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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis, characterization, antimicrobial and anti-biofilm activity of a new class of 11-bromoundecanoic acid-based betaines

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Abstract Novel betaines were synthesized from esterquats, which in turn were obtained from the reaction of 11-bromo undecanoic acid, different alkyl amines, and methyl iodide. The synthesized betaines were characterized by fourier transform infrared, proton nuclear magnetic resonance, carbon-13 nuclear magnetic resonance, and mass spectral analysis. These betaines were synthesized in four steps; in the first step, 11-bromo undecanoic acid was converted into methyl 11-bromoundecanoate followed by the synthesis of secondary amine monoester, and tertiary amine mono and diesters by the reaction of 11-bromoundecanoate with different aliphatic amines (hexyl, dodecyl, octadecyl, dioctyl, and dicyclohexyl amine). In the third step, the prepared secondary amine monoesters, tertiary amine mono, and diesters were converted into monoesterquats and diesterquats by reacting with methyl iodide. The resultant esterquats were converted into betaines by saponification reaction using $LiOH.H_2O$ in water and tetrahydrofuran. The synthesized compounds (**5a–h**) were studied for their antimicrobial activity. Some of the compounds showed good to moderate antibacterial activity with minimum inhibitory concentration values ranging between 3.9–31.2 μg mL^{-1} and antifungal activity with minimum inhibitory concentration values ranging between 7.8–62.4 μg mL^{-1}. Further, some of the betaines also showed good anti-biofilm activity with IC_{50} values ranging between 2.1–25.3 μg mL^{-1} on the tested pathogenic microbial and fungal strains.

Keywords 11-Bromoundecanoic acid · Esterquats · Betaines · Antimicrobial activity · Anti-biofilm activity

Introduction

Betaines are the quaternary ammonium-derived significant zwitterionic compounds having diverse applications in medicine, pharmacy, and biology due to their biocidal properties (Baaman 1978; Lindstedt et al. 1990). The betaines are extensively used as antimicrobial (Birnie et al. 2000; Peddie et al. 2003) and anti-biofilm agents in toothpaste formulations for the treatment of oral diseases to control dental plaque without producing an esthetically unacceptable discoloration of tooth surfaces since they have zwitterionic structure with a non-exchangeable anion with chemical structure of R_1 R_2 R_3 $N^+(CH_2)_n$ COO^- (R_1 = chain length C_{12} to C_{18}, R_2, and R_3 = methyl group, $n = 7$ to 15) (Robert et al. 1988; Kowalczyk 2008). Peddie and coworkers reported the synthesis and antimicrobial activity of aromatic and semi-aromatic betaines (Peddie et al. 2003). Similarly, Cosquer and coworkers studied the antibacterial activity of glycine betaines on different types of pathogenic Gram-positive and Gram-negative bacterial strains, and observed that some of the compounds showed good antibacterial activity (Cosquer et al. 2004). Several authors also reported the effective osmoprotectant property of amino

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acid based betaines like glycine and proline betaines for bacteria (Abdel-Ghany et al. 1993; Amin et al. 1995).

Betaines show unique amphoteric surfactant properties since they contain a hydrophobic carbon chain and find applications mostly in the toiletries and personal care products niche areas (Kumar et al. 2007; Quan et al. 2012; Domingo 1990; Zhou et al. 2016). Further, many of these compounds display interesting physical properties, exhibiting phase transitions with ferroelectric, antiferroelectric, and ferroelastic behavior as well as phases with commensurate and incommensurate superstructures (Schaack 1990). Betaines play a significant role in various living systems as osmoprotectors, osmoregulators and methyl transfer agents. Complexes of these bases can be important as model systems due to the role of the hydrogen bonds in many biologically important reactions (Godzisz et al. 2002). The present study describes the synthesis of novel betaines by employing 11-bromoundecanoic acid, various aliphatic amines and methyl iodide in new synthetic approach. The synthesized betaines were characterized and were further studied for their antimicrobial and anti-biofilm activities.

Materials and methods

Materials

The raw materials needed for the synthesis of betaines, such as 11-bromoundecanoic acid, hexyl, dodecyl, octadecyl, dioctyl, and dicyclohexyl amines were procured from Sigma-Aldrich Chemicals (USA). Anhydrous potassium carbonate (K_2CO_3), *p*-toluenesulfonic acid (PTSA), dimethylformamide (DMF) and other solvents were obtained from S.D. Fine Chemicals, Mumbai (India). All solvents and chemicals were of reagent grade and were used directly without further purification. Silica gel (60–120 mesh) for column chromatography was bought from Acme Synthetic Chemicals, Mumbai, India. Precoated thin-layer chromatography (TLC) plates were purchased from Merck, Darmstadt, Germany. All microbial strains were procured from Microbial Type Culture Collection (MTCC) and Gene Bank, CSIR-Institute of Microbial Technology, Chandigarh, India.

Analytical methods

IR spectra were recorded on a Perkin-Elmer (Model: Spectrum BX) FT-IR spectrometer using $CHCl_3$ and KBr. All proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded on 300 MHz (Brucker) and 500 MHz (Varian) spectrometers, respectively. All carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectra were recorded on 125 MHz (Brucker) spectrometer. ESI-MS spectra were recorded on Waters (Model: Q-STAR XL, Applied Biosystems, USA) Mass Spectrometer equipped with an Electrospray Ionization source. HRMS data were recorded on a Thermo Scientific Exactive Orbitrap mass spectrometer (Germany) and are given in mass units (*m*/*z*).

Synthesis of methyl 11-(alkylamino) undecanoate (3a–c)

Initially, 11-bromoundecanoic acid (**1**) was converted into 11-bromoundecanoate (11-BUME) (**2**) by reacting with methanol in presence of PTSA. Later, 11-BUME (1.0 eq) and amine (hexyl, dodecyl, and octadecyl amine; 1.1 eq) were dissolved in DMF solvent and stirred for 10 min followed by the addition of anhydrous K_2CO_3 (2 eqs to 11-BUME), and the reaction was continued at 90–110 °C for 12 h. The progress of the reaction was monitored by TLC using $CHCl_3$ and methanol (90:10 v/v) as a solvent system. After completion of the reaction, the catalyst K_2CO_3 was filtered from the crude reaction product and extracted with ethyl acetate followed by washing with water to remove the DMF solvent. The crude reaction product was dried by passing through anhydrous sodium sulfate and the ethyl acetate solvent was removed under reduced pressure by using rotary evaporator. Pure methyl 11-(alkylamino) undecanoate was separated out from the dried crude reaction product by column chromatography using silica gel (60–120 mesh) with chloroform and methanol (98:2, v/v) as eluent. Column chromatography was monitored by TLC using chloroform and methanol (9:1, v/v) solvent system and the product was identified by iodine vapor. The detailed characterization of methyl 11-(alkylamino) undecanoates (**3a–c**) has been reported by us previously (Yasa et al. 2016). The isolated yields were in the range of 85.0–90.0%.

Synthesis of dimethyl 11,11′-(alkylazanediyl) diundecanoate (3d–f)

11-BUME (1 eq) and amine (0.55 eq) were dissolved in DMF solvent and stirred for 10 min followed by the addition of K_2CO_3, base catalyst (2 eqs to 11-BUME), and the reaction continued at 90–110 °C for 12 h. After completion of the reaction, the catalyst K_2CO_3 was filtered from crude reaction product and extracted with ethyl acetate followed by washing with water to remove DMF solvent. Pure dimethyl 11,11′-(alkylazanediyl)diundecanoate was separated out from the crude product by column chromatography using silica gel (60–120 mesh) with chloroform and methanol (99:1, v/v) as eluent. The detailed characterization of dimethyl 11,11′-(alkylazanediyl) diundecanoates (**3d–f**) has been reported by us previously (Yasa et al. 2016). The isolated yields were in the range of 88.0–91.5%.

Synthesis of methyl 11-(dialkylamino) undecanoate (3g–h)

11-BUME (1.0 eq) and dialkylamine (1.1 eq) were dissolved in DMF solvent and stirred for 10 min followed by the addition of K_2CO_3 (2 eqs to 11-BUME) at room temperature and continued the reaction at 90–110 °C for 12 h. After completion of the reaction, the catalyst K_2CO_3 was filtered from crude reaction product and extracted with ethyl acetate followed by washing with water to remove DMF solvent. The crude reaction product was dried by passing through anhydrous sodium sulfate and the ethyl acetate solvent was removed under reduced pressure by using rotary evaporator. The pure methyl 11-(dialkylamino) undecanoate was separated out from the crude reaction product by silica gel (60–120 mesh) column chromatography using chloroform and methanol (98:2, v/v) as eluent. Column chromatography was monitored by TLC using chloroform and methanol (9:1, v/v) solvent system and the product was identified by iodine vapor. The detailed characterization of methyl 11-(dialkylamino) undecanoates (**3g** and **3h**) was reported by us previously (Yasa et al. 2016). Isolated yields were in the range of 95.0–96.0%.

Synthesis of esterquats (4a–h)

The synthesized secondary amine monoesters (**3a–c**), tertiary amine monoesters (**3g–h**) and diesters (**3d–f**) were converted into esterquats (**4a–h**) by reacting with methyl iodide using the following reported procedure (Srinivas et al. 2012). Amine ester (1 g) was dissolved in 2 mL chloroform and 5 mL methyl iodide was added to the solution followed by the addition of catalytic amount of K_2CO_3. The reaction mixture was stirred at room temperature for 12 h and the solvent and excess methyl iodide were removed under reduced pressure by using rotary evaporator. The crude product was purified on silica gel (60–120 mesh) column chromatography using 2.5–3.0% methanol in chloroform (v/v) as eluent to afford the pure esterquats (**4a–h**) in good yields (95.0–98.5%).

Synthesis of betaines (5a–h)

The esterquat (**4a–h**) (1 g) was dissolved in 10 mL of tetrahydrofuran (THF): H_2O (1:1, v/v) and added 3 eqs of $LiOH.H_2O$. The reaction mixture was stirred at room temperature for 12 h. After completion of the reaction, the THF solvent was removed under reduced pressure using rota evaporator at 40 °C followed by neutralization using 1 N HCl and the crude reaction mixture was extracted using ethyl acetate and washed with water to remove acid traces. The organic layer was dried over anhydrous sodium sulfate and concentrated on rotary evaporator. The pure product was separated by silica gel column chromatography using a solvent using 3.0% methanol in chloroform (v/v) as eluent. A 3% solution of the above silica gel column purified carboxylic acid quaternary compound with iodide counterion form in methanol was passed through a column of anion exchange resin in hydroxide form (Dowex 1-X8) to get pure betaine containing hydroxide counter ion. The betaines **5c** and **5f** were recovered from the residue by recrystallization using water, acetonitrile and aqueous acetone; whereas the rest of the compounds were not recrystallized since they were in liquid form. All the compounds **5a–h** were characterized by spectral techniques and the obtained yields were in the range of 97.5–99.0%.

Characterization of betaines (5a–h)

*11-(hexyldimethylammonio) undecanoate (**5a**)*

Light brown liquid, yield 98.0%; IR (Neat, cm^{-1}): 3424, 2925, 2855, 1727, 1463, 1184; ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 3.38–3.25 (4H, m, $-\underline{H}_2C-N^+-C\underline{H}_2-$), 3.11 (6H, s, $-N^+-(C\underline{H}_3)_2$), 2.24 (2H, t, $J = 7.15$ Hz, $-\underline{H}_2C-C=O$), 1.79–1.64 (4H, m), 1.62–1.48 (2H, m), 1.43–1.22 (18H, m), 0.91 (3 H, t, $J = 6.60$ Hz); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 174.73 ($-\underline{C}=O$), 63.34 ($-\underline{H}_2C-N^+-\underline{C}H_2-$), 50.14 ($-N^+-(\underline{C}H_3)_2$), 33.60 ($-\underline{H}_2C-C=O$), 30.58, 28.64, 28.42, 25.67, 25.33, 24.33, 21.82, 13.54 ($-\underline{C}H_3$); ESI-MS (*m/z*) $(M + H)^+$ 314.44; HRMS-ESI (*m/z*) calculated for $(C_{19}H_{40}NO_2)^+$, $(M + H)^+$ is 314.3054, found at 314.3055.

*11-(dodecyldimethylammonio) undecanoate (**5b**)*

Light brown semi-solid, yield 98.0%; IR (Neat, cm^{-1}): 3423, 2923, 2851, 1718, 1467, 1180; ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 3.36–3.19 (4H, m, $-\underline{H}_2C-N^+-C\underline{H}_2-$), 3.09 (6H, s, $-N^+-(C\underline{H}_3)_2$), 2.21 (2H, t, $J = 7.15$ Hz, $-\underline{H}_2C-C=O$), 1.78–1.62 (2H, m), 1.61–1.40 (4H, m), 1.39–1.14 (30H, m), 0.87 (3H, t, $J = 6.60$ Hz); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 174.63 ($-\underline{C}=O$), 63.05 ($-\underline{H}_2C-N^+-\underline{C}H_2-$), 50.20 ($-N^+-(\underline{C}H_3)_2$), 37.21 ($-\underline{H}_2C-C=O$), 31.40, 29.11, 28.81 X 2, 28.63, 28.56, 26.45, 25.83, 24.56, 22.19, 21.81, 13.98; ESI-MS (*m/z*) $(M + H)^+$ 398.31, $(M + Na)^+$ 420.33; HRMS-ESI (*m/z*) calculated for $(C_{25}H_{52}NO_2)^+$, $(M + H)^+$ is 398.3993, found at 398.3999.

*11-(octadecyldimethylammonio) undecanoate (**5c**)*

Light brown solid, yield 97.5%, melting point 158.2–158.4 °C; IR (KBr, cm^{-1}): 3422, 2919, 2850, 1711, 1468, 1181; ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 3.38–3.17 (4H, m, $-\underline{H}_2C-N^+-C\underline{H}_2-$), 3.10 (6H, s, $-N^+-(C\underline{H}_3)_2$), 2.23 (2H, t, $J = 7.15$ Hz, $-\underline{H}_2C-C=O$), 1.79–1.61(2H, m), 1.60–1.41 (4H, m), 1.40–1.10 (30H, m), 0.87 (3H, t, $J = 6.60$ Hz); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 174.56 ($-\underline{C}=O$), 63.02 ($-\underline{H}_2C-N^+-$

$\underline{C}H_2$–), 50.24 (–N^+–($\underline{C}H_3)_2$), 37.15 (–$H_2\underline{C}$–C=O), 31.51, 29.15 X 2, 28.84 X 2, 28.63 X 2, 28.56, 26.49 X 2, 25.92, 24.62, 22.27, 21.85, 13.99; ESI-MS (*m/z*) $(M + H)^+$ 482.71, $(M + Na)^+$ 504.66; HRMS-ESI (*m/z*) calculated for $(C_{31}H_{64}NO_2)^+$, $(M + H)^+$ is 482.4932, found at 482.4913.

*11,11′-(hexyl(methyl)ammonio)diundecanoate (**5d**)*

Light brown gummy liquid, yield 99.0%; IR (Neat, cm^{-1}): 3423, 2925, 2857, 1714, 1468, 1167; ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 3.27 (6H, br.t, $J = 7.7$ Hz, –N^+($\underline{C}H_2)_3$), 3.06 (3H, s, –N–$\underline{C}H_3$), 2.24 (2H, t, $J = 7.30$ Hz, –$\underline{H}_2$C–C=O), 1.76–1.64 (4H, m), 1.63–1.49 (6H, m), 1.43–1.22 (30H, m), 0.88 (3H, t, $J = 6.8$ Hz); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 174.83 (–$\underline{C}$=O), 61.05 (–N^+($\underline{C}H_2)_3$), 47.85 (–N–$\underline{C}H_3$), 33,68 (–$H_2\underline{C}$–C=O), 30.62, 28.99, 28.67, 28.50, 25,78, 25.46, 24.38, 21.87, 21.63, 13.56; ESI-MS (*m/z*) $(M + 2H)^+$ 484.40; HRMS-ESI (*m/z*) calculated for $(C_{29}H_{58}NO_4)^+$ is 484.4360, found at 484.4368.

*11,11′-(dodecyl(methyl)ammonio)diundecanoate (**5e**)*

Light brown gammy liquid, yield 98.5%; IR (Neat, cm^{-1}): 3421, 2926, 2855, 1710, 1461, 1164; ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 3.42–3.30 (6H, m, –N^+($\underline{C}H_2)_3$), 3.22 (3H, s, –N–$\underline{C}H_3$), 2.33 (2H, t, $J = 7.47$ Hz, –$\underline{H}_2$C–C=O), 1.77–1.67 (4H, m), 1.66–1.53 (6H, m), 1.45–1.14 (42H, m), 0.88 (3H, t, $J = 6.8$ Hz); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 175.19 (–$\underline{C}$=O), 61.15 (–N^+($\underline{C}H_2)_3$), 48.05 (–N–$\underline{C}H_3$), 33.83 (–$H_2\underline{C}$–C=O), 31.43, 29.15, 28.95, 29.04, 28.84, 28.79, 28.62, 25.89, 24.50, 22.22, 21.76, 13.85; ESI-MS (*m/z*) $(M + 2H)^+$ 568.69; HRMS-ESI (*m/z*) calculated for $(C_{35}H_{70}NO_4)^+$ is 568.5299, found at 568.5308.

*11,11′-(octadecyl(methyl)ammonio)diundecanoate (**5f**)*

Light brown solid, yield 99.40%, melting point 78.6–78.8 °C; IR (KBr, cm^{-1}): 3426, 2921, 2851, 1730, 1468, 1161; ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 3.45–3.27 (6H, m, -N^+($\underline{C}H_2)_3$), 3.18 (3H, s, –N–$\underline{C}H_3$), 2.31 (2H, t, $J = 7.47$ Hz, –$\underline{H}_2$C–C=O), 1.80–1.65 (4H, m), 1.64–1.54 (6H, m), 1.45–1.14 (54H, m), 0.88 (3H, t, $J = 7.0$ Hz); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 174.60 (–$\underline{C}$=O), 60.85 (–N^+($\underline{C}H_2)_3$), 47.73 (–N–$\underline{C}H_3$), 33.65 (–$H_2\underline{C}$–C=O), 31.27, 29.03, 28.89, 28.79, 28.69, 28.48, 25.75, 24.36, 22.05, 21.56, 13.73; ESI-MS (*m/z*) $(M + 2H)^+$ 652.73; HRMS-ESI (*m/z*) calculated for $(C_{41}H_{82}NO_4)^+$ is 652.6238, found at 652.6229.

*11-(dioctyl(methyl)ammonio)undecanoate (**5g**)*

Light brown liquid, yield 99.0%; IR (Neat, cm^{-1}): 3428, 2927, 2856, 1725, 1453, 1175; ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 3.37–3.21 (6H, m, –N^+($\underline{C}H_2)_3$), 3.07 (3H, s, –N–$\underline{C}H_3$), 2.23 (2H, t, $J = 7.43$ Hz, –$\underline{H}_2$C–C=O), 1.79–1.62 (6H, m), 1.61–1.50 (2H, m), 1.47–1.18 (32H, m), 0.89 (6H, t, $J = 6.60$ Hz); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 174.78 (–$\underline{C}$=O), 60.94 (–N^+($\underline{C}H_2)_3$), 47.84 (–N–$\underline{C}H_3$), 33.65 (–$H_2\underline{C}$–C=O), 31.05, 28.64, 28.42, 25.73, 24.33, 21.97, 21.59, 13.66; ESI-MS (*m/z*) $(M)^+$ 440.44, $(M + Na)^+$ 462.39; HRMS-ESI (*m/z*) calculated for $(C_{28}H_{58}NO_2)^+$, $(M + H)^+$ is 440.4462, found at 440.4458.

*11-(dicyclohexyl(methyl)ammonio)undecanoate (**5h**)*

Light brown liquid, yield 98.0%; IR (Neat, cm^{-1}): 3425, 2927, 2855, 1721, 1448, 1170; ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 3.55 (2H, t, $J = 11.2$ Hz, –$\underline{H}_2$C–N^+–$C\underline{H}_2$–, dioctyl chain methylene protons adjacent to nitrogen), 3.31–3.17 (2H, m, N^+–($\underline{C}H_2$), undecyl chain methylene protons adjacent to nitrogen), 2.99 (3H, s, –N–$\underline{C}H_3$), 2.22 (2H, t, $J = 7.15$ Hz, –$\underline{H}_2$C–C=O), 2.18–2.06 (2H, m) 2.05–1.91 (4H, m), 1.80–1.51 (10H, m), 1.50–1.19 (20H, m); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 174.56 (–$\underline{C}$=O), 69.95 (–N^+–($\underline{C}H)_2$), 56.82 (N^+–($\underline{C}H_2$)), 44.60 (–N–$\underline{C}H_3$), 33.63 (–$H_2\underline{C}$–C=O), 28.71, 28.61, 28.46, 28.31, 26.85, 26.60, 26.28, 25.37, 25.20, 24.53, 24.34, 23.34; ESI-MS (*m/z*) $(M + H)^+$ 380.38, $(M + Na)^+$ 402.33; HRMS-ESI (*m/z*) calculated for $(C_{24}H_{46}NO_2)^+$, $(M + H)^+$ is 380.3523, found at 380.3533.

Antimicrobial activity

Antimicrobial activity of the synthesized compounds (**5a–h**) was determined using well diffusion method (Amsterdam 1996) against different pathogenic reference strains, such as *Micrococcus luteus* MTCC 2470, *Bacillus subtilis* MTCC 121, *Staphylococcus aureus* MTCC 96, *S. aureus* MLS16 MTCC 2940, *Klebsiella planticola* MTCC 530, *Escherichia coli* MTCC 739, *Pseudomonas aeruginosa* MTCC 2453 and different *Candida* strains such as *Candida albicans* MTCC 183, *C. albicans* MTCC 227, *C. albicans* MTCC 854, *C. albicans* MTCC 1637, *C. albicans* MTCC 3017, *C. albicans* MTCC 3018, *C. albicans* MTCC 3958, *C. albicans* MTCC 4748, *C. albicans* MTCC 7315, *C. parapsilosis* MTCC 1744, *C. aaseri* MTCC 1962, *C. glabrata* MTCC 3019, *C. krusei* MTCC 3020, *Issatchenkia hanoiensis* MTCC 4755 procured from the MTCC, CSIR-Institute of Microbial Technology, Chandigarh, India. The pathogenic reference strains were seeded on the surface of the media Petri plates, containing Muller–Hinton agar with 0.1 mL of earlier prepared microbial suspensions individually containing 1.5×10^7 cfu mL^{-1} (equal to 0.5 McFarland standard). Wells of 6.0 mm diameter were prepared in the media plates using a cork borer and the synthesized betaines at a dose range of 125–0.9 µg per well were added in each well under sterile conditions in a laminar air flow chamber.

A standard antibiotic solution of ciprofloxacin (bacterial strains) and miconazole (*Candida* strains) at a dose range of 125–0.9 µg perwell and the well-containing methanol served as positive and negative controls, respectively. The plates were incubated for 24 h at 37 °C for bacterial strains and 30 °C for different *Candida* strains and the well containing the least concentration showing the inhibition zone was considered as the minimum inhibitory concentration (MIC). All experiments were carried out in duplicates and the mean values are represented.

Anti-biofilm activity

The synthesized betaines (**5a–h**) were screened in sterile 96-well polystyrene microtiter plates using the modified biofilm inhibition assay against a panel of pathogenic bacterial strains including *S. aureus* MTCC 96, *S. aureus* MLS16 MTCC 2940, *B. subtilis* MTCC 121, *Micrococcus luteus* MTCC 2470, *Klebsiella planticola* MTCC 530 and *C. albicans* MTCC 4748 which were cultured overnight in tryptone soy broth (supplemented with 0.5% glucose) (Olson 2015). The test compounds of predetermined concentrations ranging from 0 to 125 µg mL^{-1} were mixed with the bacterial suspensions having an initial inoculum concentration of 5×10^5 cfu mL^{-1}. Aliquots of 100 µL were distributed in each well and then incubated at 37 °C for 24 h under static conditions. The medium was then discarded and washed with phosphate buffered saline to remove the non-adherent bacteria. Each well of the microtiter plate was stained with 100 µL of 0.1% crystal violet solution followed by 30 min incubation at room temperature. Later the crystal violet solution from the plates was discarded, thoroughly washed with distilled water for three to four times, and air dried at room temperature. The crystal violet-stained biofilm was solubilized in 95% ethanol (100 µL) and the absorbance was recorded at 540 nm using TRIAD multimode reader (Dynex Technologies, Inc, Chantilly, VA, USA). Blank wells were employed as a background check. The inhibition data were interpreted from the dose-response curves, where the IC_{50} value is defined as the concentration of inhibitor required to inhibit 50% of biofilm formation under the above assay conditions. All the experiments were carried out in triplicates and the values are indicated as mean ± S.D.

Detection of intracellular reactive oxygen species (ROS) accumulated in *C. albicans* MTCC 4748 biofilms

The intracellular ROS accumulated in *C. albicans* MTCC 4748 sessile cells was detected using 2,7-dichlorofluorescein diacetate (DCFH-DA), which is converted to highly fluorescent dichlorofluorescein in the presence of intracellular ROS (Madeo et al. 1999). Briefly, the *C. albicans* MTCC 4748 strain was cultured in 24-well microtitre plates. After biofilm formation, the mature biofilms were washed with 300 µL of 0.9% (*w*/*v*) NaCl, treated with compound **5g** and Miconazole (control) was run in parallel and incubated for 24 h at 30 °C. Later, the biofilms were incubated with 10 µM DCFH-DA dye, simultaneously with the treated test sample. After 24-h incubation, the fluorescence was measured on an Infinite M200 Pro microtitre plate reader (Tecan, Switzerland) at excitation and emission wavelengths of 485 and 535 nm, respectively. The entire content in each well was removed and the cells were separated from the supernatant by centrifugation at 5000×*g* for 10 min. The fluorescence of the supernatant and that of the resuspended sessile cells in phosphate buffered saline was measured separately to determine the generated fluorescence in either intracellular or extracellular environment. The level of ROS accumulation was quantified in triplicate and the values are indicated as mean ± S.D.

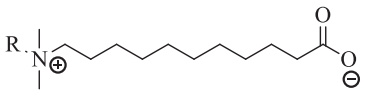
Results and discussion

Synthesis

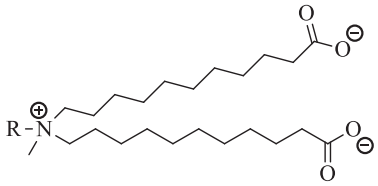
Three types of betaines were prepared (Fig. 1) and the synthetic route for each class involved four steps as shown in Scheme 1. In the first step, 11-bromo undecanoic acid (**1**) was converted into 11-BUME (**2**) by esterification reaction in presence of methanol and PTSA, followed by the synthesis of methyl-11-(alkylamino) undecanoates (**3a–c**, secondary amine monoesters and **3g**, **h**, tertiary amine monoesters) and dimethyl-11, 11′-(alkylazanediyl) diundecanoates (**3d–f**, tertiary amine diesters) by the reaction of 11-BUME with different aliphatic amines in the presence of K_2CO_3 in DMF. In the third step, the synthesized secondary amine monoesters (**3a–c**), tertiary amine mono (**3g–h**) and diesters (**3d–f**) were quaternized by using methyl iodide in the presence of K_2CO_3 to monoesterquats (**4a–c** and **4g**, **h**) and diesterquats (**4d–f**). The resultant esterquats (**4a–h**) were converted to the final compounds, i.e., betaines (**5a–h**) by saponification reaction with $LiOH.H_2O$ in water: tetrahydrofuran (1:1, v/v).

All the synthesized compounds were characterized by using different spectral techniques like fourier transform infrared (FT-IR), ^{1}H-NMR, ^{13}C-NMR, and electrospray ionization-high resolution mass spectrometry (ESI-HRMS). ^{1}H-NMR spectra of compound **5a** showed a multiplet at δ 3.38–3.25 ppm,which indicates the presence of four protons on methylene carbons adjacent to quaternary ammonium group ($-N^+-(CH_2)_2$), a singlet at δ 3.11 ppm reveals the six protons of two methyl groups were attached to quaternary ammonium group and a triplet at δ 2.24 ppm shows two carbon protons adjacent to carbonyl of carboxyl group. In

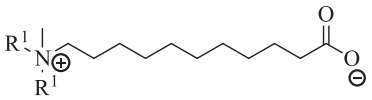
Fig. 1 Molecular structures of the synthesized betaines (**5a–h**)



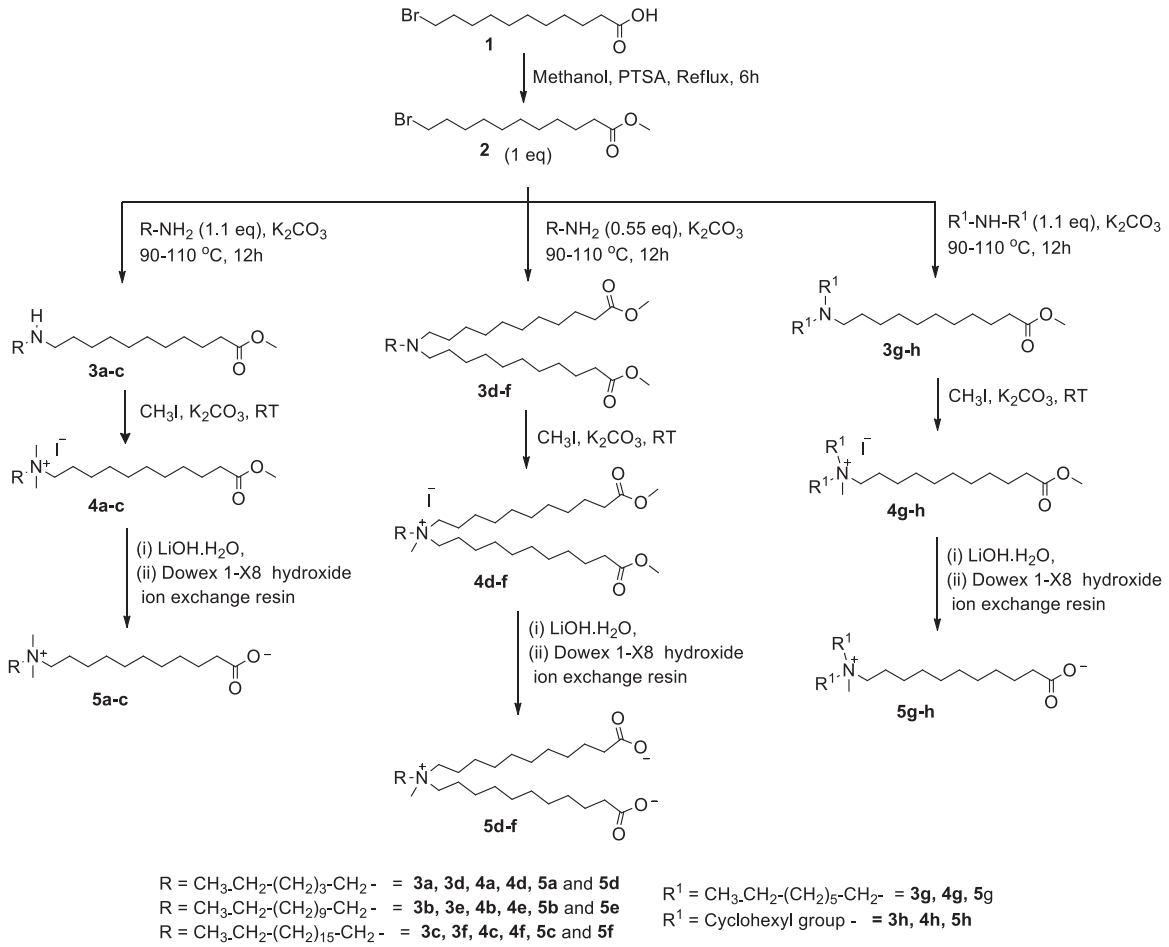
11-(alkylldimethylammonio)undecanoate
(R = C_6 (5a), C_{12} (5b) and C_{18} (5c) alkyl chain)



11,11'-(hexyl(methyl)ammonio)diundecanoate
(R = C_6 (5d), C_{12} (5e) and C_{18} (5f) alkyl chain)



11-(dialkyl(methyl)ammonio)undecanoate
(R^1 = Dioctyl group (5g) and cyclohexyl ring (5h))



Scheme 1 Schematic representation for the synthesis of betaines (**5a–h**)

^{13}C-NMR spectra of compound **5a** showed some important characteristic peaks: δ 174.73 ppm indicates the carbonyl carbon (–C=O), δ 63.34 ppm shows the methylene group carbons adjacent to quaternary ammonium group (–H_2C–N^+–CH_2–), the peak at δ 50.14 ppm shows the two methyl carbons (–N^+–(CH_3)$_2$) and at δ 33.60 ppm indicates the carbon adjacent to carbonyl functionality (–H_2C–C=O). Mass spectral studies (ESI-HRMS) also showed that the

Table 1 Antimicrobial activity of the synthesized betaines (**5a–h**)

Test compounds	Minimum inhibitory concentration (μg mL^{-1})							
	[a]*S.a*	[a]*B.s*	[a]*S.m*	[a]*M.l*	[b]*K.p*	[b]*E.c*	[b]*P.a*	[c]*C.a*
5a	15.6	31.2	15.6	7.8	15.6	>125	>125	31.2
5b	15.6	15.6	7.8	7.8	15.6	>125	>125	15.6
5c	>125	>125	>125	>125	>125	>125	>125	>125
5d	>125	>125	>125	>125	>125	>125	>125	>125
5e	>125	>125	>125	>125	>125	>125	>125	>125
5f	>125	>125	>125	>125	>125	>125	>125	>125
5g	15.6	15.6	7.8	3.9	15.6	>125	>125	7.8
5h	>125	>125	>125	>125	>125	>125	>125	>125
Ciprofloxacin	0.9	0.9	0.9	0.9	0.9	0.9	0.9	–
Miconazole	–	–	–	–	–	–	–	7.8

The minimum inhibition concentration values are mean of three determinations.

[a] Gram-positive bacteria

[b] Gram-negative bacteria

[c] fungus; S. a. (*Staphylococcus aureus* MTCC 96); B. s. (*Bacillus subtilis* MTCC 121); S. m. (*Staphylococcus aureus* MLS16 MTCC 2940); M. l. (*Micrococcus luteus* MTCC 2470); K. p. (*Klebsiella planticola* MTCC 530); E. c. (*Escherichia coli* MTCC 739); P. a. (*Pseudomonas aeruginosa* MTCC 2453); C. a. (*Candida albicans* MTCC 3017)

base peak at 314.3054 $(C_{19}H_{39}NO_2+H)^+$ corresponding to the molecular weight of the compound which further confirmed the formation of desired product, i.e., **5a**. In the FT-IR spectra, the compound (**5a**) showed a strong absorption band at 3424 cm^{-1} indicating the asymmetric stretching mode of O–H of carboxyl group; the two strong bands at 2925 and 2855 cm^{-1} show the alkyl chain (–C–H) stretching vibration and a strong band at 1727 cm^{-1} indicates the carbonyl functionality of carboxyl group.

Antimicrobial activity

The synthesized betaines (**5a–h**) were screened for their antimicrobial activity against both Gram-positive and Gram-negative bacterial strains as well as against *C. albicans*, a fungal strain. It was observed that the majority of compounds such as **5a**, **5b** and **5g** showed good antimicrobial activity in quaternary amino monocarboxylate form with MIC values ranging between 3.9–31.2 μg mL^{-1}, whereas the compound **5c** prepared using long chain alkyl group, i.e., octadecyl amine did not exhibit any activity on the tested strains even up to the maximum tested concentration of >125 μg mL^{-1} as shown in Table 1. Earlier reports also suggested that the betaines with long alkyl chains (above C_{14} carbon chain) did not show antimicrobial activity, indicating that the antimicrobial activity was dependent on the alkyl chain length (Birnie et al. 2000). While the compounds **5d–f** having dicarboxylate ions in their structure did not exhibit antimicrobial activity on the tested strains even up to the maximum tested concentration of >125 μg mL^{-1}, this could be due to more hydrophobicity of monoalkyl methyl quaternary ammonium dicarboxylates (**5d–f**), synthesized by the reaction of two moles of 11-bromo methylundecanoate and primary amine. The compound **5h** prepared using dicyclohexyl amine also showed no antimicrobial activity even up to the maximum tested concentration of >125 μg mL^{-1}. The compound **5h** didn't show any antimicrobial activity, this could be due to more steric hindrance on quaternary ammonium group by two cyclohexyl rings. Literature revealed that the antimicrobial activity of quaternary ammonium compounds is due to electrostatic interactions between the positively charged quaternary ammonium compounds and the negatively charged bacterial cellular membrane, which is followed by permeation of the carbon chain of quaternary ammonium compound into the intramembrane region, ultimately leading to leakage of cytoplasmic material and cellular lysis followed bacterial cell death (Jennings et al. 2015).

The results from the present study showed that the betaines **5a** and **5b** in monocarboxylate form synthesized using hexyl and dodecyl amines showed antimicrobial activity on Gram-positive bacteria with MIC values ranging between 7.8–62.4 μg mL^{-1}, while the compound **5g** synthesized using dioctyl amine also showed good antimicrobial activity on Gram-positive bacteria with MIC values ranging between 3.9–15.6 μg mL^{-1}. Whereas, the intermediate compounds such as amine esters (**3a**, **3d**, **3g**, and **3h**) also showed moderate antimicrobial activity was reported in our previous work (Yasa et al. 2016).

Anti-fungal activity

The betaines **5a**, **5b**, and **5g** showed good-to-moderate antifungal activity against *C. albicans* MTCC 3017 (Table 1), which prompted us to further test these compounds against other fungal strains and the results to this regard are tabulated in Table 2. As evident from the results, the three compounds **5a**, **5b**, and **5g** showed excellent-to-good antifungal activity with MIC values ranging between 7.8–62.5 μg mL^{-1} on tested fungal strains. The compound **5g** was found to be most effective among the all tested compounds and exhibited excellent antifungal activity identical to the standard Miconazole with MIC value of 7.8 μg mL^{-1} on different fungal strains, except on some strains like *C. albicans* MTCC 227, *C. albicans* MTCC 3018, *C. parapsilosis* MTCC 1744 and *C. glabrata* MTCC 3019, which showed good antifungal activity with MIC value of 15.6 μg mL^{-1}. The other compounds **5a** and **5b** synthesized using hexyl and dodecyl amines, respectively, showed good to moderate antifungal activity with MIC values ranging between 15.6–62.5 μg mL^{-1}. Based on the antifungal screening results, it was concluded that the compounds (**5d–f**) in quaternary ammonium dicarboxylate ion form, and prepared from above C_{12}-alkyl amines did not show any antifungal activity even up to the maximum tested concentration of >125 μg mL^{-1}.

Table 2 Antifungal activity of synthesized betaines (**5a**, **5b**, and **5g**)

Fungal strain	Minimum inhibitory concentration (μg mL^{-1}) of test compounds			
	5a	**5b**	**5g**	Miconazole
Candida albicans MTCC 183	62.5	15.6	7.8	7.8
C. albicans MTCC 227	31.2	31.2	15.6	7.8
C. albicans MTCC 854	62.5	62.5	7.8	7.8
C. albicans MTCC 1637	125	62.5	7.8	7.8
C. albicans MTCC 3018	31.2	31.2	15.6	7.8
C. albicans MTCC 3958	31.2	31.2	7.8	7.8
C. albicans MTCC 4748	15.6	15.6	7.8	7.8
C. albicans MTCC 7315	31.2	31.2	7.8	7.8
C. parapsilosis MTCC 1744	31.2	15.6	15.6	7.8
C. aaseri MTCC 1962	31.2	15.6	7.8	7.8
C. glabrata MTCC 3019	15.6	15.6	15.6	7.8
C. krusei MTCC 3020	31.2	31.2	7.8	7.8
Issatchenikia hanoiensis MTCC 4755	15.6	31.2	7.8	7.8

Anti-biofilm assay

Biofilms are complex communities of bacteria that exist in a self-produced matrix of polysaccharides, proteins, and extracellular DNA responsible for the growing resistance in bacteria towards various antibiotics (Olson 2015). Several clinically significant pathogens producing biofilms are implicated in more than 80% of the bacterial infections (Davies 2003). Therefore, the antimicrobial active compounds **5a**, **5b**, and **5g** were studied for their biofilm inhibitory activity against five bacterial strains, namely *S. aureus* MTCC 96, *B. subtilis* MTCC 121, *S. aureus* MLS16 MTCC 2940, *Micrococcus luteus* MTCC 2470 and *Klebsiella planticola* MTCC 530, and one fungal strain like *Candida albicans* MTCC 4748.

The results of the biofilm inhibitory activity for the betaines, such as **5a**, **5b**, and **5g** are shown in Table 3, which suggests that these compounds exhibited good-to-moderate anti-biofilm activity against all the six tested strains. In particular, the compound **5g** demonstrated good anti-biofilm activity with IC_{50} values of 8.2, 10.1, 4.1, 2.1, 10.1, and 3.7 μg mL^{-1} against *S. aureus* MTCC 96, *B. subtilis* MTCC 121, *S. aureus* MLS16 MTCC 2940, *Micrococcus luteus* MTCC 2470, *Klebsiella planticola* MTCC 530 and *C. albicans* MTCC 4748, respectively. Other compounds, such as **5a** (IC_{50} values ranging between 4.2–25.3 μg mL^{-1}) and **5b** (IC_{50} values ranging between 3.8–11.1 μg mL^{-1}) showed moderate biofilm inhibitory activity on all the tested strains. Particularly, the compound **5g** showed excellent biofilm inhibitory activity on *C. albicans* MTCC 4748 with IC_{50} value of 3.7 μg mL^{-1}, while the standard Miconazole value showed IC_{50} value of

Table 3 Antibiofilm activity of synthesized betaines (**5a**, **5b**, and **5g**)

Test compounds	IC_{50} values in (μg mL^{-1}) of test compounds				
	5a	**5b**	**5g**	Ciprofloxacin	Miconazole
Staphylococcus aureus MTCC 96	10.1 ± 0.36	9.8 ± 0.41	8.2 ± 0.25	0.7 ± 0.09	–
Bacillus subtilis MTCC 121	25.3 ± 0.32	8.6 ± 0.18	10.1 ± 0.22	0.6 ± 0.09	–
S. aureus MLS16 MTCC 2940	12.1 ± 0.16	3.8 ± 0.11	4.1 ± 0.21	0.7 ± 0.11	–
Micrococcus luteus MTCC 2470	4.2 ± 0.22	4.1 ± 0.18	2.1 ± 0.14	0.8 ± 0.09	–
Klebsiella planticola MTCC 530	10.5 ± 0.16	11.1 ± 0.18	10.1 ± 0.22	0.6 ± 0.12	–
Candida albicans MTCC 4748	11.1 ± 0.35	9.8 ± 0.36	3.7 ± 0.11	–	2.8 ± 0.12

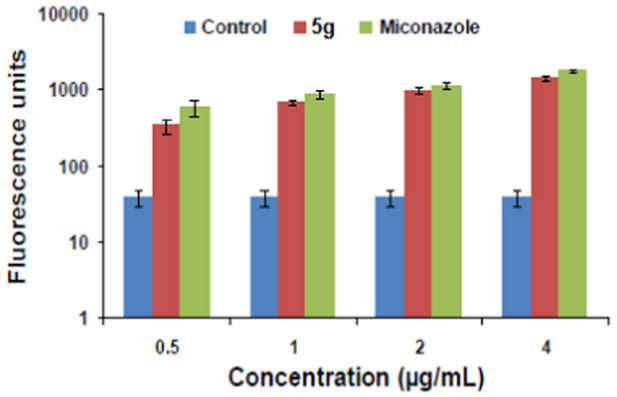


Fig. 2 Intracellular ROS accumulation in *Candida albicans* MTCC 4748 of compound **5g**

2.8 µg mL^{-1}. It is important to note that the ability to inhibit biofilm formation of these compounds to a maximum extent which is in coherence with the antimicrobial activity against the respective strain.

Detection of intracellular ROS in mature Candida biofilms

Reactive oxygen species (ROS) is an important inducer of apoptosis in bacteria, which may cause oxidative damage to cellular compounds and lead to cellular dysfunction or cell death (Madeo et al. 1999). In order to elucidate whether oxidative stress is involved in the apoptotic cell death, the intracellular ROS accumulation within the sessile cells of *C. albicans* MTCC 4748 mature biofilms was measured using 2,7-dichlorofluorescein diacetate dye, in which the dye conversion depends on the state of the metabolically active cells in the biofilm (Kumar and Poornachandra 2015). After treatment of *C. albicans* MTCC 4748 cells with different concentrations of the test compound **5g**, a significantly increased level of intracellular ROS accumulation was observed in the tested *C. albicans* MTCC 4748 strain. At a concentration of 1, 2, and 4 µg mL^{-1}, the compound **5g**-treated biofilms showed increased levels of intracellular ROS accumulation, which was closer to the standard drug Miconazole. The ROS measurements were also carried out separately in both sessile cells and in the supernatant (data not shown). The ROS-induced increase in fluorescence was observed only for the sessile cells, suggesting that the ROS accumulation was of intracellular origin (Fig. 2). This accumulated ROS may also be responsible for the fungicidal activity. Oxygen-containing free radical molecules and their precursors formed in biological systems are collectively termed as ROS, comprising of superoxides (O_2^-), peroxides (H_2O_2 and ROOH) and free radicals ($HO^{\cdot}$ and $RO^{\cdot}$). Some of the antifungal agents stimulate free radical formation via the Fenton reaction (Halliwell and Aruoma 1991; Kobayashi et al. 2002). Recent studies suggest that when yeast cells are subjected to oxidative stress, these free radicals contribute to arrest in cell growth or result in damage of the specific essential metabolic enzymes (cellular respiratory chain), disrupting cellular membrane, and DNA damage ultimately causing cell lysis and death (Dixon and Stockwell 2013).

Conclusion

In conclusion, a new type of betaine (**5a–h**) was synthesized using a simple and convenient synthetic approach with good yields by employing various aliphatic amines and 11-bromoundecanoic acid. The synthesized betaines (**5a–h**) were evaluated for antibacterial, antifungal and anti-biofilm activities. Some of the synthesized betaines showed good-to-moderate antimicrobial activity. Further, the several compounds also displayed excellent-to-good antifungal activity against different *Candida* strains. Particularly, the compound **5g** was found to exhibit excellent antifungal activity against *C. albicans* MTCC 183, *C. albicans* MTCC 854, *C. albicans* MTCC 1637, *C. albicans* MTCC 3958, *C. albicans* MTCC 4748, *C. albicans* MTCC 7315, *C. aaseri* MTCC 1962, *C. krusei* MTCC 3020 and *I. hanoiensis* MTCC strains with 7.8 µg mL^{-1}. Further, it was observed that among the synthesized betaines the antimicrobial activity of the compounds prepared using above C_{12}-alkyl amine, and the compounds **5d–f** with dicarboxylate ion form did not show any activity. The betaines **5a**, **5b**, and **5g** showed anti-biofilm activity. Among the synthesized betaines, the compound **5g** displayed promising anti-biofilm activity against *C. albicans* MTCC 4748 with IC_{50} value of 3.7 µg mL^{-1}, while the standard Miconazole showed IC_{50} value of 2.8 µg mL^{-1}. Moreover, the compound **5g** was identified as a promising lead molecule for further investigation.

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References

Abdel-Ghany YS, Ihnat MA, Miller DD, Kunin CM, Tong HH (1993) Structure-activity relationship of glycine betaine analogues on osmotolerance of enteric bacteria. J Med Chem 36:784–789

Amin US, Lash TD, Wilkinson BJ (1995) Proline betaine is a highly effective osmoprotectant for Staphylococcus aureus. Arch Microbiol 163:138–142

Amsterdam D (1996) Susceptibility testing of antimicrobials in liquid media. In: Loman V (ed) Antibiotics in laboratory medicine, 4th edn. Williams and Wilkins, Baltimore, MD, p 52–111

Baaman RA (1978) Anti-plaque agents. US Patent 4130637
Birnie CR, Malamud D, Schnaare RL (2000) Antimicrobial evaluation of *N*-alkyl betaines and *N*-Alkyl-*N*,*N*-dimethylamine oxides with variations in chain length. Antimicrob Agents Chemother 44:2514–2517
Cosquer A, Ficamos M, Jebbar M, Corbel JC, Choquet G, Fontenelle C, Uriac P, Bernard T (2004) Antibacterial activity of glycine betaine analogues: involvement of osmoporters. Bioorg Med Chem Lett 14:2061–2065
Davies D (2003) Understanding biofilm resistance to antibacterial agents. Nat Rev Drug Disc 2:114–122
Dixon SJ, Stockwell BR (2013) The role of iron and reactive oxygen species in cell death. Nat Chem Biol 10:9–17
Domingo X (1990) Betaines. In: Lomax EG (ed) Amphoteric Surfactants. Marcel Dekker, New York, NY, p 76–190
Godzisz D, Ilczyszyn MM, Ilczyszyn M (2002) Classification and nature of hydrogen bonds to betaine. X-ray, 13C CP MAS and IR description of low barrier hydrogen bonds. J Mol Struct 606:123–137
Halliwell B, Aruoma OI (1991) DNA damage by oxygen-derived species. Its mechanism and measurement in mammalian systems. FEBS Lett 281:9–19
Jennings MC, Minbiole KP, Wuest WM (2015) Quaternary ammonium compounds: An antimicrobial mainstay and platform for innovation to address bacterial resistance. ACS Infect Dis 1:288–303
Kobayashi D, Kondo K, Uehara N, Otokozawa S, Tsuji N, Yagihashi A, Watanabe N (2002) Endogenous reactive oxygen species is an important mediator of miconazole antifungal effect. Antimicrob Agents Chemother 46:3113–3117
Kowalczyk I (2008) Synthesis, molecular structure and spectral properties of quaternary ammonium derivatives of 1,1-dimethyl-1,3-propylenediamine. Molecules 13:379–390
Kumar CG, Poornachandra Y (2015) Biodirected synthesis of miconazole-conjugated bacterial silver nanoparticles and their applications as antifungal agents and drug delivery vehicles. Colloids Surf B Biointerfaces 125:110–119
Kumar R, Kalur GC, Ziserman L, Danino D, Raghavan SR (2007) Wormlike micelles of a C22-tailed zwitterionic betaine surfactant: from viscoelastic solutions to elastic gels. Langmuir 23:12849–12856
Lindstedt M, Allenmark S, Thompson RA, Edebo L (1990) Antimicrobial activity of betaine esters, quaternary ammonium amphiphiles which spontaneously hydrolyze into nontoxic components. Antimicrob Agents Chemother 34:1949–1954
Madeo F, Frohlich E, Ligr M, Grey M, Sigrist SJ, Wolf DH, Frohlich KU (1999) Oxygen stress: a regulator of apoptosis in yeast. J Cell Biol 145:757–767
Olson I (2015) Biofilm-specific antibiotic tolerance and resistance. Eur J Clin Microbiol Infect Dis 34:877–886
Peddie BA, Wood JE, Lever M, Happer DA, de Zwart F, Chambers ST (2003) Assessment of antimicrobial activity of hydrophilic betaines in osmotically stressed bacteria. Antonie Van Leeuwenhoek 83:175–181
Quan H, Zhang X, Lu H, Huang Z (2012) Synthesis and acid solution properties of a novel betaine zwitterionic surfactant. Cent Eur J Chem 10:1624–1632
Robert J, Ferlauto Jr, Yuhasz KM (1988) Stable antiplaque dentifrice. US Patent 4774077
Schaack G (1990) Experimental results on phase transitions in betaine compounds. Ferroelectrics 104:147–158
Srinivas R, Garu A, Moku G, Agawane SB, Chaudhuri A (2012) A long-lasting dendritic cell DNA vaccination system using lysinylated amphiphiles with mannose-mimicking head-groups. Biomaterials 33:6220–6229
Yasa SR, Kaki SS, Poornachandra Y, Kumar CG, Penumarthy V (2016) Synthesis, characterization, antimicrobial and biofilm inhibitory studies of new esterquats. Bioorg Med Chem Lett 26:1978–1982
Zhou M, Huang Z, Yu S, Yang Y, Huang Y, Qiu D, Zhao J (2016) Synthesis and surface active properties of novel oligomer betaine surfactants. Tenside Surfactants Deterg 53:134–139