Med Chem Res (2011) 20:1068–1073
DOI 10.1007/s00044-010-9432-0

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis of novel porphyrin-based lipids and their antibacterial activity

Amarnath Velidandi · Kranthi Kumar Gadidasu · V. Srilakshmi Patri

Received: 12 May 2010 / Accepted: 13 September 2010 / Published online: 6 October 2010

Abstract The purpose of this study was to investigate the antibacterial activity of newly developed cationic amphiphilic lipids having porphyrin as head group against two types of gram-positive bacteria and three types of gram-negative bacteria. The antibacterial activity of quantitatively prepared porphyrin-based amphiphilic lipids is evaluated by the disc-diffusion method against *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae and Proteus vulgaris.* These results are compared with the standard antibacterial activity of chloramphenicol. Both lipids showed antibacterial behaviour against gram-positive and gram-negative bacteria.

Keywords Porphyrin · Cationic lipid · Antibiotic · Antibacterial activity

Abbreviations

DCM	Dichloromethane
DDQ	2,3-Dichloro-5,6-dicyano-1,4-benzoquinone
ROS	Reactive oxygen species
S.A	*Staphylococcus aureus*
E.C	*Escherichia coli*
P.V	*Proteus vulgaris*
K.P	*Klebsiella pneumoniae*
B.S	*Bacillus subtilis*
MPs	Metalloporphyrins

A. Velidandi · V. S. Patri (✉)
Department of Chemistry, National Institute of Technology Warangal, Warangal 506004, Andhra Pradesh, India
e-mail: pv_slakshmi@yahoo.com

K. K. Gadidasu
Department of Biotechnology, Kakatiya University, Warangal 506009, Andhra Pradesh, India

Introduction

The growth of infectious diseases has become exponential in the recent past. The problem tends to be more complex because of the increase of bacterial resistance (Boyce, 1990) against the broadly used agents. Taking into account a great number of infections resulting from different bacterial species and fungi, the development of compounds with high antibacterial, antifungal activities with novel mechanism of action is an urgent need. Natural and synthetic porphyrins have relatively low toxicity in vitro and in vivo. Porphyrins are capable of effecting microbial and viral pathogens through the large number of different mechanisms. In addition, their ability for numerous chemical modifications place porphyrins into a group of compounds that present an outstanding source for discovery of novel procedures, materials and agents active against a wide range of pathogenic microorganisms (Stojiljkovic *et al*., 2001).

Antimicrobial and antiviral activities of porphyrins are based on their ability to catalyse peroxidase–oxidase reactions, generate reactive oxygen species (ROS) by absorbing photons and partition into lipids of bacterial membranes. Light-dependent, photodynamic activity of natural and synthetic porphyrins (Mariusz *et al*., 2007; Lasocki *et al*., 1999; Lambrechts *et al*., 2005) against gram-positive and gram-negative bacteria has been well demonstrated. Some non-iron metalloporphyrins (MPs) (Ghazaryan *et al*., 2008; Stojiljkovic *et al*., 1999; Xu *et al*., 2008) possess a powerful light-independent antimicrobial activity that is based on the ability of these compounds to increase the sensitivity of bacteria to ROS or directly produce ROS. MPs mimic haem

in their molecular structure and are actively accumulated by bacteria via high affinity haem-uptake systems. The same uptake systems can be used to deliver antibiotic-porphyrin and antibacterial peptide–porphyrin conjugates. Haemin (Everse and Hsia, 1997; Stojiljkovik and Hantke, 1994), the most well-known natural porphyrin, possesses a significant antibacterial activity that is augmented by the presence of physiological concentrations of hydrogen peroxide or a reducing agent.

The antibiotics must bind to the receptor located within the cell and must be taken up by the bacterial cells to exhibit the antibacterial activity. The binding capacity of porphyrins to eukaryotic cells is dependent on their hydrophobicity/hydrophilicity accountable to their side chains on the tetra-pyrrole ring, as well as to the structure of the cellular targets such as membranes and proteins (Moan *et al*., 1989). The cell wall of bacterial cells creates a physical and chemical barrier that may prevent sensitizer binding to bacterial membrane or proteins. One widely postulated hypothesis is that the positively charged peptides interact with the negatively charged outer leaflet of the cytoplasmic membranes (of both gram-negative and gram-positive bacteria) and disrupt the membranes via either a "barrel-stave" or a "carpet" mechanism (Bechinger, 1999; Oren and Shai, 1998; Shai, 1999). Over the years, cationic lipids (Felgner *et al*., 1994; Gao and Hui 2001; Ghosh *et al*., 2002; Jeremy *et al*., 2009; Mahidhar *et al*., 2004) have been developed to facilitate transport through membranes. The cationic lipids formulated as cationic liposomes have found applications as drug delivery systems against diseases (Banerjee *et al*., 1999; Srilakshmi *et al*., 2002; Sen and Chaudhuri, 2005) and gene transfection (Kumar *et al*., 2003; Rajesh *et al*., 2009). In addition, cationic lipids, including dodine (Cebral, 1992), benzalkonium chlorides (Jono *et al*., 1986), sphingosine (Drake *et al*., 2008 and fatty amines (Kitahara *et al*., 2004), chlorohexidine (Cookson *et al*., 1991), cationic polymers (Kügler *et al*., 2005) and other cationic amphiphiles (Lambert and Smith, 1977; Tomlinson *et al*., 1977) are known to exhibit broad spectrum antibacterial activities. Several mechanisms have been proposed (Davis, 1987; Hancock, 1981; Mingeot-Leclercq *et al*., 1999; Moazed *et al*., 1987; Shaw *et al*., 1993), including disruption of the bacterial envelope induced by removal of divalent positively charged counter ions by ammonium ions (Kügler *et al*., 2005). Moreover, coadministration of antibiotics with lipid bilayer permeabilizing agents including ionic lipids has been shown to induce synergistic effects resulting in enhanced antimicrobial activity (Shelburne *et al*., 2004). To this end, we developed and synthesized a series of porphyrin-based lipids which are having antibiotic activity and tend to enhance the uptake into the bacterial cells.

Materials and methods

Experimental section

General procedures and materials

Mass spectral data were acquired by using a commercial LCQ ion trap mass spectrometer (ThermoFinnigan, San Jose, CA, USA) equipped with an ESI source. ^{1}H NMR spectra were recorded on a Varian FT 300 MHz NMR Spectrometer. All the starting materials were obtained from Aldrich or Fluka and used as received. The progress of the reaction was monitored by thin-layer chromatography using 0.25-mm silica gel plates. Column chromatography was performed with silica gel (Acme Synthetic Chemicals, India; finer than 200 and 60–120 mesh).

Antibacterial activity

The antibacterial cell susceptibility testing was performed by agar disc-diffusion technique according to Bauer *et al*. (1966) against two gram-positive bacteria, *Staphylococcus aureus*, S.A (MTCC-96) and *Bacillus subtilis*, B.S (MTCC-619) and three gram-negative bacteria, *Escherichia coli*, E.C (MTCC-722), *Klebsiella pneumoniae*, K.P (MTCC-109) and *Proteus vulgaris*, P.V (MTCC-1771). Pure cultures of the organisms were obtained from Department of Biotechnology, Kakatiya University, India. A standard inoculum, 1–2 × 107 cfu/ml 0.5 Mc Farland standards (Stemper and Matsen, 1970) was introduced onto the surface of sterile Nutrient agar plate and evenly distributed by using a sterile glass spreader. Sterilized antibiotic discs (6 mm in diameter, prepared using Whatmann No. 1 paper) were placed over the medium. To find out antibacterial activity 75 μg of the compounds (initially dissolved in chloroform) were transferred to each disc with the help of a micropipette, simultaneously maintaining chloramphenicol (30 μg) disc as a standard. After overnight incubation at 37°C the diameters of zones of growth inhibition in mm were measured with a ruler and compared with standard antibiotic. Control measurements were carried out with chloroform. All the experiments were performed in triplicates and the average zones of inhibition was recorded.

*Synthesis of Lipid **1** and **2***

Synthesis of 2-N,N′-dihexadecylamino-ethanol To a solution of 2-aminoethanol (0.5 g, 8.1 mmol) in 50 ml of ethylacetate, 1-bromohexadecane (7.5 g, 24.5 mmol) and potassium carbonate (1.38 g, 8.1 mmol) were added and refluxed it for 48 h. The solvent was evaporated under reduced pressure and the residue was taken in chloroform and washed with water 2 × 50 ml. The organic layer was

separated and dried over anhydrous sodium sulphate, filtered and evaporated under reduced pressure to obtain 7.2 g (yield 57.55% $R_f = 0.4$, 50:50 ethylacetate:hexane (v/v)) of 2-dihexadecylamino-ethanol.

Synthesis of 2-N,N′-dihexadecylamino-acetaldehyde To a solution of pyridinium chloro chromate (3.9 g, 18.2 mmol) dissolved in 50 ml of anhydrous DCM added in portions a solution of 2-dihexadecylamino-ethanol (6.2 g, 12 mmol) in 10 ml of anhydrous DCM and stirred for one and half hour. To the reaction mixture 50 ml of dry ether was added and the supernatant solution decanted from black gum. The residue was extracted thoroughly with 3 × 50 ml of anhydrous ether and the combined organic solvents were dried over anhydrous sodium sulphate and the solvent removed by evaporation under reduced pressure to obtain 4 g (yield 64.8% $R_f = 0.5$, 50% ethylacetate in hexane) of dihexadecylamino-acetaldehyde.

*Synthesis of N,N′-dihexadecyl-porphyrin-5″-ylmethyl-amine (Lipid **1**)* A solution of pyrrole (1.05 g, 15.7 mmol), formaldehyde (0.35 g, 11.8 mmol) and dihexadecylamino-acetaldehyde (2.1 g, 3.94 mmol) in anhydrous DCM was purged with nitrogen gas for 15 min and to this anhydride zinc chloride is added. The reaction mixture was stirred for 1 h at room temperature followed by filtration to separate the catalyst. To the filtrate DDQ (0.895 g, 3.94 mmol) was added and the mixture was refluxed for an additional 1 h. Solvent was removed under reduced pressure. The product obtained was recrystallized from chloroform/methanol (85:15) followed by flash chromatography using ethyl acetate:hexane as eluent to obtain 0.26 g (yield 8% $R_f = 0.5$, 50% ethylacetate in hexane) of dihexadecyl-porphyrin-5-ylmethyl-amine. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.96 (t, terminal-CH_3 × 2, 6H, 16 and 16′), 1.33 (m, (–CH_2–)$_{14}$ × 2, 56H, 2–15 and 2′–15′), 2.1 (t, (–CH_2–N–CH_2–), 4H, 1 and 1′), 3.3 (s, =C–CH_2–N, 2H, 21″), 4.3 (s, =CH– × 3, 3H, 10″,15″ and 20″), 5.0 (s, pyrrole-NH– × 2, 2H), 7.3 (s, pyrrole –CH=CH– × 4, 8H, 2″, 3″, 7″, 8″, 12″, 13″, 17″, 18″). ESI–MS: *m/z* $M^+[C_{53}H_{81}N_5]^+$, calcd 787.65, found 789. Anal. Calcd for $C_{53}H_{81}N_5$: C, 80.76; H, 10.36; N, 8.88. Found: C, 80.66; H, 10.48; N, 8.86.

Synthesis of octadecylaldehyde To a solution of pyridinium chloro chromate (4.7 g, 2.1 mmol) dissolved in 200 ml of anhydrous DCM, a solution of steryl alcohol (4 g, 1.4 mmol) in 20 ml of anhydrous DCM is added in one portion and stirred the mixture for one and half an hour. To this mixture 200 ml of dry ether is added and the supernatant solution decanted from block gum. The residue was extracted thoroughly with 3 × 50 ml of anhydrous ether and the combined organic solvents were dried over anhydrous sodium sulphate and the solvent removed by evaporation under reduced pressure to obtain 4.22 g (yield 72% $R_f = 0.5$, 50% ethylacetate in hexane) of octadecylaldehyde.

*Synthesis of 5″,20″-diheptadecyl-porphyrin (Lipid **2**)* The preparation is same as described in the "Synthesis of *N,N′*-dihexadecyl-porphyrin-5″-ylmethyl-amine (Lipid **1**)" section, except that instead of dihexadecylamino-acetaldehdye, octadecylaldehyde is used here. The product obtained was recrystallized from chloroform/methanol (85:15) followed by flash chromatography using ethyl acetate:hexane as eluent, distillation to obtain 0.86 g (yield 7% $R_f = 0.5$, 50% ethylacetate in hexane) of 5,20-diheptadecyl-porphyrin. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.96 (t, terminal-CH_3 × 2, 6H, 17 and 17′), 1.33 (m, (–CH_2–)$_{15}$ × 2, 60H, 2–16 and 2′–16′), 1.9 (t, = C–CH_2 × 2, 4H, 1 and 1′), 4.3 (s, = CH– × 2, 2H, 10″ and 15″), 5.0 (s, pyrrole-NH– × 2, 2H), 7.3 (s, pyrrole CH = CH × 4, 8H, 2″, 3″, 7″, 8″, 12″, 13″, 17″ and 18″). ESI–MS: *m/z* $M^+[C_{53}H_{81}N_5]^+$, calcd 786.65, found 786. Anal. Calcd for $C_{54}H_{82}N_4$: C, 82.38; H, 10.50; N, 7.12. Found: C, 82.35; H, 10.40; N, 7.25.

Results and discussion

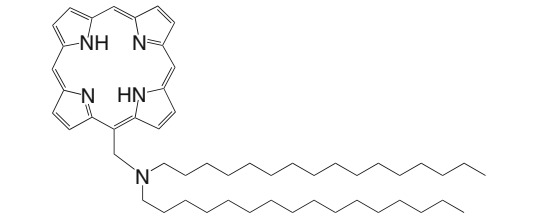
Synthesis of lipids

The key structural elements common to the porphyrin-based lipids **1** and **2** described herein include (a) the presence of hydrophobic chains as anchoring groups (i) linked to the positively charged nitrogen atom directly which in turn attached to the porphyrin group through a spacer or (ii) linked directly to the porphyrin group (b) the presence of porphyrin group which is having positively charged nitrogen atom. The details of the synthetic procedures for the novel porphyrin-based lipids **1** and **2** shown in Chart 1 are described in the "Experimental section". As outlined in Schemes 1 and 2, the chemistry involved in preparing these new lipids are straightforward. However, given their antibacterial activity, the overall yields of these lipids need to be improved in future. Scheme 1 outlines the general synthetic strategies adopted for preparing lipid **1**. The steps involved were (a) reacting hexadecyl bromide with ethanol amine to produce an intermediate tertiary amine alcohol. (b) Oxidizing the intermediate tertiary amine alcohol to aldehyde of tertiary amine using pyridinium chloro chromate. (c) The tertiary amine aldehyde intermediate is treated with formaldehyde and pyrrole in presence of acid catalyst to get the lipid **1**. Synthesis of lipid **2** essentially consists of similar steps as in synthesis of lipid **1** except that instead of reacting with tertiary amine

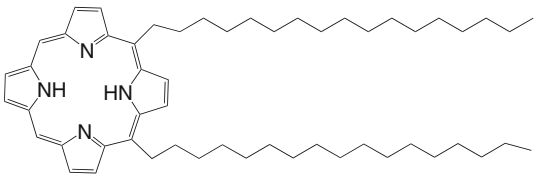
aldehyde directly taken the aliphatic aldehyde and treated with formaldehyde and pyrrole in presence of acid catalyst as in Scheme 2 to get lipid **2**.

Antibacterial activity

It is well known that porphyrins possess significant antibacterial activity. Cationic lipids are very well known as delivery systems and also they increase the uptake of the antibiotic into the bacterial cell. The essential attributes for an antibiotic to have efficient antibacterial activity are (i) possess significant antibacterial activity and (ii) uptake by the bacterial cell. To incorporate these attributes in a single molecule we developed a series of porphyrin-based cationic lipids containing two units (i) porphyrin as the head group which is responsible for the antibacterial activity and (ii) anchoring groups impart lipid like structure to the molecule, (iii) the cationic charge to enhance the uptake by the bacterial cell.



Lipid 1

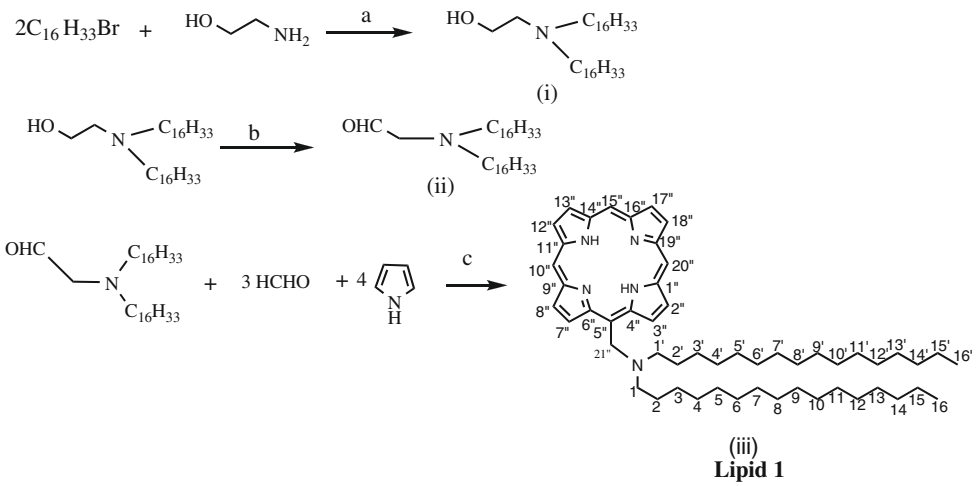


Lipid 2

Chart 1 Structures of new antibiotics

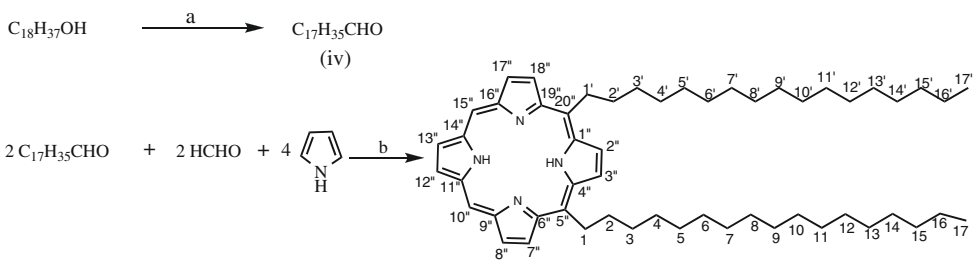
Here, we are reporting the antibacterial activities of porphyrin-based lipids **1** and **2** (shown in Chart 1) compared with those of chloramphenicol against gram-positive bacteria *Staphylococcus aureus*, Sa (MTCC-96) and *Bacillus subtilis*, BS (MTCC-619) and gram-negative bacteria, *Escherichia coli*, E.C (MTCC-722), *Klebsiella pneumoniae*, K.P (MTCC-109) and *Proteus vulgaris*, P.V (MTCC-1771). The results are shown in Table 1. Both the lipids **1** and **2** showed antibacterial behaviour against *Staphylococcus aureus, Bacillus subtilis* and *Escherichia coli*, whereas these lipids showed less antibacterial activity against *Klebsiella pneumoniae*. The results indicated that the Lipid **1** is inhibiting the growth of bacteria slightly better than Lipid **2.** The better activity of lipid **1** may be due to the additional positively charged nitrogen linked to

Scheme 1 Synthesis of lipid **1**. Reagents: (*a*) K_2CO_3, ethyl acetate, 48 h, reflux; (*b*) pyridinium chloro chromate, anhydrous DCM, 1.5 h, r.t.; (*c*) $ZnCl_2$, 2,3-dichloro-5,6-dicyano-1,4-benzoquinone, anhydrous DCM, 2.5 h, reflux



(iii)
Lipid 1

Scheme 2 Synthesis of lipid **2**. Reagents: (*a*) pyridinium chloro chromate, anhydrous DCM, 1.5 h , r.t.; (*b*) $ZnCl_2$, 2,3-dichloro-5,6-dicyano-1,4-benzoquinone, anhydrous DCM, 2.5 h, reflux



(v)
Lipid 2

Table 1 Antibiotic susceptibility

S. no.	Antibiotics	E.C	SA	P.V	K.P	B.S
1	Lipid **1**	14	15	14	12	16
2	Lipid **2**	13	13	12	10	14
3	Chloramphenicol	31	23	14	24	23

For abbreviations used see "Experimental section". The concentration of lipids is 75 μg/ml and the concentration of chloramphenicol is 30 μg/ml. The resistance pattern was determined with the disc-diffusion method (numbers present the diameters of zones of growth inhibition in mm)

the porphyrin group through a methylene spacer in its structure. Based on the above evidences we can conclude that the cationic lipids containing porphyrin as head group and long chain hydrophobic chains as anchoring groups seems to be useful as antibacterial drugs. Though the activity of these lipids is little lower than the antibacterial activity of standard chloramphenicol, these lipids can be used as antibacterial drugs as lower dosage drugs. There is still a need of further structure activity relation studies on these porphyrin-based lipids.

Conclusions

In summary, we have developed and synthesized an efficient and novel series of porphyrin-based cationic lipids for use in antibacterial activity. The present synthetic protocols should, in principle, be applicable in synthesizing various porphyrin-based structural analogues of lipids **1** and **2** for use in antibacterial activity. The antibacterial activity of these new lipids were studied and found that these lipids are active against both gram-positive bacteria and gram-negative bacteria; however, these lipids are less active against bacteria *Klebsiella pneumoniae*.

Acknowledgment Financial supports for this work from Council of Scientific and Industrial Research, CSIR (to P. V. Srilakshmi.), Government of India, New Delhi (the doctoral research fellowship to V. Amarnath) is gratefully acknowledged. We sincerely acknowledge the experimental assistance for the studies of antibacterial activity received from G. Kranthi Kumar.

References

Banerjee RK, Das PK, Srilakshmi GV, Chaudhuri A, Rao NM (1999) A novel series of non-glycerol based cationic transfection lipids for use in liposomal gene delivery. J Med Chem 42:4292–4299

Bauer AW, Kirby WM, Sherris JC, Turck M (1966) Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol 45:493–496

Bechinger B (1999) The structure, dynamics and orientation of antimicrobial peptides in membranes by multidimensional solid-state NMR spectroscopy. Biochim Biophys Acta 1462:157–183

Boyce (1990) Increasing prevalence of methicillin-resistant *Staphylococcus aureus* in the United States. J M Infect Contro Hosp Epidemiol 11:639–642

Cebral JPS (1992) Mode of antibacterial action of dodine (dodecylguanidine monoacetate) in *Pseudomonas syringae*. Can J Microbiol 38:115–123

Cookson BD, Bolton MC, Platt JH (1991) Chlorhexidine resistance in methicillin-resistant *Staphylococcus aureus* or just an elevated MIC? An in vitro and in vivo assessment. Antimicrob Agents Chemother 35:1997–2002

Davis BD (1987) Mechanism of bacterial action of aminoglycosides. Microbiol Rev 51:341–350

Drake DR, Brogden KA, Dawson DV, Wertz PA (2008) Thematic review series: skin lipids. Antimicrobial lipids at the skin surface. J Lipid Res 49:4–11

Everse J, Hsia N (1997) The toxicities of native and modified hemoglobins. Fr Rad Biol Med 22:1075–1099

Felgner JH, Kumar R, Sridhar CN, Wheeler CJ, Tsai YJ, Border R, Ramsey P, Martin M, Felgner PL (1994) Enhanced gene delivery and mechanism studies with a novel series of cationic lipid formulations. J Biol Chem 269:2550–2561

Gao H, Hui KM (2001) Synthesis of a novel series of cationic lipids that can act as efficient gene delivery vehicles through systematic heterocyclic substitution of cholesterol derivatives. Gene Ther 8:855–863

Ghazaryan RK, Sahakyan LA, Tovmasyanl AG, Movsisyan LD, Hambardzumyan ADz (2008) Novel antimicrobial agents on the base of natural and synthetic metalloporphyrins. New Arm Med J 2:40–48

Ghosh YK, Viswesaraiah SS, Bhattacharya S (2002) Advantage of the ether linkage between the positive charge and the cholesteryl skeleton in cholesterol-based amphiphiles as vectors for gene delivery. Bioconjug Chem 13:378–384

Hancock EWR (1981) Aminoglycoside uptake and mode of action—with special reference to streptomycin and gentamicin: II. Effects of aminoglycosides on cells. J Antimicrob Chemother 8:429–445

Jeremy AB, Marquita MQ, Dorota I, Robert HH, Srilakshmi PV, Jong-Mok K, David HT (2009) Cytoplasmic delivery of liposomal contents mediated by an acid-labile cholesterol-vinyl ether-PEG conjugate. Bioconjug Chem 20:47–59

Jono K, Takayama T, Kuno M, Higashide E (1986) Effect of alkyl chain length of benzalkonium chloride on the bactericidal activity and binding to organic materials. Chem Pharm Bull 34:4215–4224

Kitahara T, Koyama N, Matsuda J, Aoyama Y, Hirakata Y, Kamishira S, Kohno S, Nakashima M, Sasaki H (2004) Antimicrobial activity of saturated fatty acids and fatty amines against methicillin-resistant *Staphylococcus aureus*. Biol Pharm Bull 27:1321–1326

Kügler R, Boulassa O, Rondelez F (2005) Evidence of a charge-density threshold for optimum efficiency of biocidal cationic surfaces. Microbiology 151:1341–1348

Kumar VV, Pichon C, Refregiers M, Guerin B, Midoux P, Chaudhuri A (2003) Non-viral vectors in cystic fibrosis gene therapy: progress and challenges. Gene Ther 10:1206–1215

Lambert PA, Smith ARW (1977) The mode of action of N-(n = Dodecyl)diethanolamine with particular reference to the effect of protonation on uptake by *Escherichia coli*. J Gen Microbiol 103:367–374

Lambrechts SAG, Demidova TN, Aalders MCG, Hasan T, Hamblin MR (2005) Photochem Photobiol Sci 4:503–509

Lasocki K, Szpakowska M, Grzybkowski J, Graczyk A (1999) Pharmacol Res 39:181–184

Mahidhar YV, Rajesh M, Chaudhuri A (2004) Spacer-arm modulated gene delivery efficacy of novel cationic glycolipids: design,

synthesis, and in vitro transfection biology. J Med Chem 47:3938–3948

Mariusz G, Bozena Katarzyna, Alfreda G (2007) Bactericidal effect of photo dynamic therapy against methicillin-resistant *Staphylococcus aureus* strain with the use of various porphyrin photosensitizers. Acta Biochim Pol 54:665–670

Mingeot-Leclercq MP, Glupczynski Y, Tulkens PM (1999) Aminoglycosides: activity and resistance. Antimicrob Agents Chemother 43:727–737

Moan J, Berg K, Kvam E, Western A, Malik Z, Ruck A, Snhneckenburger H (1989) Intracellular localization of photosensitizers. Photosensitizing compounds: their chemistry, biology and clinical use. Wiley, New York, pp 95–111

Moazed D, Noller HF (1987) Interaction of antibiotics with functional sites in 16S ribosomal RNA. Nature 327:389–394

Oren Z, Shai Y (1998) Mode of action of linear amphipathic alpha-helical antimicrobial peptides. Biopolymers 47:451–463

Rajesh M, Srujan M, Mahidhar YV, Gangamodi NV, Ramakrishna S, Chaudhuri A (2009) Cationic glycolipids with cyclic and open galactose head groups for the selective targeting of genes to mouse liver. Biomaterials 30:2369–2384

Sen J, Chaudhuri A (2005) Design, syntheses, and transfection biology of novel non-cholesterol-based guanidinylated cationic lipids. J Med Chem 48:812–820

Shai Y (1999) Mechanism of the binding, insertion and destabilization of phospholipid bilayer membranes by alpha-helical antimicrobial and cell nonselective membrane-lytic peptides. Biochim Biophys Acta 1462:55–70

Shaw KJ, Rather PN, Hare RS, Miller GH (1993) Molecular genetics of aminoglycoside resistance genes and familial relationships of the aminoglycoside-modifying enzymes. Micorbiol Rev 57: 138–163

Shelburne SA, Musher DM, Hulte K, Ceasar H, Lu YM, Bhaila I, Hamill JR (2004) In vitro killing of community-associated methicillin-resistant *Staphylococcus aureus* with drug combinations. Antimicrob Agents Chemother 48:4016–4019

Srilakshmi GV, Sen J, Chaudhuri A, Ramdas Y, RaO NM (2002) Anchor-dependent lipofection with non-glycerol based cytofectins containing single 2-hydroxyethyl head groups. Biochim Biophys Acta 1559:87–95

Stemper JE, Matsen JM (1970) Device for turbidity standardization of cultures for antibiotic sensitivity testing. Appl Microbiol 19: 1015–1016

Stojiljkovic I, Kumar V, Srinivasan N (1999) Non-iron metalloporphyrins: potent antibacterial compounds that exploit haem/Hb uptake systems of pathogenic bacteria. Mol Microbiol 31: 429–442

Stojiljkovic I, Evavold BD, Kumar V (2001) Antimicrobial properties of porphyrins. Expert Opin Investig Drug 10:309–320

Stojiljkovik K, Hantke K (1994) Transport of haemin across the cytoplasmic membrane through a haemin-specific periplasmic binding-protein-dependent transport system in *Yersinia enterocolitica*. Mol Microbiol 13:719–732

Tomlinson E, Brown MRW, Davies SS (1977) Effect of colloidal association on the measured activity of alkylbenzyldimethylammonium chlorides against *Pseudomonas aeruginosa*. J Med Chem 20:1277–1282

Xu X-J, Xue Z, Xiao Q, Hou AX, Liu Y (2008) Antibacterial activities of novel diselenide-bridged bis(porphyrin)s on *Staphylococcus aureus* investigated by microcalorimetry. Biol Trace Elem Res 125:185–192