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Dedication

In memory of Dr. Jiayi (Jane) Wang.

Abstract

The combination of increased incidence of drug-resistant strains of bacteria and a lack of novel drugs in development creates an urgency for the search for new antimicrobials. Initial screening of compounds from an in-house library identified two 6-bromoindolglyoxylamide polyamine derivatives that exhibited intrinsic antimicrobial activity towards Gram-positive bacteria, *Staphylococcus aureus* and *S. intermedius* with one of the compounds also displaying *in vitro* antibiotic enhancing properties against the resistant Gram-negative bacterium *Pseudomonas aeruginosa*. A series of 6-bromo derivatives were prepared and biologically evaluated, identifying analogues with enhanced antibacterial activity towards *Escherichia coli* and with moderate to excellent antifungal properties. The mechanism of action of one of the compounds was attributed to rapid membrane permeabilisation and depolarisation in both Gram-positive and Gram-negative bacteria.

Keywords

Antibiotics; antimicrobial; bromoindole; polyamine; potentiation

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1. Introduction

Antibiotics have been the cornerstone of modern medicine saving lives by virtue of being able to cure infectious diseases and to prevent infections in those that are immune compromised.[1–4] Their intense use has led to a surging increase in the incidence of antibiotic-resistant bacteria resulting in a desperate need for antibiotics with new mechanisms of action. A strategy for overcoming bacterial resistance is to identify compounds that can circumvent the drug-resistant phenotype, enhancing or restoring the activity of antibiotics that are currently ineffective.[1–3] Polyamine-containing derivatives have been previously reported to enhance the activity of antibiotics, with simple unsubstituted polyamines, such as spermine and spermidine, having been found to act synergistically with a range of antibiotics to enhance the kill of a number of different Gram-positive and Gram-negative bacteria, but at an extraordinarily high dose of 1 mM.[5,6] A recent study on polyamine-containing natural products identified squalamine (**1**) and ianthelliformisamine C (**2**) (Fig. 1), as being intrinsically antimicrobial and also being able to enhance the activity of antibiotics.[7–9] Ianthelliformisamine C (**2**) was shown to exhibit antimicrobial activity against *Staphylococcus aureus* and *Klebsiella pneumoniae* (MIC 12.5 and 12.5 μM, respectively) and to enhance the activity of doxycycline against both microbes (MIC 12.5 and 12.5, respectively) as well as *Pseudomonas aeruginosa* (MIC 3.12 μM). Mechanism of action studies suggest squalamine (**1**) functions by disruption of the outer-membrane of Gram-negative bacteria while ianthelliformisamine C (**2**), in contrast, inhibits efflux pumps.[7,10] Being suitably intrigued by these findings and given our ongoing interest in biologically active polyamine derivatives,[11–14] we screened an in-house library of polyamine alkaloids related to the natural product ianthelliformisamine C (**2**)) for antimicrobial and antifungal properties and for the ability to restore the antibiotic activity of doxycycline towards *P. aeruginosa*. Herein we present this preliminary data, a structure-activity relationship study that explores the influence of the polyamine core on observed intrinsic antimicrobial properties and the ability to potentiate the action of doxycycline, and mechanism of action studies that evaluated compound influence on membrane permeability and polarization.

2. Results and Discussion

Our screening identified two 6-bromoindolglyoxylamide compounds, spermine **3**[13] and spermidine **4**[13] (Fig. 1) that exhibited good antimicrobial activity against two Gram-positive strains bacteria *Staphylococcus intermedius* (MIC 3.125 and 3.125 μM, respectively) and *S. aureus* (MIC 6.25 and 3.125 μM, respectively) and moderate to strong antifungal activity towards *Candida albicans* (MIC 17.2 μM for **3**) and *Cryptococcus neoformans* (MIC 1.1 and 50 μM, respectively) (Table 1). These two hits were then evaluated for the ability to enhance the antibiotic activity of doxycycline towards *Pseudomonas aeruginosa* ATCC 27853. While doxycycline alone is ineffectual against this bacterial strain (MIC 64 μg/mL), a combination of doxycycline at 2 μg/mL with **3** at 6.25 μM was able to restore the action of the antibiotic (Table 2). Somewhat surprisingly, the closely related spermidine hit compound **4** was found to be completely ineffective at restoring the action of doxycycline. In an effort to further expand on these initial findings, we prepared a series of analogues **7**–**15** (Fig. 2) that explored the influence of the polyamine core on the observed biological activities.

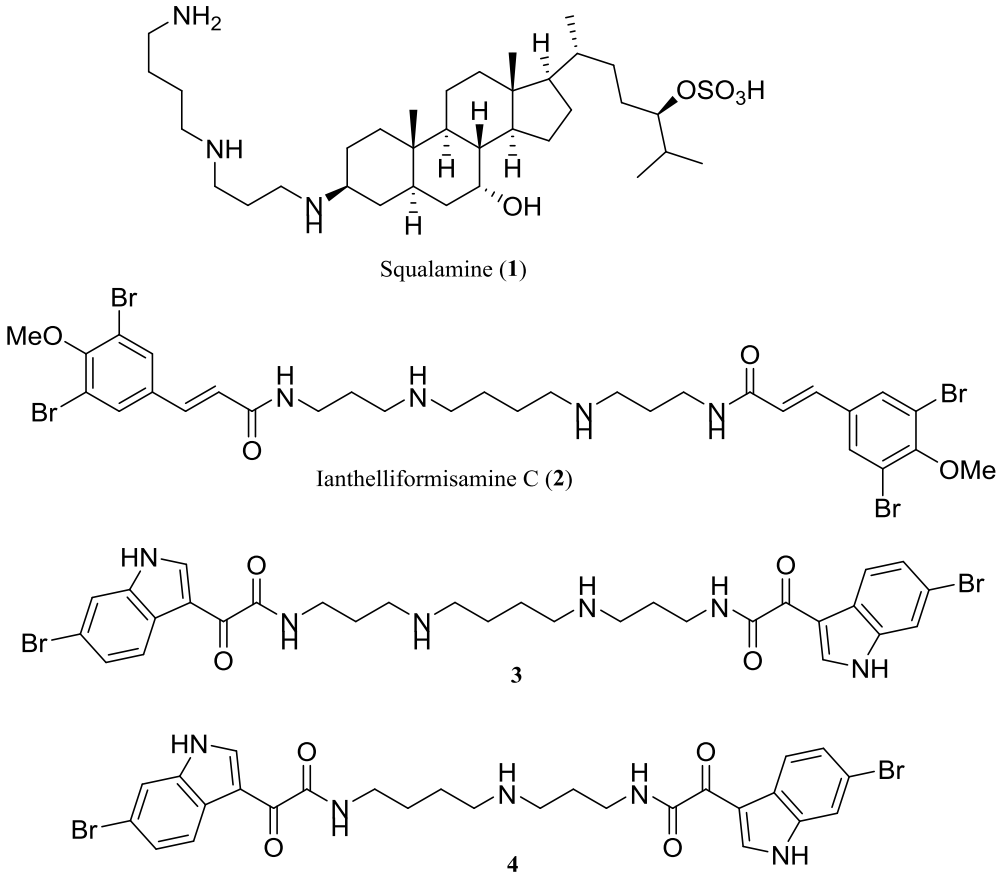


Fig. 1. Structures of polyamines **1**–**4**.

2.1 Chemistry

Synthesis of the target 6-bromoindolglyoxylamide derivatives **5**, **6**, **8** and **9** (Fig. 2) was achieved by a two-step sequence starting with reaction of 2-(6-bromo-1*H*-indol-3-yl)-2-oxoacetyl chloride (**16**)[15] with di-*tert*-butyl hexane-1,6-diylbis((3-aminopropyl)carbamate),[14] di-*tert*-butyl heptane-1,7-diylbis((3-aminopropyl)carbamate),[14] di-*tert*-butyl decane-1,10-diylbis((3-aminopropyl)carbamate)[14] or di-*tert*-butyl dodecane-1,12-diylbis((3-aminopropyl)carbamate)[14] to afford a series of Boc protected intermediates (Scheme 1). These crude reaction intermediates were then directly subjected to Boc group deprotection, with TFA in CH_2Cl_2, to give tetraamine diamides **5**, **6**, **8** and **9** as their corresponding di-TFA salts (Scheme 1). The synthesis of **7** has been reported previously.[13]

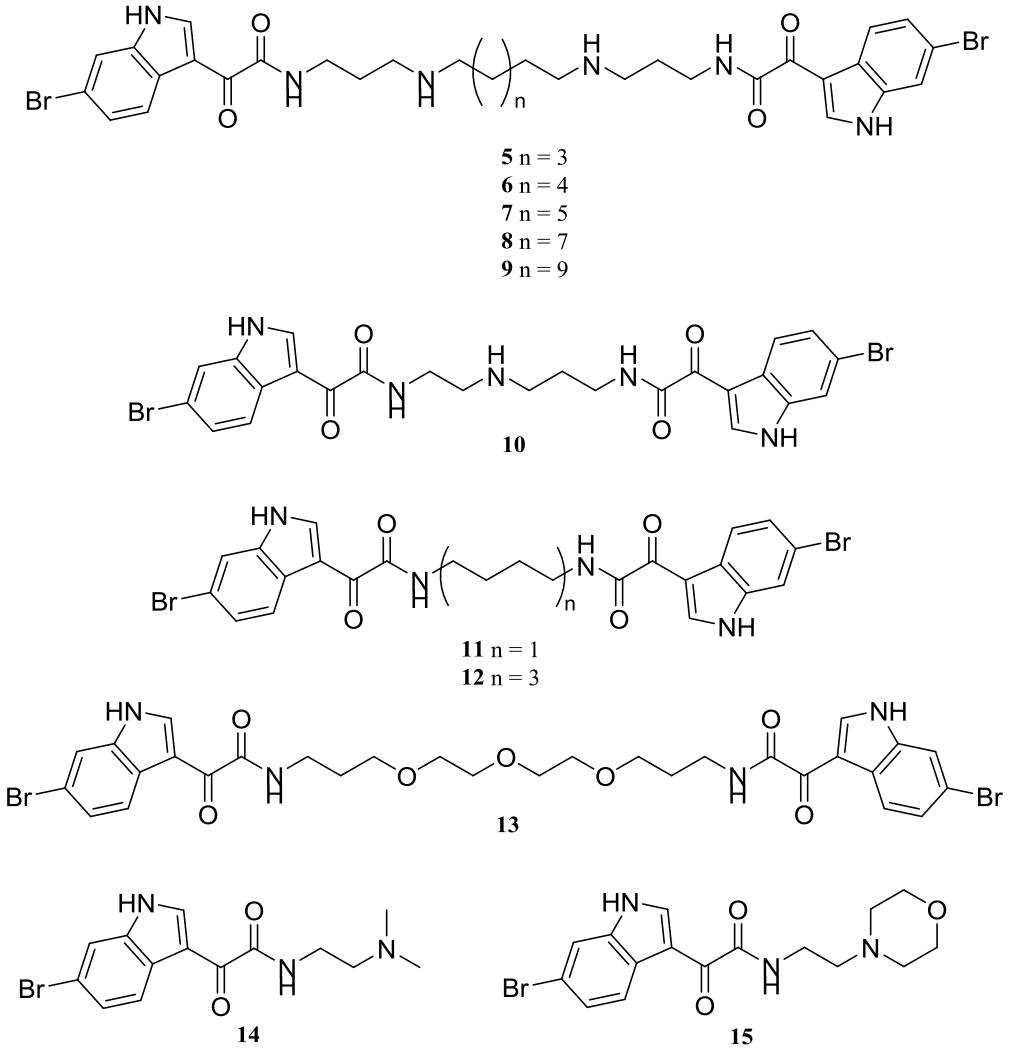
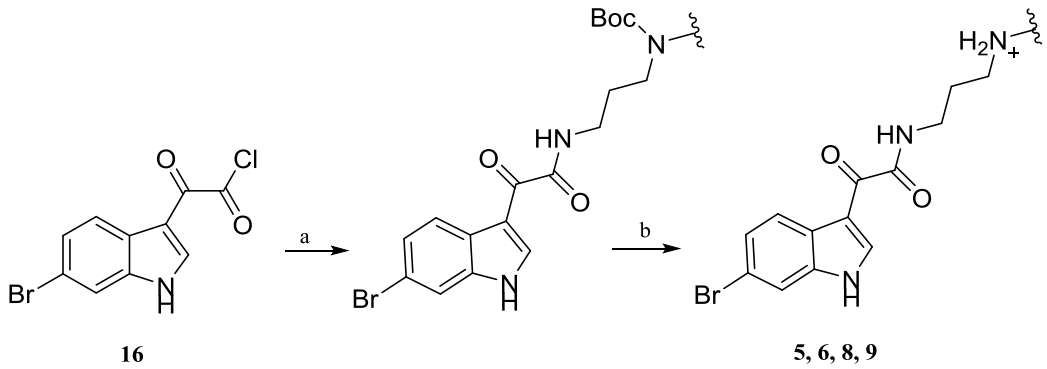


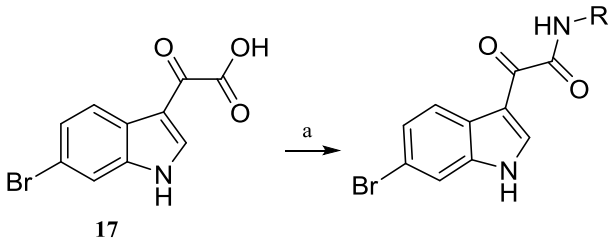
Fig. 2. Structures of the indolglyoxylamide derivatives **5**–**15**.



Scheme 1 General method for the preparation of analogues **5**, **6**, **8** and **9**.

Reagents and conditions: a) Boc-protected polyamine (0.5 eq.), DIPEA (3 eq.), DMF, r.t., 48 h; b) TFA (0.2 mL), CH_2Cl_2 or MeOH (2 mL), r.t., 3 h.

Analogues **10**–**15** were prepared by PyBOP-mediated coupling of 6-bromoindole-3-glyoxylic acid (**17**)[11,13] with spermidine, 1,4-diaminobutane, 1,12-diaminododecane, 4,7,10-trioxa-1,13-tridecanediamine, *N*,*N*-dimethylethylenediamine or 2-morpholinoethylamine (Scheme 2).



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Scheme 2 General method for the preparation of analogues **10**–**15**.

Reagents and conditions: a) RNH_2 (1 eq.), PyBOP (1 eq.), Et_3N (6 eq.), DMF, r.t., 24 h, 20–82%.

3. Results and Discussion

The library of analogues were evaluated for activity against a variety of Gram-negative (*Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae* and *Acinetobacter baumannii*) and Gram-positive bacteria (*Staphylococcus aureus* and *Staphylococcus intermedius*) as well as two yeast strains (*Candida albicans* and *Cryptococcus neoformans*) (Table 1). As a general trend, those analogues with a polyamine core (**3**, **4**, **5**-**9**) exhibited more pronounced activity than the non-polyamine (**13**) or shorter chained (**10,11,12,14,15**) derivatives. Within the polyamine examples, the presence of only one mid-chain nitrogen (**4**) reduced potency towards yeast, while the length of the polyamine mid-section gave maximal potency at PA3-10-3, with **8** exhibiting relatively broad-spectrum activity.

Table 1. Antibacterial and antifungal activities of indolglyoxylamide derivatives **3**–**15**.

	MIC (μM)							
	P. a[a]	***E. c***[b]	***S. a***[c]	***S. i***[d]	***K. p***[e]	***A. b***[f]	***C. a***[g]	***C. n***[h]
3	100	100	6.25	3.125	>34 [i]	>34 [i]	17.2	1.1
4	>200	50	3.125	3.125	>50 [i]	>50 [i]	>50 [i]	50
5	52.2	>200	6.5	n.t.[j]	>32	>32	8.35	33.4
6	51.4	51.4	6.4	n.t.	>32	>32	16.5	>32
7	50	6.25	50	12.5	>32	>32	8.11	32.4
8	24.6	24.6	12.3	n.t.	31.5	31.5	1.97	<0.25
9	>200	>200	>200	n.t.	>32	>32	7.67	7.67
10	>200	>200	12.5	50	>52 [i]	>52 [i]	>52 [i]	>52 [i]
11	>200	>200	>200	>200	>54 [i]	>54 [i]	>54 [i]	>54 [i]
12	>200	>200	>200	100	>46 [i]	>46 [i]	>46 [i]	>46 [i]
13	>200	>200	>200	12.5	>44 [i]	>44 [i]	>44 [i]	>44 [i]
14	>200	>200	>200	12.5	>95 [i]	>95 [i]	>95 [i]	>95 [i]
15	>200	>200	>200	12.5	>84 [i]	>84 [i]	>84 [i]	>84 [i]

[a] *Pseudomonas aeruginosa* ATCC 27853 with streptomycin (MIC 21.5 μM) and colistine (MIC 1 μM) used as positive controls and values presented as the mean (n = 3).
[b] *Escherichia coli* ATCC25922 with streptomycin (MIC 21.5 μM) and colistine (MIC 2 μM) used as positive controls and values presented as the mean (n = 3).
[c] *Staphylococcus aureus* ATCC 25923 with streptomycin (MIC 21.5 μM) and chloramphenicol (MIC 1.5–3 μM) used as positive controls and values presented as the mean (n = 3).
[d] *Staphylococcus intermedius* 1051997 with streptomycin (MIC 10.7 μM) and chloramphenicol (MIC 3–6 μM) used as positive controls and values presented as the mean (n = 3).
[e] *Klebsiella pneumoniae* ATCC 700603 with values presented as the mean (n = 2).
[f] *Acinetobacter baumanii* ATCC 19606 with values presented as the mean (n = 2).
[g] *Candida albicans* ATCC 90028 with values presented as the mean (n = 2).
[h] *Cryptococcus neoformans* ATCC 208821 with values presented as the mean (n = 2).
[i] Not active at a single dose test of 32 μg/mL.
[j] Not tested.

We next evaluated the library of compounds for the ability to enhance the activity of doxycycline towards *P. aeruginosa* (Table 2). In the presence of doxycycline (2 μg/mL), polyamines **5** and **8** were found to have strong enhancing effects (6.52 μM and 6.16 μM, respectively), with **6** and **7** being slightly less efficient. The absence of potentiating activity for not only non-polyamine analogues **10**–**15** but also polyamine analogue **9** and spermidine **4** implies narrow structural specificity for the observed activity.

Table 2. Doxycycline potentiation activity of indolglyoxylamide derivatives **3**–**15**.

Compound	Conc (μM) for potentiation[a]
3	6.25
4	>200
5	6.52
6	12.9
7	25
8	6.16
9	>200
10	>200
11	>200
12	>200
13	>200
14	>200
15	>200

[a] Concentration (μM) required to restore doxycycline activity at 2 μg/mL (4.5 μM) against *P. aeruginosa* ATCC 27853.

Analogue **3** was also found to strongly potentiate the activity of doxycycline towards Gram-negative bacteria *Escherichia coli* ATCC25922, *Klebsiella pneumoniae* ST258 and *Acinetobacter baumannii* AYE (Table 3). Variable levels of potentiation were observed for other antibiotics, with the action of the hydrophobic antibiotic erythromycin being improved against *E. coli*, *K. pneumoniae* and *A. baumannii* and nalidixic acid exhibiting enhanced activity against *P. aeruginosa* and *K. pneumoniae*. Negligible potentiation was observed against the test organisms for chloramphenicol.

Table 3. Antibiotic potentiating activity of **3**.

Antibiotic	Concentration (μM) for potentiation[a]			
	***P. a*[b]**	***E. c*[c]**	***K. p*[d]**	***A. b*[e]**
Doxycycline	12.5	6.25	6.25	25
Erythromycin	200	50	50	25
Chloramphenicol	>200	>200	200	>200
Nalidixic acid	50	>200	25	200

[a] Concentration (μM) of compound required to restore antibiotic activity at 2 μg/mL concentration of antibiotic.
[b] *Pseudomonas aeruginosa* ATCC27853. Positive controls were doxycycline (MIC 50 μM), erythromycin (MIC >200 μM), chloramphenicol (MIC >200 μM) and nalidixic acid (MIC >200 μM). Values presented are the mean (n = 3).
[c] *Escherichia coli* ATCC25922. Positive controls were doxycycline (MIC 25 μM) erythromycin (MIC >200 μM), chloramphenicol (MIC >200 μM) and nalidixic acid (MIC >200 μM). Values presented are the mean (n = 3).
[d] *Klebsiella pneumoniae* ST258. Positive controls were doxycycline (MIC 25 μM) erythromycin (MIC >200 μM), chloramphenicol (MIC 50 μM) and nalidixic acid (MIC 100 μM). Values presented are the mean (n = 3).
[e] *Acinetobacter baumannii* AYE. Positive controls were doxycycline (MIC 12.5 μM) erythromycin (MIC 200 μM), chloramphenicol (MIC >200 μM) and nalidixic acid (MIC >200 μM). Values presented are the mean (n = 3).

The cytotoxicity of compounds **3**–**15** were evaluated against rat skeletal muscle (L6) and human embryonic kidney (HEK-293) cell lines, finding moderate cytotoxicity to both cell lines for all of the polyamine derivatives **3**–**9** with IC_{50} values ranging from 1.55 to 17.1 μM (Table 4). The other compounds in the library were only weakly cytotoxic. Human red blood cell haemolytic activity was observed for three (**5**, **8**, **9**) of the seven polyamines, with **3**, **4**, **6**, **7** having HC_{10} values typically greater than 32 μM.

Table 4. Cytotoxicity and haemolytic activities of indolglyoxylamide derivatives **3**–**15**.

Compound	Cytotoxicity		HC_{10} (μM)[c]
	L6[a] IC_{50} (μM)	HEK-293[b] CC_{50} (μM)	
3	7.7[d]	5.06	>34[e]
4	8.7	2.32	>50[e]
5	5.7	17.1	18.00
6	4.4	10.6	>33[e]
7	5.6	6.20	>32[e]
8	12.4	6.03	7.88
9	1.6	9.72	2.18
10	89.3	>52[e]	n.d.[f]
11	>150	>54[e]	n.d.
12	89.1	>46[e]	n.d.
13	75.4	>44[e]	n.d.
14	99.5	>95[e]	n.d.
15	156.9	>84[e]	n.d.

[a] L6 rat skeletal myoblast cell line with podophyllotoxin as the positive control (IC_{50} 0.018 μM) and values presented as the mean (n = 2).

[b] Concentration of compound at 50% cytotoxicity on HEK293 human embryonic kidney cells and values presented as the mean (n = 2).
[c] Concentration of compound at 10% haemolytic activity on human red blood cells and values presented as the mean (n = 2).
[d] Data taken from Wang *et al*.[13]
[e] Not active at a single dose test of 32 µg/mL.
[f] Not determined.

Lipophilic polyamines are noted for their abilities to increase the permeability of bacterial outer membranes, with examples including squalamine,[10] ianthelliformisamine derivatives,[7] claramine,[16] and naphthylacetylspermine.[17] An increase in membrane permeability is linked to bacterial cell death (antimicrobial activity) and also with the ability to enhance the action of hydrophobic antibiotics.[17]

The effect of **3** on bacterial membrane integrity of Gram-negative (*Pseudomonas aeruginosa* PAO1) and Gram-positive (*Staphylococcus aureus*) bacteria was assessed by measuring intracellular ATP release using a bioluminescence method. Brief (1 min) exposure of cells to **3** led to dose-dependent increases in extracellular ATP. In the case of *P. aeruginosa* (Fig. 3), rapid ATP leakage was detected with a higher response than that observed for the positive control, surfactant cetyltrimethylammonium bromide (CTAB), while the polyamine negative control, spermine, showed no such effect. Experiments using **3** (at ~10 µg/mL) and an increased concentration of CTAB (1%) revealed that 10% of the intracellular ATP was released after only a few seconds of contact (data not shown) suggesting a rapid disruption of the membrane barrier of the Gram-negative bacterium.

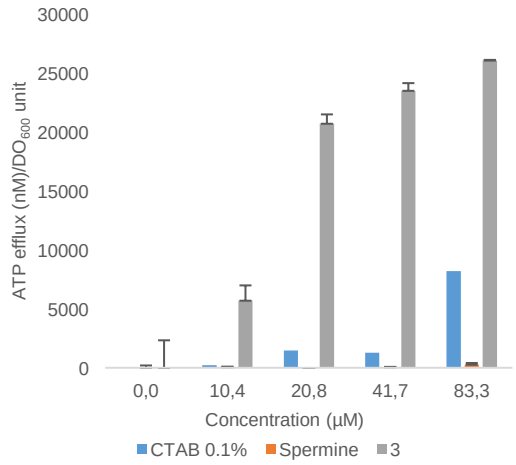

Fig. 3. Dose-dependent ATP release in *P. aeruginosa* (PAO1) exhibited by **3**.

Similar studies were conducted against the Gram-positive *S. aureus* strain where bromide **3** also caused rapid ATP leakage in a dose dependent manner (Fig. 4) albeit at a lower rate than the positive control, CTAB. Between them, the ATP leakage assays established that **3** can disrupt the integrity of bacterial membranes.

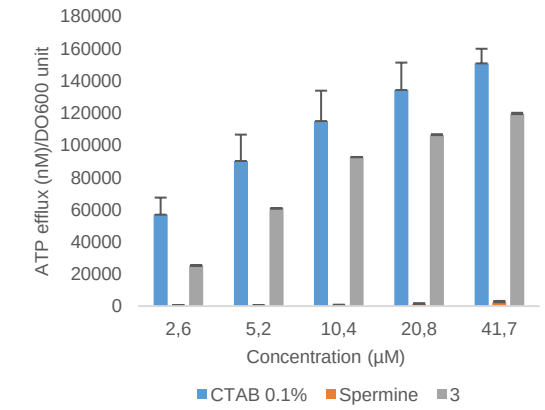

Fig. 4. Dose-dependent ATP release in *S. aureus* exhibited by **3**.

The noted ability of **3** to enhance the antibiotic action of doxycycline towards a range of Gram negative bacteria (Table 3) may arise from the polyamines ability to inhibit drug efflux systems. In particular, efflux pumps are often associated with the inherent resistance of *P. aeruginosa*.[18,19] In order to actively pump foreign molecules including antibiotics against their concentration gradient, an energy source such as a proton concentration gradient is required. Perturbation of the inner membrane through depolarisation causes loss of the proton concentration gradient resulting in the loss of the energy needed to efflux foreign compounds.[18,19] To better understand whether **3** can act as a disruptor of the transmembrane potential, a real-time efflux assay was utilised.[20] This assay makes use of the membrane-potential-sensitive probe $DiSC_3(5)$, which when preloaded into bacteria, concentrates in the inner membrane and quenches its own fluorescence. Upon membrane depolarisation, the dye is released and an increase in fluorescence (measured in relative fluorescence units) is observed. In a single-dose (10.4 µM) 15 min incubation experiment, strong depolarization was observed against both *P. aeruginosa* PAO1 and *S. aureus* ATCC25923 with a particularly strong response from the Gram-positive strain (Fig. 5). Negligible effect was observed for the negative control spermine.

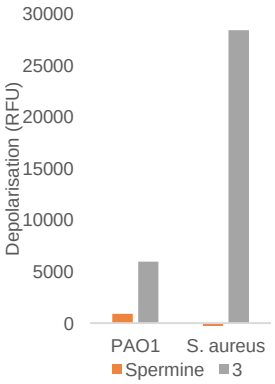

Fig. 5. Membrane depolarisation of *P. aeruginosa* (PAO1) and *S. aureus* by **3** at a single dose of 10.4 µM as measured using $DiSC_3(5)$ fluorescent dye release.

Closer examination of the membrane depolarization of *S. aureus* exhibited by **3** identified a dose-dependent response, with a rapid and strong in fluorescence (RFU), as also observed for the positive control, squalamine (Fig. 6).

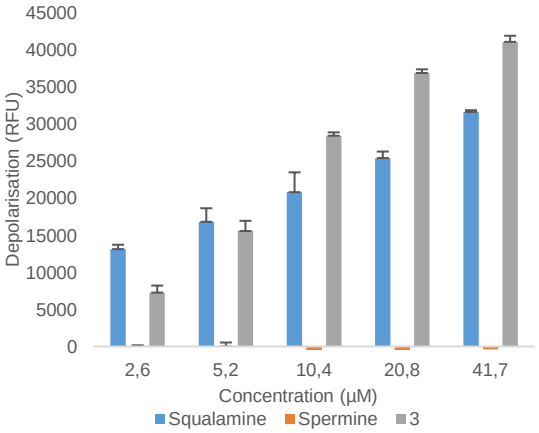

Fig. 6. Dose-dependent membrane depolarisation of *S. aureus* exhibited by **3**.

Taken together these results suggest that the intrinsic antimicrobial activity of **3** may arise from rapid disruption of cell membrane integrity, resulting in the loss of cell wall rigidity, leading to cell death while the antibiotic potentiating ability may arise from indirect efflux pump inhibition via depolarisation of the membrane.

4. Conclusion

Our initial screening identified 6-bromoindolglyoxylamides **3** and **4** as modest to moderately active antimicrobial agents with **3** also exhibiting the ability to enhance the antibiotic action of doxycycline and erythromycin towards a number of the Gram-negative bacteria including *Pseudomonas aeruginosa*, *Escherichia coli* and *Acinetobacter baumannii*. Preliminary exploration of the findings identified that longer polyamine chain analogues could exhibit more pronounced antimicrobial properties, and this peaked with the polyamine PA3-8-3 core for antibacterial activity and for the PA3-10-3 core for antifungal properties. Analogues that lacked a polyamine core were essentially devoid of antimicrobial activity. Antibiotic potentiating activity required the presence of a polyamine scaffold, with **3**, **5** and **8** being the most potent enhancers. Mechanism of action studies identified **3** as being able to disrupt the integrity of, and depolarize, bacterial membranes. The therapeutic potential of this class of compound is however limited by the observation of cytotoxicity, overcoming which is the focus of current studies.

5. Experimental

5.1. General remarks

Infrared spectra were recorded on a Perkin-Elmer spectrometer 100 Fourier Transform infrared spectrometer equipped with a universal ATR accessory. Mass spectra were acquired on a Bruker micrOTOF Q II

spectrometer. ^{1}H and ^{13}C NMR spectra were recorded at 298 K on a Bruker AVANCE 400 spectrometer at 400 and 100 MHz, respectively using standard pulse sequences. Proto-deutero solvent signals were used as internal references (DMSO-d_6: δ_H 2.50, δ_C 39.52; $CDCl_3$: δ_H 7.26, δ_C 77.16). For ^{1}H NMR, the data are quoted as position (δ), relative integral, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad), coupling constant (*J*, Hz), and assignment to the atom. The ^{13}C NMR data are quoted as position (δ), and assignment to the atom. Silica gel column chromatography was carried out using Davisil silica gel (40–60 μm) or Merck silica gel (15–40 μm). Column chromatography was also conducted on Merck Diol bonded silica (40–63 μm). Gel filtration flash chromatography was carried out on Sephadex LH-20 (Pharmacia). Thin layer chromatography was conducted on Merck DC-plastikfolien Kieselgel 60 F254 plates. All solvents used were of analytical grade or better and/or purified according to standard procedures. Chemical reagents used were purchased from standard chemical suppliers and used as purchased. All samples were determined to >95% purity. Compounds **3**,[13] **4**,[13] **7**,[13] **16**,[15] **17**,[11] di-*tert*-butyl hexane-1,6-diylbis((3-aminopropyl)carbamate),[14] di-*tert*-butyl heptane-1,7-diylbis((3-aminopropyl)carbamate),[14] di-*tert*-butyl decane-1,10-diylbis((3-aminopropyl)carbamate)[14] and di-*tert*-butyl dodecane-1,12-diylbis((3-aminopropyl)carbamate)[14] were synthesised by literature procedures.

5.2. Synthesis of compounds

5.2.1. General procedure for the preparation of compounds 5, 6, 8 and 9.

A two-step sequence was used for the preparation of these target compounds. In the first step, reaction of 2-(6-bromo-1H-indol-3-yl)-2-oxoacetyl chloride **16**[15] (2 eq.) and the appropriate Boc protected polyamine[14] (1 eq.) in DMF (1 mL) with DIPEA (6 eq.) were stirred for 48 h before solvent removal under reduced pressure. The crude reaction product was then subjected to Boc deprotection without any purification. A solution of crude *tert*-butyl-carboxylate derivative in CH_2Cl_2 (2 mL) and TFA (0.2 mL) was stirred at room temperature under N_2 for 2 h followed by solvent removal under reduced pressure. The reaction product was purified using C_8 reversed-phase column chromatography [0%–50% MeOH/H_2O (0.05% TFA)] to afford derivatives **5**, **6**, **8** and **9** as their di-TFA salt.

5.2.1.1. *N*^{1},*N*^{6}-Bis(3-(2-(6-bromo-1*H*-indol-3-yl)-2-oxoacetamido)propyl)hexane-1,6-diaminium 2,2,2-trifluoroacetate (5)

Following the general procedure, to a solution of 2-(6-bromo-1*H*-indol-3-yl)-2-oxoacetyl chloride[15] (0.072 g, 0.25 mmol) in DMF (2 mL) was added DIPEA (0.13 mL, 0.74 mmol) and di-*tert*-butyl hexane-1,6-diylbis((3-aminopropyl)carbamate)[14] (0.053 g, 0.12 mmol). The reaction mixture was stirred at room temperature for 48 h under N_2. The crude product was dissolved in CH_2Cl_2 (20 mL), washed with brine (2 x 20 mL) and the solvent removed in vacuo to afford di-*tert*-butyl hexane-1,6-diylbis((3-(2-(6-bromo-1*H*-indol-3-yl)-2-oxoacetamido)propyl)carbamate) as an orange oil (0.017 g, 15%). A sub-sample of this material (0.008 g, 0.008 mmol) was then deprotected by reaction with TFA (0.2 mL) in CH_2Cl_2 (2 mL) to give a crude product that was

purified by C_8 reversed-phase column chromatography [0%–50% MeOH (+0.05% TFA)/H_2O] to afford **5** as a pale yellow oil (0.003 g, 33%).

IR (ATR) ν_{max} 3439, 2981, 1675, 1629, 1442, 1201, 1132, 1024, 1000, 725, 799 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 12.35 (2H, br s, NH-1, NH-1'), 8.92 (2H, t, *J* = 5.6 Hz, NH-10, NH-10'), 8.79 (2H, s, H-2, H-2'), 8.47–8.35 (4H, m, NH_2-14, NH_2-14'), 8.16 (2H, d, *J* = 8.6 Hz, H-4, H-4'), 7.76 (2H, d, *J* = 1.5 Hz, H-7, H-7'), 7.42 (2H, dd, *J* = 8.6, 1.5 Hz, H-5, H-5 '), 3.33–3.28 (4H, m, H_2-11, H_2-11'), 3.00–2.92 (4H, m, H_2-13, H_2-13'), 2.92–2.86 (4H, m, H_2-15, H_2-15'), 1.89–1.83 (4H, m, H_2-12, H_2-12'), 1.60–1.54 (4H, m, H_2-16, H_2-16'), 1.33–1.30 (4H, m, H_2-17, H_2-17'); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 181.8 (C-8, C-8'), 163.4 (C-9, C-9'), 139.3 (C-2, C-2'), 137.2 (C-7a, C-7a'), 125.5 (C-5, C-5'), 125.2 (C-3a, C-3a'), 122.9 (C-4, C-4'), 116.0 (C-6, C-6'), 115.4 (C-7, C-7'), 112.0 (C-3, C-3'), 46.7 (C-15, C-15'), 44.7 (C-13, C-13'), 35.8 (C-11, C-11'), 25.7 (C-12, C-12'), 25.5 (C-17, C-17'), 25.4 (C-16, C-16'); (+)-HRESIMS $[M+H]^+$ *m/z* 729.1416 (calcd for $C_{32}H_{39}{}^{79}Br_2N_6O_4$, 729.1394), 731.1395 (calcd for $C_{32}H_{39}{}^{79}Br^{81}BrN_6O_4$, 731.1376), 733.1383 (calcd for $C_{32}H_{39}{}^{81}Br_2N_6O_4$, 733.1364).

5.2.1.2. *N*^{1},*N*^{7}-Bis(3-(2-(6-bromo-1*H*-indol-3-yl)-2-oxoacetamido)propyl)heptane-1,7-diaminium 2,2,2-trifluoroacetate (6)

Following the general procedure, to a solution of 2-(6-bromo-1*H*-indol-3-yl)-2-oxoacetyl chloride[15] (0.072 g, 0.25 mmol) in DMF (2 mL) was added DIPEA (0.13 mL, 0.74 mmol) and di-*tert*-butyl heptane-1,7-diylbis((3-aminopropyl)carbamate)[14] (0.055 g, 0.12 mmol). The reaction mixture was stirred at room temperature for 48 h under N_2. The crude product was dissolved in CH_2Cl_2 (20 mL), washed with brine (2 x 20 mL) and the solvent removed in vacuo to afford di-*tert*-butyl heptane-1,7-diylbis((3-(2-(6-bromo-1*H*-indol-3-yl)-2-oxoacetamido)propyl)carbamate) as a red-orange oil (0.021 g, 18%). A sub-sample of this material (0.020 g, 0.022 mmol) was then deprotected by reaction with TFA (0.2 mL) in CH_2Cl_2 (2 mL) to give a crude product that was purified by C_8 reversed-phase column chromatography [0%–50% MeOH (+0.05% TFA)/H_2O] to afford **6** as a pale yellow oil (0.006 g, 29%).

IR (ATR) ν_{max} 3349, 2947, 2834, 1552, 1450, 1416, 1204, 1113 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 12.38 (2H, br s, NH-1, NH-1'), 8.91 (2H, t, *J* = 6.1 Hz, NH-10, NH-10'), 8.78 (2H, s, H-2, H-2'), 8.47–8.44 (4H, m, NH_2-14, NH_2-14'), 8.15 (2H, d, *J* = 8.5 Hz, H-4, H-4'), 7.75 (2H, d, *J* = 1.6 Hz, H-7, H-7'), 7.41 (2H, dd, *J* = 8.5, 1.6 Hz, H-5, H-5'), 3.31–3.28 (4H, m, H_2-11, H_2-11'), 2.96–2.88 (8H, m, H_2-13, H_2-13', H_2-15, H_2-15'), 1.88–1.83 (4H, m, H_2-12, H_2-12'), 1.58–1.54 (4H, m, H_2-16, H_2-16'), 1.28–1.24 (6H, m, H_2-17, H_2-17', H_2-18); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 181.8 (C-8, C-8'), 163.4 (C-9, C-9'), 139.3 (C-2, C-2'), 137.2 (C-7a, C-7a'), 125.4 (C-5, C-5'), 125.2 (C-3a, C-3a'), 122.9 (C-4, C-4'), 116.0 (C-6, C-6'), 115.4 (C-7, C-7'), 112.0 (C-3, C-3'), 46.7 (C-15, C-15'), 44.7 (C-13, C-13'), 35.8 (C-11, C-11'), 28.0 (C-18), 25.8 (C-12, C-12'), 25.7 (C-17, C-17'), 25.4 (C-16, C-16'); (+)-HRESIMS $[M+H]^+$ *m/z* 743.1534 (calcd for $C_{33}H_{41}{}^{79}Br_2N_6O_4$, 743.1551), 745.1517 (calcd for $C_{33}H_{41}{}^{79}Br^{81}BrN_6O_4$, 745.1533), 747.1504 (calcd for $C_{33}H_{41}{}^{81}Br_2N_6O_4$, 747.1521).

5.2.1.3. *N*^{1},*N*^{10}-bis(3-(2-(6-bromo-1*H*-indol-3-yl)-2-oxoacetamido)propyl)decane-1,10-diaminium 2,2,2-trifluoroacetate (8)

Following the general procedure, to a solution of 2-(6-bromo-1*H*-indol-3-yl)-2-oxoacetyl chloride[15] (0.076 g, 0.27 mmol) in DMF (2 mL) was added DIPEA (0.14 mL, 0.80 mmol) and di-*tert*-butyl decane-1,10-diylbis((3-aminopropyl)carbamate)[14] (0.063 g, 0.13 mmol). The reaction mixture was stirred at room temperature for 48 h under N_2. The crude product was dissolved in CH_2Cl_2 (20 mL), washed with brine (2 x 20 mL) and the solvent removed in vacuo to afford di-*tert*-butyl decane-1,10-diylbis((3-(2-(6-bromo-1*H*-indol-3-yl)-2-oxoacetamido)propyl)carbamate) as a dark orange oil (0.42 g, 33%). A sub-sample of this material (0.014 g, 0.014 mmol) was then deprotected by reaction with TFA (0.2 mL) in CH_2Cl_2 (2 mL) to give a crude product that was purified by C_8 reversed-phase column chromatography [0%–50% MeOH (+0.05% TFA)/H_2O] to afford **8** as a pale yellow oil (0.006 g, 55%).

IR (ATR) ν_{max} 3394, 2259, 1654, 1024, 993, 825, 762 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 12.46 (2H, br s, NH-1, NH-1'), 8.98 (2H, t, *J* = 6.2 Hz, NH-10, NH-10'), 8.84 (2H, d, *J* = 3.1 Hz, H-2, H-2'), 8.79 (4H, br s, NH_2-14, NH_2-14'), 8.22 (2H, d, *J* = 8.5 Hz, H-4, H-4'), 7.82 (2H, d, *J* = 2.0 Hz, H-7, H-7'), 7.47 (2H, dd, *J* = 8.5, 2.0 Hz, H-5, H-5'), 3.36 (4H, dt, *J* = 6.4, 6.2 Hz, H_2-11, H_2-11'), 3.06–2.91 (8H, m, H_2-13, H_2-13', H_2-15, H_2-15'), 1.94–1.84 (4H, m, H_2-12, H_2-12'), 1.65–1.58 (4H, m, H_2-16, H_2-16'), 1.36–1.29 (12H, m, H_2-17, H_2-17', H_2-18, H_2-18', H_2-19, H_2-19'); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 181.8 (C-8, C-8'), 163.5 (C-9, C-9'), 138.8 (C-2, C-2'), 137.2 (C-7a, C-7a'), 125.8 (C-3a, C-3a'), 125.4 (C-5, C-5'), 122.9 (C-4, C-4'), 117.9 (C-6, C-6'), 115.3 (C-7, C-7'), 113.8 (C-3, C-3'), 46.8 (C-15, C-15'), 44.7 (C-13, C-13'), 35.8 (C-11, C-11'), 28.7 (C-18, C-18'), 28.5 (C-19, C-19'), 25.9 (C-12, C-12'), 25.6 (C-17, C-17'), 25.4 (C-16, C-16'); (+)-HRESIMS $[M+H]^+$ *m/z* 785.2031 (calcd for $C_{36}H_{47}{}^{79}Br_2N_6O_4$, 785.2020), 787.2060 (calcd for $C_{36}H_{47}{}^{79}Br^{81}BrN_6O_4$, 787.2003), 789.2015 (calcd for $C_{36}H_{47}{}^{81}Br_2N_6O_4$, 789.1992).

5.2.1.4. N^1,N^{12}-bis(3-(2-(6-bromo-1*H*-indol-3-yl)-2-oxoacetamido)propyl)dodecane-1,12-diaminium 2,2,2-trifluoroacetate (9)

Following the general procedure, to a solution of 2-(6-bromo-1*H*-indol-3-yl)-2-oxoacetyl chloride[15] (0.078 g, 0.27 mmol) in DMF (2 mL) was added DIPEA (0.14 mL, 0.82 mmol) and di-*tert*-butyl dodecane-1,12-diylbis((3-aminopropyl)carbamate)[14] (0.070 g, 0.14 mmol). The reaction mixture was stirred at room temperature for 48 h under N_2. The crude product was dissolved in CH_2Cl_2 (20 mL), washed with brine (2 x 20 mL) and the solvent removed in vacuo to afford di-*tert*-butyl dodecane-1,12-diylbis((3-(2-(6-bromo-1*H*-indol-3-yl)-2-oxoacetamido)propyl)carbamate) as a dark orange oil (0.041 g, 28.9%). A sub-sample of this material (0.019 g, 0.019 mmol) was then deprotected by reaction with TFA (0.2 mL) in CH_2Cl_2 (2 mL) to give a crude product that was purified by C_8 reversed-phase column chromatography [0%–50% MeOH (+0.05% TFA)/H_2O] to afford **9** as a pale yellow oil (0.016 g, quantitative).

IR (ATR) ν_{max} 3350, 2928, 2840, 1678, 1634, 1503, 1441, 1203, 1137, 1032, 799, 722 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 12.47 (2H, br s, NH-1, NH-1'), 8.91 (2H, t, *J* = 6.0 Hz, NH-10, NH-10'), 8.77 (2H, d, *J* = 2.8 Hz, H-2, H-2'), 8.56–8.54 (4H, m, NH_2-14, NH_2-14'), 8.15 (2H, d, *J* = 8.5 Hz, H-4, H-4'), 7.75 (2H, d, *J* = 1.3 Hz, H-7, H-7'), 7.40 (2H, dd, *J* = 8.5, 1.3 Hz, H-5, H-5'), 3.32–3.27 (4H, m, H_2-11, H_2-11'), 2.93–2.89 (8H, m, H_2-13, H_2-13', H_2-15, H_2-15'), 1.87–1.83 (4H, m, H_2-12, H_2-12'), 1.55–1.53 (4H, m, H_2-16, H_2-16'), 1.27–1.21 (16H, m, H_2-17, H_2-17', H_2-18, H_2-18', H_2-19, H_2-19', H_2-20, H_2-20'); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 181.8 (C-8, C-

8'), 163.5 (C-9, C-9'), 139.3 (C-2, C-2'), 137.2 (C-7a, C-7a'), 125.4 (C-3a, C-3a'), 125.3 (C-5, C-5'), 122.9 (C-4, C-4'), 116.0 (C-6, C-6'), 115.4 (C-7, C-7'), 112.1 (C-3, C-3'), 46.8 (C-15, C-15'), 44.7 (C-13, C-13'), 35.8 (C-11, C-11'), 28.9 (C-18, C-18'), 28.8 (C-19, C-19'), 28.5 (C-20, C-20'), 25.9 (C-12, C-12'), 25.6 (C-17, C-17'), 25.5 (C-16, C-16'); (+)-HRESIMS $[M+H]^+$ *m/z* 813.2346 (calcd for $C_{38}H_{51}{}^{79}Br_2N_6O_4$, 813.2333), 815.2331 (calcd for $C_{38}H_{51}{}^{79}Br^{81}BrN_6O_4$, 815.2317), 817.2316 (calcd for $C_{38}H_{51}{}^{81}Br_2N_6O_4$, 817.2306).

5.2.2 General procedure for the preparation of analogues 10–15.

To a stirred solution of 2-(6-bromo-1*H*-indol-3-yl)-oxoacetic acid (**17**)[11] (1 eq.) and diamine (1 eq.) in DMF (2 mL) was added PyBOP (1 eq.) and triethylamine (3 eq.). The reaction mixture was stirred for 24 h at room temperature under N_2. To the crude reaction product was added CH_2Cl_2 (30 mL) and the organic layer washed with aq. K_2CO_3 (1%, 2 x 30 mL) followed by water (2 x 30 mL). The organic extract was concentrated under reduced pressure and subjected to purification (as described below) to afford the target compound.

5.2.2.1. 2-(6-Bromo-1*H*-indol-3-yl)-*N*-(3-((2-(2-(6-bromo-1*H*-indol-3-yl)-2-oxoacetamido)ethyl)amino)propyl)-2-oxoacetamide (10)

Following the general procedure, **17**[11] (0.087 g, 0.324 mmol) was reacted with aminoethyl-1,3-propandiamine (0.020 mL, 0.162 mmol), PyBOP (0.169 g, 0.324 mmol) and triethylamine (0.135 mL, 0.972 mmol) in DMF for 24 h. The crude reaction product was concentrated under reduced pressure and washed with CH_2Cl_2 followed by MeOH/water (1:4) to afford **10** as a yellow solid (0.058 g, 58%).

R_t 10.82 mins; m.p. > 250 °C; IR (ATR) ν_{max} 3287, 3178, 2953, 1655, 1597, 1505, 1234, 658 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 12.40 (2H, br s, NH-1, NH-26), 8.94 (1H, t, *J* = 5.8 Hz, NH-17), 8.92 (1H, t, *J* = 6.3 Hz, NH-10), 8.83 (1H, s, H-2/H-25), 8.78 (1H, s, H-2/H-25), 8.15 (1H, d, *J* = 8.5 Hz, H-4/H-23), 8.14 (1H, d, *J* = 8.5 Hz, H-4/H-23), 7.75 (1H, d, *J* = 1.8 Hz, H-7/H-20), 7.74 (1H, d, *J* = 8.5 Hz, H-7/H-20), 7.40 (1H, dd, *J* = 8.5, 1.8 Hz, H-5/H-22), 7.39 (1H, dd, *J* = 8.5, 1.8 Hz, H-5/H-22), 3.57–3.52 (2H, m, H_2-11), 3.31–3.28 (2H, m, H_2-16), 3.10 (2H, t, *J* = 6.0 Hz, H_2-12), 2.97 (2H, t, *J* = 7.4 Hz, H_2-14), 1.92–1.83 (2H, m, H_2-15), NH-13 not observed; ^{13}C NMR (DMSO-d_6, 100 MHz) δ 181.9 (C-8/C-19), 181.1 (C-8/C-19), 163.44 (C-9/C-18), 163.36 (C-9/C-18), 139.4 (C-2/C-25), 139.2 (C-2/C-25), 137.21 (C-7a/C-26a), 137.16 (C-7a/C-26a), 125.5 (C-5, C-22), 125.2 (C-3a, C-23a), 122.9 (C-4, C-23), 116.0 (C-6, C-21), 115.4 (C-7, C-20), 112.03 (C-3/C-24), 111.96 (C-3/C-24), 46.1 (C-12), 44.8 (C-14), 35.9 (C-11), 35.3 (C-16), 25.8 (C-15); (+)-HIRESIMS *m/z* 616.0168 $[M+H]^+$ (calcd for $C_{25}H_{24}Br_2N_5O_4$, 616.0190).

5.2.2.2. *N,N'*-(Butane-1,4-diyl)bis(2-(6-bromo-1*H*-indol-3-yl)-2-oxoacetamide) (11)

Following the general procedure, **17**[11] (0.091 g, 0.340 mmol) was reacted with 1,4-diaminobutane (0.015 g, 0.170 mmol), PyBOP (0.177 g, 0.340 mmol) and triethylamine (0.142 mL, 1.02 mmol) in DMF for 24 h. The crude reaction product was concentrated under reduced pressure and washed with MeOH/water (4:1) to afford **11** as a brown solid (0.058 g, 58%).

R_t 8.06 mins; m.p. > 250 °C; IR (ATR) ν_{max} 3241, 2931, 2872, 1652, 1605, 1416, 1239, 661 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 12.26 (2H, d, *J* = 2.0 Hz, NH-1, NH-1′), 8.76 (2H, t, *J* = 5.6 Hz, NH-10, NH-10′), 8.76 (2H, d, *J* = 3.0 Hz, H-2, H-2′), 8.14 (2H, d, *J* = 8.5 Hz, H-4, H-4′), 7.72 (2H, d, *J* = 1.3 Hz, H-7, H-7′), 7.39 (2H, dd, *J* = 8.5, 1.3 Hz, H-5, H-5′), 3.27–3.21 (4H, m, H_2-11, H_2-11′), 1.57–1.52 (4H, m, H_2-12, H_2-12′); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 182.4 (C-8, C-8′), 163.3 (C-9, C-9′), 139.2 (C-2, C-2′), 137.2 (C-7a, C-7a′), 125.3 (C-5, C-5′), 125.2 (C-3a, C-3a′), 122.9 (C-4, C-4′), 115.9 (C-6, C-6′), 115.2 (C-7, C-7′), 112.1 (C-3, C-3′), 38.2 (C-11, C-11′), 26.3 (C-12, C-12′); (+)-HIRESIMS *m/z* 608.9714 $[M+Na]^+$ (calcd for $C_{24}H_{20}Br_2N_4NaO_4$, 608.9744).

5.2.2.3. *N,N*'-(Dodecane-1,12-diyl)bis(2-(6-bromo-1*H*-indol-3-yl)-2-oxoacetamide) (12)

Following the general procedure, **17**[11] (0.077 g, 0.286 mmol) was reacted with 1,12-diaminododecane (0.029 g, 0.143 mmol), PyBOP (0.149 g, 0.286 mmol) and triethylamine (0.119 mL, 0.857 mmol) in DMF for 24 h. The crude reaction product was concentrated under reduced pressure and washed with MeOH/water (4:1) to afford **12** as an off-white solid (0.080 g, 80%).

R_t 7.98 mins; m.p. > 250 °C; IR (ATR) ν_{max} 3304, 3234, 2921, 1604, 1505, 1421, 1128, 799 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 12.26 (2H, br s, NH-1, NH-1′), 8.75 (2H, s, H-2, H-2′), 8.70 (2H, t, *J* = 5.9 Hz, NH-10, NH-10′), 8.14 (2H, d, *J* = 8.5 Hz, H-4, H-4′), 7.73 (2H, d, *J* = 1.0 Hz, H-7, H-7′), 7.39 (2H, dd, *J* = 8.5, 1.0 Hz, H-5, H-5′), 3.22–3.15 (4H, m, H_2-11, H_2-11′), 1.53–1.45 (4H, m, H_2-12, H_2-12′), 1.27–1.22 (16H, m, H_2-13, H_2-14, H_2-15, H_2-16, H_2-13′, H_2-14′, H_2-15′, H_2-16′); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 182.4 (C-8, C-8′), 163.2 (C-9, C-9′), 139.2 (C-2, C-2′), 137.2 (C-7a, C-7a′), 125.3 (C-5, C-5′), 125.2 (C-3a, C-3a′), 122.9 (C-4, C-4′), 115.9 (C-6, C-6′), 115.3 (C-7, C-7′), 112.1 (C-3, C-3′), 38.5 (C-11, C-11′), 29.0 (C-12/C-13/C-14/C-15/C-16/C-12′/C-13′/C-14′/C-15′/C-16′), 28.73 (C-12/C-13/C-14/C-15/C-16/C-12′/C-13′/C-14′/C-15′/C-16′), 28.68 (C-12/C-13/C-14/C-15/C-16/C-12′/C-13′/C-14′/C-15′/C-16′), 26.4 (C-12/C-13/C-14/C-15/C-16/C-12′/C-13′/C-14′/C-15′/C-16′); (+)-HIRESIMS *m/z* 721.0973 $[M+Na]^+$ (calcd for $C_{32}H_{36}Br_2N_4NaO_4$, 721.0996).

5.2.2.4. *N,N′*-(((Oxybis(ethane-2,1-diyl))bis(oxy))bis(propane-3,1-diyl)) bis(2-(6-bromo-1*H*-indol-3-yl)-2-oxoacetamide) (13)

Following the general procedure, **17**[11] (0.075 g, 0.278 mmol) was reacted with 4,7,10-trioxa-1,13-tridecanediamine (0.030 mL, 0.139 mmol), PyBOP (0.145 g, 0.278 mmol) and triethylamine (0.116 mL, 0.833 mmol) in DMF for 24 h. Solvent was removed under reduced pressure and the crude reaction product was subjected to purification by a combination of silica gel (CH_2Cl_2/5%MeOH) and Sephadex LH20 (MeOH) column chromatography, to afford **13** as a white solid (0.024 g, 24%).

R_f (CH_2Cl_2/MeOH, 9:1) 0.51; m.p. 189.6–191.2 °C; IR (ATR) ν_{max} 3216, 2922, 2866, 1655, 1598, 1503, 1134, 1107, 742, 659 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 12.23 (2H, br s, NH-1, NH-1′), 8.76 (2H, s, H-2, H-2′), 8.71 (2H, t, *J* = 5.9 Hz, NH-10, NH-10′), 8.14 (2H, d, *J* = 8.5 Hz, H-4, H-4′), 7.72 (2H, d, *J* = 1.4 Hz, H-7, H-7′), 7.39 (2H, dd, *J* = 8.5, 1.4 Hz, H-5, H-5′), 3.56–3.47 (8H, m, H_2-14, H_2-15, H_2-14′, H_2-15′), 3.44 (4H, t, *J* = 6.1 Hz, H_3-13, H_3-13′), 3.29–3.24 (4H, m, H_2-11, H_2-11′), 1.78–1.70 (4H, m, H_2-12, H_2-12′); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 182.3 (C-8, C-8′), 163.2 (C-9, C-9′), 139.2 (C-2, C-2′), 137.2 (C-7a, C-7a′), 125.3 (C-5, C-5′),

125.2 (C-3a, C-3a′), 122.9 (C-4, C-4′), 115.9 (C-6, C-6′), 115.2 (C-7, C-7′), 112.1 (C-3, C-3′), 69.8 (C-14/C-15/C-14′/C-15′), 69.6 (C-14/C-15/C-14′/C-15′), 68.3 (C-13, C-13′), 36.2 (C-11, C-11′), 29.0 (C-12, C-12′); (+)-HIRESIMS *m/z* 741.0533 $[M+Na]^+$ (calcd for $C_{30}H_{32}Br_2N_4NaO_7$, 741.0530).

5.2.2.5. 2-(6-Bromo-1*H*-indol-3-yl)-*N*-(2-(dimethylamino)ethyl)-2-oxoacetamide (14)

Following the general procedure, **17**[11] (0.079 g, 0.296 mmol) was reacted with *N*,*N*-dimethylethylenediamine (0.032 mL, 0.296 mmol), PyBOP (0.154 g, 0.296 mmol) and triethylamine (0.124 mL, 0.887 mmol) in DMF for 24 h. Purification by diol-bonded silica gel column chromatography (CH_2Cl_2/3% MeOH) afforded **14** as a white solid (0.041 g, 41%).

R_f (CH_2Cl_2/MeOH, 9:1) 0.11; m.p. 209.1–210.5 °C; IR (ATR) ν_{max} 3345, 3129, 2779, 1615, 1534, 1482, 1231, 816, 681 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 12.28 (1H, br s, NH-1), 8.80 (1H, s, H-2), 8.56 (1H, t, *J* = 5.8 Hz, NH-10), 8.14 (1H, d, *J* = 8.3 Hz, H-4), 7.73 (1H, d, *J* = 1.8 Hz, H-7), 7.40 (1H, dd, *J* = 8.3, 1.8 Hz, H-5), 3.34–3.27 (2H, m, H_2-11), 2.39 (2H, t, *J* = 6.7 Hz, H_2-12), 2.18 (6H, s, $2H_3$-13); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 182.1 (C-8), 163.0 (C-9), 139.4 (C-2), 137.2 (C-7a), 125.4 (C-5), 125.2 (C-3a), 122.9 (C-4), 115.9 (C-6), 115.3 (C-7), 112.1 (C-3), 57.6 (C-12), 45.1 (C-13), 36.6 (C-11); (+)-HIRESIMS *m/z* 338.0498 $[M+H]^+$ (calcd for $C_{14}H_{16}BrN_3O_2$, 338.0499).

5.2.2.6. 2-(6-Bromo-1*H*-indol-3-yl)-*N*-(2-morpholinoethyl)-2-oxoacetamide (15)

Following the general procedure, **17**[11] (0.071 g, 0.263 mmol) was reacted with 2-morpholinoethylamine (0.034 g, 0.263 mmol), PyBOP (0.137 g, 0.263 mmol) and triethylamine (0.110 mL, 0.789 mmol) in DMF for 24 h. Purification by diol-bonded silica gel column chromatography (CH_2Cl_2/3% MeOH) afforded **15** as a white solid (0.046 g, 46%).

R_f (CH_2Cl_2/MeOH, 9:1) 0.54; m.p. 200.5–201.4 °C; IR (ATR) ν_{max} 3304, 3255, 2818, 1659, 1598, 1504, 1110, 661 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 9.04 (1H, d, *J* = 3.0 Hz, H-2), 9.03 (1H, br s, NH-1), 8.29 (1H, d, *J* = 8.7 Hz, H-4), 7.87 (1H, t, *J* = 5.0 Hz, NH-10), 7.60 (1H, d, *J* = 1.6 Hz, H-7), 7.44 (1H, dd, *J* = 8.7, 1.6 Hz, H-5), 3.75 (4H, t, *J* = 4.7 Hz, $2H_2$-14), 3.51–3.46 (2H, m, H_2-11), 2.59 (2H, t, *J* = 6.1 Hz, H_2-12), 2.53–2.49 (4H, m, $2H_2$-13); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 180.9 (C-8), 162.3 (C-9), 138.4 (C-2), 136.6 (C-7a), 126.8 (C-5), 125.7 (C-3a), 123.9 (C-4), 117.8 (C-6), 114.8 (C-7), 113.6 (C-3), 67.1 (C-14), 57.0 (C-12), 53.6 (C-13), 35.8 (C-11); (+)-HIRESIMS *m/z* 380.0602 $[M+H]^+$ (calcd for $C_{16}H_{19}BrN_3O_3$, 380.0604).

5.3. Antimicrobial assays

5.3.1. Bacterial strains

Bacterial strains used in this study were: *S. aureus* (ATCC25923), *S. intermedius* (1051997), *E. coli* (ATCC25922) and *P. aeruginosa* (ATCC27853 and PAO1). Strains were maintained at -80 °C in 15% (v/v) glycerol for cryoprotection. Bacteria were routinely grown in Mueller-Hinton (MH) broth at 37 °C.

5.3.2. Antibiotics

Doxycycline was purchased from TCI Europe and dissolved in water.

5.3.3. Antimicrobial evaluation

The susceptibility of bacterial strains to antibiotics and compounds was determined in microplates using the standard broth dilution method in accordance with the recommendations of the Comité de l'AntibioGramme de la Société Française de Microbiologie (CA-SFM). Briefly, the minimal inhibitory concentrations (MICs) were determined with an inoculum of 10^5 CFU in 200 µL of MH broth containing two-fold serial dilutions of each drug. The MIC was defined as the lowest concentration of drug that completely inhibited visible growth after incubation for 18 h at 37 °C. To determine all MICs, the measurements were independently repeated in triplicate.

5.4. Determination of the MICs of antibiotics in the presence of synergising compounds

Briefly, restoring enhancer concentrations were determined with an inoculum of 5 10^5 CFU in 200 µL of MH broth containing two-fold serial dilutions of each derivative in the presence of doxycycline at 2 µg/mL. The lowest concentration of the polyamine adjuvant that completely inhibited visible growth after incubation for 18 h at 37 °C was determined. These measurements were independently repeated in triplicate.

5.5. Measurement of ATP efflux

General procedure illustrated with CTAB (0.1%)

CTAB solutions (0.1%) were prepared in doubly distilled water at different concentrations. A suspension of growing *S. aureus* (ATCC25923) or *P. aeruginosa* (PAO1) to be studied in MH broth was prepared and incubated at 37 °C. This suspension (90 µL) was added to 10 µL of CTAB solution and vortexed for 1 sec. Luciferin-luciferase reagent (Yelen, France; 50 µL) was immediately added to the precedent mix and luminescent signal quantified with an Infinite M200 microplate reader (Tecan) for five secs. ATP concentration was quantified by internal sample addition. A similar procedure was used for negative control spermine (100 µg/mL) and for for test compound **3** (4 times the MIC).

5.6. Membrane depolarisation assays

Bacteria were grown in MH broth for 24 h at 37 °C and centrifuged at 10000 rpm at 20 °C. The supernatant was discarded and the bacteria were washed twice with buffered sucrose solution (250 mM) and magnesium sulfate solution (5 mM). The fluorescent dye 3,3′-diethylthiacarbocyanine iodide was added to a final concentration of 3 µM and allowed to penetrate into bacterial membranes during 1 h of incubation at 37 °C. Bacteria were then washed to remove the unbound dye before adding compound at different concentrations. Fluorescence measurements were performed using a Jobin Yvon Fluoromax 3 spectrofluorometer with slit widths of 5/5 nm.

The maximum RCF was considered to be that recorded with a pure solution of the fluorescent dye in buffer (3 μM).

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Supplementary information

Supplementary data related to this article can be found at

References

1. Alekshun MN, Levy SB. Molecular Mechanisms of Antibacterial Multidrug Resistance. *Cell*. 2007;128:1037–1050. doi:10.1016/j.cell.2007.03.004.
2. Wright GD. Solving the Antibiotic Crisis. *ACS Infect Dis*. 2015;1:80–84. doi:10.1021/id500052s.
3. Anon. A three-step plan for antibiotics. *Nature*. 2014;509:533.
4. Payne DJ, Gwynn MN, Holmes DJ, Pompliano DL. Drugs for bad bugs: confronting the challenges of antibacterial discovery. *Nat Rev Drug Discov*. 2007;6:29–40. doi:10.1038/nrd2201.
5. Blanchet M, Borselli D, Brunel JM. Polyamine derivatives: a revival of an old neglected scaffold to fight resistant Gram-negative bacteria? *Future Med. Chem.* 2016;8:963–973. doi:10.4155/fmc-2016-0011.
6. Kwon D-H, Lu C-D. Polyamine Effects on Antibiotic Susceptibility in Bacteria. *Antimicrob Agents Chemother*. 2007;51:2070–2077. doi:10.1128/AAC.01472-06.
7. Pieri C, Borselli D, Di Giorgio C, De Méo M, Bolla J-M, Vidal N, Combes S, Brunel JM. New Ianthelliformisamine Derivatives as Antibiotic Enhancers against Resistant Gram-Negative Bacteria. *J Med Chem*. 2014;57:4263–4272. doi:10.1021/jm500194e.
8. Salmi C, Loncle C, Vidal N, Letourneux Y, Fantini J, Maresca M, Taïeb N, Pagès J-M, Brunel JM. Squalamine: An Appropriate Strategy against the Emergence of Multidrug Resistant Gram-Negative Bacteria? Romesberg F, ed. *PLoS ONE*. 2008;3:e2765. doi:10.1371/journal.pone.0002765.
9. Lavigne J-P, Brunel J-M, Chevalier J, Pagès J-M. Squalamine, an original chemosensitizer to combat antibiotic-resistant Gram-negative bacteria. *J Antimicrob Chemother*. 2010;65:799–801. doi:10.1093/jac/dkq031.
10. Alhanout K, Malesinki S, Vidal N, Peyrot V, Rolain JM, Brunel JM. New insights into the antibacterial mechanism of action of squalamine. *J Antimicrob Chemother*. 2010;65:1688–1693. doi:10.1093/jac/dkq213.
11. Finlayson R, Pearce AN, Page MJ, Kaiser M, Bourguet-Kondracki M-L, Harper JL, Webb VL, Copp BR. Didemnidines A and B, Indole Spermidine Alkaloids from the New Zealand Ascidian *Didemnum* sp. *J Nat Prod*. 2011;74:888–892. doi:10.1021/np1008619.
12. Liew LPP, Pearce AN, Kaiser M, Copp BR. Synthesis and in vitro and in vivo evaluation of antimalarial polyamines. *Euro J Med Chem*. 2013;69:22–31. doi:10.1016/j.ejmech.2013.07.055
13. Wang J, Kaiser M, Copp BR. Investigation of Indolglyoxamide and Indolacetamide Analogues of Polyamines as Antimalarial and Antitrypanosomal Agents. *Mar Drugs*. 2014;12:3138–3160. doi:10.3390/md12063138.
14. Pearce AN, Kaiser M, Copp BR. Synthesis and antimalarial evaluation of artesunate-polyamine and trioxolane-polyamine conjugates. *Euro J Med Chem*. 2017;140:595–603. doi:10.1016/j.ejmech.2017.09.040

15. Galcera C, Sidhu A, Roubert P, Thurieau C. 5-Sulphanyl-4h-1,2,4-Triazole Derivatives and Their Use as Medicine. WO03045926 (A1). 2003.
16. Blanchet M, Borselli D, Rodallec A, Peiretti F, Vidal N, Bolla J-M, Digiorgio C, Morrison KR, Wuest WM, Brunel JM. Claramines: A New Class of Broad-Spectrum Antimicrobial Agents With Bimodal Activity. *Chem Med Chem*. 2018;13:1018–1027. doi: 10.1002/cmdc.201800073.
17. Yasuda K, Ohmizo C, Katsu T. Mode of action of novel polyamines increasing the permeability of bacterial outer membrane. *Int. J. Antimicrob. Agents* 2004;24:67–71. doi:10.1016/j.ijantimicag.2004.01.006.
18. Schweizer HP. Understanding efflux in Gram-negative bacteria: opportunities for drug discovery. *Expert Opin Drug Discov*. 2012;7:633–642. doi:10.1517/17460441.2012.688949.
19. Pagès J-M, James CE, Winterhalter M. The porin and the permeating antibiotic: a selective diffusion barrier in Gram-negative bacteria. *Nat Rev Microbiol*. 2008;6:893–903. doi:10.1038/nrmicro1994.
20. Borselli D, Lieutaud A, Thefenne H, Garnotel E, Pagès J-M, Brunel JM, Bolla J-M. Polyamino-Isoprenic Derivatives Block Intrinsic Resistance of P. aeruginosa to Doxycycline and Chloramphenicol In Vitro. *PLOS ONE*. 2016;11:e0154490. doi:10.1371/journal.pone.0154490.