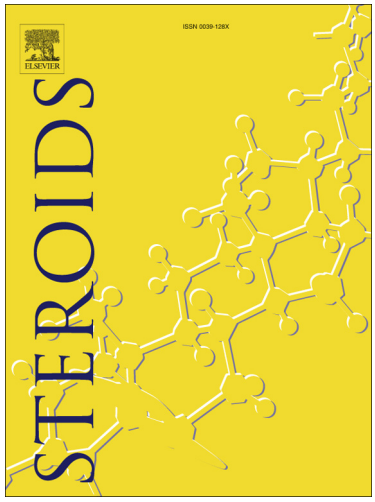

Synthesis, cytotoxic effects and tubulin polymerization inhibition of 1,4-disubstituted 1,2,3-triazole analogs of 2-methoxyestradiol

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PII: S0039-128X(14)00133-0
DOI: http://dx.doi.org/10.1016/j.steroids.2014.05.020
Reference: STE 7582

To appear in: *Steroids*

Received Date: 14 March 2014
Revised Date: 16 May 2014
Accepted Date: 25 May 2014

Please cite this article as: Solum, E.J., Vik, A., Hansen, T.V., Synthesis, cytotoxic effects and tubulin polymerization inhibition of 1,4-disubstituted 1,2,3-triazole analogs of 2-methoxyestradiol, *Steroids* (2014), doi: http://dx.doi.org/10.1016/j.steroids.2014.05.020

Graphical Abstract

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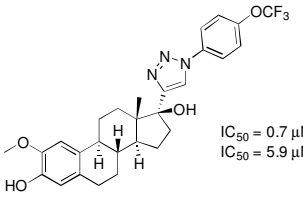

IC_{50} = 0.7 µM (cytotoxicity)
IC_{50} = 5.9 µM (tubulin inhibition)

Synthesis, cytotoxic effects and tubulin polymerization inhibition of 1,4-disubstituted 1,2,3-triazole analogs of 2-methoxyestradiol

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Abstract:
Thirteen 1,4-disubstituted 1,2,3-triazole analogs of 2-methoxyestradiol were prepared and tested for their cytotoxic and tubulin polymerization inhibition effects. Two compounds, **11j** and **11k**, exhibited anti-proliferative effects at low micromolar concentrations. The two analogs **11j** and **11k** also inhibited tubulin assembly with IC_{50} values of 8.1 and 5.9 μM, respectively.

Keywords: estrogens; 2-methoxyestradiol, 1,2,3-triazoles; cytotoxicity; tubulin inhibition; anti-cancer.

1. Introduction

Several steroids have been reported to exhibit anti-cancer effects [1] and some examples are depicted in Figure 1. One such example is 2-methoxyestradiol (2-ME, **1**), the endogen metabolite of 17β-estradiol. The estrogen **1** is oxidized by cytochrome P450 (CYP1A2 and CYP3A) to 2-hydroxyestradiol, followed by 2-*O*-methylation by catechol *O*-methyltransferase (COMT) [2]. It was previously believed that **1** was inactive. However, in 1994 D´Amato, Folkman and co-workers demonstrated that 2-ME (**1**) inhibits tubulin polymerization by interfering with the colcichine binding-site [3]. It has been reported that these effects lead to inhibition of angiogenesis in several cancer cell lines [4]. 2-ME (**1**) also showed potent *in vivo* anti-angiogenetic effects leading to inhibition of proliferation, migration and invasion of several cancer cell lines [5], [6]. In addition, it was also reported that 2-ME (**1**) down regulates hypoxia-inducible factor-1 α (HIF-1 α) resulting in potent cytotoxic effects in prostate and breast cancer cells [7]. These observations were later related to intrinsic and extrinsic apoptotic pathways [8] that were confirmed *in vivo* [9]. In addition, no toxic effects of 2-methoxyestradiol (**1**) have been observed in phase I/II clinical trials [10].

The aforementioned activities have spurred a great interest in using 2-ME (**1**) as a lead compound for the development of new anti-cancer agents [11]. The compounds **2**-**4** depicted in Figure 1 are examples of such analogs.

[Insert Figure 1 Here]

Fig. 1. Some examples of steroids exhibiting anti-cancer effects.

Most analogs of **1** have been prepared with the aim of reducing the metabolic oxidation of the hydroxyl group in the C-17 position, but also avoiding secondary

conjugation reactions at this and at the phenolic position [11], [12]. In connection with our interest in the synthesis of potential new anti-cancer agents, several 1,2,3-triazole analogs displayed tubulin polymerization inhibition, cytotoxic and anti-vascular effects in the high nanomolar range [13a], [13b], [13c], [13d]. We wanted to investigate if the introduction of a 1,4-disubstituted 1,2,3-triazole in the C-17 position of 2-methoxyestradiol (**1**) would induce cell death by inhibiting tubulin assembly. Such analogs should also reduce metabolic transformations. Modifications in this position have been reported to afford compounds, such as **3** and **4**, with anti-cancer activities [1a]. The regioselective copper(I) catalyzed cycloaddition reaction between terminal alkynes and azides affords 1,4-disubstituted-1,2,3-triazoles in high yields [14]. This reaction has found many applications in medicinal chemistry [15], including modifications of natural products [16]. Herein, the synthesis and the biological evaluations of 13 1,4-disubstituted-1,2,3-triazoles analogs of the steroid **1** are reported.

2. Experimental

2.1. General

All reagents and solvents were used as purchased without further purification unless stated otherwise. Melting points are uncorrected. Analytical TLC was performed using silica gel 60 F254 aluminum plates (Merck). Flash column chromatography was performed on silica gel 60 (40-63 μm) produced by Merck. NMR spectra were recorded on a Bruker Avance DPX-300 MHz, DPX-400 MHz or DPX-600 MHz spectrometer for ^{1}H NMR, and 75 MHz, 101 MHz or 151 MHz for ^{13}C NMR. Coupling constants (J) are reported in hertz, and chemical shifts are reported in parts per million relative to $CDCl_3$ (7.26 ppm for ^{1}H and 77.0 ppm for ^{13}C). Mass spectra were recorded at 70 eV with Fison's VG Pro spectrometer. High- resolution mass spectra were performed with a VG Prospec mass spectrometer and with a Micromass Q-TOF-2^{TM}. The HPLC analyses were performed on an Agilent Technologies 1200 Series instrument with an Eclipse XDB-C18 (5 mm 4.6 x 150 mm) column. The chemical purity of the products **11a**-**11k** and **12a**-**12b** was determined by HPLC, see Supporting information for details.

2.2. 2-Methoxyestrone

2-Methoxyestradiol (**1**) (2.42 g, 8.0 mmol) was placed in a flame dried 250 mL two-necked round-bottomed flask equipped with a 5 mL flame dried Dean-Stark trap and a reflux condenser. Toluene (80 mL) was added to dissolve the starting material. Aluminum *iso*-propoxide (8.17 g, 40.0 mmol) and cyclohexanone (33.1 mL, 320.0 mmol) were added, and the reaction mixture was heated at reflux for 20 h. The reaction mixture was allowed to cool to room temperature. Then water (100 mL) and 1 M HCl (45 mL) were added. The organic material was extracted with ethyl acetate (3 x 150 mL). The aqueous emulsion was acidified with 1 M HCl (50 mL) until the emulsion separated. The aqueous layer was extracted once more with ethyl acetate (100 mL). The combined organic extracts were dried ($MgSO_4$) and evaporated *in vacuo*. The residue was triturated with hexane to give the desired product in 88% yield (2.11 g). Mp 188-190 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.79 (s, 1 H), 6.66 (s, 1H), 5.48 (s, 1H), 3.86 (s, 3H), 2.88 – 2.72 (m, 2H), 2.51 (dd, J = 18.9, 8.6 Hz, 1H), 2.42 – 2.32 (m, 1H), 2.30 – 2.21 (m, 1H), 2.20 – 1.84 (m, 4H), 1.72 – 1.28 (m, 6H), 0.92 (s, 3H); ^{13}C NMR (101MHz, $CDCl_3$) δ 220.9, 144.7, 143.7, 131.1, 129.3, 114.7, 108.1, 56.1, 50.4, 48.0, 44.3, 38.4, 35.9, 31.6, 28.9, 26.6, 26.2, 21.6, 13.9.

*2.3. 3-Tert-butyldimethylsiloxy-2-methoxyestrone (**5**)*

2-Methoxyestrone (1.51 g, 5.0 mmol) was placed in a 100 mL round bottomed flask and dissolved in dry DMF (25 mL). Imidazole (0.85 g, 12.5 mmol) and TBSCl (1.13 g, 7.5 mmol) were added. The reaction mixture was stirred at room temperature under argon atmosphere for 5 h. Saturated aqueous NaCl (20 mL) was added, and the mixture was extracted with ethyl acetate (3 x 20 mL). The combined organic extracts were washed with brine (10 mL), aqueous AcOH (10%, 10 mL) and aqueous $NaHCO_3$ (10 mL) before it was dried ($MgSO_4$) and evaporated *in vacuo*. The product was purified by flash chromatography (silica gel, 20% ethyl acetate in hexane, R_f = 0.43) to give the pure product in 92% yield (1.91 g). Mp 163-166 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.77 (s, 1H), 6.56 (s, 1H), 3.77 (s, 3H), 2.83 – 2.74 (m, 2H), 2.50 (dd, *J* = 18.3, 8.4 Hz, 1H), 2.40 – 1.86 (m, 5H), 1.70 – 1.32 (m, 6H), 0.99 (s, 9H), 0.92 (s, 3H), 0.15 (s, 6H) ; ^{13}C NMR (101 MHz, $CDCl_3$) δ 221.1, 149.0, 143.3, 132.7, 128.9, 121.2, 110.0, 56.0, 50.7, 48.2, 44.5, 38.5, 36.1, 31.8, 28.9, 26.9, 26.3, 25.9, 21.8, 18.6, 14.1, -4.4, -4.3.

*2.4. 3-Tert-butyldimethylsiloxy-2-methoxyethynylestradiol (**6**)*

The TBS-protected estrone **5** (829 mg, 2.0 mmol) was dissolved in THF (20 mL) and cooled to 0 °C. A 0.5 M solution of ethynylmagnesiumbromide in THF (20.0 mL, 6.7 mmol) was added dropwise to the mixture over 15 min. The reaction mixture was stirred at 0 °C for additional 30 min., then brought to room temperature, and stirred until no sign of starting material (TLC) (approx. 4 h.). Upon completion saturated aqueous NH_4Cl (2 M, 20 mL) was added, and the mixture was extracted with ethyl acetate (3 x 30 mL). The combined organic phases were washed with brine, dried ($MgSO_4$), and evaporated *in vacuo*. The product was purified by flash chromatography (20% ethylacetate in hexane R_f =0.57) to give the pure product in 88% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.79 (s, 1H), 6.56 (s, 1H), 3.78 (s, 3H), 2.75 (dd, *J* = 11.1, 5.7 Hz, 2H), 2.60 (s, 1H), 2.39 – 2.30 (m, 2H), 2.28 – 2.16 (m, 1H), 2.09 – 1.68 (m, 7H), 1.56 – 1.24 (m, 4H), 1.00 (s, 9H), 0.91 (s, 3H), 0.17 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 148.7, 143.0, 133.1, 129.0, 121.0, 110.1, 87.6, 79.9, 74.0, 55.9, 49.5, 47.2, 43.9, 39.4, 39.1, 32.8, 28.9, 27.4, 26.6, 25.8, 22.8, 18.5, 12.8, -4.5, -4.6.

2.5. General procedure for the Cu(I) mediated 1,3-dipolar cycloaddition

TBS protected ethynylestradiol **6** (0.3 – 0.6 mmol) was placed in a 25 mL round-bottomed flask and dissolved in *t*-BuOH (4 – 6 mL) and water (2 – 3 mL). Copper(II) sulfate (0.1 equiv.), sodium ascorbate (0.2 equiv.) and the azide (**7a-7k)** or (**8a-8b)** (1.2 equiv.) were added. The reaction mixture was heated to 50 °C and stirred for 24 hours. Saturated aqueous NH_4Cl (20 mL) was added, and the mixture was extracted with ethyl acetate (3 x 20 mL). The combined organic extract were washed with brine (10 mL) and dried ($MgSO_4$). The solvent was removed *in vacuo*. The crude product was purified by flash chromatography (silica gel, 20% ethyl acetate in hexane) to give the pure product in 47 - 86% yield.

*2.6. 3-Tert-butyldimethylsiloxy-2-methoxyestra-17-(1-phenyl-1H-1,2,3-triazol-4-yl)diol (**9a**)*

Light yellow oil (231 mg, 83%), eluent: 20% EtOAc in hexane, R_f = 0.27. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 (s, 1H), 7.76 (d, *J* = 7.7 Hz, 2H), 7.52 (t, *J* = 7.8 Hz, 2H), 7.44 (d, *J* = 7.4 Hz, 1H), 6.68 (s, 1H), 6.53 (s, 1H), 3.72 (s, 3H), 2.94 (s, 1H), 2.80 – 2.64 (m, 2H), 2.60 – 2.41 (m, 1H), 2.24 – 2.09 (m, 2H), 2.06 – 1.85 (m, 3H), 1.78 – 1.41 (m, 5H), 1.42 – 1.24 (m, 1H), 1.10 (s, 3H), 0.99 (s, 9H), 0.86 – 0.74 (m, 1H),

0.14 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.6, 148.7, 143.0, 137.2, 133.2, 129.8, 129.1, 128.6, 121.0, 120.5, 119.5, 110.1, 82.6, 56.0, 48.7, 47.6, 43.8, 39.5, 38.2, 33.2, 29.0, 27.6, 26.5, 25.9, 23.6, 18.5, 14.5, -4.5, -4.5.

*2.7. 3-Tert-butyldimethylsiloxy-2-methoxyestra-17-(1-(4-methylphenyl)-1H-1,2,3-triazol-4-yl)diol (**9b**)*

Light yellow oil (103 mg, 61%), eluent: 20% EtOAc in hexane, R_f = 0.27. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84 (s, 1H), 7.64 (d, *J* = 8.4 Hz, 2H), 7.32 (d, *J* = 8.2 Hz, 2H), 6.68 (s, 1H), 6.53 (s, 1H), 3.72 (s, 3H), 2.82 – 2.69 (m, 2H), 2.43 (s, 4H), 2.24 – 2.10 (m, 2H), 2.09 – 1.82 (m, 3H), 1.76 – 1.27 (m, 6H), 1.09 (s, 3H), 0.98 (s, 9H), 0.84 – 0.73 (m, 1H), 0.14 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.3, 148.8, 143.1, 139.0, 134.9, 133.2, 130.4, 129.2, 121.1, 120.5, 119.5, 110.1, 82.6, 56.0, 48.8, 47.6, 43.9, 39.6, 38.2, 33.2, 29.1, 27.7, 26.6, 25.9, 23.6, 21.3, 18.6, 14.5, -4.4, -4.4.

*2.8. General procedure for **7c** and **7d***

Sodium azide (1.2 mmol, 76 mg) and copper(II) sulfate (0.1 mmol, 16 mg) were added to a 25 mL round-bottomed flask and dissolved in methanol (3 mL). Then 4-*tert*-butylphenylboronicacid (1 mmol, 0.178 g) or 4-hydroxyphenylboronicacid (1 mmol, 138 mg) was added. The reaction mixture was heated to 40 °C, and stirred until no sign of starting material (TLC), ca. 2 h. Upon completion the reaction mixture was cooled to room temperature, and the solvent was evaporated and the crude product was used in the corresponding cycloaddition reactions, with no further purification.

*2.9. 3-Tert-butyldimethylsiloxy-2-methoxyestra-17-(1-(4-(tert-butyl)phenyl)-1H-1,2,3-triazol-4-yl)diol (**9c**)*

Light yellow oil (181 mg, 49% over two steps), eluent: 20% EtOAc in hexane, R_f = 0.30. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.87 (s, 1H), 7.68 (d, *J* = 8.7 Hz, 2H), 7.53 (d, *J* = 8.8 Hz, 2H), 6.68 (s, 1H), 6.53 (s, 1H), 3.72 (s, 3H), 2.81 – 2.65 (m, 2H), 2.56 – 2.44 (m, 1H), 2.23 – 2.10 (m, 2H), 2.04 – 1.95 (m, 2H), 1.95 – 1.87 (m, 1H), 1.72 – 1.43 (m, 5H), 1.36 (s, 9H), 1.10 (s, 3H), 0.98 (s, 9H), 0.89 (t, *J* = 7.0 Hz, 1H), 0.80 (td, *J* = 12.9, 4.0 Hz, 1H), 0.14 (d, *J* = 2.0 Hz, 6H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 154.3, 152.1, 148.7, 143.0, 134.8, 133.2, 129.1, 126.7, 121.0, 120.3, 119.5, 110.0, 82.6, 56.0, 47.5, 43.8, 39.5, 38.2, 34.9, 33.2, 31.4, 29.0, 27.6, 26.5, 25.9, 23.6, 22.8, 18.5, 14.5, -4.4, -4.5.

*2.10. 3-Tert-butyldimethylsiloxy-2-methoxyestra-17-(1-(4-hydroxyphenyl)-1H-1,2,3-triazol-4-yl)diol (**9d**)*

Red oil (162 mg, 47% over two steps), eluent: 20% EtOAc in hexane, R_f = 0.30. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.83 (s, 1H), 7.49 (d, *J* = 8.6 Hz, 2H), 6.98 (d, *J* = 8.7 Hz, 2H), 6.66 (s, 1H), 6.52 (s, 1H), 3.71 (s, 3H), 2.82 – 2.60 (m, 2H), 2.56 – 2.39 (m, 1H), 2.24 – 2.16 (m, 1H), 2.18 – 2.10 (m, 1H), 2.02 – 1.93 (m, 2H), 1.92 – 1.85 (m, 1H), 1.72 (d, *J* = 12.4 Hz, 1H), 1.64 – 1.55 (m, 2H), 1.54 – 1.41 (m, 2H), 1.34 – 1.24 (m, 1H), 1.10 (s, 3H), 0.97 (s, 9H), 0.84 – 0.70 (m, 1H), 0.13 (s, 6H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 157.2, 153.9, 148.6, 142.9, 133.0, 129.7, 129.0, 122.3, 121.0, 120.2, 116.6, 110.0, 82.6, 56.0, 48.7, 47.5, 43.7, 39.3, 38.0, 33.1, 28.9, 27.4, 26.4, 25.8, 18.4, 14.4, -4.6.

*2.11. 3-Tert-butyldimethylsiloxy-2-methoxyestra-17-(1-(4-flourophenyl)-1H-1,2,3-triazol-4-yl)diol (**9e**)*

Light yellow oil (237 mg, 81%), eluent: 20% EtOAc in hexane, R_f = 0.28. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.87 (s, 1H), 7.80 – 7.63 (m, 2H), 7.25 – 7.13 (m, 2H), 6.68 (s, 1H), 6.53 (s, 1H), 3.72 (s, 3H), 2.85 – 2.66 (m, 3H), 2.57 – 2.43 (m, 1H), 2.23 – 2.10 (m, 2H), 2.05 – 1.86 (m, 3H), 1.75 – 1.27 (m, 6H), 1.09 (s, 3H), 0.98 (s, 9H), 0.86 – 0.71 (m, 1H), 0.14 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.7, 161.2, 154.8, 148.7, 143.0, 133.5 (d, *J* = 3.1 Hz), 133.2, 129.1, 122.5, 122.4, 121.1, 119.7, 116.9, 116.7, 110.1, 82.6, 56.0, 48.7, 47.5, 43.8, 39.5, 38.2, 33.2, 29.0, 27.6, 26.5, 25.9, 23.6, 18.5, 14.4, -4.5, -4.5.

*2.13. 3-Tert-butyldimethylsiloxy-2-methoxyestra-17-(1-(4-chlorophenyl)-1H-1,2,3-triazol-4-yl)diol (**9f**)*

Light yellow oil (233 mg, 78%), eluent: 20% EtOAc in hexane, R_f = 0.28. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88 (s, 1H), 7.72 (d, *J* = 8.9 Hz, 2H), 7.49 (d, *J* = 8.9 Hz, 2H), 6.67 (s, 1H), 6.53 (s, 1H), 3.72 (s, 3H), 2.81 – 2.66 (m, 2H), 2.65 – 2.54 (m, 1H), 2.50 (ddd, *J* = 14.5, 9.5, 5.6 Hz, 1H), 2.23 – 2.08 (m, 2H), 2.04 – 1.95 (m, 2H), 1.96 – 1.86 (m, 1H), 1.72 – 1.24 (m, 6H), 1.09 (s, 3H), 0.98 (s, 9H), 0.84 – 0.73 (m, 1H), 0.14 (d, *J* = 1.1 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.9, 148.8, 143.1, 135.7, 134.6, 133.2, 130.0, 129.2, 121.7, 121.1, 119.4, 110.1, 82.6, 56.0, 48.8, 47.6, 43.8, 39.5, 38.3, 33.2, 29.0, 27.6, 26.5, 25.9, 23.6, 18.5, 14.5, -4.4, -4.5.

*2.14. 3-Tert-butyldimethylsiloxy-2-methoxyestra-17-(1-(4-bromophenyl)-1H-1,2,3-triazol-4-yl)diol (**9g**)*

Light yellow oil (254 mg, 80%), eluent: 20% EtOAc in hexane, R_f = 0.28. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.89 (s, 1H), 7.64 (s, 4H), 6.67 (s, 1H), 6.53 (s, 1H), 3.72 (s, 3H), 2.83 – 2.58 (m, 2H), 2.53 – 2.38 (m, 1H), 2.26 – 2.07 (m, 3H), 2.08 – 1.84 (m, 3H), 1.76 – 1.23 (m, 8H), 1.09 (s, 3H), 0.98 (s, 9H), 0.14 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 154.9, 148.7, 143.0, 136.1, 133.1, 133.00, 129.1, 122.3, 121.9, 121.1, 119.3, 110.0, 82.6, 56.0, 48.7, 47.6, 43.8, 39.5, 38.2, 33.2, 29.0, 27.6, 26.5, 25.9, 23.6, 22.8, 18.5, 14.4, -4.5, -4.5. Eluent: 20% EtOAc in hexane, R_f=0.28, yield (254 mg, 80%).

*2.15. 3-Tert-butyldimethylsiloxy-2-methoxyestra-17-(1-(4-methoxyphenyl)-1H-1,2,3-triazol-4-yl)diol (**9h**)*

Light yellow oil (239 mg, 81%), eluent: 20% EtOAc in hexane, R_f = 0.23. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80 (s, 1H), 7.65 (d, *J* = 9.0 Hz, 2H), 7.02 (d, *J* = 9.0 Hz, 2H), 6.67 (s, 1H), 6.52 (s, 1H), 3.86 (s, 3H), 3.72 (s, 3H), 2.86 – 2.66 (m, 2H), 2.56 – 2.39 (m, 2H), 2.31 – 2.07 (m, 2H), 2.04 – 1.84 (m, 3H), 1.74 – 1.22 (m, 6H), 1.09 (s, 3H), 0.98 (s, 9H), 0.81 (td, *J* = 12.7, 3.8 Hz, 1H), 0.13 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.9, 154.3, 148.8, 143.0, 133.2, 130.7, 129.2, 122.2, 121.1, 119.6, 114.9, 110.1, 82.6, 56.0, 55.8, 48.8, 47.6, 43.8, 39.6, 38.2, 33.2, 29.0, 27.6, 26.6, 25.9, 23.6, 18.5, 14.5, -4.4, -4.5.

*2.16. 3-Tert-butyldimethylsiloxy-2-methoxyestra-17-(1-(3,4,5-trimethoxyphenyl)-1H-1,2,3-triazol-4-yl)diol (**9i**)*

Light yellow oil (224 mg, 69%), eluent: 20% EtOAc in hexane, R_f = 0.17. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84 (s, 1H), 6.97 (s, 2H), 6.67 (s, 1H), 6.53 (s, 1H), 3.93 (s, 6H), 3.89 (s, 3H), 3.72 (s, 3H), 2.81 – 2.63 (m, 2H), 2.61 – 2.40 (m, 2H), 2.25 – 2.09 (m, 2H), 2.09 – 1.86 (m, 3H), 1.78 – 1.20 (m, 6H), 1.09 (s, 3H), 0.98 (s, 9H), 0.83 (dt, *J* = 21.7, 10.4 Hz, 1H), 0.14 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.5, 154.1, 148.8, 143.1, 138.5, 133.2, 133.1, 129.2, 121.1, 119.8, 110.1, 98.6, 82.7, 61.2, 56.6, 56.1, 48.8, 47.6, 43.8, 39.6, 38.3, 33.2, 29.0, 27.7, 26.6, 25.9, 23.7, 18.6, 14.5, -4.4, -4.5.

*2.17. 3-Tert-butyldimethylsiloxy-2-methoxyestra-17-(1-(4-(trifluoromethyl)phenyl)-1H-1,2,3-triazol-4-yl)diol (**9j**)*

Light yellow oil (228 mg, 73%), eluent: 20% EtOAc in hexane, R_f = 0.26. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.97 (s, 1H), 7.93 (d, *J* = 8.5 Hz, 2H), 7.80 (d, *J* = 8.6 Hz, 2H), 6.67 (s, 1H), 6.53 (s, 1H), 3.72 (s, 3H), 2.82 – 2.67 (m, 2H), 2.61 – 2.42 (m, 1H), 2.24 – 2.07 (m, 3H), 2.06 – 1.83 (m, 3H), 1.78 – 1.23 (m, 8H), 1.10 (s, 3H), 0.98 (s, 9H), 0.14 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 155.2, 148.8, 143.1, 133.1, 129.2, 127.3, 127.2, 121.1, 120.4, 119.3, 110.1, 82.7, 56.0, 48.8, 47.6, 43.8, 39.6, 38.4, 33.2, 31.7, 29.0, 27.6, 26.5, 25.9, 23.7, 22.8, 18.56, 14.5, -4.4, -4.4.

*2.18. 3-Tert-butyldimethylsiloxy-2-methoxyestra-17-(1-(4-(trifluoromethoxyl)phenyl)-1H-1,2,3-triazol-4-yl)diol (**9k**)*

Light yellow oil (216 mg, 67%), eluent: 20% EtOAc in hexane, R_f = 0.23. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.96 (s, 1H), 7.93 (d, *J* = 8.5 Hz, 2H), 7.81 (d, *J* = 8.5 Hz, 2H), 6.67 (s, 1H), 6.53 (s, 1H), 3.72 (s, 3H), 2.81 – 2.66 (m, 2H), 2.60 – 2.43 (m, 1H), 2.25 – 2.07 (m, 2H), 2.07 – 1.87 (m, 3H), 1.78 – 1.22 (m, 8H), 1.10 (s, 3H), 0.98 (s, 9H), 0.22 – 0.03 (m, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 155.2, 148.8, 143.1, 133.1, 129.2, 127.3, 127.2, 121.1, 120.5, 119.3, 110.1, 82.7, 56.1, 48.8, 47.6, 43.8, 39.6, 38.4, 33.2, 29.0, 27.6, 26.5, 26.4, 25.9, 23.7, 22.8, 18.6, 14.5, -4.4, -4.4.

*2.19. 3-Tert-butyldimethylsiloxy-2-methoxyestra-17-(1-(4-methoxybenzyl)-1H-1,2,3-triazol-4-yl)diol (**10a**)*

Light yellow oil (184 mg, 61%), eluent: 20% EtOAc in hexane, R_f = 0.19. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.30 (s, 1H), 7.22 (d, *J* = 8.6 Hz, 2H), 6.89 (d, *J* = 8.6 Hz, 2H), 6.68 (s, 1H), 6.52 (s, 1H), 5.49 (d, *J* = 14.8 Hz, 1H), 5.44 (d, *J* = 14.8 Hz, 1H), 3.80 (s, 3H), 3.73 (s, 3H), 2.80 – 2.63 (m, 2H), 2.37 – 2.28 (m, 1H), 2.15 – 2.05 (m, 2H), 1.98 – 1.82 (m, 3H), 1.61 (dt, *J* = 12.4, 2.9 Hz, 1H), 1.55 – 1.38 (m, 4H), 1.33 – 1.23 (m, 1H), 1.03 (s, 3H), 0.98 (s, 9H), 0.65 (td, *J* = 12.9, 4.0 Hz, 1H), 0.14 (d, *J* = 1.2 Hz, 6H).^{13}C NMR (151 MHz, $CDCl_3$) δ 160.0, 154.0, 148.8, 143.0, 133.2, 129.7, 129.1, 126.7, 121.1, 121.0, 114.6, 110.0, 82.4, 56.0, 55.5, 53.9, 48.7, 47.5, 43.9, 39.5, 38.0, 33.2, 29.0, 27.6, 26.5, 25.9, 23.5, 18.6, 14.4, -4.4, -4.5.

*2.20. 3-Tert-butyldimethylsiloxy-2-methoxyestra-17-(1-(3,4,5-trimethoxybenzyl)-1H-1,2,3-triazol-4-yl)diol (**10b**)*

Light yellow oil (214 mg, 64%), eluent: 20% EtOAc in hexane, R_f = 0.16. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.38 (s, 1H), 6.65 (s, 1H), 6.52 (s, 1H), 6.46 (s, 2H), 5.49 (d, *J* = 14.9 Hz, 1H), 5.41 (d, *J* = 14.9 Hz, 1H), 3.82 (s, 3H), 3.81 (s, 6H), 3.72 (s, 3H), 2.80 – 2.65 (m, 2H), 2.42 – 2.33 (m, 1H), 2.12 – 2.02 (m, 2H), 1.96 – 1.81 (m, 3H), 1.63 – 1.58 (m, 2H), 1.57 – 1.38 (m, 4H), 1.33 – 1.24 (m, 1H), 1.04 (s, 3H), 0.97 (s, 9H), 0.66 (td, *J* = 12.8, 3.9 Hz, 1H), 0.13 (d, *J* = 1.4 Hz, 6H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 154.2, 153.8, 148.7, 143.0, 138.3, 133.1, 130.4, 129.1, 121.3, 121.1, 110.0, 105.1, 82.5, 61.0, 56.3, 56.0, 54.5, 48.7, 47.5, 43.9, 39.5, 38.1, 33.2, 29.0, 27.6, 26.5, 25.9, 23.5, 18.5, 14.4, -4.5, -4.5

2.21. General procedure for the deprotection of the TBS group

The TBS protected steroid phenol (**9a-9k** or **10a-10b**) (0.3-0.6 mmol, 1 equiv.) was placed in a dry round-bottomed flask under argon atmosphere, and dissolved in dry THF. Tetra-*n*-butylammonium fluoride (1 M in THF, 1.1 equiv.) was added dropvise. The reaction mixture was stirred at room temperature (16-18 h.). Upon completion the reaction mixture was poured into saturated aqueous $NaHCO_3$ (10 mL) and extracted with ethyl acetate (4 x 5 mL). The combined organic extract was dried ($MgSO_4$) and

the solvent evaporated *in vacuo*. The residue was purified by chromatography (silica gel, 20 – 70% ethyl acetate in hexane) to give the pure product.

*2.22. 2-Methoxyestra-17-(1-phenyl-1H-1,2,3-triazol-4-yl)diol (**11a**)*
Light yellow solid (151 mg, 83%), mp. 141-145 °C, eluent: 20% - 50% EtOAc in hexane, R_f = 0.16. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (s, 1H), 7.69 – 7.50 (m, 2H), 7.36 (t, *J* = 7.7 Hz, 2H), 7.27 (t, *J* = 7.4 Hz, 1H), 6.53 (s, 1H), 6.46 (s, 1H), 3.64 (s, 3H), 2.74 – 2.51 (m, 2H), 2.43 – 2.22 (m, 1H), 2.12 – 1.92 (m, 2H), 1.95 – 1.68 (m, 3H), 1.59 – 1.08 (m, 6H), 0.94 (s, 3H), 0.71 – 0.51 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.5, 144.7, 143.5, 137.2, 131.8, 129.9, 129.6, 128.8, 120.5, 119.5, 114.7, 108.2, 82.5, 56.2, 48.7, 47.6, 43.7, 39.6, 38.2, 33.1, 29.1, 27.5, 26.7, 23.6, 14.4. HRMS (EI^+) calcd. for $C_{27}H_{31}N_3O_3$ $[M]\bullet^+$: 445.2365. Found 445.2362.

*2.23. 2-Methoxyestra-17-(1-(4-methylphenyl)-1H-1,2,3-triazol-4-yl)diol (**11b**)*
Light yellow solid (59 mg, 72%), mp. 142-144 °C, eluent: 20% - 50% EtOAc in hexane, R_f = 0.16. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.78 (s, 1H), 7.56 (d, *J* = 8.4 Hz, 2H), 7.23 (d, *J* = 8.1 Hz, 2H), 6.61 (s, 1H), 6.54 (s, 1H), 3.72 (s, 3H), 2.76 – 2.58 (m, 2H), 2.44 – 2.37 (m, 1H), 2.34 (s, 3H), 2.14 – 2.01 (m, 2H), 1.94 – 1.87 (m, 2H), 1.86 – 1.79 (m, 1H), 1.64 – 1.35 (m, 5H), 1.29 – 1.21 (m, 1H), 1.01 (s, 3H), 0.74 (td, *J* = 12.9, 4.0 Hz, 1H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 154.2, 144.6, 143.4, 138.8, 134.8, 131.7, 130.2, 129.5, 120.3, 119.4, 114.6, 108.1, 82.4, 56.0, 48.6, 47.4, 43.6, 39.5, 38.0, 33.0, 29.0, 27.4, 26.6, 23.5, 21.1, 14.3. HRMS (EI^+) calcd. for $C_{28}H_{33}N_3O_3$ $[M]\bullet^+$: 459.2522. Found 459.2531.

*2.24. 2-Methoxyestra-17-(1-(4-(tert-butyl)phenyl)-1H-1,2,3-triazol-4-yl)diol (**11c**)*
Light yellow solid (110 mg, 76%), mp. 131-133 °C, eluent: 20% - 50% EtOAc in hexane, R_f = 0.12.^{1}H NMR (600 MHz, $CDCl_3$) δ 7.87 (s, 1H), 7.69 (d, *J* = 8.8 Hz, 2H), 7.54 (d, *J* = 8.8 Hz, 2H), 6.69 (s, 1H), 6.62 (s, 1H), 3.81 (s, 3H), 2.85 – 2.69 (m, 2H), 2.54 – 2.40 (m, 1H), 2.24 – 2.07 (m, 2H), 2.02 – 1.94 (m, 2H), 1.95 – 1.86 (m, 1H), 1.75 – 1.67 (m, 1H), 1.68 – 1.54 (m, 2H), 1.55 – 1.41 (m, 2H), 1.36 (s, 9H), 1.34 – 1.25 (m, 2H), 1.09 (s, 2H), 0.80 (td, *J* = 12.9, 4.0 Hz, 1H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 154.2, 152.3, 144.7, 143.6, 134.7, 131.8, 129.6, 126.8, 120.3, 119.5, 114.7, 108.2, 82.5, 56.2, 48.8, 47.6, 43.7, 39.6, 38.2, 34.9, 33.2, 31.4, 29.2, 27.6, 26.7, 23.6, 14.4. HRMS (EI^+) calcd. for $C_{31}H_{39}N_3O_3$ $[M]\bullet^+$: 501.2991. Found 501.2989.

*2.25. 2-Methoxyestra-17-(1-(4-hydroxyphenyl)-1H-1,2,3-triazol-4-yl)diol (**11d**)*
Red solid (144 mg, 87%), mp. 167-169 °C, eluent: 20% - 50% EtOAc in hexane, R_f = 0.16.^{1}H NMR (600 MHz, $CDCl_3$) δ 7.63 (s, 1H), 7.40 (d, *J* = 8.8 Hz, 2H), 6.84 (d, *J* = 8.8 Hz, 2H), 6.52 (s, 1H), 6.45 (s, 1H), 5.29 (s, 1H), 3.63 (s, 3H), 2.72 – 2.51 (m, 2H), 2.40 – 2.24 (m, 1H), 2.08 – 1.96 (m, 2H), 1.87 – 1.77 (m, 2H), 1.78 – 1.65 (m, 1H), 1.53 (d, *J* = 12.6 Hz, 1H), 1.49 – 1.40 (m, 2H), 1.34 – 1.28 (m, 2H), 1.19 – 1.12 (m, 1H), 0.92 (s, 3H), 0.65 (td, *J* = 12.8, 3.8 Hz, 1H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 156.8, 154.0, 144.5, 143.4, 131.7, 130.1, 129.5, 122.3, 119.8, 116.5, 114.6, 108.0, 82.5, 56.0, 48.6, 47.4, 43.6, 39.4, 38.0, 33.0, 29.0, 27.4, 26.5, 23.4, 14.3. HRMS (EI^+) calcd. for $C_{28}H_{33}N_3O_3$ $[M]\bullet^+$: 461.2315. Found 461.2319.

*2.26. 2-Methoxyestra-17-(1-(4-flourophenyl)-1H-1,2,3-triazol-4-yl)diol (**11e**)*
Light yellow solid (144 mg, 75%), mp. 119-124 °C, eluent: 20% - 50% EtOAc in hexane, R_f = 0.17.^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88 (s, 1H), 7.74 (dd, *J* = 9.0, 4.6 Hz, 2H), 7.24 – 7.15 (m, 2H), 6.69 (s, 1H), 6.62 (s, 1H), 3.80 (s, 3H), 2.85 – 2.65 (m, 2H), 2.58 – 2.39 (m, 1H), 2.24 – 2.09 (m, 2H), 2.03 – 1.86 (m, 3H), 1.73 – 1.20 (m,

6H), 1.09 (s, 3H), 0.81 (td, *J* = 12.9, 4.0 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.6, 161.1, 154.6, 144.6, 143.5, 133.4 (d, *J* = 3.1 Hz), 131.7, 129.5, 122.4, 122.3, 119.6, 116.8, 116.6, 114.7, 108.1, 82.5, 56.1, 48.6, 47.5, 43.6, 39.5, 38.1, 33.1, 29.0, 27.4, 26.6, 23.5, 14.3. HRMS (EI^+) calcd. for $C_{27}H_{30}FN_3O_3$ $[M]\bullet^+$: 463.2271. Found 463.2268.

*2.27. 2-Methoxyestra-17-(1-(4-chlorophenyl)-1H-1,2,3-triazol-4-yl)diol (**11f**)*
Light yellow solid (149 mg, 79%), mp. 125-124 °C, eluent: 20% - 50% EtOAc in hexane, R_f = 0.15.^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88 (d, *J* = 1.2 Hz, 1H), 7.83 – 7.63 (m, 2H), 7.58 – 7.41 (m, 2H), 6.69 (s, 1H), 6.62 (s, 1H), 3.80 (s, 3H), 2.89 – 2.65 (m, 2H), 2.59 – 2.37 (m, 1H), 2.25 – 2.09 (m, 2H), 2.09 – 1.85 (m, 3H), 1.79 – 1.24 (m, 6H), 1.09 (s, 3H), 0.81 (td, *J* = 12.7, 3.5 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.7, 144.6, 143.5, 135.6, 134.5, 131.6, 129.9, 129.5, 121.6, 119.3, 114.6, 108.0, 82.5, 56.0, 48.6, 47.5, 43.6, 39.5, 38.1, 33.0, 29.0, 27.4, 26.6, 23.5, 14.3. HRMS (EI^+) calcd. for $C_{27}H_{30}ClN_3O_3$ $[M]\bullet^+$: 479.1976. Found 479.1967.

*2.28. 2-Methoxyestra-17-(1-(4-bromophenyl)-1H-1,2,3-triazol-4-yl)diol (**11g**)*
Light yellow solid (152 mg, 74%), mp. 137-140 °C, eluent: 20% - 50% EtOAc in hexane, R_f = 0.15. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88 (s, 1H), 7.67 (s, 4H), 6.69 (s, 1H), 6.62 (s, 1H), 3.81 (s, 3H), 2.85 – 2.68 (m, 2H), 2.57 – 2.36 (m, 1H), 2.21 – 2.08 (m, 2H), 2.07 – 1.87 (m, 3H), 1.80 – 1.27 (m, 6H), 1.09 (s, 3H), 0.87 – 0.72 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.9, 144.7, 143.6, 136.2, 133.1, 131.8, 129.7, 122.5, 121.9, 119.3, 114.7, 108.2, 82.7, 56.2, 48.8, 47.6, 43.8, 39.6, 38.3, 33.2, 29.2, 27.6, 26.7, 23.6, 14.4. HRMS (EI^+) calcd. for $C_{27}H_{30}BrN_3O_3$ $[M]\bullet^+$: 523.1461. Found 523.1461.

*2.29. 2-Methoxyestra-17-(1-(4-methoxyphenyl)-1H-1,2,3-triazol-4-yl)diol (**11h**)*
Light yellow solid (161 mg, 82%), mp. 121-127 °C, eluent: 20% - 50% EtOAc in hexane, R_f = 0.14. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 (s, 1H), 7.66 (d, *J* = 8.9 Hz, 2H), 7.02 (d, *J* = 8.9 Hz, 2H), 6.69 (s, 1H), 6.62 (s, 1H), 3.87 (s, 3H), 3.81 (s, 3H), 2.77 (dd, *J* = 14.9, 4.8 Hz, 2H), 2.54 – 2.41 (m, 1H), 2.27 – 2.10 (m, 2H), 2.04 – 1.86 (m, 3H), 1.78 – 1.18 (m, 6H), 1.09 (s, 3H), 0.82 (td, *J* = 12.9, 3.8 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 160.0, 154.2, 144.7, 143.6, 131.8, 130.6, 129.7, 122.2, 119.7, 114.9, 114.7, 108.2, 82.6, 56.2, 55.8, 48.8, 47.6, 43.8, 39.6, 38.2, 33.2, 29.2, 27.6, 26.7, 23.6, 14.4. HRMS (EI^+) calcd. for $C_{28}H_{33}N_3O_4$ $[M]\bullet^+$: 475.2471. Found 475.2455.

*2.30. 2-Methoxyestra-17-(1-(3,4,5-trimethoxyphenyl)-1H-1,2,3-triazol-4-yl)diol (**11i**)*
Light yellow solid (133 mg, 71%), mp. 113-116 °C, eluent: 20% - 50% EtOAc in hexane, R_f = 0.11.^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88 (s, 1H), 7.26 (s, 1H), 6.67 (s, 1H), 6.59 (s, 1H), 3.90 (s, 6H), 3.86 (s, 3H), 3.78 (s, 3H), 2.73 (dd, *J* = 15.9, 5.1 Hz, 4H), 2.50 (ddd, *J* = 14.6, 9.5, 5.8 Hz, 2H), 2.14 (ddq, *J* = 13.2, 9.1, 5.6, 4.5 Hz, 3H), 2.01 – 1.93 (m, 3H), 1.92 – 1.85 (m, 2H), 1.75 – 1.19 (m, 8H), 1.07 (s, 3H), 0.81 (td, *J* = 12.9, 3.9 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.5, 153.9, 144.6, 143.5, 138.3, 133.0, 131.7, 129.5, 119.8, 114.7, 108.2, 98.4, 82.5, 61.1, 56.5, 56.1, 48.5, 47.4, 43.6, 39.5, 38.1, 33.1, 29.0, 27.4, 26.6, 23.6, 14.3. HRMS (EI^+)calcd. for $C_{30}H_{37}N_3O_6$ $[M]\bullet^+$: 535.2682. Found 535.2670.

*2.31. 2-Methoxyestra-17-(1-(4-(trifluoromethyl)phenyl)-1H-1,2,3-triazol-4-yl)diol (**11j**)*

Light yellow solid (108 mg, 59%), mp. 146-149 °C, eluent: 20% - 50% EtOAc in hexane, R_f = 0.18. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97 (s, 1H), 7.93 (d, *J* = 8.1 Hz, 2H), 7.80 (d, *J* = 8.1 Hz, 2H), 6.69 (s, 1H), 6.62 (s, 1H), 3.80 (s, 3H), 2.82 – 2.69 (m, 2H), 2.58 – 2.46 (m, 1H), 2.17 (t, *J* = 14.0 Hz, 2H), 2.08 – 1.88 (m, 3H), 1.81 – 1.28 (m, 6H), 1.10 (s, 3H), 0.81 (t, *J* = 12.5 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 155.0, 144.6, 143.5, 139.5, 131.6, 130.6 (q, *J* = 33.2 Hz), 129.5, 127.1, 127.1, 124.9, 122.2, 120.3, 119.2, 114.6, 108.0, 82.6, 56.0, 48.6, 47.5, 43.6, 39.5, 38.2, 33.0, 29.0, 27.4, 26.6, 23.5, 14.2. HRMS (EI+) calcd. for $C_{28}H_{30}F_3N_3O_3$ $[M]^{\bullet+}$: 513.2239. Found 535.2235.

2.32. 2-Methoxyestra-17-(1-(4-(trifluoromethoxyl)phenyl)-1H-1,2,3-triazol-4-yl)diol ***(11k)***

Light yellow solid (116 mg, 65%), mp. 128-130 °C, eluent: 20% - 50% EtOAc in hexane, R_f = 0.17. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.18 – 7.89 (m, 3H), 7.81 (d, *J* = 8.2 Hz, 2H), 6.69 (s, 1H), 6.63 (s, 1H), 5.42 (s, 1H), 3.81 (s, 3H), 2.84 – 2.71 (m, 2H), 2.58 – 2.46 (m, 1H), 2.26 – 2.07 (m, 2H), 2.09 – 1.89 (m, 3H), 1.75 – 1.32 (m, 6H), 1.10 (s, 3H), 0.81 (t, *J* = 12.5 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 155.0, 144.5, 143.5, 139.5, 131.6, 130.8, 130.5, 129.5, 127.1, 127.1 (q, *J* = 3.6 Hz), 120.3, 119.2, 114.6, 108.0, 82.6, 56.0, 48.6, 47.5, 43.6, 39.5, 38.2, 33.0, 29.0, 27.4, 26.6, 23.5, 14.3. HRMS (EI^+) calcd. for $C_{28}H_{30}F_3N_3O_4$ $[M]^{\bullet+}$: 529.2188. Found 529.2183.

*2.33. 2 Methoxyestra-17-(1-(4-methoxybenzyl)-1H-1,2,3-triazol-4-yl)diol (**12a**)*

Light yellow solid (118 mg, 81%), mp. 106-108 °C, eluent: 20% - 50% EtOAc in hexane, R_f = 0.13. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33 (s, 1H), 7.21 (d, *J* = 8.6 Hz, 2H), 6.88 (d, *J* = 8.6 Hz, 2H), 6.70 (s, 1H), 6.61 (s, 1H), 5.48 (d, *J* = 14.7 Hz, 1H), 5.42 (d, *J* = 14.8 Hz, 1H), 3.80 (s, 3H), 3.79 (s, 3H), 2.80 – 2.66 (m, 2H), 2.38 – 2.27 (m, 1H), 2.15 – 2.05 (m, 2H), 1.98 – 1.80 (m, 3H), 1.65 – 1.57 (m, 1H), 1.56 – 1.37 (m, 4H), 1.32 – 1.22 (m, 1H), 1.03 (s, 3H), 0.67 (td, *J* = 12.8, 3.9 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.9, 154.0, 144.6, 143.5, 131.7, 129.5, 126.7, 121.0, 114.7, 114.5, 108.2, 105.2, 82.3, 56.1, 55.3, 53.7, 48.5, 47.3, 43.7, 39.4, 37.9, 33.0, 29.0, 27.5, 26.6, 23.4, 14.3. HRMS (EI^+) calcd. for $C_{29}H_{35}N_3O_4$ $[M]^{\bullet+}$: 489.2628. Found 489.2617.

*2.34. 2-Methoxyestra-17-(1-(3,4,5-trimethoxybenzyl)-1H-1,2,3-triazol-4-yl)diol (**12b**)*

Light yellow solid (137 mg, 78%), mp. 111-113 °C, eluent: 20 % - 50 % EtOAc in hexane, R_f = 0.12. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 (s, 1H), 6.67 (s, 1H), 6.60 (s, 1H), 6.47 (s, 2H), 5.49 (d, *J* = 14.9 Hz, 1H), 5.41 (d, *J* = 14.9 Hz, 1H), 3.82 (s, 3H), 3.81 (s, 9H), 2.83 – 2.63 (m, 2H), 2.47 – 2.32 (m, 1H), 2.19 – 2.02 (m, 2H), 2.00 – 1.80 (m, 3H), 1.62 – 1.27 (m, 6H), 1.03 (s, 3H), 0.67 (td, *J* = 12.8, 4.0 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.2, 153.8, 144.7, 143.6, 138.3, 131.6, 130.4, 129.6, 121.3, 114.7, 108.1, 105.1, 82.5, 61.0, 56.3, 56.2, 54.4, 48.6, 47.4, 43.8, 39.5, 38.1, 33.1, 29.1, 27.5, 26.6, 23.5, 14.4. HRMS (EI^+) calcd. for $C_{31}H_{39}N_3O_6$ $[M]^{\bullet+}$: 549.2839. Found 549.2826.

2.35 Cancer Cell Growth Inhibition

The method applied was that described by Edmondson and coworkers [17] and is exemplified for the K562 cell line. K562 human chronic myelogeneous leukemia cells were cultivated in RMPI medium, free of antibiotics and containing 2-mercaptoethanol (2 μM) and L-glutamine (2 mM), supplemented with foetal calf serum (FCS) (10% v/v). The cells were adjusted to a concentration depending on their

observed doubling time, (ca. 40000 cells/ml), in RPMI medium supplemented with FCS (10% v/v). The candidate drug was dissolved in DMSO. A drug solution of 100 l in medium was added to 100 µl of cell solution (40000 cells/ml) in a 96-well microtitre testplate (4 µl of the compound solution diluted in medium in order to reach decreasing concentrations). This series of dilutions was continued to afford samples at different concentrations leaving one cell solution free of drug acting as a control. The plates were incubated at 37 °C (5% CO_2 in air) for 5 days. The plate was then removed from the incubator and 50 µl of a solution of MTT (3 mg/ml in PBS) was added to each well. After incubation (37 °C, 5% CO_2 in air, 3 h) the medium was carefully removed from each well by suction and the resulting formazan precipitate re-dissolved in 200 µl DMSO. The optical density of each well was read at two wavelengths (λ 540 and 690 nm) using a Titretek Multiscan MCC/340 plate reader. After processing and analysis through the application of an 'in-house' software package, the results enabled the calculation of the drug dose required to inhibit cell growth by 50% (IC_{50} value), determined by graphical means as percentage of the control growth.

2.36 Inhibition of Tubulin Assembly

The method applied was that described by Lawrence and coworkers [18]. Tubulin was isolated from porcine brain and stored at –78 °C. Samples were prepared directly in a 96-well microtitre testplate that was preincubated at 4 ºC in the fridge for 30 min and contained Mes buffer [128 µl (0.1 M Mes, 1 mM EGTA, 0.5 mM $MgCl_2$, distilled water, pH 6.6)], GTP (20 µl, 5 mM in Mes buffer), tubulin (50 µl, 11 mg/ml in Mes buffer) and the candidate drug (20 µl, Csample in DMSO). The tubulin/drug samples were immediately placed in a 96-well plate reader, alongside blank samples containing Mes buffer (198 µl) and the analogs (10 µl, same concentration). The absorbance (λ 350 nm) was recorded at 25 °C temperature for a period of 60 min, and the results were compared to untreated controls to evaluate the relative degree of change in optical density.

3. Results

3.1 Chemistry

The synthesis of the analogs started with the preparation of the steroid **6** as outlined in Scheme 1. After chromatography, only one stereoisomer was detected by ^{1}H NMR and HPLC analyses. The depicted stereochemistry at the C-17 position is in accord with literature [19a], [19b]. Steroid **6** was obtained in 64% yield over the three-step protocol. Then, under Cu(I) catalysis, different commercially available azides were reacted with the alkyne **6** affording the 1,2,3-triazoles **9a**-**9k** and **10a**-**10b** in 47-83% yield. Then deprotection of the TBS-group with excess tetra-*n*-butyl ammonium fluoride in THF [20] afforded the desired 1,4-disubstituted 1,2,3-triazoles **11a**-**11k** and **12a**-**12b**. Deprotection prior to the cycloaddition proved troublesome. The spectral data of all new products were in accord with their assigned structures.

[INSERT SCHEME 1 HERE]

Scheme 1. Synthesis of triazole analogs of 2-ME (**1**).

3.2 Biological evaluations

The target triazoles were submitted to the MTT assay for the evaluation of their cytotoxic effects against the K562 human myeloid leukemia cancer cell line. These results showed that in the K562 cancer cell line, the two most potent compounds were 2-ME (**1**) and analog **11k** with IC_{50}-values of 0.8 and 0.7 μM, respectively. Almost as potent with IC_{50} = 1.0 μM was triazole **11j**, while **11e** and **12a** were slightly less potent than **1** and **11k** (IC_{50} = 1.8 and IC_{50} = 1.4 μM). The other analogs that also exhibited cytotoxic effects in the K562 cell line had IC_{50}-values between 2.4 and 3.5 μM. The data has been compiled in Table 1.

Table 1. Biological evaluations of 2-ME (**1**) and triazoles **11a**-**11k**, **12a**-**12b**

Compound	K562 Cell assay IC_{50} (μM)[a]	OVCAR-3 Cell assay IC_{50} (μM)[a]	WM35 Cell assay IC_{50} (μM)[a]	Tubulin inhibition IC_{50}(μM)[b]
2-ME (**1**)	0.8	1.2	2.1	2.2
11a	2.8	2.6	5.8	n.d.[c]
11b	2.4	2.2	6.1	n.d.
11c	3.5	5.5	7.3	n.d.
11d	> 10	> 10	>10	n.d.
11e	1.8	1.6	2.2	> 10
11f	3.5	3.4	5.0	n.d.
11g	3.2	3.4	4.9	n.d.
11h	2.1	2.6	4.0	> 10
11i	> 10	> 10	> 10	n.d.
11j	1.0	1.4	1.9	8.1
11k	0.7	0.9	2.9	5.9
12a	1.4	2.2	3.8	> 10
12b	> 10	> 10	> 10	n.d.

[a]Results of three experiments performed as triplicates
[b]Results of three experiments performed as duplicates
[c]n.d. = not determined

Basically, the same trends were observed in the ovarian cancer cell line OVCAR-3. Analog **11k** was the most potent compound (IC_{50} = 0.9 μM) also in this assay. Again, this analog was slightly more potent than the lead compound **1** (IC_{50} = 1.2 μM). The two analogs **11e** and **11j**, with the 4-trifluoromethyl and the 4-fluoro-substituent, respectively, were potent inhibitors with IC_{50}-values of 1.6 and 1.4 μM in this cell line. In the WM36 melanoma cell line compound **11j** was the most potent (IC_{50} = 1.9 μM) with slightly higher cytotoxic effects than 2-ME (**1**) (IC_{50} = 2.1 μM) and compound **11e** (IC_{50} = 2.2 μM) In general, the above-mentioned trends were also observed in this cell line; however, all compounds were slightly less potent with IC_{50}-values between 2.9-7.3 μM. The two analogs **11i** and **12b** with a 3,4,5-trimethoxyphenyl and a 3,4,5-trimethoxybenzyl substituent, respectively, on the 1,2,3-triazole did not show any anti-proliferative effects in any of the three cancer cell lines. The same result was observed for triazole **11d** with a 4-hydroxyphenyl substituent.

2-ME (**1**) and the five most cytotoxic triazoles from the MTT-assay were subjected to a tubulin assembly inhibition assay; in this assay the most potent compound was 2-ME (**1**) with IC_{50} = 2.2 μM, similar to literature values [2]. Among the five 1,2,3-triazoles tested as tubulin inhibitors, the most potent inhibitor was **11k** with the 4-trifluoromethoxy substituent with a IC_{50}-value of 5.9 μM. Also triazole **11j** showed activity as a tubulin inhibitor (IC_{50} = 8.1 μM), but the three analogs **11e**, **11h** and **12a** were all inactive (IC_{50} > 10 μM).

Microtubules are cylindrical protein polymers composed of α- and β-tubulin heterodimers. Microtubules are essential for many cellular processes, *i.e.* maintenance of cellular shape, intracellular transport and mitotic spindle assembly during cell division [21]. Inhibition of microtubule formation leads to mitotic arrest and promotes vascular disruption leading to cell death. Hence, tubulin is one of the most common biological targets for the development of new anticancer drugs [22].

The most potent 1,4-disubstituted 1,2,3-triazole analogs in the cytotoxicity assays and in the tubulin polymerization inhibition assays, were compounds **11j** and **11k**. Apparently, the presence of a trifluoro group is important for both tubulin polymerization inhibition and cytotoxic activity. Moreover, this functional group also enhances the lipophilicity of **11j** and **11k**. The presence of a fluorine atom in the 4-position, as in compound **11e**, contributed to a higher cell death than the other prepared triazole analogs of the steroid **1**. However, this analog did not inhibit the assembly of tubulin. Similar effects were also observed for the two analogs with a trimethoxy group in the 4-position, both compound **11h** and **12a**, the latter derived from 4-methoxybenzyl azide, showed cytotoxic activities in all cancer cell lines. However, both compounds were inactive in the tubulin polymerization inhibition assay. Since 2-ME (**1**) was the most potent tubulin inhibitor, the antimitotic effects of the triazoles **11j** and **11k** were not investigated further.

4. Conclusions

Structure-activity relationship (SAR) studies have shown that 2-ME (**1**) is amendable for changes in the C-17 position. The introduction of a 1,4-disubstituted 1,2,3-triazole ring in this position gave a few analogs that were slightly more cytotoxic than 2-ME (**1**). Compound **11k** exhibited potent cytotoxic activity in all three cancer cell lines as well as moderate inhibition of tubulin polymerization. However, this analog was a less potent tubulin inhibitor than lead compound **1**. Hence, this analog only in part exhibits its cytotoxic effects due to inhibition of tubulin. The steroid **1** has also been reported to display other mechanisms of actions [5], [6]. Determination if such effects are mediated by the triazoles reported herein will be investigated.

Acknowledgements

The School of Pharmacy, University of Oslo, is gratefully acknowledged for Ph.D.-scholarship to E. J. S.

Appendix A. Supplementary Data

Supplementary data associated with this article can be found, in the online version, at [to be inserted by Editorial office]

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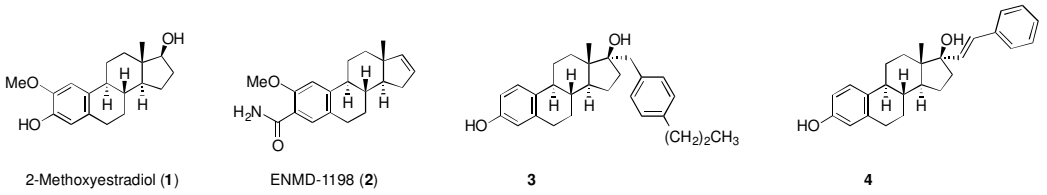
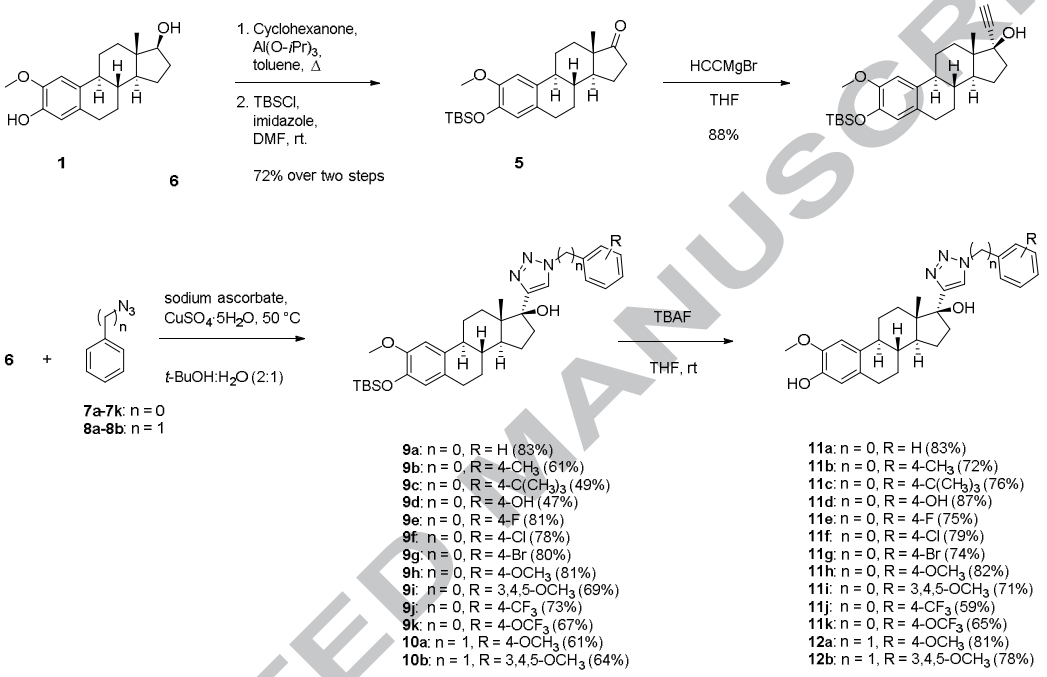


Fig. 1. Some examples of steroids exhibiting anti-cancer effects.



Scheme 1. Synthesis of triazole analogs of 2-ME (**1**).

Highlights

- Analogs of the steroid metabolite 2-methoxyestradiol were prepared as potential new anti-cancer agents
- Cytotoxic effects in the low micromolar range were observed for some analogs
- The most cytotoxic and interesting analogs also exhibited tubulin inhibition
- The 1,4-disubstitued-1,2,3-analogs were obtained with excellent diastero- and regioselectivity