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Bile acid amides derived from chiral amino alcohols: novel antimicrobials and antifungals

Braja G. Hazra,[a,*] Vandana S. Pore,[a,*] Sanjeev Kumar Dey,[a,†] Suchitra Datta,[a,†] Mahendra P. Darokar,[b] Dharmendra Saikia,[b] S. P. S. Khanuja[b] and Anup P. Thakur[a]

[a]*Organic Chemistry Synthesis Division, National Chemical Laboratory, Pune 411 008, India*
[b]*Central Institute of Medicinal and Aromatic Plants, Near Kukrail Picnic Spot, Lucknow 226 015, India*

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Abstract—Cholic and deoxycholic acid amides **10**–**17** have been synthesised from (1*R*,2*R*)-1-phenyl-2-amino-1,3-propanediol **2**, (1*S*,2*S*)-1-phenyl-2-amino-1,3-propanediol **4**, (1*R*,2*R*)-1-*para*-nitrophenyl-2-amino-1,3-propanediol **3**, (1*S*,2*S*)-1-*para*-nitrophenyl-2-amino-1,3-propanediol **5**. Amide **12** derived from *N*-succinimidyl ester **9** of deoxycholic acid and (1*R*,2*R*)-1-phenyl-2-amino-1,3-propanediol **2**, found to be active against *Cryptococcus neoformans* and the amide **17** obtained from *N*-succinimidyl ester **9** of deoxycholic acid and (1*S*,2*S*)-1-*para*-nitrophenyl-2-amino-1,3-propanediol **5**, is found to be potent against various gram-positive bacteria.

1. Introduction

Due to the increasing number of drug-resistant bacteria, the need for new antibacterial agents is well recognised. Recently a group of antimicrobial agents that has received considerable attention is, membrane active cationic peptide antibiotics.[1] These compounds disrupt or permeabilise the bacterial membrane and sensitise these organisms to hydrophobic antibiotics.

Peptide antibiotics are relatively large (>20 amino acids) and are difficult to derivatise and purify. Therefore, attention has been made to develop simpler and smaller compounds that display activities similar to those of endogenous peptide antibiotics for potential clinical use and to aid in the elucidation of how the simple and small molecules disrupt the bacterial cell wall. The modification of steroids with polyamines, hydroxy groups and sulphonic acid functionalities in the development of cationic peptide antibiotic mimics are well documented.[2,3]

Cholic acid **6** and deoxycholic acid **7** have attracted significant attention due to availability and the orientation of the hydroxy groups, that may be exploited in podant-type receptors,[4] linear dimeric hosts[5] or facial amphiphiles.[6] In addition, bile acids are natural ligands specifically recognised by hepatitic cells and are amphiphilic molecules that undergo a biological recycling during enterohepatic circulation.[7] Several cholic acid derived facial amphiphiles have been reported that improves the permeability of membranes such as bacterial cell wall.[8] Novel steroid-based antimicrobials have been prepared[2] as mimics of squalamine, a steroidal polyamine isolated[9] from dogfish shark. Very recently there are two reports[1,10] of antimicrobial activity of cholic acid derivatives.

Chloramphenicol **1** is the first broad-spectrum antibiotic[11] isolated from aerobic broth cultures of an actinomycete, *Steptomyces venezuelae*. It is microbially active against a wide range of gram-positive and gram-negative bacteria and is used for the treatment of typhoid, dysentery and bacterial infections in the eye. In this paper, we would like to disclose the synthesis of eight new amides **10**–**17** derived from chiral amino alcohols **2**–**5** and cholic acid **6** and deoxy cholic acid **7** (Scheme 1). The antibacterial and antifungal activities of these novel amides **10**–**17** are also presented. Some of these easily accessible amides show moderate antibacterial and antifungal activities.

We have replaced the dichloroacetamido moiety (–NHCOCHCl$_2$) of chloramphenicol **1** and its enantiomer with

* Corresponding author. Tel./Fax: +91-20-5893153; e-mail: hazra@ems.ncl.res.in
† Indian Institute of Technology, Kharagpur 721 302, India.

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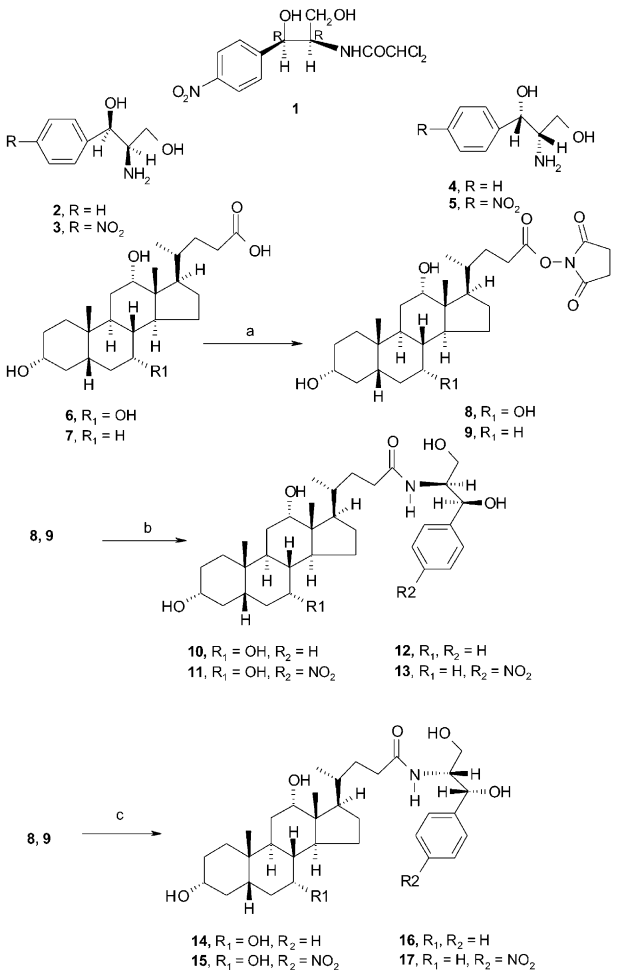

Scheme 1. Reagents and conditions: (a) *N*-Hydroxy succinimide, DCC, THF–CH_3CN, 25 °C, 18 h, **8**, 97% yield **9**, 84% yield; (b) (1*R*,2*R*)-1-phenyl-2-amino-1,3-propanesiol, DMF, 25 °C, 1.5 h (**10**, 92% yield, **12**, 88% yield), or (1*R*,2*R*)-1-*para*-nitrophenyl-2-amino-1,3-propanediol, DMF, 25 °C, 1.5 h (**11**, 86% yield, **13**, 81% yield); (c) (1*S*,2*S*)-1-phenyl-2-amino-1,3-propanediol, DMF, 25 °C, 1.5h (**14**, 90% yield, **16**, 92% yield), or (1*S*,2*S*)-1-*para*-nitrophenyl-2-amino-1,3-propanediol, DMF, 25 °C, 1.5 h (**15**, 79% yield, **17**, 82% yield).

the amide derived from C-24 carboxylic group of cholic acid **6** and deoxycholic acid **7**. Activation of the C-24 carboxylic acid moiety of cholic acid **6** and deoxycholic acid **7** has been carried out with *N*-hydroxysuccinimide, DCC in THF to afford the *N*-succinimidyl esters **8** in 97% yield and **9** in 84% yield. Formation of the amides **10**–**17** from the activated esters **8** and **9** with amines **2** to **5** is facile and take place in DMF at 28 °C in 1.5 h in very good yield. It was not necessary to protect the amino alcohols **2**–**5**. Thus, the reaction of amino alcohols (−)-(1*R*,2*R*)-1-*para*-nitrophenyl-2-amino-1,3-propanediol **3** and its enantiomer (+)-(1*S*,2*S*)-1-*para*-nitrophenyl-2-amino-1,3-propanediol **5**, with the activated cholic acid ester **8** furnished the amides **11** and **15** and with the activated deoxycholic acid ester **9** afforded amides **13** and **17**. Similarly amino alcohol **2** and its enantiomer **4**, which do not have *p*-nitro group in the phenyl ring were also used[11] for the formation of amide with compounds **8** to give products **10** and **14**. Reaction of the same amino alcohols **2** and **4** with compound **9** furnished the amides **12** and **16**.

2. Biological activity of compounds 10–17

The data in Table 1 shows that some of the newly synthesised amides are active against gram-positive bacteria. Among them compound **17**, synthsised from deoxycholic acid **7** and amino alcohol **5** shows moderate antibacterial activity against most of the gram positive bacteria while compounds **10**, **13**, **14**, **15** and **16** are active against some of the gram positive bacteria.

All the new compounds **10**–**17** were tested for antifungal activity against pathogenic fungi *Aspergillus niger*, *Aspergillus flavus*, *Trichophyton rubrum*, *Microsporum gypseum*, *Cryptococcus neoformans*, *Candida albicans*, *Sporothrix schenckii*, *Histoplasma capsulatum* using Amphotericin-B, Nystatin, Clotrimazol as standard antifungal drugs. Only compound **12**, which was synthesised from deoxycholic acid **7** and amino alcohol **2** is found to be active (IC_{50}, 62.5 μg/mL) against pathogenic fungus *C. neoformans* (Table 2).

We report here efficient synthesis of eight new amides **10**–**17** derived from chiral amino alcohols **2**–**5** and *N*-succinimidyl esters of bile acids **8** and **9** by simple coupling in DMF. The new amides **10, 13, 14, 15, 16** and **17** show moderate antibacterial and compound **12** shows antifungal activity. These new compounds might lead to the development of new drugs.

3. Experimental

All solvents and reagents used were of commercial grade. Reactions were monitored by TLC using TLC aluminium sheets, silica gel $60F_{254}$ precoated, Merck, Germany and locating the spots spraying with ethanolic solution of phosphomolybdic acid followed by heating. FTIR-8400, Shimadzu. ^1H NMR: Bruker AC 200 (200 MHz) and MSL 300 (300 MHz). For ^1H NMR, $CDCl_3$ as solvent and TMS as an internal standard and *J* values are given in Hz. ^{13}C NMR: Bruker AC 200 (50 MHz), MSL 300 (75 MHz) and DRX 500 (125 MHz). MS: Finnigan Mat 1020C (70 ev). Optical rotations: JASCO-181 Digital polarimeter using a sodium light (λ = 5893 Å) source. Elemental analyses were carried out in the Analytical Section of the Department. Melting points (uncorrected): Yanaco Micro melting point apparatus. Yields refer to crystallised material or homogeneous products (TLC) obtained by column chromatography.

3.1. Typical procedure for the synthesis of *N*-succinimidyl ester 8 of cholic acid 6 and the ester 9 of deoxycholic acid 7

To a solution of cholic acid **6** (0.2 g, 0.489 mmol) in dry THF (4 mL) and dry acetonitrile (1 mL) *N*-hydroxy succinimide (0.067 g, 0.589 mmol) was added. To the resulting homogeneous solution dicyclohexylcarbodiimide (0.1g, 0.489 mmol) in dry THF (2 mL) was added dropwise at 10–15 °C. The mixture was stirred at 25 °C for 18 h and the precipitated *N*,*N*-dicyclohexyl urea was removed by filtration. THF was removed

Table 1. Antibacterial activity: inhibitory concentration (IC_{50} in μg/mL) of compounds **10**–**17** against pathogenic bacteria determined by two-fold Micro-broth dilution assay

Compound No.	Bacterial strains								
	SA	SE	SM	EF	EC	ST	STM	EA	SF
10	>250	**20**	60	**40**	>250	>250	>250	>250	>250
11	75	>250	**40**	>250	>250	>250	>250	>250	>250
12	>250	220	60	>250	>250	>250	>250	>250	>250
13	>250	>250	**30**	**10**	>250	>250	>250	>250	>250
14	>250	**40**	**40**	60	>250	>250	>250	>250	>250
15	75	100	**06**	60	>250	>250	>250	>250	>250
16	**40**	**40**	**25**	60	>250	>250	>250	>250	>250
17	**7.5**	**7.5**	**04**	**15**	>250	>250	>250	>250	>250
Standard-1 (Vancomycin)	0.12	0.3	0.12	0.06	>250	>250	>250	>250	>250
Standard-2 (Streptomycin)	0.3	0.12	0.12	1.25	1.25	1.25	1.25	1.25	1.25

Gram-positive bacteria:
Staphylococcus aureus (SA)
Staphylococcus epidermidis (SE)
Streptococcus mutans (SM)
Enterococcus faecalis (EF)

Gram-negative bacteria:
Escherichia coli (EC)
Salmonella typhi (ST)
Salmonella typhimurium (STM)
Enterobacter aerogens (EA)
Shigella flexenerii (SF)

Table 2. Antifungal activity: inhibitory concentration (IC_{50}) in μg/mL of **10**–**17** against pathogenic fungi determined by two-fold micro-broth dilution assay

Compd	Fungal strains							
	AN	AF	TR	MG	CN	CA	SS	HC
10	>250	>250	>250	>250	>250	>250	>250	>250
11	>250	>250	>250	>250	>250	>250	>250	>250
12	>250	>250	>250	>250	**62.5**	>250	>250	>250
13	>250	>250	>250	>250	>250	>250	>250	>250
14	>250	>250	>250	>250	>250	>250	>250	>250
15	>250	>250	>250	>250	>250	>250	>250	>250
16	>250	>250	>250	>250	>250	>250	>250	>250
17	>250	>250	>250	>250	>250	>250	>250	>250
Amphotericin-B	2.5	5.0	2.5	2.5	—	0.0625	5.0	—
Nystatin	2.5	2.5	2.5	3.125	—	0.25	3.125	—
Clotrimazol	5.0	0.5	0.625	0.625	—	0.03125	0.3125	

AN, *Aspergillus niger*; AF, *Aspergillus flavus*; TR, *Trichophyton rubrum*; MG, *Microsporum gypseum*; CN, *Cryptococcus neoformans*; CA, *Candida albicans*; SS, *Sporothrix schenckii*; HC, *Histoplasma capsulatum*.

under reduced pressure and the residue was extracted with ethyl acetate (3×20 mL) and the extract was washed successively with aqueous $NaHCO_3$, water and then with brine. The extract was dried over Na_2SO_4 and ethyl acetate was removed under reduced pressure to get crude solid. This was crystallised from ethyl acetate–hexane (**8**, 0.241 g, 97%), mp 118 °C (lit.[12] mp 119–120 °C), IR (Nujol) cm^{-1} 3325 (OH), 1626, 1574 (amide), ^1H NMR ($CDCl_3$), 200 MHz, δ, ppm: 0.68 (s, 3H, 18-H), 0.87 (s, 3H, 19-H), 1.02 (d, 3H, 21-H, $J=4.2$ Hz), 2.61 (m, 2H, 23-H), 2.82 (s, 4H), 3.42 (m, 1H, 3-H), 3.83 (m, 1H, 7-H), 3.98 (m, 1H, 12-H).

In a similar manner, deoxycholic acid **7** (3.92 g, 10 mmol), *N*-hydroxy succinimide (1.63 g, 14 mmol) afforded the ester **9** (4.13 g, 84%) which was recrystallised from ethanol, mp 194–196 °C, IR (Nujol) cm^{-1} 3325 (OH), 1625 (amide), ^1H NMR ($CDCl_3$), 200 MHz, δ, 0.68 (s, 3H, 18-H), 0.89 (s, 3H, 19-H), 1.01 (d, 3H, 21-H, $J=4.2$ Hz), 2.63 (m, 2H), 2.82 (s, 4H), 3.60 (m, 1H), 3.98 (m, 1H), Mass m/z 489 (M^+), 472, 453, 438, 423, 399, 373, 357, 341, 330, 313, 291, 273, 255, 115, 81, 54 (100%), Anal. found C, 68.48, H, 8.92, N, 2.97%; $C_{28}H_{43}NO_6$ requires: C, 68.71, H, 8.79, N, 2.86%.

3.2. Typical procedure for the synthesis of amides 10–17 from *N*-succinimidyl ester 8 of cholic acid, ester 9 of deoxycholic acid and aminodiols 2–5

N-Succinimide ester **8** of cholic acid (0.505 g, 1.0 mmol) was dissolved in dry DMF (2 mL) and to this homogenous solution (1*R*,2*R*)-1-phenyl-2-amino-1,3-propanediol **2** (0.267 g, 1.6 mmol) in dry DMF (1 mL) was added. The reaction mixture was stirred at 25 °C for 1.5 h and the mixture was poured on crushed ice. The solid product was filtered and was washed with water. The crude product was purified by flash chromatography over deactivated alumina to give pure product **10** (0.509 g, 92%) which was crystallised from methanol, mp 235–237 °C, IR (Nujol) cm^{-1} 3280 (OH), 1651 (amide), ^1H NMR ($CDCl_3 + CD_3OD$), 200 MHz, δ, 0.39 (s, 3H, 18-H), 0.61 (s, 3H, 19-H), 0.67 (d, 3H, 21-H, $J=4.0$ Hz), 3.09 (m, 1H), 3.42 (s, 1H), 3.55 (m, 1H), 3.65 (m, 1H), 3.77 (m, 1H), 3.93 (m, 1H), 4.69 (m, 1H, benzylic-H), 7.03 (m, 5H, Ar–H), ^{13}C NMR (DMSO-d_6) 13.05,

17.79, 23.31, 23.56, 27.13, 27.97, 29.19, 29.22, 31.01, 31.02, 32.57, 33.12, 35.58, 35.88, 38.85, 40.28, 41.35, 42.09, 42.20, 46.98, 56.90, 61.20, 67.01, 70.50, 71.24, 71.79, 126.93 (two carbons), 127.12, 128.36 (two carbons), 144.31, 173.79, Mass *m/z* ($C_{33}H_{51}NO_6$, 557) 539 (M^+-18), 521, 509, 482, 472, 454, 433, 400, 378, 355, 337, 314, 295, 271, 204 (100%), 119, 91, 77, $[\alpha]_D^{32}$ +3.19 (MeOH, *c* 2.2). Anal. found C, 70.82, H, 9.29, N, 2.72%; $C_{33}H_{51}NO_6$ requires: C, 71.09, H, 9.15, N, 2.51%.

Spectral and other data of the amides **11**–**17** is given in ref 13.

Protocol:

A broth microdilution method was used for all the compounds and standard antibiotics.[14] The inoculum was prepared by diluting the cells to obtain 10^6 cfu/mL in phosphate buffered saline. The cells were then inoculated in the microtitre plate to the final titre of 10^3 cells per 150 μL of medium. 2-fold dilutions of the test compounds were performed and added to the microtitre plate already inoculated with the test micro-organisms to produce the range of dilutions. Also included on the microtitre plate were controls containing 100 μL of medium and 100 μL of inoculums, a solvent control containing an amount of solvent equivalent to that of the highest concentration in plate. The microtitre plate was then incubated at 28 and 37 °C for fungal and bacterial strains respectively. The plates were then read at 600 nm using SpectraMAX 190 (Molecular Devices) to obtain the OD. The IC_{50} concentration is that concentration of the compound where the growth of the microorganism is 50% that of the control inoculum.

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13. Spectral and other data of compounds **11**–**17**.
(1*R*,2*R*)-1-*para*-Nitophenyl-2-cholicacetamidopropane-1,3-diol (11): Yield 86%, mp 203–204 °C (from methanol–chloroform), IR (Nujol) cm^{-1} 3330 (broad, OH, NH), 1643 (amide), ^1H NMR (DMSO-d_6), 200 MHz, δ 0.38 (s, 3H, 18-H), 0.66 (1s and 1d, 6H, 19 and 21-H), 3.97 (m, 4H), 4.30 (m, 1H), 4.85 (m, 2H), 5.74 (d, 1H, $J=6.6$ Hz), 7.44 and 8.02 (AB q, 4H, Ar–H), ^{13}C NMR (DMSO-d_6) 12.61, 17.35, 22.90, 23.12, 26.54, 27.50, 28.86, 30.69, 32.16, 32.72, 34.70, 35.22, 35.44, 35.62, 39.77, 41.65, 41.87, 46.06, 46.50, 56.06, 60.87, 66.68, 69.77, 70.87, 71.46, 79.44, 123.07 (two carbons), 127.70 (two carbons), 146.59, 152.33, 173.39, Mass *m/z* ($C_{33}H_{50}N_2O_8$, 602) 568 (M^+-34), 372, 271, 199, 150, 91 (100%), 77, $[\alpha]_D^{32}$ +17.2 (MeOH, $c=1.8$). Anal. found C, 65.59, H, 8.43, N, 4.72%; $C_{33}H_{50}N_2O_8$ requires C, 65.78, H, 8.31, N, 4.65%.
(1*R*,2*R*)-1-Phenyl-2-deoxycholicacetamidopropane-1,3-diol (12): Yield 88%, mp 238–239 °C (from methanol–chloroform), IR (Nujol) cm^{-1} 3362 (broad, OH, NH), 1643 (amide), ^1H NMR ($CDCl_3+CD_3OD$), 300 MHz, δ 0.57 (s, 3H, 18-H), 0.84 (s, 3H, 19-H), 0.86 (d, 3H, 21-H, $J=4.0$ Hz), 3.26 (m, 1H), 3.47 (m, 1H), 3.61 (m, 1H), 3.84 (m, 1H), 3.98 (m, 1H), 4.92 (d, 1H, $J=2$ Hz), 7.20 (m, 5H), ^{13}C NMR ($CDCl_3+$DMSO-d_6) 10.82, 15.43, 21.47, 21.92, 24.49, 25.43, 25.56, 27.01, 28.60, 30.10, 30.92, 31.36, 32.25, 33.47, 33.56, 34.08, 34.68, 40.09, 44.38, 44.57, 45.88, 54.62, 59.04, 68.38, 68.48, 69.57, 124.56 (two carbons), 124.84, 125.90 (two carbons), 142.01, 171.34, Mass *m/z* 491 (M^+-50), 453, 417, 398, 380, 356, 339, 316, 273, 255, 228, 191, 91 (100%), 77, $[\alpha]_D^{32}$ +27.92 (MeOH, *c* 1.2). Anal. found C, 73.09, H, 9.59, N, 2.72%; $C_{33}H_{51}NO_5$ requires C, 73.20, H, 9.42, N, 2.59%.
(1*R*,2*R*)-1-*para*-Nitophenyl-2-deoxycholicacetamidopropane-1,3-diol (13): Yield 81%, mp 238–239 °C (from methanol–chloroform), IR (Nujol) cm^{-1} 3355 (OH), 1645 (amide), ^1H NMR (DMSO-d_6), 200 MHz, δ 0.50 (s, 3H, 18-H), 0.80 (1s and 1d, 6H, 19 and 21-H), 3.72 (m, 1H), 3.93 (m, 1H), 4.12 (m, 1H), 4.45 (m, 1H), 4.76 (m, 1H), 5.03 (m, 1H), 5.74 (d, 1H, $J=4$ Hz), 7.57 and 8.09 (AB q, 4H, Ar–H), ^{13}C NMR (200 MHz, DMSO-d_6) 12.17, 16.76, 22.83, 23.23, 25.88, 26.80, 28.38, 30.00, 31.58, 32.09, 32.75, 33.60, 34.77, 34.82, 35.47, 36.06, 40.55, 41.46, 45.77, 46.02, 47.20, 55.54, 60.39, 69.33, 69.80, 70.87, 122.44 (2 carbons), 127.11 (2 carbons), 146.08, 151.74, 172.73, Mass *m/z* 552 (M^+-34), 416, 375, 356, 301, 273, 255 (100%), 204, 121, 91, 77, $[\alpha]_D^{32}$ +31.43 (MeOH, *c* 1.4). Anal. found C, 67.31, H, 8.93, N, 4.72%; $C_{33}H_{50}N_2O_7$ requires C, 67.58, H, 8.53, N, 4.78%.
(1*S*,2*S*)-1-Phenyl-2-cholicacetamidopropane-1,3-diol (14): Yield 90%, mp 215–217 °C (from methanol), IR (Nujol) cm^{-1} 3591, 3344 (OH), 1658 (amide), ^1H NMR ($CDCl_3+CD_3OD$), 200 MHz, δ, 0.48 (s, 3H, 18-H), 0.73 (s, 3H, 19-H), 0.77 (d, 3H, 21-H, $J=4.0$ Hz), 3.15 (m,

1H), 3.22 (m, H), 3.46 (m, 1H), 3.62 (m, 1H), 3.72 (m, 1H), 3.77 (m, 1H), 3.88 (m, 1H), 4.82 (m, 1H), 7.18 (m, 5H, Ar–H), ^{13}C NMR (500 MHz, DMSO-d_6) 13.25, 17.87, 23.46, 23.73, 27.06, 28.21, 29.32, 31.12, 32.68, 33.47, 35.27, 35.69, 36.04, 36.13, 40.26, 40.35, 42.23, 42.35, 46.64, 47.21, 57.05, 61.45, 67.31, 70.88, 71.46, 72.13, 127.07 (two carbons), 127.28, 128.56 (two carbons), 144.27, 174.14, Mass m/z 540 (M^+-17), 482, 400, 355, 314, 253, 204, 161, 119 (100%), 91, 77, $[\alpha]_D^{32}$ +46.6 (MeOH, c 2.5). Anal. found C, 70.90, H, 9.33, N, 2.78%. $C_{33}H_{51}NO_6$ requires C, 71.09, H, 9.15, N, 2.51%.

(1*S*,2*S*)-1-*para*-*Nitro*phenyl-2-cholicacetamidopropane-1,3-diol (15): Yield 79%, mp 163–164 °C (from chloroform–methanol), IR (Nujol) cm^{-1} 3344 (OH), 1645 (amide), ^{1}H NMR (DMSO-d_6), 200 MHz, δ, 0.40 (s, 3H, 18-H), 0.78 (1s and 1d, 6H, 19 and 21-H), 3.70 (m, 1H), 4.04 (m, 1H), 4.39 (m, 1H), 4.87 (m, 1H), 5.00 (m, 1H), 5.84 (m, 1H), 7.53 and 8.11 (AB q, 4H, Ar–H), ^{13}C NMR (DMSO-d_6) 12.32, 17.09, 22.72, 22.90, 26.36, 27.31, 28.67, 30.55, 31.72, 32.53, 34.52, 35.00, 35.47, 39.63, 39.74, 41.47, 41.69, 45.84, 46.54, 55.87, 60.76, 66.50, 69.41, 70.65, 71.27, 79.25, 122.81 (2 carbons), 127.44 (2 carbons), 146.44, 152.14, 172.87, Mass m/z 568 (M^+-34), 539, 354, 295, 271, 253, 226, 150 (100%), 121, 91, 77, $[\alpha]_D^{32}$ +42.0 (MeOH, c 1.5). Anal. found C, 65.57, H, 8.67, N, 4.32%. $C_{33}H_{50}N_2O_8$ requires C, 65.78, H, 8.31, N, 4.65%.

(1*S*,2*S*)-1-Phenyl-2-deoxycholicacetamidopropane-1,3-diol (16): Yield 92%, mp 98–99 °C (from methanol), IR (Nujol) cm^{-1} 3330 (OH), 1643 (amide), ^{1}H NMR ($CDCl_3$), 500 MHz, δ 0.62 (s, 3H, 18-H), 0.88 (s, 3H, 19-H), 0.90 (d, 3H, 21-H, J = 4.0 Hz), 3.42 (m, 1H), 3.57 (m, 1H), 3.70 (m, 1H), 3.77 (m, 1H), 3.91 (m, 1H), 4.11 (m, 1H), 5.02 (m, 1H), 6.71 (bs, 1H), 7.30 (m, 5H, Ar–H), ^{13}C NMR ($CDCl_3$) 12.88, 17.63, 23.32, 23.90, 25.15, 25.82, 26.38, 27.33, 27.80, 30.63, 31.52, 32.89, 33.82, 35.41, 35.48, 36.13, 36.44, 42.29, 46.48, 48.50, 56.84, 61.03, 63.35, 71.98, 73.56, 77.47, 126.27 (2 carbons), 127.91, 128.63 (2 carbons), 141.59, 175.68, Mass m/z 524 (M^+-OH), 490, 417, 380, 340, 314, 273, 204, 119 (100%), 91, 77, $[\alpha]_D^{32}$ +3.75 (MeOH, c 2.8). Anal. found C, 72.91, H, 9.64, N, 2.52%. $C_{33}H_{51}NO_5$ requires C, 73.20, H, 9.42, N, 2.59%.

(1*S*,2*S*)-1-*para*-*Nitro*phenyl-2-deoxycholicacetamidopropane-1,3-diol (17): Yield 82%, mp 108–109 °C (from methanol), IR (Nujol) cm^{-1} 3311 (OH), 1647 (amide), ^{1}H NMR (DMSO-d_6), 200 MHz, δ, 0.38 (s, 3H, 18-H), 0.77 (1s and 1d, 6H, 19 and 21-H), 3.66 (m, 1H), 3.89 (m, 1H), 4.07 (m, 1H), 4.42 (m, 1H), 4.78 (m, 1H), 4.94 (m, 1H), 5.74 (m, 1H), 7.51 and 8.08 (AB q, 4H, Ar–H), ^{13}C NMR (DMSO-d_6) 12.50, 17.09, 22.61, 23.31, 23.71, 26.36, 27.24, 28.82, 30.44, 31.80, 32.46, 33.16, 35.03, 35.40, 35.91, 36.47, 40.95, 41.87, 46.13, 46.65, 47.64, 55.95, 60.80, 69.69, 70.28, 71.35, 122.88 (two carbons), 127.51 (two carbons), 146.48, 152.22, 172.95, Mass m/z 552 (M^+-34), 356, 301, 273, 255, 205, 181, 150, 121, 105, 84 (100%), 77, 55, $[\alpha]_D^{32}$ +4.12 (MeOH, c 1.7). Anal. found C, 67.41, H, 8.71, N, 4.92%. $C_{33}H_{50}N_2O_7$ requires C, 67.58, H, 8.53, N, 4.78%.

14. Oakley, K. L.; Moore, C. B.; Denning, D. W. *Antimicrob. Agents Chemother*. **1998**, *42*, 2726.