Steroids 112 (2016) 74–80

Contents lists available at ScienceDirect

Steroids

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

Synthesis and biological evaluation of 3β-androsta-5,8(14),15-trien-17-one derivatives as potential anticancer agents

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ARTICLE INFO

Article history:
Received 10 January 2016
Received in revised form 14 April 2016
Accepted 10 May 2016
Available online 15 May 2016

Keywords:
3β-Hydroxy-androsta-5,8(14),15-trien-17-one
Dehydroepiandrosterone
Anticancer agent
Suzuki coupling reaction
α,β-Unsaturated ketone

ABSTRACT

A novel and operationally simple method for highly efficient synthesis of promising anti-cancer 3β-hydroxy-16-arylandrosta-5,8(14),15-trien-17-ones was reported. Compounds were tested for their cytotoxic activities against A549, SKOV3, MKN-45 and MDA-MB-435 cancer cell lines. The preliminary results showed that compounds **5e**, **g** were the most active especially against cancer cell lines tested.

1. Introduction

Steroids and their derivatives have been found to possess the potential to be developed as drugs for the treatment of a large number of diseases [1–3]. Among them, some modified steroids have been extensively studied as their biological and clinical importance is now well validated. And most recently abiraterone acetate has been approved for use as anticancer drugs by the US FDA [4]. However because of the drug resistance and drug tolerance problems, the development of new compounds to improve the selectivity and to minimize side effects of steroidal drugs has been a challenge for a long time [5]. For many years, the modification of 3-hydroxysteroids has attracted considerable attention from medicinal and synthetic organic chemists. Various derivatives of steroids by modified at D ring with anticancer activity and cytotoxic activity have been reported [6–17]. Chemical modification of the steroid D-ring provides a way to alter biologically important properties of modified steroids. As an example on the modification of steroidal D ring, 3α,5α-17-phenylandrost- 16-en-3-ol is a neurosteroid antagonist [18]. 3α,5α-17-Phenylandrost-16-en-3-ol antagonizes selectively the GABA-modulatory and GABA-mimetic effects of 3α-hydroxy-5α-pregnan-20-one (allopregnanolone, 3α,5α-THPROG) and related 5α-pregnane steroids [18,19]. In view of the remarkable importance from pharmacological and synthetic point, the development of new modified D-ring steroids promising biological activity by new synthetic approaches using mild reaction conditions remains an active research area. Many previous studies proved that some steroidal compounds with α,β-unsaturated ketone core gave the potency against human cancer cell lines [20–24]. Recently, König and co-authors reported that three new steroids with extended conjugated system via $\Delta^{4,5}$, $\Delta^{6,7}$ and $\Delta^{8,14}$ were isolated from the marine sponge *Callyspongia* cf. *C. flammea*, which were found capable of preventing the enhanced production of amyloid β-42 in Aftin-5 treated cells as candidates for the treatment of neurodegenerative Alzheimer's disease [25]. We envisioned that the combination of 16-arylandroster-17-one with extended conjugated system via $\Delta^{8,14}$ and $\Delta^{15,16}$ moieties should also have cytotoxic activity. This encouraged us to further explore the structural motif responsible for the biological properties of 16-aryl multi doublet-bond androstenones. Thus in continuation of our program, we herein present the synthesis of 3β-hydroxy-androsta-5,8(14),15-trien-17-one derivatives and their biological evaluation for anticancer activity against SKOV3, A549, MKN-45, and MDA-MB-435 cell lines in vitro.

2. Experimental

2.1. General remarks

All melting points were determined in a SGW X-4 melting point apparatus and are uncorrected. IR spectra were recorded in a

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http://dx.doi.org/10.1016/j.steroids.2016.05.004
0039-128X/

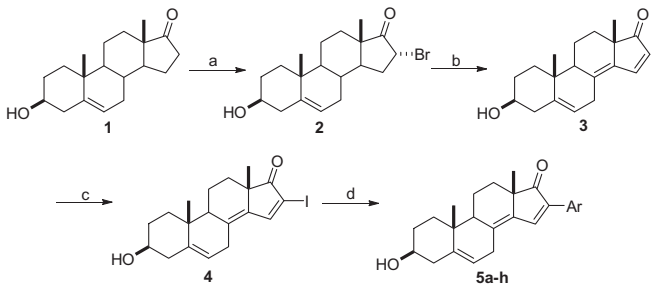
Nicolet FT-IR 5DX spectrometer. The ^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz) spectra were recorded in a Bruker AV-600 spectrometer with TMS as internal reference in DMSO-d_6 or $CDCl_3$ solutions. The *J* values are given in hertz. Only discrete or characteristic signals for the ^{1}H NMR are reported. The MS spectra were obtained on a ZAB-HS mass spectrometer with 70 eV. High-resolution ESI mass spectra were obtained on a UHR-TOF maXis (ESI) mass spectrometer. X-ray crystallographic analysis was performed with a SMART APEX-II diffractometer. The elemental analyses were performed in a Perkin-Elmer 240C instrument. Flash chromatography was performed on silica gel (230–400 mesh) eluting with ethyl acetate-hexanes mixture. All reactions were monitored by thin layer chromatography (TLC). All reagents and solvents were purchased from commercial sources and purified commonly before used.

2.2. Preparation of 16α-bromo-3β-hydroxyandrost-5-en-17-one (**2**) (Scheme 1)

The mixture of dehydroepiandrosterone (**1**) (2.88 g, 0.01 mol) and copper bromide (5.6 g, 0.025 mol) in methanol (30 mL) was stirred under reflux for 8 h, and the completion of reaction was confirmed by TLC (Hexanes/EtOAc, 1:1). After removal of methanol by reduced pressure the residues was added with water (20 mL) and was extracted with dichloromethane (50 mL × 2). The organic phase was washed with water (20 mL) and brine (15 mL), and dried over anhydrate sodium sulfate. After removal of dichloromethane, the crude product was purified via recrystallization with PE/AcOEt to afford a white solid product (**2**) (3.5 g, 82%), mp 178–179 °C (PE/AcOEt). IR (KBr, cm^{-1}): 3287, 2936, 2859, 1749, 1454, 1375, 1195, 1133, 1103, 910, 884, 608; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 5.42 (d, 1H, *J* = 5.4 Hz, 6-H), 4.56 (t, 1H, *J* = 5.4 Hz, 16-H), 3.55–3.52 (m, 1H, 3α-H), 3.45–3.20 (m, 2H, 4-H), 2.25–2.22 (m, 2H, 7-H), 1.04 (s, 3H, C18-Me), 0.93 (s, 3H, C19-Me); MS(EI) (*m*/*z*): 366.07 [M$^+$] (82.0%), 368.10 [(M+2)$^+$] (100%); Anal. Calcd for $C_{19}H_{27}BrO_2$: C, 62.13; H, 7.41; Found: C, 62.02; H, 7.33.

2.3. Preparation of 3β-hydroxyandrosta-5,8(14),15-trien-17-one (**3**)

The mixture of 16α-bromo-3β-hydroxyandrost-5-en-17-one (3.67 g, 0.01 mol), LiBr·H_2O (3.67 g, 0.035 mol) and Li_2CO_3 (2.96 g, 0.04 mol) in DMF (30 mL) was refluxed under nitrogen for 8 h, and the completion of reaction was confirmed by TLC (Hexanes/EtOAc, 1:1). After the mixture was cooled to room temperature, to the mixture was added ice-water (15 g). A yellow solid was filtered and dissolved with dichloromethane (20 mL). The solution was washed with water (20 mL) and brine (15 mL), and dried over anhydrate sodium sulfate. After removal of dichloromethane, the crude product was purified by flash chromatography (silica gel, DCM:EA = 20:1) to give the desirable product (0.88 g, 31%) as a white solid. Mp 194–195 °C (PE/AcOEt); IR (KBr, cm^{-1}): 3312, 2930, 2858, 1709, 1643, 1519, 1453, 1219, 1108, 1009, 940, 865, 700; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 7.81 (d, *J* = 5.7 Hz, 1H), 5.94 (d, *J* = 5.7 Hz, 1H), 5.28 (s, 1H), 3.53–3.51 (m, 1H), 3.47–3.22 (m, 2H, 4-H), 2.25–2.23 (m, 2H, 7-H), 1.12 (s, 3H), 0.95 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 212.2, 152.69, 141.65, 139.34, 133.78, 128.06, 118.87, 70.98, 48.28, 45.75, 41.59, 38.87, 36.34, 31.50, 28.97, 27.63, 22.95, 19.50, 18.82; MS(EI) (*m*/*z*): 285.43 [(M+H)$^+$] (100%); Anal. Calcd for $C_{19}H_{24}O_2$: C, 80.24; H, 8.51; Found: C, 80.19; H, 8.42.



Scheme 1. Preparation of target compounds **5a**–**h**. Reagents and conditions: (a) $CuBr_2$, CH_3OH, reflux, 8 h, 82%; (b) LiBr, Li_2CO_3, DMF, reflux, 31%; (c) I_2, DMAP, pyridine, CCl_4, 0 °C, 80%; (d) $Pd(PPh_3)_2Cl_2$/CuI, $ArB(OH)_2$, 2 mol/L Na_2CO_3, THF, CH_3OH, reflux, 70–91%.

2.4. Preparation of 3β-hydroxy-16-iodoandrosta-5,8(14),15-trien-17-one (**4**)

The mixture of 3β-hydroxyandrosta-5,8(14),15-trien-17-one (0.50 g, 1.74 mmol), iodine (0.89 g, 3.48 mmol), DMAP (0.01 g, 0.087 mmol) and pyridine (6 mL, 0.07 mmol) in CCl_4 (15 mL) was stirred at 0 °C for 12 h. After the completion of reaction was confirmed by TLC (Hexanes/EtOAc, 1:1), the solvent was removed by reduced pressure. The residues were extracted with dichloromethane (20 mL) and the organic phase was washed with saturated sodium thiosulfate (2 × 10 mL), 20% HCl (10 mL) and brine (10 mL), and dried over anhydrate sodium sulfate. After removal of dichloromethane, the crude product was purified by flash chromatography (silica gel, DCM:EA = 20:1) to give the desirable product (0.88 g, 80%) as a light brown solid. Mp 135–136 °C (PE/AcOEt); IR(KBr): 3468, 2929, 2858, 1700, 1657, 1513, 1450, 1370, 1276, 1211, 1062, 1004, 937, 918, 846, 734; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 8.16 (s, 1H), 5.28 (s, 1H), 3.68–3.39 (m, 1H), 1.15 (s, 3H), 0.94 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 204.62, 158.00, 140.59, 138.94, 133.13, 117.62, 97.47, 69.95, 47.06, 43.82, 40.52, 37.88, 35.24, 30.46, 28.06, 26.81, 22.11, 18.52, 17.70; HRMS (ESI) calcd for $C_{19}H_{23}IO_2$ [M+H]$^+$ 412.0822; Found 411.0818; Anal. Calcd for $C_{19}H_{23}IO_2$: C, 55.62; H, 5.65; Found: C, 55.48; H, 5.60.

2.5. General procedure for the preparation of 16-aryl-3β-hydroxyandrosta-5,8(14),15-trien-17-ones (**5a**–**h**)

To the mixture of 3β-hydroxy-16-iodoandrosta-5,8(14),15-trien-17-one (0.50 g, 1.2 mmol), arylboric acid (1.82 mmol), $Pd(PPh_3)_2Cl_2$ (17 mg, 0.024 mmol), CuI (2.4 mg, 0.024 mmol) in THF (20 mL) and methanol (4 mL) was added 2 mol/L sodium carbonate solution (4.8 mmol, 2.4 mL). The resultant mixture was stirred under reflux for 10 h. After the completion of reaction was confirmed by TLC (Hexanes/EtOAc, 1:2), the solvent was removed by reduced pressure. The residues were extracted with dichloromethane (20 mL) and the organic phase was washed with water (2 × 10 mL) and brine (10 mL), and dried over anhydrate sodium sulfate. After removal of dichloromethane, the crude product was purified by flash chromatography (silica gel, DCM:EA = 10:1) to give the desirable product **5a**–**h**.

2.5.1. 3β-Hydroxy-16-phenylandrosta-5,8(14),15-trien-17-one (**5a**)

Light yellow solid, yield 91%, mp: 175–176 °C (PE/AcOEt); IR (KBr, cm^{-1}): 3462, 2929, 2856, 1684, 1450,1339, 1292, 1176, 1059, 927, 795, 750, 654; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 7.96 (s, 1H), 7.77 (d, *J* = 7.7 Hz, 2H), 7.31 (dd, *J* = 7.6 Hz and 7.2 Hz, 2H), 7.24 (dd, *J* = 7.3 Hz and 7.2 Hz, 1H), 5.31 (s, 1H), 3.53 (m, 1H), 1.19 (s, 3H), 0.96 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 209.53, 146.57, 141.62, 137.77, 136.78, 133.70, 132.22, 128.49, 128.29, 127.12, 119.04, 71.05, 48.53, 47.50, 41.61, 38.98, 36.33, 31.52, 29.11, 27.87, 23.36, 19.58, 18.87; HRMS (ESI) calcd for $C_{25}H_{28}O_2$ [M+H]$^+$ 361.2168; Found 361.2164; Anal. Calcd for $C_{25}H_{28}O_2$: C, 83.29; H, 7.83; Found: C, 83.16; H, 7.89.

*2.5.2. 3β-Hydroxy-16-(pyridin-3-yl)androsta-5,8(14),15-trien-17-one (**5b**)*

Light yellow solid, yield 85%, mp: 167–168 °C (PE/AcOEt); IR (KBr, cm^{-1}): 3472, 2928, 2857, 1697, 1647, 1458, 1415, 1376, 1299, 1066, 933, 808; ^1H NMR (600 MHz, $CDCl_3$) δ (ppm): 8.91 (s, 1H), 8.46 (d, J = 4.4 Hz, 1H), 8.17 (d, J = 7.9 Hz, 1H), 8.06 (s, 1H), 7.28–7.23 (m, 1H), 5.32 (s, 1H), 3.58–3.51 (m, 1H), 1.98 (s, 1H), 1.20 (s, 3H), 0.98 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 208.10, 147.92, 147.00, 146.13, 140.75, 136.75, 134.59, 133.43, 132.83, 122.32, 119.83, 117.80, 69.99, 47.61, 46.25, 40.57, 38.05, 35.31, 30.49, 29.90, 26.80, 22.26, 18.57, 17.80; HRMS (ESI) calcd for $C_{24}H_{27}NO_2$ $[M+H]^+$ 362.2121; Found 362.2112; Anal. Calcd for $C_{24}H_{27}NO_2$: C, 79.74; H, 7.53; N, 3.87; Found: C, 79.78; H, 7.44; N, 3.82.

*2.5.3. 16-(2-Fluorophenyl)-3β-hydroxyandrosta-5,8(14),15-trien-17-one (**5c**)*

Light yellow solid, yield 86%, mp: 164–165 °C (PE/AcOEt); IR (KBr, cm^{-1}): 3491, 3043, 2930, 2859, 1691, 1562, 1480, 1449, 1274, 1213, 1110, 1055, 930, 875, 800, 758, 722; ^1H NMR (600 MHz, $CDCl_3$) δ (ppm): 8.17 (s, 1H), 8.01 (dd, J = 7.2 Hz and 7.2 Hz, 1H), 7.22–7.17 (m, 2H), 7.10 (dd, J = 7.5 Hz and 6.1 Hz, 1H), 5.31 (s, 1H), 3.55–3.52 (m, 1H), 1.20 (s, 3H), 0.97 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 209.43, 159.53 (d, J = 249.6 Hz), 149.42 (d, J = 10.2 Hz), 140.49, 137.02, 133.70, 130.16, 129.91, 128.32 (d, J = 8.5 Hz), 123.03 (d, J = 3.3 Hz), 119.13 (d, J = 12.3 Hz), 118.02, 114.62 (d, J = 22.3 Hz), 70.02, 47.58, 45.45, 40.58, 37.98, 35.32, 30.49, 28.05, 26.82, 22.31, 18.54, 17.84; HRMS (ESI) calcd for $C_{25}H_{27}FO_2$ $[M+H]^+$ 379.2074; Found 379.2074; Anal. Calcd for $C_{25}H_{27}FO_2$: C, 79.34; H, 7.19; Found: C, 79.32; H, 7.22.

*2.5.4. 16-(3-Fluorophenyl)-3β-hydroxyandrosta-5,8(14),15-trien-17-one (**5d**)*

Light yellow solid, yield 84%, mp: 190–191 °C (PE/AcOEt); IR (KBr, cm^{-1}): 3500, 3075, 2928, 2857, 1679, 1577, 1426, 1340, 1263, 1185, 1058, 886, 790, 681; ^1H NMR (600 MHz, $CDCl_3$) δ (ppm): 7.97 (s, 1H), 7.55 (dd, J = 7.7 and 7.2 Hz, 2H), 7.27 (dd, J = 7.3 and 7.8 Hz, 1H), 6.93 (dd, J = 7.6 and 7.6 Hz, 1H), 5.32 (s, 1H), 3.55–3.52 (m, 1H), 1.19 (s, 3H), 0.97 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 207.90, 161.88 (d, J = 245.6 Hz), 146.03, 140.69, 136.62, 134.57 (d, J = 2.1 Hz), 133.75, 133.32, 128.89 (d, J = 8.1 Hz), 121.69 (d, J = 2.8 Hz), 117.90, 114.04 (d, J = 21.3 Hz), 112.96 (d, J = 22.8 Hz), 70.05, 47.64, 46.57, 40.63, 38.02, 35.35, 30.56, 28.13, 26.88, 22.32, 18.56, 17.84; HRMS (ESI) calcd for $C_{25}H_{27}FO_2$ $[M+H]^+$ 379.2074; Found 379.2074; Anal. Calcd for $C_{25}H_{27}FO_2$: C, 79.34; H, 7.19; Found: C, 79.28; H, 7.20.

*2.5.5. 3β-Hydroxy-16-(6-fluoropyridin-3-yl)androsta-5,8(14),15-trien-17-one (**5e**)*

Light yellow solid, yield 90%, mp: 180–181 °C (PE/AcOEt); IR (KBr, cm^{-1}): 3497, 2696, 2930, 2851, 2818, 1680, 1587, 1472, 1376, 1297, 1176, 1120, 1069, 1022, 928, 834, 744, 670; ^1H NMR (600 MHz, $CDCl_3$) δ (ppm): 8.56 (s, 1H), 8.26 (dd, J = 7.2 and 7.2 Hz, 1H), 8.02 (s, 1H), 6.89 (d, J = 6.6 Hz, 1H), 5.32 (s, 1H), 3.55–3.52 (m, 1H), 1.20 (s, 3H), 0.98 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 207.92, 162.11 (d, J = 238.5 Hz), 145.84, 145.04 (d, J = 14.7 Hz), 140.67, 138.64 (d, J = 8.0 Hz), 136.54, 134.65, 131.76 (d, J = 2.9 Hz), 125.34, 117.74, 108.24 (d, J = 37.3 Hz), 70.00, 47.61, 46.19, 40.57, 38.05, 35.31, 30.49, 28.17, 26.78, 22.25, 18.56, 17.79; HRMS (ESI) calcd for $C_{24}H_{26}FNO_2$ $[M+H]^+$ 380.2027; Found 380.2012; Anal. Calcd for $C_{24}H_{26}FNO_2$: C, 75.96; H, 6.91; N, 3.69; Found: C, 76.08; H, 6.82; N, 3.76.

*2.5.6. 3β-Hydroxy-16-(pyrimidin-5-yl)androsta-5,8(14),15-trien-17-one (**5f**)*

Light yellow solid, yield 87%, mp: 203–204 °C (PE/AcOEt); IR (KBr, cm^{-1}): 3487, 3034, 2940, 2861, 2799, 1701, 1644, 1558, 1406, 1299, 1144, 1009, 932, 721; ^1H NMR (600 MHz, $CDCl_3$) δ (ppm): 9.11 (s, 2H), 9.07 (s, 1H), 8.13 (s, 1H), 5.32 (s, 1H), 3.59–3.50 (m, 1H), 1.21 (s, 3H), 0.98 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 207.56, 156.63, 153.76, 146.98, 140.77, 136.67, 136.43, 129.91, 125.53, 117.52, 69.89, 47.69, 46.10, 40.54, 38.16, 35.32, 30.46, 28.26, 26.73, 22.19, 18.60, 17.75; HRMS (ESI) calcd for $C_{23}H_{26}N_2O_2$ $[M+H]^+$ 363.2073; Found 363.2068; Anal. Calcd for $C_{23}H_{26}N_2O_2$: C, 76.21; H, 7.23; N, 7.73; Found: C, 76.14; H, 7.20; N, 7.62.

*2.5.7. 16-(3-Trifluoromethylphenyl)-3β-hydroxyandrosta-5,8(14),15-trien-17-one (**5g**)*

Light yellow solid, yield 93%, mp: 195–196 °C (PE/AcOEt); IR (KBr, cm^{-1}): 3489, 3032, 2939, 2894, 2827, 1680, 1587, 1475, 1419, 1322, 1123, 1006, 805; ^1H NMR (600 MHz, $CDCl_3$) δ (ppm): 8.04 (s, 1H), 8.02 (s, 1H), 7.99 (d, J = 7.8 Hz, 1H), 7.49 (d, J = 7.7 Hz, 1H), 7.43 (dd, J = 7.2 and 7.8 Hz, 1H), 5.32 (s, 1H), 3.56–3.52 (m, 1H), 1.20 (s, 3H), 0.98 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 207.94, 146.41, 140.64, 136.56, 134.31 (d, J = 8.5 Hz), 131.99, 129.87 (q, J = 32.6 Hz), 129.28, 127.91, 123.98, 123.69 (q, J = 3.7 Hz), 123.07 (q, J = 271.5 Hz), 122.74 (q, J = 3.5 Hz), 117.83, 70.02, 47.63, 46.50, 40.58, 38.03, 35.31, 30.50, 28.17, 26.82, 22.28, 18.56, 17.81; HRMS (ESI) calcd for $C_{26}H_{27}F_3O_2$ $[M+H]^+$ 429.2042; Found 429.2039; Anal. Calcd for $C_{26}H_{27}F_3O_2$: C, 72.88; H, 6.35; Found: C, 72.72; H, 6.32.

*2.5.8. 3β-Hydroxy-16-(5-methoxypyridin-3-yl)androsta-5,8(14),15-trien-17-one (**5h**)*

Dark yellow solid, yield 74%, mp: 193–194 °C (PE/AcOEt); IR (KBr, cm^{-1}): 3488, 2930, 2872, 1686, 1610, 1521, 1450, 1370, 1296, 1119, 1008, 940; ^1H NMR (600 MHz, $CDCl_3$) δ (ppm): 8.49 (s, 1H), 8.16 (s, 1H), 8.07 (s, 1H), 7.79 (s, 1H), 5.32 (s, 1H), 3.82 (s, 3H), 3.57–3.50 (m, 1H), 1.20 (s, 3H), 0.97 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 208.27, 154.50, 146.50, 140.67, 139.05, 136.60, 136.17, 134.84, 132.37, 127.83, 117.76, 117.45, 69.95, 54.57, 47.64, 46.32, 40.57, 38.07, 35.32, 30.48, 28.19, 26.80, 22.26, 18.57, 17.80; HRMS (ESI) calcd for $C_{25}H_{29}NO_3$ $[M+H]^+$ 392.2226; Found 392.2229; Anal. Calcd for $C_{25}H_{29}NO_3$: C, 76.70; H, 7.47; N, 3.58; Found: C, 76.62; H, 7.42; N, 3.52.

2.6. Biology

2.6.1. Cell lines and culture conditions

Human lung carcinoma cell line A549, human ovarian carcinoma cell line SKOV3, human gastric adenocarcinoma cell line MKN-45 and human breast carcinoma cell line MDA-MB-435 used in this work, were purchased from Shanghai Institute of Cell Biology, Chinese Academy of Science. The cell lines were grown in RPMI 1640 medium with 10% newborn calf serum. It was maintained in a humidified incubator with an atmosphere of 95% air and 5% CO_2 at 37 °C. The cells were continuously passaged once every 3–4 days. Growing cells were collected on experiments.

2.6.2. Cell viability assay (SRB)

The anticancer activity in vitro was measured using the SRB assay. The assay was carried out according to previous study. DMSO was used as latent solvent with the highest concentration less than 0.1% in solution of the drug. The control groups of blank (1640) and DMSO solvent were set up at the same time. Proliferative activity was evaluated by colorimetric sulforhodamine B (SRB) assay. Briefly, cells were plated in 96-well plates. After cell adhering, they were treated with different compounds in a

dose-dependent way for 44 h. Then the cells were fixed by 10% TDA for 1 h and stained by SRB for 10 min. After washed with acetic acid to remove the excess dye, protein bounding dye were dissolved in 10 mM Tris and detected by a Model Elx 800 Autoplate reader (Bio-Tek Instruments, U.S.A).

2.6.3. Data analysis

Cell survival was calculated using the formula: Survival (%) = [(absorbance of treated cells – absorbance of culture medium)/(absorbance of untreated cells – absorbance of culture medium)] × 100. The experiment was done in eight replicates and the inhibitory concentration (IC) values were calculated from a dose response curve. IC_{50} is the concentration in μM required for 50% inhibition of cell growth as compared to that of untreated control. IC_{50} values were determined from the linear portion of the curve by calculating the concentration of agent that reduced absorbance in treated cells, compared to control cells, by 50%. Evaluation is based on mean values from three independent experiments, each comprising at least six microcultures per concentration level. All the data of the experiment were compiled and analyzed according to SPSS 15.0 software. Measurement data were expressed as the mean ± S.D.

3. Results and discussion

D-ring modified steroids have attracted considerable attentions from synthetic organic chemists and pharmaceuticals researchers. Modifications at the D-ring of steroids are considerable important and as per the accepted trend as such alterations result in effective receptor binding or the increased bioavailability. In keeping with our continuing interest for the modification of natural steroids as effective cytotoxic agents, herein we report an efficient and simple synthesis of 3β-hydroxy-16-arylandrosta-5,8(14),15-trien-17-ones. The synthetic strategies are discussed below.

3.1. 3β-Hydroxy-16-arylandrosta-5,8(14),15-trien-17-one

In this part, we design to synthesize a series of 3β-hydroxy-16-arylandrosta-5,8(14),15-trien-17-ones with dehydroepiandrosterone (**1**) as the starting material according to reported method [26] (Scheme 1). Compound **2** was synthesized with the standard procedure in our laboratory, by treating **1** with copper bromide in methanol under reflux with a good yield. Compound **3** was prepared by treating compound **2** with lithium bromide and lithium carbonate in DMF and separated by column chromatography. Yield was not very well which is only 31%. The molecular structure of compound **3** was elucidated from its spectroscopic analyses as described herein for compound **3**. In the IR spectrum of compound **3**, one sharp absorption band at 1709 cm^{-1}, three bands at 1643, 1519 and 1453 cm^{-1}, could be related to *CO* and *C*═*C* stretching frequencies. The mass spectrum of compound **3** displayed the molecular ion peak at m/z = 285.43 $(M+1)^+$, which is in good agreement with the proposed structure. The ^1H NMR spectrum of compound **3** exhibited two doublet signals at 7.81 (d, J = 5.7 Hz, 1H), 5.94 (d, J = 5.7 Hz, 1H) for C16-*H* and C15-*H* of the double bond, respectively, one singlet signal at 5.28 (s, 1H) and one multipeak at 3.53–3.51 (m, 1H) for C6-*H* and C3α-*H* respectively. Characteristic ^1H chemical shift of C16-*H*, C15-*H*, C6-*H* and C3α-*H* unequivocally indicated the exclusive chemical environment of compound **3** protons. The ^1H-decoupled ^{13}C NMR spectrum of compound **3** showed 19 distinct signals in agreement with the suggested structure. The important peaks were related to the *C*O and three *C*═*C* double bonds which appeared at δ = 212.20, 152.69, 141.65, 139.34, 133.78, 128.06, and 118.87 ppm.

Compound **4** was synthesized by treating compound **3** with the mixture of I_2, DMAP and pyridine in CCl_4 in 80% yield. IR spectrum of compound **4**, one sharp absorption band at 1700 cm^{-1}, three bands at 1657, 1513 and 1450 cm^{-1}, could be related to *CO* and *C*═*C* stretching frequencies. The high resolution mass spectrum of compound **4** displayed the molecular ion peak at m/z = 411.0818 $(M+1)^+$, which is in good agreement with the proposed structure. The ^1H NMR spectrum of compound **4** showed two singlet signals at 8.16 (s, 1H), 5.28 (s, 1H) for C15-*H* and C6-*H* of the double bond, respectively, the characteristic ^1H chemical shift of C15-*H* unequivocally indicated the proton of C16 was replaced by iodine. The important peaks of ^{13}C NMR were related to the *C*O and three *C*═*C* double bonds which appeared at δ = 204.62, 158.00, 140.59, 138.94, 133.13, 117.62, and 97.47 ppm.

α-Iodo-α,β-unsaturated carbonyl compounds served as an activated iodoolefin having a great potential for the construction of Csp^2–Csp^2 by Suzuki coupling reaction. The same was done for the preparation of 16-aryl-3β-hydroxyandrosta-5,8(14),15-trien-17-ones (Table 1, **5a**–**h**) by employing the Suzuki coupling reaction of intermediate **4** with appropriately substituted aromatic boric acids in presence of the catalyst system: $Pd(PPh_3)_2Cl_2/CuI/Na_2CO_3$. The analytical and spectral data of compounds **5a**–**h** were in agreement with their structures. IR spectrum of compound **5a**–**h**, one sharp absorption band at 1680–1700 cm^{-1} could be related to *CO* stretching frequency. The ^1H NMR spectrum of the 16-aryl-3β-hydroxyandrosta-5,8(14),15-trien-17-ones in $CDCl_3$ shows the characteristic signals of the androsta-5,8(14),15-triene nucleus. The multiplet of 3α-H is to be found at 3.60–3.50 ppm. Furthermore, two olefinic signals are observed at 5.30 and 8.10 ppm, respectively, and can be assigned to C5-*H* and C15-*H*. However, the chemical shift of the C5-*H* and C15-*H* singlets differs significantly in two olefinic signals. This indicates that C5-*H* has a normal chemical shift as an olefinic proton. The chemical shift of the C15-*H* singlet moves to low field because it conjugates with a C═C double bond, a carbonyl and an aromatic ring. The corresponding C16-aromatic ring shows low-field ^1H NMR signals from 6.80 to 9.11 ppm. The important peaks of ^{13}C NMR were related to the *C*═*O* and HO-*C*H which appeared at δ = ca 208 and 70 ppm, respectively. Three *C*═*C* double bonds appeared at δ = ca 146, 141, 137, 135, 125, and 117 ppm. Taking compound **5e** as an example, in the ^{13}C NMR spectrum compound **5e** displayed signals of the carbonyl group, hydroxyalkyl group and three double bonds at 207.92 (O═C17), 70.00 (HO-C3), 145.84 (C15), 140.67 (C14), 136.54 (C8), 134.65 (C5), 125.34 (16), and 117.74 (C6). Because the signals of carbon atoms are spilt by coupling to ^{19}F, the corresponding C16-aromatic ring shows five distinctive doublets of aromatic ring carbons at 162.11 (d, $^1J_{CF}$ = 238.5 Hz), 145.04 (d, $^3J_{CF}$ = 14.7 Hz), 138.64 (d, $^3J_{CF}$ = 8.0 Hz), 131.76 (d, $^4J_{CF}$ = 2.9 Hz), 108.24 (d, $^2J_{CF}$ = 37.3 Hz) ppm. The structures of the compounds were additionally confirmed by X-ray crystal data. We obtained two X-ray crystal structures of **5a** and **5b** that were presented in Figs. 1 and 2. Crystallographic data for **5a** and **5b** have been deposited with the Cambridge Crystallographic Data Centre with the deposition number CCDC 935129 (**5a**), 937201(**5b**). These data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax (+44) 1223 336033, e-mail: deposit@ccdc.cam.ac.uk].

Both molecules consist of four six-membered rings and one five-membered ring. The absolute configurations of both compound **5a** and **5b** are C3S, C9S, C10R, and C13S, which is inferred from the known stereochemistry of dehydroepiandrosterone (3β-hydroxyandrost-5-en-17-one). The A, B, and C rings for both molecules **5a** and **5b** are chair, twist-half-chair, and twist-half-chair conformations. However, the chair conformation of A ring is slightly disturbed by the presence of exocyclic double

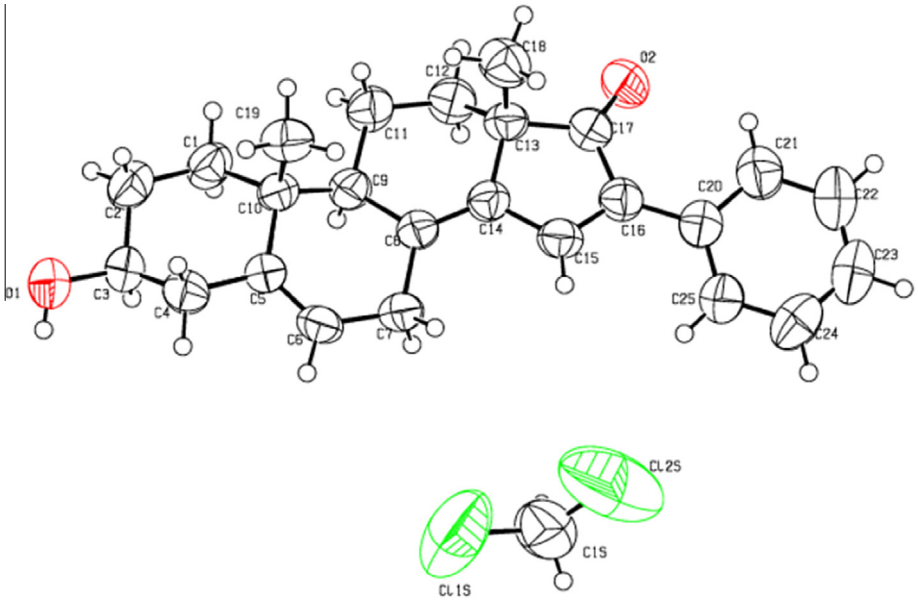


Fig. 1. Molecular structure of compound **5a** (containing CH_2Cl_2).

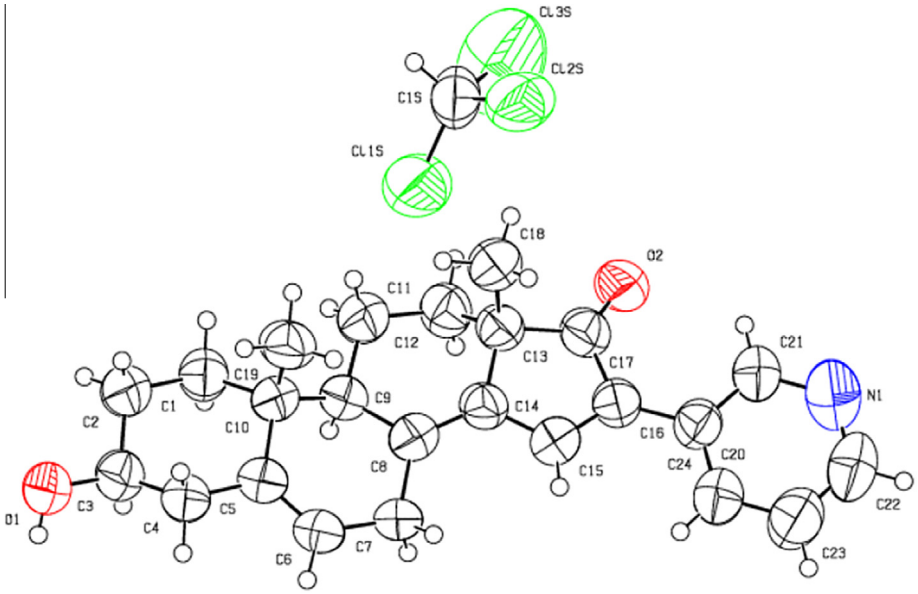


Fig. 2. Molecular structure of compound **5b** (containing $CHCl_3$).

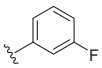
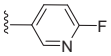
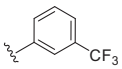
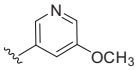
bond. The D ring and C16-aromatic ring for both molecules **5a** and **5b** are near-plane conformations. All of the bond lengths and angles are normal. In the A, B, and C rings of compound **5a** and **5b** the average magnitude of the Csp^3–Csp^3 distances 1.53 Å, the Csp^2–Csp^2 distances 1.33 Å, and the Csp^3–Csp^2 distances 1.50 Å (Table 2). It is worth noting that the average magnitude of C (15)–C(14)–C(13) angles 107.2°, the D ring with C16-aromatic ring is leaning at a low angle from the A, B, and C rings of the steroid structure.

3.2. Biology

α,β-Unsaturated ketones of natural and synthetic steroids are considered as resourceful molecules as for as their pharmacological activities are concerned [23]. In reference to those natural and synthetic steroids with α,β-unsaturated ketone core, we in our earlier communications [27] have shown their therapeutic potential. As we are interested in thorough pharmacological studies of various steroidal analogs, we examined the anticancer potential of 3β-hydroxy-16-arylandrosta-5,8(14),15-trien-17-ones and the results are described in Table 1.

The newly synthesized compounds **5a**–**h** and **3** were evaluated for their anticancer activity towards human tumor cell lines derived from various human cancer types: A549 (human lung carcinoma), SKOV3 (human ovarian carcinoma), MKN-45 (human gastric adenocarcinoma), MDA-MB-435 (human breast carcinoma) cell lines. The results are summarized as IC_{50} values in l mol/L in Table 1. From the data shown in Table 1, most of the newly synthesized compounds showed a measurable anti-cancer activity against A549 (human lung carcinoma), SKOV3 (human ovarian carcinoma), MKN-45 (human gastric adenocarcinoma), MDA-MB-435 (human breast carcinoma) cell lines tested. Although this is a

Table 1
Preparation and the in vitro cytotoxic activity (IC_{50}, in μmol L^{-1}) of **5a**–**h**.

Compd.	–Ar	Yield%[a]	Cancer cell lines[b]			
			A549	*SKOV3*	*MKN-45*	*MDA-MB-435*
5a		91	32.1 ± 3.0	12.5 ± 1.8	70.2 ± 2.2	20.2 ± 1.6
5b		85	22.5 ± 1.6	10.3 ± 1.5	21.2 ± 0.6	18.2 ± 2.2
5c		86	14.3 ± 2.6	9.6 ± 1.3	17.2 ± 1.6	12.6 ± 2.4
5d		84	12.1 ± 1.6	8.9 ± 1.2	15.3 ± 2.2	9.7 ± 2.6
5e		90	11.5 ± 2.0	7.7 ± 0.4	18.4 ± 1.2	8.8 ± 1.5
5f		87	20.2 ± 0.8	10.1 ± 1.0	21.6 ± 2.6	34.6 ± 1.9
5g		93	10.9 ± 1.8	7.5 ± 2.5	16.6 ± 3.2	8.3 ± 2.8
5h		74	20.9 ± 2.8	14.5 ± 1.5	23.6 ± 2.2	32.3 ± 1.6
3	H		42.1 ± 2.2	30.5 ± 2.0	80.2 ± 3.6	55.4 ± 2.8

[a] Isolated yields.
[b] The results are the average mean of eight replicate determinations ± SD; Used as reference *A549*: human lung carcinoma, *SKOV3*: human ovarian carcinoma, *MKN-45*: human gastric adenocarcinoma, *MDA-MB-435*: human breast carcinoma.

Table 2
Selected bond lengths [Å] and angles [°] for compounds **5a** and **5b**.

Bond	Distance (Å) of **5a**	Distance (Å) of **5b**
C(5)–C(6)	1.312(6)	1.322(9)
C(5)–C(4)	1.513(6)	1.487(8)
C(5)–C(10)	1.517(6)	1.534(8)
C(13)–C(14)	1.501(6)	1.486(8)
C(13)–C(12)	1.510(6)	1.537(10)
C(13)–C(17)	1.528(6)	1.533(8)
C(15)–C(16)	1.356(6)	1.344(8)
C(15)–C(14)	1.439(6)	1.414(9)
C(9)–C(8)	1.508(6)	1.543(9)
C(9)–C(10)	1.544(6)	1.534(8)
C(9)–C(11)	1.545(6)	1.543(9)
C(6)–C(7)	1.496(6)	1.469(9)
C(10)–C(1)	1.546(6)	1.547(9)
C(7)–C(8)	1.517(6)	1.509(8)
C(14)–C(8)	1.337(5)	1.337(8)
C(16)–C(20)	1.459(6)	1.462(9)
C(16)–C(17)	1.483(6)	1.503(9)
C(2)–C(1)	1.530(6)	1.539(9)
Angle	(°) of **5a**	(°) of **5b**
C(6)–C(5)–C(4)	120.6(4)	121.7(6)
C(6)–C(5)–C(10)	122.9(4)	121.0(5)
C(4)–C(5)–C(10)	116.5(3)	117.3(5)
C(16)–C(15)–C(14)	113.0(4)	114.5(6)
C(5)–C(6)–C(7)	125.8(4)	127.0(6)
C(6)–C(7)–C(8)	112.7(4)	112.4(5)
C(8)–C(14)–C(15)	128.9(4)	128.4(6)
C(8)–C(14)–C(13)	123.6(4)	124.0(6)
C(15)–C(14)–C(13)	107.2(4)	107.3(5)
C(15)–C(16)–C(17)	106.9(4)	106.0(6)
C(3)–C(4)–C(5)	112.1(4)	113.4(6)
C(14)–C(8)–C(9)	122.0(4)	121.6(6)
C(14)–C(8)–C(7)	123.0(4)	123.9(6)
C(9)–C(8)–C(7)	113.5(4)	113.1(5)
C(2)–C(3)–C(4)	109.7(4)	109.5(6)
C(12)–C(11)–C(9)	114.3(4)	114.0(5)
C(16)–C(17)–C(13)	107.9(4)	106.7(5)
C(3)–C(2)–C(1)	110.7(4)	110.0(5)

preliminary screening, compounds **5a**–**h** exhibited different levels of anticancer properties, the results showed all steroids tested showed a higher cytotoxicity against SKOV3 cells than against A549, MKN-45, and MDA-MB-435 cell lines. Compounds **5a**–**h** with same 3-hydroxyl structure and 5,8(14),15-trien-17-one motif, different types of substituted group of aromatic ring, showed a distinct difference in their cytotoxicity against these cancer cells. However, the compound **3** without an aromatic ring at C16 position decreased cytotoxicity. It was found that retaining the α,β-Unsaturated C17 carbonyl group in D-ring of androstene skeleton lead to reduction in growth inhibition as observed for compound of **5a**–**h** and **3** which was also in accordance with previous reports [10]. The reported results indicated that there were weak correlation between molecular function of the proteins and the puckering conformation of the steroid molecules. Both nuclear receptors and G-protein coupled receptors prefer to bind steroid molecules in the flat structure. Immunoglobulins also tend to bind steroid molecule in flat, but enzyme tends to prefer steroid molecules in half-chair [28]. The studies showed also clearly demonstrates that protein–steroid interactions are hydrophobic in both flat and half-chair steroid molecules. In both flat and half-chair cases, Asp, Glu, Arg and Lys were less represented in the interface of steroid molecule compared with the surface of proteins, and hydrophobic residues, especially aromatic residues, were highly used in the interface [28]. Thus, the near-plane conformations of D ring and C16-aromatic ring are helpful for the interface of steroid molecule compared with the surface of proteins. It was also evident from the data that the changes of substituents on the aromatic ring had a significant influence on the cytotoxicity. Compound **5c**, **d**, **e** and **g** with a fluoro-substituted group of aromatic ring had a better cytotoxicity than compounds **5a**, **b**, **f**, and **h** against SKOV3, A549, MKN-45, and MDA-MB-435 cell lines. The analogs **5a** with a phenyl remarkably decreased its cytotoxic activity against A549, SKOV3, MKN-45, and MDA-MB-435 cells in comparison with the analogs **5b**, **f**, and **h**, which have a heterocycle containing nitrogen. Compound **5e** containing a fluoro-substituted group of heterocycle containing nitrogen was found the best active to SKOV3, A549,

MKN-45, and MDA-MB-435 cells tested. On the other hand, substitution with a trifluoromethyl group as strong electron-withdrawing group of the phenyl ring (compound **5g**) resulted in a better anticancer activity. Comparison of the results obtained from Table 1 indicated that the fluoro groups and the heterocycle containing nitrogen were relatively beneficial for anticancer activity. Furthermore, it appears that an appropriately fluoro-substituted group of the heterocyclic ring containing nitrogen at C-16 could serve as a promising launch point for the further design of this type of steroidal anticancer agent.

4. Conclusion

In summary, we have successfully developed a novel and operationally simple method for highly efficient synthesis of promising anti-cancer 3β-hydroxy-16-arylandrosta-5,8(14),15-trien-17-ones. The preliminary results showed that those 3β-hydroxy-16-arylandrosta-5,8(14),15-trien-17-ones possessing an appropriately fluoro-substituted group of the heterocyclic ring containing nitrogen at C-16 have significant impact on inhibiting human lung carcinoma cell line A549, human ovarian carcinoma cell line SKOV3, human gastric adenocarcinoma cell line MKN-45 and human breast carcinoma cell line MDA-MB-435. Compounds **5e**, **g** were found to be more potent compounds, especially the compound **5e**. Further research on the structure–activity relationship, their possible mechanism of inhibiting proliferation of cancer cell lines and the development of 3β-hydroxy-16-arylandrosta-5,8(14),15-trien-17-ones as promising anticancer agents are ongoing.

Acknowledgements

Financial support of this research by the National Natural Science Foundation of China (NNSFC 21173181) is gratefully acknowledged by authors. A Project was Funded by the Priority Academic Program Development of Jiangsu Higher Education Institutions. This work is also supported by grant by TG201422 from the Taizhou science and technology support project and by BRA2014182 from the Jiangsu 333 talents project funds to L. Wang.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.steroids.2016.05.004.

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