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Lithocholic acid and derivatives: Antibacterial activity

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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.

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 Investigated and unpublished results showed that among the bile acids, lithocholic acid showed significant antibacterial activity.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., 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

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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 sulfuric 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 × 30 mL). The combined extracts were washed with H_2O (3 × 60 mL), Na_2CO_3 solution 20% (3 × 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): δ3.58–3.63 (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): δ36.70 (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): δ3.57–3.65 (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): δ35.58 (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): δ3.56–3.67 (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): δ35.55 (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 × 30 mL). The combined extracts were washed with H_2O (3 × 20 mL) and brine (1 × 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; ^1H NMR ($CDCl_3$, 300 MHz): δ4.67–4.77 (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): δ30.99 (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): δ4.65–4.76 (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): δ31.21 (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): δ4.66–4.77 (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): δ31.20 (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