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Synthesis and cytotoxicity of A-homo-lactam derivatives of cholic acid and 7-deoxycholic acid

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ABSTRACT

Using cholic acid and deoxycholic acid as starting materials, a series of 3-aza-A-homo-4-one bile acid and 7-deoxycholic acid derivatives were synthesized by the esterification, oxidation, reduction, oximation and Beckman rearrangement etc. The cytotoxicity of the synthesized compounds against MGC 7901 (human ventriculi carcinoma cell line), hela (human cervical carcinoma cell line), SMMC 7404 (human liver carcinoma cell line) were investigated. The results showed that bile acid and 7-deoxycholic-acid derivatives with 3-aza-A-homo-4-one configuration bearing a 6-hydroximino or 12-hydroximino group displayed a distinct cytotoxicity to Hela tumor cell line. In particular, the IC_{50} values of the compounds **6** and **13** were 14.3 and 24.3 μmol/L against Hela human tumor cell line respectively. The information obtained from the studies may be useful for the design of novel chemotherapeutic drugs.

1. Introduction

The synthesis of some aza-homosteroid compounds with unusual and interesting structures have been reported recently [1–5]. These compounds exhibit valuable biological activities such as cytotoxicity, antibacterium and antileukemic activity. Researches of azahomosteroids indicated that 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 [6,7]. In other way, bile acids have been considered very useful in the preparation of new pharmaceutical drugs because of their inherent chemical and biological properties [8,9]. They are pharmacological interesting as potential carriers of liver-specific drugs, absorption enhancers and cholesterol lowering agent. The facial amphiphilic structure of bile acids is the potential to be antibiotic and polymyxin [10–12]. Recently, researches found that bile acid conjugates synthesized or obtained from nature have exhibit activity in the respect of antibiotics, ionophores and molecular carriers [13,14]. However, the investigations of this kind of compounds mainly focus on bile acids conjugates which were modified at side chain carboxyl or hydroxyl on position-3. Compounds modified on the steroidal nucleus of cholic acid or deoxycholic acid have rarely been reported. In our previous work, we found that a polyhydroxysterol or a steroidal oxime has an excellent cytotoxicity when it has the cholesteric side chain and the 3- or 6-position on steroidal nucleus is substituted by the hydroxyl or hydroximino [15–17].

In order to evaluate the antitumor activity of new steroidal derivatives, we designed and synthesized some new steroidal compounds with the introduction of N atom on A-ring using cholic acid and 7-deoxycholic acid as starting materials. The cytotoxicity of synthesized compounds was tested in vitro against three tumor cell lines: MGC 7901 (human ventriculi carcinoma cell line), Hela (human cervical carcinoma cell line) and SMMC 7404 (human liver carcinoma cell line). The results reveal that the compounds have a better cytotoxicity against Hela human tumor cell line.

2. Experimental

2.1. Chemistry

The sterols and $NaBH_4$ were purchased from the Merck Co. Compounds **4** and **10** were prepared according to the procedures in the literature [18]. All chemicals and solvents were analytical grade and solvents were purified by general methods before being used. Melting points were determined on an X_4 apparatus and were uncorrected. Infrared spectra were measured with a Nicolet FT-360 Spectrophotometer. The ^1H and ^{13}C NMR spectra were

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recorded in $CDCl_3$ on a Bruker AV-600 spectrometer at working frequencies 600 and 150 MHz and a Bruker AV-300 spectrometer at working frequencies 300 and 75 MHz, respectively. Chemical shifts are expressed in ppm (δ) values and coupling constants (J) in Hz. LRESIMS were recorded on a Thermo-DSQ instrument. The cell proliferation assay was undertaken by a MTT method using 96-well plates on Biocell ELIASA analysis spectrometer.

*2.1.1. The synthesis of methyl 4,7,12-trioxo-3-aza-A-homo-cholicate (**5**)*

To a solution of oxime **4** (300 mg, 0.74 mmol) in dry THF (12 mL) the solution of thionyl chloride (1.4 mL) in 4 mL dry THF was added under argon. The solution was stirred under anhydrous conditions for 30 min at 0 °C. Then the reaction was terminated and water was added to the solution. The solution was neutralized with ammonia and the product was extracted with CH_2Cl_2. The combined extract was washed with water and saturated brine, dried over anhydrous Na_2SO_4, and evaporated under reduced pressure to give a crude product which was chromatographed on silica gel (elution: $V_{methanol}$:$V_{dichloromethane}$ = 1:50) to give 180 mg of **5** as a white solid. Yield: 60.0%, θ_{mp} 221–223 °C. IR(KBr) ν/cm^{-1}: 3382, 2949, 2872, 1732, 1703, 1654, 1142, 1380, 1331; ^{1}H NMR($CDCl_3$, 600 MHz) δ: 0.861(3H, d, J = 6.6, 21-CH_3), 1.069(3H, s, 19-CH_3), 1.389(3H, s, 18-CH_3), 2.088(1H, dd, J = 12.6, 5.4, C_9-αH), 2.159(1H, dd, J = 12.6, 2.4, C_6-βH), 2.27-2.22(1H, m, C_{23}-H), 2.293(1H, dd, J = 9.0, 7.2, C_{11}-αH), 2.415(1H, ddd, J = 15.6, 9.6, 5.4, C_{23}-H), 2.614(1H, dd, J = 15.6, 12.6, C_{4a}-αH), 2.788(1H, t, J = 12.6, C_8-H), 2.838(1H, t, J = 11.4, C_5-H), 2.990(1H, dd, J = 12.6, 5.4, C_{4a}-βH), 3.059–3.107(1H, m, C_2-βH), 3.237(1H, ddd, J = 15.6, 10.2, 5.4, C_2-αH), 3.686(3H, s, –OCH_3), 5.979(1H, brs, –NH); ^{13}C NMR($CDCl_3$, 75 MHz) δ: 212.1(12-C), 208.5(7-C), 175.6(4-C), 174.5(24-C), 56.8(8-C), 51.59(17-C), 51.51(O–CH_3), 48.9(14-C), 47.5(9-C), 46.7(13-C), 45.6(6-C), 43.2(1-C), 39.4(5-C), 38.64(4a-C), 38.60(11-C), 38.4(2-C), 36.3(10-C), 35.5(20-C), 31.3(23-C), 30.4(22-C), 27.6(15-C), 25.0(16-C), 22.9(21-C), 18.6(18-C), 11.8(19-C); LRESIMS(m/z): 432(M^++1).

2.1.2. The synthesis of methyl 4,12-dioxo-7-hydroxyimino-3-aza-A-homo-cholicate(6)

$CH_3COONa{\cdot}3H_2O$ (31.5 mg, 0.23 mmol) and NH_2OH.HCl (16.1 mg, 0.23 mmol) were added to the solution of **5** (100 mg, 0.23 mmol) in 30 mL 95% ethanol. After the solution was heated to 60 °C, the mixture was stirred at the temperature for 40 min. Then the reaction was terminated and the majority of solvent was evaporated under reduced pressure. Distilled water was added into the reaction mixture, and the product was extracted with ethyl acetate. The combined extracts were washed with saturated brine, dried, and evaporated under reduced pressure. The residue was subjected to chromatography using methanol/dichloromethane (1:40) as the eluent to give 83 mg of **6** (80%) as a white solid, θ_{mp} 198–200 °C. IR(KBr) ν/cm^{-1}: 3395, 2925, 2872, 1736, 1707, 1662, 1433, 1388, 1164, 976; ^{1}H NMR($CDCl_3$, 600 MHz) δ: 0.841 (3H, d, J = 6.6, 21-CH_3), 1.068(3H, s, 19-CH_3), 1.233(3H, s, 18-CH_3), 2.10-2.16(2H, m, C_{11}-βH, C_{4a}-βH), 2.274(1H, dd, J = 9.6, 6.6, C_{11}-αH), 2.260(1H, ddd, J = 15.6, 9.6, 6.6, C_{23}-H), 2.350(1H, brd, J = 15.6, C_6-βH), 2.395(1H, ddd, J = 15.6, 10.2, 5.4, C_{23}-H), 2.592(1H, t, J = 11.4, C_8-H), 2.659(1H, dd, J = 15.6, 12.0, C_{4a}-αH), 2.693(1H, t, J = 12.6, C_5-H), 3.056-3.006(1H, m, C_2-βH), 3.283(1H, brd, J = 10.2, C_6-αH), 3.206(1H, ddd, J = 15.0, 9.6, 4.8, C_2-αH), 3.669(3H, s, –OCH_3), 6.078(1H, brs, –NH), 7.927(1H, brs, =NOH); ^{13}C NMR($CDCl_3$, 75 MHz) δ: 213.9(12-C), 177.0(4-C), 174.7(24-C), 157.2(7-C), 57.1(17-C), 52.5(O–CH_3), 51.5(13-C), 48.5(14-C), 45.8(9-C), 41.7(1-C), 41.6(8-C), 39.5(4a-C), 39.0(10-C), 38.4(11-C), 37.4(5-C), 36.5(2-C), 35.6(20-C), 31.3(23-C), 30.4(22-C), 29.3(6-C), 27.6(15-C), 25.6(16-C), 22.9(21-C), 18.6(19-C), 11.7(18-C); LRESIMS(m/z): 447(M^++1).

*2.1.3. The synthesis of methyl 4,12-dioxy-3-aza-A-homo-7-deoxycholicate (**11**)*

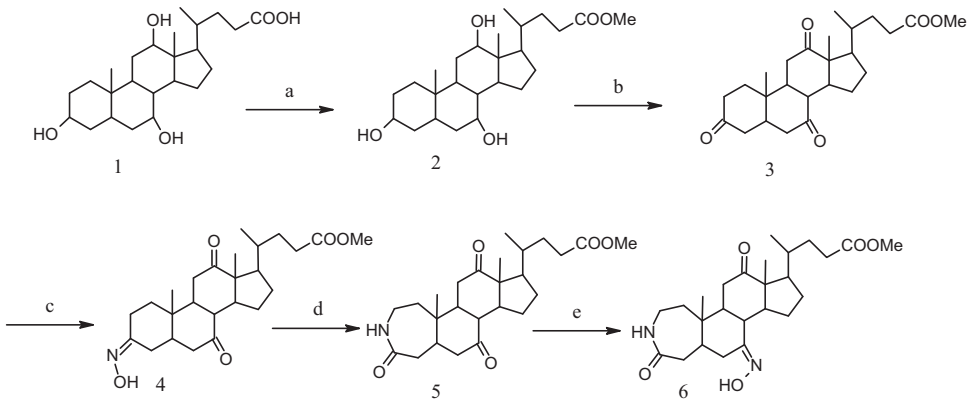
Freshly distilled thionyl chloride (1.4 mL) in 4 mL dry THF was added dropwise to a solution of methyl (Z)-3-hydroxyimino-12-oxo-deoxycholicate (**10**, 300 mg, 0.72 mmol) in 12 mL dry THF cooled to 0 °C, while maintaining the temperature at 0 °C. After this addition, the reaction mixture was stirred at 0–10 °C for 90 min under a argon atmosphere. The mixture was then poured into ice–water, neutralized with a solution of NH_3, and extracted with DCM. The organic layer was washed with water and saturated brine, dried over anhydrous Na_2SO_4. Evaporation of the solvent under reduced pressure gave a solid residue, which was chromatographed over silica gel using petroleum ether (60–90 °C)/EtOAc (1:3) as the eluent to give 220 mg (73%) of **11** as a white solid, m.p. 213–215 °C; IR(KBr) ν/cm^{-1}: 3322, 2937, 2864, 1744, 1708, 1671, 1446, 1380, 1335, 1266, 1192; ^{1}H NMR($CDCl_3$, 600 MHz) δ: 0.875(3H, d, J = 6.6, 21-CH_3), 1.058(3H, s, 19-CH_3), 1.105(3H, s, 18-CH_3), 2.26-2.22(1H, m, C_{23}-H), 2.294(1H, dd, J = 9.6, 6.6, C_{11}-αH), 2.416(1H, ddd, J = 15.6, 9.6, 5.4, C_{23}-H), 2.551(1H, dd, J = 15.0, 9.6, C_{11}-βH), 2.583(1H, dd, J = 12.0, 5.4, C_{4a}-αH), 2.981(1H, dd, J = 15.0, 12.0, C_{4a}-βH), 3.074(1H, ddd, J = 15.0, 7.8, 7.2, C_2-βH), 3.256(1H, ddd, J = 15.0, 9.6, 5.4, C_2-αH), 3.686(3H, s, –OCH_3), 5.878(1H, brs, –NH); ^{13}C NMR($CDCl_3$, 75 MHz,) δ: 214.2(12-C), 177.2(4-C), 174.6(24-C), 58.3(9-C), 58.2(17-C), 57.5(14-C), 51.5(O–CH_3), 46.5(13-C), 40.1(5-C), 39.3(1-C), 38.6(4a-C), 38.5(2-C), 37.9(11-C), 36.7(8-C), 35.7(10-C), 35.6(20-C), 31.3(23-C), 30.5(22-C), 29.8(7-C), 27.4(6-C), 25.7(16-C), 24.2(15-C), 22.6(21-C), 18.6(19-C), 11.7(18-C); LRESIMS(m/z): 418(M^++1).

*2.1.4. The synthesis of menthyl 12-hydroxy-4-oxy-3-aza-A-homo-deoxycholicate (**12**)*

To the stirred solution of **11** (200 mg, 0.48 mmol) in CH_3OH (50 mL) was added $NaBH_4$ (63 mg, 1.7 mmol) in 10 min at room temperature. After 20 min, the reaction was stopped. The solution was neutralized with 1 M HCl. After evaporation of the majority of MeOH under reduced pressure, the residue was extracted with ethyl acetate. The organic layer was washed with cold water and saturated brines. After drying over anhydrous sodium sulfate, the solvent was removed under reduced pressure to obtain a crude product, which was chromatographed on silica gel (elution: $V_{methanol}$:$V_{dichloromethane}$ = 1:40) to give 104 mg of **12** (52%) as a white solid. m.p 105–107 °C; IR(KBr) ν/cm^{-1}: 3432, 3215, 2945, 2868, 2353, 2320, 1732, 1663, 1437, 1360, 1245, 1168, 1012, 788; ^{1}H NMR($CDCl_3$, 600 MHz) δ: 0.761(3H, s, 18-CH_3), 1.028(3H, d, J = 6.6, 21-CH_3), 1.029(3H, s, 19-CH_3), 2.213(1H, dd, J = 15.6, 9.0, C_{17}-H), 2.289(1H, ddd, J = 15.6, 9.0, 7.2, C_{23}-H), 2.407(1H, ddd, J = 15.6, 9.6, 5.4, C_{23}-H), 2.551(1H, dd, J = 15.6, 12.0, C_{4a}-αH), 3.031(1H, dd, J = 15.0, 12.0, C_{4a}-βH), 3.09-3.04(1H, m, C_2-βH), 3.245(1H, ddd, J = 15.0, 9.6, 4.8, C_2-αH), 3.487(1H, dd, J = 15.6, 4.8, C_{12}-αH), 3.684(3H, s, -OCH_3), 4.034(1H, brs, –OH), 5.972(1H, brs, –NH); ^{13}C NMR($CDCl_3$, 75 MHz,) δ: 177.8(4-C), 174.7(24-C), 73.1(12-C), 51.5(O–CH_3), 47.9(17-C), 47.3(14-C), 46.5(13-C), 45.5(9-C), 40.2(1-C), 39.7(5-C), 38.7(4a-C), 36.9(8-C), 36.7(2-C), 36.0(8-C), 35.1(20-C), 33.8(10-C), 31.1(22-C), 30.9(23-C), 29.7(11-C), 29.0(7-C), 27.4(6-C), 25.7(16-C), 23.5(15-C), 17.3(21-C), 12.8(19-C), 12.7(18-C); LRESIMS(m/z): 420(M^++1).

*2.1.5. The synthesis of methyl 4-oxy-12-hydroxyimino-3-aza-A-homo-deoxycholicate (**13**)*

Compound **11** (80 mg, 0.19 mmol) was dissolved in 30 mL of 95% CH_3CH_2OH. After the mixture was heated to 70 °C, $CH_3COONa{\cdot}3H_2O$ (77 mg, 0.57 mmol) and $NH_2OH{\cdot}HCl$ (40 mg, 0.57 mmol) were added into the solution in 10 min. The mixture was stirred for 3 h at 70 °C. Then the reaction was



Scheme 1. Reagents and conditions: (a) CH_3OH/HCl; (b) PCC/CH_2Cl_2, 9 h; (c) NH_2OH·HCl/95% CH_3CH_2OH, 60 °C; (d) THF/$SOCl_2$; (e) NH_2OH·HCl/95% CH_3CH_2OH.

terminated and the majority of solvent was evaporated under reduced pressure. Distilled water was added into the reaction mixture, and the product was extracted with ethyl acetate. The combined extracts were washed with water and saturated brine, dried with anhydrous sodium sulfate, and evaporated under reduced pressure. The residue was subjected to chromatography (elution: $V_{methanol}$:$V_{dichloromethane}$ = 1:40) to produce 68 mg of **13** (82%) as a white solid, m.p. 212–213 °C; IR(KBr) ν/cm^{-1}: 3310, 3239, 2937, 2868, 1740, 1663, 1645, 1433, 1380, 1168, 923, 735; ^{1}H NMR($CDCl_3$, 600 MHz) δ: 0.945(3H, s, 19-CH_3), 0.947(3H, d, J = 6.0, 21-CH_3), 1.083(3H, s, 18-CH_3), 2.120(1H, dd, J = 18.6, 9.0, C_{4a}-βH), 2.28-2.24(1H, m, C_{4a}-αH), 2.271(1H, ddd, J = 15.6, 9.6, 6.6, C_{23}-H), 2.408(1H, ddd, J = 15.6, 10.2, 4.8, C_{23}-H), 3.029(1H, dd, J = 15.0, 12.0, C_{11}-βH), 3.102(1H, ddd, J = 15.0, 7.8, 6.0, C_2-βH), 3.271(1H, dd, J = 15.6, 9.0, C_{11}-αH), 3.325(1H, ddd, J = 15.0, 9.6, 4.8, C_2-αH), 3.685(3H, s, –OCH_3), 6.066(1H, brs, –NH), 7.789(1H, brs, N–OH); ^{13}C NMR($CDCl_3$, 75 MHz,) δ: 177.8(4-C), 174.8(24-C), 165.3(12-C), 59.2(14-C), 51.5(-OCH_3), 49.8(17-C), 47.0(13-C), 45.2(9-C), 43.9(1-C), 40.4(5-C), 39.6(4a-C), 38.6(10-C), 37.7(8-C), 36.7(2-C), 35.9(20-C), 31.5(23-C), 30.7(22-C), 29.7(7-C), 28.0(6-C), 25.8(15-C), 24.1(16-C), 22.7(11-C), 20.4(21-C), 19.1(19-C), 12.1(18-C); LRES-IMS(m/z): 433(M^++1).

2.2. Antiproliferative activity

2.2.1. Material and methods

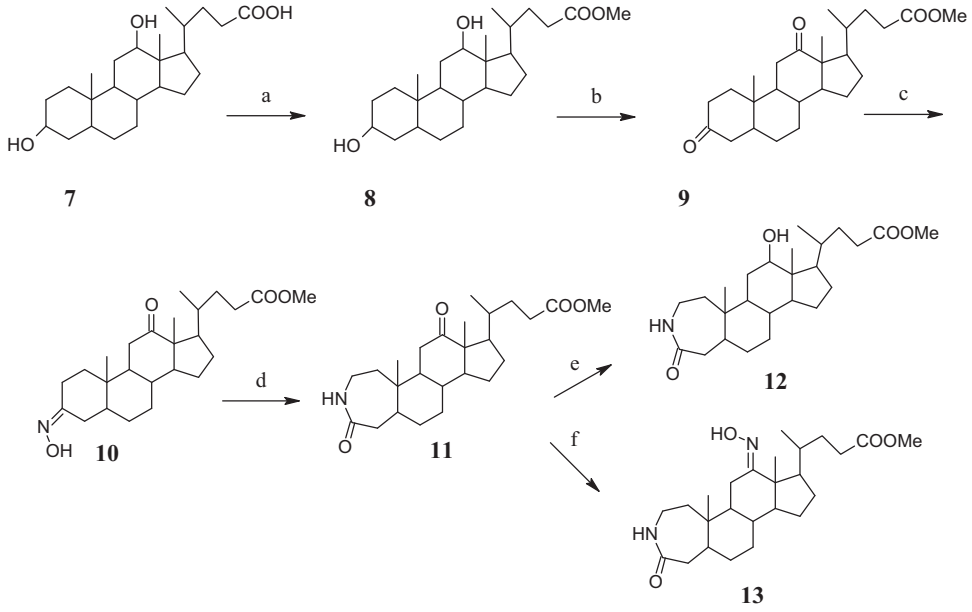
Stock solutions of the compounds were prepared in sterile dimethyl sulfoxide (DMSO) (Sigma) at a concentration of 10 mg/mL and afterwards diluted with complete nutrient medium (RPMI-1640) supplemented with 10% heat inactivated fetal bovine serum and 0.1 g/L penicillin G + 0.1 g/L streptomycin sulfate.

2.2.2. Cell culture

SMMC 7404, Hela and MGC 7901 cells were cultured in the medium(RPMI-1640) supplemented with 10% fetal bovine serum and 0.1 g/L penicillin G + 0.1 g/L streptomycin sulfate in a humidified atmosphere of 5% CO_2 at 37 °C.

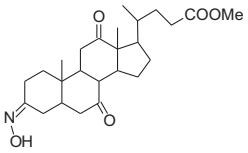
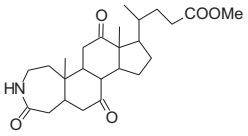
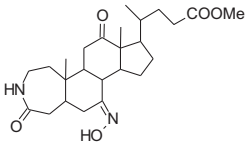
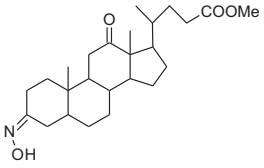
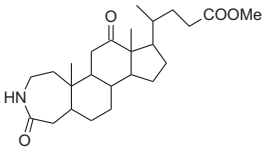
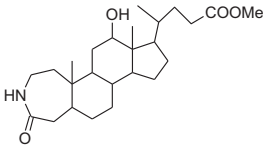
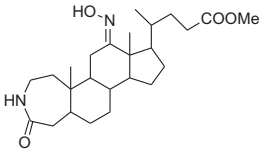
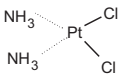
2.2.3. Treatment of cancer cells

Cancer cells (1–2 × 10^4 cells/mL, 200 μL) were seeded into each well of a 96-well microtiterplate. After incubation for 24 h, the compounds with a series of concentrations (range 1–80 μg/mL) were added to the cells. An equal amount of DMSO was added to the cells used as negative controls. All were treated in triplicate.



Scheme 2. Reagents and conditions: (a) CH_3OH/HCl; (b) PCC/CH_2Cl_2; (c) NH_2OH·HCl/95% C_2H_5OH, 60 °C; (d) THF/$SOCl_2$; (e) $NaBH_4$/anhydrous CH_3OH; (f) NH_2OH·HCl/95% C_2H_5OH, 70 °C.

Table 1
In vitro antitumor activities (IC_{50} in μmol/L) of the bile acids' A-lactam derivatives.

Compounds	Structure	7901	Hela	7404
4		>200	>200	>200
5		>200	48.7 ± 4.1	>200
6		150 ± 10	14.3 ± 1.2	>200
10		>200	>200	>200
11		96 ± 7.5	68.7 ± 5.3	>200
12		52.0 ± 4.8	44.8 ± 3.6	50.9 ± 6
13		56.2 ± 5.1	24.3 ± 1.5	74.0 ± 4.6
Cisplatin		13.7 ± 1.2	20.6 ± 1.8	11.6 ± 0.9

2.2.4. Determination of cell viability

MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] dye reduction assay was used to determine the cell viability. The cancer cells at approximately 80% confluence (i.e. logarithmically growing cells) were selected for trypsinization, and stained with trypan blue and their numbers were recorded. The cells (200 μL) were seeded in 96-well microtiterplates after the cell concentrations were adjusted to $1–2 \times 10^4$ cells/mL RPMI-1640 culture medium, and incubated at 37 °C and 5% CO_2 for 24 h. The cells were treated with the compounds dissolved in DMSO in different concentrations (range 1.0–80 μg/mL) and reincubated for 72 h. After the cells were washed with sterile phosphate buffer saline (PBS), 190 μL of RPMI-1640 and 10 μL of the tetrazolium dye (MTT) (5 mg/mL) solution were added to each well, and the cells were incubated for an additional 4 h. The medium was discarded and 200 μL of DMSO was added to dissolve the purple formazan crystals formed. The absorbance (A) at 550 nm was measured using a Biocell ELIASA analysis spectrometer. The IC_{50} value was calculated as the concentration of drug yielding 50% cell survival.

3. Result and discussion

3.1. Chemistry

Schemes 1 and 2 outline the synthetic procedures of compounds **5**–**6** and **11**–**13** respectively. First, cholic acid (**1**) is transformed into the corresponding methyl 4,7,12-trioxycholicate (**3**) via esterification in methanol and oxidation with PCC in CH_2Cl_2. Next, the compound **4** was synthesized by the oximation of **3** with ration of $NH_2OH{\cdot}HCl = 1:1$ [18]. Compound **5** was generated by Beckmann rearrangement of **4** with $SOCl_2$/THF at 0 °C in 60% yield. The structure of **5** was confirmed by analysis of the ^1H and ^{13}C NMR chemical

shifts. In the NMR spectrum, the resonances showing of N–H at 5.979 ppm(brs) and C-4 at 175.6 ppm demonstrated a formation of lactam in **5**. Resonances showing of C_2-αH at 3.237 ppm (ddd, J = 15.6, 10.2, 5.4) demonstrated a position of 3-NH in the compound **5** (If there is a 4-NH in **5**, a dd-peak would been appeared on C_{4a}-H). The compound **6** was obtained by the treatment of **5** with hydroxylamine hydrochloride (1:1.1) in ethanol in the presence of NaOAc in 80% yield. The structure of **6** was confirmed by analysis of its IR and NMR. The downfield chemical shift of 18-CH_3 at 1.233 ppm for **6** confirmed which 12-carbonyl group was held.

Compound **11** was prepared similarly according to the procedure of **5** using 7-deoxycholic acid as a starting material. The structure of **11** was confirmed by comparing IR and NMR spectra in the similar method of compound **5** analyzed. The compound **12** was obtained by a reduction of the carbonyl group at position 12 in **11** using $NaBH_4$ as a reductive agent in CH_3OH in 52% yield. The structure of compound **12** was deduced from its analytical and spectral data. In the ^1H NMR spectrum, the resonances showing of C_{12}-αH at 3.487 ppm (dd, J = 15.6, 4.8) and 12-C at 73.1 ppm demonstrated a presence of 12-hydroxy. In IR spectrum, the appearance of absorption peak at 3215 cm^{-1} and disappearance of absorption peak at 1707 cm^{-1} showed that 12-carbonyl in the **11** had been converted to 12-hydroxy in **12**.

The compound **13** was produced in a yield of 82% by the reaction of **11** with hydroxylamine hydrochloride in ethanol in the presence of NaOAc. In the IR spectra, the absorption of 1708 cm^{-1} for the original carbonyl group in **11** was absent and replaced by a new absorption at 1645 cm^{-1} (C=N) indicated the presence of a hydroximino group in **13**. In the NMR spectrum the resonances showing of 11-βH at 3.271 ppm (dd, J = 15.6, 9.0 Hz) and 12-C at 165.3 ppm demonstrated a position of (12E)-hydroximino in **13**.

3.2. In vitro evaluation of the cytotoxic activity

The cytotoxic activities of compounds **4**–**6** and **10**–**13** were determined in vitro on MGC 7901 (human ventriculi carcinoma), Hela (human cervical carcinoma) and SMMC 7404 (human liver carcinoma) tumor cell lines. The MTT method was used to analyze the antiproliferative activity. The results were summarized as IC_{50} values in μmol/L in Table 1.

As showed in Table 1, the compounds with A-homo-lactam structure have a better cytotoxicity than corresponding steroidal oximes compounds. The compounds **5**-**6** and **11**–**13** displayed a distinct cytotoxicity against Hela tumor cell line relative to SMMC 7404 and MGC 7901, and the compound **6** showed a better cytotoxicity than the cisplatin (as a standard) to Hela. The cytotoxicity of methyl 4-oxy-3-aza-A-homo-cholicate derivates against Hela tumor cell line was higher than that of corresponding methyl 4-oxy-3-aza-A-homo-7-deoxycholicate derivates, but a reverse result was observed for SMMC 7404 and MGC 7901. In our previous investigation, we found that the steroidal compounds bearing hydroxy or hydroximino exhibited higher cytotoxic activities than the same compounds bearing carbonyl [16,17], the result had been comfirmed again in present research. For example, the IC_{50} values of compounds **6**, **12**, **13** with the hydroxy or hydroximino at 7-position or 12-position against Hela tumor cell line were 14.3, 44.8, 24.3 μmol/L respectively (comparing the IC_{50} values of the compounds **5** (IC_{50}: 48.7) and **11** (IC_{50}: 68.7)).

In conclusion, we have prepared a new series of A-homo-lactam derivatives of cholic acid and 7-deoxycholic acid which were proved to be potent antitumor activities against Hela tumor cell line. Compounds **6**, **12**, **13** were found to be better potent compounds, especially the compound **6**, therefore, the further structural modification of **6** and antitumor activity study of the compounds in vivo would be in progress. Our findings could provide new evidence showing the relationship between the chemical structure and biological activity and may be useful for the design of novel chemotherapeutic drugs.

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