Steroids 76 (2011) 1141–1148

Contents lists available at ScienceDirect

Steroids

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

Synthesis of novel steroidal 17α-triazolyl derivatives via Cu(I)-catalyzed azide-alkyne cycloaddition, and an evaluation of their cytotoxic activity *in vitro*

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ARTICLE INFO

Article history:
Received 1 February 2011
Received in revised form 28 March 2011
Accepted 1 May 2011
Available online 10 May 2011

Keywords:
Regioselectivity
Cycloaddition
Triazoles
Cytotoxic activity

ABSTRACT

Regioselective Cu(I)-catalyzed 1,3-dipolar cycloaddition of steroidal 17α-azides with different terminal alkynes afforded novel 1,4-disubstituted triazolyl derivatives in good yields in both the estrone and the androstane series. The antiproliferative activities of the structurally related triazoles were determined *in vitro* on three malignant human cell lines (HeLa, MCF7 and A431), with the microculture tetrazolium assay.

1. Introduction

In recent years, considerable interest has been focused on steroidal heterocycles in view of the broad spectrum of their biological activities. Several novel synthesized compounds have been described as potent inhibitors of 17α-hydroxylase-$C_{17,20}$-lyase ($P450_{17\alpha}$) which can block androgen synthesis at an early stage, and may therefore be useful in the treatment of prostatic carcinoma [1–3]. Moreover, some steroidal heterocycles have also been found to exert inhibitory effects on 5α-reductases [4] and to display considerable cytotoxic activity [5]. Although a number of diverse triazolyl derivatives have been reported to exhibit biological activity, including antibacterial [6], antiallergic [7] and anti-HIV [8] effects, steroids containing this kind of structural moiety have received less attention from both synthetic and pharmacological aspects [9,10].

Since the first reports [11,12], Cu-catalyzed azide-alkyne 1,3-dipolar cycloaddition (CuAAC) has found numerous applications across a wide variety of disciplines, including polymer chemistry, materials research and pharmaceutical sciences, as evidenced by a huge number of related articles and several reviews [13–15]. The certain advantageous properties (versatility, regiospecific reactions, the lack of by-products and high conversions) have made 'click' chemistry [16] an ideal tool for the synthesis of libraries for initial screening and for structure–activity profiling.

To the best of our knowledge, relatively few examples are to be found in the literature in which Huisgen 1,3-dipolar cycloaddition is applied to steroid azides [11,17], though it provides convenient facilities for the construction of triazoles in which the hetero ring is attached to the steroid nucleus through a nitrogen atom. Banday and co-workers recently reported the syntheses of 21-triazolyl derivatives of pregnenolone as potential anticancer agents through use of the 'click' chemistry approach [18], but without any proposal concerning their mode of action. Since some steroid-type compounds are known to exert hormone receptor-independent antiproliferative activity by the inhibition of angiogenesis, tubulin polymerization, and the upregulation of apoptotic pathways [19–21], we set out to prepare novel steroidal 17α-triazoles via CuAAC, untinged by the structural features necessary for effective binding to the hormone receptors [22,23]. Although determination of the affinities to the hormonal receptors did not fall within the scope of the present work, in the absence of a hydroxy or keto functional group at position 3, the newly prepared triazolyl derivatives are considered to have no estrogenic or androgenic effects. Nevertheless, all compounds were screened *in vitro* for their activities against a panel of three human cancer cell lines (HeLa, MCF7 and A431).

2. Experimental

2.1. General

Melting points (Mps) were determined on a Kofler block and are uncorrected. EI mass spectra were recorded with a Varian MAT

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doi:10.1016/j.steroids.2011.05.002

311A spectrometer at an ionization energy of 70 eV. ^{1}H NMR spectra were obtained in $CDCl_3$ solution (if not otherwise stated) at 500 MHz (Bruker DRX 500), and the ^{13}C NMR spectra at 125 MHz with the same instrument. Chemical shifts are reported relative to TMS; *J* values are given in Hz. ^{13}C NMR spectra are ^{1}H-decoupled. For determination of the multiplicities, the J-MOD pulse sequence was used. Elemental analyses were carried out with a Perkin-Elmer CHN Analyzer (Model 2400). All solvents were distilled and dried prior to use. Reagents and materials were obtained from commercial suppliers and were used without purification. The reactions were monitored by TLC on Kieselgel-G (Merck Si 254F) layers (0.25 mm thick); solvent systems (ss) (A) CH_2Cl_2/hexane (70:30, v/v); (B) CH_2Cl_2/hexane (30:70, v/v); (C) CH_2Cl_2; (D) EtOAc/CH_2Cl_2 (2:98, v/v); (E) EtOAc/CH_2Cl_2 (5:95, v/v). The spots were detected by spraying with 5% phosphomolybdic acid in 50% aqueous H_3PO_4. The R_f values were determined for spots observed by illumination at 254 and 365 nm. Flash chromatography: silica gel 60, 40–63 μm.

2.2. Synthesis of 17β-estradiol-3-benzyl ether 17-tosylate (**5**)

17β-Estradiol-3-benzyl ether **3** (11.0 g, 30.3 mmol) was dissolved in pyridine (100 mL) and *para*-toluenesulfonyl chloride (12.0 g, 62.9 mmol) was added portionwise. The mixture was stirred for 72 h at room temperature, then poured onto a mixture of ice and concentrated H_2SO_4 (80 mL). The precipitate that formed was filtered off, washed until neutral with water and dried. The crude product was purified by flash chromatography (CH_2Cl_2/hexane = 50:50, v/v) to give **5** (14.9 g, 95%) as a white solid. Mp 115–117 °C; R_f = 0.34 (ss A). Anal. Calcd. for $C_{32}H_{36}O_4S$: C, 74.39; H, 7.02. Found: C, 74.52; H, 7.11. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.84 (s, 3H, 18-H_3), 1.14 (m, 2H), 1.31 (m, 1H), 1.41 (m, 3H), 1.58–1.85 (overlapping m, 4H), 1.99 (m, 1H), 2.14 (m, 1H), 2.23 (m, 1H), 2.47 (s, 3H, 4″-H_3), 2.82 (m, 2H, 6-H_2), 4.35 (t, 1H, *J* = 8.6 Hz, 17-H), 5.03 (s, 2H, O-CH_2), 6.71 (d, 1H, *J* = 2.3 Hz, 4-H), 6.78 (dd, 1H, *J* = 8.6 Hz, *J* = 2.3 Hz, 2-H), 7.16 (d, 1H, *J* = 8.6 Hz, 1-H), 7.30–7.43 (overlapping m, 7H, 2′-H, 3′-H, 4′-H, 5′-H, 6′-H, 3″-H and 5″-H), 7.81 (d, 2H, *J* = 8.2 Hz, 2″-H and 6″-H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 11.7 (C-18), 21.6 (4″-CH_3), 23.0 (CH_2), 25.9 (CH_2), 27.0 (CH_2), 29.7 (CH_2), 29.6 (CH_2), 36.0 (CH_2), 38.4 (CH), 43.3 (C-13), 43.6 (CH), 49.0 (CH), 69.9 (O-CH_2), 89.8 (C-17), 112.3 (C-2), 114.8 (C-4), 126.3 (C-4′), 127.4 (2C, C-2′ and C-6′), 127.8 (3C, C-4′, C-2″ and C-6″), 128.5 (2C, C-3′ and C-5′), 129.7 (2C, C-3″ and C-5″), 132.4 (C-10), 134.2 (C-1″), 137.2 and 138.0: C-5 and C-1′, 144.4 (C-4″), 156.6 (C-3) ppm. EI-MS (70 eV) *m*/*z* (%): 516 [M^+] (26), 91 (100).

2.3. Synthesis of 3-benzyloxyestra-1,3,5(10)-triene-17α-azide (**7**)

Compound **5** (5.4 g, 10.5 mmol) was dissolved in *N*,*N*-dimethylformamide (80 mL) and NaN_3 (5.4 g, 83.1 mmol) was added. The mixture was stirred for 48 h at 100 °C, and then poured into water (50 mL) and extracted with CH_2Cl_2 (3× 50 mL). The combined organic phases were dried with Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by flash chromatography (CH_2Cl_2/hexane = 20:80, v/v) to give **7** (3.3 g, 82%) as a white solid. Mp 78–79 °C; R_f = 0.34 (ss B). Anal. Calcd. for $C_{25}H_{29}N_3O$: C, 77.48; H, 7.54. Found: C, 77.34; H, 7.65. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.79 (s, 3H, 18-H_3), 1.28–1.57 (overlapping m, 6H), 1.69–1.92 (overlapping m, 4H), 2.23 (m, 2H), 2.37 (m, 1H), 2.86 (m, 2H, 6-H_2), 3.60 (d, 1H, *J* = 6.4 Hz, 17-H), 5.04 (s, 2H, O-CH_2), 6.74 (d, 1H, *J* = 2.1 Hz, 4-H), 6.79 (dd, 1H, *J* = 8.6 Hz, *J* = 2.1 Hz, 2-H), 7.23 (d, 1H, *J* = 8.6 Hz, 1-H), 7.33 (t-like m, 1H, 4′-H), 7.39 (t-like m, 2H, 3′-H and 5′-H), 7.44 (d, 2H, *J* = 7.2 Hz, 2′-H and 6′-H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 17.7 (C-18), 24.3 (CH_2), 26.2 (CH_2), 28.0 (CH_2), 28.7 (CH_2), 29.8 (CH_2), 32.6 (CH_2), 39.0 (CH), 43.4 (CH), 46.0 (C-13), 48.5 (CH), 69.9 (O-CH_2), 71.5 (C-17), 112.2 (C-2), 114.8 (C-4), 126.4 (C-4′), 127.4 (2C, C-2′ and C-6′), 127.8 (C-1), 128.5 (2C, C-3′ and C-5′), 132.8 (C-10), 137.3 and 137.9: C-5 and C-1′, 156.7 (C-3) ppm. EI-MS (70 eV) *m*/*z* (%): 387 [M^+] (35), 91 (100).

2.4. General procedure for the synthesis of triazoles (**10a–j** and **11a–j**)

3-Benzyloxyestra-1,3,5(10)-triene-17α-azide **7** (388 mg, 1.00 mmol) or 5α-androst-2-ene-17α-azide **8** (299 mg, 1.00 mmol) was dissolved in CH_2Cl_2 (20 mL), and CuI (19.0 mg, 0.10 mmol), triphenylphosphine (52 mg, 0.20 mmol) and substituted acetylene derivative (**9a–j**, 1.00 mmol) were added. The mixture was stirred under reflux for 24 h, and then diluted with water (20 mL) and extracted with CH_2Cl_2 (2× 20 mL). The combined organic phases were dried over Na_2SO_4, and evaporated *in vacuo*. The crude product was purified by flash chromatography, using EtOAc/CH_2Cl_2 (2:98, v/v) as eluent.

2.4.1. Synthesis of 3-benzyloxy-17α-[4-phenyl-1H-1,2,3-triazol-1-yl]estra-1,3,5(10)-triene (**10a**)

Compound **7** and phenylacetylene (**9a**, 0.11 mL) were used for the synthesis as described in Section 2.4. After purification, **10a** was obtained as a white solid (416 mg). Mp 169–171 °C; R_f = 0.52 (ss D). Anal. Calcd. for $C_{33}H_{35}N_3O$: C, 80.95; H, 7.20. Found: C, 81.13; H, 7.12. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.56 (m, 1H), 1.01 (s, 3H, 18-H_3), 1.27 (m, 1H), 1.43–1.63 (overlapping m, 4H), 1.85 (m, 1H), 1.98 (m, 1H), 2.09 (m, 1H), 2.20 (m, 2H), 2.40 (m, 1H), 2.59 (m, 1H), 2.87 (m, 2H, 6-H_2), 4.69 (dd, 1H, *J* = 8.2 Hz, *J* = 1.0 Hz, 17-H), 5.02 (s, 2H, Bn-CH_2), 6.73 (d, 1H, *J* = 2.3 Hz, 4-H), 6.75 (dd, 1H, *J* = 8.5 Hz, *J* = 2.3 Hz, 2-H), 7.10 (d, 1H, *J* = 8.5 Hz, 1-H), 7.30–7.46 (overlapping m, 8H, 2′-H, 3′-H, 4′-H, 5′-H, 6′-H, 3‴-H, 4‴-H and 5‴-H), 7.73 (s, 1H, 5″-H), 7.88 (d, 2H, *J* = 7.3 Hz, 2‴-H and 6‴-H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 18.7 (C-18), 24.9 (CH_2), 25.9 (CH_2), 27.9 (CH_2), 28.7 (CH_2), 29.8 (CH_2), 32.6 (CH_2), 39.1 (CH), 43.1 (CH), 46.6 (C-13), 48.8 (CH), 69.9 (Bn-CH_2), 70.4 (C-17), 112.2 (C-2), 114.4 (C-4), 119.9 (C-5″), 125.6 (2C, C-2‴ and C-6‴), 126.2 (C-1), 127.4 (2C, C-2′ and C-6′), 127.8 (C-4′), 128.0 (C-4‴), 128.5 (2C, C3′ and C-5′), 128.8 (2C, C-3‴ and C-5‴), 130.7 (C-1‴), 132.5 (C-10), 137.2 (C-5), 137.8 (C-1′), 146.9 (C-4″), 156.7 (C-3) ppm. EI-MS (70 eV) *m*/*z* (%): 489 [M^+] (51), 91 (100).

2.4.2. Synthesis of 3-benzyloxy-17α-[4-(4-methoxyphenyl)-1H-1,2,3-triazol-1-yl]estra-1,3,5(10)-triene (**10b**)

Compound **7** and 4-methoxyphenylacetylene (**9b**, 132 mg) were used for the synthesis as described in Section 2.4. After purification, **10b** was obtained as a white solid (437 mg). Mp 187–189 °C; R_f = 0.45 (ss E). Anal. Calcd. for $C_{34}H_{37}N_3O_2$: C, 78.58; H, 7.18. Found: C, 78.70; H, 7.32. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.57 (m, 1H), 1.00 (s, 3H, 18-H_3), 1.42–1.62 (overlapping m, 5H), 1.86 (m, 1H), 1.98 (m, 1H), 2.10 (m, 1H), 2.19 (m, 2H), 2.39 (m, 1H), 2.58 (m, 1H), 2.86 (m, 2H, 6-H_2), 3.85 (s, 3H, 4‴-OMe), 4.67 (dd, 1H, *J* = 8.3 Hz, *J* = 1.1 Hz, 17-H), 5.02 (s, 2H, Bn-CH_2), 6.72 (d, 1H, *J* = 2.3 Hz, 4-H), 6.74 (dd, 1H, *J* = 8.6 Hz, *J* = 2.3 Hz, 2-H), 6.97 (d, 2H, *J* = 8.7 Hz, 3‴-H and 5‴-H), 7.01 (d, 1H, *J* = 8.6 Hz, 1-H), 7.31 (m, 1H, 4′-H), 7.37 (m, 2H, 3′-H and 5′-H), 7.42 (d, 2H, *J* = 7.3 Hz, 2′-H and 6′-H), 7.63 (s, 1H, 5″-H), 7.80 (d, 2H, *J* = 8.7 Hz, 2‴-H and 6‴-H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 18.7 (C-18), 24.9 (CH_2), 26.0 (CH_2), 27.9 (CH_2), 28.7 (CH_2), 29.8 (CH_2), 32.7 (CH_2), 39.2 (CH), 43.1 (CH), 46.6 (C-13), 48.9 (CH), 55.3 (4‴-OMe), 69.9 (Bn-CH_2), 70.4 (C-17), 112.3 (C-2), 114.2 (2C, C-3‴ and C-5‴), 114.8 (C-4), 119.1 (C-5″), 123.6 (C-1‴), 126.2 (C-1), 126.9 (2C, C-2‴ and C-6‴), 127.4 (2C, C-2′ and C-6′), 127.8 (C-4′), 128.5 (2C, C3′ and C-5′), 132.6 (C-10), 137.3 (C-5), 137.8 (C-1′), 146.8 (C-

4″), 156.8 (C-3), 159.5 (C-4‴) ppm. EI-MS (70 eV) *m*/*z* (%): 519 [M$^+$] (17), 491 (20), 91 (100).

2.4.3. Synthesis of 3-benzyloxy-17α-[4-(4-fluorophenyl)-1H-1,2,3-triazol-1-yl]estra-1,3,5(10)-triene (**10c**)

Compound **7** and 4-fluorophenylacetylene (**9c**, 0.11 mL) were used for the synthesis as described in Section 2.4. After purification, **10c** was obtained as a white solid (431 mg). Mp 189–192 °C; R_f = 0.17 (ss C). Anal. Calcd. for $C_{33}H_{34}FN_3O$: C, 78.08; H, 6.75. Found: C, 78.19; H, 6.92. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.57 (m, 1H), 1.01 (s, 3H, 18-H_3), 1.43–1.62 (overlapping m, 5H), 1.86 (m, 1H), 1.98 (m, 1H), 2.10 (m, 1H), 2.19 (m, 2H), 2.39 (m, 1H), 2.59 (m, 1H), 2.87 (m, 2H, 6-H_2), 4.68 (dd, 1H, *J* = 8.3 Hz, *J* = 1.2 Hz, 17-H), 5.02 (s, 2H, Bn-CH_2), 6.72 (d, 1H, *J* = 2.3 Hz, 4-H), 6.75 (dd, 1H, *J* = 8.6 Hz, *J* = 2.3 Hz, 2-H), 7.10 (d, 1H, *J* = 8.6 Hz, 1-H), 7.12 (dd, 2H, *J* = 15.6 Hz, *J* = 8.5 Hz, 3‴-H and 5‴-H), 7.31 (m, 1H, 4′-H), 7.37 (m, 2H, 3′-H and 5′-H), 7.42 (d, 2H, *J* = 7.1 Hz, 2′-H and 6′-H), 7.68 (s, 1H, 5″-H), 7.84 (dd, 2H, *J* = 8.5 Hz, *J* = 5.4 Hz, 2‴-H and 6‴-H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 18.7 (C-18), 24.9 (CH_2), 25.9 (CH_2), 27.9 (CH_2), 28.7 (CH_2), 29.8 (CH_2), 32.7 (CH_2), 39.1 (CH), 43.1 (CH), 46.6 (C-13), 48.9 (CH), 69.9 (Bn-CH_2), 70.5 (C-17), 112.3 (C-2), 114.8 (C-4), 115.7 (d, 2C, *J* = 21.7 Hz, C-3‴ and C-5‴), 119.6 (C-5″), 126.2 (C-1), 127.0 (C-1‴), 127.3 (d, 2C, *J* = 7.7 Hz, C-2‴ and C-6‴), 127.4 (2C, C-2′ and C-6′), 127.8 (C-4′), 128.5 (2C, C3′ and C-5′), 132.5 (C-10), 137.3 (C-5), 137.8 (C-1′), 146.1 (C-4″), 156.8 (C-3), 162.6 (d, *J* = 247.3 Hz, C-4‴) ppm. EI-MS (70 eV) *m*/*z* (%): 507 [M$^+$] (32), 254 (12), 91 (100).

2.4.4. Synthesis of 3-benzyloxy-17α-[4-(4-tolyl)-1H-1,2,3-triazol-1-yl]estra-1,3,5(10)-triene (**10d**)

Compound **7** and 4-tolylacetylene (**9d**, 0.12 mL) were used for the synthesis as described in Section 2.4. After purification, **10d** was obtained as a white solid (428 mg). Mp 216–218 °C; R_f = 0.54 (ss D). Anal. Calcd. for $C_{34}H_{37}N_3O$: C, 81.08; H, 7.40. Found: C, 81.17; H, 7.23. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.55 (m, 1H), 1.00 (s, 3H, 18-H_3), 1.27 (m, 1H), 1.43–1.54 (overlapping m, 4H), 1.85 (m, 1H), 1.97 (m, 1H), 2.09 (m, 1H), 2.18 (m, 2H), 2.38 (s, 3H, 4‴-H_3), 2.39 (m, 1H), 2.59 (m, 1H), 2.86 (m, 2H, 6-H_2), 4.68 (dd, 1H, *J* = 8.3 Hz, *J* = 1.2 Hz, 17-H), 5.01 (s, 2H, Bn-CH_2), 6.72 (d, 1H, *J* = 2.3 Hz, 4-H), 6.74 (dd, 1H, *J* = 8.6 Hz, *J* = 2.3 Hz, 2-H), 7.01 (d, 1H, *J* = 8.6 Hz, 1-H), 7.24 (d, 2H, *J* = 8.0 Hz, 3‴-H and 5‴-H), 7.31 (m, 1H, 4′-H), 7.37 (m, 2H, 3′-H and 5′-H), 7.41 (d, 2H, *J* = 7.1 Hz, 2′-H and 6′-H), 7.67 (s, 1H, 5″-H), 7.75 (d, 2H, *J* = 8.0 Hz, 2‴-H and 6‴-H) ppm. EI-MS (70 eV) *m*/*z* (%): 503 [M$^+$] (23), 91 (100).

2.4.5. Synthesis of 3-benzyloxy-17α-[4-(4-ethylphenyl)-1H-1,2,3-triazol-1-yl]estra-1,3,5(10)-triene (**10e**)

Compound **7** and 4-ethylphenylacetylene (**9e**, 0.13 mL) were used for the synthesis as described in Section 2.4. After purification, **10e** was obtained as a white solid (430 mg). Mp 149–152 °C; R_f = 0.52 (ss D). Anal. Calcd. for $C_{35}H_{39}N_3O$: C, 81.20; H, 7.59. Found: C, 81.08; H, 7.67. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.56 (m, 1H), 1.00 (s, 3H, 18-H_3), 1.27 (t, 3H, *J* = 7.6 Hz, 4‴-$CH_2\underline{CH_3}$), 1.42–1.62 (overlapping m, 5H), 1.86 (m, 1H), 1.98 (m, 1H), 2.09 (m, 1H), 2.19 (m, 2H), 2.40 (m, 1H), 2.58 (m, 1H), 2.69 (q, 2H, *J* = 7.6 Hz, 4‴-$\underline{CH_2}CH_3$), 2.86 (m, 2H, 6-H_2), 4.67 (dd, 1H, *J* = 8.3 Hz, *J* = 1.2 Hz, 17-H), 5.02 (s, 2H, Bn-CH_2), 6.72 (d, 1H, *J* = 2.3 Hz, 4-H), 6.74 (dd, 1H, *J* = 8.6 Hz, *J* = 2.3 Hz, 2-H), 7.10 (d, 1H, *J* = 8.6 Hz, 1-H), 7.27 (d, 2H, *J* = 8.1 Hz, 3‴-H and 5‴-H), 7.31 (m, 1H, 4′-H), 7.37 (m, 2H, 3′-H and 5′-H), 7.42 (d, 2H, *J* = 7.1 Hz, 2′-H and 6′-H), 7.68 (s, 1H, 5″-H), 7.79 (d, 2H, *J* = 8.1 Hz, 2‴-H and 6‴-H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 15.5 (4‴-$CH_2\underline{CH_3}$), 18.7 (C-18), 24.9 (CH_2), 26.0 (CH_2), 28.0 (CH_2), 28.7 (2C, 2 × CH_2), 29.8 (CH_2), 32.7 (CH_2), 39.2 (CH), 43.1 (CH), 46.6 (C-13), 48.9 (CH), 69.9 (Bn-CH_2), 70.4 (C-17), 112.3 (C-2), 114.8 (C-4), 119.6 (C-5″), 125.7 (2C, C-3‴ and C-5‴), 126.2 (C-1), 127.4 (2C, C-2′ and C-6′), 127.8 (C-4′), 128.2 (C-1‴), 128.3 (2C, C-2‴ and C-6‴), 128.5 (2C, C3′ and C-5′), 132.6 (C-10), 137.3 (C-5), 137.8 (C-1′), 144.2 (C-4‴), 147.0 (C-4″), 156.8 (C-3) ppm. EI-MS (70 eV) *m*/*z* (%): 517 [M$^+$] (25), 91 (100).

2.4.6. Synthesis of 3-benzyloxy-17α-[4-(4-propylphenyl)-1H-1,2,3-triazol-1-yl]estra-1,3,5(10)-triene (**10f**)

Compound **7** and 4-propylphenylacetylene (**9f**, 0.16 mL) were used for the synthesis as described in Section 2.4. After purification, **10f** was obtained as a white solid (463 mg). Mp 136–138 °C; R_f = 0.34 (ss D). Anal. Calcd. for $C_{36}H_{41}N_3O$: C, 81.32; H, 7.77. Found: C, 81.46; H, 7.64. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.54 (m, 1H), 0.96 (t, 3H, *J* = 7.0 Hz, 4″-$CH_2CH_2\underline{CH_3}$), 1.00 (s, 3H, 18-H_3), 1.28 (m, 1H), 1.47–1.69 (overlapping m, 6H), 1.84 (m, 1H), 1.97 (m, 1H), 2.08 (m, 1H), 2.19 (m, 2H), 2.42 (m, 1H), 2.58 (m, 1H), 2.61 (t, 2H, *J* = 7.0 Hz, 4″-$\underline{CH_2}CH_2CH_3$), 2.86 (m, 2H, 6-H_2), 4.72 (dd, 1H, *J* = 8.3 Hz, *J* = 1.2 Hz, 17-H), 5.02 (s, 2H, Bn-CH_2), 6.71 (d, 1H, *J* = 2.3 Hz, 4-H), 6.74 (dd, 1H, *J* = 8.6 Hz, *J* = 2.3 Hz, 2-H), 7.10 (d, 1H, *J* = 8.6 Hz, 1-H), 7.26 (d, 2H, *J* = 8.1 Hz, 3‴-H and 5‴-H), 7.31 (m, 1H, 4′-H), 7.37 (m, 2H, 3′-H and 5′-H), 7.42 (d, 2H, *J* = 7.1 Hz, 2′-H and 6′-H), 7.68 (s, 1H, 5″-H), 7.85 (d, 2H, *J* = 8.1 Hz, 2‴-H and 6‴-H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 13.7 (4‴-$CH_2CH_2\underline{CH_3}$), 18.7 (C-18), 24.4 (CH_2), 24.9 (CH_2), 25.9 (CH_2), 27.9 (CH_2), 28.7 ($\underline{CH_2}$), 29.8 (CH_2), 32.7 (CH_2), 37.8 (CH_2), 39.1 (CH), 43.1 (CH), 46.5 (C-13), 48.9 (CH), 69.9 (Bn-CH_2), 70.7 (C-17), 112.3 (C-2), 114.8 (C-4), 119.3 (C-5″), 125.5 (2C, C-2‴ and C-6‴), 126.2 (C-1), 127.4 (2C, C-2′ and C-6′), 127.8 (C-4′), 128.2 (C-1‴), 128.5 (2C, C-3‴ and C-5‴), 129.0 (2C, C3′ and C-5′), 132.5 (C-10), 137.3 (C-5), 137.8 (C-1′), 142.8 (C-4‴), 147.0 (C-4″), 156.8 (C-3) ppm. EI-MS (70 eV) *m*/*z* (%): 531 [M$^+$] (22), 91 (100).

2.4.7. Synthesis of 3-benzyloxy-17α-[4-(4-tert-butylphenyl)-1H-1,2,3-triazol-1-yl]estra-1,3,5(10)-triene (**10g**)

Compound **7** and 4-*tert*-butylphenylacetylene (**9g**, 0.18 mL) were used for the synthesis as described in Section 2.4. After purification, **10g** was obtained as a white solid (458 mg). Mp 157–159 °C; R_f = 0.40 (ss D). Anal. Calcd. for $C_{37}H_{43}N_3O$: C, 81.43; H, 7.94. Found: C, 81.60; H, 8.07. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.53 (m, 1H), 1.01 (s, 3H, 18-H_3), 1.34 (s, 9H, 3 × *t*Bu-CH_3), 1.45–1.61 (overlapping m, 5H), 1.84 (m, 1H), 1.98 (m, 1H), 2.08 (m, 1H), 2.19 (m, 2H), 2.45 (m, 1H), 2.63 (m, 1H), 2.86 (m, 2H, 6-H_2), 4.75 (bs, 1H, 17-H), 5.02 (s, 2H, Bn-CH_2), 6.71 (d, 1H, *J* = 2.3 Hz, 4-H), 6.74 (dd, 1H, *J* = 8.5 Hz, *J* = 2.3 Hz, 2-H), 7.10 (d, 1H, *J* = 8.5 Hz, 1-H), 7.31 (m, 1H, 4′-H), 7.37 (m, 2H, 3′-H and 5′-H), 7.42 (d, 2H, *J* = 7.1 Hz, 2′-H and 6′-H), 7.49 (d, 2H, *J* = 8.1 Hz, 3‴-H and 5‴-H), 7.68 (s, 1H, 5″-H), 7.91 (d, 2H, *J* = 8.1 Hz, 2‴-H and 6‴-H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 18.7 (C-18), 24.9 (CH_2), 25.9 (CH_2), 27.9 (CH_2), 28.8 (CH_2), 29.8 (CH_2), 31.2 (3C, 3 × *t*Bu-CH_3), 32.7 (CH_2), 34.7 (4‴-*t*Bu-C), 39.1 (CH), 43.1 (CH), 46.5 (C-13), 48.9 (CH), 69.9 (Bn-CH_2), 70.2 (C-17), 112.3 (C-2), 114.8 (C-4), 119.5 (C-5″), 125.3 (2C, C-3‴ and C-5‴), 125.7 (2C, C-2‴ and C-6‴), 126.2 (C-1), 127.4 (2C, C-2′ and C-6′), 127.8 (C-4′), 128.1 (C-1‴), 128.5 (2C, C3′ and C-5′), 132.5 (C-10), 137.3 (C-5), 137.8 (C-1′), 147.0 (C-4″), 151.3 (C-4‴), 156.8 (C-3) ppm. EI-MS (70 eV) *m*/*z* (%): 545 [M$^+$] (17), 91 (100).

2.4.8. Synthesis of 3-benzyloxy-17α-[4-cyclopropyl-1H-1,2,3-triazol-1-yl]estra-1,3,5(10)-triene (**10h**)

Compound **7** and cyclopropylacetylene (**9h**, 0.09 mL) were used for the synthesis as described in Section 2.4. After purification, **10h** was obtained as a white solid (399 mg). Mp 74–76 °C; R_f = 0.21 (ss D). Anal. Calcd. for $C_{30}H_{35}N_3O$: C, 79.43; H, 7.78. Found: C, 79.26; H, 7.92. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.45 (m, 1H), 0.95 (s, 3H,

18-H_3), 0.96 (m 2H), 1.42–1.56 (overlapping m, 6H), 1.76 (m, 1H), 1.95 (m, 3H), 2.05–2.20 (overlapping m, 3H), 2.27 (m, 1H), 2.51 (m, 1H), 2.85 (m, 2H, 6-H_2), 4.58 (dd, 1H, J = 8.3 Hz, J = 1.0 Hz, 17-H), 5.02 (s, 2H, Bn-CH_2), 6.70 (d, 1H, J = 2.2 Hz, 4-H), 6.75 (dd, 1H, J = 8.6 Hz, J = 2.2 Hz, 2-H), 7.11 (d, 1H, J = 8.6 Hz, 1-H), 7.19 (s, 1H, 5″-H), 7.31 (m, 1H, 4′-H), 7.37 (m, 2H, 3′-H and 5′-H), 7.42 (d, 2H, J = 7.2 Hz, 2′-H and 6′-H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 6.7 (C-1‴), 7.7 (2C, C-2‴ and C-3‴), 18.6 (C-18), 24.8 (CH_2), 25.9 (CH_2), 27.9 (CH_2), 28.6 (CH_2), 29.8 (CH_2), 32.5 (CH_2), 39.1 (CH), 43.1 (CH), 46.4 (C-13), 48.8 (CH), 69.9 (Bn-CH_2), 70.1 (C-17), 112.2 (C-2), 114.7 (C-4), 120.0 (C-5″), 126.2 (C-1), 127.4 (2C, C-2′ and C-6′), 127.8 (C-4′), 128.5 (2C, C3′ and C-5′), 132.5 (C-10), 137.2 (C-5), 137.8 (C-1′), 149.3 (C-4″), 156.7 (C-3) ppm. EI-MS (70 eV) m/z (%): 453 [M^+] (30), 91 (100).

2.4.9. Synthesis of 3-benzyloxy-17α-[4-cyclopentyl-1H-1,2,3-triazol-1-yl]estra-1,3,5(10)-triene (**10i**)

Compound **7** and cyclopentylacetylene (**9i**, 0.12 mL) were used for the synthesis as described in Section 2.4. After purification, **10i** was obtained as a white solid (385 mg). Mp 105–107 °C; R_f = 0.35 (ss E). Anal. Calcd. for $C_{32}H_{39}N_3O$: C, 79.79; H, 8.16. Found: C, 79.95; H, 8.26. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.45 (m, 1H), 0.97 (s, 3H, 18-H_3), 0.40–1.57 (overlapping m, 5H), 1.69–1.87 (overlapping m, 7H), 1.97 (m, 1H), 2.07–2.21 (overlapping m, 5H), 2.33 (m, 1H), 2.53 (m, 1H), 2.86 (m, 2H, 6-H_2), 3.23 (m, 1H), 4.60 (d, 1H, J = 7.8 Hz, 17-H), 5.02 (s, 2H, Bn-CH_2), 6.71 (d, 1H, J = 2.3 Hz, 4-H), 6.75 (dd, 1H, J = 8.5 Hz, J = 2.3 Hz, 2-H), 7.11 (d, 1H, J = 8.5 Hz, 1-H), 7.26 (s, 1H, 5″-H), 7.31 (m, 1H, 4′-H), 7.37 (m, 2H, 3′-H and 5′-H), 7.42 (d, 2H, J = 7.2 Hz, 2′-H and 6′-H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 18.7 (C-18), 24.9 (CH_2), 25.1 (CH_2), 25.9 (CH_2), 27.9 (CH_2), 28.6 (2C, 2 × CH_2), 29.8 (CH_2), 32.6 (CH_2), 33.3 (2C, 2 × CH_2), 36.5 (C-1‴), 39.1 (CH), 43.1 (CH), 46.5 (C-13), 48.8 (CH), 69.9 (Bn-CH_2), 70.5 (C-17), 112.3 (C-2), 114.8 (C-4), 120.4 (C-5″), 126.2 (C-1), 127.4 (2C, C-2′ and C-6′), 127.8 (C-4′), 128.5 (2C, C3′ and C-5′), 132.5 (C-10), 137.3 (C-5), 137.8 (C-1′), 151.4 (C-4″), 156.7 (C-3) ppm. EI-MS (70 eV) m/z (%): 481 [M^+] (47), 228 (18), 91 (100).

2.4.10. Synthesis of 3-benzyloxy-17α-[4-cyclohexyl-1H-1,2,3-triazol-1-yl]estra-1,3,5(10)-triene (**10j**)

Compound **7** and cyclohexylacetylene (**9j**, 0.13 mL) were used for the synthesis as described in Section 2.4. After purification, **10j** was obtained as a white solid (392 mg). Mp 120–122 °C; R_f = 0.35 (ss E). Anal. Calcd. for $C_{33}H_{41}N_3O$: C, 79.96; H, 8.34. Found: C, 80.08; H, 8.49. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.45 (m, 1H), 0.97 (s, 3H, 18-H_3), 1.29 (m, 1H), 1.38–1.56 (overlapping m, 9H), 1.74 (m, 1H), 1.81 (m, 3H), 1.97 (m, 1H), 2.08 (m, 3H), 2.17 (m, 2H), 2.33 (m, 1H), 2.52 (m, 1H), 2.78 (m, 1H), 2.86 (m, 2H, 6-H_2), 4.59 (dd, 1H, J = 8.3 Hz, J = 1.1 Hz, 17-H), 5.02 (s, 2H, Bn-CH_2), 6.72 (d, 1H, J = 2.3 Hz, 4-H), 6.75 (dd, 1H, J = 8.6 Hz, J = 2.3 Hz, 2-H), 7.11 (d, 1H, J = 8.6 Hz, 1-H), 7.19 (s, 1H, 5″-H), 7.31 (m, 1H, 4′-H), 7.37 (m, 2H, 3′-H and 5′-H), 7.42 (d, 2H, J = 7.2 Hz, 2′-H and 6′-H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 18.7 (C-18), 24.9 (CH_2), 26.0 (CH_2), 26.1 (3C, 3 × CH_2), 27.9 (CH_2), 28.6 (CH_2), 29.8 (CH_2), 32.5 (CH_2), 33.1 (2C, 2 × CH_2), 35.3 (C-1‴), 39.1 (CH), 43.1 (CH), 46.5 (C-13), 48.8 (CH), 69.9 (Bn-CH_2), 70.1 (C-17), 112.3 (C-2), 114.8 (C-4), 119.6 (C-5″), 126.2 (C-1), 127.4 (2C, C-2′ and C-6′), 127.8 (C-4′), 128.5 (2C, C3′ and C-5′), 132.6 (C-10), 137.3 (C-5), 137.8 (C-1′), 152.8 (C-4″), 156.7 (C-3) ppm. EI-MS (70 eV) m/z (%): 495 [M^+] (51), 242 (17), 91 (100).

2.4.11. Synthesis of 17α-[4-phenyl-1H-1,2,3-triazol-1-yl]-5α-androst-2-ene (**11a**)

Compound **8** and phenylacetylene (**9a**, 0.11 mL) were used for the synthesis as described in Section 2.4. After purification, **11a** was obtained as a white solid (329 mg). Mp 192–193 °C; R_f = 0.35 (ss D). Anal. Calcd. for $C_{27}H_{35}N_3$: C, 80.75; H, 8.78. Found: C, 80.63; H, 8.91. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.29 (m, 1H), 0.60 (m, 1H), 0.73 (s, 3H, 19-H_3), 0.96 (s, 3H, 18-H_3), 1.03 (m, 1H), 1.29 (m, 1H), 1.26–1.47 (overlapping m, 7H), 1.51–1.70 (overlapping m, 3H), 1.73–1.87 (overlapping m, 3H), 2.08 (m, 1H), 2.29 (m, 1H), 2.52 (m, 1H), 4.63 (dd, 1H, J = 7.2 Hz, J = 1.2 Hz, 17-H), 5.55 (m, 2H, 2-H and 3-H), 7.32 (t-like m, 1H, 4″-H), 7.42 (t-like m, 2H, 3″-H and 5″-H), 7.67 (s, 1H, 5′-H), 7.86 (d-like m, 2H, 2″-H and 6″-H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 11.6 (C-19), 18.6 (C-18), 20.2 (CH_2), 25.2 (CH_2), 28.6 (2C, 2 × CH_2), 30.2 (CH_2), 31.9 (CH_2), 32.6 (CH_2), 34.6 (C-10), 35.9 (CH), 39.5 (CH_2), 41.2 (CH), 46.2 (C-13), 49.9 (CH), 53.1 (CH), 70.4 (C-17), 119.7 (C-5′), 125.6 (2C, C-2″ and C-6″), 125.7 (2C, C-2 and C-3), 128.0 (C-4″), 128.8 (2C, C-3″ and C-5″), 130.8 (C-1″), 146.8 (C-4′) ppm. EI-MS (70 eV) m/z (%): 401 [M^+] (40), 372 (71), 358 (44), 145 (100), 117 (41), 93 (45), 91 (62), 79 (51), 67 (37), 55 (27).

2.4.12. Synthesis of 17α-[4-(4-methoxyphenyl)-1H-1,2,3-triazol-1-yl]-5α-androst-2-ene (**11b**)

Compound **8** and 4-methoxyphenylacetylene (**9b**, 132 mg) were used for the synthesis as described in Section 2.4. After purification, **11b** was obtained as a white solid (345 mg). Mp 243–245 °C; R_f = 0.46 (ss E). Anal. Calcd. for $C_{28}H_{37}N_3O$: C, 77.92; H, 8.64. Found: C, 78.08; H, 8.76. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.30 (m, 1H), 0.60 (m, 1H), 0.73 (s, 3H, 19-H_3), 0.96 (s, 3H, 18-H_3), 1.04 (m, 1H), 1.15–1.88 (overlapping m, 14H), 2.08 (m, 1H), 2.28 (m, 1H), 2.52 (m, 1H), 3.84 (s, 3H, 4″-OMe), 4.62 (d, 1H, J = 7.5 Hz, 17-H), 5.55 (m, 2H, 2-H and 3-H), 6.96 (d, 2H, J = 8.7 Hz, 3″-H and 5″-H), 7.58 (s, 1H, 5′-H), 7.78 (d, 2H, J = 8.7 Hz, 2″-H and 6″-H) ppm. ^{13}C NMR (125 MHz, MeOD/$CDCl_3$ = 10:90): δ = 11.1 (C-19), 18.0 (C-18), 17.7 (CH_2), 24.6 (CH_2), 28.1 (2C, 2 × CH_2), 29.7 (CH_2), 31.5 (CH_2), 32.1 (CH_2), 34.1 (C-10), 35.4 (CH), 39.1 (CH_2), 40.8 (CH), 45.8 (C-13), 49.5 (CH), 52.8 (CH), 54.8 (4″-OMe), 70.1 (C-17), 113.8 (2C, C-3″ and C-5″) 118.8 (C-5′), 122.6 (C-1″), 125.2 (2C, C-2 and C-3), 126.5 (2C, C-2″ and C-6″), 146.3 (C-4′), 159.1 (C-4″) ppm. EI-MS (70 eV) m/z (%): 431 [M^+] (63), 403 (95), 388 (76), 282 (31), 175 (55), 147 (63), 132 (100), 91 (57), 79 (50), 67 (35), 55 (31).

2.4.13. Synthesis of 17α-[4-(4-fluorophenyl)-1H-1,2,3-triazol-1-yl]-5α-androst-2-ene (**11c**)

Compound **8** and 4-fluorophenylacetylene (**9c**, 0.11 mL) were used for the synthesis as described in Section 2.4. After purification, **11c** was obtained as a white solid (352 mg). Mp 184–187 °C; R_f = 0.24 (ss C). Anal. Calcd. for $C_{27}H_{34}FN_3$: C, 77.29; H, 8.17. Found: C, 77.13; H, 8.28. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.29 (m, 1H), 0.61 (m, 1H), 0.74 (s, 3H, 19-H_3), 0.97 (s, 3H, 18-H_3), 1.03 (m, 1H), 1.20 (m, 1H), 1.27–1.45 (overlapping m, 8H), 1.52–1.70 (overlapping m, 4H), 1.74–1.87 (overlapping m, 3H), 2.08 (m, 1H), 2.30 (m, 1H), 2.54 (m, 1H), 4.63 (d, 1H, J = 7.0 Hz, 17-H), 7.11 (m, 2H, 3″-H and 5″-H), 7.70 (s, 1H, 5′-H), 7.86 (bs, 2H, 2″-H and 6″-H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 11.6 (C-19), 18.6 (C-18), 20.2 (CH_2), 25.2 (CH_2), 28.6 (CH_2), 28.7 (CH_2), 30.2 (CH_2), 32.0 (CH_2), 32.7 (CH_2), 34.6 (C-10), 35.9 (CH), 39.6 (CH_2), 41.3 (CH), 46.2 (C-13), 49.9 (CH), 53.2 (CH), 70.8 (C-17), 115.8 (d, 2C, J = 21.7 Hz, C-3″ and C-5″), 119.6 (C-5′), 125.7 (2C, C-2 and C-3), 127.4 (d, 2C, J = 7.7 Hz, C-2″ and C-6″), 126.8 (C-1″), 146.8 (C-4′), 163.0 (d, J = 247.3 Hz, C-4″) ppm. EI-MS (70 eV) m/z (%): 419 [M^+] (46), 390 (54), 376 (42), 163 (100), 91 (49), 79 (48), 67 (33), 55 (25).

2.4.14. Synthesis of 17α-[4-(4-tolyl)-1H-1,2,3-triazol-1-yl]-5α-androst-2-ene (**11d**)

Compound **8** and 4-tolylacetylene (**9d**, 0.12 mL) were used for the synthesis as described in Section 2.4. After purification, **11d**

was obtained as a white solid (345 mg). Mp 251–253 °C; R_f = 0.31 (ss D). Anal. Calcd. for $C_{28}H_{37}N_3$: C, 80.92; H, 8.97. Found: C, 81.05; H, 8.88. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.29 (m, 1H), 0.60 (m, 1H), 0.73 (s, 3H, 19-H_3), 0.96 (s, 3H, 18-H_3), 1.04 (m, 1H), 1.20 (m, 1H), 1.26–1.47 (overlapping m, 7H), 1.51–1.70 (overlapping m, 3H), 1.74–1.88 (overlapping m, 3H), 2.08 (m, 1H), 2.28 (m, 1H), 2.37 (s, 3H, 4″-H_3), 2.52 (m, 1H), 4.63 (dd, 1H, J = 8.3 Hz, J = 1.2 Hz, 17-H), 5.55 (m, 2H, 2-H and 3-H), 7.23 (d, 2H, J = 8.0 Hz, 3″-H and 5″-H), 7.63 (s, 1H, 5′-H), 7.74 (d, 2H, J = 8.0 Hz, 2″-H and 6″-H) ppm. ^{13}C NMR (125 MHz, MeOD/$CDCl_3$ = 5:95): δ = 11.4 (C-19), 18.4 (C-18), 20.0 (CH_2), 21.0 (4″-CH_3), 25.0 (CH_2), 28.4 (2C, 2 × CH_2), 30.0 (CH_2), 31.8 (CH_2), 32.5 (CH_2), 34.4 (C-10), 35.7 (CH), 39.4 (CH_2), 41.1 (CH), 46.1 (C-13), 49.8 (CH), 53.1 (CH), 70.4 (C-17), 119.4 (C-5′), 125.4 (2C, C-2″ and C-6″), 125.6 (2C, C-2 and C-3), 127.4 (C-1″), 129.4 (2C, C-3″ and C-5″), 137.9 (C-4″), 146.8 (C-4′) ppm. EI-MS (70 eV) m/z (%): 415 [M^+] (63), 386 (82), 372 (68), 159 (100), 131 (52), 91 (65), 79 (60), 67 (45), 55 (34).

2.4.15. Synthesis of 17α-[4-(4-ethylphenyl)-1H-1,2,3-triazol-1-yl]-5α-androst-2-ene (**11e**)

Compound **8** and 4-ethylacetylene (**9e**, 0.13 mL) were used for the synthesis as described in Section 2.4. After purification, **11e** was obtained as a white solid (369 mg). Mp 214–216 °C; R_f = 0.32 (ss D). Anal. Calcd. for $C_{29}H_{39}N_3$: C, 81.07; H, 9.15. Found: C, 80.94; H, 9.23. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.29 (m, 1H), 0.60 (m, 1H), 0.73 (s, 3H, 19-H_3), 0.96 (s, 3H, 18-H_3), 1.04 (m, 1H), 1.18 (m, 1H), 1.25 (t, 3H, J = 7.6 Hz, 4″-$CH_2\underline{CH_3}$), 1.26–1.46 (overlapping m, 7H), 1.51–1.70 (overlapping m, 3H), 1.74–1.88 (overlapping m, 3H), 2.08 (m, 1H), 2.29 (m, 1H), 2.52 (m, 1H), 2.68 (q, 2H, J = 7.6 Hz, 4″-$\underline{CH_2}CH_3$), 4.63 (dd, 1H, J = 8.4 Hz, J = 1.2 Hz, 17-H), 5.54 (m, 2H, 2-H and 3-H), 7.25 (d, 2H, J = 8.0 Hz, 3″-H and 5″-H), 7.63 (s, 1H, 5′-H), 7.77 (d, 2H, J = 8.0 Hz, 2″-H and 6″-H) ppm. ^{13}C NMR (125 MHz, MeOD/$CDCl_3$ = 10:90): δ = 11.2 (C-19), 15.2 (4″-$CH_2\underline{CH_3}$), 18.2 (C-18), 19.9 (CH_2), 24.9 (CH_2), 28.3 (2C, 2 × CH_2), 28.4 (CH_2), 29.9 (CH_2), 31.8 (CH_2), 32.4 (CH_2), 34.3 (C-10), 35.7 (CH), 39.3 (CH_2), 41.0 (CH), 46.0 (C-13), 49.8 (CH), 53.0 (CH), 70.3 (C-17), 119.5 (C-5′), 125.4 (2C, C-2″ and C-6″), 125.5 (2C, C-2 and C-3), 127.5 (C-1″), 128.1 (2C, C-3″ and C-5″), 144.3 (C-4″), 146.8 (C-4′) ppm. EI-MS (70 eV) m/z (%): 429 [M^+] (48), 400 (81), 386 (76), 173 (100), 130 (47), 91 (66), 79 (61), 67 (44), 55 (33).

2.4.16. Synthesis of 17α-[4-(4-propylphenyl)-1H-1,2,3-triazol-1-yl]-5α-androst-2-ene (**11f**)

Compound **8** and 4-propylacetylene (**9f**, 0.16 mL) were used for the synthesis as described in Section 2.4. After purification, **11f** was obtained as a white solid (382 mg). Mp 192–194 °C; R_f = 0.48 (ss D). Anal. Calcd. for $C_{30}H_{41}N_3$: C, 81.21; H, 9.31. Found: C, 81.40; H, 9.22. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.28 (m, 1H), 0.59 (m, 1H), 0.73 (s, 3H, 19-H_3), 0.95 (t, 3H, J = 7.4 Hz, 4″-$CH_2CH_2\underline{CH_3}$), 0.96 (s, 3H, 18-H_3), 1.03 (m, 1H), 1.20 (m, 1H), 1.25–1.59 (overlapping m, 9H), 1.65 (m, 3H), 1.75 (m, 1H), 1.84 (m, 2H), 2.07 (m, 1H), 2.28 (m, 1H), 2.52 (m, 1H), 2.61 (t, 2H, J = 7.6 Hz, 4″-$\underline{CH_2}CH_2CH_3$), 4.62 (d, 1H, J = 8.3 Hz, 17-H), 5.54 (m, 2H, 2-H and 3-H), 7.23 (d, 2H, J = 8.0 Hz, 3″-H and 5″-H), 7.64 (s, 1H, 5′-H), 7.77 (d, 2H, J = 8.0 Hz, 2″-H and 6″-H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 11.6 (C-19), 13.8 (4″-$CH_2CH_2\underline{CH_3}$), 18.6 (C-18), 20.2 (CH_2), 24.5 (CH_2), 25.1 (CH_2), 28.6 (2C, 2 × CH_2), 30.2 (CH_2), 31.9 (CH_2), 32.6 (CH_2), 34.6 (C-10), 35.9 (CH), 37.8 (CH_2), 39.5 (CH_2), 41.2 (CH), 46.2 (C-13), 49.8 (CH), 53.1 (CH), 70.3 (C-17), 119.3 (C-5′), 125.5 (2C, C-2″ and C-6″), 125.7 (2C, C-2 and C-3), 128.2 (C-1″), 128.9 (2C, C-3″ and C-5″), 142.6 (C-4″), 146.9 (C-4′) ppm. EI-MS (70 eV) m/z (%): 443 [M^+] 60), 415 (98), 400 (92), 187 (100), 130 (52), 115 (65), 91 (73), 79 (66), 67 (47), 55 (37).

2.4.17. Synthesis of 17α-[4-(4-tert-butylphenyl)-1H-1,2,3-triazol-1-yl]-5α-androst-2-ene (**11g**)

Compound **8** and 4-*tert*-butylphenylacetylene (**9g**, 0.18 mL) were used for the synthesis as described in Section 2.4. After purification, **11g** was obtained as a white solid (384 mg). Mp 215–217 °C; R_f = 0.35 (ss D). Anal. Calcd. for $C_{31}H_{43}N_3$: C, 81.35; H, 9.47. Found: C, 81.44; H, 9.63. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.26 (m, 1H), 0.58 (m, 1H), 0.73 (s, 3H, 19-H_3), 0.96 (s, 3H, 18-H_3), 1.03 (m, 1H), 1.19 (m, 1H), 1.26–1.46 (overlapping m, 7H), 1.34 (s, 9H, 3 × *t*Bu-CH_3), 1.51–1.70 (overlapping m, 3H), 1.74–1.87 (overlapping m, 3H), 2.08 (m, 1H), 2.29 (m, 1H), 2.52 (m, 1H), 4.63 (dd, 1H, J = 8.4 Hz, J = 1.2 Hz, 17-H), 5.54 (m, 2H, 2-H and 3-H), 7.45 (d, 2H, J = 8.3 Hz, 3″-H and 5″-H), 7.65 (s, 1H, 5′-H), 7.79 (d, 2H, J = 8.3 Hz, 2″-H and 6″-H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 11.6 (C-19), 18.6 (C-18), 20.2 (CH_2), 25.2 (CH_2), 28.6 (2C, 2 × CH_2), 30.2 (CH_2), 31.3 (3C, 3 × *t*Bu-CH_3), 31.9 (CH_2), 32.6 (CH_2), 34.6 (2C, 4″-*t*Bu-C and C-10), 35.9 (CH), 39.5 (CH_2), 41.2 (CH), 46.3 (C-13), 49.8 (CH), 53.2 (CH), 70.3 (C-17), 119.4 (C-5′), 125.3 (2C) and 125.7 (2C): C-2″, C-3″, C-5″ and C-6″, 125.8 (2C, C-2 and C-3), 128.0 (C-1″), 146.7 (C-4′), 151.1 (C-4″) ppm. EI-MS (70 eV) m/z (%):457 [M^+] (99), 429 (100), 414 (75), 201 (56), 91 (42), 79 (39), 67 (27).

2.4.18. Synthesis of17α-[4-cyclopropyl-1H-1,2,3-triazol-1-yl]-5α-androst-2-ene (**11h**)

Compound **8** and cyclopropylacetylene (**9h**, 0.09 mL) were used for the synthesis as described in Section 2.4. After purification, **11h** was obtained as a white solid (285 mg). Mp 122–124 °C; R_f = 0.31 (ss E). Anal. Calcd. for $C_{24}H_{35}N_3$: C, 78.85; H, 9.65. Found: C, 78.76; H, 9.80. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.20 (m, 1H), 0.60 (m, 1H), 0.72 (s, 3H, 19-H_3), 0.85 (m, 3H), 0.91 (s, 3H, 18-H_3), 0.93 (m, 2H), 1.02 (m, 1H), 1.18 (m, 1H), 1.26–1.51 (overlapping m, 7H), 1.59–1.78 (overlapping m, 3H), 1.84 (m, 2H), 1.94 (m, 1H), 2.00 (m, 1H), 2.19 (m, 1H), 2.45 (m, 1H), 4.50 (dd, 1H, J = 8.5 Hz, J = 1.5 Hz, 17-H), 5.55 (m, 2H, 2-H and 3-H), 7.13 (s, 1H, 5′-H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 6.7 (C-1″), 7.7 (2C, C-2″ and C-3″), 11.6 (C-19), 18.5 (C-18), 20.2 (CH_2), 25.1 (CH_2), 28.5 (CH_2), 28.6 (CH_2), 30.2 (CH_2), 31.9 (CH_2), 32.5 (CH_2), 34.6 (C-10), 35.9 (CH), 39.6 (CH_2), 41.3 (CH), 46.1 (C-13), 49.8 (CH), 53.1 (CH), 70.1 (C-17), 119.9 (C-5′), 125.7 (2C, C-2 and C-3), 149.2 (C-4′) ppm. EI-MS (70 eV) m/z (%): 365 [M^+] (25), 322 (83), 108 (100), 91 (42), 79 (46), 67 (37).

2.4.19. Synthesis of 17α-[4-cyclopentyl-1H-1,2,3-triazol-1-yl]-5α-androst-2-ene (**11i**)

Compound **8** and cyclopentylacetylene **(9i**, 0.12 mL) were used for the synthesis as described in Section 2.4. After purification, **11i** was obtained as a white solid (295 mg). Mp 145–147 °C; R_f = 0.24 (ss E). Anal. Calcd. for $C_{26}H_{39}N_3$: C, 79.34; H, 9.99. Found: C, 79.46; H, 10.11. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.20 (m, 1H), 0.60 (m, 1H), 0.73 (s, 3H, 19-H_3), 0.91 (s, 3H, 18-H_3), 1.03 (m, 1H), 1.19 (m, 1H), 1.25–1.87 (overlapping m, 19H), 2.01 (m, 1H), 2.10 (m, 2H), 2.22 (m, 1H), 2.45 (m, 1H), 3.18 (m, 1H), 4.51 (dd, 1H, J = 8.5 Hz, J = 1.5 Hz, 17-H), 5.53 (m, 2H, 2-H and 3-H), 7.14 (s, 1H, 5′-H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 11.6 (C-19), 18.6 (C-18), 20.2 (CH_2), 25.1 (CH_2), 25.2 (CH_2), 28.6 (3C, 3 × CH_2), 30.2 (CH_2), 31.9 (CH_2), 32.5 (CH_2), 33.2 (CH_2), 33.3 (CH_2), 34.6 (C-10), 35.9 (CH), 36.8 (CH), 39.6 (CH_2), 41.2 (CH), 46.1 (C-13), 49.8 (CH), 53.1 (CH), 70.1 (C-17), 119.8 (C-5′), 125.7 (2C, C-2 and C-3), 151.8 (C-4′) ppm. EI-MS (70 eV) m/z (%): 393 [M^+] (56), 350 (93), 241 (53), 136 (92), 79 (100), 67 (79).

*2.4.20. Synthesis of 17α-[4-cyclohexyl-1H-1,2,3-triazol-1-yl]-5α-androst-2-ene (**11j**)*

Compound **8** and cyclohexylacetylene (**9j**, 0.13 mL) were used for the synthesis as described in Section 2.4. After purification, **11i** was obtained as a white solid (342 mg). Mp 158–160 °C; R_f = 0.45 (ss E). Anal. Calcd. for $C_{27}H_{41}N_3$: C, 79.55; H, 10.14. Found: C, 79.68; H, 10.24. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.17 (m, 1H), 0.58 (m, 1H), 0.72 (s, 3H, 19-H_3), 0.91 (s, 3H, 18-H_3), 1.02 (m, 1H), 1.14–1.53 (overlapping m, 13H), 1.59–1.88 (overlapping m, 8H), 1.94 (m, 1H), 1.99–2.10 (m, 3H), 2.20 (m, 1H), 2.46 (m, 1H), 2.74 (m, 1H), 4.52 (dd, 1H, J = 8.5 Hz, J = 1.6 Hz, 17-H), 5.52 (m, 2H, 2-H and 3-H), 7.12 (s, 1H, 5′-H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 11.6 (C-19), 18.6 (C-18), 20.2 (CH_2), 25.2 (CH_2), 26.0 (CH_2), 26.2 (2C, 2 × CH_2), 28.5 (CH_2), 28.6 (CH_2), 30.2 (CH_2), 31.9 (CH_2), 32.5 (CH_2), 33.0 (CH_2), 33.1 (CH_2), 34.6 (C-10), 34.6 (C-1″), 35.9 (CH), 39.6 (CH_2), 41.2 (CH), 46.1 (C-13), 49.8 (CH), 53.1 (CH), 70.1 (C-17), 119.5 (C-5′), 125.7 (2C, C-2 and C-3), 152.8 (C-4′) ppm. EI-MS (70 eV) m/z (%): 407 [M^+] (97), 364 (87), 241 (82), 150 (76), 107 (88), 95 (92), 81 (100), 79 (98), 67 (76), 55 (52).

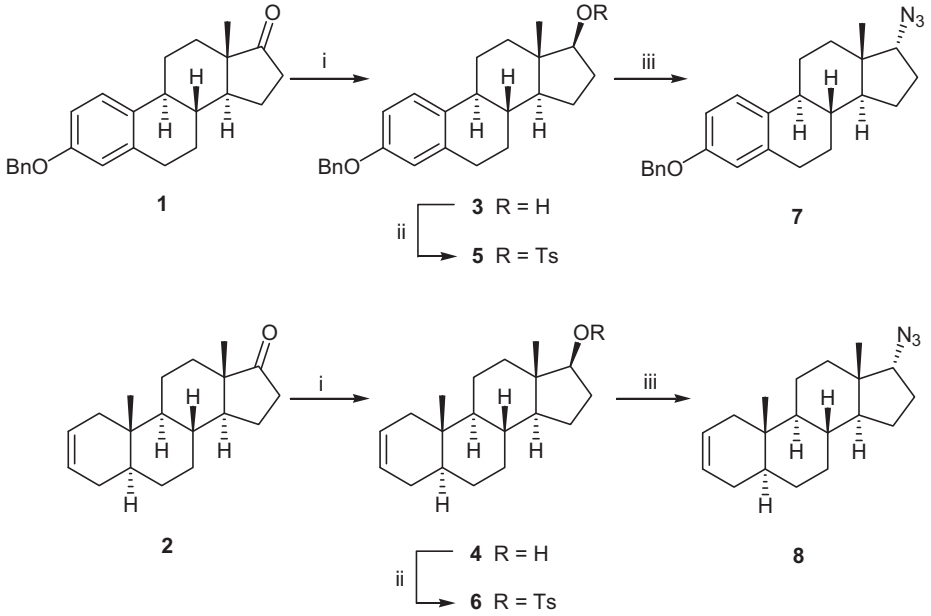
2.5. Determination of antiproliferative activities

Cytotoxic effects were measured *in vitro* on three human cell lines (ECACC; Salisbury, UK): HeLa (cervix adenocarcinoma), MCF7 (breast adenocarcinoma) and A431 (skin epidermoid carcinoma). The cells were cultivated in minimal essential medium (Sigma–Aldrich, Budapest, Hungary) supplemented with 10% fetal bovine serum, 1% non-essential amino acids and an antibiotic–antimycotic mixture. Near-confluent cells were seeded into a 96-well plate (5000 cells/well) and, after overnight standing, the medium (200 μL) containing the tested compound (at 10 or 30 μM) was added. Following a 72-h incubation in a humidified atmosphere of 5% CO_2 at 37 °C the living cells were assayed by the addition of 20 μL of 5 mg/mL MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] solution [24]. MTT was converted by intact mitochondrial reductase and precipitated as blue crystals during a 4-h contact period. The medium was then removed, the precipitated formazan crystals were solubilized in DMSO (100 μL) during a 60-min period of shaking at 25 °C, and the absorbance was read at 545 nm with a microplate reader. Wells with untreated cells were utilized as controls. All *in vitro* experiments were carried out on two microplates with at least five parallel wells. Stock solutions of the tested substances (10 mM) were prepared with DMSO. The DMSO concentration (0.3%) of the medium did not have any significant effect on cell proliferation. Cisplatin was used as reference compound.

3. Results and discussion

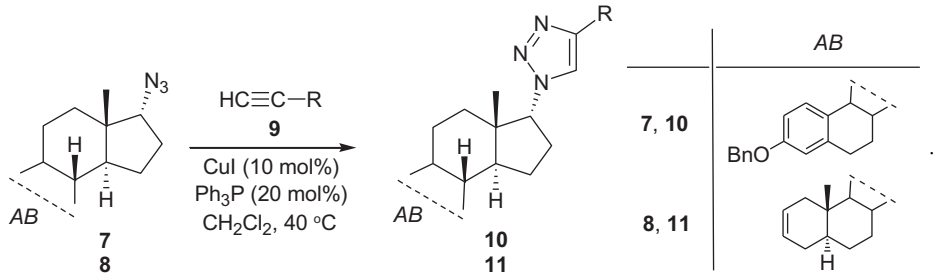
For the preparation of novel triazole derivatives, two kinds of steroidal 17α-azides (**7** and **8**), readily available from estrone-3-benzyl ether (**1**) or 5α-androst-2-en-17-one (**2**) in a three-step pathway, were used as starting materials (Scheme 1). Stereoselective reduction of the 17-keto group leading to **3** and **4** was followed by tosylation to give **5** and **6**, which then underwent facile S_N2 substitution with sodium azide in *N*,*N*-dimethylformamide to furnish the corresponding 17α-azido compounds **7** and **8** [25].

CuAAC of **7** with phenylacetylene (**9a**) was carried out in refluxing dichloromethane with CuI as catalyst (Table 1). The application of Cu(I) salts in such reactions is known to require high temperature or at least an amine base additive (DIPEA or Et_3N) for adequate formation of the Cu-acetylide complex. Moreover, certain complexing ligands (mostly TBTA or bathophenanthroline) are often employed in order to enhance the activity of the catalyst and to protect the Cu(I) from oxidation. However, complete conversion of **7** with **9a** was found to occur after 24 h in the presence of triphenylphosphine (20 mol%) instead of an amine base, and the corresponding 1,4-disubstituted triazole (**10a**) was obtained in high yield. Triphenylphosphine is assumed to accelerate the rate of the reaction and to improve the solubility of the catalyst by complexing to Cu(I), since no appreciable transformation was noted without its addition to the reaction mixture. After optimization of the reaction conditions, similar cycloadditions of **7** with different terminal acetylenes (**9b**–**j**) were performed to furnish 17α-triazolyl derivatives (**10b**–**j**) in good yields (Table 1). Analogously, a series of novel steroidal triazoles were also synthesized by reaction of **8** with alkynes (**9a**–**j**), and the products (**11a**–**j**) were isolated in yields of ~80% after purification by column chromatography.



Scheme 1. Reagents and conditions: (i) KBH_4, $MeOH/CH_2Cl_2$, rt, 8 h; (ii) TsCl, pyridine, rt, 72 h; (iii) NaN_3, DMF, 100 °C, 48 h.

Table 1
Synthesis of steroidal 1,2,3-triazoles by CuAAC.



Substrate	*Acetylene*	*R*	Product	Yield[a] (%)
7	**9a**		**10a**	85
8			**11a**	82
7	**9b**	OMe	**10b**	84
8			**11b**	80
7	**9c**	F	**10c**	85
8			**11c**	82
7	**9d**		**10d**	85
8			**11d**	83
7	**9e**		**10e**	83
8			**11e**	86
7	**9f**		**10f**	87
8			**11f**	86
7	**9g**		**10g**	84
8			**11g**	84
7	**9h**		**10h**	88
8			**11h**	78
7	**9i**		**10i**	80
8			**11i**	75
7	**9j**		**10j**	79
8			**11j**	84

[a] After purification by column chromatography.

Table 2
Antiproliferative effects of the synthetized compounds.

Triazole	Growth inhibition % ±(*SEM*)					
	HeLa		MCF7		A431	
	10 μM	30 μM	10 μM	30 μM	10 μM	30 μM
10a	<25[a]	<25	<25	<25	35 (*1.0*)	30 (*1.0*)
11a	46 (*0.8*)	72 (*0.5*)	34 (*1.3*)	47 (*0.7*)	37 (*0.9*)	58 (*0.9*)
10b	28 (*2.4*)	41 (*1.8*)	<25	33 (*1.3*)	44 (*1.80*)	48 (*2.0*)
11b	52 (*1.2*)	54 (*1.4*)	42 (*1.7*)	53 (*1.6*)	53 (*1.3*)	62 (*1.6*)
10c	<25	28 (*1.9*)	<25	<25	<25	27 (*0.9*)
11c	44 (*0.3*)	63 (*1.1*)	55 (*1.4*)	79 (*0.5*)	55 (*1.7*)	75 (*0.7*)
10d	<25	36 (*1.4*)	<25	28 (*0.1*)	<25	35 (*1.7*)
11d	33 (*1.8*)	53 (*1.7*)	<25	39 (*1.9*)	31 (*1.4*)	49 (*1.0*)
10e	<25	<25	<25	<25	<25	34 (*2.2*)
11e	30 (*0.7*)	67 (*0.7*)	<25	47 (*1.6*)	<25	51 (*1.5*)
10f	<25	27 (*1.8*)	<25	<25	<25	27 (*1.9*)
11f	60 (*1.0*)	79 (*0.4*)	35 (*1.6*)	53 (*0.5*)	69 (*0.8*)	81 (*0.1*)
10g	<25	<25	<25	<25	35 (*1.9*)	30 (*1.7*)
11g	27 (*0.7*)	46 (*0.9*)	<25	30 (*1.7*)	30 (*1.4*)	48 (*0.6*)
10h	47 (*1.8*)	43 (*2.0*)	35 (*1.5*)	42 (*1.0*)	48 (*1.9*)	50 (*2.0*)
11h	52 (*1.4*)	98 (*0.1*)	30 (*1.9*)	92 (*0.7*)	<25	82 (*0.8*)
10i	46 (*1.5*)	52 (*2.0*)	26 (*1.5*)	39 (*1.1*)	32 (*1.2*)	39 (*1.9*)
11i	40 (*1.7*)	67 (*1.3*)	<25	63 (*2.1*)	39 (*1.4*)	56 (*1.8*)
10j	35 (*1.5*)	38 (*1.6*)	<25	26 (*2.1*)	<25	28 (*1.0*)
11j	52 (*1.7*)	71 (*0.4*)	24 (*1.6*)	55 (*1.0*)	29 (*2.2*)	71 (*1.4*)
Cisplatin	43 (*2.3*)	100 (*0.3*)	53 (*2.3*)	87 (*1.2*)	89 (*0.5*)	90 (*1.8*)

[a] Compounds eliciting less than 25% inhibition of proliferation were considered ineffective and the exact results are not given, for simplicity.

In the ^{1}H NMR spectra of compounds **10a**–**g** and **11a**–**g**, the signals of the protons on the Ph ring appeared at 6.5–8.0 ppm. The 5-H singlet of the newly formed hetero ring was observed at 7.6–7.7 ppm for the aryl-substituted derivatives (**10a**–**g** and **11a**–**g**), and at 7.1–7.2 ppm for those containing a cycloalkyl substituent (**10h**–**j** and **11h**–**j**).

The novel triazolyl derivatives (**10a**–**j** and **11a**–**j**) were applied in *in vitro* pharmacological studies in order to investigate their antiproliferative effects on three human adherent malignant cell lines (Table 2). The cell-growth-inhibitory potencies of the benzyl ether derivatives (**10a**–**j**) were generally found to be lower than those of their counterparts (**11a**–**j**) from the androst-2-ene series. Compounds **10a**–**g** may be considered to be practically ineffective, while the introduction of a smaller cycloalkyl ring instead of an aromatic moiety into the triazole ring (**10h**, **10i**) resulted in a relative increase in activity on all three cell lines. However, the moderate effect was again lower for the triazole containing a cyclohexyl group on the hetero ring (**10j**). Derivatives with an unsaturated ring A proved to possess higher activity. *Para*-substitution of the phenyl ring with an F or OMe group (**11b**, **11c**) enhanced the inhibition of the growth of at least the MCF7 and A431 cells at both applied concentrations, while extension of the carbon chain on the Ph ring also resulted in increased activity at 30 μM (in the sequence Me < Et < Pr). Furthermore, compound **11g**, with a *tert*-butyl group on the Ph ring, exhibited limited efficacy, not attaining 50% proliferation inhibition even at 30 μM. Compound **11h** was the most potent of the tested derivatives, causing 82–98% growth inhibition on all malignant cell lines at 30 μM, and therefore comparable to the reference compound cisplatin. Since most of the other compounds displayed substantially lower activity, cyclopropyl-substituted triazole is considered to be a favorable structural moiety in the development of more potent steroidal antiproliferative agents.

4. Conclusions

In view of the lack of structural characteristics of estrogenic and androgenic steroids contributing to their binding to the corresponding hormone receptors, the major aim of the present work was to synthetize novel steroidal heterocycles in order to investigate their cytostatic activities. The syntheses were carried out efficiently from the corresponding azides with terminal acetylenes by CuAAC, triphenylphosphine being applied as additive. All compounds were tested *in vitro* as concerns their antiproliferative activities on three malignant cell lines, and the cyclopropyl-substituted triazole in the 5α-androst-2-ene series proved to exert a promising cell-growth-inhibitory effect at 30 μM. Although the antiproliferative activities of the tested compounds are moderate, the results suggest that steroidal triazoles may induce a disturbance in the cell division by a mode other than hormone receptor-based action, motivating the search for further derivatives and optimization for better activities.

Acknowledgements

The work was supported financially by the Hungarian Scientific Research Fund (OTKA PD 72403 and K 72309). The project named, "TÁMOP 4.2.1/B-09/1/KONV-2010-0005—Creating the Center of Excellence at the University of Szeged" is supported by the European Union and co-financed by the European Regional Fund. The authors thank to Mrs. Györgyi Udvarnoki (University of Göttingen, Germany) for the mass spectra and Mrs. Irén Forgó (University of Szeged, Hungary) for the technical assistance.

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