Steroids 113 (2016) 14–21

Contents lists available at ScienceDirect

Steroids

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

Synthesis and biological evaluation of 13α-estrone derivatives as potential antiproliferative agents

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ARTICLE INFO

Article history:
Received 20 December 2015
Received in revised form 25 May 2016
Accepted 31 May 2016
Available online 2 June 2016

Keywords:
13α-Estrone
Azide-alkyne cycloaddition
17-Deoxy-13α-estrone
Antiproliferative effect

ABSTRACT

13α-Estrone derivatives containing various substituents on C-3 and C-17 were synthesized, and evaluated by means of MTT assays for *in vitro* antiproliferative activity against a panel of human adherent cancer cell lines (HeLa, MCF-7, A2780 and A431). Compounds with *N*-benzyltriazolylmethoxy moieties on C-3 proved to be more potent than their 3-hydroxy or 3-ether counterparts. Some triazoles exerted substantial cytostatic effects against particular tumor cell lines, with submicromolar IC_{50} values.

1. Introduction

Anticancer drugs administered for the treatment of estrogen-dependent cancers are often analogs of natural estrogenic compounds. The development of estrone-based anticancer agents [1–6] lacking estrogenic activity is one of the major challenges in the medicinal chemistry of steroids. The literature reveals that inversion of the configuration of C-13 in estrone (E1) or 17α/β-estradiols leads to a complete loss of the estrogenic activity, which results from the conformational changes [7–9]. 13α-Estrone, which is readily available from E1 by the method of Yaremenko and Khvat [10], is an appropriate scaffold for the design of hormonally inactive agents with selective biological potency. The first reported antiproliferative 13α-estrone derivative was the 3-benzyl ether of the 16-oxime propionate, which proved to be potent against particular cancer cell lines, inducing apoptosis with high tumorselectivity [11]. We recently published the synthesis and CuAAC (azide-alkyne click reaction) of steroidal azidoalcohols with terminal alkynes [12–16]. Some of the formed triazoles displayed pronounced *in vitro* antiproliferative effects on certain human malignant cell lines. On the basis of these outstanding results, we continued the design of potentially antitumoral 13α-estrone derivatives by incorporating triazole rings, in order to enhance the solubility, the bioavailability and the stability against metabolic degradation [17]. We therefore synthesized two series of diastereomeric *trans*-16-phenyltriazolyl-17-estradiol 3-benzyl ethers of 13α-estrone. It was confirmed that the 16β,17α isomers bearing *p*-alkyl substituents on the triazolylphenyl ring displayed substantial growth-inhibitory properties against 7 cancer cell lines with apoptosis induction via the intrinsic pathway [18]. The diastereomeric 13α-estradiols have been reported to be moderate antiproliferative agents [7], and incorporation of the 3-benzyl and 16β-triazolyl functions into the 13α,17α-estradiol core significantly improved the growth-inhibitory activity. These results indicate that the introduction of structural elements such as a triazole moiety at other positions of 13α-estrone may lead to enhanced antitumor properties. Drasar et al. recently described the synthesis of 3-*O*-propargylestrone [20], using the propargylation procedure of Skorobogatyi et al. [19]. The CuAAC reaction of *O*-propargylestrone with 2,6-bis(azidomethyl)pyridine furnished a bridged steroidal homodimer. The triazolyl conjugate was tested on a panel of steroid receptor reporter cell lines to determine the capacity of the compound to modulate the transcriptional activity of different steroid receptors. The conjugate displayed moderate potency in both the estrogen receptor α (ERα) and the estrogen receptor β (ERβ) assays, these modifications of E1 therefore resulting in retained estrogenic activity. The compound exerted substantial cytostatic activity against the CEM–DNR-BULK cell line, which is a daunorubicin-resistant derivative of CCRF-CEM cells. These results indicate that introduction of the triazolyl function onto

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http://dx.doi.org/10.1016/j.steroids.2016.05.010
0039-128X/

the 3-OH of the estrane skeleton may lead to cytostatic properties, but complete loss of the estrogenic activity of the antitumoral derivative would be of primary importance.

In view of the promising literature evidences, we decided to synthesize triazolyl derivatives of 13α-estrone through incorporation of the heterocyclic moiety onto the 3-hydroxy function. We aimed to determine the *in vitro* antiproliferative activities of the newly synthetized derivatives and their precursors by means of MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assays against a panel of human adherent cancer cell lines (HeLa, MCF-7, A431 and A2780).

2. Methods and materials

2.1. Chemical synthesis

2.1.1. General methods

Compounds used as starting materials **2** [8], **3** [21] and **15** [22] were obtained by the literature methods. Debenzylation [21] of **3** led to steroid **1** [23]. Melting points (mp) were determined with a Kofler hot-stage apparatus and are uncorrected. Elemental analyses were performed with a PerkinElmer CHN analyzer model 2400. Thin-layer chromatography: silica gel 60 F254; layer thickness 0.2 mm (Merck); eluents: (A) CH_2Cl_2, (B) EtOAc/CH_2Cl_2 = 2/98, (C) EtOAc/CH_2Cl_2 = 5/95, (D) EtOAc/CH_2Cl_2 = 30/70, (E) CH_2Cl_2/hexane = 10/90; detection with iodine or UV (365 nm) after spraying with 5% phosphomolybdic acid in 50% aqueous phosphoric acid and heating at 100–120 °C for 10 min. Flash chromatography: silica gel 60, 40–63 mm (Merck). ^{1}H NMR spectra were recorded in $CDCl_3$ solution (if not otherwise stated) with a Bruker DRX-500 instrument at 500 MHz, with Me_4Si as internal standard. ^{13}C NMR spectra were recorded with the same instrument at 125 MHz under the same conditions. Full-scan mass spectra of the compounds were acquired in the range 50–800 *m/z* with an Agilent 500MS Ion trap mass spectrometer equipped with an electrospray ionization source. Analyses were performed in positive ion mode, if not otherwise stated. Capillary and needle voltages were 80 and 5000 V, respectively. RF loading was set at 88%. Nebulizing gas (N_2) and drying gas (N_2) pressures were maintained at 60 and 20 psi, respectively. Drying gas temperature was held at 300 °C. The spectra were collected by continuous infusion of the steroid solution at a concentration of 10 ng μl^{-1} in acetonitrile/5 mM ammonium formate 50/50 (v/v%) at a flow rate of 15 $\mu l\ min^{-1}$. The analytical HPLC measurements were performed on an Agilent 1260 Infinity HPLC equipped with a Micro Vacuum Degasser, Binary Pump, Standard Autosampler, Thermostated Column Compartment, and Variable Wavelength Detector. The chromatographic separation was achieved at 40 °C on a Gemini NX C-18 analytical column (3 mm, 150 × 2 mm) from Phenomenex, equipped with a C-18 guard column, using gradient elution. Mobile phase A was water (Sigma–Aldrich Ltd., Budapest, Hungary), while mobile phase B was acetonitrile (Merck Ltd., Budapest, Hungary). A linear gradient was applied from 20% B to 100% B in 10 min (holding time: 5 min), the B content was then lowered to 20% in 5 min, and finally the column was re-equilibrated for 5 min. The flow rate was set to 0.2 ml/min.

2.1.2. General procedure for the reduction of 17-ketones (**1**–**3**)

To a stirred solution of 3-hydroxy-13α-estra-1,3,5(10)-trien-17-one (**1**) (270 mg, 1.0 mmol) or 3-methoxy-13α-estra-1,3,5(10)-trien-17-one (**2**) (284 mg, 1.0 mmol) or 3-benzyloxy-13α-estra-1,3,5(10)-trien-17-one (**3**) (360 mg, 1.0 mmol) in a 1:1 mixture of MeOH and CH_2Cl_2 (5 ml), $NaBH_4$ (189 mg, 5.0 mmol) was added. The reaction mixture was stirred at room temperature for 3 h, then diluted with water and extracted with CH_2Cl_2. The combined organic phases were washed with water until neutral, dried over Na_2SO_4 and evaporated *in vacuo*. The crude product was a mixture of the 17β-hydroxy (**4**–**6**) and the 17α-hydroxy (**7**–**9**) diastereomers in a ratio of nearly 1:1.

*2.1.2.1. 3-Hydroxy-13α-estra-1,3,5(10)-trien-17β-ol (**4**) and 3-hydroxy-13α-estra-1,3,5(10)-trien-17α-ol (**7**).* As described in Section 2.1.2, ketone **1** (270 mg, 1.0 mmol) was reacted with $NaBH_4$ (189 mg, 5.0 mmol). Compounds **4** and **7** were identical with compounds described in the literature [7]. **4**: Mp 185–187 °C, R_f = 0.62 (ss D); ^{1}H NMR (DMSO-d_6): δ_H 0.84 (s, 3H, H-18); 2.64 (m, 2H, H-6); 3.62 (m, 1H, H-17); 4.40 (s, 1H, OH); 6.40 (s, 1H, H-4); 6.50 (d, *J* = 8.5 Hz, 1H, H-2); 7.02 (d, *J* = 8.5 Hz, 1H, H-1). ^{13}C NMR (DMSO-d_6): δ_C 26.0; 28.6 (2C); 29.7 (C-18); 29.8; 31.8; 32.7; 39.9; 41.3; 43.8 (C-13); 51.1; 81.2 (C-17); 112.9 (C-2); 114.4 (C-4); 126.9 (C-1); 131.3 (C-10); 137.4 (C-5); 154.5 (C-3). **7**: Mp 192–194 °C, R_f = 0.53 (ss D); ^{1}H NMR (CD_3OD): δ_H 0.94 (s, 3H, H-18); 2.74 (m, 2H, H-6); 4.20 (m, 1H, H-17); 4.40 (s, 1H, OH); 6.48 (s, 1H, H-4); 6.58 (d, *J* = 8.5 Hz, 1H, H-2); 7.15 (d, *J* = 8.5 Hz, 1H, H-1). ^{13}C NMR (CD_3OD): δ_C 22.2 (C-18); 23.6; 26.6; 28.6; 28.7; 30.2; 32.7; 42.3; 42.8; 43.3 (C-13); 50.3; 73.2 (C-17); 112.5 (C-2); 114.4 (C-4); 126.5 (C-1); 131.1 (C-10); 137.8 (C-5); 154.5 (C-3).

*2.1.2.2. 3-Methoxy-13α-estra-1,3,5(10)-trien-17β-ol (**5**) and 3-methoxy-13α-estra-1,3,5(10)-trien-17α-ol (**8**).* As described in Section 2.1.2, ketone **2** (284 mg, 1.0 mmol) was reacted with $NaBH_4$ (189 mg, 5.0 mmol). Compounds **5** and **8** were identical with compounds described in the literature [8]. **5**: Mp 75–77 °C, R_f = 0.52 (ss C); ^{1}H NMR (DMSO-d_6): δ_H 0.85 (s, 3H, H-18); 2.71 (m, 2H, H-6); 3.63 (m, 1H, H-17); 3.68 (s, 3H, 3-OMe); 4.43 (s, 1H, OH); 6.57 (s, 1H, H-4); 6.66 (d, *J* = 8.5 Hz, 1H, H-2); 7.14 (d, *J* = 8.5 Hz, 1H, H-1). ^{13}C NMR (DMSO-d_6): δ_C 26.2; 28.6; 28.7; 29.8 (18-Me); 30.1; 31.9; 32.9; 40.1; 41.3; 43.9 (C-13); 51.2; 54.9 (3-OMe); 81.3 (C-17); 111.8 (C-2); 113.0 (C-4); 127.2 (C-1); 133.1 (C-10); 137.7 (C-5); 156.8 (C-3). **8**: Mp 105–107 °C, R_f = 0.41 (ss C); ^{1}H NMR (DMSO-d_6): δ_H 0.82 (s, 3H, H-18); 2.71 (m, 2H, H-6); 3.68 (s, 3H, 3-OMe); 4.01 (m, 1H, H-17); 4.35 (s, 1H, OH); 6.58 (s, 1H, H-4); 6.66 (d, *J* = 8.5 Hz, 1H, H-2); 7.18 (d, *J* = 8.5 Hz, 1H, H-1). ^{13}C NMR (DMSO-d_6): δ_C 22.9; 23.6 (18-Me); 26.3; 28.1; 29.2; 29.9; 32.7; 41.8 (2C); 42.9 (C-13); 49.7 (C-14); 54.7 (3-OMe); 71.7 (C-17); 111.5 (C-2); 113.1 (C-4); 126.6 (C-1); 131.9 (C-10); 137.7 (C-5); 156.9 (C-3).

*2.1.2.3. 3-Benzyloxy-13α-estra-1,3,5(10)-trien-17β-ol (**6**) and 3-benzyloxy-13α-estra-1,3,5(10)-trien-17α-ol (**9**).* As described in Section 2.1.2, ketone **3** (360 mg, 1.0 mmol) was reacted with $NaBH_4$ (189 mg, 5.0 mmol). The crude product was purified by flash chromatography with EtOAc/CH_2Cl_2 = 2/98 as eluent. The first-eluted compound **6** was obtained as a white solid after evaporation of the eluent (102 mg, 28%), mp 64–66 °C, R_f = 0.58 (ss B); ^{1}H NMR: δ_H 0.95 (s, 3H, H-18); 2.79 (m, 2H, H-6); 3.83 (m, 1H, H-17α); 5.03 (s, 2H, OCH_2); 6.69 (s, 1H, H-4); 6.77 (dd, *J* = 8.6 Hz, *J* = 2.3 Hz, 1H, H-2); 7.19 (d, *J* = 8.6 Hz, 1H, H-1); 7.31 (t, *J* = 7.3 Hz, 1H, H-4′); 7.37 (t, *J* = 7.3 Hz, 2H, H-3′, H-5′); 7.42 (d, *J* = 7.3 Hz, 2H, H-2′, H-6′). ^{13}C NMR: δ_C 26.7; 28.5; 29.0; 29.8 (C-18); 30.5; 31.4; 33.2; 40.1; 42.2; 44.4 (C-13); 51.5; 69.9 (OCH_2); 83.5 (C-17); 112.6 (C-2); 114.4 (C-4); 127.4 (3C: C-1, C-3′, C-5′); 127.8 (C-4′); 128.5 (2C: C-2′, C-6′); 133.7 (C-10); 137.4 (C-1′); 138.3 (C-5); 156.5 (C-3). ESI-MS *m/z* (%): 363 (100, $[M+H]^+$), 91 (15, Bn). Anal. Calcd. for $C_{25}H_{30}O_2$: C, 82.83; H, 8.34. Found: C, 82.95; H, 8.26. Purity from HPLC: 99.1%. Continued elution yielded a mixture of alcohols **6** and **9** (119 mg, 33%) and finally compound **9** (120 mg, 33%), mp 80–84 °C, R_f = 0.45 (ss B); ^{1}H NMR: δ_H 0.95 (s, 3H, H-18); 2.79 (m, 2H, H-6); 4.21 (m, 1H, H-17β); 5.03 (s, 2H, OCH_2); 6.72 (s, 1H, H-4); 6.81 (dd, *J* = 8.6 Hz, *J* = 2.3 Hz, 1H, H-2); 7.23 (d, *J* = 8.6 Hz, 1H, H-1); 7.31 (t, *J* = 7.3 Hz, 1H, H-4′); 7.39 (t,