11-1-2011

Synthesis and Antiproliferative Activity of Some Steroidal Lactams

Yanmin Huang
Guangxi Teachers Education University

Jianguo Cui
Guangxi Teachers Education University

Sijing Chen
Guangxi Teachers Education University

Chunfang Gan
Guangxi Teachers Education University

Aimin Zhou
Cleveland State University, A.ZHOU@csuohio.edu

Synthesis and antiproliferative activity of some steroidal lactams

Yanmin Huang , Jianguo Cui , Sijing Chen , Chunfang Gan , Aimin Zhou

Introduction

Steroidal compounds display a variety of biological functions and play a very important role in life [1,2]. The steroidal drugs are widely used in traditional medicines, such as antibacterium, hormone kind medication, etc. 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. The study of natural products which isolated from marine life showed that the steroidal compounds bearing different functional groups, such as hydroxyl, hydroximino, hydrazone, sulfate groups, had excellent cytotoxicity against some tumor cells [3 7].

A variety of steroids with unusual and interesting structures have been synthesized and evaluated for their anti tumor activity [8 10]. In order to evaluate the anti tumor activity of new steroi dal derivatives, we synthesized a series of steroidal oxime deriva tives and investigated their cytotoxic activity against different types of cancer cells [11,12]. Interestingly we found that the cyto toxic activity of a steroidal oxime is dependent on the cholesteric side chain and function groups at position 3 and 6 on the steroidal nucleus.

Azahomosteroids are also a class of steroid compounds which were synthesized and modified in order to increase biological activity of steroids. These compounds have been tested success fully as anti cancer drugs against several types of cancer cells [13 17]. In order to find novel and effective anti tumor agents, we synthesized a series of 17a aza D Homo andrester 17 one, 3 aza A homo 4 one bile acid and 7 deoxycholic acid derivatives with various groups on the steroidal nucleus, and the results showed that these compounds could exhibit a high cytotoxicity to HeLa tumor cell line in vitro [18,19]. Here, some steroidal com pounds carrying lactam at A ring and a different active group on the 6 position of steroidal nucleus were synthesized and evaluated for their antiproliferative activity against some cancer cells.

Experimental

Chemistry

The sterols and $NaBH_4$ were purchased from the Merck Co. 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. Infra red spectra were measured with a Nicolet FT 360 Spectrophotom eter. The ^1H and ^{13}C NMR spectra were 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 frequen cies 300 and 75 MHz, respectively. Chemical shifts are expressed in parts per million (δ) values and coupling constants (J) in Hertz. LREIMS were recorded on a Thermo DSQ instrument. The cell pro liferation assay was undertaken by a MTT method using 96 well plates on Biocell ELISA analysis spectrometer.

(3*E*) Hydroximinocholest 6 one (**1**) was prepared according to procedures in the literature [12].

*The synthesis of 4 aza A homocholest 3,6 dione (**2**) and 3 aza A homocholest 3,6 dione (**3**)*

The solution of thionyl chloride (2.1 mL) in 5 mL dry THF was added to a solution of oxime **1** (450 mg, 0.99 mmol) in dry THF (15 mL). The solution was stirred under anhydrous conditions for 1 h 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 (20× 3 mL). The combined extract was washed with water, 5% $NaHCO_3$, and saturated brine, dried over anhydrous Na_2SO_4 and evaporated under reduced pres sure to give a crude product (750 mg) which was chromatographed on silica gel (elution: petroleum ether (60 90 °C)/EtOAc (1:5)) to give a yellow oily mixture. The oily mixture was further subjected to chromatography (methanol/dichloromethane (1:20)) to afford 184.8 mg of 4 aza A homocholest 3,7 dione (**2**) as white crystals, yield: 45%, θ_{mp} 212 213 °C; IR(KBr) ν/cm^{-1}: 3190, 3072, 2950, 2850, 2310, 1711, 1687, 1454, 1380, 816; ^{1}H NMR($CDCl_3$, 600 MHz) δ: 0.671(3H, s, 18 CH_3), 0.862(3H, s, 19 CH_3), 0.864(3H, d, J = 6.6, 26 or 27 CH_3), 0.868(3H, d, J = 6.6, 26 or 27 CH_3), 0.913(3H, d, J = 6.6, 21 CH_3), 1.998(1H, t, J = 12.6, C_1 αH), 2.054(1H, dt, J = 12.6, 3.6, C_7 βH), 2.305(1H, dd, J = 15.0, 7.8, C_2 βH), 2.332(1H, dd, J = 13.2, 4.8, C_7 αH), 2.415(1H, d, J = 9.6, C_5 αH), 2.599(1H, t, J = 13.8, C_2 αH), 3.311(1H, ddd, J = 16.2, 9.6, 4.8, C_{4a} βH), 3.445(1H, ddd, J = 16.2, 7.8, 1.2, C_{4a} αH), 6.007(1H, brt, J = 4.8, NH); ^{13}C NMR($CDCl_3$, 150 MHz) δ: 210.5(C 6), 178.1(C 3), 61.5(C 5), 56.7(C 14), 56.1(C 17), 53.7(C 9), 46.8(C 7), 44.3(C 13), 42.8(C 10), 39.5(C 12), 38.0(C 8), 36.8(C 4a), 36.1(C 22), 35.7(C 20), 34.8(C 1), 31.2(C 2), 28.00(C 25), 27.99(C 16), 23.9(C 15), 23.8(C 24), 22.8(C 23), 22.7(C 27), 22.5(C 26), 21.5(C 11), 18.6(C 21), 12.9(C 19), 12.0(C 18); ESI MS m/z: 416(M+1)$^+$.

In the reaction, 3 aza A homocholest 3,7 dione (**3**) (the isomer of **2**) was obtained as a byproduct in 21.5% yield (88 mg), θ_{mp} 235 237 °C; IR(KBr) ν/cm^{-1}: 3190, 3072, 2953, 1707, 1679, 1466, 1368; ^{1}H NMR($CDCl_3$, 600 MHz) δ: 0.666(3H, s, 18 CH_3), 0.833(3H, s, 19 CH_3), 0.860(3H, d, J = 6.6, 26 or 27 CH_3), 0.871(3H, d, J = 6.6, 26 or 27 CH_3), 0.909(3H, d, J = 6.6, 21 CH_3), 2.022(1H, dd, J = 26.4, 13.2, C_7 βH), 2.372(1H, dd, J = 12.6, 4.2, C_7 αH), 2.534(1H, dd, J = 13.8, 11.4, C_{4a} βH), 2.600(1H, d, J = 11.4, C_{4a} αH), 2.720(1H, dd, J = 14.4, 2.4, C_5 αH), 3.048(1H, dddd, J = 15.6, 7.8, 6.0, 1.8, C_2 αH), 3.391(1H, ddd, J = 15.6, 12.0, 4.2, C_2 βH), 6.040(1H, brs, NH); ^{13}C NMR($CDCl_3$, 150 MHz) δ: 209.5(C 6), 177.5(C 3), 56.7(C 14), 56.1(C 17), 54.9(C 5), 54.1(C 9), 46.9(C 7), 44.3(C 13), 42.8(C 10), 41.5(C 12), 39.52(C 1), 39.48(C 24), 38.0(C 8), 37.6(C 2), 36.1(C 22), 35.7(C 20), 30.6(C 4a), 28.00(C 25), 27.99(C 16), 23.9(C 15), 23.8(C 23), 22.8(C 27), 22.6(C 26), 21.8(C 11), 18.6(C 21), 12.9(C 19), 12.0(C 18); ESI MS m/z: 416(M+1)$^+$.

*6 Hydroxy 4 aza A homocholest 3 one (**4**)*

To the stirred solution of **2** (100 mg, 0.24 mmol) in CH_3OH (15 mL) was added $NaBH_4$ (30 mg, 0.79 mmol) in one time at room temperature. After 30 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 (3× 15 mL). The organic layer was washed with cold water and saturated brines. After drying over anhydrous sodium sulfate, the solvent was removed under reduced pressure and a crude product (90 mg) was obtained. After crystallization from methanol, the compound **4** was obtained as a white solid (81 mg, 81%), θ_{mp} 228 230 °C; IR(KBr) ν/cm^{-1}: 3411, 2933, 2864, 1662, 1466, 1372, 1262, 1094, 1041, 804; ^{1}H NMR($CDCl_3$, 600 MHz) δ: 0.695(3H, s, 18 CH_3), 0.863(3H, d, J = 6.6, 26 or 27 CH_3), 0.867(3H, d, J = 6.6, 26 or 27 CH_3), 0.906(3H, d, J = 6.6, 21 CH_3), 1.144(3H, s, 19 CH_3), 2.004(1H, dt, J = 12.6, 3.6, C_2 αH), 2.137(1H, dd, J = 15.0, 1.8, C_2 βH), 3.013 2.963(1H, m, C_{4a} βH), 3.038(1H, dd, J = 14.4, 10.8, C_{4a} αH), 3.420(1H, ddd, J = 15.6, 12.0, 4.2, C_6 βH), 3.958(1H, d, J = 1.8, NH), 5.827(1H, brs, OH); ^{13}C NMR($CDCl_3$, 150 MHz) δ: 178.1(C 3), 73.6(C 6), 56.3 (C 14), 56.1(C 17), 54.0(C 9), 45.8(C 5), 43.5(C 13), 42.4(C 10), 39.9(C 12), 39.8(C 24), 39.5(C 8), 38.8(C 7), 38.1(C 4a), 37.7(C 22), 36.2(C 20), 35.8(C 1), 29.5(C 2), 28.2(C 25), 28.0(C 16), 24.2(C 15), 23.9(C 23), 22.8(C 27), 22.6(C 26), 21.2(C 11), 18.7(C 21), 15.3(C 19), 12.1(C 18); ESI MS m/z: 418(M+1)$^+$.

*6 Hydroxy 3 aza A homocholest 3 one (**8**)*

Compound **8** was prepared similarly according to the procedure of **4**, but the compound **3** used as starting material instead of the compound **2**. Yield: 90%, θ_{mp} 245 247 °C; IR(KBr) ν/cm^{-1}: 3378, 2937, 2868, 1650, 1462, 1380, 1209, 1143, 1094, 1021; ^{1}H NMR($CDCl_3$, 600 MHz) δ: 0.697(3H, s, 18 CH_3), 0.863(3H, d, J = 6.6, 26 or 27 CH_3), 0.867(3H, d, J = 6.6, 26 or 27 CH_3), 0.909(3H, d, J = 6.6, 21 CH_3), 1.152(3H, s, 19 CH_3), 2.270(1H, dd, J = 15.0, 8.4, C_{4a} αH), 2.634(1H, t, J = 13.2, C_2 αH), 2.865(1H, dd, J = 15.6, 7.8, C_2 βH), 3.725(1H, ddd, J = 15.6, 9.3, 4.2, C_6 βH), 3.984(1H, d, J = 1.8 Hz, NH), 6.003(1H, brs, OH); ^{13}C NMR($CDCl_3$, 150 MHz) δ: 178.1(C 3), 73.6(C 6), 56.3 (C 14), 56.1(C 17), 54.0(C 9), 45.8(C 5), 43.5(C 13), 42.4(C 10), 39.9(C 12), 39.8(C 24), 39.5(C 8), 38.8(C 7), 38.1(C 4a), 37.7(C 22), 36.2(C 20), 35.8(C 1), 29.5(C 2), 28.2(C 25), 28.0(C 16), 24.2(C 15), 23.9(C 23), 22.8(C 27), 22.6(C 26), 21.2(C 11), 18.7(C 21), 15.3(C 19), 12.1(C 18); ESI MS m/z: 418 (M+1)$^+$.

*6 Hydroximino 4 aza A homocholest 3 one (**5**)*

Compound **2** (100 mg, 0.24 mmol) was dissolved in 10 mL of 95% CH_3CH_2OH. After the mixture was heated to 60 °C, $CH_3COONa{\cdot}3H_2O$ (33 mg, 0.24 mmol) and $NH_2OH{\cdot}HCl$ (20 mg, 0.27 mmol) were added into the solution in 10 min. The mixture was stirred for 1 h at 60 °C. Then 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 ex tracted with ethyl acetate. The combined extract was washed with saturated brine, dried with anhydrous sodium sulfate and evapo rated under reduce pressure. The residue was subject to chroma tography (methanol/dichloromethane (1:20)) to produce 81 mg of **5** (90%), θ_{mp} 243 245 °C; IR(KBr) ν/cm^{-1}: 3321, 3240, 2940, 2864, 1654, 1470, 1380, 968; ^{1}H NMR($CDCl_3$, 600 MHz) δ: 0.650(3H, s, 18 CH_3), 0.842(3H, s, 19 CH_3), 0.862(3H, d, J = 6.6, 26 or 27 CH_3), 0.867(3H, d, J = 6.6, 26 or 27 CH_3), 0.898(3H, d, J = 6.6, 21 CH_3), 1.865(1H, dd, J = 15.0, 6.0, C_5 αH), 2.001(1H, dt, J = 12.0, 3.0, C_2 αH), 2.333(1H, d, J = 10.8, C_8 αH), 2.611(1H, dd, J = 15.0, 11.4, C_2 βH), 3.046(1H, d, J = 13.8, C_7 αH), 3.025(1H, dd, J = 13.8, 6.0, C_7 βH), 3.377(1H, dd, J = 13.2, 4.8, C_{4a} αH), 3.419(1H, ddd, J = 15.0, 11.4, 4.2, C_{4a} βH), 6.193(1H, brs, NH), 9.094(1H, brs, NOH); ^{13}C NMR($CDCl_3$, 150 MHz) δ: 179.1(C 3), 158.7(C 6), 56.6(C 14), 56.2(C 17), 54.4(C 9), 54.0(C 5), 47.8(C 13), 42.8(C 10), 42.4(C 24), 41.3(C 4a), 39.8(C 12), 39.5(C 7), 37.8(C 8), 36.1(C 22), 35.7(C 20), 31.9(C 1), 29.9(C 2), 28.1(C 25), 28.0(C 16), 24.0(C 15), 23.8(C 23), 22.8(C 27), 22.6(C 26), 21.7(C 11), 18.6(C 21), 12.5(C 19), 12.0(C 18); ESI MS m/z: 431(M+1)$^+$.

*. 6 Hydroximino 3 aza A homocholest 3 one (**9**)*

Compound **9** was prepared similarly according to the procedure of **5** using the compound **3** as the starting material. Yield: 81%, θ_{mp} 245 257 °C; IR(KBr) ν/cm^{-1}: 3119, 2929, 2859, 1658, 1470, 1381, 1135, 959, 910; ^{1}H NMR($CDCl_3$, 600 MHz) δ: 0.589(3H, s, 18 CH_3), 0.799(3H, s, 19 CH_3), 0.791(3H, d, J = 6.6 Hz, 26 or 27 CH_3), 0.807(3H, d, J = 6.6 Hz, 26 or 27 CH_3), 0.834(3H, d, J = 6.6 Hz, 21 CH_3), 1.880(1H, dd, J = 13.5, 6.6 Hz, C_5 αH), 1.956(1H, m, C_2 αH), 2.065(1H, brt, J = 9.0 Hz, C_8 αH), 2.167(1H, td, J = 14.4, 7.8 Hz, C_8 αH), 2.572(1H, dd, J = 22.8, 13.8 Hz, C_2 βH), 3.238(1H, dd, J = 15.6,

9.6 Hz, C_{4a} αH), 3.296(1H, m, C_7 βH), 3.423(1H, dd, *J* = 15.6, 9.0 Hz, C_{4a} βH), 6.428(1H, brs, NH), 9.004(1H, brs, NOH); ^{13}C NMR($CDCl_3$, 150 MHz) δ: 179.6(C 4), 158.9(C 6), 56.5(C 14), 56.0(C 17), 53.9(C 9), 53.7(C 5), 42.6(C 13), 42.1(C 24), 39.6(C 10), 39.4(C 12), 37.9(C 1), 36.0(C 22), 35.7(C 20), 35.6(C 2), 34.4(C 8), 30.7(C 4a), 29.5(C 7), 28.0(C 25), 27.9(C 16), 23.9(C 15), 23.7(C 23), 22.6(C 27), 22.4(C 26), 21.3(C 11), 18.4(C 21), 12.2(C 19), 11.9(C 18); ESI MS *m*/*z*: 431(M+1)$^+$.

*4 aza A Homocholest 3,6 dione 6 thiosemicarbazone (**6**)*

A mixture of 4 aza A homocholest 3,6 dione (100 mg, 0.249 mmol), thiosemicarbazide (124 mg, 0.5 mmol), and a few drops of glacial acetic acid (0.5 mL) in 95% ethanol (20 mL) was stirred at 60 70 °C for 2 h. After completion of the reaction, the majority of solvent was evaporated and some water was added to this solution. The mixture was extracted with CH_2Cl_2 and the extract was washed with saturated brine, dried with anhydrous sodium sulfate and evaporated under reduce pressure. The resulting residue was chromatographed on a column of silica gel with a mixture of DCM methanol (20:1) to give compound **6** (91 mg, 72%), θ_{mp} 228 230 °C; IR(KBr) ν/cm^{-1}: 3452, 3338, 2941, 2859, 1658, 1580, 1462, 1350, 1221, 1150, 1071, 956; ^{1}H NMR($CDCl_3$, 600 MHz) δ: 0.665(3H, s, 18 CH_3), 0.823(3H, s, 19 CH_3), 0.867(3H, d, *J* = 6.6, 26 or 27 CH_3), 0.872(3H, d, *J* = 6.6, 26 or 27 CH_3), 0.907(3H, d, *J* = 6.6, 21 CH_3), 1.975(1H, dd, *J* = 14.4, 8.4, C_5 αH), 2.244(1H, d, *J* = 9.0, C_7 βH), 2.301(1H, ddd, *J* = 14.4, 6.6, 1.8, C_2 βH), 2.597(1H, t, *J* = 13.8, C_1 βH), 2.672(1H, dd, *J* = 12.6, 2.4, C_2 αH), 3.396(1H, ddd, *J* = 15.0, 9.6, 4.8, C_{4a} βH), 3.490(1H, dd, *J* = 15.0, 8.4, C_{4a} αH), 6.082(1H, brs, (C═S) NH), 6.355(1H, brs, (C═S) NH), 7.058(1H, d, *J* = 4.2, (C═O) NH), 8.756(1H, brs, N NH C); ^{13}C NMR($CDCl_3$, 150 MHz) δ: 179.1(C═S), 178.4(C 3), 155.5(C 6), 56.4(C 14), 56.1(C 17), 55.5(C 9), 54.1(C 5), 49.4(C 13), 43.2(C 4a), 42.9(C 24), 39.5(C 12), 38.5(C 8), 36.8(C 10), 36.1(C 22), 35.7(C 20), 34.8(C 1), 31.9(C 2), 30.9(C 7), 28.07(C 16), 28.02(C 25), 24.2(C 19), 23.8(C 15), 22.8(C 26), 22.6(C 27), 21.5(C 23), 18.6(C 11), 12.6(C 21), 12.0(C 18); ESI MS *m*/*z*: 489.

*4 aza A Homocholest 3,6 dione 6 semicarbazone (**7**)*

Compound **7** was prepared similarly according to the procedure of **6**, but the semicarbazide used as an attack reagent instead of the thiosemicarbazide and the reaction mixture was heated at 70 80 °C for 6 h. Yield: 62%. θ_{mp} 219 220 °C; IR(KBr) ν/cm^{-1}: 3448, 2949, 2868, 1654, 1576, 1470, 1376, 1227; ^{1}H NMR($CDCl_3$, 600 MHz) δ: 0.652(3H, s, 18 CH_3), 0.806(3H, s, 19 CH_3), 0.866(3H, d, *J* = 6.6, 26 or 27 CH_3), 0.871(3H, d, *J* = 6.6, 26 or 27 CH_3), 0.902(3H, d, *J* = 6.6, 21 CH_3), 1.880(1H, dd, *J* = 14.4, 6.0, C_7 αH), 2.021(1H, ddd, *J* = 12.6, 6.0, 3.6, C_1 αH), 2.389(1H, d, *J* = 10.8, C_5 αH), 2.656(1H, dd, *J* = 15.0, 3.6, C_2 βH), 2.827(1H, brd, *J* = 16.2, C_2 αH), 3.050(1H, m, C_{4a} βH), 3.387(1H, dd, *J* = 12.0, 4.2, C_{4a} αH), 4.908(1H, brs, NC(O) NH), 6.021(1H, brs, NC(O) NH), 6.264(1H, d, *J* = 4.2, (C═O) NH), 7.995(1H, s, N NH C); ^{13}C NMR($CDCl_3$, 150 MHz) δ: 178.0(C 3), 157.8(C 6), 152.0(NC(O)NH_2), 56.3(C 14), 56.1(C 17), 54.4(C 9), 49.2(C 5), 42.9(C 13), 42.8(C 4a), 41.4(C 10), 39.6(C 12), 39.5(C 24), 37.6(C 8), 36.2(C 2), 36.1(C 22), 35.7(C 20), 32.6(C 1), 31.7(C 7), 28.1(C 16), 28.0(C 25), 24.1(C 19), 23.8(C 15), 22.8(C 26), 22.6(C 27), 21.7(C 23), 18.6(C 11), 12.5(C 21), 12.0(C 18); ESI MS *m*/*z*: 473(M+1)$^+$.

Antiproliferative activity

Material and methods

Stock solutions of the compounds were prepared in sterile dimethyl sulfoxide (DMSO) (Sigma) at a concentration of 10 mg/mL and afterward 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.

Cell culture

SMMC 7404 (human liver carcinoma), MGC 7901 (human gastric carcinoma), HeLa (human cervical carcinoma) cancer cells were grown in the medium (RPMI 1640) supplemented with 10% cosmic calf 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.

Assay for cell viability

The viability of these cells was determined using MTT (3 (4,5 dimethylthiazol 2 yl) 2,5 diphenyltetrazolium bromide) dye reduction assay. Briefly, cells (1×10^4 cells per well) were seeded in 96 well plates. One day after seeding, the cells were treated with different concentration of each compound. An equal amount of DMSO was added to the cells used as negative controls. All were treated in triplicate. After reincubated for 72 h, the cells were washed with sterile phosphate buffer saline (PBS). The 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 492 nm was measured using a Biocell ELISA analysis spectrometer. The IC_{50} value was calculated as the concentration of drug yielding 50% cell survival. The effect of compounds on the morphology of treated human carcinoma cells was investigated by the light microscope and then photographed by Nikon(TE2000 U) inverted microscope.

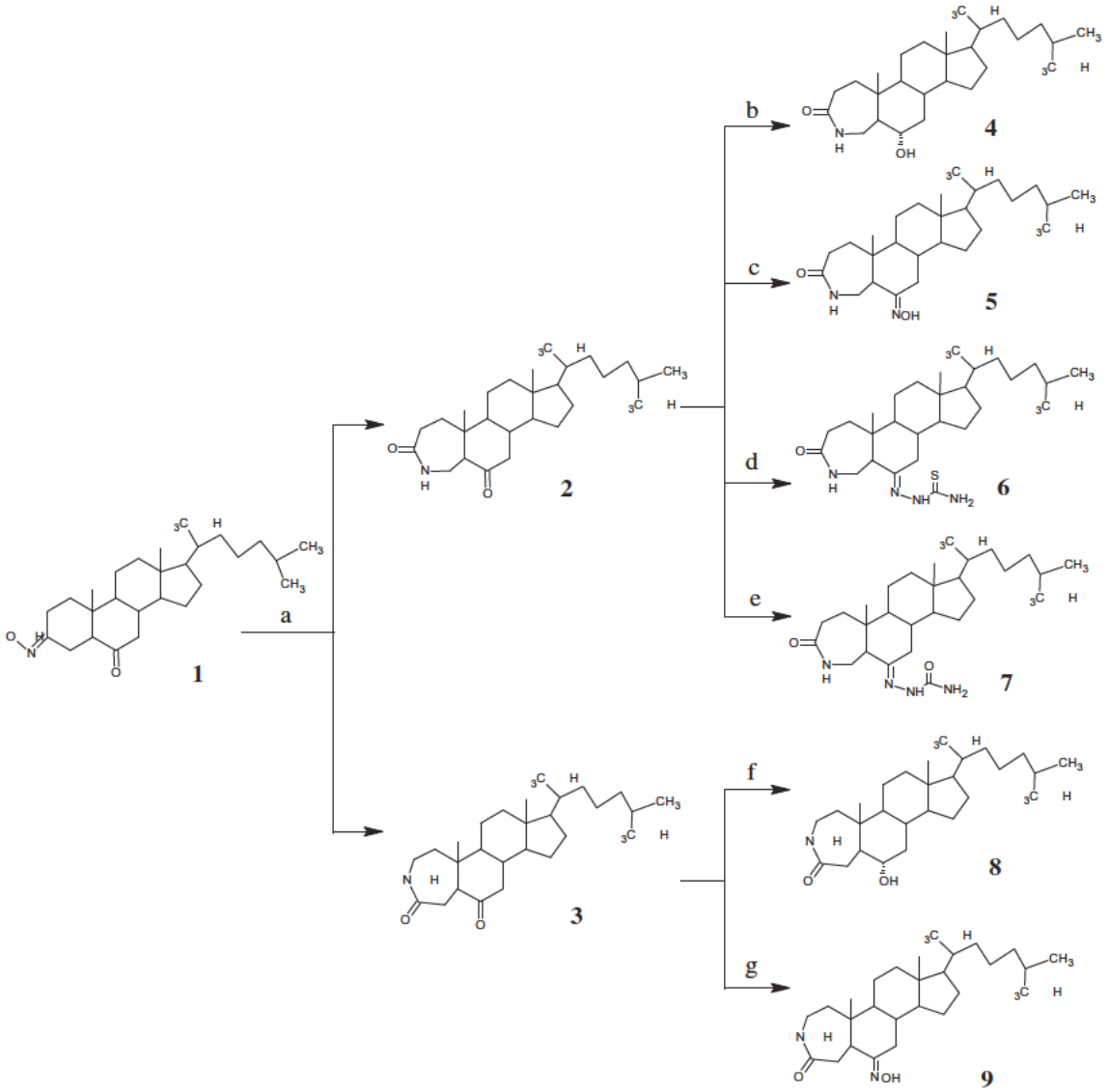
Results and discussion

Chemistry

Scheme 1 outlines the synthetic procedures of compounds **2** **9**. In our previous report [12], compound **1** was obtained in 66.5% yield by four steps using cholesterol as a starting material. The structure of compound **1** had been confirmed by its IR and NMR spectra.

The compounds **2** and **3** were synthesized by Beckmann rearrangement of **1** with $SOCl_2$/THF at 0 °C. In the reaction, the compound **2** with 4 aza structure was obtained as a major product in 45% yield. At the same time, the compound **3** with 3 aza structure was obtained as a byproduct in 21.5% yield. The structures of **2** and **3** were confirmed by analysis of the proton and carbon NMR chemical shifts at 2 C and 4a C. Resonances showing of C_{4a} βH at 3.311 ppm (ddd, *J* = 16.2, 9.6 and 4.8 Hz) and C_{4a} αH at 3.445 ppm (ddd, *J* = 16.2, 7.8, 1.2 Hz) demonstrated a position of 4 NH in the compound **2**, while the chemical shifts found for C_2 αH at 3.048 ppm (dddd, *J* = 15.6, 7.8, 6.0, 1.8 Hz) and C_2 βH at 3.391 ppm (ddd, *J* = 15.6, 12.0, 4.2 Hz) were an indicative of the 3 NH in the compound **3**. Here, the amide protons produced a coupling effect to C_4 H in **2** and C_2 H in **3**, respectively. Moreover, ^{1}H NMR revealed the presence of the broad singlet at 6.007 ppm in **2** and 6.040 ppm in **3** for the amide proton.

Compounds **4** and **8** were obtained by reduction using $NaBH_4$ as reductant in CH_3OH. The structures of compounds **4** and **8** were deduced from its analytical and spectral data. In the ^{1}H NMR spectrum, the resonances showing of C_6 βH at 3.420 ppm (ddd, *J* = 15.6, 12.0, 4.2 Hz) and 6 C at 73.6 ppm for **4** and C_6 βH at 3.725 ppm (ddd, *J* = 15.6, 9.3, 4.2 Hz) and 6 C at 73.6 ppm for **8** demonstrated a position of 6 hydroxy respectively. In IR spectrum, the absorption peaks at 3411 3378 cm^{-1} showed that 6 carbonyl had been converted to 6 hydroxy in **4** and **8**.



Scheme 1. Reagents and conditions: (a) $SOCl_2$/THF, 0 °C; (b) $NaBH_4$/MeOH, rt; (c) $H_2NOH{\cdot}HCl/Na_2Ac{\cdot}3H_2O$/EtOH, reflux; (d) $H_2NC(S)NHNH_2$/EtOH, 60 °C; (e) $H_2NC(O)NHNH_2$/EtOH; (f) $NaBH_4$/MeOH, rt; (g) $H_2NOH{\cdot}HCl/Na_2Ac{\cdot}3H_2O$/EtOH, reflux.

Compounds **5** and **9** were synthesized by the oximation of **2** and **3**. The structures of **5** and **9** were confirmed by analysis of IR, ^1H and ^{13}C NMR chemical shifts at 7 C. In the IR spectra, the absorp tions of 1711 and 1707 cm^{-1} for the original carbonyl group in **2** and **3**, were absent and replaced by a new absorption at 1654 cm^{-1} (C═N) for **5** and 1658 cm^{-1} for **9**. IR spectra bands at 3321 cm^{-1} in **5** and 3119 cm^{-1} in **9** indicate the presence of a hydroximino group. In the ^1H NMR spectrum of compounds **5** and **9**, the signal for C_7 βH was shifted downfield, appearing at 3.025 ppm for **5** and 3.296 ppm for **9** due to the deshielding influ ence of the hydroxyl oxygen of the oxime which confirmed the (*E*) configuration [20]. Also, the ^1H NMR revealed the presence of broad singlet (1H) at 9.094 ppm in **5** and at 9.004 ppm in **9** for the NOH group.

Similarly, the reaction of compound **2** with thiosemicarbazide or semicarbazide using few drops of glacial acetic acid as a catalyst afforded the corresponding product **6** or **7**. In the IR spectra, the compound **6** showed intense bands in the region 1150 cm^{-1} due to the ν(C═S) stretching of the thiocarboxamide group. In addition, the absorption bands at 1580 cm^{-1} were attributed to the ν(C═N) stretching vibration, which also confirms the formation of desired thiosemicarbazone in the compound **6**. Similarly, the compound **7** showed an absorption band of the ν(C═N) stretching vibration at 1576 cm^{-1}. The compounds **6** and **7** showed additional sharp bands in the region 3452 3338 cm^{-1} due to the ν(N H) stretching vibration. In the ^1H NMR spectrum, the singlets appearing at 8.756 ppm for **6** and 7.995 ppm for **7** confirmed the presence of N NH C protons in the **6** and **7**.

In vitro evaluation of the antiproliferative activity

Structure activity relationship

To evaluate the antiproliferative activity of these compounds, the IC_{50} values were determined in SMMC 7404, HeLa and MGC 7901 cancer cells by using a MTT assay according to the manufac turer's instructions. MTT is a compound that can be taken up by viable cells and reduced by a mitochondrial dehydrogenase form ing a formazan product in living cells. The absorbance of the forma zan product at 492 nm is in linear proportion to cell numbers. The results were summarized as IC_{50} values in μmol/L in Table 1.

Table 1
In vitro antiproliferative activities (IC_{50} in μmol/L) of the compounds **2**–**9**.

Compounds	Carcinoma cell lines		
	MGC 7901	HeLa	SMMC 7404
2	26.5	42.1	25.3
3	31.8	11.3	28.9
4	15.8	17.2	16.8
5	12.8	22.8	17.6
6	15.3	6.5	40.9
7	36.6	10.6	78.3
8	>100	7.7	77
9	16.3	5.6	17.9
Cisplatin	6.7	10.1	23.2

Apparently all steroidal lactams (**2**–**9**) displayed a distinct cytotoxicity against these cancer cells. Although the cytotoxic activity against MGC 7901 and SMMC 7404 cells was not significantly different between 4 *N* lactam **2** and 3 *N* lactam **3** or **5** and **9**, 3 *N* lactams showed a higher cytotoxicity against HeLa cells than 4 *N* lactams. Interestingly 4 *N* lactam **4** exhibited a high cytotoxicity to all cancer cells tested, but the cytotoxic activity was remarkably deceased in 3 *N* lactam **8** although HeLa cells were sensitive to the compound.

Compounds **2** and **4**–**7**, with same 4 *N* lactam structure and different types of 6 substituted groups, showed a distinct difference in their cytotoxicity against these cancer cells. The analogs **4** and **5**, with a hydroxyl or a hydroximino at C 6, remarkably increased their cytotoxic activity against MGC 7901 and SMMC 7404 cells in comparison with the analogs **2** and **7**, which have a carbonyl or semicarbazone groups at C 6. Compounds **6** and **7** with a thiosemicarbazone or semicarbazone groups at C 6 had a better cytotoxicity than compounds **2**, **4** and **5** against HeLa cells. Here compounds **6**, **8**, **9** (**6**: 6.56 μmol/L; **8**: 7.76 μmol/L; **9**: 5.6 μmol/L) were even more cytotoxic than cisplatin to HeLa cells (positive contrast: 10.1 μmol/L).

Conclusion

We have prepared a series of 3 aza A homo 3 oxycholestane and 4 aza A homo 3 oxycholestane derivatives with different substituted groups at position 6 of the ring B. The antiproliferative activity of the synthesized compounds against SMMC 7404, HeLa and MGC 7901 cancer cells was investigated. All these compounds displayed a distinct cytotoxicity against these cancer cells. Our results revealed that the structures of functional groups at position 6 on the steroidal ring are crucial for the IC_{50} value of antiproliferative activities of these compounds and the cytotoxic activity against MGC 7901 and SMMC 7404 cells was not significantly different between 4 *N* lactams and 3 *N* lactams when its 6 substituted group was a carbonyl or a hydroximino, but all 3 *N* lactams showed a higher cytotoxicity against HeLa cells than 4 *N* lactams. 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.

Acknowledgments

The authors acknowledge the financial support of the Natural Science Foundation of Guangxi Province (Nos. 2010GXNSFD013 019 and 0832106).

References

[1] Festi D, Montagnani M, Azzaroli F, Lodato F, Mazzella G, Roda A, et al. Clinical efficacy and effectiveness of ursodeoxycholic acid in cholestatic liver diseases. Curr Clin Pharmacol 2007;2(2):155–77.
[2] Ifere GO, Barr E, Equan A, Gordon K, Singh UP, Chaudhary J, et al. Differential effects of cholesterol and phytosterols on cell proliferation, apoptosis and expression of a prostate specific gene in prostate cancer cell lines. Cancer Detect Prev 2009;32(4):319–28.
[3] Duha CY, Loa IW, Wang SK, Dai CF. New cytotoxic steroids from the soft coral *Clavularia viridis*. Steroids 2007;72:573–9.
[4] Malika IO, Maurice S. Recent advances in thiasteroids chemistry. Steroids 2006;71:1025–44.
[5] James RH. Steroids: partial synthesis in medicinal chemistry. Nat Prod Rep 2006;23:100–7.
[6] Chen SJ, Cui JG, Li Y, Fan LH. Recent advance of steroidal hydrazone with biological activities. Chin J Org Chem 2011;31(2):187–92.
[7] Zhang XJ, Cui JG, Li Y, Chen SJ. Recent advance in steroidal oximes with biological activity. Chin J Org Chem 2010;30(5):655–61.
[8] Javier P, Miriam R, Vanessa P, Beatriz A, Jaime R, Nélida S, et al. Synthesis and evaluation of new 6-hydroximinosteroid analogs as cytotoxic agents. Bioorg Med Chem 2007;15:4722–40.
[9] Mohamed EF, Gamal AE, Emad FE, Hanaa MR, Mohamed AT. Novel modified steroid derivatives of androstanolone as chemotherapeutic anti-cancer agents. Eur J Med Chem 2009;44:3936–46.
[10] Dominique B, Laïla EK, Monique C, Walae S, Mohamed K, Christos R, et al. Synthesis of bile acid derivatives and in vitro cytotoxic activity with pro-apoptotic process on multiple myeloma (KMS-11), glioblastoma multiforme (GBM), and colonic carcinoma (HCT-116) human cell lines. Eur J Med Chem 2010;45:2912–8.
[11] Cui JG, Fan L, Huang LL, Liu HL, Zhou AM. Synthesis and evaluation of some steroidal oximes as cytotoxic agents: structure/activity studies (I). Steroids 2009;74(1):62–72.
[12] Cui JG, Fan L, Huang YM, Xin Y, Zhou AM. Synthesis and evaluation of some steroidal oximes as cytotoxic agents: structure/activity studies (II). Steroids 2009;74(12):989–95.
[13] Venetia K, Eleftheria M, Zafiroula I, Dionysios M, Manolis F, Anna K, et al. A new approach for evaluating in vivo anti-leukemic activity using the SCE assay: an application on three newly synthesised anti-tumour steroidal esters. Mutat Res 2003;535:79–86.
[14] Dimitrios TPT. Hybrid aza-steroid alkylators in the treatment of colon cancer. Cancer Lett 2006;243:202–10.
[15] Natalija MK, Mira SB, Željko Ž, Mirjana DP, Zorica DJ, Vladimir DP. Synthesis of some steroidal oximes, lactams, thiolactams and their anti-tumor activities. Steroids 2007;72:406–14.
[16] Anna IK, Manolis AF, Evagelia SA, Athanasios P, George NP, Sotiris SN. Design, synthesis, and in vivo evaluation of the antileukemic activity of six new alkylating steroidal esters. Bioorg Med Chem 2008;16:5207–15.
[17] Dimitrios TPT, George DG, Catherine K, Athanasios P, Panayiotis K, Charalambos C. A D-homo-aza-androsterone alkylator in the treatment of breast cancer. Breast Cancer Res Treat 2006;97:17–31.
[18] Huang YM, Chen SJ, Cui JG, Gan CF, Liu ZP, Wei YL, et al. Synthesis and cytotoxicity of A-homo-lactam derivatives of cholic acid and 7-deoxycholic acid. Steroids 2011;76:690–4.
[19] Huang YM, Cui JG, Zhong ZG, Gan CF, Zhang WY, Song HC. Synthesis and cytotoxicity of 17a-Aza-D-homo-androster-17-one derivatives. Bioorg Med Chem Lett 2011;21:3641–3.
[20] Cui JG, Gan CF, Fan L. NMR spectral characteristic and conformation analysis of (3*E*)-cholest-4-en-3,6-dione-3-oxime and (6*E*)-cholest-4-en-3,6-dione-6-oxime. J Instrum Anal 2008;27:523–6 [in Chinese].