Bioorganic & Medicinal Chemistry Letters 21 (2011) 3641–3643

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis and cytotoxicity of 17a-aza-D-homo-androster-17-one derivatives

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ARTICLE INFO

Article history:
Received 26 November 2010
Revised 2 February 2011
Accepted 21 April 2011
Available online 28 April 2011

Keywords:
17a-Aza-D-homo-andrester-17-one derivatives
Synthesis
Cytotoxicity

ABSTRACT

A series of 17a-aza-D-homo-andrester-17-one derivatives, bearing hydroxyl, hydroximino, carbonyl and thiosemicarbazido groups at the position-3 or position-6 of steroidal nucleus, were prepared and evaluated in vitro against two human cell lines (Hela (human cervical carcinoma) and SMMC 7404 (human liver carcinoma)). The results showed that these compounds could exhibit a high cytotoxicity to Hela tumor cell line, especially for compounds **8** and **12**, the IC_{50} values are 15.1 and 14.0 nmol/mL, respectively. Our findings could provide new evidence showing the relationship between the chemical structure and biological activity and may be useful for the discovery of new anti-cancer drugs.

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Dehydroepaimdrosterone (DHEA) is endogenous steroidal prohormone with important physiological activities, such as anti-aging and assimilation of protein. It is also a very important intermediate in the synthesis of steroid drugs. Previously, besides applying to hormonal drugs, several modified steroid compounds, such as aza-D-homo steroids that contain the NHCO group inside D-ring, were used in the synthesis of steroids ester carrying alkylator.[1–4] The result of the investigation of aza-D-homo-androsterone alkylator (Fig. 1) indicated that the presence of characteristic group, –NH–CO–, in the homo-aza steroid molecule is very important in reducing the acute toxicity and enhance the anti-tumor activity for the steroid compounds.[5,6] However, the cytotoxicity investigation for these kinds of compounds was mainly focused on anti-leukemic activity. In our previous work, we found that a steroidal compound has excellent cytotoxicity when it has the cholesteric side chain and the 3- or 6-position on steroidal nucleus is substituted by hydroxyl, hydroximino, or sodium sulphate.[7–10] In order to find novel and effective anti-tumor agents, we designed and synthesized a series of 17a-aza-D-homo-andrester-17-one derivatives with various groups at the 3- or 6-position of the steroidal nucleus. The design was based on the knowledge of our previous SARs analysis and effective enhancing of the anti-cancer activity by transferring D-ring of 3β-hydroxy-5-androsten-17-one into lactamide.[11]

Using 3β-hydroxy-5-androsten-17-one (**1**) as a starting material, 17α-aza-D-homo-4-androsten-3,6,17-trione (**6**) was synthesized in five steps (Scheme 1). First, the compound **1** was transformed into the corresponding 3β-acetoxy-5-androsten-17-one (**2**), followed by the condensation reaction with hydroxylamine hydrochloride to offer 3β-acetoxy-5-androsten-17-oxime (**3**) according to the literature 10. Beckman rearrangement of compound **3**[12] in $SOCl_2$/THF gave 3β-acetoxy-17-aza-D-homo-5-androsten-17-one (**4**) in 78% yield. 3β-hydroxy-17-aza-D-homo-5-androsten-17-one (**5**)[13] was obtained in 81% yield by deacetylation in the aqueous solution of 13% K_2CO_3. Lastly, the oxidation of the compound **5** with Jones reagent afforded the expected product, 17α-aza-D-homo-4-androsten-3,6,17-trione (**6**).[14]

The synthetic route and the structures of target compounds **7**–**12**[15–18] were outlined in Scheme 2. Compound **6** was reduced by $NaBH_4$ in methanol into compound **7**, and **6** condensed with two equal amounts of hydroxylamine hydrochloride to afford di-oxime compound **8** and condensed with two equal amount of thiosemicarbazide to generate di-thiosemicarbazido compound **9**. The 17a-aza-D-homo-androstan-3,6,17-trione (**10**) was synthesized by the reduction of **6** with $NaBH_4$/$NiCl_2$ and oxidation with Jones reagent in two steps. Using similar procedure for preparing **7** and **8**, compounds **12** and **11** were obtained, respectively.

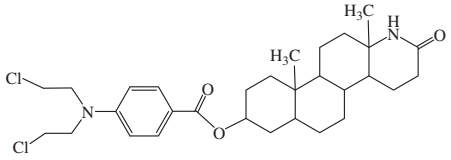

Figure 1. Chemical structure of D-homo-aza-androsterone alkylator.

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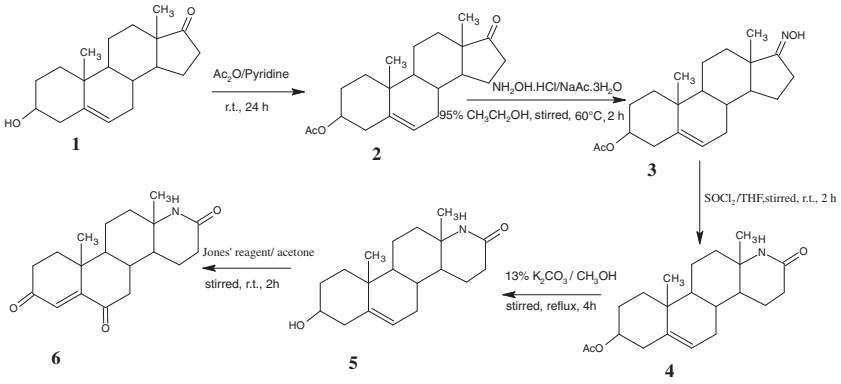
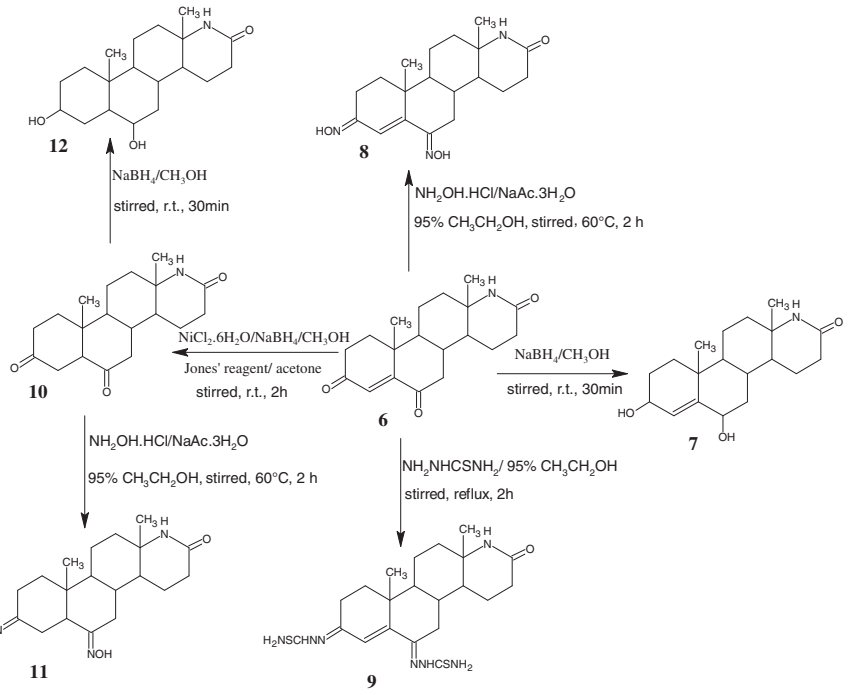
doi:10.1016/j.bmcl.2011.04.093



Scheme 1. Synthesis of 17a-aza-D-homo-4-androsten-3,6,17-trione.



Scheme 2. Syntheses of 17a-aza-D-homo-androster-17-one derivatives.

Table 1
Cytotoxicities of 17a-aza-D-homo-andrester-17-one derivatives in vitro[b] (IC_{50} in μmol/L[a])

Compound	4	5	6	7	8	9	10	11	12
Hela	37.6	65.9	102.4	18.8	15.1	28.8	53.6	75.7	14.0
SMMC 7404	>200	>200	>200	>200	>200	80.5	60.8	184	124.4

[a] Cytotoxicity as IC_{50} for each cell line is the concentration of compound which reduced by 50% the optical density of treated cells with respect to untreated cells using the MTT assay.
[b] Data represent the mean values of three independent determinations.

The cytotoxic activities of compounds **4**–**12** were determined in vitro on 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 shown in Table 1, compounds **4**–**12** displayed a distinct cytotoxicity against Hela tumor cell line, however, performed a weak activity to almost inactivity against SMMC 7404. We previously reported that the steroidal compounds bearing hydroxyl or hydroximino exhibited higher cytotoxic activities than the corresponding compounds bearing carbonyl.[9,10] The result was confirmed again in this investigation of cytotoxic activity against Hela tumor cell line. But a contrary result was observed to the cytotoxicity against SMMC 7404, such as the cytotoxic activity of compound **10** against SMMC 7404 was higher than compounds **11** and **12**. The introduction of hydroxyl or hydroxyimino into the 3- or 6-position of steroid nucleus increased the cytotoxicity to Hela tumor cell line. For example, the IC_{50} values of compounds **7**, **8** and **12** were 18.8, 15.1 and 14.0 μmol/L, respectively.

Interestingly, our results indicated that the presence of double bond between positions 4 and 5 on A-ring conferred a positive effect to the cytotoxicity against Hela tumor cell lines when both

3,6-positions of steroidal nucleus were substituted by hydroximino (comparing compound **8** (IC_{50} value: 15.1) with the compound **11** (IC_{50} value: 75.7)). Nevertheless, when the 3,6-positions were substituted by hydroxyl, the IC_{50} value was not affected regardless of the existence of the double bond ((comparing the IC_{50} values of the compound **7** (IC_{50} value: 18.8) and **12** (IC_{50} value: 14.0)).

In conclusion, we have prepared a new series of 17a-aza-D-homo-androster-17-one derivatives which were proven to be potent anti-tumor activities against Hela tumor cell line. Compounds **7**, **8** and **12** were found to be more potent compounds, therefore, the further structural modification and anti-tumor activity study 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.

Acknowledgments

The authors acknowledge the financial support of the Natural Science Foundation of Guangxi Province (No: 2010GXNSFD 013019) and the Natural Science Foundation of Guangxi Province (No: 2010GXNSFA013052).

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11. Cui, J.; Wang, H.; Huang, Y.; Zhou, A. CN: 101845075 A, 2010.09.29 [p].
12. *Procedure for the preparation of compound* **4**. To a solution of oxime **3** (1 mmol) in dry THF (15 mL) the solution of thionyl chloride (1.6 mL) in 4 mL dry THF was added under argon. The solution was stirred under anhydrous conditions for 2 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. 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: 30) to give 270 mg of **4** as a white solid. Yield: 78%. Mp 167–168 °C; IR(KBr) ν/cm^{-1}: 3475, 3178, 2945, 2868, 1744, 1658, 1455, 1249; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 1.03(3H, s, 19-CH_3), 1.13(3H, s, 18-CH_3), 1.90 (3H, s, CH_3CO–), 2.52–2.33 (3H, m, C_{16}-H, C_8-H), 4.63 (1H, m, C_3-H), 5.41 (1H, br s, C_6-H), 5.88 (1H, s, –NH); ^{13}C ($CDCl_3$, 75 MHz) δ: 171.7 (C-17), 170.5 (C=O), 139.5 (C-5), 121.1 (C-6), 73.6 (C-3), 54.2 (C-9), 49.4 (C-13), 47.9 (C-14), 37.8 (C-7), 36.8 (C-10), 36.7 (C-1), 33.3 (C-4), 32.2 (C-12), 31.2 (C-16), 30.8 (C-8), 27.6 (C-2), 22.1 (C-18), 21.4 (CH_3CO), 20.9 (C-15), 20.0 (C-11), 19.2 (C-19); ESI-MS *m*/*z*: 346 (M+1)$^+$.
13. *Procedure for the preparation of compound* **5**. K_2CO_3 solution (13%) of 15 mL was added to a solution of compound **4** (1 mmol) in CH_3OH (30 mL). The reaction mixture was heated under reflux for 4 h. After completion of the reaction as indicated by TLC, the solvent was removed under vacuum. CH_2Cl_2 (60 mL) was added to dissolve a 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 elution to give 245 mg of **5** as a white solid. Yield: 81%. Mp 168–170 °C; IR(KBr) ν/cm^{-1}: 3452, 3162, 2933, 2904, 1670, 1625, 1433, 1401, 1045; ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 1.00 (3H, s, 19-CH_3), 1.17 (3H, s, 18-CH_3), 2.35–2.31 (1H, m, C_{16}-H), 2.40–2.33 (1H, m, C_8-H), 2.48 (1H, ddd, *J* = 18.6, 7.2, 1.2, C_{16}-H), 3.57–3.51 (1H, m, C_3-H), 5.36 (1H, t, *J* = 3.0, C_6-H), 5.68 (1H, s, –NH); ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 171.6 (C-17), 140.6 (C-5), 120.8 (C-6), 71.5 (C-3), 54.2 (C-9), 49.5 (C-13), 48.1 (C-14), 41.9 (C-7), 39.9 (C-10), 36.9 (C-1), 36.7 (C-4), 32.3 (C-12), 31.5 (C-16), 31.3 (C-2), 30.8 (C-8), 22.1 (C-18), 21.0 (C-15), 20.1 (C-11), 19.3 (C-19); ESI-MS *m*/*z*: 306 (M+1)$^+$.
14. *Procedure for the preparation of compound* **6**. The Jones' reagent of 0.5 mL (0.267 mol/L) was added dropwise to the solution of **5** (1 mmol) in 20 mL of acetone in 10 min. The mixture was stirred at room temperature for 1 h and then neutralized with 10% K_2CO_3 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 (30:1) as elution to give 189 mg of **6** as a white solid. Yield: 60%. Mp 173–174 °C; IR(KBr) ν/cm^{-1}: 3476, 3182, 2953, 1687, 1654, 1458, 1249, 1323, 1053, 935, 845, 728; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 1.17 (3H, s, 19-CH_3), 1.24 (3H, s, 18-CH_3), 2.93 (1H, dd, *J* = 16.5, 3.0, C_7-αH), 2.55–2.21 (m, 5H, C_7-βH, C_2-H, C_{16}-H) 6.23 (1H, s, C_4-H), 6.77 (1H, s, –NH); ^{13}C ($CDCl_3$, 75 MHz): 200.8 (C-6), 198.9 (C-3), 171.6 (C-17), 159.2 (C-5), 125.9 (C-4), 53.8 (C-9), 50.0 (C-13), 47.7 (C-14), 45.3 (C-7), 39.2 (C-1), 39.1 (C-10), 35.2 (C-12), 34.3 (C-16), 33.8 (C-2), 30.3 (C-8), 22.0 (C-18), 20.6 (C-15), 19.8 (C-11), 17.4 (C-19); ESI-MS *m*/*z*: 316 (M+1)$^+$.
15. *Procedure for the preparation of compound* **10**. To a stirred solution of **6** (1 mmol) and $NiCl_2{\cdot}6H_2O$ (1 mmol) in CH_3OH (50 mL) was added $NaBH_4$ (3 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. The combined extracts were washed with cold water and saturated brines. After drying over anhydrous sodium sulfate, the solvent was removed under reduced pressure. The resulting solid was dissolved in 30 mL of acetone. The Jones' reagent was gradually added into the solution of acetone at 0–5 °C until the orange of solution was not faded, and evaporated under reduced pressure. The residue was subjected to chromatography (methanol/dichloromethane = 30:1) to give 145 mg of **10** as a white solid, Yield: 46%. Mp 176–178 °C; IR(KBr) ν/cm^{-1}: 3440, 3170, 2962, 2851, 1707, 1650, 1402, 1392, 1245, 1217, 1172; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 0.96 (3H, s, 19-CH_3), 1.21 (3H, s, 18-CH_3), 2.91 (1H, dd, *J* = 16.2, 4.2, C_5-H), 6.33 (1H, s, –NH); ^{13}C ($CDCl_3$, 75 MHz): 210.5 (C-3), 208.0 (C-6), 171.5 (C-17), 54.2 (C-5), 53.7 (C-9), 52.1 (C-13), 47.2 (C-14), 40.5 (C-4), 39.2 (C-7), 38.1 (C-10), 37.2 (C-1), 35.7 (C-12), 34.3 (C-16), 33.6 (C-2), 30.5 (C-8), 22.1 (C-18), 21.4 (C-15), 19.9 (C-11), 12.4 (C-19); ESI-MS *m*/*z*: 318 (M+1)$^+$.
16. *General procedure for preparation of compounds* **7** *and* **12**. $NaBH_4$ (4 mmol) was added to a solution of **6** or **10** (1 mmol) in CH_3OH (50 mL) 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 (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 crystallization from methanol, the compound **7** or **12** was obtained as a white solid. Compound **7**, yield: 78%. Mp 201–203 °C; IR(KBr) ν/cm^{-1}: 3411, 2937, 2855, 1638, 1446, 1401, 1384, 1352, 1286, 1061, 1041; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 1.06 (3H, s, 19-CH_3), 1.19 (3H, s, 18-CH_3), 2.40 (1H, dd, *J* = 10.2, 8.1, C_7-H), 2.50 (1H, dd, *J* = 18.3, 6.6, C_{16}-H), 4.21 (2H, m, C_3-H, C_6-H), 5.71 (1H, s, C_4-H), 5.92 (1H, s, –NH); ^{13}C ($CDCl_3$, 75 MHz): 171.6 (C-17), 147.9 (C-5), 120.4 (C-4), 68.2 (C-6), 67.6 (C-3), 54.3 (C-13), 53.6 (C-14), 47.1 (C-7), 40.6 (C-10), 39.9 (C-1), 37.8 (C-12), 35.8 (C-16), 34.8 (C-2), 30.5 (C-8), 28.9 (C-18), 22.1 (C-15), 20.0 (C-11), 19.7 (C-19); ESI-MS *m*/*z*: 320 (M+1)$^+$.
17. *General procedure for preparation of compounds* **8** *and* **11**. Compound **6** or **10** (1 mmol) was dissolved in 40 mL 95% CH_3CH_2OH. After the mixture was heated to 60 °C, $CH_3COONa{\cdot}3H_2O$ (1.2 mmol) and $NH_2OH{\cdot}HCl$ (1.3 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 extracted with ethyl acetate. The combined extract was washed with saturated brine, dried with anhydrous sodium sulfate, and evaporated under reduce pressure. The residue was subject to chromatography (methanol/dichromethane (1:30)) to produce **8** or **11**. Compound **8**, yield: 80%. Mp 220–222 °C; IR(KBr) ν/cm^{-1}: 3407, 2925, 2847, 1634, 1446, 1401, 1352, 1220, 1286, 1061, 963; ^{1}H NMR (CD_3OD, 300 MHz) δ: 1.01 (3H, s, 19-CH_3), 1.21 (3H, s, 18-CH_3), 2.38 (1H, dd, *J* = 9.0, 8.4, C_7-αH), 2.44 (1H, dd, *J* = 18.6, 6.0, C_{16}-H), 3.09 (1H, ddd, *J* = 17.5, 4.8, 1.8, C_2-βH), 3.59 (1H, dd, *J* = 15.6, 4.8, C_7-βH), 6.32 (1H, s, C_4-H), 6.52 (1H, s, –NH), 6.81 (1H, s, 6-NOH), 6.88 (1H, s, 3-NOH); ^{13}C (CD_3OD, 75 MHz): 172.1 (C-17), 155.6 (C-6), 155.4 (C-3), 145.1 (C-5), 118.5 (C-4), 53.8 (C-9), 51.4 (C-13), 39.1 (C-1), 38.9 (C-10), 36.1 (C-12), 34.4 (C-16), 32.2 (C-2), 31.4 (C-8), 22.1 (C-18), 20.9 (C-15), 19.9 (C-11), 17.8 (C-19); ESI-MS *m*/*z*: 346 (M+1)$^+$.
18. *Procedure for the preparation of compound* **9**. A mixture of compound **6** (1 mmol), thiosemicarbazide (2 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 327 mg of compound **9** as a yellow solid. Yield: 71%. Mp 230–231 °C; IR(KBr) ν/cm^{-1}: 3452, 3338, 2941, 2859, 1638, 1580, 1462, 1350, 1221, 1150, 1071, 956; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 0.92 (3H, s, 19-CH_3), 1.07 (3H, s, 18-CH_3), 2.86 (1H, dd, *J* = 18.0, 2.4, C_2-βH), 3.11 (1H, dd, *J* = 17.0, 4.5, C_7-βH), 6.65 (1H, s, C_4-H), 7.57(1H, s, –NH), 7.61 (1H, br s, –(C=S)–NH), 7.76 (1H, br s, –(C=S)–NH), 8.25 (1H, br s, –(C=S)–NH), 8.34 (1H, br s, –(C=S)–NH), 10.32 (1H, br s, –N–NH–C), 10.67 (1H, br s, –N–NH–C); ^{13}C (CD_3OD, 75 MHz): 179.3 (–C=S), 178.9 (–C=S), 170.3 (C-17), 149.6 (C-6), 148.4 (C-3), 148.3 (C-5), 123.4 (C-4), 53.8 (C-9), 48.8 (C-13), 47.9 (C-14), 37.4 (C-1), 33.4 (C-16), 32.6 (C-2), 31.6 (C-8), 21.2 (C-18), 21.1 (C-15), 19.8 (C-11), 18.2 (C-19); ESI-MS *m*/*z*: 462 (M+1)$^+$.