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Synthesis and cytotoxic activity of some 4,6-diaza-A,B-dihomo-steroid bilactams

Jianguo Cui, Qifu Lin, Chunfang Gan, Qiucui Yao, Wei Su, Yanmin Huang *

College of Chemistry and Life Science, Guangxi Teachers Education University, Nanning 530001, PR China

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ABSTRACT

Using cholesterol, stigmasterol and sitosterol as starting materials, some 4,6-diaza-A,B-dihomo-steroid bilactams were synthesized via two different synthetic routes by oxidation, reduction, oximation, Beckman rearrangement, etc. The cytotoxic activity of the synthesized compounds against SGC 7901 (human ventriculi carcinoma), Bel-7404 (human liver carcinoma), HeLa (human cervical carcinoma) and HT-29 (colonic carcinoma) cancer cells were investigated. The results showed that compounds **2** and **7b** displayed a good cytotoxic activity to the SGC 7901, Bel 7404 and HeLa tumor cell lines with the IC_{50} values of 11.6, 16.4, 13.9 and 13.1, 21.8, 13.1 μmol/L, respectively. Their cytotoxic activity is almost same as cisplatin to these cells. The information obtained from the studies may be useful for the design of novel chemotherapeutic drugs.

1. Introduction

Steroidal compounds display a variety of biological functions and play a very important role in life [1–4]. The steroidal drugs are widely used in traditional medicines, such as antibacterium, hormone kind medication, etc. The introduction of heteroatom or replacement of carbon atoms by a heteroatom in steroids often affects the chemical properties of the steroidal molecule and results in alterations of biological activities [5–7].

Azahomosteroids are a class of steroid compounds which were synthesized and modified in order to increase biological activity of steroids. The synthesis of some aza-homosteroid compounds with unusual and interesting structures had been reported recently [8–12]. These compounds exhibit valuable biological activities such as cytotoxicity, antibacterium, antileukemic activity, antiandrogenic activities, etc. 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 anti-tumour activity of the compound in cancer research [13–14].

In order to find novel and effective anti-tumor agents, we synthesized a series of novel steroidal lactams, and investigated their cytotoxic activity against different types of cancer cells [15–19]. The results showed that some A-homo steroidal lactams with a cholesteric side chain and the 6-hydroxyl or hydroximino group displayed a good cytotoxic activity against some cancer cells in vitro and induced cancer cell apoptosis.

Steroidal compounds possessing double lactams had rarely been reported in literature. Morzycki et al. reported the synthesis of a series of 4,17-diazasteroids with the structure of A, D-ring bilactams and the evaluation of their activity against human 5α-reductase [20]. Koutsourea et al. reported the preparation of another type steroidal bilactams possessing the structure of 7α,17α-diaza-B,D-dihomo-5-androsten-7,17-dione [21].

In the present study, as an extension of our previous studies, some steroidal A,B-dihomo bilactams with different side chain were synthesized via two unlike precedures and evaluated for their cytotoxic activity against some cancer cells.

2. 2. Experimental

2.1. Chemistry

The sterols were purchased from the Sinopharm Chemical Reagent Co., Ltd., Shanghai, China. All chemicals and solvents were analytical grade. Melting points were determined on an X_4 apparatus (Beijing Tech Instrument Co. Ltd., Beijing, China) and were uncorrected. Infrared spectra were measured with a Nicolet FT-360 Spectrophotometer (Thermo Scientific, America). The ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ on a Bruker AV-300 spectrometer (Bruker Corporation, Ameica) at working frequencies 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 (Thermo Fisher

* Corresponding author. Tel.: +86 13977159868.
E-mail address: huangyanmin828@163.com (Y. Huang).

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Scientific, America), while HREIMS were measured on a Agilent 6210 TOFMS instrument (Agilent Technologies, America). The cell proliferation assay was undertaken by a MTT method using 96-well plates on MLLTISKAN MK3 analysis spectrometer (Thermo Scientific, Shanghai, China).

The compound **1** was prepared according to procedures in the literature [17] and the compounds **3a**–**3b** were obtained according to procedures in the literature [22].

*2.1.1. The preparation of 4,6-diaza-A,B-dihomo-cholest-3,7-dione (**2**)*

To a solution of **1** (118 mg, 0.27 mmol) in dry THF (5 mL) the solution of thionyl chloride (1 mL) in 3 mL dry THF was added under argon. The mixture was stirred under anhydrous conditions for 4 h at 0 °C. Then the reaction was terminated and proper water was added. 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 provide a crude product which was chromatographed on silica gel (elution: $V_{\text{petroleum ether}}$:-$V_{\text{ethyl acetate}}$ = 1:3) to give 78 mg of **2** as a white solid. Yield: 66%, θ_{mp} 290–292 °C. IR(KBr) ν/cm^{-1}: 3432, 2958, 2864, 1646, 1262, 800; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.687 (s, 3H, 18-CH_3), 0.871 (d, 3H, J = 6.6 Hz, 21-CH_3), 0.882 (s, 3H, 19-CH_3), 0.892 (d, 6H, J = 6.0 Hz, 26- and 27-CH_3), 2.41–2.27 (m, 3H, C_2–H and C_7–αH), 3.048 (dd, 1H, J = 15.6, 6.3 Hz, C_7–βH), 3.26–3.15 (m, 2H, 4α–CH_2), 3.797 (dd, 1H, J = 11.4, 6.0 Hz, C_5–αH), 6.495 (d, 1H, J = 6.0 Hz, 6-NH–), 6.980 (br s, 1H, 4-NH–); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 11.9 (18-C), 14.8 (19-C), 18.5 (21-C), 22.6 (27-C), 22.8 (26-C), 23.5 (11-C), 23.8 (15-C), 25.5 (23-C), 27.7 (25-C), 28.0 (16-C), 29.7 (2-C), 34.6 (1-C), 35.7 (8-C), 35.9 (10-C), 36.9 (20-C), 39.3 (22-C), 39.4 (4a-C), 39.6 (24-C), 39.9 (12-C), 41.3 (13-C), 42.5 (7a-C), 54.7 (9-C), 55.1 (5-C), 55.9 (17-C), 56.5 (14-C), 173.4 (3-C), 176.1 (7-C); HREIMS: m/z 431.3637 [M+H]$^+$ (calcd for $C_{27}H_{47}N_2O_2$, 431.3638).

*2.1.2. General procedure for the preparation of compounds **4a**–**4b***

Thionyl chloride (2.5 mL) in 5 mL dry THF was added to a solution of the oxime **3** (0.99 mmol)) in dry THF (16 mL). The mixture was stirred under anhydrous condition for 4 h at 0 °C. Then the reaction was terminated and an appropriate water was added. 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 pressure. The crude product was chromatographed on silica gel (elution: CH_2Cl_2/MeOH = 40/1)) to give **4** as white solid.

3-Hydroxy-6-aza-B-homo-24-ethylcholest-22-en-7-one (**4a**): Yield: 75%, θ_{mp} 252–254 °C; IR(KBr) ν/cm^{-1}: 3428, 3244, 2958, 2872, 1646, 1565, 1454, 1414, 1066; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.707 (s, 3H, 18-CH_3), 0.806 (d, 3H, J = 6.3, 26- or 27-CH_3), 0.810 (t, 3H, J = 7.5, 29-CH_3), 0.853 (d, 3H, J = 6.3, 26- or 27-CH_3), 0.860 (s, 3H, 19-CH_3), 1.011 (d, 3H, J = 6.6, 21-CH_3), 2.37–2.22 (m, 2H, C_{7a}–H), 3.42–3.35 (m, 1H, C_5–αH), 3.67–3.56 (m, 1H, C_3–αH), 5.027 (dd, 1H, J = 15.3, 8.4, C_{22}–H), 5.144 (dd, 1H, J = 15.0, 8.4, C_{23}–H), 5.377 (br s, 1H, –NH); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 12.0 (29-C), 12.3 (18-C), 12.5 (19-C), 19.0 (21-C), 21.1 (26-C), 21.1 (27-C), 23.0 (11-C), 25.4 (15-C), 25.7 (28-C), 28.3 (16-C), 30.9 (2-C), 31.9 (25-C), 34.6 (8-C), 35.7 (1-C), 38.56 (10-C), 38.60 (4-C), 39.8 (12-C), 40.3 (13-C), 40.5 (20-C), 42.3 (7a-C), 51.2 (9-C), 55.8 (5-C), 56.3 (24-C), 56.9 (17-C), 58.8 (14-C), 68.8 (3-C), 129.6 (23-C), 137.9 (22-C), 176.3 (7-C); HREIMS: m/z 444.3839 [M+H]$^+$ (calcd for $C_{29}H_{45}NO_2$, 444.3842).

3-Hydroxy-6-aza-B-homo-24-ethylcholest-7-one (**4b**): Yield: 72%, θ_{mp} 274–276 °C. IR(KBr) ν/cm^{-1}: 3318, 3232, 2954, 2860, 1642, 1442, 1377, 1136, 1050, 952, 784; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.656 (s, 3H, 18-CH_3), 0.789 (d, 3H, J = 6.6, 26- or 27-CH_3), 0.813 (s, 3H, 19-CH_3), 0.815 (d, 3H, J = 6.6, 26- or 27-CH_3), 0.822 (t, 3H, J = 7.5, 29-CH_3), 0.878 (d, 3H, J = 6.3, 21-CH_3), 2.30–2.18(m, 2H, C_{7a}–H), 3.40–3.28 (m, 1H, C_5–αH), 3.58–3.47 (m, 1H, C_3–αH), 6.022 (d, 1H, J = 4.8, –NH); ^{13}C NMR(75 MHz, $CDCl_3$) δ: 11.8 (29-C), 12.0 (18-C), 12.5 (19-C), 18.6 (21-C), 19.0 (27-C), 19.8 (26-C), 23.0 (11-C), 25.6 (15-C), 26.0 (28-C), 27.6 (23-C), 29.1 (16-C), 30.7 (25-C), 32.4 (2-C), 33.7 (8-C), 34.6 (1-C), 35.7 (22-C), 36.1 (20-C), 38.5 (10-C), 38.8 (4-C), 40.0 (12-C), 40.2 (13-C), 42.5 (7a-C), 45.8 (24-C), 55.6 (9-C), 56.4 (5-C), 57.1 (17-C), 58.8 (14-C), 68.5 (3-C), 176.7 (7-C); HREIMS: m/z 446.3979 [M+H]$^+$ (calcd for $C_{29}H_{52}NO_2$, 446.3998).

*2.1.3. General procedure for the preparation of compounds **5a**–**5b***

Pyridinium chlorochromate (PCC) (3.75 mmol) was added to a solution of **4** (0.59 mmol) in 50 mL of dried CH_2Cl_2 in one portion at room temperature. The reaction was completed in 6 h. The suspension was poured over a silica gel column and eluted with CH_2Cl_2. The resulting solution was washed with cold water and saturated brines. After drying over anhydrous sodium sulfate, the solvent was removed under reduced pressure, and the resulting crude product was purified by chromatography on silica gel using petroleum ether (60–90 °C)/EtOAc (1:1) as eluent to give **5** as white solid.

6-Aza-B-homo-24-ethylcholest-22-en-3,7-dione (**5a**): Yield: 58%. θ_{mp} 225–227 °C. IR (KBr) ν/cm^{-1}: 3432, 2966, 2888, 1712, 1659, 1450, 1389, 1283, 1107, 984; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.714 (s, 3H, 18-CH_3), 0.787 (d, 3H, J = 6.3, 26- or 27-CH_3), 0.791 (t, 3H, J = 7.2 Hz, 29-CH_3), 0.836 (d, 3H, J = 6.3, 26- or 27-CH_3), 0.998 (d, 3H, J = 6.6, 21-CH_3), 1.054 (s, 3H, 19-CH_3), 2.33–2.17(m, 4H, C_2–H and C_4–H), 2.48–2.38 (m, 2H, C_{7a}–H), 3.73–3.66 (m, 1H, C_5–αH), 5.011 (dd, 1H, J = 15.3, 8.4, C_{22}–H), 5.129 (dd, 1H, J = 15.3, 8.4, C_{23}–H), 6.638 (d, 1H, J = 4.8, –NH); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 12.0 (29-C), 12.3 (18-C), 12.3 (19-C), 19.0 (21-C), 21.1 (26-C), 21.2 (27-C), 23.6 (11-C), 25.4 (15-C), 25.7 (28-C), 28.2 (16-C), 31.9 (25-C), 34.7 (8-C), 36.1 (1-C), 36.9 (2-C), 39.0 (10-C), 39.7 (12-C), 40.2 (4-C), 40.5 (20-C), 42.4 (7a-C), 43.6 (13-C), 51.2 (5-C), 55.7 (24-C), 56.2 (9-C), 58.0 (17-C), 58.5 (14-C), 129.6 (23-C), 137.8 (22-C), 176.7 (7-C), 208.1 (3-C); HREIMS: m/z 442.3682 [M+H]$^+$ (calcd for $C_{29}H_{48}NO_2$, 442.3685).

6-Aza-B-homo-24-ethylcholest-3,7-dione (**5b**): Yield: 65%, θ_{mp} 206–208 °C. IR(KBr) ν/cm^{-1}: 3354, 2954, 2860, 1716, 1659, 1454, 1385, 1348, 1283, 1250, 1115, 968; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.694 (s, 3H, 18-CH_3), 0.798 (d, 3H, J = 6.3, 26-CH_3 or 27-CH_3), 0.810 (d, 3H, J = 6.3, 26-CH_3 or 27-CH_3), 0.831 (t, 3H, J = 6.9, 29-CH_3), 0.893 (d, 3H, J = 6.3, 21-CH_3), 1.049 (s, 3H, 19-CH_3), 2.458 (d, 2H, J = 9.3, C_{7a}–H), 3.73–3.64 (m, 1H, C_5–αH), 6.713 (br s, 1H, –NH–); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 11.8 (29-C), 12.0 (18-C), 12.2 (19-C), 15.4 (21-C), 18.6 (27-C), 19.0 (26-C), 19.8 (11-C), 23.0 (15-C), 23.6 (28-C), 25.6 (23-C), 26.0 (16-C), 27.6 (25-C), 29.1 (8-C), 33.7 (22-C), 34.8 (1-C), 36.1 (20-C), 36.8 (2-C), 39.0 (10-C), 39.8 (12-C), 40.2 (4-C), 42.5 (13-C), 43.5 (7a-C), 45.8 (24-C), 55.6 (5-C), 56.3 (9-C), 58.0 (17-C), 58.5 (14-C), 176.7 (7-C), 208.1 (3-C); HREIMS: m/z 444.3856 [M+H]$^+$ (calcd for $C_{29}H_{50}NO_2$ 444.3842).

*2.1.4. General procedure for the preparation of compounds **6a**–**6b***

After the compound **5** (0.23 mmol) was dissolved in 20 mL of 95% ethanol, $CH_3COONa{\cdot}3H_2O$ (0.24 mmol) was added. Then the mixture was heated to 60 °C, hydroxylamine hydrochloride (0.25 mmol) were added and stirred for 1 h until no starting material. The majority of ethanol was evaporated under reduced pressure. The 10 mL of water was added to the mixture and extracted by CH_2Cl_2 (20 mL × 3), and the organic layer was washed with water and saturated brine. After drying over anhydrous sodium sulfate, solvent was removed under reduced pressure, and the resulting crude product was purified by chromatography on silica gel using petroleum ether (60–90 °C)/EtOAc (8:1) as eluent to give the corresponding target product **6**.