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Antitumor activity of newly synthesized oxo and ethylidene derivatives of bile acids and their amides and oxazolines

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ABSTRACT

Bile acid derivatives with modifications in side chain and modifications on steroid skeleton were synthetized and their antitumor activity against five human cancer cell lines was investigated. Modifications in side chain include amid group, formed in reaction with 2-amino-2-methylpropanol, and 4,4-dimethyloxazoline group, obtained after cyclization of amides. In the steroid skeleton oxo groups were introduced in position 7 (**2**, **2a**, **2b**) and 7,12 (**3**, **3a**, **3b**). Ethylidene groups were introduced regio- and stereoselectively on C-7, and/or without stereoselectivity on C-3 by Wittig reaction. By combination of these modifications, a series of 19 bile acid derivatives were synthesized. Compounds containing both C-7 ethylidene and C-12 carbonyl groups (**6**, **6a**, **6b**) shown very good antitumor activity with $IC_{50} < 5$ μM. Altering carboxylic group to amide or oxazoline group has positive effect on cytotoxicity. Different molecular descriptors were determined *in silico* and after principal component analysis was found that molecular descriptor BLTF96 can be used for fast assessment of experimental cytotoxicity of bile acid derivatives.

1. Introduction

For long time bile acids (BAs) were considered as detergent-like molecules derived from cholesterol with important role in absorption of cholesterol, fat-soluble vitamins, and lipids from the intestines. New established role of BAs as endogenous ligand for Farnesoid X receptor (FXR) [1–3] in 1999 and discovery of BAs membrane-type, G protein coupled receptor TGR5 [4,5] in 2002 initiated renaissance of BAs research which profusely supplemented knowledge of bile acid physiology and chemistry. Hence, BAs were recognized as signaling molecules with various endocrine and paracrine functions involved in cholesterol homeostasis, lipid and carbohydrate metabolism, and regulations of immune system [6].

Primary BAs, cholic acid (**1**) and chenodeoxycholic acid (CDCA) are synthesized in liver and conjugated with glycine or taurine in corresponding *N*-acyl conjugates. As conjugates, they are secreted into bile that is evacuated in intestine. In small intestine and in colon BAs conjugates are unconjugated and 7-dehydroxylated by enteric bacteria to produce secondary bile acids, deoxycholic acid (DCA) and lithocholic acid (LCA) from cholic acid (**1**) and CDCA, respectively [7–10].

Diets with high fat intake promote increased secretion of BAs which leads to elevated content of secondary BAs in intestine. Secondary BAs are cytotoxic and correlated with colon tumor [11–13]. Mechanism by which secondary BAs promote colon tumorigenesis and induce cytotoxicity could be connected with their ability to alter stability of membrane bilayer [14]. Also, increased hydrophobicity of secondary BAs, DCA and LCA enable greater capacity to partly digest or perturb the structure of cell membranes [15]. While DCA and LCA at higher concentrations (≥250 μM) induce necrosis and at lower concentrations apoptosis [16–18], ursodeoxycholic acid (UDCA) have chemoprotective effect on normal cells [19]. These finding that small changes in structure of BAs greatly influence biological activity prompted us to synthesize new derivatives. Since hitherto research included only effect of modification of steroid side chain of natural BAs [20–23] we investigated influence of derivatives with structural changes both in side chain and in steroid nucleus on antitumor activity.

2. Experimental

Dehydrocholic acid (**4**) was obtained from Sigma. 3α-Hydroxy-7,12-dioxo-5β-cholanoic acid (**3**) was synthesized as described

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previously [24]. Compounds **1a** and **1b** were synthesized according to method described in [25], while **2**, **2a** and **2b** according to [26]. Compounds **5**, **6**, **8**, **8′**, **9** and **9′** were synthesized as described in our previous publication [27]. Flash chromatography was performed on silica gel 60 (0.04–0.063 mm, Merck). Melting points were determined using a Nagema Boeitus melting point apparatus and the results are uncorrected. IR spectra were recorded on a Nexus 670 FT-IR spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC 250 apparatus (^{1}H 250 MHz and ^{13}C 62.5 MHz), Bruker Avance III HD 400 (400 MHz ^{1}H, 101 MHz ^{13}C) apparatus using tetramethylsilane as the internal standard. HRMS spectra (TOF) were recorded on a 6210 Time-of-Flight LC/MS Agilent Technologies (ESI+) instrument. The colorimetric MTT assay was carried out following the reported procedure [28].

2.1. 2-(3α-Hydroxy-7,12-dioxo-5β-cholan-24-amido)-2-methyl-1-propanol (**3a**)

To suspension of compound **3** (1.7121 g; 4.23 mmol) in ethyl-acetate (60 mL) were added: triethylamine (1 mL), 2-amino-2-methyl-1-propanol (50% solution in ethyl acetate 5 mL; 13.10 mmol), EEDQ (2.08 g; 8.41 mmol) and water (7 mL). Reaction mixture was refluxed for 8 h. After cooling to room temperature, the reaction mixture was washed successively with 3 M HCl (1 × 10 mL), water (1 × 10 mL), 10% $NaHCO_3$ (2 × 10 mL) and then with water to neutrality (3 × 10 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered, and evaporated in vacuum to give an oily residue which was purified by flash column chromatography ($CHCl_3$/Acetone 4:1). Pure **3a** was obtained after recrystallization from acetone as white crystals (mp 190 °C) in yield of 60% (1.2100 g).

^{1}H NMR (400 MHz, DMSO-d_6, ppm): 0.75 (d, J = 6.1 Hz, 3H, H-21), 0.98 (s, 3H, H-18), 1.16 (s, 6H, 2CH_3 on amide), 1.25 (s, 3H, H-19), 2.75 (t, J = 12.7 Hz, 1H, H-11), 2.98 (m, 2H, H-8 and CH_2), 3.36 (s, 2H, CH_2 from amide), 4.47 (d, J = 4.5 Hz, 1H, OH from amide), 4.89 (s, 1H, OH on C-3), 7.24 (s, 1H, NH), ^{13}C NMR (101 MHz, DMSO-d_6, ppm): 11.86 (C-18), 19.32 (C-21), 22.54 (C-19), 24.17 (2CH_3 from amide), 25.11, 27.71, 30.11, 31.66, 33.71, 34.10, 35.48, 35.90, 37.88, 38.55 (C-11), 44.95, 45.51, 45.62, 45.71, 48.40 (C-8), 52.50, 54.59, 56.72, 68.12 (CH_2 from amide), 69.23 (C-3), 173.14 (C-24), 210.49 (C-7), 213.03 (C-12). HRMS: calculated for $C_{28}H_{45}NO_5$ $(M+H)^+$: 476.33705; found: 476.33702. IR (cm^{-1}): 3366, 2932, 2874, 1705, 1648, 1545, 1461, 1389, 1272, 1066, 734.

2.2. 2-Methyl-2-(3,7,12-trioxo-5β-cholan-24-amido)-1-propanol (**4a**)

Compound **4a** was prepared in similar manner as **3a**. Dehydrocholic acid (**4**) (0.5275 g; 1.31 mmol), ethyl acetate (13 mL), triethylamine (0.3 mL), 2-amino-2-methyl-1-propanol (0.5 mL; 5.24 mmol), EEDQ (0.60 g; 2.43 mmol) and water (8 mL). Reaction mixture was refluxed for 5 h. Crude product was purified by flash column chromatography (CH_2Cl_2/Acetone 5:1). Pure **4a** was obtained as solid (mp 252 °C after recrystallization from acetone) in 68% yield (0.4814 g).

^{1}H NMR (400 MHz, $CDCl_3$, ppm): 0.86 (d, J = 6.4 Hz, 3H, H-21), 1.08 (s, 3H, CH_3), 1.30 (s, 6H, 2CH_3 from amide), 1.41 (s, 3H, CH_3), 3.58 (s, 2H, CH_2 from amide), 4.99 (bs, 1H, OH from amide), 5.61 (s, 1H, NH). ^{13}C NMR (101 MHz, $CDCl_3$, ppm): 11.85, 18.78, 21.90, 24.79, 24.89, 25.12, 27.60, 31.12, 34.07, 35.26, 35.38, 36.01, 36.48, 38.64, 42.79, 44.98, 45.47, 45.53, 46.82, 48.98, 51.78, 56.21, 56.91, 70.92, 174.50 (C-24), 208.76, 209.12, 212.09. HRMS: calculated for $C_{28}H_{43}NO_5$ $(2M+H)^+$: 947.63552; found: 947.63476. IR (cm^{-1}): 3307, 2927, 1705, 1652, 1557, 1471, 1387.

2.3. 2-((E)-7-Ethylidene-3α,12α-dihydroxy-5β-cholan-24-amido)-2-methyl-1-propanol (**5a**)

Compound **5a** was prepared in similar manner as **3a**. (*E*)-7-Ethylidene-3α,12α-dihydroxy-5β-cholanoic acid (**5**, 0.7803 g; 1.86 mmol), ethyl-acetate (16 mL), triethylamine (0.3 mL), 2-amino-2-methyl-1-propanol (50% solution in ethyl acetate 0.5 mL; 2.62 mmol), EEDQ (0.62 g; 2.55 mmol), without addition of water. Crude product was purified by flash chromatography ($CHCl_3$/Acetone 1:1) yielded 0.6930 g (76%) of pure **5a** in form of oil.

^{1}H NMR (400 MHz, DMSO-d_6, ppm): 0.61 (s, 3H, H-18), 0.94 (d, J = 6.3 Hz, 3H, H-21), 0.98 (s, 3H, H-19), 1.16 (s, 6H, 2CH_3 from amide), 1.53 (d, J = 6.5 Hz, 3H, CH_3 from ethylidene), 3.37 (s, 2H, CH_2 from amide), 3.82 (s, 1H), 4.21 (d, J = 3.2 Hz, 1H), 4.42 (d, J = 4.3 Hz, 1H), 4.91 (t, J = 5.7 Hz, 1H, OH from amide), 5.23 (q, J = 6.2 Hz, 1H, CH from ethylidene), 7.24 (s, 1H, NH). ^{13}C NMR (101 MHz, DMSO-d_6, ppm): 13.17, 13.37, 17.85, 23.86, 24.18 (2CH_3 from amide), 25.18, 27.67, 29.09, 30.55, 31.89, 32.13, 33.55, 35.22, 35.29, 35.67, 36.30, 36.61, 41.11, 42.93, 45.10, 46.06, 46.63, 54.58, 68.18, 70.01, 70.80, 114.08 (CH from ethylidene), 140.15 (C-7), 173.35 (C-24). HRMS: calculated for $C_{30}H_{51}NO_4$ $(M+K)^+$: 528.34497; found: 528.34503. IR (cm^{-1}): 3322, 2938, 2867, 1647, 1552, 1455, 1379.

2.4. 2-((E)-7-Ethylidene-3α-hydroxy-12-oxo-5β-cholan-24-amido)-2-methyl-1-propanol (**6a**)

Compound **6a** was prepared in similar manner as **3a**. (*E*)-7-Ethylidene-3α-hydroxy-12-oxo-5β-cholanoic acid (**6**) (0.1552 g; 0.37 mmol), ethyl-acetate (3 mL), triethylamine (0.1 mL), 2-amino-2-methyl-1-propanol (50% solution in ethyl acetate 0.2 mL; 2.51 mmol), EEDQ (0.12 g; 0.49 mmol), without addition of water. Flash chromatographically purified crude product ($CHCl_3$/Acetone 4:1) gave 0.1135 g (62%) of pure **6a** (as oil).

^{1}H NMR (400 MHz, $CDCl_3$, ppm): 0.84 (d, J = 6.5 Hz, 3H, H-21), 1.06 (s, 3H, CH_3), 1.16 (s, 3H, CH_3), 1.29 (s, 6H, 2CH_3 from amide), 1.56 (d, J = 6.6 Hz, 3H, CH_3 from ethylidene), 3.57 (s, 2H, CH_2 from amide), 3.62 (m, 1H, H-3), 5.22 (q, J = 6.4 Hz, 1H, CH from ethylidene), 5.58 (s, 1H, NH). ^{13}C NMR (101 MHz, $CDCl_3$, ppm): 11.85, 13.08, 18.73, 23.09, 24.82, 24.89, 25.90, 27.57, 30.23, 31.09, 31.65, 34.11, 34.89, 35.49, 36.10, 36.64, 38.22, 42.57, 44.25, 45.45, 46.92, 53.81, 56.22, 57.00, 70.94, 71.04, 116.05 (CH from ethylidene), 137.52 (C-7), 174.67 (C-24), 215.10 (C-12). HRMS: calculated for $C_{30}H_{49}NO_4$ $(M+H)^+$: 488.37344; found: 488.37325. IR (cm^{-1}): 3324, 2928, 2244, 1704, 1644, 1549, 1453, 1383.

2.5. 2-(3α-Hydroxy-7,12-dioxo-5β-cholano-23-yl)-4,4-dimethyloxazoline (**3b**)

Freshly distilled $SOCl_2$ (1.4 mL, 19.3 mmol), was added dropwise to the stirred ice-cooled solution of compound **3a** (2.1550 g; 4.53 mmol) in THF (30 mL) and the reaction mixture was stirred further for 90 min. A saturated $NaHCO_3$ solution (50 ml) was added with vigorous stirring. Reaction mixture was extracted with ethyl acetate (4 × 15 mL). The organic extracts were combined, washed with water (2 × 10 mL), dried over anhydrous Na_2SO_4 and evaporated to dryness. Crude product was purified by flash chromatography (CH_2Cl_2/Acetone 3:1), and recrystallized from acetone to afford white crystals (mp 185 °C) of pure **3b** (1.7300 g; 83%).

^{1}H NMR (400 MHz, DMSO-d_6, ppm): 0.77 (d, J = 5.8, 3H, H-21), 0.98 (s, 3H, H-18), 1.14 (s, 6H, 2CH_3 on C-4′), 1.25 (s, 3H, H-19), 2.74 (t, J = 12.7 Hz, 1H, H-11β), 2.97 (m, 2H, H-6β), 3.35 (m, 1H, H-3), 3.84 (s, 2H, H-5′), 4.47 (d, J = 5.4, 1H, OH on C-3).^{13}C NMR (101 MHz, DMSO-d_6, ppm): 11.83 (C-18), 19.14 (C-21), 22.54 (C-19), 24.17, 25.15, 27.72, 28.70 (CH_3 on C-4′), 28.78 (CH_3 on C-4′),