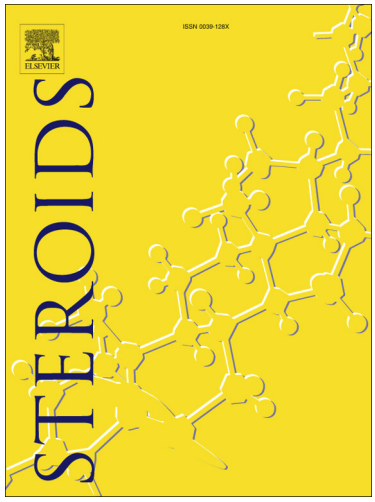


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# Lithocholic Acid and Derivatives: Antibacterial Activity

Patrícia G.G. do Nascimento[a], Telma L.G. Lemos[a,*], Macia C.S. Almeida[a], Juliana M.O. de Souza[a], Ayla M.C. Bizerra[a], Gilvandete M.P. Santiago[b], José G.M. da Costa[c], Henrique D.M. Coutinho[c]

[a]*Departamento de Química Orgânica e Inorgânica, Universidade Federal do Ceará, Campus do Pici, 60021-940, Fortaleza-CE, Brazil*

[b]*Departamento de Farmácia, Universidade Federal do Ceará, Rua Capitão Francisco Pedro N$^{o}$ 1210, Campus do Porangabussu, 60430-370, Fortaleza-CE, Brazil*

[c]*Departamento de Química Biológica, Universidade Regional do Cariri, 63105-000, Crato-CE, Brazil*

[*]Corresponding author at: Departamento de Química Orgânica e Inorgânica, Universidade Federal do Ceará, Campus do Pici, 60021-940, Fortaleza-CE, Brazil. Tel.: +55 85 33669366; fax: +55 85 33669782

*E-mail address*: tlemos@dqoi.ufc.br

## ABSTRACT

In order to develop bioactive lithocholic acid derivatives, we prepared fifteen semi-synthetic compounds through modification at C-3 and/or C-24. The reactions showed yields ranging from 37 to 100%. The structures of all compounds obtained were identified on the basis of their spectral data (IR, MS, 1D- and 2D-NMR). The activity of lithocholic acid and derivatives was evaluated against the growth of *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus* and *Pseudomonas aeruginosa*. The derivative 3α-formyloxy-5β-cholan-24-oic acid (**LA-06**) showed the best activity, with MIC values of 0.0790 mM against *E. coli* (Ec 27) and *B. cereus* in both cases, and 0.0395 mM against *S. aureus* (ATCC 12692). Lithocholic acid and the derivatives with MIC ≤ 1.2 mM were evaluated on the susceptibility of some bacterial pathogens to the aminoglycoside antibiotics neomycin, amikacin and gentamicin was evaluated. There are no previously reported studies about these compounds as modifiers of the action of antibiotics or any other drugs.


Keywords: bile acids, lithocholic acid derivatives, antibacterial activity

## 1. Introduction

The bile acids are formed from cholesterol in the liver of mammals [1]. They have a physiological function to help in the digestion of lipids and lipophilic vitamins reabsorption [2], and also are used in the coupling with drugs used in conventional cancer treatment via covalent bonds and, for this reason, various synthetic derivatives of bile acids have been developed [1].

Lithocholic acid (**LA**), one of major bile acids excreted by mammals, is formed in the metabolism by the bacterial 7-α-dehydroxylation of the primary bile acid, chenodeoxycholic acid, in the colon [1]. The biological properties of this compound and derivatives have been extensively studied, among them antimicrobial [3], membrane probe [4], tumor promotion or inhibition [5], vitamin D receptor modulation [6], antiproliferative and pro-apoptotic effect on human cancer cell lines [1] and proteasome inhibitors [7]. Investigated and unpublished results showed that among the bile acids, lithocholic acid showed significant antibacterial activity.

Microbial resistance against antibiotics is a serious health problem. This happens by the indiscriminate use of such chemotherapeutic agents, making it difficult to control species of bacteria of medical sanitary interest [8]. Pathogenic bacteria such as *Staphylococcus aureus*, *Staphylococcus epidermis*, *Salmonella sp.*, *Salmonella enteritidis* and *Salmonella enteritidis*, already have strains resistant to conventional antibiotics, making their presence in food establishments and commercial potential health threat [8,9].

The aim of this study was to synthesize derivatives of this acid (**LA**), and to investigate their antibacterial activity, and also to evaluate the influence of the derivatives with

MIC ≤ 1.2 mM on the aminoglycosides antibiotics neomycin, amikacin, kanamycin and gentamicin susceptibility of several Gram-positive and Gram-negative bacteria.

## 2. Experimental

### 2.1. General methods

Lithocholic acid (**LA**) was purchased from Sigma-Aldrich (St. Louis, MO). Melting points were determined on a digital Mettler Toledo FP82HT apparatus and are uncorrected. The IR spectra were measured in KBr pellets using a Perkin-Elmer FT-IR Spectrum 1000. A Bruker® Avance DPX 300 spectrometer, operating at 300 MHz for $^{1}$H-NMR, and 75 MHz for $^{13}$C-NMR was used for experiments 1D and 2D with chemical shifts given in ppm. The spectra were run using $CDCl_3$ as the solvent. Chemical shifts, measured on the δ scale. The HRESIMS spectra were acquired using an LCMSIT-TOF spectrometer (Shimadzu, Japan). The positive ion mass spectra were recorded in the range *m/z* 300-700 Da by using a potential of 4.5 V on the capillary and He as collision gas. For the MS/MS scanning mode, the percentage of collision energy was 50%. Optical rotations were measured on a Perkin Elmer 341 digital polarimeter (USA). Silica gel 60 (70–230 mesh) was used for column chromatography, and thin layer chromatography (TLC) was performed on precoated silica gel G60 F254 by detection by spraying with vanillin in perchloric acid/ethanol. All solvents used for chromatography were from Synth. The microbiological culture media were purchased from Fundação Oswaldo Cruz-FIOCRUZ (Rio de Janeiro, Brazil).

2.2. Chemical modifications

2.2.1. General procedure for the preparation of LA(a), LA(b) and LA(c)

Lithocholic acid (**LA**, 1.0 g, 2.65 mmol) was refluxed with 150 mL of methanol, ethanol or isopropanol in presence of sulphuric acid (1 mL) for 24 h. After this period the solvent was evaporated, followed by addition of water (100 mL) and extraction with dichloromethane (3 x 30 mL). The combined extracts were washed with $H_2O$ (3 x 60 mL), $Na_2CO_3$ solution 20% (3 x 60 mL) and brine, dried over anhydrous $Na_2SO_4$, and evaporated under reduced pressure. The residues were purified by column chromatography on silica gel (30 g) with *n*-hexane: ethyl acetate (80:20) (v/v).

*2.2.1.1. Methyl 3α-hydroxy-5β-cholan-24-oate (**LA(a)**).* Amorphous white powder; 0,95 g; yield 92%; m.p. 110-112 °C (literature [10] m.p. 116-117 °C); $[\alpha]_D^{20}$ +25.5 (*c* 0.01; $CHCl_3$) (literature [11] $[\alpha]_D$ +34.45 (*c* 0.72; $CHCl_3$)); IR (KBr, $cm^{-1}$): 3518, 2932, 2861, 1712, 1444, 1384, 1239; $^1H$ NMR ($CDCl_3$; 300 MHz): $\delta$3.58 - 3.63 (m, 1H, H-3β), 2.18 - 2.26 (m, 1H, CH-23), 2.30 - 2.40 (m, 1H, CH-23). 3.65 (s, 3H, $OCH_3$), 0.63 (s, 3H, $CH_3$-18); 0.91 (s, 3H, $CH_3$-19); 0.89 (d, $J$ = 5.4, 3H, $CH_3$-21); $^{13}C$ NMR ($CDCl_3$, 75 MHz): $\delta$36.70 ($CH_2$-1), 30.78 ($CH_2$-2), 72.08 (CH-3), 36.70 ($CH_2$-4), 42.34 (CH-5), 27.41 ($CH_2$-6), 26.63 ($CH_2$-7), 36.08 (CH-8), 40.68 (CH-9), 34.79 (C-10), 21.04 ($CH_2$-11), 40.40 ($CH_2$-12), 42.96 (C-13), 56.72 (CH-14), 24.41 ($CH_2$-15), 28.38 ($CH_2$-16), 56.20 (CH-17), 12.24 ($CH_3$-18), 23.57 ($CH_3$-19), 35.58 (CH-20), 18.47 ($CH_3$-21), 31.29 ($CH_2$-22), 31.23 ($CH_2$-23), 174.95 (C-24), 51.63 ($OCH_3$). The NMR data are in agreement with the literature values [12]; positive HRESIMS *m/z* 373.3112 $[M - H_2O]^+$ (calcd for $C_{25}H_{42}O_3$, 390.3134).

*2.2.1.2. Ethyl 3α-hydroxy-5β-cholan-24-oate (**LA(b)**).* Amorphous white powder; 0.87 g; yield 81%; m.p. 81-83 °C; $[\alpha]_D^{20}$ +25.7 (*c* 0.01; $CHCl_3$); IR (KBr, $cm^{-1}$): 3302 (OH), 2925, 2863, 1732, 1446, 1366, 123;. $^1H$ NMR ($CDCl_3$; 300 MHz): $\delta$3.57 - 3.65 (m, 1H, H-3β), 2.14 - 2.24 (m, 1H, CH-23), 2.28 - 2.38 (m, 1H, CH-23), 4.07 (q, *J* = 7.1 Hz, 2H, $OCH_2$), 1.22 (t, *J* = 7.1 Hz, 3H, $CH_3$), 0.63 (s, 3H, $CH_3$-18), 0.91 (s, 3H, $CH_3$-19, 0.89 (d, *J* = 4.6 Hz, 3H, $CH_3$-21); $^{13}C$ NMR ($CDCl_3$,75 MHz): $\delta$35.58 ($CH_2$-1), 30.75 ($CH_2$-2), 72.03 (CH-3), 36.66 ($CH_2$-4), 42.33 (CH-5), 27.41 ($CH_2$-6), 26.63 ($CH_2$-7), 36.07 (CH-8), 40.67 (CH-9), 34.79 (C-10), 21.04 ($CH_2$-11), 40.39 ($CH_2$-12), 42.95 (C-13), 56.72 (CH-14), 24.41 ($CH_2$-15), 28.37 ($CH_2$-16), 56.20 (CH-17), 12.24 ($CH_3$-18), 23.58 ($CH_3$-19), 35.55 (CH-20), 18.48 ($CH_3$-21), 31.54 ($CH_2$-22), 31.21 ($CH_2$-23), 174.54 (C-24), 60.36 ($OCH_2$), 14,45 ($CH_3$); positive HRESIMS *m/z* 387.3266 $[M - H_2O]^+$ (calcd for $C_{26}H_{44}O_3$, 404.3290).

*2.2.1.3. Isopropyl 3α-hydroxy-5β-cholan-24-oate (**LA(c)**).* Amorphous white powder; 0.90 g; yield 81%; m.p. 78-80 °C; $[\alpha]_D^{20}$ +23.8 (*c* 0.01; $CHCl_3$); IR (KBr, $cm^{-1}$): 3300, 2927, 2864, 1729, 1447, 1373, 1252; $^1H$ NMR ($CDCl_3$, 300 MHz): $\delta$3.56 - 3.67 (m, 1H, H-3β), 2.11 - 2.22 (m, 1H, CH-23), 2.25 - 2.33 (m, 1H, CH-23), 4.94 - 5.03 (m, 1H, OCH), 1.21 (d, *J* = 6.2 Hz, 6H, $2CH_3$), 0.63 (s, 3H, $CH_3$-18), 0.91 (s, 3H, $CH_3$-19), 0.89 (d, *J* = 5.0 Hz, 3H, $CH_3$-21); $^{13}C$ NMR ($CDCl_3$, 75 MHz): $\delta$35.55 ($CH_2$-1), 30.71 ($CH_2$-2), 72.01 (CH-3), 36.63 ($CH_2$-4), 42.30 (CH-5), 27.39 ($CH_2$-6), 26.61 ($CH_2$-7), 36.04 (CH-8), 40.63 (CH-9), 34.76 (C-10), 21.01 ($CH_2$-11), 40.37 ($CH_2$-12), 42.92 (C-13), 56.69 (CH-14), 24.38 ($CH_2$-15), 28.36 ($CH_2$-16), 56.19 (CH-17), 12.21 ($CH_3$-18), 23.56 ($CH_3$-19), 35.50 (CH-20), 18.45 ($CH_3$-21), 31.85 ($CH_2$-22), 31.21 ($CH_2$-23), 174.04 (C-24), 67.49 (OCH), 22.04 ($2CH_3$); positive HRESIMS *m/z* 401.3435 $[M - H_2O]^+$ (calcd for $C_{27}H_{46}O_3$, 418.3447).

2.2.2. General procedure for the preparation of LA-04, LA(a)-04, LA(b)-04 and LA(c)-04

To a solution of lithocholic acid (**LA**), methyl 3α-hydroxy-5β-cholan-24-oate (**LA(a)**), ethyl 3α-hydroxy-5β-cholan-24-oate (**LA(b)**) or isopropyl 3α-hydroxy-5β-cholan-24-oate (**LA(c)**) (1 mmol) in pyridine (2 mL) was added $Ac_2O$ (4 mL, 2.10 mmol) and catalytic amount of 4-(dimethylamino)pyridine (DMAP) (60 mg). After stirring at room temperature for 24 h, the reaction mixture was quenched with saturated $CuSO_4$ (20 mL) and extracted with EtOAc (3 x 30 mL). The combined extracts were washed with $H_2O$ (3 x 20 mL) and brine (1 x 20 mL), dried over anhydrous $Na_2SO_4$, and evaporated under reduced pressure. The residues were purified by column chromatography on silica gel (10 g) with *n*-hexane: ethyl acetate (80:20) (v/v).

*2.2.2.1. 3α-Acetoxy-5β-cholan-24-oic acid (**LA-04**).* Amorphous white powder; 0.40 g; yield 96%; m.p. 156-158 °C (literature [1] m.p. 167 °C); $[\alpha]_D^{20}$ +41.6 (*c* 0.01; $CHCl_3$); IR (KBr, $cm^{-1}$): 2923, 2868, 1731, 1707, 1449, 1375, 1244; $^1$H NMR ($CDCl_3$, 300 MHz): $\delta$4.67 - 4.77 (m, 1H, H-3β), 2.20 - 2.31 (m, 1H, CH-23), 2.35 - 2.45 (m, 1H, CH-23), 2.03 (s, 3H, $CH_3CO$), 0.65 (s, 3H, $CH_3$-18), 0.93 (s, 3H, $CH_3$-19), 0.91 (d, $J$ = 4.3 Hz, 3H, $CH_3$-21); $^{13}$C NMR ($CDCl_3$, 75 MHz): $\delta$30.99 ($CH_2$-1), 26.54 ($CH_2$-2), 74.66 (CH-3), 35.26 ($CH_2$-4), 42.12 (CH-5), 27.24 ($CH_2$-6), 26.85 ($CH_2$-7), 36.02 (CH-8), 40.65 (CH-9), 34.80 (C-10), 21.05 ($CH_2$-11), 40.37 ($CH_2$-12), 42.97 (C-13), 56.71 (CH-14), 24.39 ($CH_2$-15), 28.37 ($CH_2$-16), 56.22 (CH-17), 12.26 ($CH_3$-18), 23.54 ($CH_3$-19), 35.52 (CH-20), 18.46 ($CH_3$-21), 31.22 ($CH_2$-22), 32.47 ($CH_2$-23), 180.32 (C-24), 170.94 (C=O), 21.66 ($CH_3CO$). The NMR data are in agreement with the literature values [1]; negative HRESIMS m/z 417.3083 $[M - H]^-$ (calcd for $C_{26}H_{42}O_4$, 418.3083).

*2.2.2.2. Methyl 3α-acetoxy-5β-cholan-24-oate (**LA(a)-04**).* Crystal white powder; 0.42 g; yield 97%; m.p. 122-123 °C; $[\alpha]_D^{20}$ +41.3 (*c* 0.01; $CHCl_3$); IR (KBr, $cm^{-1}$): 2929, 2865, 1730, 1435, 1376, 1244; $^1H$ NMR ($CDCl_3$, 300 MHz): $\delta$4.65 - 4.76 (m, 1H, H-3β), 2.15 - 2.25 (m, 1H, CH-23), 2.29 - 2.39 (m, 1H, CH-23), 2.01 (s, 3H, $CH_3CO$), 3.65 (s, 3H, $OCH_3$), 0.63 (s, 3H, $CH_3$-18), 0.91 (s, 3H, $CH_3$-19), 0.89 (d, *J* = 6.0 Hz, 3H, $CH_3$-21); $^{13}C$ NMR ($CDCl_3$, 75 MHz): $\delta$31.21 ($CH_2$-1), 26.52 ($CH_2$-2), 74.59 (CH-3), 35.24 ($CH_2$-4), 42.10 (CH-5), 27.22 ($CH_2$-6), 26.83 ($CH_2$-7), 36.00 (CH-8), 40.62 (CH-9), 34.78 (C-10), 21.03 ($CH_2$-11), 40.35 ($CH_2$-12), 42.94 (C-13), 56.70 (CH-14), 24.38 ($CH_2$-15), 28.37 ($CH_2$-16), 56.21 (CH-17), 12.23 ($CH_3$-18), 23.52 ($CH_3$-19), 35.56 (CH-20), 18.46 ($CH_3$-21), 31.25 ($CH_2$-22), 32.45 ($CH_2$-23), 174.92 (C-24), 170.82 (C=O), 21.64 ($CH_3CO$), 51.64 ($OCH_3$); positive HRESIMS *m/z* 455.3144 $[M + Na]^+$ (calcd for $C_{27}H_{44}O_4$, 432.3240).

*2.2.2.3. Ethyl 3α-acetoxy-5β-cholan-24-oate (**LA(b)-04**).* Crystal white powder; 0.37 g; yield 91%; m.p. 93-95 °C; $[\alpha]_D^{20}$ +37.06 (*c* 0.00623; $CHCl_3$); IR (KBr, $cm^{-1}$): 2930, 2865, 1735, 1449, 1376, 1241; $^1H$ NMR ($CDCl_3$, 300 MHz): $\delta$4.66 - 4.77 (m, 1H, H-3β), 2.14 - 2.25 (m, 1H, CH-23), 2.28 - 2.38 (m, 1H, CH-23), 4.08 (q, *J* = 6.0 Hz, 2H, $OCH_2$), 1.22 (t, *J* = 6.0 Hz, 3H, $CH_3$), 2.02 (s, 3H, $CH_3CO$), 0.64 (s, 3H, $CH_3$-18), 0.92 (s, 3H, $CH_3$-19), 0.90 (d, *J* = 6.0 Hz, 3H, $CH_3$-21); $^{13}C$ NMR ($CDCl_3$, 75 MHz): $\delta$31.20 ($CH_2$-1), 26.51 ($CH_2$-2), 74.59 (CH-3), 35.23 ($CH_2$-4), 42.10 (CH-5), 27.22 ($CH_2$-6), 26.82 ($CH_2$-7), 36.00 (CH-8), 40.61 (CH-9), 34.77 (C-10), 21.03 ($CH_2$-11), 40.34 ($CH_2$-12), 42.93 (C-13), 56.69 (CH-14), 24.37 ($CH_2$-15), 28.36 ($CH_2$-16), 56.22 (CH-17), 12.22 ($CH_3$-18), 23.51 ($CH_3$-19), 35.53 (CH-20), 18.46 ($CH_3$-21), 32.45 ($CH_2$-22), 32.45 ($CH_2$-23), 174.49 (C-24), 170.81 (C=O), 60.34 ($OCH_2$), 14.44

($CH_3$), 21.63 ($CH_3CO$); positive HRESIMS *m/z* 469.3312 $[M + Na]^+$ (calcd for $C_{28}H_{46}O_4$, 446.3396).

*2.2.2.4. Isopropyl 3α-acetoxy-5β-cholan-24-oate (**LA(c)-04**).* Crystal white powder; 0.46 g; yield 100%; m.p. 96-97 °C; $[\alpha]_D^{20}$ +35 (*c* 0.01, $CHCl_3$); IR (KBr; $cm^{-1}$): 2928, 2869, 1733, 1453, 1378, 1240; $^1H$ NMR ($CDCl_3$, 300 MHz): $\delta$4.66 - 4.77 (m, 1H, H-3β), 2.12 - 2.22 (m, 1H, CH-23), 2.25 - 2.36 (m, 1H, CH-23), 4.95 - 5.04 (m, 1H, OCH), 1.21 (d, *J* = 6.2 Hz, 6H, $2CH_3$), 2.02 (s, 3H, $CH_3CO$), 0.64 (s, 3H, $CH_3$-18), 0.92 (s, 3H, $CH_3$-19), 0.90 (d, *J* = 7.2 Hz, 3H, $CH_3$-21); $^{13}C$ NMR ($CDCl_3$,75 MHz): $\delta$31.23 ($CH_2$-1), 26.53 ($CH_2$-2), 74.62 (CH-3), 34.78 ($CH_2$-4), 42.10 (CH-5), 27.22 ($CH_2$-6), 26.83 ($CH_2$-7), 36.00 (CH-8), 40.62 (CH-9), 34.78 (C-10), 21.03 ($CH_2$-11), 40.36 ($CH_2$-12), 42.94 (C-13), 56.70 (CH-14), 24.38 ($CH_2$-15), 28.37 ($CH_2$-16), 56.25 (CH-17), 12.22 ($CH_3$-18), 23.52 ($CH_3$-19), 35.52 (CH-20), 18.47 ($CH_3$-21), 31.88 ($CH_2$-22), 32.46 ($CH_2$-23), 174.06 (C-24), 170.87 (C=O), 67.52 (OCH), 21.65 ($CH_3CO$), 22.04 ($2CH_3$); positive HRESIMS *m/z* 483.3450 $[M + Na]^+$ (calcd for $C_{29}H_{48}O_4$, 460.3553).

2.2.3. General procedure for the preparation of LA-05, LA(a)-05, LA(b)-05 and LA(c)-05

To a solution of lithocholic acid (**LA**), methyl 3α-hydroxy-5β-cholan-24-oate (**LA(a)**), ethyl 3α-hydroxy-5β-cholan-24-oate (**LA(b)**) or isopropyl 3α-hydroxy-5β-cholan-24-oate (**LA(c)**) (1 mmol) in acetone/$CH_2Cl_2$ (6/4 mL) was added pyridinium chlorochromate (PCC) (600 mg, 2.78 mmol). After stirring at room temperature for 24 h, the mixture was concentrated and partitioned with $H_2O$ (10 mL) and $CH_2Cl_2$ (3 x 10 mL). The organic layer

was concentrated and purified by silica gel column chromatography (13 g) eluted with hexane: ethyl acetate (50:50) (v/v).

*2.2.3.1. 3-Oxo-5β-cholan-24-oic acid (**LA-05**)*. Amorphous white powder; 0.14 g; yield 37%; m.p. 121-123 °C (literature [10] m.p. 121-122 °C); $[\alpha]_D^{20}$ +28.1 (*c* 0.01; $CHCl_3$); IR (KBr, $cm^{-1}$): 2926 , 2879, 1698, 1447, 1376; $^1H$ NMR ($CDCl_3$, 300 MHz,): $\delta$2.21 - 2.30 (m, 1H, H-23), 2.32 - 2.44 (m, 1H, CH-23), 0.67 (s, 3H, CH-18), 1.00 (s, 3H, $CH_3$-19), 0.91 (d, *J* = 6.3 Hz, 3H, $CH_3$-21); $^{13}C$ NMR ($CDCl_3$, 75 MHz): $\delta$37.38 ($CH_2$-1), 37.20 ($CH_2$-2), 213.88 (C-3), 42.53 ($CH_2$-4), 44.51 (CH-5), 25.96 ($CH_2$-6), 26.81 ($CH_2$-7), 35.49 (CH-8), 40.94 (CH-9), 35.07 (C-10), 21.39 ($CH_2$-11), 40.25 ($CH_2$-12), 42.99 (C-13), 56.62 (CH-14), 24.35 ($CH_2$-15), 28.33 ($CH_2$-16), 56.16 (CH-17), 12.28 ($CH_3$-18), 22.83 ($CH_3$-19), 35.73 (CH-20), 18.45 ($CH_3$-21), 31.22 ($CH_2$-22), 30.94 ($CH_2$-23), 180.26 (C-24). The NMR data are in agreement with the literature values [10]; positive HRESIMS *m/z* 469.3312 $[M + Na]^+$ (calcd for $C_{28}H_{46}O_4$, 446.3396).

*2.2.3.2. Methyl 3-oxo-5β-cholan-24-oate (**LA(a)-05**)*. Crystal white powder; 0.33 g; yield 85%; m.p. 107-109 °C; $[\alpha]_D^{20}$ +9.9 (*c* 0.0017; $CHCl_3$); IR (KBr, $cm^{-1}$): 2926, 2869, 1735, 1710, 1452, 1379, 1256; $^1H$ NMR ($CDCl_3$, 300 MHz): $\delta$2.16 - 2.28 (m, 1H, H-23), 2.30 - 2.38 (m, 1H, CH-23), 3.65 (s, 3H, $OCH_3$), 0.67 (s, 3H, $CH_3$-18), 1.01 (s, 3H, $CH_3$-19), 0.90 (d, *J* = 6.3 Hz, 3H, $CH_3$-21); $^{13}C$ NMR ($CDCl_3$, 75 MHz): $\delta$37.37 ($CH_2$-1), 37.19 ($CH_2$-2), 213.46 (C-3), 42.53 ($CH_2$-4), 44.49 (CH-5), 25.94 ($CH_2$-6), 26.80 ($CH_2$-7), 35.51 (CH-8), 40.92 (CH-9), 35.06 (C-10), 21.37 ($CH_2$-11), 40.22 ($CH_2$-12), 42.95 (C-13), 56.61 (CH-14), 24.32 ($CH_2$-15), 28.31 ($CH_2$-16), 56.15 (CH-17), 12.23 ($CH_3$-18), 22.82 ($CH_3$-19), 35.71 (CH-20), 18.45

($CH_3$-21), 31.21 ($CH_2$-22), 31.15 ($CH_2$-23), 174.85 (C-24), 51.63 ($OCH_3$); positive HRESIMS *m/z* 411.2886 $[M + Na]^+$ (calcd for $C_{25}H_{40}O_3$, 388.2977).

*2.2.3.3. Ethyl 3-oxo-5β-cholan-24-oate (**LA(b)-05**).* Amorphous white powder; 0.38 g; yield 84%; m.p. 88-90 °C (literature [3f] m.p. 85-88 °C); $[\alpha]_D^{20}$ +27.5 (*c* 0.0017; $CHCl_3$); (literature [13] $[\alpha]_D$ +28.9); IR (KBr, $cm^{-1}$): 2937, 2865, 1734, 1712, 1446, 1380, 1243; $^1H$ NMR ($CDCl_3$, 300 MHz): $\delta$4.08 (q, *J* = 7.1 Hz, 2H, $OCH_2$), 1.22 (t, *J* = 7.1 Hz, 3H, $CH_3$), 0.67 (s, 3H, $CH_3$-18), 1.01 (s, 3H, $CH_3$-19), 0.90 (d, *J* = 6.3 Hz, 3H, $CH_3$-21); $^{13}C$ NMR ($CDCl_3$, 75 MHz): $\delta$37.38 ($CH_2$-1), 37.19 ($CH_2$-2), 213.51 (C-3), 42.53 ($CH_2$-4), 44.50 (CH-5), 25.95 ($CH_2$-6), 26.80 ($CH_2$-7), 35.50 (CH-8), 40.92 (CH-9), 35.06 (C-10), 21.37 ($CH_2$-11), 40.23 ($CH_2$-12), 42.95 (C-13), 56.62 (CH-14), 24.33 ($CH_2$-15), 28.32 ($CH_2$-16), 56.17 (CH-17), 12.24 ($CH_3$-18), 22.83 ($CH_3$-19), 35.71 (CH-20), 18.46 ($CH_3$-21), 31.49 ($CH_2$-22), 31.15 ($CH_2$-23), 174.43 (C-24), 60.34 ($OCH_2$), 14.43 ($CH_3$); positive HRESIMS *m/z* 425.3052 $[M + Na]^+$ (calcd for $C_{26}H_{42}O_3$, 402.3134).

*2.2.3.4. Isopropyl 3-oxo-5β-cholan-24-oate (**LA(c)-05**).* Yellow oil; 0.38 g; yield 91%; $[\alpha]_D^{20}$ +26.3 (*c* 0.0017; $CHCl_3$); IR (KBr, $cm^{-1}$): 2932, 2865, 1715, 1446, 1374, 1256; $^1H$ NMR ($CDCl_3$, 300 MHz): $\delta$4.95 - 5.01 (m, 1H, OCH), 1.21 (d, *J* = 6.3 Hz, 6H, $2CH_3$), 0.67 (s, 3H, $CH_3$-18), 1.01 (s, 3H, $CH_3$-19), 0.90 (d, *J* = 6.3 Hz, 3H, $CH_3$-21); $^{13}C$ NMR ($CDCl_3$, 75 MHz): $\delta$37.39 ($CH_2$-1), 37.20 ($CH_2$-2), 213.53 (C-3), 42.55 ($CH_2$-4), 44.51 (CH-5), 25.96 ($CH_2$-6), 26.82 ($CH_2$-7), 35.49 (CH-8), 40.94 (CH-9), 35.08 (C-10), 21.38 ($CH_2$-11), 40.25 ($CH_2$-12), 42.97 (C-13), 56.64 (CH-14), 24.34 ($CH_2$-15), 28.33 ($CH_2$-16), 56.21 (CH-17), 12.24 ($CH_3$-18), 22.84 ($CH_3$-19), 35.73 (CH-20), 18.47 ($CH_3$-21), 31.84 ($CH_2$-22), 31.19 ($CH_2$-23), 173.97

(C-24), 67.48 (OCH), 22.05 ($2CH_3$); positive HRESIMS *m/z* 439.3204 $[M + Na]^+$ (calcd for $C_{27}H_{44}O_3$, 416.3290).

2.2.4. General procedure for the preparation of LA-06, LA(a)-06, LA(b)-06 and LA(c)-06

A solution of of lithocholic acid (**LA**), methyl 3α-hydroxy-5β-cholan-24-oate (**LA(a)**), ethyl 3α-hydroxy-5β-cholan-24-oate (**LA(b)**) or isopropyl 3α-hydroxy-5β-cholan-24-oate (**LA(c)**) (1 mmol) in of ≥95% $HCO_2H$ (2 mL) and 70% perchloric acid (20 drops) was heated in an $H_2O$ bath at 60° for 4 h. The solution was removed from the bath and allowed to cool to about 40°. $Ac_2O$ was then added dropwise while the temperature was maintained between 55 and 60° until a large quantity of bubbles appeared (1 mL of $Ac_2O$ was required). The solution was then cooled to room temperature and poured into 10 mL of $H_2O$, with stirring [14]. The precipitate was filtered under vacuum, washed with $H_2O$.

*2.2.4.1. 3α-Formyloxy-5β-cholan-24-oic acid (**LA-06**).* Amorphous white powder; 0.37 g; yield 92%; m.p. 128-130 °C (literature [15] m.p. 127-128 °C); $[\alpha]_D^{20}$ +38.5 (*c* 0.01; $CHCl_3$); (literature [14] $[\alpha]_D^{23}$ +38.5 (*c* 10; $CHCl_3$)); IR (KBr, $cm^{-1}$): 2936, 2866, 1718, 1704, 1448, 1379, 1249; $^1H$ NMR ($CDCl_3$, 300 MHz): $\delta$8.03 (s, 1H, HC=O), 4.81 - 4.86 (m, 1H, H-3β), 2.20 - 2.30 (m, 1H, CH-23), 2.34 - 2.43 (m, 1H, CH-23), 0.65 (s, 3H, $CH_3$-18), 0.93 (s, 3H, $CH_3$-19), 0.91 (d, *J* = 7.4 Hz, 3H, $CH_3$-21); $^{13}C$ NMR ($CDCl_3$, 75 MHz): $\delta$30.96 ($CH_2$-1), 26.51 ($CH_2$-2), 74.64 (CH-3), 35.17 ($CH_2$-4), 42.12 (CH-5), 27.19 ($CH_2$-6), 26.84 ($CH_2$-7), 36.00 (CH-8), 40.67 (CH-9), 34.78 (C-10), 21.05 ($CH_2$-11), 40.33 ($CH_2$-12), 42.96 (C-13), 56.67 (CH-14), 24.38 ($CH_2$-15), 28.35 ($CH_2$-16), 56.17 (CH-17), 12.26 ($CH_3$-18), 23.52 ($CH_3$-19), 35.50 (CH-20), 18.45 ($CH_3$-21), 31.24 ($CH_2$-22), 32.43 ($CH_2$-23), 180.60 (C-24), 161.05

(HC=O). The NMR data are in agreement with the literature values [15]; negative HRESIMS *m/z* 403.2885 $[M - H]^-$ (calcd for $C_{25}H_{40}O_4$, 404.2927).

*2.2.4.2. Methyl 3α-formyloxy-5β-cholan-24-oate (**LA(a)-06**)*. Amorphous white powder; 0.38 g; yield 91%; m.p 106-108 °C; $[\alpha]_D^{20}$ +43.1 (*c* 0.0051; $CHCl_3$); IR (KBr, $cm^{-1}$): 2926, 2866, 1719, 1444, 1378, 1251; $^1$H NMR ($CDCl_3$, 300 MHz): $\delta$8.04 (s, 1H, HC=O), 4.85 (m, 1H, H-3β), 2.18 - 2.27 (m, 1H, CH-23), 2.30 - 2.39 (m, 1H, CH-23), 3.66 (s, 3H, $OCH_3$), 0.65 (s, 3H, $CH_3$-18), 0.92 (s, 3H, $CH_3$-19), 0.90 (d, *J* = 4.9 Hz, 3H, $CH_3$-21); $^{13}$C NMR ($CDCl_3$, 75 MHz): $\delta$31.21 ($CH_2$-1), 26.51 ($CH_2$-2), 74.61 (CH-3), 35.17 ($CH_2$-4), 42.13 (CH-5), 27.18 ($CH_2$-6), 26.84 ($CH_2$-7), 36.00 (CH-8), 40.67 (CH-9), 34.78 (C-10), 21.04 ($CH_2$-11), 40.33 ($CH_2$-12), 42.94 (C-13), 56.67 (CH-14), 24.37 ($CH_2$-15), 28.36 ($CH_2$-16), 56.19 (CH-17), 12.24 ($CH_3$-18), 23.52 ($CH_3$-19), 35.55 (CH-20), 18.47 ($CH_3$-21), 31.22 ($CH_2$-22), 32.43 ($CH_2$-23), 174.93 (C-24), 160.97 (HC=O), 51.65 ($OCH_3$); positive HRESIMS *m/z* 441.2999 $[M + Na]^+$ (calcd for $C_{26}H_{42}O_4$, 418.3083).

*2.2.4.3. Ethyl 3α-formyloxy-5β-cholan-24-oate (**LA(b)-06**)*. Crystal white powder; 0.25 g; yield 57%; m.p. 65-66 °C; $[\alpha]_D^{20}$ +37.6 (*c* 0.0029; $CHCl_3$); IR (KBr, $cm^{-1}$): 2925, 2864, 1737, 1720, 1449, 1376, 1245; $^1$H NMR ($CDCl_3$, 300 MHz): $\delta$8.03 (s, 1H, HC=O), 4.85 (m, 1H, H-3β), 2.15 - 2.25 (m, 1H, CH-23), 2.29 - 2.34 (m, 1H, CH-23), 4.08 (q, *J* = 7.1 Hz, 2H, $OCH_2$), 1.23 (t, *J* = 7.6 Hz, 3H, $CH_3$), 0.64 (s, 3H, $CH_3$-18), 0.92 (s, 3H, $CH_3$-19), 0.91 (d, *J* = 3.9 Hz, 3H, $CH_3$-21); $^{13}$C NMR ($CDCl_3$, 75 MHz): $\delta$31.22 ($CH_2$-1), 26.53 ($CH_2$-2), 74.62 (CH-3), 35.19 ($CH_2$-4), 42.14 (CH-5), 27.20 ($CH_2$-6), 26.86 ($CH_2$-7), 36.02 (CH-8), 40.68 (CH-9), 34.80 (C-10), 21.06 ($CH_2$-11), 40.35 ($CH_2$-12), 42.96 (C-13), 56.70 (CH-14), 24.39

($CH_2$-15), 28.38 ($CH_2$-16), 56.23 (CH-17), 12.25 ($CH_3$-18), 23.53 ($CH_3$-19), 35.55 (CH-20), 18.49 ($CH_3$-21), 31.55 ($CH_2$-22), 32.45 ($CH_2$-23), 174.50 (C-24), 160.96 (HC=O), 60.36 ($OCH_2$), 14.47 ($CH_3$); positive HRESIMS *m/z* 451.2591 $[M + K]^+$ (calcd for $C_{27}H_{44}O_4$, 432.3240).

*2.2.4.4. Isopropyl 3α-formyloxy-5β-cholan-24-oate (**LA(c)-06**).* Crystal white powder; 0.21 g; yield 46%; m.p. 45-47 °C; $[\alpha]_D^{20}$ +31.5 (*c* 0.002; $CHCl_3$); $^1$H NMR ($CDCl_3$, 300 MHz): $\delta$8.04 (s, 1H, HC=O), 4.80 - 4.89 (m, 1H, H-3β), 2.12 - 2.23 (m, 1H, CH-23), 2.26 - 2.36 (m, 1H, CH-23), 4.96 - 5.04 (m, 1H, OCH), 1.22 (t, *J* = 6.3 Hz, 3H, $2CH_3$), 0.65 (s, 3H, $CH_3$-18), 0.94 (s, 3H, $CH_3$-19), 0.90 (d, *J* = 6.5 Hz, 3H, $CH_3$-21); $^{13}$C NMR ($CDCl_3$, 75 MHz): $\delta$31.25 ($CH_2$-1), 26.54 ($CH_2$-2), 74.63 (CH-3), 35.20 ($CH_2$-4), 42.16 (CH-5), 27.22 ($CH_2$-6), 26.87 ($CH_2$-7), 36.03 (CH-8), 40.69 (CH-9), 34.81 (C-10), 21.07 ($CH_2$-11), 40.36 ($CH_2$-12), 42.97 (C-13), 56.71 (CH-14), 24.40 ($CH_2$-15), 28.39 ($CH_2$-16), 56.26 (CH-17), 12.26 ($CH_3$-18), 23.54 ($CH_3$-19), 35.55 (CH-20), 18.50 ($CH_3$-21), 31.90 ($CH_2$-22), 32.46 ($CH_2$-23), 174.03 (C-24), 160.98 (HC=O), 67.52 (OCH), 22.07, 22.08 ($2CH_3$).

2.3. Antibacterial Activity and Minimal Inhibitory Concentration

The antibacterial activity of lithocholic acid and its derivatives was investigated employing a microdilution method, recommended by National Committee for Clinical and Laboratory Standards M7-A6 [16]. In tests were used three standard strains of Gram (-) and three Gram (+), and two clinical isolates of multidrug-resistant *Escherichia coli* (Ec 27) (from sputum) and *Staphylococcus aureus* (Sa 358) of the surgical wound. The brain heart infusion (BHI 3.8%) broth was used for the bacterial growth (24 h, 35 ± 2°C). The inoculum was an

overnight culture of each bacterial species in the BHI broth diluted in the same medium to a final concentration of approximately $1 \times 10^8$ CFU/mL (0.5 NTU – McFarland scale). After this, the suspension was diluted to $1 \times 10^6$ CFU/mL in 10% BHI. A total of 100 µL of each dilution was distributed in 96-well plates plus substance, achieving $5 \times 10^5$ CFU/mL as the final concentration of the inoculums [17-19].

The initial solutions of the lithocholic acid and its derivatives were prepared using dimethyl sulfoxide (DMSO). From this concentration, several dilutions were made in distilled water in order to obtain a stock solution of each compound corresponding with 1024 µg/mL (varying between 2.22 and 2.63 mM for each compound). Further serial dilutions were performed by the addition of the BHI broth. All experiments were performed in triplicate and the most common result was used. The microdilution trays were incubated at 35 ± 2°C for 24 h. The antibacterial activity was detected using a colorimetric method by adding 25 µL of the resauzurin staining (0.01%) aqueous solution in each well at the end of the incubation period [20]. The minimal inhibitory concentration (MIC) was defined as the lowest extract concentration able to inhibit the bacteria growth, as indicated by resauzurin staining (dead bacterial cells are not able to change the staining color by visual observation – blue to red).

2.4. Evaluation of the modulatory activity by direct contact

In the investigation of the lithocholic acid and derivatives as modulators of antibiotic resistance, the MICs of aminoglycosides antibiotics neomycin, amikacin, and gentamicin against the analyzed strains were determined in the presence or absence of the samples using the microdutiltion test. Subinhibitory concentrations (MIC 1/8) in 10% BHI were used. The antibiotic solutions corresponding with 1024 µg/mL (varying between 1.66 and 2.14 mM for

each antibiotic) were prepared in distilled water for use on the same day. A total of 100 µL of the antibiotic solution, using serial dilutions (1:2), was added to the wells containing 10% BHI and the diluted bacterial suspension (1:10). Microplates were incubated at 35 ± 2°C for 24 h and the antibacterial activity was determined as described before [21]. All experiments were performed in triplicate and the most common result was used.

## 3. Results and Discussion

### 3.1. Chemistry

The lithocholic acid derivatives were synthesized through modification at the oxygenated carbon C-3 and/or at the carboxyl carbon C-24. Initially, lithocholic acid (**LA**) was transformed into its methyl, ethyl and isopropyl esters under general esterification conditions, with the appropriate alcohol in presence of sulphuric acid ($H_2SO_4$). The synthesis of others derivatives was accomplished by treating lithocholic acid (**LA**), methyl 3α-hydroxy-5β-cholan-24-oate (**LA(a)**), ethyl 3α-hydroxy-5β-cholan-24-oate (**LA(b)**) or isopropyl 3α-hydroxy-5β-cholan-24-oate with acetic anhydride in the presence of pyridine and 4-(dimethylamino)pyridine (DMAP); pyridinium chlorochromate (PCC) and $HCO_2H$ in the presence of perchloric acid (Figure 1). The structures of all compounds were established by 1D $^{1}H$ and $^{13}C$ (and DEPT) and 2D HSQC and HMBC NMR spectral data.

### 3.2. Biological evaluations

Lithocholic acid (**LA**) and its derivatives were tested for antibacterial activity against six bacterial strains (two strains of *Staphylococcus aureus*, two strains of *Escherichia coli*,

*Bacillus cereus* and *Pseudomonas aeruginosa*), by employing the microdilution method. The values of minimum inhibitory concentration (MIC) of lithocholic acid (**LA**) and its derivatives are shown in Table 1. According to the results (Table 1), it was found that the derivative **LA-06** (3α-formyloxy-5β-cholan-24-oic acid) showed the best results, with MIC values ≤ 0.3162 mM against all tested bacterial strains. The results found against *E. coli* (Ec 27) and *B. cereus* showed MIC value of 0.0790 mM in both cases, and against *S. aureus* (ATCC 12692) representing the most significant result, with a MIC value of 0.0395 mM. Interestingly, among all evaluated compounds in this study, the derivative **LA-04** (3α-acetoxy-5β-cholan-24-oic acid) was inactive against all bacterial strains evaluated, with MIC ≥ 2.44 mM.

The association results of the lithocholic acid (**LA**) **LA(a)**, **LA(b)**, **LA(c)**, **LA(a)-04**, **LA(b)-04**, **LA-05** and **LA-06** with aminoglycosides antibiotics amikacin, gentamicin and neomycin on MIC values are shown in Table 2. In these assays were selected the compounds that showed MIC ≤ 1.2 mM.

Modifier of antibiotic activity is a term used for substances that modulate or even reverse bacterial resistance to certain antibiotics, where it can alter the microbial susceptibility to antibiotics by inhibition of the resistance mechanisms [22]. When the substance utilized in combination intervenes in a positive way, it is considered increasing the activity of the antibiotic, is considered to have a synergistic effect. On the contrary, when there is a decrease in the action or inactivation of the antibiotic, the added substance has an antagonistic effect [23].

The association of lithocholic acid (**LA**) with aminoglycosides antibiotics increased the activity of all antibiotics tested against *E. coli* (ATCC 25922), while in the effect against *S. aureus* (ATCC 12692), **LA** showed a synergistic effect on the activity of amikacin. **LA(a)**

(methyl 3α-hydroxy-5β-cholan-24-oate) showed the potentiation of the activity of antibiotics when tested against *E. coli* (Ec 27). Already in the effect on *B. cereus* (ATCC 33018), **LA(a)** showed an antagonistic action on the activity of amikacin and neomycin. The association of **LA(b)** (ethyl 3α-hydroxy-5β-cholan-24-oate) with the aminoglycosides antibiotics increased the effect of amikacin and neomycin against *E. coli* (Ec 27). **LA(c)** (isopropyl 3α-hydroxy-5β-cholan-24-oate) associated to the aminoglycosides antibiotics enhanced the activity of amikacin and neomycin against *S. aureus* (ATCC 12692), and showed an antagonistic action on the activity of amikacin against *E. coli* (ATCC 25922).

Compound **LA(a)-04** (methyl 3α-acetoxy-5β-cholan-24-oate), having a acetyl group at C-3, showed potentiation of the activity of amikacin and neomycin against the strain *S. aureus* (ATCC 12692). It was observed that **LA(a)-04** showed an antagonistic action on the activity of gentamicin against the strain *E. coli* (ATCC 25922). A little change at carbon size in the ester derivative **LA(b)-04** (ethyl 3α-acetoxy-5β-cholan-24-oate) potentiated the activity of neomycin against *S. aureus* (ATCC 12692) and *E. coli* (ATCC 25922).

The results of the association of the derivative oxidized at C-3 **LA-05** (3-oxo-5β-cholan-24-oic acid) with the aminoglycosides antibiotics showed the increase of the activity of antibiotics neomycin and amikacin against the strain *S. aureus* (ATCC 12692). On the strain *P. aeruginosa* (ATCC 15442), **LA-05** also demonstrated a synergistic action on the activity of amikacin. Finally, it was found that the association of the compound having a formyl group at C-3 **LA-06** (3α-formyloxy-5β-cholan-24-oic acid) with the aminoglycosides antibiotics increases the activity of antibiotics against *P. aeruginosa* (ATCC 15442) with significant results when compared to the MIC of the antibiotic in the absence of the substance, and may highlight the neomycin reduction in MIC of 0.0129 to 0.008 mM (94%) and

reduction with amikacin a MIC of 0.0543 to 0.0135 mM. Already in interaction with *S. aureus* strain (ATCC 12692), **LA-06** showed synergistic action on the activity of aminoglycosides antibiotics.

The gram-negative bacteria *E. coli* and *P. aeruginosa* have higher amounts of lipids in their structure, which can explain the favorable results seen in modulation [24,25], demonstrating the greater affinity between the derivatives of the lithocholic acid and the own acid with the cell membrane.

The Figure 1 shows the formation of the lithocholic acid and its derivatives, and all these compounds demonstrating a nonpolar behavior. Lipophilic substances such as the lithocholic acid and its derivatives allow perturbations in the bacterial membrane, resulting in damage to essential elements for the integrity of the membrane such as: reduction in membrane potential and loss of ions, cytochrome C, proteins and radicals, followed by the collapse of proton pumps and decrease in ATP [26,27].

Besides that lithocolic acid and its derivatives can interact with the lipid bilayer of the cell membrane and affect the respiratory chain and energy production of bacteria [28], it can also make the cell more permeable to antibiotics, leading to interruption of vital cell activity and interference with the enzyme systems of bacteria, which is also a potential mechanism of action [29,30]. In the Table 2, we can see that the main compound with modulatory activity is the own lithocholic acid. Beside the other compounds be lipophilic, none of them demonstrate the same activity of the original compound, which suggest that the modifications in C3 and in carboxyl group in C17 have not increased this effect.

The bacterial resistance modifying effect of lithocholic acid and its derivatives is due to these compounds being capable of acting through a lipophilic action in the cell envelope,

resulting in an imbalance in the fluid mosaic nature of the bacterial membrane [31] and makes the modulation more effective. The outer and inner membranes can be subject to permeabilization, facilitating the entrance of antibiotics, besides causing cell lysis and necrosis [32,33]

Cholesterol is an important component of biological membranes of eukaryotes, and is absent from the membrane of bacteria. They are responsible for maintaining the permeability of membranes. The cell can control its fluidity through the regulation of cholesterol levels or the degree of saturation of the hydrocarbon chains of phospholipids [34]. Due to the lipophilic behavior, compounds analogous to the cholesterol, such as lithocolic acid and its derivatives demonstrated an effect over the bacterial membrane.

There are no previously reported studies of the utilization of bile acid and 15 derivatives as modifiers of the action of antibiotics or any other drugs, where this is the first study to be conducted with this aim.

## 4. Conclusions

The results show that these compounds exhibit significant antibacterial activity and some of them potentiate the effect of antibiotics such as amikacin, gentamicin and neomycin. Since the parent compound lithocholic acid (**LA**) is part of the mammalian metabolism, and therefore without toxicity, these compounds are promising antibiotic agents.

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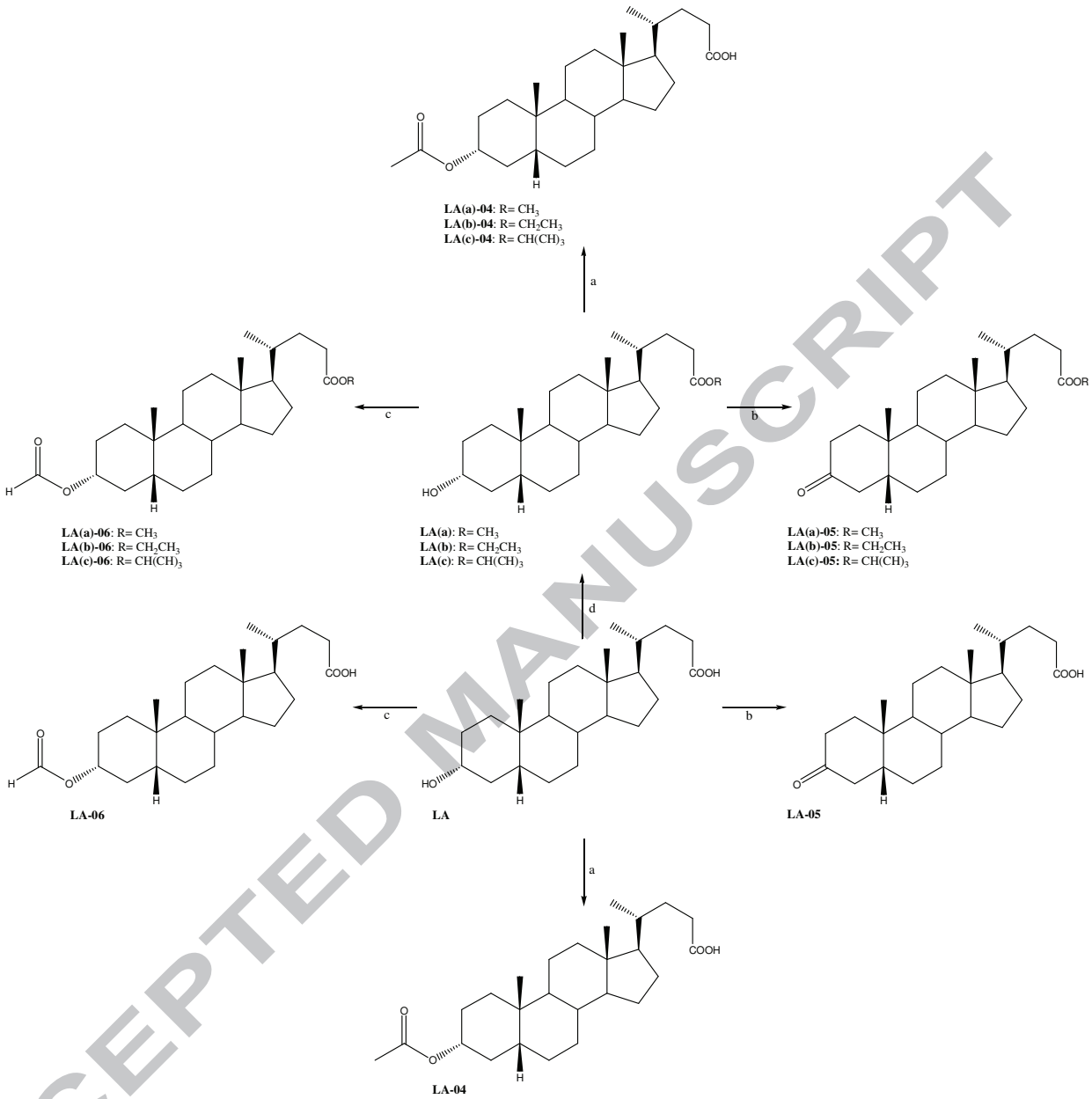



Reagents and conditions: (a) $Ac_2O$/pyridine, DMAP (4-(dimethylamino)pyridine), rt, 24 h; (b) PCC (pyridinium chlorochromate), rt, 24 h; (c) $HCO_2H$, perchloric acid, 60 °C, 4 h (d) Methanol, ethanol or isopropanol, $H_2SO_4$, reflux, 24 h

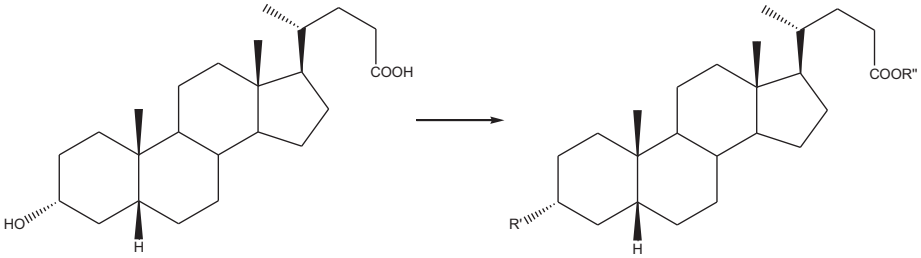
**Table 1.** Values of the minimal inhibitory concentration (MIC) of lithocholic acid and derivatives.

| **Compounds** | **Bacterial strains MIC (mM)** | | | | | |
|---|---|---|---|---|---|---|
| | ***S. aureus*** **(ATCC 12692)** | ***S. aureus*** **(Sa 358)** | ***E. coli*** **(ATCC 25922)** | ***E. coli*** **(Ec 27)** | ***B. cereus*** **(ATCC 33018)** | ***P. aeruginosa*** **(ATCC 15442)** |
| LA | 1.36 | 1.36 | 1.36 | 1.36 | 1.36 | 1.36 |
| LA (a) | 1.31 | ≥ 2.62 | 1.31 | 0.655 | 0.655 | 1.31 |
| LA (b) | 1.265 | ≥ 2.53 | 0.6325 | 0.6325 | 1.265 | 1.265 |
| LA (c) | 1.22 | ≥ 2.44 | 0.61 | 1.22 | ≥ 2.44 | ≥ 2.44 |
| LA -04 | ≥ 2.44 | ≥≥ 2.44 | ≥2.44 | ≥2.44 | ≥2.44 | ≥2.44 |
| LA (a)-04 | 1.185 | ≥ 2.37 | 1.185 | 1.185 | 1.185 | 1.185 |
| LA (b)-04 | 0.2862 | ≥ 2.29 | 0.5725 | 0.5725 | 1.145 | 1.145 |
| LA (c)-04 | 1.11 | ≥ 2.22 | 1.11 | 1.11 | 1.11 | ≥ 2.22 |
| LA -05 | 0.5725 | 1.145 | 1.145 | 0.5725 | 1.145 | 0.5725 |
| LA (a)-05 | 1.315 | 0.6575 | ≥ 2.63 | ≥ 2.63 | 1.315 | ≥ 2.63 |
| LA (b)-05 | 0.635 | ≥ 2.54 | 1.27 | 1.27 | ≥ 2.54 | ≥ 2.54 |
| LA (c)-05 | 1.23 | ≥ 2.46 | ≥ 2.46 | 1.23 | ≥ 2.46 | ≥ 2.46 |
| LA -06 | 0.0395 | 0.6325 | 0.3162 | 0.0790 | 0.0790 | 0.1581 |
| LA (a)-06 | 1.22 | 1.22 | 256 | ≥ 2.44 | 1.22 | ≥ 2.44 |
| LA (b)-06 | 1.185 | ≥ 2.37 | 1.185 | 1.185 | ≥ 2.37 | ≥ 2.37 |
| LA (c)-06 | ≥ 2.29 | ≥ 2.29 | 1.145 | 1.145 | ≥ 2.29 | ≥ 2.29 |

**Table 2.** Minimal inhibitory concentration (MIC) values for the aminoglycoside in the absence and presence of the lithocholic acid and derivatives.

| Bacterial strains | MIC (mM) | | | | | |
|---|---|---|---|---|---|---|
| | Antibiotic | | | Antibiotic + Sample | | |
| | **AMI** | **GEN** | **NEO** | **AMI** | **GEN** | **NEO** |
| *S. aureus* (ATCC 12692) | 0.2175 | 0.2675 | 0.1037 | 0.1087 | 0.2675 | 0.1037 |
| *E. coli* (ATCC 25922) | 0.1087 | 0.2675 | 0.83 | 0.0271 | 0.1337 | 0.2075 |
| | | | | | LA(a) | |
| *E. coli* (Ec 27) | 0.1087 | 0.535 | 0.0518 | 0.0543 | 0.2675 | 0.0129 |
| *B. cereus* (ATCC 33018) | 0.0543 | 0.2675 | 0.0259 | 0.1087 | 0.2675 | 0.0518 |
| | | | | | LA(b) | |
| *E. coli* (ATCC 25922) | 0.0543 | 0.2675 | 0.83 | 0.1087 | 0.2675 | 0.415 |
| *E. coli* (Ec 27) | 0.435 | 0.2675 | 0.2075 | 0.2175 | 0.2675 | 0.1037 |
| | | | | | LA(c) | |
| *S. aureus* (ATCC 12692) | 0.435 | 0.2675 | 0.415 | 0.2175 | 0.2675 | 0.1037 |
| *E. coli* (ATCC 25922) | 0.0543 | 0.2675 | 0.415 | 0.1087 | 0.1337 | 0.415 |
| | | | | | LA(a)-04 | |
| *S. aureus* (ATCC 12692) | 0.435 | 0.2675 | 0.415 | 0.2175 | 0.2675 | 0.2075 |
| *E. coli* (ATCC 25922) | 0.1087 | 0.1337 | 0.415 | 0.1087 | 0.2675 | 0.2075 |
| | | | | | LA(b)-04 | |
| *S. aureus* (ATCC 12692) | 0.2175 | 0.2675 | 0.2075 | 0.2175 | 0.2675 | 0.1037 |
| *E. coli* (ATCC 25922) | 0.0543 | 0.2675 | 0.415 | 0.0543 | 0.2675 | 0.2075 |
| | | | | | LA-05 | |
| *S. aureus* (ATCC 12692) | 0.1087 | 0.2675 | 0.415 | 0.0543 | 0.2675 | 0.0129 |
| *P. aeruginosa* (ATCC 15442) | 0.2175 | 0.2675 | 0.0129 | 0.0543 | 0.2675 | 0.0518 |
| | | | | | LA-06 | |
| *S. aureus* (ATCC 12692) | 0.1087 | 0.2675 | 0.0129 | 0.1087 | 0.2675 | 0.0129 |
| *P. aeruginosa* (ATCC 15442) | 0.0543 | 0.2675 | 0.0129 | 0.0135 | 0.1337 | 0.008 |

**AMI: Amikacin; GEN: Gentamicin; NEO: Neomycin**




**LA-04**: R' = $OCOCH_3$; R" = H
**LA(a)-04**: R' = $OCOCH_3$; R" = $CH_3$
**LA(b)-04**: R' = $OCOCH_3$; R" = $CH_2CH_3$
**LA(c)-04**: R' = $OCOCH_3$; R" = $CH(CH_3)_2$

**LA-05**: R' = O; R" = H
**LA(a)-05**: R' = O; R" = $CH_3$
**LA(b)-05**: R' = O; R" = $CH_2CH_3$
**LA(c)-05**: R' = O; R" = $CH(CH_3)_2$

**LA-06**: R' = OCOH; R" = H
**LA(a)-06**: R' = OCOH; R" = $CH_3$
**LA(b)-06**: R' = OCOH; R" = $CH_2CH_3$
**LA(c)-06**: R' = OCOH; R" = $CH(CH_3)_2$

## HIGHLIGHTS

- The mono hydroxylated bile acid, lithocholic acid, and derivatives have been evaluated as antibacterial.
- Derivatives of lithocholic acid were investigated against the growth of different microorganisms.
- Lithocholic acid and derivatives showed good inhibitory results with aminoglycosides antibiotics.