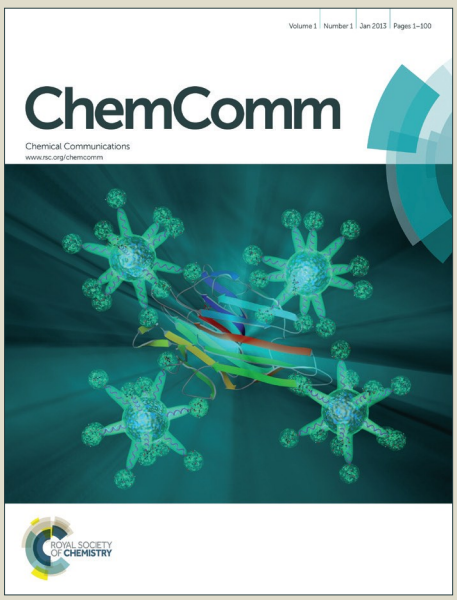
ChemComm

Accepted Manuscript



ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/chemcomm

Cite this: DOI: 10.1039/c0xx00000x

www.rsc.org/xxxxxx

COMMUNICATIONS

Selective and Broad Spectrum Amphiphilic Small Molecules to Combat Bacterial Resistance and Eradicate Biofilm

Jiaul Hoque,[a] Mohini M. Konai,[a] Sandip Samaddar,[a] Spandhana Gonuguntala,[a] Goutham B. Manjunath,[a] Chandradhish Ghosh[a] and Jayanta Haldar[a]*

Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX
DOI: 10.1039/b000000x

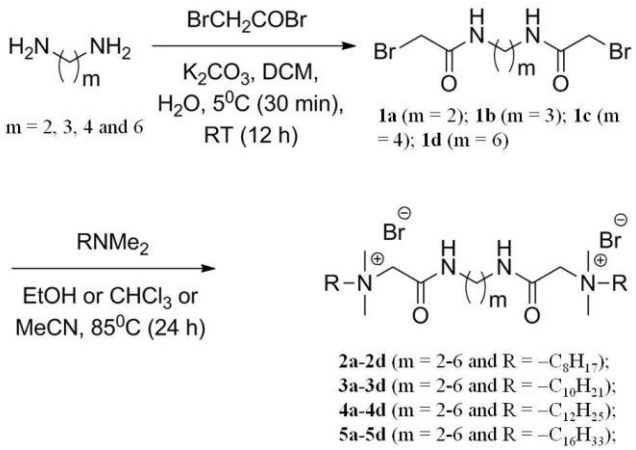
Rationally designed amphiphilic small molecules kill selectively drug-sensitive and drug-resistant bacteria over mammalian cells. The small molecules disperse preformed biofilm and reduce the viable bacterial count in the biofilm. Moreover, this class of membrane-active molecules disarm the development of bacterial resistance.

Continued emergence of drug-resistant pathogens along with declined approval of new antimicrobial drugs pose unavoidable threats to human health.[1] Drug-resistant pathogenic bacteria are empowered with the abilities to either circumvent or thwart the action of commonly used therapeutic antibiotics. To tackle this problem, it is thus necessary to develop new antibacterial agents that act on bacterial cells selectively over the mammalian cells. The bacterial cell membrane being mostly negatively charged (in sharp contrast to the zwitterionic mammalian cell membrane) is considered to be a major and inevitable target given its immense role in the survival of bacteria. Membrane-targeting antibacterial agents are thus considered to be an alternative to counter resistance development.[2]

Another major threat to public health is the formation of bacterial biofilm protected by extracellular polymeric substances (EPS). Biofilms are known to induce chronic infections and elevate bacterial resistance to antibiotics and host immune system as a result of the diffusion barrier, genetic mutation, and presence of persistent cells with slow metabolism and so on.[3] Notably, bacterial biofilms are known to cause more than 75% of microbial infections in humans.[3] Therefore, there is a pressing need for antibacterial agents which not only disarm bacterial resistance but also disperse established bacterial biofilm.

Membrane-targeting antimicrobial peptides (AMPs) and lipopeptides are known to overcome bacterial resistance and have exhibited the potency to disperse established bacterial biofilms.[4] However, high cost of manufacture, proteolytic degradation and low selectivity limit the use of these natural antimicrobials as therapeutic agents. Synthetic mimics of these natural antimicrobials such as β-peptides, γ-AApeptides, aryl-amide foldamers, peptide dendrimer, oligoacyl lysines, oligoureas, small antibacterial peptides, antimicrobial polymers, alkylated peptoids etc have widely been demonstrated to overcome the aforementioned problems.[5] Though highly effective, the applications of peptidomimetics are still limited by the synthetic complexity, availability of frameworks and difficulty of introducing a variety of functional groups.[6]

Herein, we report the development of membrane-active amphiphilic small molecules in a facile and cost effective way using commercially available diaminoalkanes as scaffold. The small molecules showed high selectivity towards bacterial cells over mammalian cells. These molecules dispersed established bacterial biofilm and reduced the viable count within the biofilm. Further, the molecules not only killed drug-resistant bacteria but also stalled development of bacterial resistance. To mimic the structural features of antimicrobial peptides or lipopeptides composed of positive charges, lipophilic moieties either from hydrophobic amino acids or fatty acid and peptide (amide) groups, we introduced two positive charges, two lipophilic moieties and two non-peptidic amide groups into the small molecules (Scheme 1). Further, to fine-tune the structure-activity relationship of the molecules, the lipophilic moieties were varied between the amine groups of the diaminoalkane scaffold. To synthesize cationic small molecules in a simple two step process, various diamides obtained from diaminoalkanes by reacting with bromoacetyl bromide were quaternized with *N,N*-dimethylamino alkanes with quantitative yield (Scheme 1, See ESI† for experimental details and characterizations).



Scheme 1. Synthesis of amphiphilic small molecules.

In order to evaluate the potential as antibacterial yet non-toxic compounds, antibacterial activity expressed as minimum inhibitory concentration (MIC) and hemolytic activity expressed as HC_{50} (concentration at which 50% hemolysis occurs) were determined. Among four sets of molecules (**2a-2d** with $-C_8H_{17}$ lipophilic chain and m = 2-6; **3a-3d** with $-C_{10}H_{21}$ chain and m = 2-6; **4a-4d** with $-C_{12}H_{25}$ lipophilic chain and m = 2-6 and **5a-5d** with $-C_{16}H_{33}$ lipophilic chain and m = 2-6), **3a-3d** and **4a-4d** were found to be more active than **2a-2d** and **5a-5d**

against both *S. aureus* and *E. coli* thus showing a parabolic relationship of activity with lipophilic chain length (Table 1, and ESI,† Fig. S1). For example, minimum inhibitory concentrations (MICs) for compounds **3a-3d** and **4a-4d** were 1.5-2 μg/mL and 1-2 μg/mL against *S. aureus* and 1.9-3.9 μg/mL for both sets against *E. coli* whereas MICs for molecules **2a-2d** and **5a-5d** were 1.9-22 μg/mL and 62.5-125 μg/mL against *S. aureus* and 3.9-125 μg/mL and >1000 μg/mL against *E. coli* respectively. The toxicity towards human erythrocytes (HC_{50}, concentration at which 50% hemolysis occurs) also increased as the lipophilic alkyl chain length increased from $–C_8H_{17}$ (HC_{50} = 780->1000 μg/mL for **2a-2d**) to $–C_{10}H_{21}$ (HC_{50} = 45-200 μg/mL for **3a-3d**) to $–C_{12}H_{25}$ (HC_{50} = 31-53 μg/mL for **4a-4d**). Further increase in length of alkyl chain to $–C_{16}H_{33}$ showed similar toxicity (HC_{50} = 32-49 μg/mL for **5a-5d**) as compounds **4a-4d** (Table 1, ESI,† Fig. S2). Thus by varying the lipophilic alkyl chain, we could tune the selectivity (HC_{50}/MIC) of the small molecules. On the other hand, with the increase in lipophilic spacer length, both antibacterial and hemolytic activities were also found to vary but differently for the molecules with a particular alkyl chain. For example, MICs of the molecules **2a** (m = 2, R = $–C_8H_{17}$), **2b** (m = 3, R = $–C_8H_{17}$), **2c** (m = 4, R = $–C_8H_{17}$) and **2d** (m = 6, R = $–C_8H_{17}$) were 22 μg/mL, 18 μg/mL, 10 μg/mL and 2 μg/mL against *S. aureus* and 125 μg/mL, 62.5 μg/mL, 31.2 μg/mL and 3.9 μg/mL against *E. coli* respectively whereas HC_{50} were >1000 μg/mL for **2a-2c** and 780 μg/mL for **2d** (Table 1, ESI,† Fig. S2). Thus by varying both lipophilic alkyl chain and spacer chain length, we could able to fine-tune the selectivity of the amphiphilic small molecules (Table 1).

Table 1. MIC and HC_{50} values of amphiphilic small molecules

Biocides	MIC (μg/mL)						HC_{50} (μg/mL)
	Drug-sensitive bacteria			Drug-resistant bacteria			
	S. aureus	*E. coli*	*P. aeruginosa*	MRSA	VRE	*K. pneumoniae*	
2a	22	125	188	20	94	500	> 1000
2b	18	62.5	188	17	125	500	> 1000
2c	7.8	31.2	125	8	62	250	> 1000
2d	1.9	3.9	31.2	3	6	31.2	780
3a	2	3.9	6	1.5	1.5	8	200
3b	1.9	3.9	6	1	1	6	140
3c	1.5	3.9	6	1	1	4	110
3d	1.5	1.9	3	1	1	3	45
4a	2	3.9	9	3.9	2	6	53
4b	1.9	2	10	3.9	1.5	6	40
4c	1.9	2	14	3.9	1.5	6	33
4d	1.0	1.9	28	8	1.5	8	31
5a	125	>1000	250	47	188	530	49
5b	94	>1000	186	47	147	517	41
5c	62.5	>1000	186	47	137	500	37
5d	62.5	>1000	250	31	94	500	32
Collistin	ND	0.4	0.4	ND	ND	1.2	> 798
Vancomycin	0.63	ND	ND	0.63	750	ND	>1000

P. aeruginosa = *Pseudomonas aeruginosa*, MRSA = Methicillin-resistant *S. aureus*, VRE = vancomycin-resistant *E. faecium*, *K. pneumoniae* = beta-lactam-resistant *Klebsiella pneumoniae*, ND = Not determined. MIC = Minimum inhibitory concentration after 24 h of treatment with the biocides (at 2-fold serial dilution) in growth media (nutrient medium/Luria-Bertani medium/brain-heart infusion medium. HC_{50} = Concentration at which 50% hemolysis (human red blood cells) occurs after 1 h of treatment with the biocides. Variation in values ≤ 5%.

Amongst all, molecules **2d** and **3a** were found to be most potent in terms of their activity and selectivity. Molecules **2d** and **3a** showed selectivity of 410 and 100 respectively against *S. aureus* and 200 and 50 respectively against *E. coli* over human erythrocytes. These molecules also showed good activity (and selectivity) against *P. aeruginosa*, a difficult-to-treat Gram-negative pathogen (Selectivity of **2d** and **3a** is 25 and 33 respectively) (Table 1). These molecules showed broad-spectrum activity not only against drug sensitive bacteria but also against various drug-resistant bacteria (Table 1). Against methicillin-resistant *S. aureus* (MRSA), molecules **2d** and **3a** displayed MIC values of 3 μg/mL and 1 μg/mL respectively thus showing the selectivity of 260 and 200. The activities of molecules **2d** and **3a** against vancomycin-resistant enterococcus (VRE) were also high as they exhibited MIC values of 6 μg/mL and 1 μg/mL respectively (Selectivity = 130 and 200 respectively). These molecules were also found to be active against *β*-lactam-resistant *Klebsiella pneumoniae*. The MICs of molecules **2d** and **3a** were 31.2 μg/mL and 8 μg/mL respectively thus showing selectivity of 25 each against this bacterium. Antibacterial activities of these molecules were also compared with a gram-positive antibiotic vancomycin and a gram-negative antibiotic colistin (Table 1). Vancomycin showed activity only against Gram-positive bacteria (MIC = 0.63 μg/mL against *S. auerus* and MRSA) except against VRE (MIC = 750 μg/mL) whereas colistin showed activity only Gram-negative bacteria (MIC = 0.4 μg/mL against *E. coli* and *P. aeruginosa* and 1.2 μg/mL against *K. pneumoniae*). In contrast the amphiphilic small molecules showed activity against both drug-sensitive and drug-resistant Gram-positive and Gram-negative bacteria.

One of the major limitations of the natural antimicrobial peptides is the loss of antibacterial efficacy in the presence of blood plasma due to enzymatic hydrolysis.[7] However, the most potent molecule **2d** did not reveal any loss in activity on incubation with 50% plasma for 0 h, 3 h and 6 h respectively prior to determining its activity against *S. aureus*. The MIC was determined to be 2 μg/mL each after 0 h, 3 h and 6 h incubation in 50% plasma (ESI,† Fig. S3a). The cationic molecule **2d** was also found to be active in 50% serum (MBC, minimum

bactericidal concentration = 3 μg/mL), 50% plasma (MBC = 6 μg/mL) and 50% blood (MBC = 12 μg/mL) against MRSA respectively. The activities in complex media compared favourably to the MBC value of 3 μg/mL in nutrient media (ESI,† Fig. S3b). The above results thus indicated that the molecule retained its antibacterial efficacy (only 2-4 fold increase in MBC) even in very complex mammalian fluids like human serum, plasma and blood. These molecules were found to show rapid bactericidal activity against both types of bacteria. Molecule **2d** killed *S. aureus* (~5 log reduction) at 240 min in growth medium and at <10 min in a relatively less complex medium HEPES-glucose buffer (1:1) respectively at 11.4 μg/mL (Fig. 1b, ESI,† Fig. S4a). On the other hand, **2d** killed *E. coli* (~5 log reduction) at 360 min in growth medium and 20 min in HEPES-glucose buffer (1:1) at 23.4 μg/mL (ESI,† Fig. S4b and Fig. S4c).

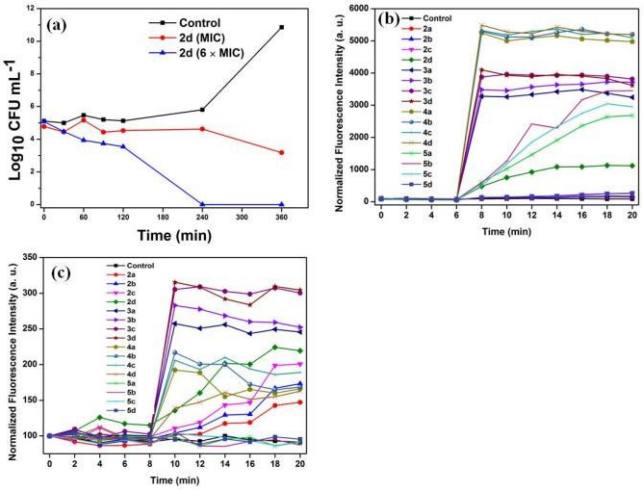


Fig. 1. Antibacterial kinetics and mechanism of action of small molecules. (a) Time-kill kinetics of **2d** aganist *S. aureus*; (b) and (c) Membrane depolarization and permeabilization by small molecules aganist *S. aureus* after treating bacteria in HEPES: glucose (1:1) buffer using potential sensitive fluorescent dye $diSC_3(5)$ and membrane impermeable fluorescent dye propidium iodide (PI).

Membrane permeabilization and disruption resulting in loss of membrane integrity of bacteria is the main mechanism of action of the cationic antimicrobials.[8] The amphiphilic small molecules were similarly found to dissipate the membrane potential of both Gram-positive and Gram-negative bacteria when bacterial suspension in HEPES: glucose (1:1) buffer was treated at a particular concentration of all the small molecules (40 μg/mL) (Fig. 1c, and ESI,† Fig. S5a). The molecules also showed membrane permeabilization against both types of bacteria (Fig. 1d, and ESI,† Fig. S5b, S5c). Interestingly, even the least active compounds **5a-5d** showed membrane permeabilization in the buffer. This could be due to less interaction of the molecules with the buffer which was verified by determining the MBCs in the above buffer where a cationic and hydrophobic molecule **5d** showed good activity (MBC = 3.9-7.8 μg/mL against *E. coli*) (ESI,† Table S1). This corroborates the findings mentioned previously regarding the dependence of activities of these compounds on the nature of media used. However, these membrane-active small molecules caused no significant leakage of intracellular K^+ ions against both types of bacteria (data not shown).

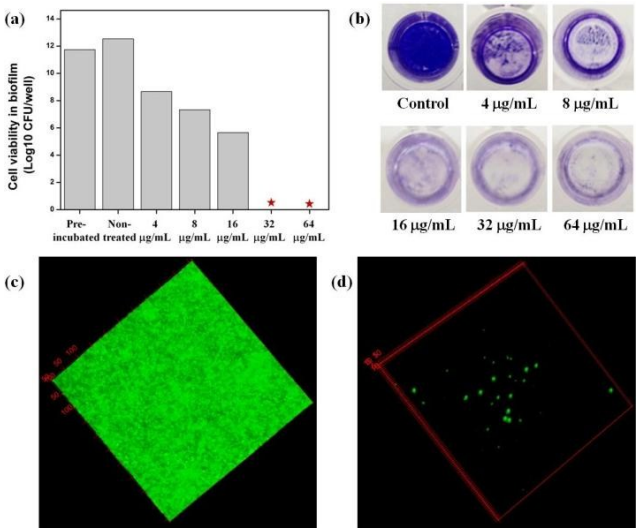


Fig. 2. Antibiofim activity of small molecule **2d**. (a) Cell viability in biofilms indicating the presence of live bacteria in the biofilm obtained after plating and counting the colonies of *S. aureus* (Star represents <50 CFU/mL); (b) Images of the treated and non-treated biofilms of *S. aureus* after staining with crystal violet; (c) and (d) CLSM images of non-treated and treated *S. aureus* biofilm (32 μg/mL of **2d**) after staining with SYTO 9.

To establish the ability of this class of small molecules to disperse the preformed bacterial biofilms, matured *S. aureus* biofilm at solid-liquid interface (developed for 24 h in flat bottom 96-well plate) having an initial count of 11.8 log_{10}CFU/well of bacteria were treated with **2d** at five different concentrations such as 4 μg/mL, 8 μg/mL, 16 μg/mL, 32 μg/mL and 64 μg/mL respectively. The molecule was found to reduce the cell viabilities in biofims (8.77, 7.45 and 5.9 log_{10}CFU/well at 4 μg/mL, 8 μg/mL, 16 μg/mL and 0 CFU/well at 32 and 64 μg/mL respectively) whereas cell viability in non-treated biofilm increased to 12.6log_{10} CFU/well. Compound **2d** at concentration of 32 μg/mL showed complete eradication of established *S. aureus* biofilm which is also evident from crystal violet staining (Fig. 2). The compound **2d** was also able to reduce the cell viabilities in matured *E. coli* biofilm at both solid-liquid and liquid-air interface (developed for 72 h in flat bottom 96-well plate) having an initial count of 21.5 log_{10}CFU/well to 19.6, 19.3, 15.3, 12.1 and 9.4 log_{10}CFU/well at 4 μg/mL, 8 μg/mL, 16 μg/mL, 32 μg/mL and 64 μg/mL respectively whereas cell viability in non-treated biofilm increased to 23.3 log_{10}CFU/well which was evident from crystal violet staining). Antibiofilm activity of compound **2d** was also evaluated against matured biofilms formed on cover slip (18 mm diameter) at the solid-liquid interface for both *S. aureus* and *E. coli*. Biocide **2d** at 32 μg/mL was found to reduce the viable count from 17.7 log_{10}CFU/mL to 5.6 log_{10}CFU/mL against *S. aureus* biofilm and completely eradicated *E. coli* biofilm having an initial bacterial count of 7.2 log_{10}CFU/mL respectively (ESI,† Fig. S6). The ability of the compound to disperse preformed biofilm was further visualized after treating with **2d** (at 32 μg/mL) by confocal laser scanning microscopy (CLSM) images of both treated and nontreated bacterial biofilm grown on cover slips (Fig. 2, ESI,† Fig. S7).

The ability of the small molecules to suppress bacterial resistance development was evaluated by challenging one of the potent molecules **2d** at sub-MICs repeatedly against both *S.*

aureus and *E. coli*. To evaluate the propensity of developing resistance, bacteria grown at the sub-MIC level (MIC/2) were used for successive MIC assay and the process was repeated for 20 passages. The cationic biocide showed no change in MIC against both the bacteria even after 20 passages, whereas around 805-fold and 250-fold increase in MIC was observed for antibiotic norfloxacin against *S. aureus* and lipopeptide colistin against *E. coli* respectively (ESI,† Fig. S8). The above results thus indicated that bacteria were less prone to develop resistance against this type of molecules.

In order to further evaluate the toxicity of the small molecules, human embryo kidney cells (HEK 293) were treated with the most potent molecule **2d** for 24 h. The half-maximal inhibitory concentration (IC_{50}) was found to be 220 μg/mL in lactate dehydrogenase (LDH) assay following the manufacturer protocol (Lactate dehydrogenase activity assay kit, Sigma-Aldrich, catalog number MAK066). Also, the treated cells, visualized by LIVE/DEAD staining method, showed green fluorescence even at 128 μg/mL (64 times of MIC) and were similar to the untreated cells whereas cell treated with triton-X were found to have completely red fluorescence (Fig. 3, ESI,† Fig. S9). These results thus indicated that these biocides are indeed non-toxic towards mammalian cells.

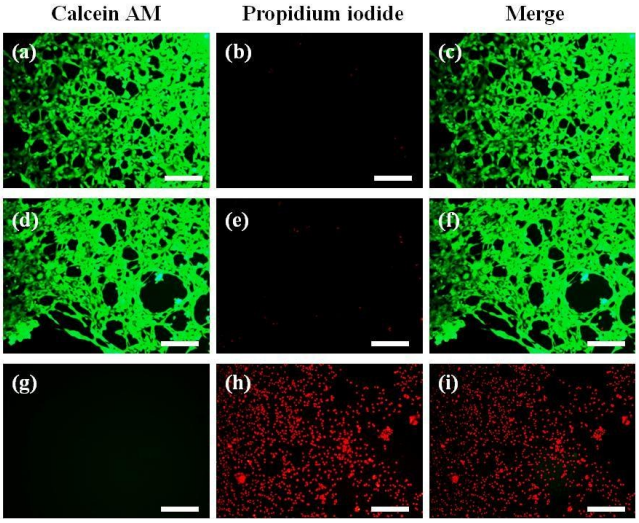


Fig. 3. Fluorescence microscopy images of HEK cells after treatment with small molecule **2d** for 24 h and staining with calcein AM and propidium iodide (PI). (a-c) Nontreated cells (negative control); (d-f) cells treated with **2d** (128 μg/mL); and (g-i) cells treated with 0.1% Triton X (positive control). Scale bar is 20 μm.

In summary, novel membrane-active amphiphilic small molecules, developed in a facile and cost-effective way, were highly active towards drug-sensitive and drug-resistance pathogenic bacteria but were less or non-toxic to human erythrocytes and human kidney cells. The molecules killed bacteria mainly by disrupting membrane integrity and hindered the propensity of developing bacterial resistance. Further, the small molecules dispersed the preformed Gram-positive and Gram-negative bacterial biofilms and reduced viable bacteria in biofilms. The structure-activity relationship, demonstrated by varying the nature of the lipophilic alkyl chain and spacer chain lengths, emphasized the role of optimum amphiphilicity in developing non-toxic yet potent membrane-active antibacterial agents.

Notes and references

We thank Prof. C. N. R. Rao, FRS (JNCASR) for his constant support and encouragement. Dr. Haldar acknowledges the Department of Science and Technology (DST), Govt. of India, for Ramanujan fellowship [SR/S2/RJN-43/2009]. Jiaul thanks JNCASR for Senior Research Fellowship (SRF). SS is grateful to Sheikh Saqr Laboratory (SSL), JNCASR for postdoctoral fellowship.

[a]*Chemical Biology and Medicinal Chemistry Laboratory, New Chemistry Unit, Jawaharlal Nehru Centre for Advanced Scientific Research, Jakkur, Bengaluru 560064, India; E-mail: jayanta@jncasr.ac.in*; Ph (+91)-080-2208-2556.

†Electronic Supplementary Information (ESI) available: Experimental procedures, synthetic protocols and characterizations of the molecules, figures of antibacterial and antibiofilm activity. See DOI: 10.1039/b000000x/

1. a) G. Taubes, *Science*, 2008, **321**, 356; b) C. Walsh, *Nature*, 2000, **406**, 775; c) M. N. Alekshun and S. B. Levy, *Cell*, 2007, **128**, 1037; d) G. D. Wright, *Chem. Commun.*, 2011, **47**, 4055.

2. a) J. Haldar, D. An, L. Alvarez de Cienfuegos, J. Chen and A. M. Klibanov, *Proc. Natl. Acad. Sci. U. S. A.*, 2006, **103**, 17667; b) J. G. Hurdle, A. J. O'Neill, I. Chopra and R. E. Lee, *Nat. Rev. Microbiol.*, 2011, **9**, 62; c) D. S. S. M. Uppu, P. Akkapeddi, G. B. Manjunath, V. Yarlagadda, J. Hoque and J. Haldar, *Chem. Commun.*, 2013, **49**, 9389; d) C. Ghosh, G. B. Manjunath, P. Akkapeddi, V. Yarlagadda, J. Hoque, D. S. S. M. Uppu, M. M. Konai and J. Haldar, *J. Med. Chem.*, 2014, **57**, 1428; e) M. M. Konai, C. Ghosh, V. Yarlagadda, S. Samaddar and J. Haldar, *J. Med. Chem.*, 2014, ***57***, 9409.

3. a) J. W. Costerton, P. S. Stewart and E. P. Greenberg, *Science*, 1999, **284**, 1318; b) L. Hall-Stoodley, W. J. Costerton and P. Stoodley, *Nat. Rev. Microbiol.*, 2004, **2**, 95; c) D. Davies, *Nat. Rev. Drug Discov.*, 2003, **2**, 114.

4. a) V. Luca, A. Stringaro, M. Colone, A. Pini and M. L. Mangoni, *Cell. Mol. Life Sci.*, 2013, **70**, 2773; b) N. Bionda, I. Pastar, S. C. Davis and P. Cudic, *Protein Pept. Lett.*, 2014, **21**, 352.

5. a) E. A. Porter, X. Wang, H. S. Lee, B. Weisblum and S. H. Gellman, *Nature*, 2000, **405**, 298; b) D. H. Liu, S. Choi, B. Chen, R. J. Doerksen, D. J. Clements, J. D. Winkler, M. L. Klein and W. F. DeGrado, *Angew. Chem. Int. Ed.*, 2004, **43**, 1158; c) H. Tang, R. J. Doerksen and G. N. Tew, *Chem. Commun.*, 2005, **28**, 1537; d) K. Hilpert, R. V.- Engert, T. Walter and R. E. W. Hancock, *Nat. Biotechnol.*, 2005, **23**, 1008; e) A. Makovitzki, D. Avrahami and Y. Shai, *Proc. Natl. Acad. Sci. U. S. A.*, 2006, **103**, 15997; f) V. Sambhy, B. R. Peterson and A. Sen, *Angew. Chem. Int. Ed.*, 2008, **47**, 1250; g) N. P. Chongsiriwatana, J. A. Patch, A. M. Czyzewski, M. T. Dohm, A. Ivankin, D. Gidalevitz, R. N. Zuckermann and A. E. Barron, *Proc. Natl. Acad. Sci.U. S. A.*, 2008, **105**, 2794; h) F. Nederberg, Y. Zhang, J. P. K. Tan, K. J. Xu, H. Y. Wang, C. Yang, S. J. Gao, X. D. Guo, K. Fukushima, L. J. Li, J. L. Hedrick, Y. Y. Yang, *Nat. Chem.*, 2011, **3**, 409; i) Y. Niu, S. Padhee, H. Wu, G. Bai, L. Harrington, W. N. Burda, L. N. Shaw, C. Cao and J. Cai, *chem. commun.*, 2011, **47**, 12197; j) C. Monteiro, M. Fernandes, M. Pinheiro, S. Maiad , C. L. Seabra, F. Ferreira-da-Silva, F. Costa, S. Reis, P. Gomes and M. C. L. Martins, *Biochim. Biophys. Acta* 2015, **1848**, 1139; k) M. Stach, T. N. Siriwardena, T. Köhler, C. van Delden, Priv.-Doz. T. Darbre, and J.-L. Reymond, *Angew. Chem. Int. Ed.* 2014, **53**, 12827.

6. a) C. M. Goodman, S. Choi, S. Shandler and W. F. DeGrado, *Nat. Chem. Biol.*, 2007, **3** 252; b) N. P. Chongsiriwatana, T. M. Miller, M. Wetzler, S. Vakulenko, A. J. Karlsson, S. P. Palecek, S. Mobashery and A. E. Barron, *Antimicrob. Agents Chemother.*, 2011, **55**, 417.

7. A. K. Marr, W. J. Gooderham and R. E. W. Hancock, *Curr. Opin. Pharmacol.*, 2006, **6**, 468.

8. a) J. Hoque, P. Akkapeddi, V. Yarlagadda, D. S. S. M. Uppu, P. Kumar and J. Haldar, *Langmuir*, 2012, **28**, 12225; b) V. Yarlagadda, P. Akkapeddi, G. B. Manjunath and J. Haldar, *J. Med. Chem.*, 2014, **57**, 4558; c) J. Hoque, P. Akkapeddi, V. Yadav, G. B. Manjunath, D. S. S. M. Uppu, M. M. Konai, V. Yarlagadda, K. Sanyal and J. Haldar, *ACS Appl. Mater. Interfaces*, 2015, **7**, 1804.