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Synthesis and antibacterial activity of new long-chain-alkyl bile acid-based amphiphiles

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ABSTRACT

The efficient synthesis of some bile acid-derived cationic amphiphiles with a flexible long hydrocarbon tail was investigated. Firstly, the modification on the side-chain carboxyl of bile acids was carried out efficiently by one-pot amidation of bile acids and a long-chain aliphatic amine in the presence of HOBt and DCC to introduce a flexible long hydrocarbon tail. Then the hydrophilic concave side of bile acids with hydroxyl groups was further modified into cationic groups for strengthening hydrophilicity. This strategy offered a very straightforward and efficient method for access to the designed amphiphiles in good overall yields. The preliminary results showed that an increase both in the length of the hydrophobic tail and in the number of charged groups resulted in a decrease in the CMC of bile acid-derived cationic amphiphiles. And the bile acid-derived cationic amphiphiles with a flexible longer hydrocarbon tail and more positive charges had the highest antibacterial and antimicrobial activity.

1. Introduction

Bile acids and their salts are widely used as a first-choice therapy in most cholestatic diseases including primary biliary cirrhosis, primary sclerosing cholangitis, cholestatic disorders in pediatric patients, pregnancy-induced cholestasis and drug-induced cholestasis. Due to their unique physicochemical properties, they become valuable in the fields of drug delivery [1–3]. Additional, because of the biological compatibility of bile acids, bile acids-derived various polymers and oligomers have shown potential biological and pharmaceutical applications [4–7]. Chemical and biological investigations showed that bile acids are powerful detergents that emulsify and dissolve lipids, vitamins, cholesterol and other molecules in the biliary tract and intestines. It has long been known that bile acids form soluble mixed micelles with lipids [8]. However, their distinguishingly facial amphiphilic properties have attracted a great deal of attention due to their amphiphilic structures, which a steroid-ring system is rigid and slightly curved, attached to the hydrophobic ring system. There are hydrophilic groups between one and three hydroxyl groups and one acidic group, bile acids and their salts thus have a facial structure with a hydrophobic side and a hydrophilic side only [9]. Due to this unusual amphiphilicity and the rigidity of the steroidal polycyclic backbone, bile acids are attractive building blocks for biomimetic materials in supra-molecular chemistry. The materials based on bile acids still keep the capabilities to self-assemble and respond to the chemical environment by exposing either their hydrophilic or hydrophobic faces [10–12]. It is known that the applications mentioned above for bile acid derivatives are directly related to their facial amphiphilicity which their polar and non-polar groups are located on opposite faces, the chemical structure and physicochemical characteristics of bile acids and their derivatives are very different from those of the common that are surfactants characterized by a hydrophilic head and a hydrophobic tail. However, the facial amphiphilicity of bile acids are weak with only one to three hydroxyl groups and carboxylate on the hydrophilic face. Recently many studies indicated that in order to increase the facial amphiphilicity, various groups including sugars, sulfates, ammoniums, and carboxylates have been introduced to the hydrophilic face of bile acid by employing different linkages including acetal, sulfate ester, ether, and ester [13–19]. To the best of our knowledge, there is no direct method available for the modification on the hydrophobic face of bile acids to affect their amphiphilicity. Hence, we envisioned the combined modifications on the hydrophilic face and the hydrophobic face of bile acids to improve their facial amphiphilicity. Firstly, the saturated hydrocarbon tail 12–18 carbon atoms in length was linked together with carboxyl of bile acids by an amido spacer on the hydrophobic face of bile acids. And then we used ester linkages exclusively in the synthesis by the nucleophilic substitution of hydroxyl of bile acids with chloroactyl chloride. Next quaternary ammoniation of chloroacetates of bile acids with pyridine functionalizes the hydrophilic face of bile acids with a range of polar groups with different ionic characteristics, so that the modified bile acid-based quaternary ammonium amphiphiles possess both positive charges on the hydrophilic face and a flexible long hydrocarbon tail on the hydrophobic face of bile acids.

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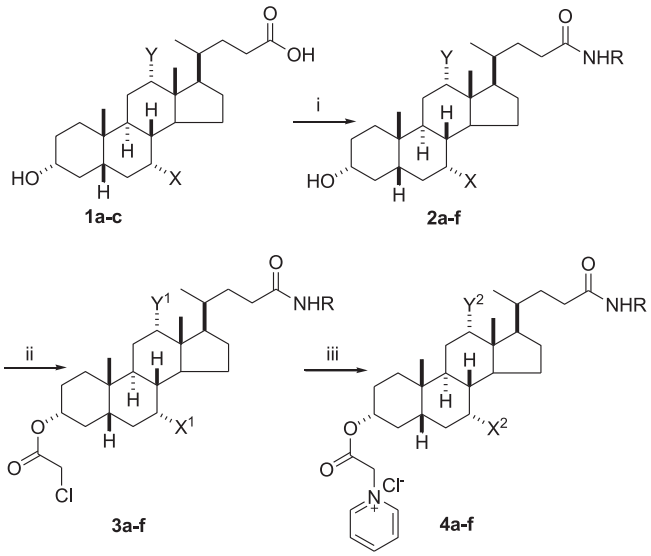


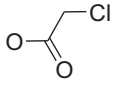
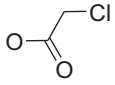
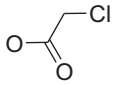
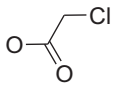
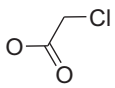
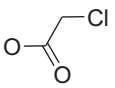
Scheme 1. Preparation of target compounds (**4a–f**). Reaction conditions: (i) RNH_2, HOBt, DCC, THF, 60 °C, 4 h; (ii) chloroacetyl chloride, CH_2Cl_2, K_2CO_3, rt, 18 h; and (iii) Pyridine, 70–80 °C, 12 h.

As been well known, cationic surfactants were shown to interfere with bacterial growth, and have been used as antibiotics in biomedical applications. Studies indicated that the bacterial action of cationic surfactants is due to their interaction with the negatively-charged phosphate groups on the surface of the bacterial cell wall, leading to disruption of the cell division and cell–cell interaction processes. In recent years, overall cationic cholic acid-derived antimicrobial agents have been reported to show potent antibacterial activity [20,21,14,22–26]. As an extension of new bile acid-based surfactants we now report the synthesis of the bile acid-based surfactants with a flexible long hydrocarbon tail from readily available starting materials (Scheme 1, and Tables 1–3). These cationic surfactants show effectively antibacterial activity. Our results may provide useful information for the design of new bile acid-based surfactants for antibacterial agents.

Table 1
Preparation of compounds **2a–f.**

Entry	X	Y	R	Comp. **2**	Yield (%)
1	OH	H	n-$C_{12}H_{25}$	**2a**	78
2	OH	H	n-$C_{14}H_{29}$	**2b**	82
3	OH	H	n-$C_{16}H_{33}$	**2c**	85
4	OH	H	n-$C_{18}H_{37}$	**2d**	75
5	OH	OH	n-$C_{14}H_{29}$	**2e**	65
6	H	H	n-$C_{12}H_{25}$	**2f**	92

Table 2
Preparation of compounds **3a–f.**

Entry	X^1	Y^1	R	Comp. **3**	Yield (%)
1		H	n-$C_{12}H_{25}$	**3a**	80
2		H	n-$C_{14}H_{29}$	**3b**	78
3		H	n-$C_{16}H_{33}$	**3c**	80
4		H	n-$C_{18}H_{37}$	**3d**	85
5			n-$C_{14}H_{29}$	**3e**	75
6	H	H	n-$C_{12}H_{25}$	**3f**	93

2. Experimental

2.1. General

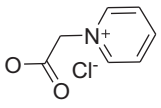
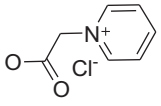
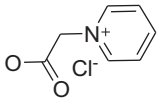
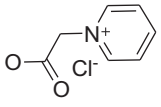
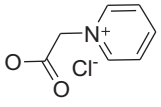
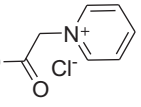
All melting points were determined in a Yanaco melting point apparatus and are uncorrected. IR spectra were recorded in a Nicolet FT-IR 5DX spectrometer. The ^1H NMR (600 MHz) and ^{13}C NMR (150 MHz) spectra were recorded in a Bruker AV-600 spectrometer with TMS as internal reference in $CDCl_3$, CD_3OD or DMSO-d_6 solutions. The *J* values are given in hertz. Only discrete or characteristic signals for the ^1H NMR are reported. The MS spectra were obtained on an Agilent 1200–6460 QQQ mass spectrometer. The elemental analyses were performed in a Perkin–Elmer 240C instrument. Flash chromatography was performed on silica gel (230–400 mesh) eluting with ethyl acetate–hexanes mixture.

2.2. Organic synthesis

2.2.1. 3α,7α-Dihydroxy-N-laurylchenodeoxycholamide (*2a*)

The mixture of lauryl amine (4.7 g, 25.4 mmol), HOBt (1.7 g, 12.6 mmol), and DCC (6.4 g, 31.1 mmol) in dried THF (40 mL) was stirred at 60 °C for 15 min. To the resultant mixture was added the solution of chenodeoxycholic acid (5 g, 14.2 mmol) in dried THF (20 mL), then the resulting mixture was stirred at 60 °C for 4 h. After the completion of reaction, the mixture was cooled in an ice bath. The white precipitate was filtered off, the filtrate was concentrated by reduced pressure distillation. The residues were dissolved with dichloromethane (25 mL), the resulting solution was washed with 10% HCl (10 mL), saturated Na_2CO_3 and brine (15 mL) respectively, dried over anhydrous Na_2SO_4, and evaporated under reduced pressure to get a residue. The residue was subjected to chromatography using ethyl acetate/acetone (5:1) as

Table 3
Preparation of compounds **4a–f.**

Entry	X^2	Y^2	R	Comp. **4**	Yield (%)
1		H	n-$C_{12}H_{25}$	**4a**	99
2		H	n-$C_{14}H_{29}$	**4b**	98
3		H	n-$C_{16}H_{33}$	**4c**	99
4		H	n-$C_{18}H_{37}$	**4d**	99
5			n-$C_{14}H_{29}$	**4e**	98
6	H	H	n-$C_{12}H_{25}$	**4f**	98

the eluent to give 5.62 g of product **2a** (78%) as a white solid, mp 62–64 °C (EtOAc); IR (KBr, cm^{-1}) ν 3302, 2926, 2854, 1648, 1549, 1413, 1375, 1078; ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 5.70 (s, 1H, CONH), 3.82 (s, 1H, 3β-H), 3.45 (s, 1H, 7β-H), 3.22 (s, 2H, NCH_2), 2.20 (s, 2H, CH_2CO), 2.02 (s, 2H), 1.95 (s, 2H) 1.82–1.64 (m, 6H), 1.55–1.25 (m, 31H), 1.33 (m, 3H), 0.91 (s, 2H), 0.89 (s, 3H, 18-CH_3), 0.87 (s, 2H), 0.64 (s, 3H, 19-CH_3); ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 172.17, 70.92, 67.41, 54.86, 49.41, 41.65, 40.53, 38.74, 38.69, 38.57, 38.42, 34.47, 34.37, 34.04, 33.63, 32.59, 31.83, 30.91, 29.62, 28.69, 28.65, 28.65, 28.60, 28.57, 27.22, 25.96, 22.68, 21.68, 19.60, 17.41, 13.12, 10.77; LC-MS *m*/*z* (%): 559.09 (M−1, 100); Anal. calcd. for $C_{36}H_{65}NO_3$: C, 77.22; H, 11.70; N, 2.50; Found C, 77.36; H, 11.58; N, 2.24.

*2.2.2. 3α,7α-Dihydroxy-N-tetradecylchenodeoxycholamide (**2b**)*

Mp 66–68 °C (EtOAc); IR (KBr, cm^{-1}) ν 3302, 2925, 2854, 1464, 1553, 1463, 1373, 1079; ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 5.68 (s, 1H, CONH), 3.76 (s, 1H, 3β-H), 3.37 (s, 1H, 7β-H), 3.15 (s, 2H, NCH_2), 2.16 (s, 2H, CH_2CO), 1.99–1.59 (m, 13H), 1.40–1.21 (m, 38H), 1.10 (s, 4H), 0.83 (s, 11H), 0.58 (s, 3H, 19-CH_3); ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 172.46, 71.00, 67.47, 55.04, 49.54, 41.76, 40.67, 38.97, 38.79, 38.60, 38.58, 34.52, 34.51, 34.47, 34.13, 33.78, 32.72, 31.97, 30.93, 29.78, 28.74, 28.69, 28.67, 28.62, 28.58, 28.35, 27.23, 25.99, 22.75, 21.82, 21.67, 19.69, 17.48, 13.07, 10.84; LC-MS *m*/*z* (%): 587.10 (M−1, 100); Anal. calcd. for $C_{38}H_{69}NO_3$: C, 77.63; H, 11.83; N, 2.38; Found C, 77.29; H, 11.90; N, 2.48.

*2.2.3. 3α,7α-Dihydroxy-N-hexadecylchenodeoxycholamide (**2c**)*

Mp 64–65 °C (EtOAc); IR (KBr, cm^{-1}) ν 3303, 2925, 2853, 1646, 1553, 1463, 1373, 1079; ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 5.78 (s, 1H, CONH), 3.76 (s, 1H, 3β-H), 3.37 (s, 1H, 7β-H), 3.15 (s, 2H, NCH_2), 2.16 (s, 2H, CH_2CO), 1.99–1.60 (m, 10H), 1.39–1.21 (m, 38H), 1.10 (s, 4H), 0.85 (s, 10H), 0.58 (s, 3H, 19-CH_3); ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 172.52, 71.00, 67.42, 55.05, 49.54, 41.75, 40.69, 39.89, 38.80, 38.61, 38.58, 34.52, 34.49, 34.13, 33.80, 32.71, 31.97, 30.97, 30.94, 29.78, 28.74, 28.71, 28.68, 28.66, 28.63, 28.60, 28.36, 27.25, 26.00, 22.75, 21.84, 21.69, 19.70, 17.49, 13.08, 10.85; LC-MS *m*/*z* (%): 615.21 (M−1, 100); Anal. calcd. for $C_{40}H_{73}NO_3$: C, 77.99; H, 11.94; N, 2.27; Found C, 78.12; H, 11.88; N, 2.20.

*2.2.4. 3α,7α-Dihydroxy-N-octadecylchenodeoxycholamide (**2d**)*

Mp 62–63 °C (EtOAc); IR (KBr, cm^{-1}) ν 3303, 2825, 2853, 1646, 1553, 1463, 1372, 1079; ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 5.59 (s, 1H, CONH), 3.77 (s, 1H, 3β-H), 3.38 (s, 1H, 7β-H), 3.14 (s, 2H, NCH_2), 2.15 (s, 2H, CH_2CO), 1.99–1.59 (m, 12H), 1.40–1.18 (m, 42H), 1.08 (s, 3H), 0.85 (s, 10H), 0.58 (s, 3H, 19-CH_3); ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 172.42, 71.02, 67.49, 55.03, 49.50, 47.77, 40.66, 38.99, 38.78, 38.59, 34.51, 34.46, 34.13, 33.77, 32.72, 31.97, 30.94, 29.78, 28.75, 28.71, 28.62, 28.58, 28.35, 27.23, 25.998, 22.76, 21.82, 21.68, 19.68, 17.47, 13.07, 10.84; LC-MS *m*/*z* (%): 643.26 (M−1, 100); Anal. calcd. for $C_{42}H_{77}NO_3$: C, 78.32; H, 12.05; N, 2.17; Found C, 78.21; H, 12.02; N, 2.26.

*2.2.5. 3α,7α,12α-Trihydroxy-N-tetradecylcholamide (**2e**)*

Mp 88–90 °C (EtOAc); IR (KBr, cm^{-1}) ν 3306, 2925, 2855, 1647, 1553, 1462, 1373, 1078; ^{1}H NMR ($CDCl_3$, 600 MHz, ppm) δ 6.12 (s, 1H, CONH), 4.01 (s, 1H, 3β-H), 3.83 (m, 1H, 7β-H), 3.43 (s, 1H, 12β-H), 3.21 (m, 5H, NCH_2, OH), 2.20 (m, 2H, CH_2 CO), 2.10 (m, 1H), 1.90–1.80 (m, 7H), 1.60–1.37 (m, 13H), 1.20–1.25 (m, 24H), 1.11 (m, 1H), 0.99 (s, 3H, *18*-CH_3), 0.88 (m, 6H), 0.67 (s, 3H, *19*-CH_3); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 174.08, 73.13, 71.86, 68.47, 46.47, 46.38, 41.58, 41.53, 39.58, 39.42, 35.38, 34.77, 34.72, 33.12, 31.92, 31.79, 30.39, 29.71, 29.68, 29.63, 29.40, 29.37, 29.10, 27.59, 27.01, 26.29, 23.29, 22.69, 22.43, 17.47, 14.13, 12.45; LC-MS *m*/*z* (%): 603.29 (M−1, 100%); Anal. calcd. for $C_{38}H_{69}NO_4$: C, 75.57; H, 11.52; N, 2.32; Found C, 75.66; H, 11.76; N, 2.16.

*2.2.6. 3α-Hydroxy-N-laurylliithocholamide (**2f**)*

Mp 88–90 °C (EtOAc); IR (KBr, cm^{-1}) ν 3435, 3332, 2928, 2859, 1658, 1547, 1452, 1040; ^{1}H NMR (600 MHz, $CDCl_3$, ppm) δ 5.49 (s, 1H, NH), 3.61–3.65 (m, 1H, 3βH), 3.22–3.25 (m, 2H, NCH_2), 0.92–0.93 (m, 6H), 0.87–0.89 (t, 3H, *J* = 7.2 Hz, CH_3CH_2), 0.64 (s, 3H, *18*-CH_3), 0.64 (s, 3H, *19*-CH_3); ^{13}C NMR ($CDCl_3$, 150 MHz, ppm) δ 173.48, 71.82, 56.50, 56.04, 42.74, 42.09, 40.42, 40.19, 39.53, 36.45, 35.84, 35.49, 35.35, 34.57, 33.73, 31.92, 31.88, 30.53, 29.66, 29.60, 29.56, 29.36, 29.32, 28.26, 27.20, 26.94, 26.42, 24.22, 23.38, 22.69, 20.82, 18.39, 14.13, 12.05; LC-MS *m*/*z* (%): 566 (M+Na, 100); Anal. calcd. for $C_{36}H_{65}NO_2$: C, 79.50; H, 12.05; N, 2.58; Found C, 79.66; H, 12.28; N, 2.63.

*2.2.7. 3α,7α-di(2-Chloroacetoyloxy)-N-laurylchenodeoxycholamide (**3a**)*

To a solution of 3α,7α-dihydroxy-*N*-laurylchenodeoxycholamide (**2a**) (1.0 g, 1.93 mmol) in dichloromethane (10 mL) was chloroacetyl chloride (1.0 mL) in the presence of anhydrous potassium carbonate. The resultant mixture was stirred at room temperature for 18 h. After the reaction was quenched with water (1.0 mL), the mixture was extracted with dichloromethane (2 × 20 mL), the organic phase was washed with saturated sodium carbonate (10 mL) and brine (10 mL) successively, dried over anhydrous sodium sulfate, and evaporated under reduced pressure to get a residue. The residue was subjected to chromatography using ethyl acetate/petroleum ether (1:5) as the eluent to give 1.02 g of product **3a** (80%) as a white solid, mp 53.5–56.5 °C (ether); IR (KBr, cm^{-1}) ν 3411, 2927, 2858, 1742, 1648; ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 6.1 (s, 1H, NH), 4.9 (s, 1H, 3β-H), 4.63–4.59 (m, 1H, 7β-H), 4.04–3.95 (m, 4H, CH_2Cl), 3.17–3.14 (m, 2H, NCH_2), 0.88 (s, 3H, *18*-CH_3), 0.85(d, 3H, *J* = 6.0 Hz, *21*-CH_3), 0.81(t, 3H, *J* = 7.2 Hz, CH_3CH_2), 0.576 (s, 3H, *19*-CH_3) ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 172.9, 166.2, 165.8, 75.5, 73.1, 55.3, 49.6, 42.1, 40.7, 40.6, 40.1, 38.9, 38.8, 37.4, 34.1, 33.4, 33.0, 31.3, 31.2, 30.6, 29.1, 29.07, 29.04, 29.01, 28.7, 27.4, 26.4, 25.9, 23.0, 22.1, 22.0, 20.0,

17.8, 13.5, 11.1; LC-MS *m*/*z* (%): 712, 78 (M+1, 60%); Anal. calcd. for $C_{40}H_{67}Cl_2NO_5$: C, 67.39; H, 9.47; 9.95; N, 1.96; Found C, 67.49; H, 9.55; N, 2.18.

*2.2.8. 3α,7α-di(2-Chloroacetoyloxy)-N-tetradecylchenodeoxycholamide (**3b**)*

Mp 54.5–55 °C (ether); IR (KBr, cm^{-1}) ν 3411, 2927, 2858, 1742, 1648; ^1H NMR ($CDCl_3$, 600 MHz) δ: 5.58 (s, 1H, NH), 4.95 (s, 1H, 3β-H), 4.64(s, 1H, 7β-H), 3.96–4.63 (m, 4H, CH_2Cl), 3.16–3.19 (m, 2H, NCH_2), 0.91(s, 3H, *18*-CH_3), 0.89 (d, 3H, *J* = 6.6 Hz, *21*-CH_3), 0.84 (t, 3H, *J* = 5.4 Hz, CH_3CH_2), 0.61(s, 3H, *19*-CH_3); ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 173.3, 166.8, 166.4, 76.1, 73.7, 55.8, 50.2, 42.7, 41.2, 41.1, 40.7, 39.5, 39.3, 38.0, 35.4, 34.73, 34.70, 34.4, 34.0, 33.6, 31.9, 31.7, 31.2, 29.6, 29.58, 29.54, 29.34, 29.30, 28.0, 26.9, 26.5, 23.5, 22.6, 22.5, 20.6, 18.3, 14.1, 11.6; LC-MS *m*/*z* (%): 740.47 (M+1, 100%); Anal. calcd. for $C_{42}H_{71}Cl_2NO_5$: C, 68.08; H, 9.66; N, 1.89; Found C, 68.24; H, 9.52; N, 1.98.

*2.2.9. 3α,7α-di(2-Chloroacetoyloxy)-N-hexadecylchenodeoxycholamide (**3c**)*

Mp 54–55.5 °C (ether); IR (KBr, cm^{-1}) ν 3411, 2927, 2858, 1742, 1648; ^1H NMR ($CDCl_3$, 600 MHz) δ: 5.63 (s, 1H, NH), 4.94 (s, 1H, 3β-H), 4.65–4.63 (m, 1H, 7β-H), 4.63–3.96 (m, 4H, CH_2Cl), 3.18–3.15 (m, 2H, NCH_2), 0.9 (s, 3H, *18*-CH_3), 0.88 (d, 3H, *J* = 6.6 Hz, *21*-CH_3), 0.83 (t, 3H, *J* = 6.6 Hz, CH_3CH_2), 0.60 (s, 3H, *19*-CH_3); ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 173.3, 166.8, 166.4, 76.1, 73.7, 55.8, 50.2, 42.7, 41.2, 41.1, 40.7, 39.5, 39.2, 38.0, 35.4, 34.7, 34.4, 34.0, 33.6, 31.9, 31.7, 31.2, 29.6, 29.59, 29.55, 29.35, 29.31, 28.0, 26.9, 26.5, 23.5, 22.6, 22.5, 20.6, 18.3, 14.1, 11.6; LC-MS *m*/*z* (%): 768.97 (M+1, 100%); Anal. calcd. for $C_{44}H_{75}Cl_2NO_5$: C, 68.72; H, 9.83; N, 1.82; Found C, 68.88; H, 9.78; N, 1.96.

*2.2.10. 3α,7α-di(2-Chloroacetoyloxy)-N-octadecylchenodeoxycholamide (**3d**)*

Mp 54.5–56 °C (ether); IR (KBr, cm^{-1}) 3411, 2927, 2858, 1742, 1648; ^1H NMR (CDCl3, 600 Hz) δ: 5.76 (s, 1H, NH), 4.90 (s, 1H, 3β-H), 4.63–4.59 (m, 1H, 7β-H), 4.01–3.99 (m, 4H, CH_2Cl), 3.16–3.12 (m, 2H, NCH_2), 0.88 (s, 3H, *18*-CH_3), 0.86 (d, 3H, *21*-CH_3), 0.81 (t, *J* = 6.6 Hz, 3H, CH_3CH_2), 0.58 (s, 3H, *19*-CH_3); ^{13}C NMR (CDCl3, 150 MHz) δ: 173.3, 166.7, 166.4, 76.1, 73.7, 55.2, 50.2, 42.7, 41.2, 41.1, 40.7, 39.5, 39.3, 38.0, 35.4, 34.72, 34.70, 34.4, 34.0, 33.6, 31.9, 31.7, 31.2, 29.6, 29.58, 29.54, 29.34, 29.30, 28.0, 26.9, 26.5, 23.5, 22.6, 22.5, 20.6, 18.3, 14.1, 11.6; LC-MS *m*/*z* (%): 796.83 (M+1, 100); Anal. calcd. for $C_{46}H_{79}Cl_2NO_5$: C, 69.32; H, 9.99; N, 1.76; Found C, 69.44; H, 9.73; N, 1.88.

*2.2.11. 3α,7α,12α-tri(2-Chloroacetoyloxy)-N-tetradecylcholamide (**3e**)*

Colorless oil, IR (KBr, cm^{-1}) 3300, 2926 2856, 1736 1647, 1544, 1462, 1290, 1062; ^1H NMR (600 MHz, $CDCl_3$, ppm) δ 5.50 (t, *J* = 5.5 Hz, 1H), 5.21 (s, 1H), 5.04 (d, *J* = 2.5 Hz, 1H), 4.66 (t, *J* = 4.5 Hz, 1H), 4.17–3.95 (m, 6H), 3.22 (dd, *J* = 13.4, 6.7 Hz, 2H), 0.94 (s, 3H), 0.88 (t, *J* = 7.0 Hz, 3H), 0.84 (d, *J* = 6.5 Hz, 3H), 0.75 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$, ppm) δ 172.00, 165.81, 165.55, 165.27, 76.37, 74.87, 72.10, 46.41, 44.19, 41.87, 40.16, 39.62, 38.54, 36.93, 33.73, 33.66, 33.21, 32.47, 30.90, 30.49, 30.19, 28.86, 28.44, 28.31, 27.51, 26.16, 25.92, 25.45, 24.20, 21.86, 21.67, 21.29, 16.64, 13.11, 11.09; LC-MS *m*/*z* (%): 854 (M+Na, 100); Anal. calcd. for $C_{44}H_{72}Cl_3NO_7$: C, 63.41; H, 8.71; N, 1.68; Found C, 63.56; H, 8.98; N, 1.73.

*2.2.12. 3α-(2-Chloroacetoyloxy)-N-laurylliithocholamide (**3f**)*

Mp 54.5–56 °C (ether); IR (KBr, cm^{-1}); ^1H NMR (600 MHz, $CDCl_3$, ppm) δ 5.57 (s, 1H), 4.95–4.68 (m, 1H), 4.03 (s, 2H), 3.23 (dd, *J* = 13.2, 6.6 Hz, 2H), 0.93 (dd, *J* = 12.2, 5.8 Hz, 6H), 0.88 (t, *J* = 6.6 Hz, 3H), 0.64 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$, ppm) δ 173.43, 166.79, 76.68, 56.45, 56.11, 42.72, 41.89), 41.21, 40.43, 40.12, 39.51, 35.76, 35.49, 34.92, 34.55, 33.73, 31.93, 29.78, 29.45, 29.32, 28.23, 26.95, 26.45, 26.21, 24.16, 23.26, 22.67, 20.83, 18.38, 14.10, 12.03; LC-MS *m*/*z* (%): 642 (M+Na, 100); Anal. calcd. for $C_{38}H_{66}ClNO_3$: C, 73.57; H, 10.72; N, 2.26; Found C, 73.70; H, 10.60; N, 2.44.

*2.2.13. (3α,5β,7α,17β)-17-(3-(N-laurycarbamoyl)-1-methylpropyl)androstan-3,7-di(oxy-carbonylmethylpyridium) dichloride (**4a**)*

The solution of 3α,7α-di(2-chloroacetoyloxy)-*N*-laurylchenodeoxycholamide (**3a**) (0.5 g, 0.74 mmol) in dried pyridine (10 mL) was stirred at 70–80 °C for 12 h. After the completion of reaction, the mixture was cooled in an ice bath. The excess pyridine was removed by reduced pressure distillation. The light yellow precipitate was filtered off, washed with ethyl acetate (2 × 15 mL), dried in air to give a product **4a** (0.6 g, 99%), which was pure enough for all analytical purposes. Mp 156–158 °C; IR (KBr, cm^{-1}) ν 3422, 3052, 2926, 2854, 1744, 1640, 1550, 1493; ^1H NMR (CD_3OD, 600 MHz) δ: 9.22 (d, 2H, *J* = 5.4 Hz, PyH), 9.19 (d, 2H, *J* = 5.4 Hz, PyH), 8.8 (t, 1H, *J* = 7.8 Hz, PyH), 8.7 (t, 1H, *J* = 7.8 Hz, PyH), 8.29 (t, 2H, *J* = 6.6 Hz, PyH), 8.21 (t, 2H, *J* = 6.6 Hz, PyH), 5.67–5.99 (m, 4H), 5.1(s, 1H), 4.8 (t, *J* = 7.6 Hz, 1H), 3.18–3.23 (m, 2H), 3.12–3.17 (m, 1H), 2.3–2.25 (m, 1H), 1.01–0.99 (m, 6H), 0.91(t, *J* = 7.2 Hz, 3H), 0.71 (s, 3H); ^{13}C NMR (CD_3OD, 150 MHz) δ: 174.6, 165.1, 164.3, 146.3, 146.1, 145.9, 145.8, 127.5, 127.2, 76.6, 74.7, 60.2, 55.4, 49.8, 42.1, 40.1, 38.9, 37.2, 34.8, 34.0, 33.9, 33.8, 33.5, 32.3, 31.4, 31.1, 30.2, 28.9, 28.87, 28.83, 28.7, 28.5, 27.2, 26.1, 25.8, 22.6, 22.3, 21.8, 21.0, 19.7. 16.9, 12.5, 10.2; LC-MS *m*/*z* (%): 834.5 (100); Anal. calcd. for $C_{50}H_{77}Cl_2N_3O_5$: C, 68.94; H, 8.91; N, 4.82; Found C, 68.86; H, 8.78; N, 4.80.

*2.2.14. (3α,5β,7α,17β)-17-(3-(N-tetradecylcarbamoyl)-1-methylpropyl)androstan-3,7-di(oxycarbonylmethylpyridium) dichloride (**4b**)*

Mp 154.5–155.5 °C; IR (KBr, cm^{-1}) ν 3422, 3052, 2926, 2854, 1744, 1640, 1550, 1493; ^1H NMR(CD_3OD, 600 Hz) δ: 9.45 (d, 2H, *J* = 5.4 Hz, PyH), 9.35 (d, 2H, *J* = 5.4 Hz, PyH), 8.82 (t, 1H, *J* = 7.8 Hz, PyH), 8.73 (t, 1H, *J* = 7.8 Hz, PyH), 8.31 (t, 2H, *J* = 6.6 Hz, PyH), 8.25 (t, 2H, *J* = 6.6 Hz, PyH), 6.2–5.78 (m, 4H), 4.88 (s, 1H), 4.65 (s, 1H), 3.04–3.08 (m, 1H), 2.94–2.99 (m, 1H), 0.90 (d, 3H, *J* = 6.6 Hz), 0.87 (s, 3H), 0.85 (t, 3H, *J* = 6.6 Hz), 0.58 (s, 3H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 172.9, 166.5, 165.5, 147.3, 147.2, 146.8, 146.7, 128.4, 128.2, 76.5, 74.8, 60.8, 60.7, 56.0, 50.2, 42.7, 38.6, 37.4, 35.2, 34.6, 34.4, 34.0, 33.0, 32.0, 31.7, 30.8, 29.5, 29.4, 29.2, 29.1, 28.0, 26.8, 26.6, 23.3, 22.5, 20.5, 18.6, 14.4, 11.9; LC-MS *m*/*z* (%): 827.21 (100); Anal. calcd. for $C_{52}H_{81}Cl_2N_3O_5$: C, 69.46; H, 9.08; N, 4.67; Found C, 69.70; H, 9.13; N, 4.78.

*2.2.15. (3α,5β,7α,17β)-17-(3-(N-hexadecylcarbamoyl)-1-methylpropyl)androstan-3,7-di(oxycarbonylmethylpyridium) dichloride (**4c**)*

Mp 155–156.5 °C; IR (KBr, cm^{-1}) ν 3422, 3052, 2926, 2854, 1744, 1640, 1550, 1493; ^1H NMR (CD_3OD, 600 Hz) δ: 9.4 (d, 2H, *J* = 5.4 Hz, PyH), 9.3 (d, 2H, *J* = 5.4 Hz, PyH), 8.82 (t, 1H, *J* = 7.8 Hz, PyH), 8.72 (t, 1H, *J* = 7.8 Hz, PyH), 8.31 (t, 2H, *J* = 6.6 Hz, PyH), 8.25 (t, 2H, *J* = 6.6 Hz, PyH), 5.77–6.10 (m, 4H), 4.88 (s, 1H), 4.6 (s, 1H), 3.08–3.04 (m, 1H), 2.94–2.98 (m, 1H), 0.90 (d, 3H, *J* = 0.6 Hz), 0.87 (s, 3H), 0.85 (t, 3H), 0.58 (s, 3H); ^{13}C NMR (CD_3OD, 150 MHz) δ: 172.9, 166.5, 165.5, 147.3, 147.2, 146.8, 146.7, 128.4, 128.2, 76.5, 74.7, 60.8, 60.7, 56.0, 50.2, 42.7, 38.6, 37.4, 35.2, 34.6, 34.4, 34.0, 32.9, 32.0, 31.7, 31.1, 30.8, 29.5, 29.4, 29.2, 29.1, 28.0, 26.8, 26.6, 23.3, 22.54, 22.51, 28.5, 18.6, 14.3, 11.9; LC-MS *m*/*z* (%): 865.08 (100); Anal. calcd. for $C_{54}H_{85}Cl_2N_3O_5$: C, 69.95; H, 9.24; N, 4.53; Found C, 69.86; H, 9.48; N, 4.66.

*2.2.16. (3α,5β,7α,17β)-17-(3-(N-octadecylcarbamoyl)-1-methylpropyl)androstan-3,7-di(oxycarbonylmethylpyridium) dichloride (**4d**)*

Mp 153–154 °C; IR (KBr, cm^{-1}) ν 3422, 3052, 2926, 2854, 1744, 1640, 1550, 1493; ^1H NMR (DMSO-d6, 600 MHz, ppm) δ: 9.18 (s, 2H), 9.14 (s, 2H), 8.7 (s, 1H, *J* = 7.8 Hz, PyH), 8.6 (s, 1H, *J* = 7.8 Hz, PyH), 8.2 (s, 2H, *J* = 6.6 Hz, PyH), 8.1 (s, 2H, *J* = 6.6 Hz, PyH), 5.6–5.9 (m, 4H), 5.03 (s, 1H), 4.7 (s, 1H), 3.1–3.16 (m, 2H), 0.93 (s, 6H), 0.85 (s, 3H), 0.65 (s, 3H); ^{13}C NMR (DMSO-d6, 150 MHz, ppm) δ: 175.1, 165.6, 164.9, 146.6, 146.4, 146.3, 128.0, 127.7, 77.1, 75.2, 60.7, 55.9, 50.3, 42.6, 40.7, 39.4, 39.0, 37.8, 35.3, 34.5, 34.4, 34.3, 34.0, 32.8, 32.0, 31.7, 30.7, 29.4, 29.3, 29.2, 29.0, 27.7, 26.6, 26.3, 23.2, 22.86, 22.83, 22.3, 21.6, 20.3, 17.5, 13.1, 10.8; LC-MS *m/z* (%): 884.12 (100); Anal. calcd. for $C_{56}H_{89}Cl_2N_3O_5$: C, 70.41; H, 9.39; N, 4.40; Found C, 70.60; H, 9.52; N, 4.32.

*2.2.17. (3α,5β,7α,12α,17β)-17-(3-(N-tetradecylcarbamoyl)-1-methylpropyl)androstan-3,7,12-tri(oxycarbonylmethylpyridium) trichloride (**4e**)*

Mp 150–152 °C; IR (KBr, cm^{-1}) ν 3412, 3062, 2924, 2856, 1743, 1645, 1554, 1483; ^1H NMR (600 MHz, $CDCl_3$, ppm) δ 9.80–9.37 (m, 6H), 9.02–8.47 (m, 3H), 8.47–7.94 (m, 6H), 7.56 (s, 1H), 5.26 (s, 1H), 5.01 (s, 1H), 4.67 (s, 1H), 3.78 (s, 6H), 3.34–3.08 (m, 2H), 0.87 (dd, *J* = 14.0, 6.7 Hz, 9H), 0.71 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$, ppm) δ 174.57, 166.25, 165.56, 164.87, 147.21, 147.11, 146.71, 146.50, 145.91, 128.73, 128.13, 127.80, 78.25, 76.50, 74.06, 61.30, 61.15, 60.82, 45.77, 45.14, 43.30, 40.24, 39.62, 37.55, 34.29, 33.96, 31.92, 31.45, 30.88, 29.70, 29.41, 28.86, 27.20, 26.23, 25.66, 22.81, 38.3, 22.02, 17.87, 14.13, 12.25; LC-MS *m/z* (%): 1033.5 (100); Anal. calcd. for $C_{59}H_{87}Cl_3N_4O_7$: C, 66.18; H, 8.19; N, 5.23; Found C, 66.22; H, 8.35; N, 5.40.

*2.2.18. (3α,5β,17β)-17-(3-(N-laurylcarbamoyl)-1-methylpropyl)androstan-3-oxycarbonyl methylpyridium chloride (**4f**)*

Mp 141–144 °C; IR (KBr, cm^{-1}) ν 3411, 3052, 2924, 2829, 1735, 1645, 1560, 1483; ^1H NMR (600 MHz, $CDCl_3$, ppm) δ 9.32 (s, 2H), 8.55 (s, 1H), 8.09 (s, 2H), 6.10 (s, 2H), 5.74 (s, 1H), 4.81 (s, 1H), 3.22 (d, *J* = 5.6 Hz, 2H), 0.98–0.86 (m, 9H), 0.64 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$, ppm) δ 173.56, 165.60, 146.71, 146.06, 127.81, 78.32, 61.22, 56.49, 56.33, 56.14, 42.72, 41.95, 40.38, 40.02, 39.54, 35.77, 35.42, 34.89, 34.54, 33.65, 31.95, 29.96, 29.46, 29.32 28.21, 26.95, 26.36, 24.17, 23.22, 22.66, 20.84, 18.42, 14.10, 12.05; LC-MS m/z (%): 663.12 (100); Anal. calcd. for $C_{43}H_{71}ClN_2O_3$: C, 73.83; H, 10.23; N, 4.00; Found C, 73.96; H, 10.38; N, 4.22.

2.3. Surface tension and critical micelle concentration

Surface tensions of aqueous solutions were measured at room temperature with a Du Nöuy tensiometer (Hurdson & Co., Kolkata) by ring detachment method. Before each experiment, the instrument was calibrated and checked by measuring the surface tension of distilled water. A stock solution of sample was made in double distilled water. Aliquot of this solution was transferred to a beaker containing known volume of water. The solution was gently stirred magnetically and allowed to stand for about 5 min and then surface tension was measured. Measurements were repeated until three successive readings gave γ values within ±0.1 mN m^{-1} difference. The CMC was obtained from the breakpoint of plot of surface tension (γ) versus ln*C*. These title compounds solutions were prepared at the different concentrations. Their surface tensions were measured at balance time between 2 and 5 h by DCATII-type surface tensiometer via the Wilhelmy plate method. The results were shown in Table 4.

Table 4
The CMC and surface tensions of these title compounds.

Compound	CMC (mmol L^{-1})	Surface tension (mN m^{-1})
Water	–	72.26
Chenodeoxycholic acid	0.1579	57.47
4a	0.0518	42.28
4b	0.0405	43.08
4c	0.0277	42.10
4d	0.0047	38.25
4e	0.0078	39.18
4f	0.0782	48.72

Table 5
Antibacterial activity of compounds **4a–f** against *B. subtilis*, *S. longisporum*, *E. coli* and *S. enteritidis*.

Compound	Organism (MIC) (μg mL^{-1})			
	B. subtilis	*S. longisporum*	*E. coli*	*S. enteritidis*
4a	30	21	26	>32
4b	28	16	24	>32
4c	22	12	8	10
4d	1.2	1.6	0.8	1.6
4e	12	10	3.6	8
4f	28	25	16	>32

2.4. Antimicrobial activity

The bacterial strains used for the analysis were *Bacillus subtilis* (ATCC 6633), *Streptosporangium longisporum* (ACCC40732), *Escherichia coli* (ATCC 11229), and *Salmonella enteritidis* (ATCC 13076). Compounds were solubilized in DMSO (2.5%, v/v) and were tested using serial dilutions ranging from 0.04 to 32 μg mL^{-1}. Test isolates were maintained as frozen stocks which were thawed and adjusted to inoculum density of approximately 5×10^5 cfu mL^{-1}. Following inoculation, microtitre assay plates were incubated at 35 °C for 24 h. The minimal inhibitory concentrations (MICs) of compounds were tested by a 2-fold microdilution method (Table 5).

3. Results and discussion

3.1. Chemistry

Because of the biological compatibility of bile acids, there is great interest in preparing bile acid-derived important amphiphilic molecules. Various bile acid-based amphiphilic molecules were reported as anti-microbial agents [27]. Investigations showed that several bile acid-derived facial amphiphiles could improve the permeability of membranes including bacterial cell wall [28]. Bile acids have attracted significant attention due to availability and the orientation of the hydroxy groups that may be exploited in facial amphiphiles [17,18]. However, there is no method available for the modification on the carbonyl group to strengthen hydrophobicity of the hydrophobic head. Although, on the hydrophobic β-face of the bile acid unit, there is a weak hydrophilic amide, the molecule is overall hydrophobic on the convex hydrophobic face of the unit during the amide coupling of carboxyl- and amino-terminated long hydrocarbon.

In general, amides could be prepared easily via the reaction between acyl halides and amine group. Thus, based on the reported method [29], firstly, the protection on hydroxyl group of bile acid was carried out as a formate and the reaction of bile acid derivatives with oxalyl chloride gave the corresponding acyl chloride. Following the reaction between acyl halide and a long-chain aliphatic amine yielded the desired product, but the total yield of the desired product was too low to apply to synthesize the designed target molecules by the multistep route.

Considering the synthesis of these bile acid-derived facial amphiphiles designed, we needed a stable amide linker with a long hydrocarbon tail as a hydrophobic end for the formation of new bile acid-derived surfactants. Fortunately, the one-pot reaction of bile acid with unprotected hydroxy groups as a starting material and a long-chain aliphatic amine in the presence of HOBt and DCC could give directly the desired amide derivatives **2a**–**f** in 65–92% yields (Scheme 1, Table 1). The structural assignment was confirmed by NMR: among other signals the ^{1}H NMR spectrum shows one proton bonded to nitrogen of amide bond as a broad single peak in 5.49–6.12 ppm. Following, treatment of steroids **2a**–**f** with chloroacetyl chloride in the presence of K_2CO_3 at room temperature for 18 h gave compounds **3a**–**f** in 75–93% yields (Scheme 1, Table 2). Subjection of chlorosteroidal esters **3a**–**f** to standard quaternary ammoniation with anhydrous pyridine at 70–80 °C overnight afforded compounds **4a**–**f** in quantitative yields (Scheme 1, Table 3).

3.2. Characterization of aggregation behavior

Amphiphiles **4a**–**f** are soluble in water and form translucent solutions. All are water-soluble in the ammonium form. The main difference between the six amphiphiles is the number of charged groups: **4a**–**d** has two ammonium cations respectively, **4e** has three ammonium cations, and **4f** has only one ammonium cation. Although their chemical structure and physicochemical characteristics are very different from those of the common that are surfactants characterized by a hydrophilic head and a hydrophobic tail, the modified bile acid derivatives are cationic and rigid biplanar amphiphiles containing a flexible long hydrophobic hydrocarbon tail and two methyl groups on the convex hydrophobic face and up to three ammonium cations on the concave hydrophilic face.

Tensiometric measurements allowed us to determine the main tensioactive properties of these amphiphiles (Table 4). For all compounds tested, the surface tension is observed to linearly decrease as amphiphile concentration increases, showing breaks corresponding to the critical micelle concentration (CMC) depending on the number of charged groups and the hydrophobic chain length, as for a conventional surfactant.

The results in Table 4 indicated that the modified amphiphiles **4a**–**f** possess tensiometric properties compared with chenodeoxycholic acid. Different behaviors were observed for **4a** to **4d** amphiphiles with the same number of charged groups as the hydrophobic tail increased in length. An increase in the length of the hydrophobic tail results in a decrease in the CMC. Comparisons between **4a** and **4f**, **4b** and **4e** at the same length of the hydrophobic tail are shown in Table 4. An increase in the number of charged groups also results in a decrease in the CMC.

3.3. Biological activity

The antimicrobial activity of these compounds was tested against Gram-positive *B. subtilis*, *S. longisporum* and Gram-negative *E. coli*, *S. enteritidis*. Ofloxacin served as the positive control. The test results are shown in Table 5. Although, from Table 5 we can deduce that the inhibitory activity of all the compounds studied is worse than that of ofloxacin, most of the six compounds demonstrated a potent antimicrobial activity against *B. subtilis, S. longisporum, E. coli* and *S. enteritidis.*

Among them, compounds **4c**, **4d** and **4e** showed similar inhibitory activity to that of ofloxacin, as they all contain up to three hydrophilic ammonium cations and a longer hydrophobic alkyl. These compounds might take effect by disrupting the integrity of the cell membrane instead of inhibiting the enzymatic activity inside the cell. The inhibitory activity of compound **4d** against *B. subtilis, S. longisporum, E. coli* and *S. enteritidis* was stronger than that of compounds **4c** and **4e**. This showed that the existence of a flexible long hydrocarbon tail and a quaternary ammonium plays a critical role in the inhibitory activity of these compounds against *B. subtilis, S. longisporum, E. coli* and *S. enteritidis*. Further, the growth suppressive effect of compounds **4d** and **4a** exhibited a significant difference because of the effect of the length of a flexible long hydrocarbon tail. Although the mode of antibacterial action of cationic amphiphiles **4a**–**f** is not known, it is known that quaternary ammonium salts have antimicrobial and antibacterial activities, which depend upon the lipophilicity of these compounds [30]. The critical micelle concentration (CMC) is one of the important parameters that describe lipophilicity of a compound. The nature of interactions between amphiphilic molecules and proteins and bacterial cells is strongly related to the CMC value. It has been found that the highest antibacterial and antimicrobial activity is observed at lower CMC. Thus, further analyses of structure–activity relationships of these compounds would make it possible to design novel synthetic antimicrobial agents. In this regard, the high activity of **4d** and **4e** against *B. subtilis, S. longisporum, E. coli* and *S. enteritidis* provides potential lead compounds for the development of new antimicrobial agents.

4. Conclusion

In summary, we have successfully developed a novel and operationally simple synthetic route for highly efficient synthesis of some bile acid-derived cationic amphiphiles with a flexible long hydrocarbon tail. Firstly, the modification on the side-chain carboxyl of bile acids was carried out efficiently by one-pot amidation of bile acids and a long-chain aliphatic amine in the presence of HOBt and DCC to introduce a flexible long hydrocarbon tail. Then the hydrophilic concave side of bile acids with hydroxyl groups was further modified into other functional groups to serve positive charges for strengthening hydrophilicity. This strategy offered a very straightforward and efficient method for access to the designed amphiphiles in good overall yields. The preliminary results showed that an increase both in the length of the hydrophobic tail and in the number of charged groups results in a decrease in the CMC of bile acid-derived cationic amphiphiles. And the bile acid-derived cationic amphiphiles with a flexible longer hydrocarbon tail and more positive charges had the highest antibacterial and antimicrobial activity. Due to the structural features of our novel compounds, this mechanism of action cannot be discarded. Further research on the structure–activity relationship, their possible mechanism of inhibiting bacteria and the development of the title compounds as promising antimicrobial agents is ongoing.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bioorg.2013.08.003.

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