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5-Alkyloxytryptamines are membrane-targeting, broad-spectrum antibiotics

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ABSTRACT

Antibiotic adjuvant therapy represents an exciting opportunity to enhance the activity of clinical antibiotics by co-dosing with a secondary small molecule. Successful adjuvants decrease the concentration of antibiotics used to defeat bacteria, increase activity (in some cases introducing activity against organisms that are drug resistant), and reduce the frequency at which drug-resistant bacteria emerge. We report that 5-alkyloxytryptamines are a new class of broad-spectrum antibacterial agents with exciting activity as antibiotic adjuvants. We synthesized 5-alkyloxytryptamine analogs and found that an alkyl chain length of 6–12 carbons and a primary ammonium group are necessary for the antibacterial activity of the compounds, and an alkyl chain length of 6–10 carbons increased the membrane permeability of Gram-positive and Gram-negative bacteria. Although several of the most potent analogs also have activity against the membranes of human embryonic kidney cells, we demonstrate that below the minimum inhibitory concentration (MIC)—where mammalian cell toxicity is low—these compounds may be successfully used as adjuvants for chloramphenicol, tetracycline, ciprofloxacin, and rifampicin against clinical strains of *Salmonella typhimurium*, *Acinetobacter baumannii* and *Staphylococcus aureus*, reducing MIC values by as much as several logs.

The juxtaposition between the slow pace of antibiotic discovery and the accelerated rate of antibacterial resistance is a current concern in the medical and research community.[1–3] Of particular concern are Gram-negative bacteria, which use multiple mechanisms to evade antibiotics, including the diffusion barrier presented by their bilayer membranes, pumps for drug efflux, and enzymes that modify and deactivate drugs.[4–7] Antibiotic adjuvant therapy is an antibacterial development strategy gaining attention in combating these three major hurdles, improving the efficacy of existing antibiotics, and suppressing the emergence of drug resistance.[8–13]

In antibiotic adjuvant therapy, two or more compounds—one of which is traditionally an antibiotic—are combined to potentiate function and improve activity. There are several successful examples of combination therapy, including Augmentin™: the clinically successful combination of a beta-lactam (e.g., amoxicillin) with clavulanic acid.[14] Several recent adjuvant approaches draw on a combination of an antibiotic with a mechanism for inhibiting resistance mechanisms,[15] inhibiting efflux pumps,[16,17] dispersing biofilms,[18] increasing reactive oxygen species production[19] and exploiting bacterial metabolism.[20,21]

Another strategy for adjuvant therapy pairs an antibiotic with a molecule that improves the transport of the compound into bacterial cells to increase the concentration and bioavailability of an antibiotic. For example, the membrane-permeabilizing properties of colistin sensitize multidrug-resistant *Acinetobacter baumannii* to vancomycin.[22] Phenylpropanoids are a family of natural products that damage membranes and create synergistic interactions with various antibiotics that use different mechanisms of action against Gram-negative and Gram-positive bacteria.[23,24] The discovery of new families of small molecules that target bacterial membranes and increase the transport of antibiotics into cells may advance this emerging area of antibiotic chemotherapy.

We discovered 5-nonyloxytryptamine (5-NOT, **1**) in a high throughput screen of small molecules that cause bacterial cells to form anucleate daughter cells (i.e., cells lacking chromosomes) (Fig. 1A).[25] **1** belongs to a family of serotonin-like compounds that bind 5-hydroxytryptamine (serotonin) receptors (5-HT), and was previously synthesized to test its selectivity in binding the human

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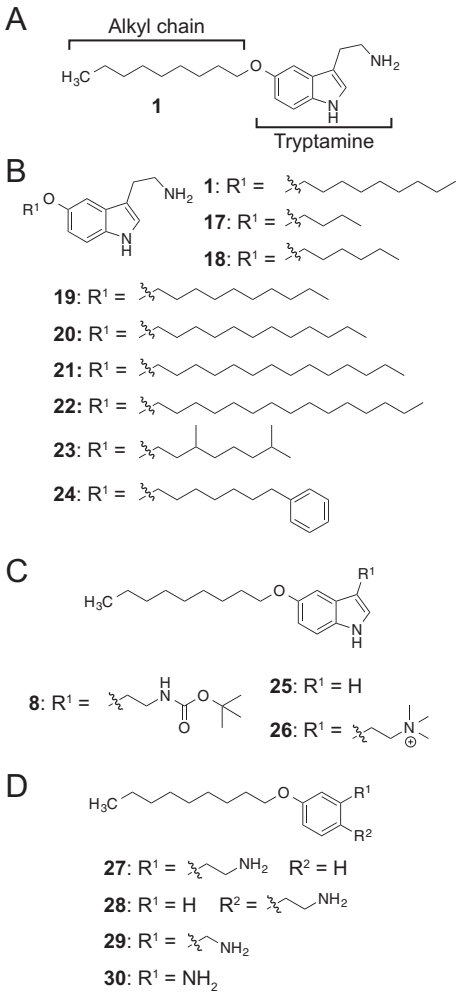

Figure 1. (A) The chemical structure of 5-nonyloxytryptamine (**1**), an *O*-alkylated derivative of serotonin. A list of analogs of **1** synthesized separated by structural modifications: (B) modification of alkyl chain, (C) modification of the substituent at 3-position and the amine, and (D) substitution of the indole ring by a phenyl ring and modification of the length of the ethylene chain.

5-HT_{1D} receptor.[26,27] Multiple variants of human 5-HT_1 receptors exist and elicit different neurological responses.[28] 5-HT_{1D} causes endothelium dependent relaxation in arteries and previous research suggests that 5-HT_{1D} receptor agonists inhibit neurotransmitter release and relax cranial arteries.[29] Sumatriptan selectively binds to the 5-HT_{1D} receptor and is an effective strategy for treating migraines.[27,30] A structure–activity relationship study of sumatriptan and other serotonin-like compounds led to the discovery of **1**, which binds to 5-HT_{1D} receptors with 300-fold selectivity over non-specific serotonin 5-HT_1 receptors.[27]

We performed minimum inhibitory concentration (MIC) assays of **1** against *Escherichia coli* BW25113 (6.0 μg/mL), *Bacillus subtilis* 168 (0.76 μg/mL), and *E. coli* BW25113 Δ*tolC* (1.5 μg/mL) (Table 1). These experiments made it possible for us to determine its potency against a model Gram-negative and Gram-positive bacterium, and a bacterium in which the AcrAB-TolC drug efflux pump system was partially disabled to potentially increase the intracellular concentration of the compound (compared to the parent *E. coli* BW25113 strain). We also measured the antibacterial activity of **1** against various BSL-2 bacterial strains; **1** was effective against all strains tested with MIC values ranging from 3.0 to 24 μg/mL (Table 1). We determined the minimum bactericidal concentration (MBC) of **1** against BSL-1 and BSL-2 strains to establish whether it was bacteriostatic or bactericidal (Table 1).[31] The ratio of MBC to MIC for **1** indicated that it was bactericidal against: *Shigella boydii, Klebsiella pneumonia, Acinetobacter baumannii, Edwardsiella tarda, Morganella morganii, Staphylococcus aureus,* and *Streptococcus pyogenes* and bacteriostatic against: *Pseudomonas aeruginosa, Salmonella typhimurium, Vibrio cholera,* and *Enterobacter aerogenes* (Table 1).

We initiated our structure–activity relationship (SAR) study by testing the MIC activity of commercially available molecules with structures related to serotonin: tryptamine (**2**), serotonin (**3**), 5-methoxytryptamine (**4**), 2-methyl-5-hydroxytryptamine (**5**), and 6-methoxytryptamine (**6**) (Fig. S1). We determined the MIC values of **2**–**6** against *E. coli* BW25113, *E. coli* BW25113 Δ*tolC*, and *B. subtilis* 168 and found they were all >56 μg/mL, consistent with the importance of the long alkyl group of **1** on its antibacterial properties (Table 2). We continued our SAR study by synthesizing new analogs of **1** in which we varied the alkyl group.

To create 5-alkyloxytryptamine analogs, we used a three-step synthesis: protection of the primary amine of serotonin, alkylation of the hydroxyl group, and deprotection of the primary amine (Fig. 2).[27] We synthesized **1** by converting serotonin to *N*-Boc-protected serotonin (**7**) in 84%. We alkylated **7** with 1-bromononane to yield the alkylated product **8** (30%) and removed the Boc protecting group to provide **1** in 85% yield. We used a similar synthesis procedure to prepare analogs **17**–**24** (Fig. 1B). Serotonin was protected with di-*tert*-butyl dicarbonate, alkylated with different bromoalkanes to yield Boc-protected intermediates **8**–**16**, and deprotected under acidic conditions to provide final products **17**–**24** (Fig. 2).

We next turned our attention to modification of the substituent at the 3-position. We synthesized the *N*-Boc-protected 5-nonyloxytryptamine analog **8** by stopping at the O-alkylation intermediate (Fig. 1C); the yield of **8** was 47% over two steps. We synthesized **25** by O-alkylation of 1*H*-indol-5-ol with 1-bromononane in 52% yield. **1** was trimethylated with methyl iodide in ethanol to produce analog **26** in yield of 27% (Fig. 1C). We synthesized analogs **27**–**30** by starting with a different scaffold and using the same reaction conditions for **1** (Fig. 1D). We produced **27** in 82% yield from 3-(2-aminoethyl)phenol. Analog **28** was synthesized from 4-(2-aminoethyl)phenol in 82% yield. We synthesized **29** from 3-(aminomethyl)phenol in 65% yield. Finally, we synthesized **30** from 4-aminophenol in 42% yield.

We used the library of 5-NOT analogs to make correlations between the structure of **1** and its antibacterial activity. First, we tested how altering the length of the alkyl chain of **1** affected antibacterial activity (Fig. 1B). The MIC value for analogs with butyl and hexyl saturated alkyl chains (**17**, **18**) displayed a decrease in antibacterial activity against *E. coli* BW25113. Butane analog **17** had an MIC value >74 μg/mL against a panel of BSL-1 and BSL-2 bacterial strains (Tables 2 and S1). The MIC value of the hexyl analog **18** was 42 μg/mL against *E. coli* BW25113, *E. coli* BW25113 Δ*tolC*, and *B. subtilis* 168 and ⩾42 μg/mL against a panel of BSL-2 bacterial strains (Tables 2 and S1). **1**, **17**, and **18** have *c*Log*P* values of 4.65, 2.38, and 3.29, respectively. Compound **19** has a 10 carbon-chain, MIC values that are comparable to **1** against *E. coli* BW25113 (MIC, 6.3 μg/mL), *E. coli* BW25113 Δ*tolC* (MIC, 3.2 μg/mL), and *B. subtilis* 168 (MIC, 1.6 μg/mL) (Table 2), and a *c*Log*P* value of 5.11, which is close to the *c*Log*P* value of **1** (Table 2). Increasing the length of the saturated alkyl groups (**20** has a 12 carbon-chain, **21** has a 14 carbon-chain, and **22** has a 15 carbon-chain) decreased the antibacterial activity of compounds (Fig. 1B). Of the analogs with longer saturated chains, **20** had the highest antibacterial activity with MIC values of 26, 6.9, and 1.7 μg/mL against *E. coli* BW25113, *E. coli* BW25113 Δ*tolC*, and *B. subtilis* 168. (Table 2). **21** had antibacterial activity only against *B. subtilis* 168 (MIC, 15 μg/mL) and *S. aureus* (MIC, 3.4 μg/mL), and **22** did not display antibacterial properties (Tables 2 and S1). *c*Log*P* values of

Table 1
Minimum inhibitory concentration (MIC) and minimum bacterial concentration (MBC) values of **1** against *E. coli* BW25113, *E. coli* BW25113 Δ*tolC*, *B. subtilis* 168, and BSL-2 bacterial strains

Bacterial strain	MBC (μg/mL)	MIC (μg/mL)	MBC/MIC	Mode
E. coli BW25113	12	6.0	2	Bactericidal
E. coli BW25113 Δ*tolC*	6	1.5	4	Bacteriostatic
B. subtilis 168	0.76	0.76	1	Bactericidal
Pseudomonas aeruginosa	96	24	4	Bacteriostatic
Salmonella typhimurium	24	6.0	4	Bacteriostatic
Vibrio cholerae	24	6.0	4	Bacteriostatic
Shigella boydii	6.0	6.0	1	Bactericidal
Klebsiella pneumoniae	6.0	6.0	1	Bactericidal
Enterobacter aerogenes	>24	6.0	>4	Bacteriostatic
Acinetobacter baumannii	6.0	6.0	1	Bactericidal
Edwardsiella tarda	12	6.0	2	Bactericidal
Morganella morganii	24	12	2	Bactericidal
Staphylococcus aureus	6.0	3.0	2	Bactericidal
Streptococcus pyogenes	24	24	1	Bactericidal

Table 2
Estimated *c*Log*P* and measured MIC (μg/mL) values of compounds **1**–**6**, **8**, **17**–**30** against *E. coli* BW25113, *E. coli* BW25113 Δ*tolC*, *B. subtilis* 168, *S. aureus*, and *A. baumannii*

Compound number	*c*Log*P*	MIC values against bacterial strains (μg/mL)				
		E. coli BW25113	*E. coli* BW25113 Δ*tolC*	*B. subtilis* 168	*S. aureus*	*A. baumannii*
1	4.65	6.0	1.5	0.76	0.6	6.0
2	1.13	>51	>51	>51	>51	>51
3	0.79	>56.4	>56.4	>56.4	>56.4	>56.4
4	1.06	>60.8	>60.8	>60.8	>60.8	>60.8
5	1.19	>60.8	>60.8	>60.8	>60.8	>60.8
6	1.06	>60.8	>60.8	>60.8	>60.8	>60.8
8	6.41	>130	>130	>130	>130	>130
17	2.38	>74.3	>74.3	>74.3	>74.3	>74.3
18	3.29	42	42	42	42	42
19	5.11	6.3	3.2	1.6	3.2	6.3
20	6.01	26	6.9	1.7	3.4	28
21	6.92	>119	>119	15	3.4	>119
22	7.38	>124	>124	>124	>124	>124
23	4.63	13	3.2	1.6	6.3	13
24	5.18	14	1.8	1.8	3.5	7.0
25	5.22	>83	>83	>83	>83	>83
26	6.55	52	6.5	52	6.5	52
27	4.61	5.3	2.6	0.66	5.3	5.3
28	4.61	5.3	1.3	0.66	2.6	5.3
29	4.18	9.4	4.7	4.7	9.4	9.4
30	4.5	>40	10	40	>80	>80

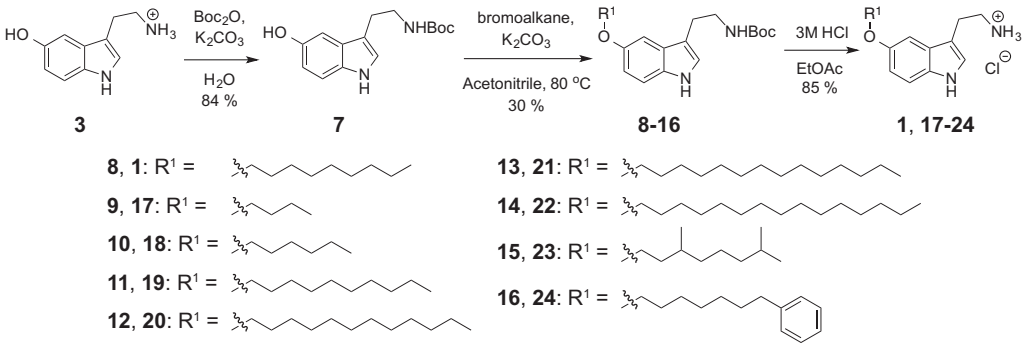

Figure 2. The general synthetic strategy for the synthesis of **1** and its analogs: Boc protection of the amine, alkylation of the hydroxyl group, and acidic deprotection to produce the HCl salt of the free amine. Percent yields included in this figure are for the synthesis of **1**.

compounds **20**–**22** increased with chain length: *c*Log*P* values were 6.01 (**20**), 6.92 (**21**), and 7.39 (**22**) (Table 2). These results suggest that the most active analogs have an optimal alkyl length of 6–12 carbon atoms and a *c*Log*P* value of ~5.

After alkane modification, we synthesized and tested two analogs with similar chain length containing various substituents: **23** has an 8 carbon branched chain bearing two methyl groups, while **24** has a 7 carbon-tail modified with a terminal phenyl group (Fig. 1B); **23** and **24** have *c*Log*P* values of 4.63 and 5.18, respectively. **23** and **24** had comparable MIC values of 13–14 μg/mL against *E. coli* BW25113 (Table 2) and displayed MIC values of 3.2–25 μg/mL against a panel of BSL-2 strains, with the exception of *P. aeruginosa* (25 and 56 μg/mL, respectively) and *M. morganii* (>101 and >112 μg/mL, respectively) (Table S1).

We next investigated the function of the primary amine on the ethylene chain located at the 3-position of the indole ring to determine whether the ammonium group was important for the antibacterial properties of these compounds. We synthesized analogs with a protected primary amine (**8**), lacking the ethylene amine chain (**25**), or with a quaternary ammonium (**26**) to determine the role of the amine on the antibacterial activity of compounds. Trimethylating the primary amine created quaternary ammonium analog **26** (Fig. 1C). Protecting the amine (**8**) and removing it completely (**25**) eliminated the antibacterial properties (MIC, >83 μg/mL) against *E. coli* BW25113, *E. coli* BW25113 Δ*tolC*, and *B. subtilis* 168 cells (Table 2). Trimethylamine analog (**26**) had decreased antibacterial activity compared to **1**: MIC values of 52 μg/mL (*E. coli* BW25113), 6.5 μg/mL (*E. coli* BW25113 Δ*tolC*), and 52 μg/mL (*B. subtilis* 168) (Table 2). These results confirm our hypothesis that the amine is important for the antibacterial activity of these compounds and that the small size and presence of acidic protons on the ammonium group are contributing factors to the antibacterial activity.

We next investigated the impact of the indole ring of **1** on its activity. We replaced the indole ring with benzene (**27**) to alter the structure, yet maintain the same spacing between the ethylene amine group and hydrophobic tail in **1** (Fig. 1D); the ethylene amine at the 3-position and *O*-alkyl chain at the 5-position on the indole ring is structurally similar to the 1 and 3-positions on the benzene ring. The MIC value of analog **27** was 5.3 μg/mL against *E. coli* BW25113, 2.6 μg/mL against *E. coli* BW25113Δ*tolC*, 0.66 μg/mL against *B. subtilis* 168, and matched the MIC value of **1** against *S. boydii, K. pneumonia, A. baumannii, E. tarda, M. morganii, P. aeruginosa, S. typhimurium, V. cholera,* and *E. aerogenes* (Tables 2 and S2). Since **1** and **27** exhibited similar antibacterial activity, we tested the position of the ethylene amine relative to the hydrophobic tail on the benzene scaffold. Instead of a 1,3 configuration of the alkyl group and ethylene amine groups, we synthesized a 1,4 configuration (**28**) (Fig. 1D). The MIC values of **28** against *E. coli* BW25113 (5.3 μg/mL), *E. coli* BW25113 Δ*tolC* (1.3 μg/mL), *B. subtilis* 168 (0.66 μg/mL) were approximately the same as for **27** (Table 2) and identical to **27** and **1** against *S. boydii, K. pneumonia, A. baumannii, S. typhimurium,* and *V. cholera*. The MIC values of **28** increased to >84 μg/mL against *P. aeruginosa*, *E. tarda,* and *M. morganii* and 10 μg/mL against *E. aerogenes* (Table S2). Both **27** and **28** have *c*Log*P* values of 4.61, which are similar to the *c*Log*P* value of **1** (Table 2). These studies suggest that the 1,3 orientation of the alkyl tail and the ethylene amine groups around the scaffold structure is not required for antibacterial activity.

Finally, we tested the contribution of the ethylene chain connecting the primary amine at the 3-position of the benzene scaffold to the antibacterial activity of this family of compounds. We synthesized two analogs that maintain the 1,3 configuration: methylene amine analog **29** and amino analog **30** (Fig. 1D). The MIC values of **29** and **30** were against *E. coli* BW25113 (9.4 and >40 μg/mL, respectively), *E. coli* BW25113 Δ*tolC* (4.7 and 10 μg/mL, respectively), and *B. subtilis* 168 (4.7 and 40 μg/mL, respectively) suggest that a distance of ~3–5 Å between the primary amine and the aromatic ring is ideal for activity (Table 2).

These results suggest that the hydrophobic tail and the charged, primary amine head of **1** are essential for its antibacterial activity, making it plausible that these compounds mimic the general structural features of lipids and lead to bacterial cell death through altering the physical properties of membranes.[32] We investigated the effect of **1** on membrane permeability using the membrane impermeable DNA probe propidium iodide (PI) to label DNA in cells in which the membrane is compromised (Fig. 3A).[33,34] We treated *E. coli* BW25113 cells with **1** at 6.0 μg/mL (1× MIC) or 12 μg/mL (2× MIC), 50% v/v ethanol (positive control), and an equivalent volume of DMSO (solvent control) and used flow cytometry to measure the fluorescence intensity of individual cells in each of the treatment populations in the presence and absence of PI (background fluorescence control). Ethanol-treated cells and cells treated with **1** at both concentrations gave a significantly higher fluorescent signal than the population treated with DMSO and untreated cells, suggesting **1** increases the membrane permeability of *E. coli* cells.

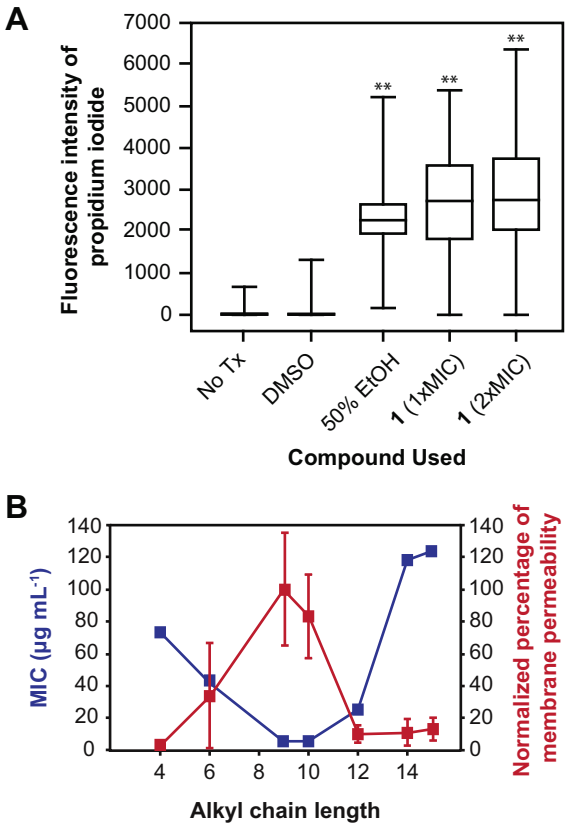

Figure 3. (A) A plot depicting the fluorescence intensity of propidium iodine (PI) after treating *E. coli* BW25113 cells with DMSO, 50% ethanol, 1× MIC of **1** (6.0 μg/mL), and 2× MIC of **1** (12.0 μg/mL), labeling using PI, and measurements using flow cytometry. Each condition includes a sample size of n >7000 cells. The three treatment conditions (50% ethanol, 1× MIC, and 2× MIC) are statistically significant compared to DMSO treatment ($^{**}p$ <0.001). A Q-test was preformed. (B) The MIC and percent of membrane permeability after treatment of *E. coli* BW25113 cells with compounds **1** and **17**–**22** are presented on the same axes. The fluorescence/absorbance ratio was normalized to the fluorescence/absorbance ratio of the 50% ethanol treatment control.

We also explored the effect of alkyl chain length on the membrane permeability of *E. coli* BW25113 cells treated with 1xMIC of **1** (6.0 μg/mL), **18** (42 μg/mL), **19** (6.3 μg/mL), and **20** (26 μg/mL) and the highest concentration of **17** (74 μg/mL), **21** (119 μg/mL), and **22** (124 μg/mL) that we tested in the SAR study (as these compounds did not display significant antibiotic activity). We treated cells with compound for 15 min, added PI, and measured the absorbance and fluorescence intensity of both labeled and unlabeled cells (the latter a background fluorescence control) using a microplate reader (Fig. 3B). When normalized to the 50% ethanol positive control, we observed 100% membrane permeability when cells were treated with **1**, 34% after treatment with **18**, 83% after treatment with **19** and 10% after treatment with **20**. Compounds **17**, **21**, and **22** displayed very little membrane permeability—3%, 11%, and 13%, respectively, which is consistent with their lack of biological activity in MIC assays. An alkyl chain length of 6–10 carbons maximizes bacterial cell membrane permeability and

Table 3
The MIC (μg/mL; ng/mL) of 4 antibiotics against *E. coli* BW25113 supplemented with **1**, **18**, **19**, **20**, and **27**. Gray shading highlights co-treatment combinations tested against BSL-2 bacterial strains, which form the basis for Table 4

Antibiotic	No analog	Analog Concentration (μg/mL)									
		1	**1**	**18**	**18**	**19**	**19**	**20**	**20**	**27**	**27**
		3.0	1.5	21	10.5	3.2	1.6	14	6.9	2.6	1.3
Chloramphenicol (ng/mL)	8	8	8	4	4	2	4	0.5	2	4	8
Tetracycline (ng/mL)	2	2	2	1	2	0.25	1	0.25	1	1	2
Ciprofloxacin (ng/mL)	13	13	13	13	13	13	13	0.8	13	25	25
Rifampicin (ng/mL)	8	8	8	1	4	1	8	0.25	1	2	4

Table 4
The MIC (μg/mL) of rifampicin and tetracycline against BSL-2 strains: *Salmonella typhimurium*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Staphylococcus aureus* supplemented with sub-MIC concentrations of **18** and **19**

Antibiotic (μg/mL) Analog/[analog] (μg/mL)	Rifampicin No analog	Rifampicin **18**/21	Tetracycline No Analog	Tetracycline **19**/3.2
Salmonella typhimurium	8	0.5	1	0.016
Acinetobacter baumannii	2	0.016	1	0.25
Pseudomonas aeruginosa	16	16	32	16
Klebsiella pneumoniae	16	8	2	2
Staphylococcus aureus	0.004	0.004	0.125	0.001*

* We used a concentration of **19** (1.6 μg/mL) with tetracycline when treating *S. aureus* to maintain 0.5× MIC of the hydroxytryptamine analog.

minimizes the MIC, suggesting that biological activity of these compounds is related to membrane permeability (Fig. 3B).

To rule out the activity of **1** also arising from binding to a protein target in cells, we performed a spontaneous resistant mutant screen. We isolated stable *E. coli* mutants that were resistant to 10× MIC of **1**, isolated genomic DNA of two mutants, deep sequenced them, and assembled their genomes. We identified loci for mutations that mapped to four genes; *pykA*, *otsA*, *thrS*, *ydeA* (Table S3). Using a multiple copy suppression assay, we demonstrated that overexpressing each protein did not reduce the MIC of *E. coli* MG1655 cells to **1** (Table S3). These results suggest that this compound does not have a specific protein target in bacteria and that its activity arises from its interaction with the membrane.

The membrane permeability activity of **1**, **18**, **19**, **20**, and **27** led us to explore these compounds as antibiotic adjuvants to facilitate antibiotic transport into bacterial cells, decrease the concentration of therapeutic antibiotics (and thereby reduce the susceptibility for bacteria developing drug resistance), and increase the susceptibility of bacteria to the drugs. We used chloramphenicol, tetracycline, ciprofloxacin and rifampicin as model clinical antibiotics that hit a range of drug targets. We treated *E. coli* BW25113 cells with a combination of each of these antibiotics and **1**, **18**, **19**, **20,** or **27** at a concentration of either 0.25× or 0.5× MIC. Analogs **18**, **19**, and **20** at 0.5× MIC significantly changed the MIC of certain antibiotics against *E. coli* BW25113, while **1** had no effect on the MIC of all four antibiotics (Table 3). Co-dosing **1**, **18**, **19**, or **27** at 0.25× MIC did not have a significant effect on the MIC of the four antibiotics. **18** and **19** (at 0.5× MIC) reduced the MIC of rifampicin by ⩾8-fold. Co-dosing **20** (at 0.5× MIC) reduced the MIC of chloramphenicol and ciprofloxacin by 16-fold, the MIC of tetracycline by 8-fold, and the MIC of rifampicin by 32-fold against *E. coli* BW25113. 10 was also active at 0.25× MIC and reduced the MIC of chloramphenicol by 4-fold and rifampicin by 8-fold (Table 3). From these data we selected **18**, **19**, rifampicin, and tetracycline to test additional drug combinations against *S. typhimurium*, *A. baumannii, P. aeruginosa, K. pneumoniae, S. aureus*.

Table 4 displays MIC data for rifampicin dosed with **18** (21 μg/mL) and tetracycline dosed with **19** (3.2 μg/mL; with the exception of *S. aureus*, for which we used 1.6 μg/mL) against all five bacterial strains. The activity of **18** and **19** as potential adjuvants was most notable against *S. typhimurium*, *A. baumannii*, and *S. aureus*. The MIC of rifampicin against *S. typhimurium* and *A. baumannii* was 8 μg/mL and 2 μg/mL, respectively. Co-dosing rifampicin with **18** (21 μg/mL) decreased the MICs to 0.5 μg/mL and 0.016 μg/mL, respectively. The MIC of tetracycline was 1 μg/mL for both *S. typhimurium* and *A. baumannii*; adding **19** (3.2 μg/mL) decreased the MIC to 0.016 μg/mL and 0.25 μg/mL, respectively. The only combination effective against *S. aureus* was **19** (1.6 μg/mL) and

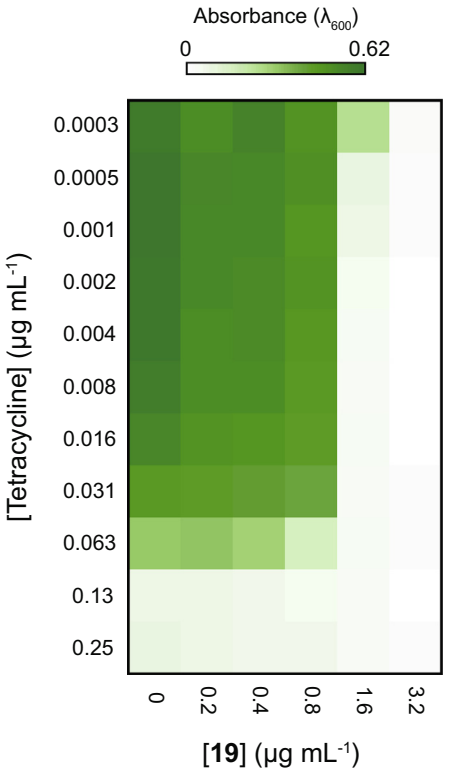

Figure 4. A plot depicting a checkerboard assay with varying concentrations of tetracycline and **19** against *S. aureus*. The MIC of tetracycline and **19** against *S. aureus* is 0.13 μg/mL and 3.2 μg/mL, respectively.

tetracycline, which reduced the MIC of tetracycline by over 100-fold to 0.001 μg/mL (Table 4, Fig. 4).

To explore 5-NOT analogs as antibiotic adjuvants from a therapeutic perspective, we investigated the cytotoxicity of several analogs against human embryonic kidney (HEK) cells. We measured HEK cell viability 24 and 48 h after treatment with **18**, **19**, **20**, and **27** at the concentrations used in the co-dosing experiments described above (Table S4). We found that **19** (0.25× MIC, 1.6 μg/mL) and **27** (0.25× MIC, 1.3 μg/mL) reduce HEK cell viability to 29.6% and 60.5% after 24 h of treatment. At this concentration, **19** reduced the susceptibility of *S. aureus* to tetracycline from 0.125 μg/mL to 0.001 μg/mL (Table 4, Fig. 4). These data suggest that **19** at sub-MIC concentrations can potentiate the activity of clinical antibiotics, however further structural modifications will be needed to reduce HEK cell cytotoxicity.

Although **1** was selected in a high throughput screen designed to detect anucleate cells after compound treatment, cells treated with this compound and the DNA-intercalating dye 4′,6-diamidino-2-phenylindole (DAPI) became fluorescent, indicating that cells contained a nucleoid. To test the hypothesis that molecules that disrupt the cell envelope are false positives in the anucleate assay, we tested three β-lactams (meropenem, aztreonam, and cefotaxime) that inhibit the biosynthesis of peptidoglycan[35,36] and an antimicrobial peptide (cecropin A) that disrupts the bacterial membrane.[37] We used rifampicin as a positive control and chloramphenicol as a negative control.[25] We treated *E. coli* SH3210 cells with each compound and the β-galactosidase substrate, DDAOG and measured the fluorescence signal indicating production of the cleaved fluorescent byproduct. Meropenem, aztreonam, cefotaxime, cecropin A, and **1** produced a significantly higher β-gal signal than the negative control, chloramphenicol and the DMSO solvent control (Fig. S2). These results confirm our reasoning that compounds that interact with the membrane or inhibit cell wall biosynthesis are false positive hits in the anucleate cell assay.

In conclusion, we report the discovery and characterization of 5-alkyloxytryptamine analogs as a new family of broad-spectrum antibiotics. Members of this family of compounds were described previously as human 5-HT_{1D} receptor agonists. We describe a series of compounds in this family that target membranes and display MIC values as low as 0.6–5 μg/mL against a range of pathogenic bacteria. At sub-MIC concentrations—toward the end of the spectrum where toxicity against HEK cells is minimized—the compounds are effective adjuvants that potentiate the activity of clinical antibiotics. For example, co-dosing *S. aureus* cells with **19** reduced the MIC of tetracycline from 0.125 μg/mL to 0.001 μg/mL. Exploring other co-dosing formulations and 5-alkyloxytryptamine analogs may increase potency while reducing toxicity.

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Supplementary data

Supplementary data (figures, tables, synthetic procedures, compound characterization data and biological methods) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2016.10.004.

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