Small Molecular Antibacterial Peptoid Mimics: The Simpler the Better!

ARTICLE *in* JOURNAL OF MEDICINAL CHEMISTRY · JANUARY 2014

Impact Factor: 5.45 · DOI: 10.1021/jm401680a · Source: PubMed

8 AUTHORS, INCLUDING:

Chandradhish Ghosh
Jawaharlal Nehru Centre for Advanced Scienti…
11 PUBLICATIONS **41** CITATIONS
SEE PROFILE

Yarlagadda Venkateswarlu
Jawaharlal Nehru Centre for Advanced Scienti…
16 PUBLICATIONS **134** CITATIONS
SEE PROFILE

Jiaul Hoque
Jawaharlal Nehru Centre for Advanced Scienti…
13 PUBLICATIONS **128** CITATIONS
SEE PROFILE

Divakara S S Murthy Uppu
Jawaharlal Nehru Centre for Advanced Scienti…
12 PUBLICATIONS **100** CITATIONS
SEE PROFILE

Journal of Medicinal Chemistry

Article

pubs.acs.org/jmc

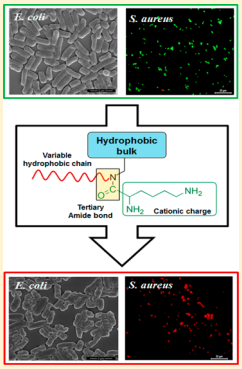
Small Molecular Antibacterial Peptoid Mimics: The Simpler the Better!

Chandradhish Ghosh, Goutham B. Manjunath, Padma Akkapeddi, Venkateswarlu Yarlagadda, Jiaul Hoque, Divakara S. S. M. Uppu, Mohini M. Konai, and Jayanta Haldar*

Chemical Biology and Medicinal Chemistry Laboratory, New Chemistry Unit, Jawaharlal Nehru Centre for Advanced Scientific Research (JNCASR), Jakkur, Bengaluru 5600064, Karnataka, India

Supporting Information

ABSTRACT: The emergence of multidrug resistant bacteria compounded by the depleting arsenal of antibiotics has accelerated efforts toward development of antibiotics with novel mechanisms of action. In this report, we present a series of small molecular antibacterial peptoid mimics which exhibit high in vitro potency against a variety of Gram-positive and Gram-negative bacteria, including drug-resistant species such as methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus faecium*. The highlight of these compounds is their superior activity against the major nosocomial pathogen *Pseudomonas aeruginosa*. Nontoxic toward mammalian cells, these rapidly bactericidal compounds primarily act by permeabilization and depolarization of bacterial membrane. Synthetically simple and selectively antibacterial, these compounds can be developed into a newer class of therapeutic agents against multidrug resistant bacterial species.



INTRODUCTION

Bacterial resistance to conventional antibiotics is one of the most serious problems facing world health today. The dearth of drugs against drug-resistant Gram positive bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococci* (VRE) as well as multidrug resistant Gram-negative bacteria such as *Pseudomonas aeruginosa* is the most important cause of concern.[1−3] In the recent past, only cationic antimicrobial peptides (AMPs) have shown some promise as potential antibiotics and several of them are undergoing clinical trials.[4] AMPs are sentinels of the innate immune system of most species and are usually the first line of defense against any infection.[5] While most of the conventional antibiotics act by targeting intracellular organelles of bacteria, AMPs and their mimics are known to act primarily by causing lysis of the bacterial cell membrane.[6−9] Consequently, unlike in the case of conventional antibiotics, where even point mutations can render them inactive, bacteria find it difficult to develop resistance against antimicrobial peptides.[7]

It is believed that AMPs display amphipathic conformation, i.e., facial segregation of positive charges and hydrophobic moieties while interacting with bacterial membranes.[5,6] AMPs act selectively toward bacterial cells over mammalian cells, which can be attributed to the differences of the lipid components of the cell membranes of bacterial and mammalian cells. While bacterial membrane is largely composed of anionic lipids, the mammalian cell membrane is mainly composed of uncharged lipids at neutral pH.[10] Despite several advantages, no AMP has yet been approved for clinical use.[4] AMPs are mainly limited by their high in vivo toxicity, lability toward proteases, and their high cost of manufacture. Consequently, several groups around the world have tried to develop strategies to counter the problems faced by AMPs. Most of these strategies, such as α-peptides,[11,12] β-peptides,[13,14] peptoids,[15] antimicrobial polymers,[16−22] oligoacyllysines,[23] oligoureas,[24] α-AA peptides,[25] and cationic amphiphiles,[26] keep the physiological properties of AMPs constant in their design and try to address the problems associated with their synthesis and degradation. Although macromolecular mimics of AMPs are well-known and abundant,[27] development of small molecular mimics of AMPs has been seldom attempted. Polymedix's aryl amide foldamers[28,29] and Lytix Biopharma's LTX 109[30,31] are successful examples of small molecular mimics of antimicrobial peptides. Some other examples include the binaphthyl based dicationic peptoids,[32] $\beta^{2,2}$-amino acid derivatives,[33] and those based on aryl scaffolds.[34,35] Although most of these compounds involve designs that are extremely efficient in their purpose, their major limitations lie in the complexity of their structures, the number of steps involved in their synthesis, the cost of their manufacture, and the presence of peptide bonds which are labile to protease degradation.

Herein we report, for the first time, the design and development of small molecular antibacterial peptoid mimics involving only three synthetic steps. In our minimalistic design, the two positive charges were contributed by an L-lysine moiety; hydrophobicity was brought about by an aromatic core (methyl anthracene, methyl naphthalene, or methyl benzene) and an alkyl chain (Figure 1). The hydrophobicity of the lipophilic alkyl chain and the bulky aromatic core was varied

Received: October 31, 2013
Published: January 30, 2014

   dx.doi.org/10.1021/jm401680a | *J. Med. Chem.* 2014, 57, 1428−1436

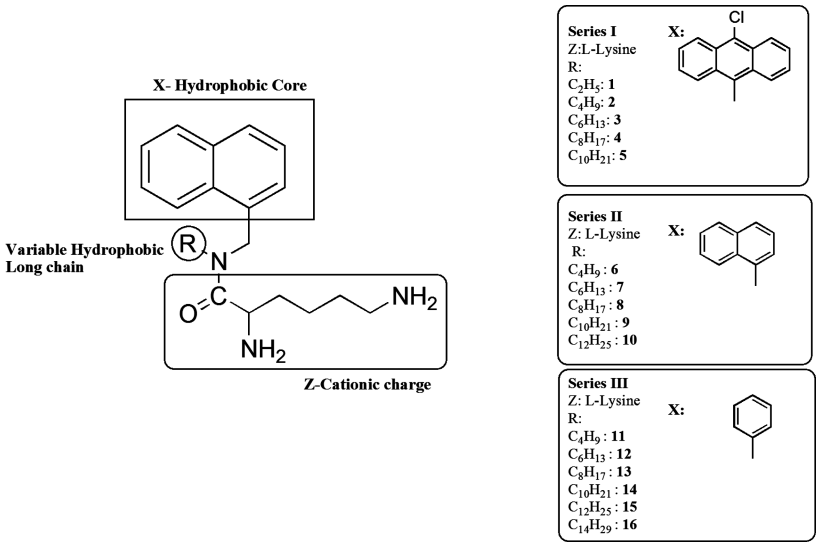
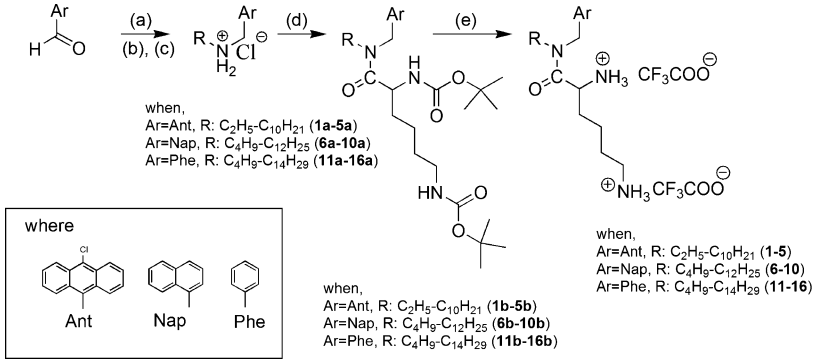


Figure 1. Structures of small molecular antibacterial peptoid mimics.

Scheme 1. General Synthetic Scheme for the Preparation of the Compounds[a]



[a](a) RNH_2 (where R is alkyl group) in MeOH, 6 h; (b) $NaBH_4$ in MeOH, 18 h; (c) HCl; (d) Boc-Lys(Boc)-OH, *N*,*N*-diisopropylethylamine (DIPEA), *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluroniumhexafluorophosphate (HBTU) in DMF/$CHCl_3$ (5:2), RT, 16−24 h; (e) 50% CF_3COOH in CH_2Cl_2, 12 h.

systematically to understand the role of these parameters toward selective antibacterial activity.

RESULTS

Synthesis. To synthesize the compounds, first, aromatic aldehydes (10-chloro-9-anthracenaldehyde, naphthaldehyde, and benzaldehyde) were reacted with alkyl amines to form Schiff's bases, followed by reduction with sodium borohydride, to give secondary amines (Scheme 1). Then secondary amines were coupled to Boc-Lys(Boc)-OH using HBTU coupling, and finally, the Boc groups were deprotected using trifluoroacetic acid to obtain the required compounds (Figure 1): methyl anthracene core (series I, compounds **1**−**5**), methyl naphthalene core (series II, compounds **6**−**10**), and methyl benzene core (series III, compounds **11**−**16**). The final compounds were purified by HPLC to more than 95% purity and characterized by ^{1}H NMR, ^{13}C NMR, IR, and HR-MS (Supporting Information, Figures S1−S64). Two significant features of these compounds are that there is no imposed structural rigidity in their design and that they include an *N*-disubstituted or tertiary amide bond.

Antibacterial Activity. The antibacterial efficacy (Table 1) of these compounds was evaluated against different Gram-positive (*Staphylococcus aureus* and *Enterococcus faecium*) and Gram-negative (*Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*) bacteria including MRSA and VRE by determination of their minimum inhibitory concentrations (MICs, the concentration of compounds required to completely inhibit the growth of bacteria).

In general, all the compounds of series I (**1**−**5**) have MICs comparable to MSI-78 (an AMP currently undergoing phase III clinical trials as a topical antibiotic).[4,36] The shortest alkyl chain derivative **1** (ethyl analogue) was found to be moderately active against both Gram-positive and Gram-negative bacteria, with MICs of 11 and 25 μg mL^{-1} against *S. aureus* and *E. coli*, respectively. Increasing the long chain to butyl improves antibacterial activity by 2-fold as was observed in **2**. Further increase in alkyl chain yielded the hexyl analogue **3**, which displayed improved MICs of 2.4 and 3.5 μg mL^{-1} against *S. aureus* and *E. coli*, respectively. The antibacterial activity was found to increase even further on increasing the long chain to octyl; **4** displayed MICs of 2.2 and 2.9 μg mL^{-1} against *S. aureus* and *E. coli*, respectively. However, further increase in alkyl chain compromised the activity of the compounds as was observed for the decyl analogue **5**. The octyl analogue **4** emerged as the most potent compound in the series whose activity could be compared to vancomycin (MIC of 0.9 μg

dx.doi.org/10.1021/jm401680a | J. Med. Chem. 2014, 57, 1428−1436

Table 1. In Vitro Antibacterial and Hemolytic Activity of the Compounds

	minimum inhibitory concentration (μg mL^{-1})								
	drug sensitive bacteria				drug resistant bacteria				
compd	*S. aureus*	*E. faecium*	*E. coli*	*P. aeruginosa*	MRSA	VRE	*K. pneumoniae*	HC_{50} (μg mL^{-1})	HPLC retention times (min)
1	11	13.6	25	4	21	7.2	31	118	11.8
2	5.3	4.5	4.8	1.9	6.3	5.3	17	91	12.4
3	2.4	3.3	3.5	1.6	2.8	5.2	16	82	13.6
4	2.2	2.5	2.9	3.8	2.3	3	4.3	64	14.6
5	7.1	4.9	26	11	4.6	5.6	7.6	71	15.6
6	>100	>100	>100	>100	>100	>100	>100	>1000	10.8
7	20	34	25	11	65	54	100	508	11.8
8	6.3	5.5	5	5.4	4.4	7	13	60	12.9
9	2.5	3.5	4	3	2.6	1.6	5.8	54	14.2
10	3	1.6	3.1	3.2	2.7	3.4	4	56	15.1
11	>100	>100	>100	>100	>100	ND[a]	>100	>1000	9.4
12	>100	>100	>100	>100	>100	ND	>100	>1000	10.6
13	46	60	51	60	>100	>100	>100	325	12.1
14	5.7	6.5	6.5	4	15.7	5.8	31	95	13.4
15	2.7	2.6	5	4	2.9	3.3	2.8	45	14.5
16	3.1	2	3.1	2.8	2.5	2.5	4	50	15.8
vancomycin	0.9	0.87	ND	ND	0.9	>100	ND	ND	ND
colistin	20	>100	0.4	0.4	54	>100	1.2	ND	ND
MSI-78	8–16[b]	64[b]*	16–32[b]	8–16[b]	16–32[b]	8[b]	8–16[b]	120[c]	ND

[a]ND stands for "not determined". [b]Literature values obtained from ref 36. * indicates value for *E. faecalis*, [c]Literature value obtained from ref 28. VRE (vancomycin-resistant *E. faecium*) and MRSA (methicillin-resistant *S. aureus*), *K. pneumoniae* is resistant to β-lactam antibiotics.

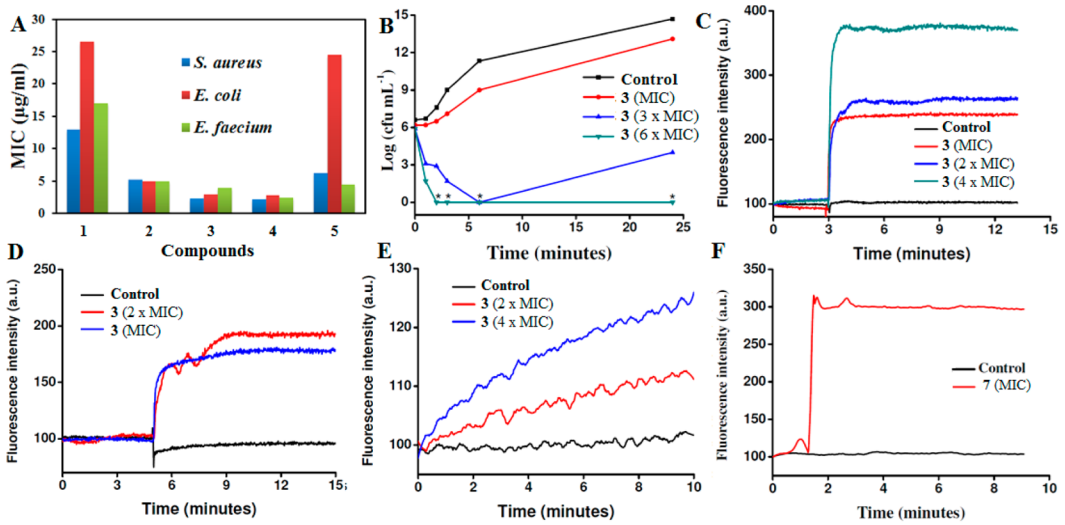


Figure 2. (A) Variation of antibacterial activity with increasing chain length in series I. (B) Time-kill kinetics of **3** against MRSA (stars indicate that bacterial count is <50 CFU mL^{-1}). (C) Cytoplasmic membrane depolarization of *S. aureus* by **3**. (D) Cytoplasmic membrane depolarization of *P. aeruginosa* by **3**. (E) *S. aureus* membrane permeabilization by **3**. (F) *E. coli* outer membrane permeabilization by **7**.

mL^{-1} against *S. aureus*) and colistin (MIC of 0.4 μg mL^{-1} against *E. coli*). Compounds **3** and **4** were around 5 times more active against *E. coli* compared to MSI-78, which emphasizes the effectiveness of the design. The structure–activity relationship (SAR) study indicates that if the anthracene core was kept constant, the optimum chain length lies between butyl to octyl (Figure 2A).

All the compounds were active against *E. faecium*; in fact, compound **1**, the least effective compound of the series also displayed moderate activity (MIC of 13.6 μg mL^{-1}) against the bacterium. Compound **4** with MIC of 2.5 μg mL^{-1} was again the most potent compound while vancomycin had MIC of 0.87 μg mL^{-1}.

The highlight of these set of compounds, however, is their superior activity toward opportunistic human pathogen *P. aeruginosa*, which is a leading cause of hospital-acquired infections and is known to show resistance to almost all clinically approved antibiotics.[37] All the compounds of series I was extremely active against *P. aeruginosa*. Against this bacterium, the minimum MIC value was displayed by **3** (MIC = 1.6 μg mL^{-1}). Compound **2** also showed excellent activity toward *P. aeruginosa*, with MIC of 1.9 μg mL^{-1}.

To elucidate the role of the hydrophobic core toward antibacterial activity, we synthesized series II where the aromatic core is methyl naphthalene (**6**–**10**). It was hypothesized that decreasing the bulkiness from anthracene

dx.doi.org/10.1021/jm401680a | J. Med. Chem. 2014, 57, 1428–1436

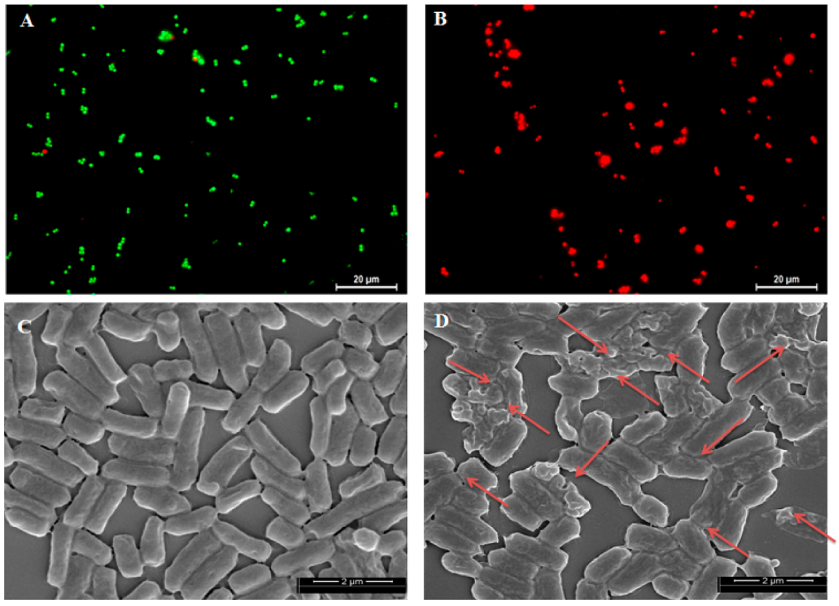


Figure 3. Fluorescence microscopy images of *S. aureus* (A) untreated and (B) treated with **3** (10 × MIC) for 1.5 h after staining with SYTO 9 and PI (scale: 20 μm). Scanning electron microscopy (SEM) images of (C) untreated *E. coli* and (D) *E. coli* treated with **3** (10 × MIC).

to naphthalene might yield a more selective antibacterial agent. Unlike in the case of series I, the small chain analogues of series II, e.g., compound **6**, was devoid of any activity. Compound **7**, the hexyl analogue, was moderately active and showed potency against all the drug sensitive bacteria, especially *P. aeruginosa* (MIC = 11 μg mL^{-1}). The octyl analogue, **8**, exhibited potent activity against all pathogens comparable to its anthracenyl counterpart, **4**, for example, MICs against *S. aureus* and *E. coli* were 6.3 and 5 μg mL^{-1}, respectively. Its activity toward *P. aeruginosa* was also commendable (MIC = 5.4 μg mL^{-1}). Compounds **9** and **10** were the most active compounds in the series (Table 1), with superior activity against all the drug sensitive bacteria (MIC ranged from 1.6 to 4 μg mL^{-1}). Activity of **10** toward *E. faecium* must be mentioned here; its MIC of 1.6 μg mL^{-1} is comparable to that of vancomycin (MIC = 0.87 μg mL^{-1}). No compound in series I was active at this low concentration.

To see the effect of a further decrease of aromatic core, series III (where the aromatic core was methyl benzene) was prepared. In this series of compounds (**11**−**16**), however, no significant activity was observed until the octyl analogue **13**. The decyl analogue, **14**, showed potent activity against all the pathogens. With MICs of 5.7 and 6.5 μg mL^{-1} against *S. aureus* and *E. coli*, respectively, its activity was comparable to **8**. Compounds **15** and **16** had comparable antibacterial activity against all the tested pathogens (Table 1). Against *S. aureus*, **15** was the most potent compound in the series with MIC of 2.7 μg mL^{-1}. Against all other pathogens, activity of **16** was slightly better, e.g., **16** displayed an MIC of 2 μg mL^{-1} compared to that of 2.6 μg mL^{-1} displayed by **15** against *E. faecium*.

To emphasize on the efficacy of the active compounds, their activity was tested against drug resistant superbugs such as VRE, MRSA, and β-lactam resistant *K. pneumoniae*. Most of these compounds possessed remarkable activity against VRE (Table 1). Compounds **4**, **9**, and **16** were the most potent compounds, with MIC values of 3, 1.6, and 2.5 μg mL^{-1}, respectively, which were far superior compared to the MIC of MSI-78 (8 μg mL^{-1}) while vancomycin remained inactive until a concentration of 100 μg mL^{-1}. Compounds **4**, **9**, and **16** were also potent against MRSA, with MICs around 2.5 μg mL^{-1} for all. In comparison, MSI-78 was active only at 16−32 μg mL^{-1}. Other compounds of series I were also active against MRSA but to a lower extent. Compound **4** was also potent against β-lactam resistant *K. pneumoniae* (MIC = 4.3 μg mL^{-1}). Compounds **10** and **16** were the most active compounds in their respective series, with MICs of 4 μg mL^{-1} each, whereas colistin showed MIC of 1.2 μg mL^{-1}.

Bactericidal Kinetics. In vitro time-kill assay with compound **3** against MRSA revealed the rapid bactericidal properties of the compounds. Kinetics of killing increased with increase in concentration, and ∼6 $\log_{10}$ (CFU/mL) reduction in the number of viable cells was observed in the first hour of incubation at a concentration of 6 × MIC. At 3 × MIC, however, bactericidal activity was observed in the sixth hour (Figure 2B). **7** too showed rapid bactericidal action at 2 × MIC against *S. aureus* (Supporting Information Figure S65), establishing the efficacy of these compounds.

Hemolytic Activity. Toxicity of these compounds toward mammalian cells was evaluated by their ability to lyse human erythrocytes and represented as their HC_{50} values (i.e., the concentration at which 50% of the red blood cells are lysed). HC_{50} of the compounds ranged from 45 μg mL^{-1} to >1000 μg mL^{-1}. Hemolytic toxicity studies reveal that these compounds are selectively toxic toward bacterial cells. In general, the HC_{50} values of these compounds were comparable to that of MSI-78 (HC_{50} = 120 μg mL^{-1}). Compound **7**, with a HC_{50} value of >500 μg mL^{-1}, has minimal toxicity toward human erythrocytes.

Antibacterial Activity in Plasma. One of the major disadvantages associated with antimicrobial peptides is that they are prone to protease degradation. This is also reflected in the subsequent loss of activity in presence of blood plasma.[23] Although the MIC of compound **7** (the least hemolytic and active compound in the series II) against *S. aureus* in 50% blood plasma was 30 μg mL^{-1}, no loss of activity was observed in physiologically relevant time frame of 3 h (Supporting Information Figure S66).

dx.doi.org/10.1021/jm401680a | *J. Med. Chem.* 2014, 57, 1428−1436

Enzyme Stability. Trypsin and chymotrypsin are well-known proteases, which are omnipresent in vertebrates. When the model compound, **7**, was incubated with trypsin, it was found to be resistant to degradation up to maximum tested time 24 h (Supporting Information Figure S67), confirming that this type of antibacterial agent was not a substrate for protease.

Mechanism of Action. Investigation into the mechanism of action of the most selective compounds **3** and **7** (model compounds) using various spectroscopic techniques revealed that these compounds primarily act by targeting the bacterial cell membrane. For example, experiments with the membrane-potential sensitive dye $DiSC_3(5)$ showed that both **3** (Figure 2C) and **7** (Supporting Information Figure S68) rapidly depolarize the membrane of *S. aureus* cells. In fact, the membranes of Gram-negative species *P. aeruginosa* and *E. coli* were also depolarized by **3** and **7**, respectively (Figure 2D and Supporting Information Figure S69). Bacterial cytoplasmic membrane permeabilization was studied using the fluorescent probe propidium iodide. Although both **3** and **7** were able to permeabilize the *S. aureus* membrane (Figure 2E and Supporting Information Figure S70), a similar effect was not observed against Gram-negative cells. However, experiments, using fluorescent probe *N*-phenylnaphthylamine with **7** showed that it could cause permeabilization of Gram-negative outer membrane at MIC (Figure 2F). The same experiment could not be performed with **3**, as it absorbs at the same frequency as the dye. The effect of the compound on the bacterial membrane was also studied using fluorescence microscopy and scanning electron microscopy. Fluorescent microscopic studies, using the "live−dead stain",[38] confirmed that on treatment with **3** and **7**, bacterial cell membranes were indeed compromised (Figure 3A,B and Supporting Information Figure S71). FESEM images (Figure 3C,D and Supporting Information Figure S72) reveal that the membranes of treated *E. coli* cells were disrupted and several dead bacteria merged with each other on treatment with **3** and **7**, respectively. Formation of blebs and holes were observed on treated *S. aureus* cells (Supporting Information Figures S73 and S74). These images are a visual proof of the membrane damaging properties of the compounds.

DISCUSSION

In pursuit of novel antibacterial agents, much attention has been focused on developing synthetic mimics of antimicrobial peptides. In this report, we have created simple small molecular antibacterial peptoid mimics where an aryl group, an alkyl group, and a lysine moiety has been assembled through a tertiary amide linkage. Although some of the successful designs in the field focus on imposing rigidity into their systems,[28,29,39] our design is devoid of any imposed rigidity. Other than conformational rigidity, the minimum required parameters are two hydrophobic bulk units and two cationic charges.[32,33] Although all the existing strategies mentioned above have effectively studied the role of amphiphilicity through subtle variation of hydrophobicity and cationic charges, we studied in our structure−activity relationship (SAR) the role of both aromatic moieties and alkyl chains together. We sought out to systematically study the effect of aromatic core individually (keeping the alkyl chain length constant) as well as the effect of long chains individually (keeping the aromatic core constant). Needless to say that in both the cases the cationic charge was kept constant at 2 (brought about by a lysine moiety).

Keeping the aromatic ring constant, the alkyl chain has been varied in all the three series. In case of series I, a parabolic pattern of chain length dependent activity was observed. In both the other cases, the activity was found to increase with increase in long chain and reached a steady state at compounds **10** and **16**, respectively. Further increase in long chain would take away the emphasis from the aromatic core and the long chain would have had more of an impact on the activity.

If the alkyl chain is kept constant, and the aromatic core is varied, a somewhat different SAR is obtained. If we consider the hexyl analogues, **3** is a potent antibacterial agent. On moving to naphthalene core, compound **7**, moderate activity is retained but the benzyl analogue **12** is devoid of activity. From this study, it is clear that the effect of the aromatic core is dominant over the alkyl chain toward antibacterial activity. On moving to the octyl analogues, we find a similar trend. Activity decreases as we move from anthracene to naphthalene to benzene. However, the activity of compound **8** is comparable to that of compound **4**, suggesting that the long chain has an important role to play toward activity and only aromaticity is not the dominant factor. On moving to the decyl analogue, a somewhat different trend is observed. While compounds **5** and **14** have comparable antibacterial activity, compound **9** is roughly 2-fold more active than both. It can be concluded that **9** has an optimum aromaticity and optimum alkyl chain length for potent antibacterial activity. Also, it becomes clear that the decyl long chain dominates over aromaticity toward antibacterial activity.

There is a paucity of drugs against difficult to treat Gram-negative pathogens, and currently there is a huge demand for antibacterial agents against Gram-negative pathogens. Although all the compounds here show excellent activity against drug-resistant Gram-positive bacteria (MRSA and VRE), the highlight of the complete set of compounds is definitely activity against Gram-negative pathogens like *E. coli*, *P. aeruginosa*, and *K. pneumoniae*. Against *P. aeruginosa*, compound **3** has a MIC, which is comparable to colistin. Compounds **9** and **16** are also extremely potent with comparable MIC values. This superior activity may open new avenues toward development of novel drugs against *P. aeruginosa*. However, selective toxicity toward Gram-negative bacteria is an important factor to consider if clinical translation is the ultimate goal. Taking *P. aeruginosa* into consideration, all the compounds show selective toxicity toward bacteria over red blood cells. In fact, **3** and **7** have a good selectivity ratio of around 50 (Supporting Information Table S1). At therapeutically relevant concentrations of 3 × MIC and 6 × MIC, these compounds show bactericidal activity within an hour. This effect is also a consequence of the membrane damaging ability of the compounds. The superior selectivity of the compounds is also displayed by the fact that even at their bactericidal concentrations, they are not hemolytic. The selectivity of the compounds has also been displayed as their therapeutic indices (HC_{50}/MIC) against both drug-sensitive Gram-positive and Gram-negative bacteria (Supporting Information Table S1). MIC of the compounds against *S. aureus* and *P. aeruginosa* is a good reflection of the activity of the compounds toward most bacteria, and thus these therapeutic indices are a good indicator of the preferential damage caused by the compounds toward bacterial cells.

The tertiary amide bond introduces an exciting new approach toward achieving plasma stability of the compounds. This bond not only provides the point of assembly for all the

three parts of the molecule but also adds to the abiotic nature. Antibacterial activity of the compounds in the presence of plasma does not change, which proves that the proteases present in the blood are unable to degrade the compound (also substantiated by trypsin stability experiment) and that the bioavailability of the compound is not hindered by interaction with any other proteins.

HPLC retention time (RT values) is an effective indicator of the overall amphipathicity of the molecules. Antibacterial activity and hemolytic activity profiles of the compounds were found to vary with respect to the RT values as observed in the HPLC data (Table 1). As expected, in each series, the hemolytic activity increases with increasing RT values. The antibacterial activity was also found to increase with increase in RT values. For series II and series III, the antibacterial activity in general increases until compounds **9** (RT = 14.2 min) and **15** (RT = 14.5 min), then remains almost constant with further increase in RT values, whereas in series I, antibacterial activity increases until compound **4** (RT = 14.6 min) and then somewhat decreases in case of compound **5**. It is also clear that in order to have some antibacterial activity the compounds must have RT value greater than 11 min.

Studies into the mechanism of action of the compounds are an indicator of the efficacy of design of the compounds as mimics of antimicrobial peptides. The experiments performed indicated that unlike in the case of Gram-positive bacteria, where both cytoplasmic membrane permeabilization and depolarization could be the possible mechanism of bactericidal action of these compounds, against Gram-negative bacteria, the effect was mostly on the outer membrane permeabilization and depolarization. Like natural AMPs and known synthetic mimics of AMPs, cytoplasmic depolarization is the dominant mechanism of action. The fact that these compounds can permeabilize the Gram-positive cell membrane opens up the question of internal targets. Although in the case of Gram-negative bacteria, only outer membrane permeabilization has been observed, other mechanisms of action cannot be ruled out. Further studies need to be performed to draw any sort of conclusion. However, membrane lysis does seem to be the primary mode of action, as the compounds were found to be rapidly bactericidal. Furthermore the SEM images are visual proof of the membrane damaging properties of the molecules.

■ CONCLUSION

In conclusion, this report illustrates a systematic way of creating highly potent, broad-spectrum small molecular compounds which emulate the efficiency of AMPs. Prepared from inexpensive starting materials in only three steps, these compounds are selectively toxic toward bacterial cells (over mammalian cells) at very low concentrations. Various spectroscopic and microscopic studies reveal that depolarization and disruption of bacterial cell membranes are the primary mechanisms of their bactericidal action. Therefore, these promising compounds can be developed into a new class of antibiotics against multidrug resistant (MDR) bacteria.

■ EXPERIMENTAL SECTION

Unless otherwise mentioned all commercially available chemicals were used as supplied. All the solvents were of reagent grade and were distilled and dried prior to use wherever required. All final compounds were purified by reverse phase HPLC using 0.1% trifluoroacetic acid (TFA) in water/acetonitrile (0−100%) as mobile phase to more than 95% purity.

Synthesis. *Synthesis of Boc-Lys(Boc)-OH.* L-Lysine hydrochloride (5 g, 27.3 mmol) was dissolved in H_2O (50 mL), and to it $NaHCO_3$ (6.9 g, 82.1 mmol) was added and stirred. To this, di-*t*-butylpyrocarbonate (Boc_2O) (7.16 g, 65.5 mmol) in 50 mL of tetrahydrofuran (THF) was added at 0 °C. Then the reaction mixture was stirred at room temperature for 12 h. After 12 h, same amount of Boc_2O (7.16 g, 65.5 mmol) was added again at 0 °C and the mixture was stirred for additional 12 h at room temperature. At the end of the reaction, THF was removed under reduced pressure and the aqueous layer was washed with diethyl ether to remove organic impurities. Then the aqueous layer was acidified to pH 4−5 using citric acid solution. The aqueous layer was then extracted with dichloromethane (DCM). The organic layer was then washed with brine and dried over anhydrous Na_2SO_4. The organic layer was removed under reduced pressure to obtain the compound in 90% yield.

General Procedure for Synthesis of *N*-Alkylaminomethylarene Hydrochlorides (1a−16a). In a typical reaction, aromatic aldehydes (2.08 mmol) and alkyl amines (2.08 mmol) were dissolved in a 1:1 mixture of dry chloroform and methanol (20 mL) and stirred at room temperature (under nitrogen atmosphere) for 6 h. The resulting clear solution was then cooled to 0 °C, and to it sodium borohydride (0.142 g, 3.75 mmol) was added. This was allowed to come to room temperature and stirred overnight. Then the solvents were evaporated under reduced pressure (not to dryness) and diluted with diethyl ether. To this, 2N NaOH (20 mL) was added and stirred for 15 min. After separation from the NaOH layer, the organic layer was subsequently washed with water twice and brine and dried over $MgSO_4$. The organic layer was then evaporated under reduced pressure, and the residue was dissolved minimum volume of methanol. To this 4N HCl (3 mL) was added and instantaneous formation of precipitate was observed. The solvent was completely removed, and the precipitate was dissolved in minimum volume of ethyl acetate (a few drops of methanol was added to dissolve completely). To this hexane was added to obtain pure crystals of the target compound (yield 65−90%). These crystals were filtered, dried, and subsequently characterized using ^{1}H NMR and mass spectrometry.

General Procedure for Amide Coupling (1b−16b). In a typical amide coupling reaction, to a stirred solution of Boc-Lys(Boc)-OH (0.2 g, 0.58 mmol) in 5:2 DMF/$CHCl_3$(7 mL) was added *N*,*N*-diisopropylethylamine (DIPEA, 250 μL, 1.44 mmol) at 0 °C. To this solution was then added HBTU (0.22 g, 0.58 mmol). This mixture was stirred for 5 min at 0 °C, and subsequently the secondary amines (**1a**−**16a**, 0.48 mmol) were added to it. The mixture was stirred at 0 °C for 30 min and subsequently at RT for 24 h typically. At the end, $CHCl_3$ was evaporated under reduced pressure and the resulting solution was diluted to 2 times its original volume by addition of ethyl acetate. This mixture was subsequently washed with 0.5 M $KHSO_4$, H_2O (thrice), and brine. After passage through anhydrous Na_2SO_4, the organic layer was evaporated under reduced pressure and the residue was purified using column chromatography (only $CHCl_3$) to obtain the product in 65%-90% yield. The purified compound was subsequently characterized using ^{1}H NMR, IR, and mass spectrometry.

General Procedure for Deprotection of Boc Groups (1−16). Typically, the Boc-Lys(Boc)-*N*-alkyl-aromatic compounds (**1b**−**16b**, 0.35 mmol) were dissolved in DCM and subsequently CF_3COOH (50% by volume) was added and stirred at RT. The reactions were monitored by TLC until complete removal of starting material was observed. All the volatile components were removed, and the product was purified by reverse phase HPLC using 0.1% trifluoroacetic acid (TFA) in water/acetonitrile (0−100%) as mobile phase to more than 95% purity. A C_{18} column (10 mm diameter, 250 mm length) and a UV detector (at 270 nm wavelength) were used. After drying the compounds in a freeze dryer, the compounds were characterized by ^{1}H NMR, ^{13}C NMR, IR, and mass spectrometry (all spectra are furnished in Supporting Information). Peptoids of naphthalene have been reported to show presence of rotamers in solution.[40] These compounds (series I and series II) too show existence of rotamers, as is evident from their NMR spectra (furnished in Supporting Information).

dx.doi.org/10.1021/jm401680a | J. Med. Chem. 2014, 57, 1428−1436

In Vitro Biological Assays. *Microorganisms and Culture Conditions.* The antibacterial activity of the small molecules was evaluated against both Gram-positive (*S. aureus*, *E. faecium*, methicillin resistant *S. aureus*, and vancomycin resistant *E. faecium*) and Gram-negative (*E. coli*, *P. aeruginosa*, and *K. pneumoniae*) bacteria. *E. coli* was cultured in Luria−Bertani broth (10 g of tryptone, 5 g of yeast extract, and 10 g of NaCl in 1000 mL of sterile distilled water (pH 7.0), while *S. aureus*, *P. aeruginosa* (ATCC 4676), and MRSA were grown in yeast−dextrose broth (1 g of beef extract, 2 g of yeast extract, 5 g of peptone, and 5 g of NaCl in 1000 mL of sterile distilled water). For *E. faecium* and VRE, brain heart infusion broth (BHI) was used. *K. pneumoniae* was grown in nutrient media (3 g of beef extract and 5 g of peptone in 1000 mL of sterile distilled water). For solid media, 5% agar was used along with the above-mentioned composition. The bacterial samples were freeze-dried and stored at −80 °C. Then 5 μL of these stocks were added to 3 mL of the nutrient broth, and the culture was grown for 6 h at 37 °C prior to the experiments.

Antibacterial Activity. Antibacterial activity was reported as minimum inhibitory concentration (MIC), i.e., the lowest concentration of the antimicrobial agent that will inhibit the growth of a microorganism after overnight incubation. MIC was determined by following previously published protocol.[22] All the final compounds were water soluble. The compounds were assayed in a modified microdilution broth format. Stock solutions were made by serially diluting the compounds using autoclaved Millipore water. Bacteria, to be tested, was grown for 6 h in the suitable media contained ∼10^9 cfu/mL (determined through dilution plate technique by spread plate method), which was then diluted to 10^5 cfu/mL using nutrient media. Then 50 μL of serially diluted compound was added to a 96-well plate containing 150 μL of bacterial solutions. Two controls were made: one containing 150 μL of media and 50 μL of compound and the other containing 200 μL of bacterial solution. The plate was then incubated at 37 °C for a period of 24 h, and MIC data was recorded by measuring the OD value at 600 nm using a TecanInfinitePro series M200 microplate reader. MIC value was determined by taking the average of triplicate OD values for each concentration and plotting it against concentration using Origin Pro 8.0 software. The data was then subjected to sigmoidal fitting. From the curve the MIC value was determined as the point in the curve where the OD was similar to that of control having no bacteria. The MIC values and errors are reported as averages and standard errors of mean of at least two independent experiments (each experiment was performed in triplicates), respectively. The error of the experiments is less than 7%.

Hemolytic Activity. Hemolytic experiments were performed with slight modification of a previously reported protocol.[22] Erythrocytes were isolated from freshly drawn, heparinized human blood and resuspended to 5 vol% in PBS (pH 7.4). In a 96-well microtiter plate, 150 μL of erythrocyte suspension was added to 50 μL of serially diluted compound. Two controls were made, one without compound and other with 50 μL of 1 vol % solution of Triton X-100. The plate was incubated for 1 h at 37 °C. The plate was then centrifuged at 3500 rpm for 5 min, 100 μL of the supernatant from each well was transferred to a fresh microtiter plate, and A_{540} was measured. Percentage of hemolysis was determined as $(A - A_0)/(A_{total} - A_0) \times 100$, where A is the absorbance of the test well, A_0 the absorbance of the negative controls (without compound), and A_{total} the absorbance of 100% hemolysis wells (with Triton X-100), all at 540 nm. The HC_{50} values and errors are reported as averages and standard errors of mean of at least two independent experiments (each experiment was performed in triplicates), respectively. The error of the experiments is less than 10%.

Bactericidal Time-Kill Kinetics. The bactericidal activity of the derivatives was assessed by the kinetics or the rate at which it affects the killing action of the compound. Briefly, MRSA was grown in yeast−dextrose broth at 37 °C for 6 h. Test compound **3**, having the final concentrations of 1 × MIC, 3 × MIC, and 6 × MIC, was inoculated with the aliquots of MRSA resuspended in fresh media at approximately 1.8 × 10^6 CFU mL^{-1}. After specified time intervals (0, 1, 2, 3, 6, and 24 h), 20 μL aliquots were serially diluted 10-fold in 0.9% saline, plated on sterile yeast−dextrose agar plates, and incubated at 37 °C overnight. The viable colonies were counted the next day and represented as $\log_{10}$ (CFU/mL). A similar protocol was followed with compound **7**, but two concentrations were considered, i.e., MIC and 2 × MIC against *S. aureus*.

Antibacterial Activity in Plasma. *S. aureus* was grown for 6 h in yeast−dextrose agar media and finally contained ∼10^9 cfu/mL (determined through dilution plate technique by spread plate method), which was then diluted to 10^5 cfu/mL using nutrient media. Fresh human blood cells were centrifuged at 3000 rpm for 5 min. The plasma, which was separated from the RBC solution, was collected carefully. The test compound **7** was dissolved in water at a concentration of 3200 μg/mL. This was diluted 2-fold into the plasma solution so that the final concentration of the compound was 1600 μg/mL. Three such test samples were preincubated in 50% plasma solution at 37 °C for 0, 3, and 6 h. Then 50 μL of 2-fold serial dilutions of this solution was added to 150 μL of the bacterial solution in a 96-well plate. This was subjected to 10-fold serial dilutions and incubated at 37 °C for 24 h. Then the MIC values were determined as mentioned in the antibacterial assay.

Enzyme Stability. Compound **7** was dissolved in 0.1 M NH_4HCO_3 to a final concentration 1 mg mL^{-1}. Then 1 mg of trypsin was dissolved in 50 mL of 0.1 M NH_4HCO_3 buffer to make a solution. Then 200 μL of freshly made trypsin solution and 200 μL of compound solution was incubated in 1600 μL of 0.1 M NH_4HCO_3 buffer at 37 °C. The 200 μL aliquots were taken at different time intervals like 0 min, 5 min, 15 min, 30 min, 1 h, 2 h, 4 h, and 24 h. This was diluted with 200 μL of water and analyzed using LC-MS. For negative control, samples without trypsin were taken at 0 min and 24 h. The LC chromatogram was an indicator of formation of new products (if there is degradation), and the total ion current represented the total concentration of each chemical species.

Cytoplasmic Membrane Depolarization Assay. Midlog phase bacterial cells (*S. aureus*, *P. aeruginosa*, or *E. coli*) were harvested, washed with 5 mM HEPES and 5 mM glucose, and resuspended in 5 mM glucose, 5 mM HEPES buffer, and 100 mM KCl solution in 1:1:1 ratio (10^8 cfu/mL). Measurements were made in a cuvette containing 2 mL of bacterial suspension and 2 μM $DiSC_3(5)$. The bacterial suspension (2 mL) was preincubated for 10 min (20 min in case of *P. aeruginosa* or *E. coli*) with $DiSC_3(5)$ dye in a fluorimeter cuvette (dye uptake, and resultant self-quenching). The fluorescence of the bacterial suspension was measured and allowed to stabilize for 2 min at room temperature before the addition of test compounds (**3** and **7**) of different concentrations, and the decrease in potential was monitored by the increase in fluorescence for further 10 min. Excitation wavelength of 622 nm (slit width: 10 nm) and emission wavelength of 670 nm (slit width: 5 nm) were chosen for the study.

Inner Membrane Permeabilization Assay. Midlog phase *S. aureus* cells were harvested (4000 rpm, 4 °C, 10 min), washed, and resuspended in 5 mM HEPES and 5 mM glucose pH 7.2. Then test compounds (**3** and **7**) of different concentrations were added to a cuvette containing 2.0 mL of cells and 15 μM propidium iodide (PI). Excitation wavelength of 535 nm (slit width: 10 nm) and emission wavelength of 617 nm (slit width: 5 nm) were chosen for the study. The uptake of PI was measured by the increase in fluorescence of PI for 10 min as a measure of inner membrane permeabilization.

Outer Membrane Permeabilization Assay. Midlog phase *E. coli* cells (grown for 6 h, 10^8 cells/mL) were harvested (4000 rpm, 4 °C, 10 min), washed, and resuspended in 5 mM glucose/5 mM HEPES buffer at pH 7.2. Then 26 μL of compound **7** in water was added to a cuvette containing 2 mL of cells and 10 μM *N*-phenylnaphthylamine, NPN (30 μL from a 500 μM stock solution in acetone). The excitation and emission wavelengths used were 350 and 420 nm, respectively (slit width was 10 nm in both cases). The uptake of NPN as a measure of outer membrane permeabilization was monitored by the increase in fluorescence of NPN for 10 min.

Fluorescence Microscopy.[26] To 1 mL of bacterial suspension containing 10^9 CFU/mL, 10 times MIC of compounds **3** or **7** was added to make a final volume of 1 mL of suspension (final concentration 1 × 10^9 cells/mL), and another 1 mL was left untreated as a control. The mixture was incubated for 1.5 h, centrifuged (12000

dx.doi.org/10.1021/jm401680a | J. Med. Chem. 2014, 57, 1428−1436

rpm for 1 min), and resuspended in 50 μL of PBS. Then 5 μL of the bacterial suspension was combined with 20 μL of a fluorescent probe mixture containing 3.0 μM green fluorescent nucleic acid stain SYTO 9 (Invitrogen, USA) and 15.0 μM red fluorescent nucleic acid stain PI (Sigma-Aldrich, USA). The mixture was incubated in the dark for 15 min, and a 5 μL aliquot was placed on a glass slide, which was then covered with a coverslip, sealed, and examined under a fluorescence microscope. Excitation was carried out for SYTO 9 at 450−490 nm and for PI at 515−560 nm. Emission was collected using a band-pass filter for SYTO 9 at 500−550 nm and a long-pass filter for PI at 590−800 nm. In all cases, a 100× objective was used with immersion oil, giving a total magnification of 1000×. Images were captured with a Leica DM 2500 fluorescence microscope.

Scanning Electron Microscopy (SEM). The bacterial cells were cultured for 6 h in suitable media (LB broth for *E. coli* and yeast−dextrose broth for *S. aureus*) at 37 °C. The cells were centrifuged and resuspended in nutrient media at pH 7.4 (10^6 cells/mL). The suspension was divided into two portions. To one portion (1 mL) was added 10 × MIC of compounds **3** and **7**. The other portion was left untreated as a control. The suspension was then incubated at 37 °C for 2 h (at 250 rpm shaking speed), and the cells from both tubes were harvested by centrifugation at 12000 rpm. After treatment, the cells were dehydrated sequentially with 30, 50, 70, 80, 90, and 100% ethanol for 15 min. Later, 5 μL of dehydrated cells was dropped on a small piece of silicon wafer and dried at room temperature. Before being imaged, the silicon wafer containing *S. aureus* was sputter coated and *E. coli* was used directly for imaging without sputtering. Images were recorded by using Quanta 3D FEG FEI field-emission scanning electron microscopy at 20 kV for *S. aureus* and 10 kV for *E. coli*.

■ ASSOCIATED CONTENT

Supporting Information

Materials and instrumentation, details of characterization, HPLC data, NMR data, mass spectra of the final compounds, and other experimental figures. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: (+91) 80-2208-2565. Fax: (+91) 80-2208-2627. E-mail: jayanta@jncasr.ac.in.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

J.H. acknowledges Department of Science and Technology (DST), Government of India, for a Ramanujan Fellowship (SR/S2/RJN-43/2009) and Jawaharlal Nehru Centre for Advanced Scientific Research for funding. We thank Prof. C. N. R. Rao (JNCASR) for his constant support and encouragement.

■ ABBREVIATIONS USED

AMP, antimicrobial peptide; MRSA, methicillin-resistant *Staphylococcus aureus*; VRE, vancomycin-resistant *Enterococci*; MIC, minimum inhibitory concentration; HC_{50}, 50% hemolytic concentration; CFU, colony forming units

■ REFERENCES

(1) Taubes, G. The bacteria fight back. *Science* **2008**, *321*, 356−361.

(2) Boucher, H. W.; Talbot, G. H.; Bradley, J. S.; Edwards, J. E.; Gilbert, D.; Rice, L. B.; Scheld, M.; Spellberg, B.; Bartlett, J. Bad Bugs, No Drugs: No ESKAPE! An Update from the Infectious Diseases Society of America. *Clin. Infect. Dis.* **2009**, *48*, 1−12.

(3) Boucher, H. W.; Talbot, G. H.; Benjamin, D. K.; Bradley, J.; Guidos, R. J.; Jones, R. N.; Murray, B. E.; Bonomo, R. A.; Gilbert, D. 10 × '20 Progress—Development of New Drugs Active Against Gram-Negative Bacilli: An Update From the Infectious Diseases Society of America. *Clin. Infect. Dis.* **2013**, *56*, 1685−1694.

(4) Fjell, C. D.; Hiss, J. A.; Hancock, R. E.; Schneider, G. Designing antimicrobial peptides: form follows function. *Nature Rev. Drug Discovery* **2012**, *11*, 37−51.

(5) Hancock, R. E. W.; Sahl, H. G. Antimicrobial and host-defense peptides as new anti-infective therapeutic strategies. *Nature Biotechnol.* **2006**, *24*, 1551−1557.

(6) Zasloff, M. Antimicrobial peptides of multicellular organisms. *Nature* **2002**, *415*, 389−395.

(7) Brogden, K. A. Antimicrobial peptides: pore formers or metabolic inhibitors in bacteria? *Nature Rev. Microbiol.* **2005**, *3*, 238−250.

(8) Tew, G. N.; Scott, R. W.; Klein, M. L.; DeGrado, W. F. De Novo Design of Antimicrobial Polymers, Foldamers, and Small Molecules: From Discovery to Practical Applications. *Acc. Chem. Res.* **2009**, *43*, 30−39.

(9) Giuliani, A.; Rinaldi, A. Beyond natural antimicrobial peptides: multimeric peptides and other peptidomimetic approaches. *Cell. Mol. Life Sci.* **2011**, *68*, 2255−2266.

(10) McPhee, J. B.; Hancock, R. E. W. Function and therapeutic potential of host defence peptides. *J. Pept. Sci.* **2005**, *11*, 677−687.

(11) Chen, Y. X.; Mant, C. T.; Farmer, S. W.; Hancock, R. E. W.; Vasil, M. L.; Hodges, R. S. Rational design of alpha-helical antimicrobial peptides with enhanced activities and specificity/therapeutic index. *J. Biol. Chem.* **2005**, *280*, 12316−12329.

(12) Won, H. S.; Jung, S. J.; Kim, H. E.; Seo, M. D.; Lee, B. J. Systematic peptide engineering and structural characterization to search for the shortest antimicrobial peptide analogue of gaegurin 5. *J. Biol. Chem.* **2004**, *279*, 14784−14791.

(13) Porter, E. A.; Wang, X.; Lee, H. S.; Weisblum, B.; Gellman, S. H. Non-haemolytic beta-amino-acid oligomers (vol 404, pg 565, 2000). *Nature* **2000**, *405*, 298−298.

(14) Hamuro, Y.; Schneider, J. P.; DeGrado, W. F. De novo design of antibacterial beta-peptides. *J. Am. Chem. Soc.* **1999**, *121*, 12200−12201.

(15) Chongsiriwatana, N. P.; Patch, J. A.; Czyzewski, A. M.; Dohm, M. T.; Ivankin, A.; Gidalevitz, D.; Zuckermann, R. N.; Barron, A. E. Peptoids that mimic the structure, function, and mechanism of helical antimicrobial peptides. *Proc. Natl. Acad. Sci. U. S. A.* **2008**, *105*, 2794−2799.

(16) Ilker, M. F.; Nusslein, K.; Tew, G. N.; Coughlin, E. B. Tuning the hemolytic and antibacterial activities of amphiphilic polynorbornene derivatives. *J. Am. Chem. Soc.* **2004**, *126*, 15870−15875.

(17) Kuroda, K.; DeGrado, W. F. Amphiphilic polymethacrylate derivatives as antimicrobial agents. *J. Am. Chem. Soc.* **2005**, *127*, 4128−4129.

(18) Gelman, M. A.; Weisblum, B.; Lynn, D. M.; Gellman, S. H. Biocidal activity of polystyrenes that are cationic by virtue of protonation. *Org. Lett.* **2004**, *6*, 557−560.

(19) Nederberg, F.; Zhang, Y.; Tan, J. P. K.; Xu, K. J.; Wang, H. Y.; Yang, C.; Gao, S. J.; Guo, X. D.; Fukushima, K.; Li, L. J.; Hedrick, J. L.; Yang, Y. Y. Biodegradable nanostructures with selective lysis of microbial membranes. *Nature Chem.* **2011**, *3*, 409−414.

(20) Haldar, J.; An, D.; Alvarez de Cienfuegos, L.; Chen, J.; Klibanov, A. M. Polymeric coatings that inactivate both influenza virus and pathogenic bacteria. *Proc. Natl. Acad. Sci. U. S. A.* **2006**, *103*, 17667−17671.

(21) Sambhy, V.; Peterson, B. R.; Sen, A. Antibacterial and hemolytic activities of pyridinium polymers as a function of the spatial relationship between the positive charge and the pendant alkyl tail. *Angew. Chem.* **2008**, *47*, 1250−1254.

(22) Uppu, D. S. S. M.; Akkapeddi, P.; Manjunath, G. B.; Yarlagadda, V.; Hoque, J.; Haldar, J. Polymers with tunable side-chain amphiphilicity as non-hemolytic antibacterial agents. *Chem. Commun.* **2013**, *49*, 9389−9391.

(23) Radzishevsky, I. S.; Rotem, S.; Bourdetsky, D.; Navon-Venezia, S.; Carmeli, Y.; Mor, A. Improved antimicrobial peptides based on acyl-lysine oligomers. *Nature Biotechnol.* **2007**, *25*, 657−659.

(24) Violette, A.; Averlant-Petit, M. C.; Semetey, V.; Hemmerlin, C.; Casimir, R.; Graff, R.; Marraud, M.; Briand, J. P.; Rognan, D.; Guichard, G. *N,N′*-Linked oligoureas as foldamers: chain length requirements for helix formation in protic solvent investigated by circular dichroism, NMR spectroscopy, and molecular dynamics. *J. Am. Chem. Soc.* **2005**, *127*, 2156−2164.
(25) Padhee, S.; Hu, Y. G.; Niu, Y. H.; Bai, G.; Wu, H. F.; Costanza, F.; West, L.; Harrington, L.; Shaw, L. N.; Cao, C. H.; Cai, J. F. Non-hemolytic alpha-AA peptides as antimicrobial peptidomimetics. *Chem. Commun.* **2011**, *47*, 9729−9731.
(26) Hoque, J.; Akkapeddi, P.; Yarlagadda, V.; Uppu, D. S.; Kumar, P.; Haldar, J. Cleavable cationic antibacterial amphiphiles: synthesis, mechanism of action, and cytotoxicities. *Langmuir* **2012**, *28*, 12225−12234.
(27) Engler, A. C.; Wiradharma, N.; Ong, Z. Y.; Coady, D. J.; Hedrick, J. L.; Yang, Y. Y. Emerging trends in macromolecular antimicrobials to fight multi-drug-resistant infections. *Nano Today* **2012**, *7*, 201−222.
(28) Liu, D. H.; Choi, S.; Chen, B.; Doerksen, R. J.; Clements, D. J.; Winkler, J. D.; Klein, M. L.; DeGrado, W. F. Nontoxic membrane-active antimicrobial arylamide oligomers. *Angew. Chem., Int. Ed.* **2004**, *43*, 1158−1162.
(29) Choi, S.; Isaacs, A.; Clements, D.; Liu, D. H.; Kim, H.; Scott, R. W.; Winkler, J. D.; DeGrado, W. F. De novo design and in vivo activity of conformationally restrained antimicrobial arylamide foldamers. *Proc. Natl. Acad. Sci. U. S. A.* **2009**, *106*, 6968−6973.
(30) Haug, B. E.; Stensen, W.; Kalaaji, M.; Rekdal, O.; Svendsen, J. S. Synthetic antimicrobial peptidomimetics with therapeutic potential. *J. Med. Chem.* **2008**, *51*, 4306−4314.
(31) Isaksson, J.; Brandsdal, B. O.; Engqvist, M.; Flaten, G. E.; Svendsen, J. S. M.; Stensen, W. A Synthetic Antimicrobial Peptidomimetic (LTX 109): Stereochemical Impact on Membrane Disruption. *J. Med. Chem.* **2011**, *54*, 5786−5795.
(32) Bremner, J. B.; Keller, P. A.; Pyne, S. G.; Boyle, T. P.; Brkic, Z.; David, D. M.; Garas, A.; Morgan, J.; Robertson, M.; Somphol, K.; Miller, M. H.; Howe, A. S.; Ambrose, P.; Bhavnani, S.; Fritsche, T. R.; Biedenbach, D. J.; Jones, R. N.; Buckheit, R. W.; Watson, K. M.; Baylis, D.; Coates, J. A.; Deadman, J.; Jeevarajah, D.; McCracken, A.; Rhodes, D. I. Binaphthyl-Based Dicationic Peptoids with Therapeutic Potential. *Angew. Chem., Int. Ed.* **2010**, *49*, 537−540.
(33) Hansen, T.; Alst, T.; Havelkova, M.; Strom, M. B. Antimicrobial Activity of Small beta-Peptidomimeties Based on the Pharmacophore Model of Short Cationic Antimicrobial Peptides. *J. Med. Chem.* **2010**, *53*, 595−606.
(34) Thaker, H. D.; Som, A.; Ayaz, F.; Lui, D. H.; Pan, W. X.; Scott, R. W.; Anguita, J.; Tew, G. N. Synthetic Mimics of Antimicrobial Peptides with Immunomodulatory Responses. *J. Am. Chem. Soc.* **2012**, *134*, 11088−11091.
(35) Thaker, H. D.; Cankaya, A.; Scott, R. W.; Tew, G. N. Role of Amphiphilicity in the Design of Synthetic Mimics of Antimicrobial Peptides with Gram-Negative Activity. *ACS Med. Chem. Lett.* **2013**, *4*, 481−485.
(36) Ge, Y. G.; MacDonald, D. L.; Holroyd, K. J.; Thornsberry, C.; Wexler, H.; Zasloff, M. In vitro antibacterial properties of pexiganan, an analog of magainin. *Antimicrob. Agents Chemother.* **1999**, *43*, 782−788.
(37) Lister, P. D.; Wolter, D. J.; Hanson, N. D. Antibacterial-Resistant *Pseudomonas aeruginosa*: Clinical Impact and Complex Regulation of Chromosomally Encoded Resistance Mechanisms. *Clin. Microbiol. Rev.* **2009**, *22*, 582−610.
(38) Lienkamp, K.; Tew, G. N. Synthetic Mimics of Antimicrobial Peptides—A Versatile Ring-Opening Metathesis Polymerization Based Platform for the Synthesis of Selective Antibacterial and Cell-Penetrating Polymers. *Chem.—Eur. J.* **2009**, *15*, 11784−11800.
(39) Thaker, H. D.; Sgolastra, F.; Clements, D.; Scott, R. W.; Tew, G. N. Synthetic Mimics of Antimicrobial Peptides from Triaryl Scaffolds. *J. Med. Chem.* **2011**, *54*, 2241−2254.
(40) Laursen, J. S.; Engel-Andreasen, J.; Fristrup, P.; Harris, P.; Olsen, C. A. Cis−Trans Amide Bond Rotamers in *β*-Peptoids and Peptoids: Evaluation of Stereoelectronic Effects in Backbone and Side Chains. *J. Am. Chem. Soc.* **2013**, *135*, 2835−2844.