7.5 Hz, 1H), 8.08 (s, 1H).

5.31. 3-(2-Phenyl-1*H*-indol-3-yl)-propylamine

$LiAlH_4$ (11.5 mL of a 1 M solution in THF) was added over a 5 min period to a solution of 3-(2-phenyl-1*H*-indol-3-yl)-propionamide (0.51 g, 1.925 mmol) in 10 mL anhydrous THF at 0 °C. The reaction was stirred at 0 °C for 40 min and then heated to reflux for 4 h. The reaction was cooled in ice and 3 mL of water was added, followed by 3 mL 15% aqueous NaOH solution, and an additional 3 mL of water. The reaction was filtered and the filtrate was partitioned between EtOAc and water. The aqueous phase was extracted two times with EtOAc and the combined organic extracts were washed with water and with brine, dried over Na_2SO_4, and concentrated to give the title compound (0.47 g, 1.88 mmol, 98% yield) as a brown gum: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.86 (quintet, J = 7.5 Hz, 2H), 2.74 (t, J = 7.1 Hz, 2H), 2.94 (t, J = 7.1 Hz, 2H), 7.10–7.25 (m, 2H), 7.33–7.43 (m, 2H), 7.47 (t, J = 7.5 Hz, 2H), 7.56 (d, J = 7.5 Hz, 2H), 7.64 (d, J = 7.5 Hz, 1H), 8.03 (s, 1H).

5.32. [3-(2-Phenyl-1*H*-indol-3-yl)-propyl]-carbamic acid *tert*-butyl ester (22)

A solution of di-*tert*-butyldicarbonate (2.07 mL, 1 M) in THF was added to a solution of 3-(2-phenyl-1*H*-indol-3-yl)-propylamine (0.47 g, 1.88 mmol) in 10 mL THF at 0 °C. The reaction was stirred at room temperature for 45 min and poured into water. The aqueous phase was extracted with EtOAc and the organic phase was washed with brine, dried over K_2CO_3, and concentrated to give an oil. The crude product was chromatographed on silica gel using 20% EtOAc–hexane, to afford a yellow solid, 0.35 g (1 mmol): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.42 (s, 9H), 1.86 (quintet, J = 7.5 Hz, 2H), 2.93 (t, J = 7.1 Hz, 2H), 3.10–3.17 (m, 2H), 4.40 (br s, 1H), 7.10–7.25 (m, 2H), 7.33–7.39 (m, 2H), 7.41–7.58 (m, 4H), 7.60 (d, J = 7.5 Hz, 1H), 8.02 (s, 1H).

5.33. 3-[3-(3-*tert*-Butoxycarbonylamino-propyl)-2-phenyl-indol-1-yl]-propionic acid methyl ester (23)

A solution of **22** (50 mg, 0.143 mmol), *n*-Bu_4NI (5 mg), KO*t*-Bu (10 μL of a 1.0 M solution in THF), and methyl

acrylate (52 μL, 0.574 mmol) in 1.4 mL toluene was heated in a microwave reactor (Smith Synthesizer, Personal Chemistry, Foxboro, MA) at 150 °C for 540 s. The reaction was checked for completeness by LC–MS. The reaction was concentrated and the crude product was dissolved in CH_2Cl_2 and washed with water. The organic phase was concentrated to give the title compound (61 mg, 0.140 mmol), which was used in the following step without further purification. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.39 (s, 9H), 1.74 (quintet, J = 7.5 Hz, 2H), 2.53 (t, J = 7.5 Hz, 2H), 2.68 (t, J = 7.5 Hz, 2H), 2.95–3.05 (m, 2H), 3.57 (s, 3H), 4.22 (br s, 1H), 4.32 (t, J = 7.5 Hz, 2H), 7.10–7.19 (m, 2H), 7.20–7.28 (m, 1H), 7.33–7.40 (m, 3H), 7.41–7.52 (m, 2H), 7.61 (d, J = 7.8 Hz, 1H).

5.34. 3-[3-(3-Amino-propyl)-2-phenyl-indol-1-yl]-propionic acid methyl ester

A solution of 1.0 mL TFA in 1.6 mL CH_2Cl_2 was added to a solution of **23** (0.287 g, 0.657 mmol) in 3 mL CH_2Cl_2 at 0 °C. The reaction was stirred at 0 °C for 20 min and at room temperature for 50 min. The reaction was concentrated and the crude product was dissolved in CH_2Cl_2 and washed with 1 N NaOH and with brine and dried over K_2CO_3. The solution was concentrated to afford 0.184 g (0.545 mmol) of the product, which was used in the following step without further purification. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.96 (t, J = 7.25 Hz, 2H), 1.81 (quintet, J = 7.25 Hz, 2H), 2.52 (t, J = 7.8 Hz, 2 H), 2.66 (q, J = 7.8 Hz, 4H), 3.55 (s, 3H), 4.32 (t, J = 7.6 Hz, 2H), 7.12 (t, J = 7.0 Hz, 1H), 7.20–7.25 (m, 1H), 7.28–7.39 (m, 3H), 7.40–7.52 (m, 3H), 7.61 (d, J = 7.8 Hz, 1H).

5.35. 3-[3-(3-Methanesulfonylamino-propyl)-2-phenyl-indol-1-yl]-propionic acid methyl ester (24)

Methanesulfonyl chloride (63 μL, 0.81 mmol) was added to a solution of 3-[3-(3-amino-propyl)-2-phenyl-indol-1-yl]-propionic acid methyl ester (0.184 g, 0.54 mmol) and triethylamine (74 μL, 0.53 mmol) in 2.5 mL CH_2Cl_2 at 0 °C. The reaction was left to stir at 0 °C for 1.5 h and then poured into water and extracted with CH_2Cl_2. The organic extracts were washed with brine, dried and concentrated to afford 0.225 g (0.54 mmol) of the product as a gum. It was carried on to the next step without further purification. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.68 (br s, 1H), 1.81 (quintet, J = 7.0 Hz, 2H), 2.53 (t, J = 7.8 Hz, 2H), 2.70–2.78 (m, 2H), 2.77 (s, 3H), 2.99 (t, J = 6.75 Hz, 2H), 3.56 (s, 3H), 4.34 (t, J = 7.6 Hz, 2H), 7.16 (t, J = 7.0 Hz, 1H), 7.20–7.30 (m, 1H), 7.3–7.41 (m, 3H), 7.43–7.56 (m, 3H), 7.59 (d, J = 7.8 Hz, 1H); m/z 415 [M+H].

5.36. 3-[3-(3-Methanesulfonylamino-propyl)-2-phenyl-indol-1-yl]-propionic acid (25)

Sodium hydroxide (2.72 mmol, 0.27 mL of 10 N solution in water) was added to a solution of **24** (0.225 g, 0.54 mmol) in 2.5 mL MeOH. The mixture was stirred at room temperature overnight. The reaction was acidified to pH 2 using 6 N HCl and extracted two times with EtOAc. The combined organic extracts were dried and concentrated to afford 0.319 g of the product (>100% yield) as a beige solid. It was characterized by ^{1}H NMR and MS and carried on to the next step without further purification. ^{1}H NMR (D_2O, 300 MHz) δ 1.25 (br s, 1H), 1.65 (quintet, J = 7.8 Hz, 2H), 2.26 (t, J = 7.8 Hz, 2H), 2.63 (t, J = 7.8 Hz, 2H), 2.68 (s, 3H), 2.70–2.78 (m, 2H), 4.20 (t, J = 7.8 Hz, 2H), 7.11 (t, J = 7.5 Hz, 1H), 7.22 (t, J = 7.1 Hz, 1H), 7.35–7.50 (m, 6H), 7.64 (d, J = 7.8 Hz, 1H); m/z 401 [M+H].

Compounds **26.a.(1–4)** were prepared from **25** following the procedure used for the preparation of compounds **6.(a–e).(1–23)** and **8.(a–b).(1–23)**.

5.37. 3-[3-(3-Methanesulfonylamino-propyl)-2-phenyl-indol-1-yl]-*N*-[2-(1-methyl-pyrrolidin-2-yl)-ethyl]-propionamide (26.a.1)

(15%) ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.30–1.55 (m, 3H), 1.60–1.70 (m, 2H), 1.75–2.05 (m, 3H), 2.05–2.16 (m, 2H), 2.165 (s, 3H), 2.32 (t, J = 7.2 Hz, 2H), 2.70–2.80 (m, 2H), 2.78 (s, 3H), 2.94–3.04 (m, 4H), 3.25 (sextet, J = 6 Hz, 1H), 4.34 (t, J = 6 Hz, 2H), 6.58 (br s, 1H), 7.14 (t, J = 7.0 Hz, 1H), 7.23 (t, J = 7.0 Hz, 1H), 7.38 (d, J = 7.0 Hz, 2H), 7.41–7.55 (m, 4H), 7.58 (d, J = 7.6 Hz, 1H). Anal. Calcd for $C_{28}H_{39}N_4O_3S^{1+}$: 511.27374. Found 511.27209 ($[M+H]^{1+}$, Δ = −1.65 mmu, ESI-FTMS).

5.38. 3-[3-(3-Methanesulfonylamino-propyl)-2-phenyl-indol-1-yl]-*N*-(2-pyrrolidin-1-yl-ethyl)-propionamide (26.a.3)

(16%) ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.72 (br s, 2H), 1.75–1.85 (m, 2H), 1.92 (br s, 2H), 2.31–2.40 (m, 7H), 2.70–2.80 (m, 2H), 2.73 (s, 3H), 2.94–3.04 (m, 2H), 3.16 (q, J = 6 Hz, 2H), 4.37 (t, J = 6 Hz, 2H), 6.10 (br s, 1H), 7.14 (t, J = 7.0 Hz, 1H), 7.24 (t, J = 7.0 Hz, 1H), 7.40 (d, J = 7.0 Hz, 2H), 7.42–7.55 (m, 4H), 7.58 (d, J = 7.6 Hz, 1H). Anal. Calcd for $C_{27}H_{37}N_4O_3S^{1+}$: 497.25809. Found 497.25688 ($[M+H]^{1+}$, Δ = −1.21 mmu, ESI-FTMS).

5.39. 3-[3-(3-*tert*-Butoxycarbonylamino-propyl)-2-phenyl-indol-1-yl]-propionic acid methyl ester (27)

Compound **27** was prepared from **23** following the procedure used for the preparation of compound **25**. ^{1}H NMR ($CDCl_3$, 300 MHz) 1.39 (s, 9H), 1.73 (quintet, J = 7.2 Hz, 2H), 2.57 (t, J = 7.2 Hz, 2H), 2.68 (t, J = 7.2 Hz, 2H), 3.00 (q, J = 6.3 Hz, 2H), 4.32 (t, J = 7.5 Hz, 2H), 7.10–7.18 (m, 1H), 7.15–7.25 (m, 1H), 7.3–7.40 (m, 3H), 7.42–7.55 (m, 3H), 7.61 (d, J = 7.6 Hz, 1H); m/z 421 (M−H).

5.40. General procedure for the preparation of compounds 28.a.(1–21)

Acid **27** was coupled to amines using the same procedure used for the preparation of compounds **6.(a–e).(1–23)** and **8.(a–b).(1–23)**. Following aqueous wash and prior to purification by RP-HPLC, solutions of

the crude products in CH_2Cl_2 were treated with 1.25 mL TFA and mixed at room temperature for 2 h. Volatiles were removed in vacuo and the residue of each reaction was redissolved in DMSO and purified by RP-HPLC.

5.40.1. 3-[3-(3-Amino-propyl)-2-phenyl-indol-1-yl]-*N*-phenyl-propionamide (28.a.17). (17%) ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.74–1.84 (m, 2H), 2.53 (t, $J = 6.8$ Hz, 2H), 2.65–2.92 (m, 4H), 4.37 (t, $J = 5.4$ Hz, 2H), 7.01 (t, $J = 7.0$ Hz, 1H), 7.09 (t, $J = 7.0$ Hz, 1H), 7.19 (t, $J = 7.2$ Hz, 2H), 7.32–7.45 (m, 9H), 7.58 (d, $J = 7.6$ Hz, 1H), 8.0 (s, 1H). Anal. Calcd for $C_{26}H_{28}N_3O_1^{1+}$: 398.22269. Found 398.22126 ($[M+H]^{1+}$, $\Delta = -1.43$ mmu, ESI-FTMS).

5.40.2. 3-[3-(3-Amino-propyl)-2-phenyl-indol-1-yl]-*N*-(4-fluoro-phenyl)-propionamide (28.a.20). (17%) ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.74–1.84 (m, 2H), 2.52 (t, $J = 6.8$ Hz, 2H), 2.60–2.90 (m, 4H), 4.35 (t, $J = 5.4$ Hz, 2H), 6.82 (t, $J = 8.4$ Hz, 2H), 7.08 (t, $J = 7.0$ Hz, 1H), 7.16 (t, $J = 7.0$ Hz, 1H), 7.267 (t, $J = 5.6$ Hz, 2H), 7.37–7.45 (m, 6H), 7.58 (d, $J = 7.6$ Hz, 1H), 8.25 (s, 1H). Anal. Calcd for $C_{26}H_{27}FN_3O_1^{1+}$: 416.21327. Found 416.21137 ($[M+H]^{1+}$, $\Delta = -1.9$ mmu, ESI-FTMS).

References and notes

1. Bramhill, D. *Ann. Rev. Cell Dev. Biol.* **1997**, *13*, 395.
2. Adinall, S. G.; Cao, C.; Lutkenhaus, J. *J. Bacteriol.* **1997**, *179*, 4277.
3. Osteryoung, K. W.; Vierling, E. *Nature* **1995**, *376*, 473.
4. Hale, C. A.; de Boer, P. A. J. *Cell* **1997**, *88*, 175.
5. Mosyak, L.; Zhang, Y.; Glasfeld, E.; Haney, S.; Stahl, M.; Seehra, J.; Somers, W. S. *EMBO J.* **2000**, *19*, 3179.
6. Stover, C. K.; Pham, X. Q., et al. *Nature* **2000**, *406*, 959, http://www.pseudomonas.com./index.html.
7. Akerly, B. J.; Rubin, E. J.; Novick, V. L.; Amaya, K.; Judson, N.; Mekalanos, J. J. *Proc. Nat. Acad. Sci. U.S.A.* **2002**, *99*, 966.
8. RayChaudhuri, R. *EMBO J.* **1999**, *18*, 2373.
9. (a) Anonymous: *Int. J. Tuberc. Lung Dis.* **1998**, *2*, 2; (b) Cappelletty, D. *Pediatr. Infect. Dis. J.* **1998**, *17*, S55; (c) Debbia, E. A.; Schito, G. C., et al. *J. Chemother.* **2001**, *13*(Spec No 1), 205.
10. (a) Palavecino, E. L. *Am. J. Managed Care* **2001**, *7*(6 Suppl.), S170; (b) Stratton, C. W. *Emer. Infect. Dis.* **2003**, *9*(1), 10.
11. Moy, F. J.; Glasfeld, E.; Powers, R. *J. Biomol. NMR* **2000**, *17*, 275.
12. Alvarez, J. C.; Tsao, D. H. H. Personal communication.
13. Jennings, L. D.; Foreman, K. W.; Rush, T. S.; Tsao, D. H. H.; Mosyak, L.; Li, Y.; Sukhdeo, M. N.; Ding, W.; Dushin, E. G.; Kenny, C. H.; Moghazeh, S. L.; Petersen, P. J.; Ruzin, A. V.; Tuckman, M.; Sutherland, A. G. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1427.
14. For an account of a different approach to the optimization of compound **1**, see: Sutherland, A. G.; Alvarez, J.; Ding, W.; Foreman, K. W.; Kenny, C. H.; Labthavikul, P.; Mosyak, L.; Petersen, P. J.; Rush, T. S.; Ruzin, A.; Tsao, D. H. H.; Wheless, K. L. *Org. Biomol. Chem.* **2003**, *1*, 4138.
15. Elizabeth G. Dushin. Personal communication.
16. Martin, E. J.; Blaney, J. M.; Siani, M. A.; Spellmeyer, D. C.; Wong, A. K.; Moos, W. H. *J. Med. Chem.* **1995**, *38*, 1431.
17. Umetrics AB, Umeå, Sweden.
18. Hutchins, S. M.; Chapman, K. T. *Tetrahedron Lett.* **1996**, *37*, 4869.
19. Kenny, C. H.; Ding, W.; Kelleher, K.; Benard, S.; Dushin, E. G.; Sutherland, A. G.; Mosyak, L.; Kriz, R.; Ellestad, G. *Anal. Biochem.* **2003**, *323*, 224.
20. (a) Brooijmans, N.; Sharp, K. A.; Kuntz, I. D. *Proteins: Structure, Function Genetics* **2002**, *48*, 645; (b) Kuntz, I. D.; Chen, K.; Sharp, K. A.; Kollman, P. A. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 9997.
21. Kansy, M.; Senner, F.; Gubernator, K. *J. Med. Chem.* **1998**, *41*, 1007.
22. 'Methods for Dilution of Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically'. Approved Standards: M7-A5; National Committee for Clinical Laboratory Standards: Wayne, PA; Vol. 20.
23. Slätt, J.; Bergman, J. *Tetrahedron* **2002**, *58*, 9187.