Synthesis and Evaluation of Some 17-Acetamidoandrostane and N,N-Dimethyl-7-deoxycholic Amide Derivatives as Cytotoxic Agents: Structure/Activity Studies

Yanmin Huang
Guangxi Teachers Education University

Jianguo Cui
Guangxi Teachers Education University

Linyi Jia
No. 37 Middel School in Nanning

Chunfang Gan
Guangxi Teachers Education University

Huacan Song
SUN YAT-SEN University

Follow this and additional works at: https://engagedscholarship.csuohio.edu/scichem_facpub

Part of the Chemistry Commons

How does access to this work benefit you? Let us know!

Recommended Citation
Huang, Yanmin; Cui, Jianguo; Jia, Linyi; Gan, Chunfang; Song, Huacan; Zeng, Chun; and Zhou, Aimin, "Synthesis and Evaluation of Some 17-Acetamidoandrostane and N,N-Dimethyl-7-deoxycholic Amide Derivatives as Cytotoxic Agents: Structure/Activity Studies" (2013). Chemistry Faculty Publications. 415.
https://engagedscholarship.csuohio.edu/scichem_facpub/415

This Article is brought to you for free and open access by the Chemistry Department at EngagedScholarship@CSU. It has been accepted for inclusion in Chemistry Faculty Publications by an authorized administrator of EngagedScholarship@CSU. For more information, please contact library.es@csuohio.edu.
Synthesis and Evaluation of Some 17-Acetamidoandrostane and N,N-Dimethyl-7-deoxycholic Amide Derivatives as Cytotoxic Agents: Structure/Activity Studies

Yanmin Huang 1, Jianguo Cui 1,*, Linyi Jia 2, Chunfang Gan 1, Huacan Song 3, Chun Zeng 4 and Aimin Zhou 4,*

1 College of Chemistry and Life Science, Guangxi Teachers Education University, Nanning 530001, China
2 No. 37 Middel School in Nanning, Nanning 530001, China
3 School of Chemistry and Chemical Engineering, SUN YAT-SEN University, Guangzhou 510275, China
4 Clinical Chemistry Program, Department of Chemistry, SI 424, Cleveland State University, Cleveland, OH 44115, USA

* Authors to whom correspondence should be addressed; E-Mails: cujig1954@126.com (J.C.); a.zhou@csuohio.edu (A.Z.); Tel.: +86-771-553-0019 (J.C.); +1-216-687-2416 (A.Z.).

Received: 9 May 2013; in revised form: 13 June 2013 / Accepted: 18 June 2013 / Published: 26 June 2013

Abstract: Using pregnenolone and 7-deoxycholic acid as starting materials, some 17-acetamidoandrostane and N,N-dimethyl-7-deoxycholic amide derivatives were synthesized. The cytotoxicity of the synthesized compounds was tested in vitro against two tumor cell lines: SGC 7901 (human gastric carcinoma) and Bel 7404 (human liver carcinoma). The result showed that the blockage of the interaction of the amide group with outside groups might cause a decrease of the cytotoxicity, and an O-benzyloximino group at the 3-position of N,N-dimethyl-7-deoxycholic amide could enhance the cytotoxic activity of the compound. The information obtained from the studies provides the structure-activity relationship for these compounds and may be useful for the design of novel chemotherapeutic drugs.

Keywords: pregnenolone; 17-acetamidoandrostane; N,N-dimethyl-7-deoxycholic amide; antiproliferative activity
1. Introduction

Steroidal compounds display a variety of biological functions and play a very important role in life [1–3]. Azahomosteroids are also a class of steroid compounds which were synthesized and modified in order to increase the biological activity of steroids. These compounds have been tested successfully as anti-cancer drugs against several types of cancer cells [4–8]. Some researchers 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 [9,10].

Several new steroidal lactams with the introduction of NHCO groups on the A-ring, C-ring or D-ring of the steroidal nucleus were synthesized and assayed in our group [11–14]. We found that these compounds displayed distinct cytotoxicity against different cancer cells, for instance, the steroidal lactam compounds I and II (Figure 1) displayed distinct cytotoxicity against some cancer cells through effective induction of cancer cell apoptosis by activation of the intrinsic pathway [15].

Figure 1. The structure of compounds I–IV.

A survey of the literature suggested steroidal compounds with a NHCO group in the side chain exhibit some valuable biological activities, such as cytotoxicity and antibacterial properties. For example, finasteride, dutasteride and PNU-157706 [16,17], used as clinical benign prostatic hyperplasia drugs, have a NHCO group in their branch chain. In order to further evaluate the antiproliferative activity of new steroidal derivatives and study their structure-activity relationships, some 17-acetamidoandrostane (Scheme 1) and N,N-dimethyl-7-deoxycholic amide derivatives (Scheme 2) with a NHCO group in their side chain were designed and synthesized. Their antiproliferative activities were tested in vitro against two tumor cell lines: SGC 7901 (human gastric carcinoma) and Bel 7404 (human liver carcinoma).

Here, the N,N-dimethyl-7-deoxycholic amide (III) has a similar atom rank of the side chain of as 24-methylenecholest-4-en-3β,6β-diol (IV), which is a polyhydroxysterol obtained from the soft coral Alcyonium patagonicum that showed potent cytotoxicity to murine leukemia cell with an IC50 value of 1 µg/mL [18], except for different atoms substituted in the 25- and 28-positions of the side chain.

Reagents and conditions: (i) Ac₂O/Pyridine/r.t., 24 h; (ii) NH₂OH.HCl/NaOAc.3H₂O/95% CH₃CH₂OH, 60 °C, 2h; (iii) SOCl₂/THF, r.t., 2 h; (iv) 13% K₂CO₃/CH₃OH/reflux, 4 h; (v) Jones’ reagent/acetone/r.t., 2 h; (vi) NaBH₄/CH₃OH, r.t., 30 min; (vii) NiCl₂·6H₂O/NaBH₄.

Scheme 2. Synthetic procedures of N,N-dimethyl-7-deoxycholic amide derivatives.

Reagents and conditions: (a) Jones’ reagent, (CH₃)₂CO, 0 °C; (b) Toluene, (CO)₂Cl₂, Py/(CH₃)₂NH (33%); (c) NaBH₄/CH₃OH; (d) Ac₂O/Pyridine/r.t., 24 h; (e) NH₂NHCSNH₂/EtOH, 80 °C.
2. Results and Discussion

2.1. Chemistry

Scheme 1 outlines the synthetic procedures for compounds 4–8. First, the 3β-hydroxy group of pregnenolone (1) was protected by acetylation, then oximation of 3β-acetoxypregnenolone (2) was performed using NH₂OH·HCl and NaOAc·3H₂O to give compound 3. Compound 4 was generated by Beckmann rearrangement of 3 with SOCl₂/THF. Afterwards, compound 5 was obtained via the deacetylation of 4 with alcoholic K₂CO₃. Compound 6 was prepared by the oxidation of 5 with Jones’ reagent and multiple extraction because of better water solubility of compound 6. Lastly, compound 6 was converted to compounds 7 and 8 by selective reduction using NaBH₄ under different conditions.

Synthetic procedures to obtain N,N-dimethyl-7-deoxycholic amide derivatives 11–14 are outlined in Scheme 2. First, 3,12-dioxo-7-deoxycholic acid (10) was obtained by oxidation of 7-deoxycholic acid (9) with Jones’ reagent. Then, the reaction of 10 with (CO)₂Cl₂ and (CH₃)₂NH in toluene gave N,N-dimethyl-3,12-dioxo-7-deoxycholic amide (11). The structure of compound 11 had been confirmed by its IR, NMR and MS spectra. In the IR spectrum, the disappearance of the broad absorption peak at 3,200–2,500 cm⁻¹ showed that the COOH functional group had been converted to another group. The chemical shifts of 2.923 and 2.985 ppm (single peak) in the ¹H-NMR confirmed the existence of the CON(CH₃)₂ moiety. Next, the functional groups on the 3-position were modified in order to compare the relative antiproliferative activities of different groups. Compound 11 was converted to N,N-dimethyl-3-hydroxy-12-oxo-7-deoxycholic amide (12) by selective reduction using NaBH₄. Finally, compound 13 was produced by the esterification of the 3-hydroxy group of 12, and compound 14 was obtained by the reaction of 12 with thiosemicarbazide.

To further determine the effect of the 3-substituent groups of N,N-dimethyl-7-deoxycholic amide derivatives on the cytotoxic activity, compounds 15–18 with different 3-substitutent groups were prepared. Similarly, the reaction of compound 11 with hydroxylamine hydrochloride, thiosemicarbazide, CH₃ONH₂·HCl or PhCH₂ONH₂·HCl afforded the corresponding products 15, 16, 17 or 18, respectively (Scheme 3).

Scheme 3. Synthetic procedures of N,N-dimethyl-7-deoxycholic amide derivatives.

**Reagents and Conditions:**
(a) NH₂OH·HCl/95%EtOH, CH₃COONa, 70 °C;
(b) NH₂NHCSNH₂/EtOH, 80 °C;
(c) CH₃ONH₂·HCl/95%EtOH, CH₃COONa, 70 °C;
(d) PhCH₂ONH₂·HCl/95% EtOH, CH₃COONa, 70 °C.
2.2. In Vitro Evaluation of the Antiproliferative Activity

2.2.1. Cell Culture and Assay for Cell Viability

SGC 7901 (human gastric carcinoma) and Bel 7404 (human liver carcinoma) cell lines were obtained from Guangxi Traditional Chinese Medical University. Cells were grown in RPMI-1640 supplemented with 10% cosmic calf serum (Hyclone, Beijing, China) and antibiotics in a humidified atmosphere of 5% CO₂ at 37 °C. The viability of these cells was determined using the colorimetric CellTiter 96 aqueous Cell Proliferation Assay (MTT) according to the instructions provided by the manufacturer (Promega, Madison, WI, USA). Briefly, cells (1–3 × 10⁴ cells per well) were seeded in 96 wells plates. An equal amount of DMSO was added to the cells used as negative controls. All were treated in triplicate. One day after seeding, the cells were treated with or without different concentration of each compound 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; 200 µL of DMSO was added to dissolve the purple formazan crystals formed. The absorbance (A) at 492 nm was measured using a MULTISCAN MK3 analysis spectrometer (Thermo Scientific, Shanghai, China). The results were summarized as IC₅₀ values in µ mol/L in Table 1.

Table 1. In vitro antiproliferative activities (IC₅₀ in µmol/L) of the compounds 3–18.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>11</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>SGC 7901</td>
<td>23.4</td>
<td>65.8</td>
<td>63.4</td>
<td>49.0</td>
<td>58.4</td>
<td>61.5</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Bel 7404</td>
<td>51.2</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>80.1</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Compounds</td>
<td>13</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>16</td>
<td>17</td>
<td>18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SGC 7901</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>15.0</td>
<td>6.7</td>
<td></td>
</tr>
<tr>
<td>Bel 7404</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>14.4</td>
<td>23.2</td>
<td></td>
</tr>
<tr>
<td>Cisplatin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

2.2.2. Structure-Activity Relationships

As shown in Table 1, compound 3 with a 20-hydroximino group displayed distinct cytotoxicity against the two studied cancer cell lines. However, after the 20-hydroximino group of 3 was converted into an amide group (compounds 4–8), the antiproliferative activity of compounds was remarkably decreased. The result could be ascribed to the blockage of the interaction of the amide group with outside groups, which may be a main reason causing a decrease of the cytotoxicity because of the steric hindrance of the steroidal nucleus [14]. Therefore, changing the hydroximino group to the amide group at position-20 did not increase the cytotoxic activities of these 17-acetamidoandrost-5-ene derivatives. Comparing the result with the earlier studies [12,13], we can see that the cytotoxicity of the steroidal amide compounds is not only dependent on the structure of the side chain, but also the position of the CONH functional group. When the compounds had a cholesteric side chain and A-ring or B-ring was modified into an A-lactam [13] or a B-lactam [19], it would display distinctive cytotoxicity against the cancer cells.

To investigate the effect of the 24-amide group on the biological function of the compounds, we synthesized some N,N-dimethyl-7-deoxycholic amide derivatives with various 3-substituent groups.
Here, compounds 11–17 were almost inactive against the cancer cells. Once again, the reason may be the steric hindrance of N,N-dimethyl group inhibiting the affinity of the amide group with outside groups. Nevertheless, a 3-O-benzylxime group played an important role in the cytotoxicity of compound 18 against these cancer cells. Compound 18 showed almost the same cytotoxicity as cisplatin to SGC 7901 and Bel 7404 cells (18: 15.0 and 14.4; cisplatin: 6.7 and 23.2 µmol/L), indicating that the presence of the 3-O-benzylxime group on the compound 18 confers a positive effect on the cytotoxicity against these cancer cells, which provides clues the seeking more powerful anti-cancer drug candidates based on O-benzylxime groups via structural optimization.

### 3. Experimental

Sterols and NaBH₄ were purchased from Merck Co. (Shanghai, China) All chemicals and solvents were analytical grade and solvents were purified by general methods before being used. Melting points were determined on an X4 apparatus and were uncorrected. Infrared spectra were measured with a Thermo Scientific IS-10 Spectrophotometer. The ¹H and ¹³C-NMR spectra were recorded in CDCl₃ on a Bruker AV-300 spectrometer at working frequencies 300 and 75 MHz. Chemical shifts are expressed in parts per million (δ) values and coupling constants (J) in Hertz. LREIMS were recorded on a Thermo-DSQ instrument, while HREIMS were measured on a Agilent 6210 TOFMS instrument. 3-Acetoxy-17-acetamidoandrost-5-ene (4) and 3,12-dioxydeoxycholic acid (10) were prepared according to the literature [6] and [20], respectively.

**Synthesis of 3-hydroxy-17-acetamidoandrost-5-ene (5).** K₂CO₃ solution (13%, 20 mL) was added to a solution of compound 4 (438 mg, 3.32 mmol) in CH₃OH (200 mL). The reaction mixture was heated under reflux for 1 h. After completion of the reaction as indicated by TLC, the solvent was removed under vacuum. CH₂Cl₂ (200 mL) was added to dissolve the solid and the resulting solution was washed with cold water and saturated brine. 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 methanol/dichloromethane (30:1) as eluent to give 709 mg (76%) of 5 as a white solid. θ mp 196–197 °C; IR (KBr) ν/cm⁻¹: 3354, 2962, 2937, 1646, 1556, 1433, 1372, 1315; ¹H-NMR (CDCl₃, 300 MHz) δ: 0.721 (3H, s, 18-CH₃), 1.028 (3H, s, 19-CH₃), 2.002 (3H, s, N-COCH₃), 2.36–2.24 (2H, m, C₄-H), 3.58–3.51 (1H, m, C₃-H), 3.908 (1H, q, J = 9.0, C₁₇-H), 5.302 (1H, brd, J = 9.9, N-H), 5.367 (1H, dd, J = 3.6, 1.8, C₆-H); ¹³C-NMR (CDCl₃, 75 MHz) δ: 169.9 (-NHCOCH₃), 140.9 (5-C), 121.3 (6-C), 71.7 (3-C), 58.9 (17-C), 52.8 (9-C), 50.1 (13-C), 42.7 (4-C), 42.2 (14-C), 37.2 (10-C), 36.8(1-C), 36.7 (12-C), 32.0 (8-C), 31.6 (7-C), 31.5 (16-C), 28.7 (2-C), 27.7 (15-C), 23.6 (21-C), 20.6 (11-C), 19.4 (19-C), 11.9 (18-C); ESI-MS m/z: 332 (M+1)⁺.

**Synthesis of 17-acetamidoandrost-4-en-3,6-dione (6).** Jones’ reagent (0.7 mL, 0.267 mol/L) was added dropwise to the solution of 5 (709 mg) in acetone (30 mL) in 20 min. The mixture was stirred at room temperature for 1 h and then neutralized with 10% K₂CO₃ solution. The suspension was poured over a silica gel column and eluted with ethyl acetate. The solvent was removed under reduced pressure. The residue was purified by chromatography on silica gel using methanol/dichloromethane (20:1) as elution to give 590 mg (80%) of 6 as white solid. θ mp 198–199 °C; IR (KBr) ν/cm⁻¹: 3321, 2941, 2864, 1691, 1654, 1556, 1442, 1384; ¹H-NMR (CDCl₃, 300 MHz) δ: 0.728 (3H, s, 18-CH₃), 1.150 (3H, s,
19-CH₃), 1.976 (3H, s, N-COCH₃), 2.17–2.10 (2H, m, C₇-βH and C₂-αH), 2.495 (1H, dd, J = 13.8, 5.1, C₇-αH), 2.662 (1H, dd, J = 15.9, 3.9, C₂-βH), 3.937 (1H, q, J = 9.0, C₁⁷-H), 5.582 (1H, d, J = 9.0, N-H), 6.149 (1H, s, C₄-H); ¹³C-NMR (CDCl₃, 300 MHz) δ: 201.8 (6-C), 199.3 (3-C), 170.1 (-NHCOCH₃), 160.7 (5-C), 125.6 (4-C), 58.5 (14-C), 52.7 (17-C), 50.9 (9-C), 46.3 (13-C), 43.0 (10-C), 39.7 (1-C), 36.3 (12-C), 35.5 (7-C), 34.3 (8-C), 33.9 (16-C), 28.2 (2-C), 23.4 (15-C), 23.2 (21-C), 20.5 (11-C), 17.6 (19-C), 11.9 (18-C); ESI-MS m/z: 344 (M+1)+.

Synthesis of 3,6-dihydroxy-17-acetamidoandrost-4-en-7-one (7). NaBH₄ (42 mg, 1.1 mmol) was added at room temperature to a solution of 6 (124 mg, 0.36 mmol) in CH₃OH (50 mL) in one portion. 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 (15 mL × 3). 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 was obtained. After recrystallization from methanol, 95 mg (78%) of compound 7 was obtained as a white solid. θ mp 198–200 ºC; IR (KBr) ν/cm⁻¹: 3331, 2941, 2866, 1654, 1442, 1384, 1217, 1131; ¹H-NMR (CDCl₃, 300 MHz) δ: 0.703 (3H, s, 18-CH₃), 1.008 (3H, s, 19-CH₃), 1.988 (3H, s, N-COCH₃), 3.57–3.47 (1H, m, C₃-H), 3.93–3.80 (1H, m, C₆-H), 4.13 (1H, dd, J = 7.0, 1.5, C₁⁷-H), 5.36 (1H, d, J = 2.1, C₄-H), 5.40 (1H, br s, N-H); ¹³C-NMR (CDCl₃, 75 MHz) δ: 170.0 (-NHCO), 140.9 (5-C), 121.3 (4-C), 71.6 (6-C), 68.8 (3-C), 58.9 (17-C), 52.8 (14-C), 50.1 (9-C), 42.7 (13-C), 42.2 (7-C), 37.2 (10-C), 36.8 (1-C), 36.5 (12-C), 32.1 (8-C), 31.6 (2-C), 31.5 (16-C), 28.7 (15-C), 23.6 (21-C), 20.6 (11-C), 19.4 (19-C), 12.0 (18-C); ESI-MS m/z: 348 (M+1)+.

Synthesis of 3-hydroxy-17-acetamidoandrost-4-en-6-one (8). NaBH₄ (42 mg, 1.1 mmol) was added to a solution of 6 (124 mg, 0.36 mmol) and NiCl₂·6H₂O (87 mg, 0.37 mmol) in CH₃OH (15 mL) in the interval of 5 min at room temperature. After 5 min, the reaction was stopped. The solution was neutralized with 1 M HCl. After evaporation of the majority of MeOH under reduced pressure, water of 15 mL was added. Then the residue was extracted with ethyl acetate (15 × 3). 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 flash chromatography on silica gel using methanol/dichloromethane (20:1) as the eluent. Compound 8 was obtained as white solid (75 mg, 60%), θ mp 187–188 ºC; IR(KBr) ν/cm⁻¹: 3330, 2942, 2865, 1675, 1442, 1385, 1218; ¹H-NMR (CDCl₃, 300 MHz) δ: 0.747 (3H, s, 18-CH₃), 1.196 (3H, s, 19-CH₃), 2.004 (3H, s, N-COCH₃), 2.22–2.14 (2H, m, C₁₆-H), 2.418 (1H, dd, J = 12.9, 4.5, C₁₇-H), 3.929 (1H, dd, J = 11.4, 4.2, 1.2, C₃-βH), 5.330 (1H, br d, J = 7.5, N-H), 6.196 (1H, d, J = 1.2, C₄-H); ¹³C-NMR (CDCl₃, 75 MHz) δ: 199.3 (6-C), 170.0 (-NHCO), 169.9 (5-C), 119.9 (4-C), 119.9 (4-C), 68.5 (3-C), 58.6 (17-C), 53.7 (9-C), 51.7 (14-C), 42.9 (13-C), 40.9 (7-C), 39.0 (10-C), 36.5 (8-C), 36.3 (1-C), 34.3 (12-C), 33.7 (2-C), 28.6 (16-C), 23.6 (15-C), 23.5 (21-C), 20.6 (11-C), 18.3 (19-C), 12.1 (18-C); ESI-MS m/z: 346 (M+1)+.

Synthesis of N,N-dimethyl-3,12-dioxo-7-deoxycholic amide (11). Oxalyl chloride (200 µL, 2.23 mmol) was added to a solution of 10 (535 mg, 1.38 mmol) and pyridine (150 µL, 1.80 mmol) in toluene (25 mL) at ice bath temperature. The mixture was stirred for 2 h. After completion of the reaction as
indicated by TLC, a solution of dimethyl amine (540 µL) was added dropwise. The solution was stirred for 10 min at 0 °C and 10 mL ice water was added to the solution. The solution was extracted with dichloromethane (30 × 3 mL). The organic solution was washed with cold water and saturated brine. 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/ethyl acetate (1:2) as the eluent. Compound 11 was obtained as a white solid (480 mg, 71%), \( \theta_{\text{mp}} \) 271–272 °C; IR(KBr) \( \nu/cm\^-1: \) 2929, 2859, 1695, 1634; \(^1\)H-NMR (CDCl\(_3\), 300 MHz) \( \delta \): 0.797 (3H, d, \( J = 5.7, 21-\text{CH}_3 \)), 1.000 (3H, s, 18-\text{CH}_3), 1.050 (3H, s, 19-\text{CH}_3), 2.13–2.04 (2H, m), 2.37–2.18 (3H, m), 2.58–2.48 (2H, m, \( \text{C}_{11}\text{-H} \)), 2.872 (3H, s, \( \text{NCH}_3 \)), 2.955 (3H, s, \( \text{NCH}_3 \)); \(^{13}\)C-NMR (CDCl\(_3\), 75 MHz) \( \delta \): 214.2 (12-C), 212.1 (3-C), 173.4 (24-C), 58.5 (14-C), 57.5 (17-C), 46.5 (9-C), 44.2 (13-C), 43.6 (5-C), 42.1 (4-C), 38.3 (8-C), 37.3 (11-C), 36.8 (1-C), 36.7 (2-C), 35.6 (10-C), 35.5 (N-C), 35.4 (N-C), 35.3 (20-C), 30.6 (7-C), 30.2 (23-C), 27.4 (22-C), 26.5 (15-C), 25.4 (6-C), 24.3 (16-C), 22.1 (21-C), 18.7 (19-C), 11.7 (18-C); ESI-MS \( m/z \): 416 (M+1)+.

**Synthesis of N,N-dimethyl-3-hydroxy-12-oxo-7-deoxycholic amide (12).** Compound 12 was prepared according to the procedure of 7. Yield 30%, \( \theta_{\text{mp}} \) 279–280 °C; IR(KBr) \( \nu/cm\^-1: \) 3465, 2925, 1707, 1621; \(^1\)H-NMR (CDCl\(_3\), 300 MHz) \( \delta \): 0.843 (3H, d, \( J = 6.3, 21-\text{CH}_3 \)), 0.986 (3H, s, 19-\text{CH}_3), 0.995 (3H, s, 18-\text{CH}_3), 2.26–2.17 (3H, m), 2.49–2.32 (2H, m, \( \text{C}_{11}\text{-H} \)), 2.912 (3H, s, \( \text{NCH}_3 \)), 2.994 (3H, s, \( \text{NCH}_3 \)); \(^{13}\)C-NMR (CDCl\(_3\), 75 MHz) \( \delta \): 215.2 (12-C), 173.7 (24-C), 71.2 (3-C), 58.7 (14-C), 57.5 (17-C), 46.4 (13-C), 44.1 (5-C), 41.5 (9-C), 38.1 (8-C), 37.3 (11-C), 36.3 (1-C), 35.6 (4-C), 35.4 (10-C), 35.3 (N-C), 35.3 (N-C), 35.2 (20-C), 30.6 (2-C), 30.3 (23-C), 30.2 (22-C), 27.5 (6-C), 27.1 (15-C), 26.1 (16-C), 24.3 (7-C), 22.8 (21-C), 18.8 (18-C), 11.7 (19-C); ESI-MS \( m/z \): 418 (M+1)+.

**Synthesis of N,N-dimethyl-3-acetoxy-12-oxo-7-deoxycholic amide (13).** Compound 13 was prepared by the esterification of 12 using acetic anhydride in pyridine. Yield: 78%, \( \theta_{\text{mp}} \) 153–154 °C; IR(KBr) \( \nu/cm\^-1: \) 3440, 2953, 1730, 1656, 1465, 1447; \(^1\)H-NMR (CDCl\(_3\), 300 MHz) \( \delta \): 0.885 (3H, d, \( J = 6.3, 21-\text{CH}_3 \)), 1.035 (6H, br s, 18-CH\(_3\) and 19-CH\(_3\)), 2.025 (3H, s, \( \text{CH}_3\text{CO-} \)), 2.951 (3H, s, \( \text{NCH}_3 \)), 3.025 (3H, s, \( \text{NCH}_3 \)), 4.659–4.763 (1H, m, \( \text{C}_3\text{-H} \)), 3.659 (1H, m, \( \text{C}_3\text{-H} \)); \(^{13}\)C-NMR (CDCl\(_3\), 75 MHz) \( \delta \): 214.9 (12-C), 173.6 (24-C), 170.6 (\( \text{CH}_3\text{CO-} \)), 73.7 (3-C), 58.7 (14-C), 57.6 (17-C), 46.5 (13-C), 44.1 (5-C), 41.4 (9-C), 38.1 (8-C), 37.3 (11-C), 35.7 (1-C), 35.4 (4-C), 35.3 (N-C), 34.9 (N-C), 32.1 (20-C), 30.7 (2-C), 30.3 (23-C), 27.5 (6-C), 26.9 (22-C), 26.4 (6-C), 26.4 (15-C), 24.4 (16-C), 22.7 (7-C), 21.4 (21-C), 18.8 (18-C), 11.7 (19-C); ESI-MS \( m/z \): 460 (M+1)+.

**Synthesis of N,N-dimethyl-3-hydroxy-12-thiosemicarbazone-7-deoxycholic amide (14).** The compound 12 (100 mg, 0.24 mmol) was dissolved in 15 mL of CH\(_3\)CH\(_2\)OH and the solution was adjusted to pH ≈ 3–5 by adding a few drops of glacial acetic acid. After the solution was heated to 80 °C, and thiosemicarbazide (32 mg, 0.36 mmol) was added. The mixture was stirred for 10 h at 80 °C until no starting material (the progress of the reaction was monitored by TLC). Then the reaction was terminated and the majority of solvent was evaporated under reduced pressure. Water (10 mL) was added to the mixture which was then extracted with dichloromethane. The organic layer was washed with water and saturated brine. After drying over anhydrous sodium sulfate, the solvent was removed
under reduced pressure, and the resulting crude product was purified by flash chromatography on silica gel using dichloromethane/methanol (40:1) as the eluent to give 56 mg of 14 as a white solid (yield: 49%), \( \theta_{\text{mp}} 294–295 \, ^\circ\text{C} \); IR (KBr) \( \nu/cm^{-1} \): 3427, 2921, 1634, 1503, 1401; \( ^1\)H-NMR (CDCl\(_3\), 300 MHz): 0.843 (3H, d, \( J = 6.3 \), 21-CH\(_3\)), 0.986 (3H, s, 18-CH\(_3\)), 0.995 (3H, s, 19-CH\(_3\)), 2.912 (3H, s, N-CH\(_3\)), 2.994 (3H, s, N-CH\(_3\)), 3.577 (1H, m, C\(_3\)-H), 6.576 (1H, br s, -NH\(_2\)), 7.234 (1H, br s, -NH\(_2\)), 9.284 (1H, s, -NH-); \( ^{13}\)C-NMR (CDCl\(_3\), 75 MHz): 178.8 (C=S), 173.6 (24-C), 162.6 (12-C), 71.1 (3-C), 58.7 (14-C), 57.5 (17-C), 46.4 (13-C), 44.1 (5-C), 41.6 (9-C), 38.1 (8-C), 37.4 (4-C), 36.3 (11-C), 35.7 (10-C), 35.6 (1-C), 35.4 (N-C), 35.3 (20-C), 35.1 (N-C), 30.7 (2-C), 30.3 (22-C), 30.2 (23-C), 27.5 (6-C), 27.1 (15-C), 26.1 (16-C), 24.4 (7-C), 22.8 (21-C), 18.8 (18-C), 11.7 (19-C); ESI-MS m/z: 491(M+1)+.

**Synthesis of N,N-dimethyl-3-hydroximino-12-oxo-7-deoxycholic amide (15).** Compound 11 (91 mg, 0.22 mmol) was dissolved in 95% CH\(_3\)CH\(_2\)OH (15 mL). After the mixture was heated to 70 °C, CH\(_3\)COONa·3H\(_2\)O (30 mg, 0.22 mmol) and NH\(_2\)OH·HCl (18 mg, 0.26 mmol) were added. The mixture was stirred for 3 h at 70 °C. 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 dichloromethane. The combined extracts were washed with water and saturated brine, dried with anhydrous sodium sulfate, and evaporated under reduced pressure. The residue was purified by flash chromatography on silica gel using dichloromethane/methanol (40:1) as the eluent. Compound 15 was obtained as a white solid (56 mg, 60%). \( \theta_{\text{mp}} 232–233 \, ^\circ\text{C} \); IR (KBr) \( \nu/cm^{-1} \): 3440, 1691, 1437, 1392; \( ^1\)H-NMR (CDCl\(_3\), 300 MHz): 0.853 (3H, d, \( J = 6.3 \), 21-CH\(_3\)), 1.025 (3H, s, 18-CH\(_3\)), 1.035 (3H, s, 19-CH\(_3\)), 2.534 (2H, m, C\(_{11}\)-H), 2.923 (3H, s, N-CH\(_3\)), 2.999 (3H, s, N-CH\(_3\)), 3.070 (1H, dd, \( J = 14.4 \), 3.9, C\(_2\)-βH); \( ^{13}\)C-NMR (CDCl\(_3\), 75 MHz): 214.9 (12-C), 173.7 (24-C), 160.2 (3-C), 58.6 (14-C), 57.6 (17-C), 46.4 (9-C), 44.2 (5-C), 44.0 (13-C), 43.3 (10-C), 41.9 (8-C), 38.3 (11-C), 37.4 (N-C), 36.9 (N-C), 36.1 (20-C), 35.6 (7-C), 35.5 (4-C), 30.6 (23-C), 30.3 (6-C), 27.4 (22-C), 26.6 (2-C), 26.5 (1-C), 25.6 (15-C), 24.8 (16-C), 24.3 (21-C), 22.6 (19-C), 18.8 (18-C); ESI-MS m/z: 431(M+1)+.

**Synthesis of N,N-dimethyl-3-thiosemicarbazone-12-oxo-7-deoxycholic amide (16).** Compound 16 was prepared similarly to 14, but from the compound 11. Yield: 69%, \( \theta_{\text{mp}} 282–283 \, ^\circ\text{C} \); IR (KBr) \( \nu/cm^{-1} \): 3440, 1691, 1601, 1437, 1392; \( ^1\)H-NMR (CDCl\(_3\), 300 MHz): 0.853 (3H, d, \( J = 6.3 \), 21-CH\(_3\)), 1.025 (3H, s, 18-CH\(_3\)), 1.035 (3H, s, 19-CH\(_3\)), 2.534 (2H, m, C\(_{11}\)-H), 2.923 (3H, s, N-CH\(_3\)), 2.999 (3H, s, N-CH\(_3\)), 3.070 (1H, dd, \( J = 14.4 \), 3.9, C\(_2\)-βH); \( ^{13}\)C-NMR (CDCl\(_3\), 75 MHz): 214.5 (12-C), 178.7 (S=C), 173.6 (24-C), 160.2 (3-C), 58.6 (14-C), 57.6 (17-C), 46.4 (9-C), 44.2 (5-C), 44.0 (13-C), 43.3 (10-C), 41.9 (8-C), 38.3 (11-C), 37.4 (N-C), 36.9 (N-C), 36.1 (20-C), 35.6 (7-C), 35.5 (4-C), 30.6 (23-C), 30.3 (6-C), 27.4 (22-C), 26.6 (2-C), 26.5 (1-C), 25.6 (15-C), 24.8 (16-C), 24.3 (21-C), 22.6 (19-C), 18.8 (18-C); ESI-MS m/z: 489(M+1)+.

**Compounds 17 and 18** were prepared similarly according to the procedure of 15, but CH\(_3\)ONH\(_2\)-HCl and PhCH\(_2\)ONH\(_2\)-HCl were used as reagents instead of NH\(_2\)OH·HCl.

**N,N-dimethyl-3-O-methyloximino-12-oxo-7-deoxycholic amide (17).** Yield: 70%, \( \theta_{\text{mp}} 188–189 \, ^\circ\text{C} \); IR (KBr) \( \nu/cm^{-1} \): 3436, 2855, 1703, 1654, 1446, 1041; \( ^1\)H-NMR (CDCl\(_3\), 300 MHz): 0.875 (3H, d, \( J = 6.3 \), 21-CH\(_3\)), 1.043 (3H, s, 18-CH\(_3\)), 1.054 (3H, s, 19-CH\(_3\)), 2.55–2.40 (2H, m, C\(_{11}\)-H), 2.940 (3H, s, 29.9 (2-C), 27.4 (22-C), 26.7 (1-C), 25.6 (15-C), 24.3 (16-C), 22.5 (21-C), 18.8 (19-C), 11.8 (18-C); ESI-MS m/z: 489(M+1)+.

Compounds 17 and 18 were prepared similarly according to the procedure of 15, but CH\(_3\)ONH\(_2\)-HCl and PhCH\(_2\)ONH\(_2\)-HCl were used as reagents instead of NH\(_2\)OH·HCl.
N, N-dimethyl-3-O-benzyloximino-12-oxo-7-deoxycholic amide (18). Yield: 72%, \( \theta_{\text{mp}} \) 148–149 °C. IR (KBr) \( \nu / \text{cm}^{-1} \): 2970, 2868, 1707, 1625, 1450, 1397; \(^1\)H-NMR (CDCl\(_3\), 300 MHz): 0.882 (3H, d, \( J = 6.3 \), 21-CH\(_3\)), 1.048 (3H, s, 18-CH\(_3\)), 1.056 (3H, s, 19-CH\(_3\)), 2.35–2.45 (1H, m, C\(_{11-}\)\( \beta \)H), 2.553 (1H, t, \( J = 12.6 \), C\(_{11-}\)\( \alpha \)H), 2.947 (3H, s, N-CH\(_3\)), 3.019 (3H, s, N-CH\(_3\)), 5.052 (2H, s, O-CH\(_2\)-Ph), 7.36–7.29 (5H, m, -C\(_6\)H\(_5\)); \(^13\)C-NMR (CDCl\(_3\), 75 MHz): 214.7 (12-C), 173.5 (24-C), 160.5 (3-C), [138.2, 128.3, 127.9, 127.8, 127.6 (-C\(_6\)H\(_5\))], 75.2 (O-C), 58.6 (14-C), 57.5 (17-C), 46.5 (9-C), 44.3 (13-C), 44.0 (5-C), 43.5 (10-C), 41.9 (8-C), 38.3 (11-C), 37.3 (N-C), 36.1 (N-C), 36.0 (20-C), 35.6 (2-C), 35.4 (7-C), 30.6 (23-C), 30.3 (6-C), 27.4 (1-C), 26.6 (22-C), 25.8 (4-C), 25.6 (15-C), 24.3 (16-C), 22.6 (21-C), 18.8 (19-C), 11.8 (18-C); ESI-MS \( m/z \): 521 (M+1)\(^+\).

4. Conclusions

We have prepared some 17-acetamidoandrostane and N,N-dimethyl-7-deoxycholic amide derivatives with different substituent groups on different positions of the steroidal nucleus. The antiproliferative activity of the synthesized compounds against the SGC 7901 and Bel 7404 cell lines was assayed. The results showed that the blockage of the interaction of the amide group with outside groups might cause a decrease of the cytotoxicity, and an O-benzyloxime group on the 3-position of N,N-dimethyl-7-deoxycholic amide could enhance the cytotoxic activity of the compound. Our result revealed some structure-activity relationships of the compounds and although some displayed indistinctive cytotoxicity against these cancer cells, this information may be useful for the design of novel chemotherapeutic drugs with better activity.

Acknowledgments

The authors acknowledge the financial support of the Natural Science Foundation of Guangxi Province (No. 2010GXNSFA013019, No. 2010GXNSFJD013052) and Natural Science Fund of Education Department of Guangxi Province (No. 201202ZD059).

Conflict of Interest

The authors declare no conflict of interest.

References


*Sample Availability:* Samples of the compound 4, 5, 11, 12 are available from the authors.

© 2013 by the authors; licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution license (http://creativecommons.org/licenses/by/3.0/).