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Synthesis and cytotoxicity of 17E-(2-aryl-2-oxo-1-ethylidene)-5α-androstane derivatives

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ABSTRACT

The efficient synthesis of some 17E-(2-aryl-2-oxo-1-ethylidene)-5α-androstan-3β-ols was investigated. 17-Alkynyl-3,17-androstanediols were prepared through the nucleophilic addition of epiandrosterone using the corresponding 1-alkynes in the presence of a strong base *n*-BuLi firstly. The Meyer–Schuster rearrangement of 17-alkynyl-3,17-androstanediols was carried out efficiently catalyzed by 10% H_2SO_4 and $HgSO_4$ in THF. This strategy offered a very straightforward and efficient method for access to conjugated α,β-unsaturated ketone 17E,5α-androstan-3β-ols from the 17-alkynyl-3,17-androstanediols in good overall yields, which are key intermediates for the preparation of some biologically important modified 17-side chain steroids. Evaluation of the synthesized compounds for cytotoxicity against A549, SKOV3, MKN-45 and MDA-MB-435 cell lines showed that 17E-(2-aryl-2-oxo-1-ethylidene)-5α-androstanes possessing a hydroxyl groups at C-3 and fluoro-substituted group of aromatic ring in the side chain have significant inhibition activity.

1. Introduction

The modifications of the natural steroids and the studies on the biological activity of these steroidal derivatives have attracted considerable attentions from synthetic organic chemists and pharmaceuticals researchers. The modified steroidal derivatives have been a rich source of candidates with potential pharmaceutical applications that have encouraged the design and synthesis of new analogs with increased pharmacological activity. Recently, in the field of chemical modifications about the natural steroids, studies have revealed that a number of biologically important properties of modified steroids are dependent upon structural features of the steroid D-ring [1–10]. Chemical modification of the steroid D-ring provides a way to alter the functional groups, sizes, and stereochemistry of the D-ring, and numerous structure–activity relationships have been established by such synthetic alterations. For example, some 16E-arylidene androstene derivatives modified in D-ring have exhibited a broad range of biological activities as potent antimicrobial agents and anticancer agents [5–10].

The preliminary studies on the mechanism of antitumor activities of the modified steroid derivatives revealed that compounds containing a α,β-unsaturated carbonyl function can form adducts with reactive thiol groups of proteins to induce protein modification and mis-folding, which might be responsible for the observed antitumor activities [11]. Although the mechanism of their antitumor activities is not fully understood, a great number of the modified steroids containing a α,β-unsaturated carbonyl function as anticancer agents were described [12–14]. Moreover, α,β-unsaturated carbonyl steroids fused a heterocyclic ring such as a pyrazole and an isoxazole ring were indicated as an aromatase inhibitors to resist breast cancers [15].

The modified D-ring steroid containing a α,β-unsaturated carbonyl function are differ from the modified A, B or C-ring steroids. Having such varied pharmacological activities, on the synthesis of D ring substituted steroidal analogs containing a α,β-unsaturated carbonyl function, these compounds mainly possess an exocyclic double bond. Some researches proved 16-exocyclic double bond of the steroid ring was achieved easily [5–10]. On the contrary, the researches about 17E alkylidene androstane have rarely been reported [16–18]. It is known that the Meyer–Schuster rearrangement is a means of preparing α,β-unsaturated carbonyl compounds as part of a two-stage olefination strategy [19,20]. Due to the important application in the synthesis of the complex compounds, the research and development of the Meyer–Schuster rearrangement were further promoted [21–23]. Based on this above information, the C-17 alkylidene group and the α,β-unsaturated carbonyl function of ring D were designed and five 17E-(2-aryl-2-oxo-1-ethylidene)-5α-androstan-3β-ols and two 3β-acetoxy-17E-(2-aryl-2-oxo-1-ethylidene)-5α-androstanes were synthesized via the Meyer–Schuster rearrangement of 17-substituted ethynyl steroidal 17-ols as a key reaction to investigate their cytotoxic effects on tumor cell lines. In the present paper, we describe a good yielding, convergent strategy for the synthesis of the17E alkylidene

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androstanes with a α,β-unsaturated carbonyl function and evaluate the anticancer activity of these compounds. Our results may provide useful information for the design of chemotherapeutic drugs.

2. Experimental

2.1. General

All melting points were determined in a Yanaco melting point apparatus and are uncorrected. IR spectra were recorded in a 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 $CDCl_3$ 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. 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.

2.2. Organic synthesis

*2.2.1. Preparation of 17α-(2-aryl-1-ethynyl)-5α-androstane-3β,17β-diols (**2a–e**) (Scheme 1, Table 1)*

*2.2.1.1. 17α-(2-Phenyl-1-ethynyl)-5α-androstane-3β,17β-diol (**2a**).* To a solution of 1-ethynylbenzene (0.66 mL, 6 mmol) in dried THF (10 mL) was added *n*-BuLi (2.7 M THF solution, 2.22 mL, 6 mmol) at −20 °C under nitrogen. After the resultant mixture was cooled to −78 °C, the 3β-hydroxy-5α-androstan-17-one (0.58 g, 2 mmol) in dried THF (20 mL) was added drop wise. Then the reaction mixture was warmed to room temperature, the mixture was stirred at this temperature for 3 h and was monitored on TLC. After completion of the reaction, the cold water (20 mL) was poured into the reaction mixture and the majority of solvent was evaporated under reduced pressure. The product was extracted with methylene chloride (2 × 15 mL). The combined extracts were washed with water and saturated brine, dried over Na_2SO_4, and evaporated under reduced pressure to give a residue. The residue was purified by preparative TLC (EtOAc:hexanes = 1:10) to furnish compound **2a** as a white solid (0.61 g, 78%); mp 115–118 °C (EtOAc–hexanes); IR (KBr, cm^{-1}) *ν* 3430, 2956, 2926, 2856, 2202, 1602, 1460, 1379, 1263, 1039, 741, 698; ^{1}H NMR ($CDCl_3$, 600 MHz) *δ* (ppm): 7.37 (d, *J* = 6.6 Hz, 2H), 7.26–7.24 (m, 3H), 3.54–3.49 (m, ^{1}H, 3α-H), 0.81 (s, 3H), 0.76 (s, 3H); ^{13}C NMR ($CDCl_3$, 150 MHz) *δ* (ppm): 130.63 (2C), 127.24 (2C), 127.17, 122.01, 91.89, 84.74, 79.32, 70.29, 53.02, 49.71, 46.39, 43.82, 38.03, 37.15, 35.98, 35.19, 34.54, 32.02, 30.58, 30.48, 27.56, 22.24, 19.96, 12.00, 11.34; MS (EI): 393 (M+1, 38%); Anal. Calcd. for $C_{27}H_{36}O_2$: C, 82.61; H, 9.24; found C, 82.83; H, 9.10.

*2.2.1.2. 17α-(2-(4-Methoxyphenyl)-1-ethynyl)-5α-androstane-3β,17β-diol (**2b**).* Following the above procedure using 1-ethynyl-4-methoxybenzene as a starting material, the title compound **2b** (85% in yield) was obtained as a white solid, mp 81–83 °C (EtOAc–hexanes); IR (KBr, cm^{-1}) *ν* 3333, 2931, 2855, 2219, 1571, 1510, 1445, 1383, 1247, 1132, 1035, 831; ^{1}H NMR ($CDCl_3$, 600 MHz) *δ* (ppm): 7.30 (d, *J* = 6.6 Hz, 2H), 6.76 (d, *J* = 6.6 Hz, 2H), 3.73 (s, 3H, Ar–OMe), 3.52–3.47 (m, ^{1}H, 3α-H), 0.80 (s, 3H), 0.74 (s, 3H); ^{13}C NMR ($CDCl_3$, 150 MHz) *δ* (ppm): 158.51, 132.07 (2C), 114.28, 112.90 (2C), 90.66, 84.52, 79.27, 70.24, 54.29, 53.08, 49.70, 46.37, 43.87, 38.12, 37.16, 36.03, 35.24, 34.56, 32.07, 30.61, 30.48, 27.61, 22.26, 20.00, 12.04, 11.34; MS (EI): 423 (M+1, 52%); Anal. Calcd. for $C_{28}H_{38}O_3$: C, 79.58; H, 9.06; found C, 79.80; H, 9.17.

*2.2.1.3. 17α-(2-(4-Methylphenyl)-1-ethynyl)-5α-androstane-3β,17β-diol (**2c**).* Following the above procedure using 1-ethynyl-4-methylbenzene as a starting material, the title compound **2c** (81% in yield) was obtained as a white solid, mp 93–95 °C (EtOAc–hexanes); IR (KBr, cm^{-1}) *ν* 3400, 2928, 2857, 2220, 1578, 1510, 1451, 1379, 1283, 1136, 1043, 817; ^{1}H NMR ($CDCl_3$, 600 MHz) *δ* (ppm): 7.33 (d, *J* = 6.6 Hz, 2H), 7.12 (d, *J* = 6.6 Hz, 2H), 3.61–3.56 (m, ^{1}H, 3α-H), 2.35 (s, 3H, Ar–CH_3), 0.88 (s, 3H), 0.83 (s, 3H); ^{13}C NMR ($CDCl_3$, 150 MHz) *δ* (ppm): 137.27, 130.54 (2C), 128.00 (2C), 119.00, 91.21, 84.87, 79.37, 70.32, 53.09, 49.72, 46.41, 43.89, 38.10, 37.23, 36.04, 35.25, 34.58, 32.05, 30.61, 30.55, 27.61, 22.25, 20.43, 20.00, 12.00, 11.35; MS (EI): 407 (M+1, 28%); Anal. Calcd. for $C_{28}H_{38}O_2$: C, 82.71; H, 9.42; found C, 82.63; H, 9.55.

*2.2.1.4. 17α-(2-(4-Fluorophenyl)-1-ethynyl)-5α-androstane-3β,17β-diol (**2d**).* Following the above procedure using 1-ethynyl-4-fluorobenzene as a starting material, the title compound **2d** (72% in yield) was obtained as a white solid, mp 87–89 °C (EtOAc–hexanes); IR (KBr, cm^{-1}) *ν* 3400, 2930, 2857, 2218, 1591, 1507, 1452, 1380, 1227, 1144, 1043, 837; ^{1}H NMR ($CDCl_3$, 600 MHz) *δ* (ppm): 7.36–7.34 (m, 2H), 6.94 (t, *J* = 7.8 Hz, 2H), 3.54–3.49 (m, ^{1}H, 3α-H), 0.81 (s, 3H), 0.76 (s, 3H); ^{13}C NMR ($CDCl_3$, 150 MHz) *δ* (ppm): 161.42 (d, *J* = 247.5 Hz, 1C), 132.52 (d, *J* = 7.5 Hz, 2C), 118.07, 114.50 (d, *J* = 22.5 Hz, 2C), 91.55, 83.73, 79.33, 70.28, 53.04, 49.76, 46.38, 43.84, 38.05, 37.16, 35.99, 35.19, 34.56, 32.05, 30.58, 30.49, 27.55, 22.24, 19.95, 11.98, 11.34; MS (EI): 411 (M+1, 23%); Anal. Calcd. for $C_{27}H_{35}FO_2$: C, 78.99; H, 8.59; found C, 79.20; H, 8.50.

*2.2.1.5. 17α-(2-(3-Fluorophenyl)-1-ethynyl)-5α-androstane-3β,17β-diol (**2e**).* Following the above procedure using 1-ethynyl-4-fluorobenzene as a starting material, the title compound **2e** (70% in yield) was obtained as a white solid, mp 139–141 °C (EtOAc–hexanes); IR (KBr, cm^{-1}) *ν* 3373, 2934, 2858, 2221, 1578, 1475, 1440, 1377, 1290, 1140, 1045, 871, 782, 681; ^{1}H NMR ($CDCl_3$, 600 MHz) *δ* (ppm): 7.22–7.19 (m, ^{1}H), 7.15 (d, *J* = 7.2 Hz, ^{1}H), 7.06 (d, *J* = 9.0 Hz, ^{1}H), 6.96–6.93 (m, ^{1}H), 3.54–3.49 (m, ^{1}H, 3α-H), 0.81 (s, 3H), 0.76 (s, 3H); ^{13}C NMR ($CDCl_3$, 150 MHz) *δ* (ppm): 162.34 (*J* = 244.5 Hz, 1C), 129.83 (*J* = 9.0 Hz, 1C), 127.55, 124.89 (*J* = 10.5 Hz, 1C), 118.47 (*J* = 22.5 Hz, 1C), 115.52 (*J* = 21.0 Hz, 1C), 93.95, 84.61, 80.32, 71.30, 54.04, 50.83, 47.46, 44.84, 39.04, 38.16, 36.99, 36.20, 35.57, 33.08, 31.61, 31.50, 28.57, 23.27, 20.96, 13.00, 12.36; MS (EI): 411 (M+1, 36%); Anal. Calcd. for $C_{27}H_{35}FO_2$: C, 78.99; H, 8.59; found C, 79.13; H, 8.42.

*2.2.2. Preparation of 17E-(2-aryl-2-oxo-1-ethylidene)-5α-androstan-3β-ols (**3a–e**)*

*2.2.2.1. 17E-(2-phenyl-2-oxo-1-ethylidene)-5α-androstan-3β-ol (**3a**).* To the mixture of 17α-(2-phenyl-1-ethynyl)-5α-androstane-3β,17β-diol (**2a**) (0.392 g, 1 mmol) in THF (10 mL) was added 10% H_2SO_4 (0.6 mL, 0.6 mmol) and $HgSO_4$ (30 mg, 0.1 mmol). The resulting mixture was stirred at 30 °C for 48 h and was monitored on TLC. Then the reaction was terminated and the majority of solvent was evaporated under reduced pressure. Distilled water (15 mL) was added into the reaction mixture, and the product was extracted with methylene chloride (2 × 15 mL). The combined extracts were washed with water, saturated $NaHCO_3$ and saturated brine, dried over Na_2SO_4, and evaporated under reduced pressure to give a residue. The residue was subjected to chromatography using ethyl acetate/dichloromethane (1:30) as the eluent to give 0.225 g of product **3a** (65%) as a white solid, mp 206–209 °C (EtOAc); IR (KBr, cm^{-1}) *ν* 3475, 3069, 2924, 2860, 1660, 1610, 1447, 1372, 1259, 1220, 1040, 706, 676; ^{1}H NMR ($CDCl_3$, 600 MHz) *δ* (ppm): 7.86 (d, *J* = 7.8 Hz, 2H), 7.45–7.43 (t, *J* = 7.8 Hz, ^{1}H), 7.38–7.36

(t, J = 7.8 Hz, 2H), 6.63 (s, ^{1}H, 20-H), 3.56–3.51 (m, ^{1}H, 3α-H), 2.98–2.81 (m, 2H, 16-CH_2), 0.81 (s, 3H), 0.78 (s, 3H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ (ppm): 190.17, 177.32, 138.69, 131.01, 127.41 (2C), 126.99 (2C), 111.19, 70.24, 53.60, 52.60, 45.87, 43.94, 37.23, 36.08, 34.68 (2C), 34.36, 30.97, 30.61, 30.54, 27.62, 23.55, 20.25, 17.67, 11.36; EI-MS: 393 (M+1, 35%); Anal. Calcd. for $C_{27}H_{36}O_2$: C, 82.61; H, 9.24; found C, 82.59; H, 9.08.

*2.2.2.2. 17E-(2-(4-methoxyphenyl)-2-oxo-1-ethylidene)-5α-androstan-3β-ol (**3b**).* Following the above procedure using 17α-(2-(4-methoxyphenyl)-1-ethynyl)-5α-androstane-3β,17β-diol (**2b**) as a starting material, the title compound **3b** (63% in yield) was obtained as a white solid, mp 198–200 °C (EtOAc); IR (KBr, cm^{-1}) ν 3476, 3080, 2925, 2858, 1659, 1608, 1510, 1440, 1350, 1265, 898, 706, 675; ^{1}H NMR ($CDCl_3$, 600 MHz) δ (ppm): 7.93 (d, J = 8.4 Hz, 2H), 6.93 (d, J = 8.4 Hz, 2H), 6.67 (s, ^{1}H, 20-H), 3.87 (s, 3H, Ar–OCH_3), 3.64–3.58 (m, ^{1}H), 3.04–2.86 (m, 2H, 16-CH_2), 0.87 (s, 3H), 0.85(s, 3H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ (ppm): 188.80, 176.22, 169.58, 161.62, 131.80, 129.02 (2C), 112.80 (2C), 112.00, 70.14, 54.52, 53.50, 46.88, 44.80, 38.17, 37.12, 35.64, 35.10, 31.92, 31.68, 31.33, 30.91, 28.60, 24.48, 21.26, 18.60, 12.32; EI-MS: 423 (M+1, 29%); Anal. Calcd. for $C_{28}H_{38}O_3$: C, 79.58; H, 9.06; found C, 79.59; H, 9.14.

*2.2.2.3. 17E-(2-(4-methylphenyl)-2-oxo-1-ethylidene)-5α-androstan-3β-ol (**3c**).* Following the above procedure using 17α-(2-(4-methylphenyl)-1-ethynyl)-5α-androstane-3β,17β-diol (**2c**) as a starting material, the title compound **3c** (65% in yield) was obtained as a white solid, mp 167–170 °C (EtOAc); IR (KBr, cm^{-1}) ν 3472, 3080, 2926, 2850, 1660, 1606, 1510, 1445, 1352, 1270, 898, 710, 678; ^{1}H NMR ($CDCl_3$, 600 MHz) δ (ppm): 7.83 (d, J = 7.8 Hz, 2H), 7.24 (d, J = 7.8 Hz, 2H), 6.68 (s, ^{1}H, 20-H), 3.65–3.58 (m, ^{1}H), 3.04–2.86 (m, 2H, 16-CH_2), 0.88 (s, 3H), 0.85(s, 3H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ (ppm): 189.56, 176.60, 169.60, 141.52, 136.10, 128.18 (2C), 127.10 (2C), 111.16, 70.59, 53.48, 52.02, 45.88, 43.72, 35.68, 34.70, 34.20, 33.00, 30.78, 30.36, 27.46, 26.48, 23.50, 20.44, 20.12, 17.60, 11.26; EI-MS: 407 (M+1, 47%); Anal. Calcd. for $C_{28}H_{38}O_2$: C, 82.71; H, 9.42; found C, 82.60; H, 9.38.

*2.2.2.4. 17E-(2-(4-fluorophenyl)-2-oxo-1-ethylidene)-5α-androstan-3β-ol (**3d**).* Following the above procedure using 17α-(2-(4-fluorophenyl)-1-ethynyl)-5α-androstane-3β,17β-diol (**2d**) as a starting material, the title compound **3d** (50% in yield) was obtained as a white solid, mp 211–213 °C (EtOAc); IR (KBr, cm^{-1}) ν 3685, 2929, 2853, 1664, 1603, 1509, 1453, 1372, 1224, 1157, 1041, 840; ^{1}H NMR ($CDCl_3$, 600 MHz) δ (ppm): 7.90–7.87 (m, 2H), 7.06–7.03 (m, 2H), 6.59 (s, ^{1}H, 20-H), 3.58–3.53 (m, ^{1}H, 3α-H), 2.97–2.80 (m, 2H, 16-CH_2), 0.81 (s, 3H), 0.79 (s, 3H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ (ppm): 189.55, 178.96, 165.16 (d, J = 252 Hz, 1C), 135.88, 130.51 (d, J = 9 Hz, 2C), 115.44 (d, J = 22.5 Hz, 2C), 117.73, 71.24, 54.49, 53.50, 46.92, 44.86, 38.16, 37.01, 35.64, 35.28, 31.93, 31.68, 31.48, 30.94, 28.56, 24.52, 21.20, 18.65, 12.36; EI-MS: 411 (M+1, 15%); Anal. Calcd. for $C_{27}H_{35}FO_2$: C, 78.99; H, 8.59; found C, 78.82; H, 8.58.

*2.2.2.5. 17E-(2-(3-fluorophenyl)-2-oxo-1-ethylidene)-5α-androstan-3β-ol (**3e**).* Following the above procedure using 17α-(2-(3-fluorophenyl)-1-ethynyl)-5α-androstane-3β,17β-diol (**2e**) as a starting material, the title compound **3e** (49% in yield) was obtained as a white solid, mp 205–208 °C (EtOAc); IR (KBr, cm^{-1}) ν 3477, 2930, 2853, 1664, 1611, 1585, 1445, 1372, 1262, 1161, 1043, 882, 811, 725; ^{1}H NMR ($CDCl_3$, 600 MHz) δ (ppm): 7.64 (d, J = 7.8 Hz, ^{1}H), 7.53 (d, J = 7.8 Hz, ^{1}H), 7.35 (s, ^{1}H), 7.14 (t, J = 7.8 Hz, ^{1}H), 6.57 (s, ^{1}H, 20-H), 3.56–3.51 (m, ^{1}H, 3α-H), 2.97–2.80 (m, 2H, 16-CH_2), 0.81 (s, 3H), 0.78 (s, 3H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ (ppm): 188.59, 178.74, 165.10 (d, J = 252 Hz, 1C), 140.75, 129.02 (d, J = 7.5 Hz, 1C), 122.63, 117.97 (d, J = 21.0 Hz, 1C), 113.81 (d, J = 22.5 Hz, 1C), 110.75, 70.21, 53.50, 52.50, 46.00, 43.86, 37.16, 36.02, 34.64, 34.57, 34.28, 30.92, 30.81, 30.48, 27.56, 23.50, 20.19, 17.63, 11.35; EI-MS: 411 (M+1, 16%); Anal. Calcd. for $C_{27}H_{35}FO_2$: C, 78.99; H, 8.59; found C, 78.89; H, 8.66.

*2.2.3. Esterification of 17E-(2-aryl-2-oxo-1-ethylidene)-5α-androstan-3β-ols (**3b–c**)*

*2.2.3.1. 17E-(2-(4-methoxyphenyl)-2-oxo-1-ethylidene)-3β-acetyloxy-5α-androstane (**4b**).* The mixture of 17E-(2-(4-methoxyphenyl)-2-oxo-1-ethylidene)-5α-androstan-3β-ol (**3b**) (0.422 g, 1 mmol) and acetic anhydride (0.204 g, 2 mmol) in dried pyridine (3.5 mL) was stirred for 12 h at room temperature. After completion of the reaction, the cold water (20 mL) was poured into the reaction mixture. The resulting mixture was extracted with methylene chloride (2 × 15 mL). The combined extracts were washed with water and saturated brine, dried over Na_2SO_4, and evaporated under reduced pressure to give a residue. The residue was purified by preparative TLC (EtOAc:hexanes = 1:3) to furnish compound **4b** as a white solid (0.441 g, 95%); mp 105–109 °C (EtOAc/hexanes); IR (KBr, cm^{-1}) ν 3684, 2943, 2878, 1731, 1655, 1602, 1369, 1242, 1169, 1027, 838; ^{1}H NMR ($CDCl_3$, 600 MHz) δ (ppm): 7.87 (d, J = 7.2 Hz, 2H), 6.86 (d, J = 7.2 Hz, 2H), 6.60 (s, ^{1}H, 20-H), 4.65–4.60 (m, ^{1}H, 3α-H), 3.80 (s, 3H, Ar–OMe), 2.96–2.80 (m, 2H, 16-CH_2), 1.96 (s, 3H, 3β-OAc), 0.80 (s, 6H, 2XCH_3); ^{13}C NMR ($CDCl_3$, 150 MHz) δ (ppm): 188.84, 176.07, 169.63, 161.79, 131.56, 129.19 (2C), 112.59 (2C), 111.00, 72.60, 54.40, 53.42, 52.46, 45.71, 43.70, 35.79, 34.64 (2C), 34.29, 33.00, 30.84, 30.40, 27.47, 26.47, 23.53, 20.42, 20.17, 17.65, 11.25; EI-MS: 465 (M+1, 35%); Anal. Calcd. for $C_{30}H_{40}O_4$: C, 77.55; H, 8.68; found C, 77.43; H, 8.60.

*2.2.3.2. 17E-(2-(4-methylphenyl)-2-oxo-1-ethylidene)-3β-acetyloxy-5α-androstane (**4c**).* Following the above procedure using 17E-(2-(4-methylphenyl)-2-oxo-1-ethylidene)-5α-androstan-3β-ol (**3c**) as a starting material, the title compound **4c** (93% in yield) was obtained as a white solid; mp 103–107 °C (EtOAc/hexanes); IR (KBr, cm^{-1}) ν 3687, 2944, 2878, 1736, 1661, 1611, 1511, 1451, 1370, 1243, 1180, 1034, 838; ^{1}H NMR ($CDCl_3$, 600 MHz) δ (ppm): 7.76 (d, J = 7.8 Hz, 2H), 7.17 (d, J = 7.8 Hz, 2H), 6.61 (s, ^{1}H, 20-H), 4.65–4.60 (m, ^{1}H, 3α-H), 2.97–2.80 (m, 2H, 16-CH_2), 2.33 (s, 3H, Ph–Me), 1.95 (s, 3H, 3β-OAc), 0.81 (s, 3H), 0.80 (s, 3H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ (ppm): 189.84, 176.62, 169.63, 141.63, 136.07, 128.09 (2C), 127.11 (2C), 111.18, 72.59, 53.41, 52.45, 45.76, 43.69, 35.79, 34.64, 34.60, 34.29, 33.00, 30.84, 30.50, 27.46, 26.47, 23.53, 20.55, 20.42, 20.16, 17.64, 11.25; EI-MS: 449 (M+1, 18%); Anal. Calcd. for $C_{30}H_{40}O_3$: C, 80.31; H, 8.99; found C, 80.49; H, 9.20.

2.2.4. Cytotoxic activity against 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

All tumor cell lines tested were purchased from Shanghai Institute of Cell Biology, Chinese Academy of Science. The cell lines were cultured in RPMI 1640 medium with 10% newborn calf serum 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. 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, USA). 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.

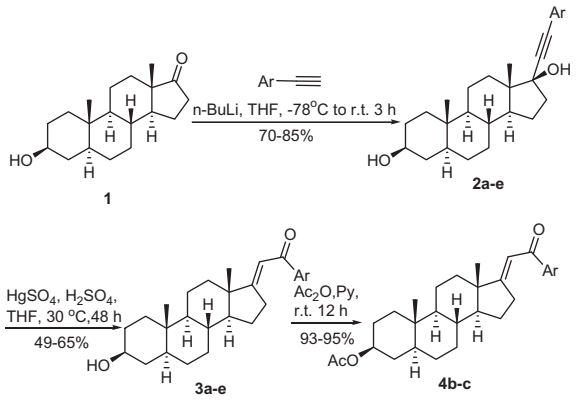
3. Results and discussion

3.1. Chemistry

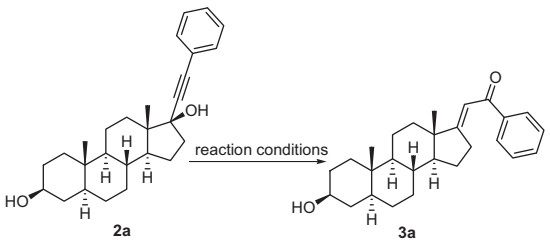
In general, α,β-unsaturated ketones could be prepared via aldol condensation from ketones or aldehydes with an active α-methylene group. Thus, firstly, the aldol reaction of (3β,5α)-3-hydroxyandrostan-17-one and acetophenone was carried out to synthesize the desired product in the presence of sodium hydroxide or sodium hydride at room temperature or under refluxing, but the desired 17E-(2-phenyl-2-oxo-1-ethylidene)-5α-androstan-3β-ol (**3a)** was not afforded. Considering the synthesis of these complex steroids, we needed an active intermediate for the formation of steroidal 17E(20)-alkenes. Fortunately, the Meyer–Schuster rearrangement of an alkyne with α-hydroxy group as a starting material in the presence of Lewis acid could give the desired α,β-unsaturated ketone.

To initiate our studies, we first prepared five 17-alkynyl-3, 17-androstanediols (**2a**–**e**) in 70–85% yields through the nucleophilic addition of epiandrosterone using the corresponding 1-alkynes in the presence of a strong base *n*-BuLi (Schemes 1 and 2, Table 1).

To achieve this goal for the formation of the designed α,β-unsaturated ketone, at first, the Lewis acids were used for the preparation of compound **3a** from the model compound **2a**. We observed the formation of the desired product **3a** (Table 2) when the reaction was carried out using compound **2a**, 10% H_2SO_4 (0.6 mL, 0.6 mmol) and acetone (5 mL) in the presence of various catalysts at 60 °C for 48 h. A comparison of the method using $Hg(OAc)_2$ or $HgSO_4$ as a catalyst (Table 2, entry 3, 46%; and entry 7, 45% in yield), with selected other Lewis acid catalyst such as $Co(OAc)_2$, $Zn(OAc)_2$, $Cu(OAc)_2$, $Ni(OAc)_2$ and $Fe_2(SO_4)_3$ (Table 2, entry 1, 10% in yield; entry 2, 16% in yield; entry 4, 10% in yield; entry 5, trace; entry 6, trace, respectively) that were examined is collected in Table 2 demonstrated that the method using $Hg(OAc)_2$ or $HgSO_4$ as a catalyst is indeed superior to several of the other protocols. Thus, $Hg(OAc)_2$ and $HgSO_4$ were found to be the better choice for this reaction.



Scheme 1. Preparation of target compounds (**3a–e**, **4b–c**).



Scheme 2. Various reaction conditions for the Meyer–Schuster rearrangement of compound **2a**.

Table 1
Preparation of target compounds (**3a–e**, **4b–c**).

Compd.	Ar	Yield%[a]
2a	C_6H_5	78
2b	*p*-$MeOC_6H_4$	85
2c	*p*-MeC_6H_4	81
2d	*p*-FC_6H_4	72
2e	*m*-FC_6H_4	70
3a	C_6H_5	65
3b	*p*-$MeOC_6H_4$	63
3c	*p*-MeC_6H_4	65
3d	*p*-FC_6H_4	50
3e	*m*-FC_6H_4	49
4b	*p*-$MeOC_6H_4$	95
4c	*p*-MeC_6H_4	93

Table 2
Various catalysts effect on the reaction.[a]

Entry	Catalyst	Yield%[b]
1	$Co(OAc)_2$	10
2	$Zn(OAc)_2$	16
3	$Hg(OAc)_2$	46
4	$Cu(OAc)_2$	10
5	$Ni(OAc)_2$	Trace
6	$Fe_2(SO_4)_3$	Trace
7	$HgSO_4$	45

[a] Reaction conditions: 10% mol catalyst, 10% H_2SO_4, acetone, 60 °C, 48 h.
[b] Isolated yields.

To optimize the reaction conditions, reaction time, reaction temperature and solvent were varied. Firstly the reaction time in the preparation of compound **3a** from the model compound **2a**, and 10% H_2SO_4 in the presence of $Hg(OAc)_2$ (0.10 mmol) was varied. Among the reaction time tested (Table 3, entries 1–5), the reaction time from 48 to 60 h gave the best result. The result showed that the reaction time at 48 and 60 h gave the product **3a** in 45% yield.

In the second set of experimental, the model reaction with compound **2a**, and 10% H_2SO_4 in the presence of $Hg(OAc)_2$ (0.10 mmol) in acetone was carried out by varied reaction temperature. After some experimentation (Table 4, entries 1–4), it was found that the model reaction using reaction temperature 30 °C produced the corresponding compound **3a** in 56% yield.

Furthermore, among the solvents tested (Table 5, entries 1–5), THF and acetone gave the best result. The result showed that THF, acetone gave the product **3a** in 65%, 56% yield, respectively.

Table 3
Various reaction time effect on the reaction.[a]

Entry	Time h	Yield%[b]
1	12	<5
2	24	18
3	36	33
4	48	45
5	60	45

[a] Reaction conditions: 10% mol $HgSO_4$, 10% H_2SO_4, acetone, 60 °C.
[b] Isolated yields.

Table 4
Various reaction temperature effect on the reaction.[a]

Entry	*T* °C	Yield%[b]
1	10	40
2	30	56
3	40	48
4	60	45

[a] Reaction conditions: 10% mol $HgSO_4$, 10% H_2SO_4, acetone, 48 h.
[b] Isolated yields.

Table 5
Various solvents effect on the reaction.[a]

Entry	Solvent	Yield%[b]
1	Dioxane	40
2	THF	65
3	Acetone	56
4	Methanol	Trace
5	Ethanol	Trace

[a] Reaction conditions: 10% mol $HgSO_4$, 10% H_2SO_4, 30 °C, 48 h.

Thus, with these results in hand, we synthesized five 17E-(2-aryl-2-oxo-1-ethylidene)-5α-androstan-3β-ols (**3a–e)** in yield varying from 49% to 65% by the Meyer–Schuster rearrangement of 17-alkynyl-3,17-androstanediols (**2a**–**e**) (1.0 mmol), H_2SO_4 (0.6 mL, 0.6 mmol) and $HgSO_4$ (0.10 mmol) in THF (10 ml) at 30 °C for 48 h (Scheme 1, Table 1).

We obtained an X-ray crystal structure of **4b** that is presented in Fig. 1. The configuration at C-17 has been assigned E. Crystallographic data for **4b** have been deposited with the Cambridge Crystallographic Data Centre with the deposition number CCDC 808601. 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].

In order to study the effect on the cytotoxicity of a hydroxyl or acetoxy at C-3 of steroidal 17(20)E-alkenes, esterification of compound **3b–c** was carried out by treatment with acetic anhydride and pyridine to furnish the corresponding esters **4b–c** in 93–95% yields (Scheme 1, Table 1).

3.2. Biological activity

Recently some studies showed those natural and synthetic steroids with α,β-unsaturated ketone core gave the potency against human cancer cell lines [12,24–26]. Thus, all compounds **3a–e** and **4b–c** synthesized as described above were subjected to in vitro cytotoxic evaluation against 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 μmol/L in Table 6.

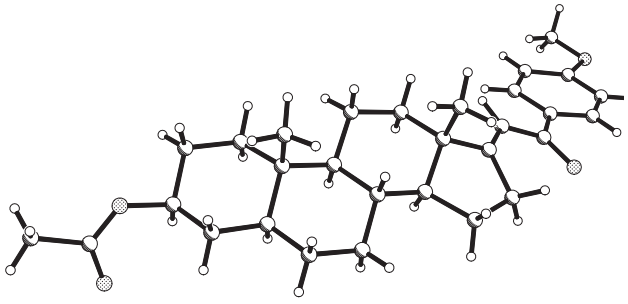


Fig. 1. X-ray crystal structure of **4b**.

Table 6
The in vitro cytotoxic activity (IC_{50}, in μmol/L) of compounds **3a**–**e**, **4b**–**c**.[a]

Compound	Cancer cell lines[b]			
	A549	SKOV3	MKN-45	MDA-MB-435
3a	58.1 ± 3.4	16.5 ± 1.6	74.5 ± 4.2	27.7 ± 1.3
3b	38.5 ± 1.6	12.3 ± 0.8	32.2 ± 0.6	28.2 ± 2.6
3c	42.3 ± 4.6	15.6 ± 2.2	42.2 ± 1.8	36.6 ± 3.2
3d	22.1 ± 1.8	3.4 ± 0.2	34.3 ± 2.0	16.7 ± 1.6
3e	41.5 ± 2.3	4.7 ± 0.4	13.1 ± 1.2	28.6 ± 0.8
4b	45.0 ± 0.8	20.1 ± 1.4	101.6 ± 5.6	44.6 ± 3.8
4c	205.9 ± 8.8	74.5 ± 4.5	343.6±8.2	104.3 ± 6.8
Cisplatin	9.14	10.22	18.25	33.20

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

From the data shown in Table 6, most of the new compounds showed a measurable anti-cancer activity against A549, SKOV3, MKN-45, and MDA-MB-435 cell lines tested. Although this is a preliminary screening, 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 **3a–e**, with same 3-hydroxyl structure and different types of substituted group of aromatic ring in the side chain, showed a distinct difference in their cytotoxicity against these cancer cells. Compound **3d** and **e** with a fluoro-substituted group of aromatic ring in the side chain had a better cytotoxicity than compounds **3a**–**3c** against SKOV3 cells, and also had a better cytotoxicity than cisplatin which has been introduced into clinical use for couples of years. The analogs **4b** and **c**, with an acetoxy at C-3, remarkably decreased their cytotoxic activity against A549, SKOV3, MKN-45, and MDA-MB-435 cells in comparison with the analogs **3b** and **c**, which have a hydroxyl groups at C-3. Compound **4c** containing an acetoxy at C-3 and 4-methylphenyl substituted group in the side chain was found inactive to A549, MKN-45, and MDA-MB-435 cells tested (IC_{50} value >80 μmol/L). The above results indicated that a hydroxyl groups at C-3 must be present to retain cytotoxic activity. This may indicate that the anticancer properties depend not only on a hydroxyl groups at C-3 but also on the moieties attached to the side chain.

4. Conclusion

In summary, we have successfully developed a novel and operationally simple reaction for highly efficient synthesis of 17E-(2-aryl-2-oxo-1-ethylidene)-5α-androstan-3β-ols. Firstly, 17-alkynyl-3,17-androstanediols were prepared through the nucleophilic addition of epiandrosterone using the corresponding 1-alkynes in the presence of a strong base *n*-BuLi. The Meyer–Schuster rearrangement reaction of 17-alkynyl-3,17-androstanediols was carried out efficiently catalyzed by H_2SO_4 and $HgSO_4$ in THF. This strategy offered a very straightforward and efficient method for access to conjugated α,β-unsaturated ketone 17E,5α-androstan-3β-ols from the 17-alkynyl-3,17-androstanediols in good overall yields, which can be key intermediates for the preparation of some biologically important modified 17-side chain steroids. The preliminary results showed that those 17E-(2-aryl-2-oxo-1-ethylidene)-5α-androstanes possessing a hydroxyl groups at C-3 and different types of

substituted group of aromatic ring in the side chain 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 **3d**, **e** were found to be more potent compounds, especially the compound **3d**. Due to the structural features of our novel compounds, the mechanism of action cannot be discerned. Further research on the structure–activity relationship, their possible mechanism of inhibiting proliferation of cancer cell lines and the development of 17E-(2-aryl-2-oxo-1-ethylidene)-5α-androstanes as promising anticancer agents are ongoing.

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References

[1] Levinaa IS, Pokrovskayab EV, Kulikovaa LE, Kamernitzkya AV, Kachalaa VV, Smirnovb AN. 3- and 19-Oximes of 16α, 17α-cyclohexanoprogesterone derivatives: synthesis and interactions with progesterone receptor and other proteins. Steroids 2008;73:815–27.
[2] Boonananwong S, Kongkathip B, Kongkathip N. First synthesis of 3,16,20-polyoxygenated cholestanes, new cytotoxic steroids from the gorgonian *Leptogorgia sarmentosa*. Steroids 2008;73:1123–7.
[3] Romanoa D, Ferrarioa V, Moraa D, Lennab R, Molinaria F. Enantioselective production of 3-hydroxy metabolites of tibolone by yeast reduction. Steroids 2008;73:112–5.
[4] Banday AH, Zargar MI, Ganaie BA. Synthesis and antimicrobial studies of chalconyl pregnenolones. Steroids, in press. doi: 10.1016/j.steroids.2011.07.001.
[5] Chattopadhaya R, Jindal DP, Minu M, Gupta R. Synthesis and cytotoxic studies of hydroximino derivatives of some 16E-arylidenosteroids. Arzneimittel Forschung 2004;54:551–6.
[6] Bansal R, Guleria S. Synthesis of 16E-[3-methoxy-4-(2-aminoethoxy)-benzylidene]androstene derivatives as potent cytotoxic agents. Steroids 2008;73:1391–9.
[7] Dubey S, Piplani P, Jindal DP. Synthesis and in vitro antineoplastic evaluation of certain 16-(4-substituted benzylidene) derivatives of androst-5-ene. Chem Biodiver 2004;1:1529–36.
[8] Dubey S, Kaur P, Jindal DP, Satyanarayan YD, Piplani P. Synthesis and evaluation QSAR studies of 16-(4 and 3,4-substituted) benzylidene androstene derivatives as anticancer agents. Med Chem 2008;4:229–36.
[9] Guo H, Wu H, Yang J, Xiao Y, Altenbach H, Qiu G, Hu H, Wu Z, He X, Zhou D, Hu X. Synthesis, characterization and biological evaluation of some 16E-arylidene androstane derivatives as potential anticancer agents. Steroids 2011;76:709–72.
[10] Moreira VM, Salvador JAR, Bejad AM, Paixaod JA. 16-dien-3β-yl-acetate: synthesis and structural elucidation of novel 16-azolylmethylene-17-oxoandrostanes. Steroids 2011;76:582–7.
[11] Bradshaw TD, Matthews CS, Cookson J, Chew E-H, Shah M, Bailey K, et al. Elucidation of thioredoxin as a molecular target for antitumor quinols. Cancer Res 2005;65:3911–9.
[12] Jiang C, Huang C, Feng B, Li J, Gong J, Kurtánc T, Guo Y. Synthesis and antitumor evaluation of methyl spongoate analogs. Steroids 2010;75:1153–63.
[13] Carney JR, Yoshida WY, Scheuer PJ. Kiheisterones, new cytotoxic steroids from a Maui sponge. J Org Chem 1992;57:6637–43.
[14] Yan XH, Lin LP, Ding J, Guo YW. Methyl spongoate, a cytotoxic steroids from the Sanya soft coral *Spongodes* sp.. Bioorg Med Chem Lett 2007;17:2661–3.
[15] Yadav MR, Sabale PM, Rajani G, Zimmer C, Haupenthal J, Hartmann RW. Synthesis of some novel androstanes as potential aromatase inhibitors. Steroids 2011;76:464–70.
[16] Chowdhury P, Borah JM, Goswamil P, Das AM. A convenient synthesis of the side chain of loteprednol etabonate-An ocular soft corticosteroid from 20-oxopregnanes using metal-mediated halogenation as a key reaction. Steroids 2011;76:497–501.
[17] Gyermek L, Iriarte J, Crabbe P. Structure–activity relation of some steroidal hypnotic agents. J Med Chem 1968;11(1):117–25.
[18] Holt DA, Yamashita DS, Gleason JG, Darcy MG. Preparation of 5 α-redustase inhibiting 17β-substituted 3-carboxy steroids. 1995; WO 9528413.
[19] Li JJ. In Meyer–Schuster rearrangement, name reactions: a collection of detailed reaction mechanisms. Berlin: Springer; 2006. p. 380–1.
[20] Engel DA, Dudley GB. Org Biomol Chem 2009;7:4149–58.
[21] Tanaka K, Shoji T, Hirano M. Cationic rhodium(I)/bisphosphane complex-catalyzed isomerization of secondary propargylic alcohols to α,β-enones. Eur J Org Chem 2007;16:2687–99.
[22] Liao WW, Mueller Thomas JJ. Sequential coupling-isomerization-coupling reactions. A novel three-component synthesis of aryl chalcones. Synlett 2006;20:3469–73.
[23] Cadierno Victorio, Garcia-Garrido SE, Gimeno J. Isomerization of propargylic alcohols into α,β-unsaturated carbonyl compounds catalyzed by the 16-electron allyl-ruthenium(II) complex [Ru(2-C_3H_4Me)(CO)(dppf)] [SbF6]. Adv Syn Catal 2006;348(1–2):101–10.
[24] Li C, Qiu W, Yang Z, Luo J, Yang F, Liu M, Xie J, Tang J. Stereoselective synthesis of some methyl-substituted steroid hormones and their in vitro cytotoxic activity against human gastric cancer cell line MGC-803. Steroids 2010;75:859–69.
[25] Wang C, Liu L, Xu H, Zhang Z, Wang X, Liu H. Novel and efficient synthesis of 22-alkynyl-13, 24(23)-cyclo-18, 21-dinorchol-22-en-20(23)-one analogues. Steroids 2011;76:491–6.
[26] Cui J, Fan L, Huang L, Liu H, Zhou A. Synthesis and evaluation of some steroidal oximes as cytotoxic agents: structure/activity studies (I). Steroids 2009;74:62–72.