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Synthesis of B- and C-ring-modified lithocholic acid analogues as potential sialyltransferase inhibitors

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ABSTRACT

In order to identify structural features of lithocholic acid (LCA) critical for inhibition of the enzyme sialyltransferase (ST) novel analogues with modifications of the skeleton (**7**–**9**, **16**–**18** and **20**) were designed and synthesized. Methyl 3α-acetoxy-7-oxo-cholanate (**1**), methyl 3α-acetoxy-12-oxo-cholanate (**2**) and methyl 3α,7α-diacetoxy-12-oxo-cholanate (**3**) were subjected to Baeyer-Villiger oxidation to provide homolactones (**7**–**9**) or to the Beckmann rearrangement of the corresponding oximes to give homolactams (**16**–**18**). Both reactions proceed regio- and stereoselectively. Ring B homolog of lithocholic acid (**20**) was efficiently synthesized. Among these compounds, **7**, **9** and **16** were found to have the significant activity, with IC_{50} values ⩽3 μM against α-2,6-(*N*)-ST selectively, which are 5-fold lower than that of Lith-*O*-Asp. Given the reality that LCA and its analogue, Lith-*O*-Asp, have been revealed to improve inhibitory efficacy of ST and to have a wide range of antimetastatic activities in different human cancer cells, the up-to-date findings have noteworthy pharmacological significance as they open a promising path to the improvement of a prospective molecular targeted application of modified LCA analogues as agents for the treatment of cancer metastasis.

1. Introduction

Lithocholic acid (LCA) and its analogues are considered as a new class of sialyltransferase (ST) inhibitors. With increasing attention to the mode of action at physiological and molecular levels and the receptors, the structure-activity relationships of lithocholic acid analogues are being studied in our group [1]. Among bile acids, lithocholic acid exhibits the highest activity in various sialyltransferase inhibition assays, which is generally ascribed to the 3-α-hydroxy, as well as the A/B *cis* ring junction and side chain [1]. Some structural modifications on the side chain and 3-α-hydroxy have been done so far, it is still obscure whether the steroid skeleton is really crucial for the high activity or not. The introduction of heteroatom or replacement of one or more carbon atoms in steroidal molecule by a heteroatom affects the chemical properties of the steroidal molecule and often results in alterations of its biological activities [2]. For example, the presence of the characteristic group (—NH—CO—) in the aza-homosteroid molecule had been proven to be important in lowering the acute toxicity and improving antitumour activity of the compound in cancer research [3].

We thought that ring expansion and/or insertion of —COO— or —CONH— to rings B or C should be a good modification to probe the structural requirements of LCA activity toward sialyltransferase inhibition. Accordingly, rings B and C homolog, homolactones and homolactams were synthesized and characterized as novel analogues of LCA. Evidence made in this effort reveal that the presence of a lactone/lactam or hydroxyl substituent in LCA analogues at rings B or C leads to considerably enhanced efficacy against α-2,6-(*N*)-ST rather than α-2,3-(*O*)-ST and suggest that compounds **7**, **9** and **16** may be effective molecular targeted agents for the treatment of cancer metastasis.

2. Experimental

2.1. General

All chemicals were obtained from commercial sources and used as purchased. ^{1}H NMR and ^{13}C NMR spectra were recorded with

Abbreviations: CMP-Neu5Ac, cytidine monophosphate-N-acetylneuraminic acid; EDTA, ethylenediaminetetraacetic acid; LCA, lithocholic acid; Lith-*O*-Asp, aspartic acid ester of lithocholic acid at 3-OH; MES, 2-(N-morpholino)ethanesulfonic acid; $TMSCHN_2$, trimethylsilyldiazomethane; ST, sialyltransferase.

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Bruker AMX400 or 500 MHz. Proton chemical shifts are reported in parts per million (ppm) relative to the singlet at 7.24 ppm for residual $CHCl_3$ in deuterochloroform or the quintet at 3.30 ppm for residual CHD_2OD in the methanol-d_4. Carbon chemical shifts are reported in parts per million (ppm) relative to the internal ^{13}C signals in $CDCl_3$ (77.0 ppm) and CD_3OD-d_4 (49.0 ppm). Mass spectra were obtained with a FAB JMS-700 double focusing mass spectrometer (JEOL, Tokyo, Japan). Melting points (m.p.) were recorded with a capillary melting point apparatus (Electrothermal MEL-TEMP). Analytical thin layer chromatography (TLC) was performed on silica gel 60 F254 (Merck). Flash column chromatography was performed with silica gel 60 (230–400 mesh) (Merck).

2.2. General procedure for Baeyer-Villiger oxidation for synthesis of (**4**–**6**)

Methyl ketocholanates **1**–**3** (0.66 mmol) in dichloromethane (12 mL) was added at 0 °C to a solution of trifluoroperacetic acid in dichloromethane prepared by adding trifluoroacetic anhydride (4.5 mL) to 30% aqueous hydrogen peroxide (0.75 mL) in dichloromethane (12 mL) at 0 °C. The mixture was stirred at this temperature for 2 h then was warmed to room temperature and maintained for 4 h. The reaction mixture was diluted with dichloromethane (30 mL), and the resulting solution was washed with saturated sodium bicarbonate solution, brine solution, dried over anhydrous sodium sulphate, and concentrated. The residue was chromatographed (eluting with ethyl acetate/hexane; 2/1) to afford the lactones **4**–**6** as white solids.

2.3. Methyl 3α-acetoxy-8-oxa-7-oxo-B-homo-5β-cholanate (**4**)

Gave 250.40 mg (82% yield), R_f = 0.25 (hexane/EtOAc, 2/1). ^1H NMR (400 MHz, $CDCl_3$) δ 4.62–4.58 (m, H-3 ax.), 4.11 (dd, J = 9.2 and 10.0 Hz, H-8 ax), 3.55 (s, CH_3O), 2.95 (br. d, J = 15.0 Hz, H-6), 2.30–2.05 (m, 3H), 1.95–1.07 (m, 24H, steroidal CH, CH_2), 0.98 (s, CH_3-19), 0.81 (d, J = 6.8 Hz, CH_3-21), 0.56 (s, CH_3-18). ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.07 (C-24), 173.62 (C-7), 170.03 (AcO), 79.23 (C-8), 72.39 (C-3), 55.43 (C-17), 54.18 (C-14), 51.17 (CH_3—O), 42.90 (C-13), 41.23 (C-5), 39.69 (C-9), 38.15 (C-12), 37.27 (C-10), 35.23 (C-1), 35.08 (C-6), 34.72 (C-20), 31.48 (C-4), 30.68 (C-23), 30.53 (C-22), 27.48 (C-16), 26.20 (C-2), 24.77 (C-15), 23.14 (C-19), 22.26 (C-11), 20.95, 17.93 (C-21), 11.25 (C-18).

2.4. Methyl 3α-acetoxy-13-oxa-12-oxo-C-homo-5β-cholanate (**5**)

Gave 250.00 mg (82% yield), R_f = 0.30 (hexane/EtOAc, 2/1). ^1H NMR (400 MHz, $CDCl_3$) δ 4.67–4.59 (m, H-3 ax.), 3.57 (s, CH_3O), 2.52 (br. d, J = 12.0 Hz, H-11), 2.37–2.25 (m, 3H), 1.92 (s, 3H, CH_3-CO), 1.84–1.02 (m, 24H, steroidal CH, CH_2, CH_3-18), 0.95 (d, J = 6.5 Hz, CH_3-21), 0.80 (s, CH_3-19). ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.21 (C-24), 174.25 (C-12), 170.49 (AcO), 87.24 (C-13), 73.44 (C-3), 55.15, 54.95, 51.25, 40.83, 38.86, 37.33, 36.29, 35.27, 34.55, 33.91, 31.67, 30.52, 29.95, 26.31, 26.25, 25.85, 25.49, 23.86, 22.11, 21.06, 20.53, 20.32, 16.99, 14.39.

2.5. Methyl 3α,7α-diacetoxy-13-oxa-12-oxo-B-homo-5β-cholanate (**6**)

Gave 281.80 mg (82% yield), R_f = 0.30 (hexane/EtOAc, 2/1). ^1H NMR (400 MHz, $CDCl_3$) δ 4.60 (br. d, J = 2.0 Hz, H-7) 4.46–4.39 (m, 1H, H-3 ax.), 3.49 (s, CH_3O), 2.52 (br. d, J = 13.2 Hz, H-11), 2.37–2.25 (m, 4H), 2.10–0.99 (m, 30H, steroidal CH, CH_2, CH_3-18, 2 Ac), 0.88 (d, J = 6.6 Hz, CH_3-21), 0.76 (s, CH_3-19). ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.95 (C-24), 170.17 (C-12), 169.70 (AcO), 86.28 (C-13), 73.14 (C-3),71.02, 55.03, 51.16, 49.84, 40.66, 39.85, 35.64, 35.17, 34.69, 34.26, 33.80, 32.75, 30.65, 30.40, 29.91, 26.35, 24.72, 23.69, 21.71, 21.17, 21.04, 17.01, 14.23.

2.6. General procedure for saponification of lactone for synthesis of (**7**–**9**)

A mixture of lactone (0.11 mmol) and barium hydroxide octahydrate (powdered 3.5 g) in MeOH (5 mL) was stirred at room temperature for 97 h. The heavy white suspension was diluted with brine (3 ml) and acidified with 1 M HCl to give a clear solution of pH = 3–4. This aqueous solution was extracted with EtOAc ($2 \times 50 + 2 \times 20$) and the combined organic extracts washed with brine (20 mL), dried, concentrated in vacuo, washed with chloroform to give the deprotected lactones **7**–**9** as white solids.

2.7. 3α-Hydroxy-8-oxa-7-oxo-B-homo-5β-cholanoic acid (**7**)

Gave 40.20 mg (90% yield), R_f = 0.23 (dichloromethane/MeOH, 5/1). Mp. 212–213 °C ^1H NMR (400 MHz, acetone-d_6) δ 4.40 (dd, J = 9.2 and 10.0 Hz, H-8 ax), 3.59–3.54 (m, H-3 ax.), 3.20 (br. d, J = 14.8 Hz, H-6), 2.35–2.19 (m, 3H), 1.99–1.26 (m, 22H, steroidal CH and CH_2), 1.12 (s, CH_3-19), 0.96 (d, J = 6.4 Hz, CH_3-21), 0.72 (s, CH_3-18). ^{13}C NMR (100 MHz, acetone-d_6) δ 175.06 (C-24), 173.89 (C-7), 79.55 (C-8), 70.93 (C-3), 56.75 (C-17), 55.69 (C-14), 44.03 (CH_3—O), 42.66 (C-13), 41.18 (C-5), 39.48 (C-9), 38.39 (C-12), 37.08 (C-10), 36.58 (C-1), 36.38 (C-20), 35.89 (C-4), 31.74 (C-6), 31.37 (C-23), 31.24, 28.45 (C-16), 25.71 (C-15), 23.92 (C-19), 23.27 (C-11), 18.65 (C-21), 11.93 (C-18). HRMS calcd for $C_{24}H_{37}O_5$ $(M-H)^-$, 405.2641; found, 405.2635.

2.8. 3α-Hydroxy-13-oxa-12-oxo-C-homo-5β-cholanoic acid (**8**)

Gave 35.70 mg (80% yield), R_f = 0.21 (dichloromethane/MeOH, 5:1). Mp. 102–103 °C. ^1H NMR (400 MHz, MeOH-d_4) δ 3.56–3.50 (m, H-3 ax.), 2.37–2.17 (m, 4H), 2.00–1.20 (m, 20H, steroidal CH and CH_2), 1.05 (d, J = 5.6 Hz, CH_3-21), 0.93 (s, CH_3-19), 0.82 (s, CH_3-18). ^{13}C NMR (100 MHz, MeOH-d_4) δ 179.04 (C-24), 178.31 (C-12), 82.28 (C-13), 72.39 (C-3), 54.41, 52.92, 43.21, 37.68, 37.23, 36.54, 35.71, 32.10, 31.92, 31.74, 28.10, 26.62, 25.81, 23.94, 20.53, 17.82, 16.73. HRMS calcd for $C_{24}H_{37}O_5$ $(M+Na)^+$, 429.2617; found, 429.2613.

2.9. 3α,7α-Dihydroxy-13-oxa-12-oxo-C-homo-5β-cholanoic acid (**9**)

Gave 39.5 mg (85% yield), R_f = 0.2 (dichloromethane/MeOH, 5:1). Mp. 99–101 °C. ^1H NMR (400 MHz, MeOH-d_4) δ 3.70 (d, J = 2.4 Hz, H-7) 3.44–3.39 (m, H-3 ax.), 2.7.–2.5 (m, 2H), 2.40–1.26 (m, 26H, steroidal CH and CH_2, CH_3-19), 1.05 (d, J = 6.4 Hz, CH_3-21), 0.91 (s, CH_3-18). ^{13}C NMR (100 MHz, MeOH-d_4) δ 178.14 (C-12 and C-24), 88.91 (C-13), 72.53 (C-3), 68.76 (C-7), 57.01, 51.70, 43.75, 42.49, 40.40, 37.33, 36.61, 36.39, 35.87, 35.56, 33.78, 32.31, 31.96, 31.80, 26.56, 26.07, 25.16, 22.60, 17.83, 14.78. HRMS calcd for $C_{24}H_{38}O_6$ $(M-H)^-$, 421.2590; found, 421.2583.

2.10. General procedure for oxime synthesis (**10**–**12**)

Sodium acetate (1.03 g, 13.42 mmol, 5.75 equiv.) and hydroxylamine hydrochloride (0.288 g, 4.13 mmol, 2 equiv) were added to a solution of ketone (1.0 g, 1 equiv) in methanol (20 mL). The reaction mixture was stirred at reflux for 6 h. The solvent was removed under vacuum and the residue dissolved in CH_2Cl_2. The organic solution was washed with water then dried over Na_2SO_4. The solvent was evaporated under reduced pressure and the crude product recrystallized from aq. ethanol to afford chemically pure oximes **10**–**12** as white solids.