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

Yang Li [a], Jinliang Liu [a], Lizhong Wang [b], Xushun Qing [a], Cunde Wang [a,*]

[a] School of Chemistry and Chemical Engineering, Yangzhou University, 180 Siwangting Street, Yangzhou 225002, PR China
[b] School of Pharmacy, Taizhou Polytechnic College, Taizhou 225300, PR China

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

* Corresponding author.
E-mail address: wangcd@yzu.edu.cn (C. Wang).

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

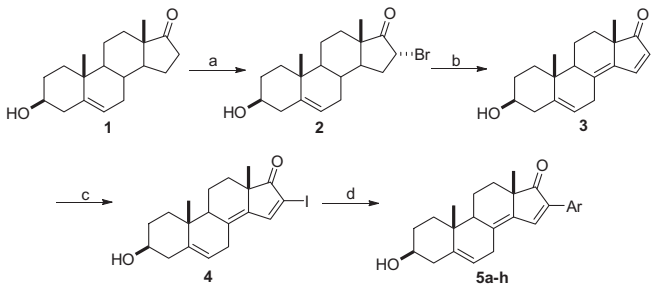
Nicolet FT-IR 5DX spectrometer. The ^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz) spectra were recorded in a Bruker AV-600 spectrometer with TMS as internal reference in DMSO-d_6 or $CDCl_3$ solutions. The *J* values are given in hertz. Only discrete or characteristic signals for the ^{1}H NMR are reported. 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.