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Synthesis of novel 17-(5′-iodo)triazolyl-3-methoxyestrane epimers *via* Cu(I)-catalyzed azide-alkyne cycloaddition, and an evaluation of their cytotoxic activity *in vitro*

Gyula Schneider [a,*], Tamás Görbe [a,1], Erzsébet Mernyák [a], János Wölfling [a], Tamás Holczbauer [b], Mátyás Czugler [b], Pál Sohár [c], Renáta Minorics [d], István Zupkó [d,*]

[a] Department of Organic Chemistry, University of Szeged, Dóm tér 8., H-6720 Szeged, Hungary
[b] Institute of Organic Chemistry, Research Center of Natural Sciences, Hungarian Academy of Sciences, Pusztaszeri út 59-67., H-1025 Budapest, Hungary
[c] Institute of Chemistry, Eötvös Lóránd University, Pázmány Péter sétány 1/A., H-1117 Budapest, Hungary
[d] Department of Pharmacodynamics and Biopharmacy, University of Szeged, Eötvös u.6., H-6720 Szeged, Hungary

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Dedicated to Professor emerita Irene Vincze on the occasion of her 85th birthday.

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ABSTRACT

The regioselective Cu(I)-catalyzed 1,3-dipolar cycloaddition of 3-methoxyestrane 17α- and 17β-azide epimers (**3** and **5**) with different terminal alkynes afforded novel 1,4-substituted triazolyl derivatives (**8a–c** and **10a–c**). If the Ph_3P in the classical CuAAC process was replaced by Et_3N, the formation of small quantities of 5-iodotriazoles (**9a–c** and **11a–c**) was observed. For the preparation of 5-iodo-1,2,3-triazoles (**9a–c** and **11a–c**), an improved method was developed, directly from steroidal azides and terminal alkynes, in reactions mediated by CuI and ICl as iodinating agents. The antiproliferative activities of the structurally related triazoles were determined *in vitro* with the microculture tetrazolium assay on six malignant human cell lines of gynecological origin (HeLa, A2780, MCF7, MB-231, MB-361 and T47D). X-ray analysis revealed the presence of the iodo substituent on the 1,2,3-triazole ring.

1. Introduction

Steroid derivatives in which ring D is modified with *exo*-heterocycles exhibit numerous forms of biological activity and are attractive for medicine [1]. Several novel synthesized compounds containing five- or six-membered 17β-*exo*-heterocycles are 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 prostate carcinoma [2–5]. A new family of 17-azolyl-Δ^{16}-steroids was recently reported by Brodie et al. [6]. Although a few Δ^{16}-17-azole-androstane steroids are known in which the azole is attached to the steroid nucleus through a carbon atom of the heterocycle [7], the isomeric compounds in which the azole group is attached to the steroid nucleus through a nitrogen of the azole appear to constitute a new class of compounds. A number of diverse triazolyl derivatives have been reported to exhibit biological activity, including antibacterial [8], antiallergic [9] and anti-HIV [10] effects, but steroids containing this kind of structural moiety have received less attention from both synthetic and pharmacological aspects [6,11].

The Huisgen 1,3-dipolar cycloaddition of organic azides and terminal alkynes has been of considerable interest in recent years following the independent introduction of Cu(I) catalysis in 2002 by the research groups of Sharpless [12] and Meldal [13]. The presence of the catalyst dramatically improves both the rate and the regioselectivity of the reaction, leading exclusively to the 1,4-disubstituted 1,2,3-triazole [14].

To the best of our knowledge, relatively few examples are to be found in the literature in which Cu(I)-catalyzed 1,3-dipolar azide-alkyne cycloaddition (CuAAC) is applied to steroid azides [15,16], 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 et al. recently reported the synthesis of 21-triazolyl derivatives of pregnenolone as potential anticancer agents through use of the click chemistry approach,

* Corresponding authors. Fax: +36 (62) 544200 (G. Schneider), +36 (62) 546424 (I. Zupkó).
E-mail addresses: schneider@chem.u-szeged.hu (G. Schneider), zupko@pharm.u-szeged.hu (I. Zupkó).
[1] Present address: Organic Chemistry, Arrhenius Laboratory, Stockholm University, 106 91 Stockholm, Sweden.

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but without any suggestion concerning their mode of action [17]. The most frequent synthetic modifications are introduced at the position adjacent to the existing C-2 or C-17 functional groups, where substitution is facilitated [18,19]. Substitution on C-1 or C-15 of the sterane skeleton has proved to be more difficult, necessitating several reaction steps, and is therefore rarely performed [20,21].

The position and the steric orientation of the azido group are determined by the synthetic method applied. The C-17 azido group is generally in the α position, which leads to the nucleophilic exchange reaction of 17β-tosylate or mesylate by sodium azide with Walden inversion.

Frank et al. recently reported the regioselective synthesis of steroidal 17α-azides with different terminal alkynes by CuAAC, furnishing novel 1,4-disubstituted triazolyl derivatives in good yields in both the estrane and the androstane series [19]. The antiproliferative activities of the structurally related triazoles were determined *in vitro* on three malignant human cell lines (HeLa, MCF7 and A431). Although the antiproliferative activities of the tested compounds were 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.

We set out to synthesize not only the 17α-azides, but a novel series of 17β-azide epimers, as starting materials for CuAACs in order to obtain novel 1,4-disubstituted triazolyl estrone derivatives with different terminal alkynes as reagents. By means of minor modifications of earlier reported procedures, we found a new type of compound, the 5′-iodotriazolyl derivative. To the best of our knowledge, no examples are to be found in the literature in which steroidal 5′-iodotriazolyl by-products appear during a CuAAC.

We set out to obtain answers to the following questions: (1) How is the CuAAC process influenced by the steric structure of the steroidal azides? (2) How can the formation of 5′-iodotriazolyl derivatives be influenced? (3) How do the antiproliferative activities in the C-17 epimer series differ?

2. Experimental

2.1. General

Melting points (mp) were determined on a Kofler block and are uncorrected. Specific rotations were measured in $CHCl_3$ (*c* 1) at 20 °C with a POLAMAT-A (Zeiss-Jena) polarimeter and are given in units of 10^{-1} deg cm^2 g^{-1}. Elementary analysis data were determined with a PerkinElmer CHN analyzer model 2400. The reactions were monitored by TLC on Kieselgel-G (Merck Si 254 F) layers (0.25 mm thick); solvent systems (ss): (A) CH_2Cl_2, (B) CH_2Cl_2/hexane (1:1, v/v), (C) acetone/toluene/hexane (30:35:35, v/v). The spots were detected by spraying with 5% phosphomolybdic acid in 50% aqueous phosphoric acid. The R_f values were determined for the spots observed by illumination at 254 and 365 nm. Flash chromatography: silica gel 60, 40–63 μm. All solvents were distilled immediately prior to use. NMR spectra were recorded on a Bruker DRX 500 instrument at 500 (^{1}H NMR) or 125 MHz (^{13}C NMR). Chemical shifts are reported in ppm (δ scale), and coupling constants (*J*) in Hz. For the determination of multiplicities, the *J*-MOD pulse sequence was used.

2.2. 3-Methoxyestra-1,3,5(10)-triene-17β-tosylate (**2b**)

3-Methoxyestra-1,3,5(10)trien-17β-ol (**2a**, 14.32 g, 50 mmol) was dissolved in pyridine (100 ml), and a solution of *p*-toluenesulfonyl chloride (14.25 g, 75 mmol) in pyridine (50 ml) was added during cooling with ice. The reaction mixture was allowed to stand for 24 h and then poured onto a mixture of ice (500 g) and concentrated H_2SO_4 (50 ml). The crystalline precipitate that separated out was filtered off, washed thoroughly with water, and recrystallized from a mixture of acetone and water, resulting in **2b** (19.2 g, 87%). Mp 162–163 °C (Ref. [22]: 162–164 °C).

2.3. 3-Methoxyestra-1,3,5(10)-trien-17α-azide (**3**)

Compound **2b** (8.80 g, 20 mmol) was dissolved in *N*,*N*-dimethylformamide (200 ml), and NaN_3 (5.2 g, 80 mmol) was added. The mixture was stirred for 48 h at 100 °C, and then poured into water (400 ml). The precipitate that formed was filtered off and washed with water. The residue obtained was dissolved in CH_2Cl_2 and chromatographed on silica gel with CH_2Cl_2/hexane (1:1, v/v) to yield **3** (5.20 g, 83.6%) as a white solid. Mp 43–45 °C; R_f = 0.70 (ss B); $[\alpha]_D^{20}$ + 14 (*c* = 1 in $CHCl_3$). (Found: C, 73.41; H, 7.92. $C_{19}H_{25}N_3O$ requires: C, 73.28; H, 8.09%.)

^{1}H NMR 0.79 (s, 3H, 18-H_3), 2.86 (m, 2H, 6-H_2), 3.59 (d, 1H, *J* = 6.5 Hz, 17-H), 3.78 (s, 3H, 3-OCH_3), 6.64 (d, 1H, *J* = 2.2 Hz, 4-H), 6.73 (dd, 1H, *J* = 8.6 Hz, *J* = 2.2 Hz, 2-H), 7.22 (d, 1H, *J* = 8.6 Hz, 1-H). ^{13}C NMR 17.7 (C-18), 24.3, 26.2, 28.0, 28.7, 29.9, 32.6, 39.1, 43.4, 46.3, 48.6, 55.2 (3-OCH_3), 71.6 (C-17), 111.5 (C-2), 113.8 (C-4), 126.3 (C-1), 132.5 (C-10), 137.9 (C-5), 157.5 (C-3).

2.4. 3-Methoxyestra-1,3,5(10)-trien-17α-iodide (**4**)

Compound **2a** (17.18 g, 60 mmol), Ph_3P (15.78 g, 60.15 mmol) and imidazole (4.10 g, 60.15 mmol) were dissolved in toluene (250 ml), and I_2 (15.26 g, 60.15 mmol) was added in two portions. The reaction mixture was stirred at 80 °C for 2 h and allowed to cool to room temperature. A saturated aqueous Na_2SO_3 solution (150 ml) was then added and the resulting mixture was stirred until all the solids had dissolved. EtOAc (100 ml) was added, and the organic phase was washed with saturated aqueous $NaHCO_3$ (2 × 100 ml) and brine (100 ml), dried over Na_2SO_4 and evaporated *in vacuo*. The residue was subjected to column chromatography on silica gel in CH_2Cl_2/hexane (1:3, v/v) to yield **4** (19.6 g, 82.4%) as a white solid. Mp 95–97 °C; R_f = 0.75 (ss B); $[\alpha]_D^{20}$ –71 (*c* 1 in $CHCl_3$). (Found: C, 57.42; H, 6.48. $C_{19}H_{25}IO$ requires: C, 57.58; H, 6.36%.)

^{1}H NMR 0.87 (s, 3H, 18-H_3), 2.87 (m, 2H, 6-H_2), 3.78 (s, 3H, 3-OCH_3), 4.43 (d, 1H, *J* = 6.9 Hz, 17-H), 6.64 (d, 1H, *J* = 2.2 Hz, 4-H), 6.72 (dd, 1H, *J* = 8.6 Hz, *J* = 2.2 Hz, 2-H), 7.21 (d, 1H, *J* = 8.6 Hz, 1-H). ^{13}C NMR 15.8 (C-18), 24.6, 27.4, 28.0, 29.9, 36.8, 39.6, 40.8, 43.2, 45.7, 48.2, 48.3, 55.2 (3-OCH_3), 111.5 (C-2), 113.8 (C-4), 126.4 (C-1), 132.4 (C-10), 137.9 (C-5), 157.5 (C-3).

2.5. 3-Methoxyestra-1,3,5(10)-trien-17β-azide (**5**) and 3-methoxyestra-1,3,5(10),16-tetraene (**6**)

Compound **4** (12.45 g, 30 mmol) was dissolved in *N*,*N*-dimethylformamide (200 ml), and NaN_3 (7.46 g, 120 mmol) was added. The mixture was stirred for 24 h at 60 °C, and then poured onto ice (600 g). The resulting emulsion was extracted with CH_2Cl_2 (3 × 150 ml). The CH_2Cl_2 phase was washed with water, dried over Na_2SO_4, evaporated *in vacuo* and subjected to chromatographic separation on silica gel in CH_2Cl_2/hexane (1:3 v/v) to yield **6** (4.20 g, 49.35%) as a slowly-crystallizing colorless oil. Mp 65–67 °C; (Ref. [23]: mp 66–68 °C), R_f=0.80 (ss B); $[\alpha]_D^{20}$ + 109 (*c* 1 in $CHCl_3$). (Found: C, 84.91; H, 9.17. $C_{19}H_{24}O$ requires: C, 85.03; H, 9.01%.) Continued elution resulted in **5** (3.83 g, 41.00%) as a white solid. Mp 117–119 °C; R_f = 0.65 (ss B); $[\alpha]_D^{20}$ + 42 (*c* 1 in $CHCl_3$). (Found: C, 73.37; H, 8.15. $C_{19}H_{25}N_3O$ requires: C, 73.28; H, 8.09%.) ^{1}H NMR (δ, ppm): 0.80 (s, 3H, 18-H_3), 2.85 (m, 2H, 6-H_2), 3.42 (t, 1H, *J* = 9.0 Hz, 17-H), 3.78 (s, 3H, 3-OCH_3), 6.64

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(d, 1H, J = 2.2 Hz, 4-H), 6.72 (dd, 1H, J = 8.6 Hz, J = 2.2 Hz, 2-H), 7.20 (d, 1H, J = 8.6 Hz, 1-H). ^{13}C NMR (δ, ppm): 12.3 (C-18), 23.3, 26.2, 27.0, 27.4, 29.7, 37.2, 38.8, 43.8, 44.7, 51.2, 55.2 (3-OCH_3), 71.3 (C-17), 111.5 (C-2), 113.8 (C-4), 126.3 (C-1), 132.3 (C-1P0), 137.8 (C-5), 157.5 (C-3).

2.6. 3-Methoxy-17α-(4-phenyl-1H-1,2,3-triazol-1-yl)estra-1,3,5(10)-triene (**8a**)

3-Methoxyestra-1,3,5(10)-trien-17α-azide (**3**) (312 mg, 1.00 mmol) was dissolved in CH_2Cl_2 (20 ml), and CuI (19 mg, 0.10 mmol), Ph_3P (52 mg, 0.20 mmol) and phenylacetylene (**7a**, 0.22 ml, 2 mmol) were added. The mixture was stirred under reflux for 24 h, and then diluted with water (30 ml) and extracted with CH_2Cl_2 (2 × 30 ml). The combined organic phase was dried over Na_2SO_4, and evaporated *in vacuo*. The crude product was purified by flash chromatography with CH_2Cl_2/hexane (1:1 v/v) to yield pure **8a** (215 mg, 52%) as a white solid. Mp 230–232 °C; R_f = 0.50 (ss C); $[\alpha]_D^{20}$ + 57 (c 1 in $CHCl_3$). (Found C, 78.61; H, 7.43. $C_{27}H_{31}N_3O$ requires C, 78.42; H, 7.56%.) ^{1}H NMR (δ, ppm): 1.00 (s, 3H, 18-H_3), 2.87 (m, 2H, 6-H_2), 3.76 (s, 3H, 3-OCH_3), 4.68 (d, 1H, J = 7.8 Hz, 17-H), 6.62 (d, 1H, J = 2.2 Hz, 4-H), 6.67 (dd, 1H, J = 8.6 Hz, J = 2.2 Hz, 2-H), 7.10 (d, 1H, J = 8.6 Hz, 1-H), 7.33 (t, 1H, J = 7.3 Hz, 4′-H), 7.43 (d, 2H, J = 7.3 Hz, 3′-and 5′-H), 7.72 (s, 1H, C = CH), 7.87 (d, 2H, J = 7.3 Hz, 2′- and 6′-H). ^{13}C NMR (δ, ppm): 18.7 (C-18), 24.9, 26.0, 28.0, 28.7, 29.8, 32.7, 39.2, 43.1, 46.6, 48.9, 55.2 (3-OCH_3), 70.5 (C-17), 111.4 (C-2), 113.8 (C-4), 119.9 (C = CH), 125.6 and 128.8 (2x2C: C-2′, -3′, -5′, -6′), 126.2 (C-1), 128.0 (C-4′), 130.8 (C-1′), 132.2 (C-10), 137.8 (C-5), 146.9 (C = CH), 157.5 (C-3).

2.7. General procedure for the synthesis of triazoles (**8a–d**, **9a–d**, **10a–d** and **11a–d**) in the presence of Et_3N

3-Methoxyestra-1,3,5(10)-trien-17α-azide (**3**) (312 mg, 1.00 mmol) or 3-methoxyestra-1,3,5(10)-trien-17β-azide (**5**) (312 mg, 1.00 mmol) was dissolved in CH_2Cl_2 (20 ml), and CuI (19 mg, 0.10 mmol), Et_3N (0.2 ml, 2 mmol) and substituted acetylene derivative (**7a–d**, 2 mmol) were added. The mixture was stirred under reflux for 24 h, and then diluted with water (30 ml) and extracted with CH_2Cl_2 (2 × 30 ml). The combined organic phases were dried over Na_2SO_4, and evaporated *in vacuo*. The crude product was purified by flash chromatography, using CH_2Cl_2/hexane (1:3 v/v), CH_2Cl_2/hexane (1:1 v/v) or CH_2Cl_2/hexane (2:1 v/v).

2.8. 3-Methoxy-17α-(4′-phenyl-1H-1,2,3-triazol-1-yl)estra-1,3,5(10)-triene (**8a**) and 3-methoxy-17α-(4′-phenyl-5′-iodo-1H-1,2,3-triazol-1-yl)estra-1,3,5(10)-triene (**9a**)

Compound **3** and phenylacetylene (**7a**, 0.22 ml) were used for the synthesis as described in Section 2.4. The crude product was chromatographed on silica gel with CH_2Cl_2/hexane (1:3 v/v) to yield pure **9a** (35 mg, 6.5%) as a white solid. Mp 226–228 °C; R_f = 0.55 (ss C); $[\alpha]_D^{20}$ + 153 (c 1 in $CHCl_3$). (Found: C, 59.98; H, 5.78. $C_{27}H_{30}IN_3O$ requires C, 60.11; H, 5.61%.). ^{1}H NMR (δ, ppm): 1.07 (s, 3H, 18-H_3), 2.87 (m, 2H, 6-H_2), 3.77 (s, 3H, 3-OCH_3), 4.81 (dd, 1H, J = 8.3 Hz, J = 1.8 Hz, 17-H), 6.63 (d, 1H, J = 2.2 Hz, 4-H), 6.68 (dd, 1H, J = 8.6 Hz, J = 2.2 Hz, 2-H), 7.10 (d, 1H, J = 8.6 Hz, 1-H), 7.40 (t, 1H, J = 7.3 Hz, 4′-H), 7.48 (d, 2H, J = 7.3 Hz, 3′- and 5′-H), 7.98 (d, 2H, J = 7.3 Hz, 2′- and 6′-H). ^{13}C NMR (δ, ppm): 18.7 (C-18), 25.2, 26.1, 27.9, 29.2, 29.9, 32.8, 39.1, 42.9, 47.3, 48.6, 55.2 (3-OCH_3), 70.0 (C-17), 79.1 (C = CI), 111.5 (C-2), 113.8 (C-4), 126.2 (C-1), 127.6 (2C) and 128.4 (3C): C-2′, -3′, -4′, -5′, -6′), 130.5 (C-1′), 132.4 (C-10), 137.9 (C-5), 148.7 (C = CI), 157.4 (C-3).

Continued elution with CH_2Cl_2/hexane (1:1) resulted in **8a** (252 mg, 61%) as a white solid. The physical data were the same as described in Section 2.5.

2.9. 3-Methoxy-17α-[4′-(4‴-tolyl)-1H-1,2,3-triazol-1-yl]estra-1,3,5(10)-triene (**8b**) and 3-methoxy-17α-[4′-(4‴-tolyl)-5′-iodo-1H-1,2,3-triazol-1-yl]estra-1,3,5(10)-triene (**9b**)

Compound **3** and 4-tolylacetylene (**7b**, 0.22 ml) were used for the synthesis as described in Section 2.4. The crude product was chromatographed on silica gel with CH_2Cl_2/hexane (1:3 v/v) as eluent and crystallized from acetone to afford colorless crystalline **9b** (42 mg, 7.6%). Mp 212–214 °C; R_f = 0.70 (ss C); $[\alpha]_D^{20}$ + 106 (c 1 in $CHCl_3$). (Found: C, 60.85; H, 5.72. $C_{28}H_{32}IN_3O$ requires C, 60.76; H, 5.83%). ^{1}H NMR (δ, ppm): 1.06 (s, 3H, 18-H_3), 2.41 (s, 3H, tolyl-CH_3), 2.86 (m, 2H, 6-H_2), 3.76 (s, 3H, 3-OCH_3), 4.79 (d, 1H, J = 8.2 Hz, 17-H), 6.63 (d, 1H, J = 2.2 Hz, 4-H), 6.67 (dd, 1H, J = 8.6 Hz, J = 2.2 Hz, 2-H), 7.10 (d, 1H, J = 8.6 Hz, 1-H), 7.28 (d, 2H, J = 7.9 Hz, 3′- and 5′-H), 7.86 (d, 2H, J = 7.9 Hz, 2′- and 6′-H). ^{13}C NMR (δ, ppm): 18.7 (C-18), 21.3 (tolyl-CH_3), 25.2, 26.1, 27.9, 29.2, 29.9, 32.8, 39.1, 42.9, 47.3, 48.6, 55.2 (3-OCH_3), 70.0 (C-17), 78.8 (C = CI), 111.5 (C-2), 113.7 (C-4), 126.2 (C-1), 127.5 and 129.1 (2 × 2C: C-2′, -3′, -5′, -6′), 127.6 (C-1′), 132.4 (C-10), 137.9 and 138.3 (C-5 and C-4′), 148.8 (C = CI), 157.4 (C-3).

Continued elution with CH_2Cl_2/hexane (1:1 v/v) resulted in **8b** (193 g, 45%) as a white solid. Mp 224–226 °C; R_f = 0.60 (ss C); $[\alpha]_D^{20}$ + 33 (c 1 in $CHCl_3$). (Found C, 78.51; H, 7.92. $C_{28}H_{33}N_3O$ requires C 78.65; H, 7.78%.) ^{1}H NMR (δ, ppm): 1.00 (s, 3H, 18-H_3), 2.38 (s, 3H, tolyl-CH_3), 2.87 (m, 2H, 6-H_2), 3.76 (s, 3H, 3-OCH_3), 4.68 (dd, 1H, J = 8.4 Hz, J = 1.3 Hz, 17-H), 6.62 (d, 1H, J = 2.2 Hz, 4-H), 6.67 (dd, 1H, J = 8.6 Hz, J = 2.2 Hz, 2-H), 7.10 (d, 1H, J = 8.6 Hz, 1-H), 7.23 (d, 2H, J = 7.9 Hz, 3′- and 5′-H), 7.67 (s, 1H, C = CH), 7.75 (d, 2H, J = 7.9 Hz, 2′- and 6′-H). ^{13}C NMR (δ, ppm): 18.7 (C-18), 21.3 (tolyl-CH_3), 24.9, 26.0, 28.0, 28.7, 29.8, 32.7, 39.2, 43.1, 46.6, 48.9, 55.2 (3-OCH_3), 70.4 (C-17), 111.4 (C-2), 113.8 (C-4), 119.5 (C = CH), 125.6 and 129.5 (2 × 2C: C-2′, -3′, -5′, -6′), 126.3 (C-1), 128.0 (C-1′), 132.3 (C-10), 137.8 (2C: C-5 and C-4′), 147.0 (C = CH), 157.5 (C-3).

2.10. 3-Methoxy-17α-(4′-benzoyloxymethyl-1H-1,2,3-triazol-1-yl)estra-1,3,5(10)-triene (**8c**) and 3-methoxy-17α-(4′-benzoyloxymethyl-5′-iodo-1H-1,2,3-triazol-1-yl)estra-1,3,5(10)-triene (**9c**)

Compound **3** and propargyl benzoate (**7c**, 0.20 ml) were used for the synthesis as described in Section 2.4. The crude product was chromatographed on silica gel with CH_2Cl_2/hexane (1:1 v/v) to yield pure **9c** (39 mg, 5.5%) as a white solid. Mp 106–108 °C; R_f = 0.70 (ss C); $[\alpha]_D^{20}$ + 136 (c 1 in $CHCl_3$). (Found C, 58.05; H, 5.54. $C_{29}H_{32}IN_3O_3$ requires C 58.30; H, 5.40%.) ^{1}H NMR (δ, ppm): 1.04 (s, 3H, 18-H_3), 2.86 (m, 2H, 6-H_2), 3.76 (s, 3H, 3-OCH_3), 4.70 (dd, 1H, J = 7.9 Hz, J = 2.1 Hz, 17-H), 5.45 (d, 2H, J = 3.8 Hz, OCH_2), 6.63 (d, 1H, J = 2.2 Hz, 4-H), 6.67 (dd, 1H, J = 8.6 Hz, J = 2.2 Hz, 2-H), 7.09 (d, 1H, J = 8.6 Hz, 1-H), 7.44 (d, 2H, J = 7.7 Hz, 3′- and 5′-H), 7.56 (t, 1H, J = 7.7 Hz, 4′-H), 8.07 (d, 2H, J = 7.7 Hz, 2′- and 6′-H). ^{13}C NMR (δ, ppm): 18.7 (C-18), 25.1, 26.0, 27.9, 29.2, 29.8, 32.7, 39.1, 42.9, 47.3, 48.5, 55.2 (3-OCH_3), 58.3 (OCH_2), 70.3 (C-17), 83.3 (C = CI), 111.5 (C-2), 113.8 (C-4), 126.2 (C-1), 128.3 and 129.8 (2x2C: C-2′, -3′, -5′, -6′), 129.7 (C-1′), 132.3 (C-10), 133.1 (C-4′), 137.9 (C-5), 145.7 (C = CI), 157.4 (C-3), 166.2 (OCO).

Continued elution with CH_2Cl_2/hexane (2:1 v/v) resulted in **8c** (290 mg, 61.5%) as a white solid. Mp 160–162 °C; R_f = 0.60 (ss C); $[\alpha]_D^{20}$ + 33 (c 1 in $CHCl_3$). (Found C, 73.98; H, 6.92. $C_{29}H_{33}N_3O_3$ requires C 73.86; H, 7.05%). ^{1}H NMR (δ, ppm): 0.98 (s, 3H, 18-H_3), 2.85 (m, 2H, 6-H_2), 3.76 (s, 3H, 3-OCH_3), 4.64 (d, 1H,

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J = 8.1 Hz, 17-H), 5.49 (s, 2H, OCH_2), 6.62 (d, 1H, J = 2.2 Hz, 4-H), 6.67 (dd, 1H, J = 8.6 Hz, J = 2.2 Hz, 2-H), 7.09 (d, 1H, J = 8.6 Hz, 1-H), 7.43 (t, 2H, J = 7.7 Hz, 3′- and 5′-H), 7.55 (t, 1H, J = 7.7 Hz, 4′-H), 7.65 (s, 1H, C = CH), 8.05 (d, 2H, J = 7.7 Hz, 2′- and 6′-H). ^{13}C NMR (δ, ppm): 18.7 (C-18), 24.9, 25.9, 27.9, 28.7, 29.8, 32.6, 39.1, 43.1, 46.6, 48.8, 55.2 (3-OCH_3), 58.2 (OCH_2), 70.5 (C-17), 111.5 (C-2), 113.7 (C-4), 124.3 (C = CH), 126.2 (C-1), 128.4 and 129.8 (2 × 2C: C-2′, -3′, -5′, -6′), 129.7 (C-1′), 132.2 (C-10), 133.1 (C-4′), 137.8 (C-5), 142.1 (C = CH), 157.5 (C-3), 166.5 (OCO).

2.11. 3-Methoxy-17α-[4′-(4‴-toluoyloxymethyl)-1H-1,2,3-triazol-1-yl]estra-1,3,5(10)-triene (**8d**) and 3-methoxy-17α-[4′-(4‴-toluoyloxymethyl)-5′-iodo-1H-1,2,3-triazol-1-yl]estra-1,3,5(10)-triene (**9d**)

Compound **3** and propargyl 4-methylbenzoate (**7d**, 0.20 ml) were used for the synthesis as described in Section 2.4. The crude product was chromatographed on silica gel with CH_2Cl_2/hexane (1:1 v/v) to yield pure **9d** (61 mg, 7.8%) as a white solid. Mp 192–194 °C; R_f = 0.75 (ss C); $[\alpha]_D^{20}$ + 128 (c 1 in $CHCl_3$). (Found C, 59.06; H, 5.72. $C_{30}H_{34}IN_3O_3$ requires C 58.92; H, 5.60%.) ^{1}H NMR (δ, ppm): 1.04 (s, 3H, 18-H_3), 2.40 (s, 3H, tolyl-CH_3), 2.86 (m, 2H, 6-H_2), 3.76 (s, 3H, 3-OCH_3), 4.70 (m, 1H, 17-H), 5.43 (d, 2H, J = 3.0 Hz, OCH_2), 6.63 (d, 1H, J = 2.2 Hz, 4-H), 6.67 (dd, 1H, J = 8.6 Hz, J = 2.2 Hz, 2-H), 7.09 (d, 1H, J = 8.6 Hz, 1-H), 7.23 (d, 2H, J = 7.9 Hz, 3′- and 5′-H), 7.96 (d, 2H, J = 7.9 Hz, 2′- and 6′-H). ^{13}C NMR 18.7 (C-18), 21.7 (tolyl-CH_3), 25.2, 26.0, 27.9, 29.2, 29.8, 32.7, 39.1, 42.9, 47.3, 48.5, 55.2 (3-OCH_3), 58.2 (OCH_2), 70.2 (C-17), 83.3 (C = CI), 111.5 (C-2), 113.8 (C-4), 126.1 (C-1), 127.0 (C-1′), 129.0 and 129.9 (2 × 2C: C-2′, -3′, -5′, -6′), 132.3 (C-10), 137.9 (C-5), 143.8 (C-4′), 145.8 (C = CI), 157.4 (C-3), 166.3 (OCO).

Continued elution with CH_2Cl_2/hexane (2:1 v/v) yielded pure **8d** (306 mg, 63%) as a white solid. Mp 136–138 °C; R_f = 0.65 (ss C); $[\alpha]_D^{20}$ + 30 (c 1 in $CHCl_3$). (Found C, 74.08; H, 7.41%. $C_{30}H_{35}N_3O_3$ requires C, 74.20; H, 7.26%.) ^{1}H NMR (δ, ppm): 0.97 (s, 3H, 18-H_3), 2.39 (s, 3H, tolyl-CH_3), 2.86 (m, 2H, 6-H_2), 3.76 (s, 3H, 3-OCH_3), 4.64 (d, 1H, J = 8.0 Hz, 17-H), 5.48 (s, 2H, OCH_2), 6.62 (d, 1H, J = 2.2 Hz, 4-H), 6.67 (dd, 1H, J = 8.6 Hz, J = 2.2 Hz, 2-H), 7.09 (d, 1H, J = 8.6 Hz, 1-H), 7.21 (d, 2H, J = 7.9 Hz, 3′- and 5′-H), 7.64 (s, 1H, C = CH), 7.93 (d, 2H, J = 7.9 Hz, 2′- and 6′-H). ^{13}C NMR (δ, ppm): 18.7 (C-18), 21.6 (tolyl-CH_3), 24.9, 25.9, 27.9, 28.7, 29.8, 32.6, 39.1, 43.1, 46.6, 48.8, 55.2 (3-OCH_3), 58.1 (OCH_2), 70.5 (C-17), 111.5 (C-2), 113.8 (C-4), 124.3 (C = CH), 126.2 (C-1), 127.1 (C-1′), 129.1 and 129.8 (2 × 2C: C-2′, -3′, -5′, -6′), 132.1 (C-10), 137.8 (C-5), 142.3 (C = CH), 143.9 (C-4′), 157.5 (C-3), 166.6 (OCO).

2.12. 3-Methoxy-17β-(4′-phenyl-1H-1,2,3-triazol-1-yl)estra-1,3,5(10)-triene (**10a**) and 3-methoxy-17β-(4′-phenyl-5′-iodo-1H-1,2,3-triazol-1-yl)estra-1,3,5(10)-triene (**11a**)

Compound **5** and phenylacetylene (**7a**, 0.22 ml) were used for the synthesis as described in Section 2.4. The crude product was chromatographed on silica gel with CH_2Cl_2/hexane (1:1 v/v) to yield pure **11a** (42 mg, 7.7%) as a white solid. Mp 148–150 °C; R_f = 0.60 (ss C); $[\alpha]_D^{20}$ -85 (c 1 in $CHCl_3$). (Found C, 60.23; H, 5.43. $C_{27}H_{30}IN_3O$ requires C, 60.11; H, 5.61%.) ^{1}H NMR (δ, ppm): 0.83 (s, 3H, 18-H_3), 2.89 (m, 2H, 6-H_2), 3.78 (s, 3H, 3-OCH_3), 4.69 (t, 1H, J = 9.3 Hz, 17-H), 6.65 (d, 1H, J = 2.2 Hz, 4-H), 6.71 (dd, 1H, J = 8.6 Hz, J = 2.2 Hz, 2-H), 7.19 (d, 1H, J = 8.6 Hz, 1-H), 7.40 (t, 1H, J = 7.3 Hz, 4′-H), 7.48 (d, 2H, J = 7.3 Hz, 3′- and 5′-H), 7.95 (d, 2H, J = 7.3 Hz, 2′- and 6′-H). ^{13}C NMR (δ, ppm): 13.0 (C-18), 23.3, 26.3, 27.2, 27.4, 29.7, 38.3, 38.8, 43.7, 46.1, 52.5, 55.2 (3-OCH_3), 70.2 (C-17), 78.6 (C = CI), 111.5 (C-2), 113.8 (C-4), 126.2 (C-1), 127.8 (2C) and 128.4 (3C): C-2′, -3′, -4′, -5′, -6′), 130.5 (C-1′), 132.1 (C-10), 137.8 (C-5), 149.2 (C = CI), 157.5 (C-3).

Continued elution with CH_2Cl_2 (3:1 v/v) yielded pure **10a** (236 mg, 57%) as a white solid. Mp 295–296 °C; R_f = 0.50 (ss C); $[\alpha]_D^{20}$ + 25 (c 1 in $CHCl_3$). (Found C, 78.33; H, 7.62. $C_{27}H_{31}N_3O$ requires C, 78.42; H, 7.56%.) ^{1}H NMR (δ, ppm, C_6D_6): 0.31 (s, 3H, 18-H_3), 2.72 (m, 2H, 6-H_2), 3.42 (s, 3H, 3-OCH_3), 3.99 (t, 1H, J = 9.6 Hz, 17-H), 6.72 (d, 1H, J = 2.2 Hz, 4-H), 6.79 (dd, 1H, J = 8.6 Hz, J = 2.2 Hz, 2-H), 7.00 (m, 2H) and 7.25–7.31 (overlapping multiplets, 5H): 1-H, C = CH, 3′-, 4′- and 5′-H; 8.02 (d, 2H, J = 7.5 Hz, 2′- and 6′-H). ^{13}C NMR (δ, ppm): 16.7 (C-18), 21.3, 23.0, 27.0, 27.7, 29.8, 32.7, 39.2, 40.1, 44.6, 49.9, 55.0 (3-OCH_3), 70.7 (C-17), 111.8 (C-2), 114.2 (C-4), 120.9 (C = CH), 127.8 and 129.8 (2 × 2C: C-2′, -3′, -5′, -6′), 127.2 (C-1), 129.0 (C-4′), 133.8 (C-1′), 134.2 (C-10), 139.1 (C-5), 148.9 (C = CH), 158.5 (C-3).

2.13. 3-Methoxy-17β-[4′-(4‴-tolyl)-1H-1,2,3-triazol-1-yl]estra-1,3,5(10)-triene (**10b**) and 3-methoxy-17β-[4′-(4‴-tolyl)-5′-iodo-1H-1,2,3-triazol-1-yl)estra-1,3,5(10)-triene (**11b**)

Compound **5** and 4-tolylacetylene (**7b**, 0.22 ml) were used for the synthesis as described in Section 2.4. The crude product was chromatographed on silica gel with CH_2Cl_2/hexane (2:1 v/v) to yield pure **11b** (34 mg, 6.2%) as a white crystalline form. Mp 188–190 °C; R_f = 0.75 (ss C); $[\alpha]_D^{20}$ -71 (c 1 in $CHCl_3$). (Found C, 60.47; H, 5.94. $C_{28}H_{32}IN_3O$ requires C, 60.76; H, 5.83%.) ^{1}H NMR (δ, ppm): 0.83 (s, 3H, 18-H_3), 2.41 (s, 3H, tolyl-CH_3), 2.90 (m, 2H, 6-H_2), 3.78 (s, 3H, 3-OCH_3), 4.67 (t, 1H, J = 9.4 Hz, 17-H), 6.65 (d, 1H, J = 2.2 Hz, 4-H), 6.70 (dd, 1H, J = 8.6 Hz, J = 2.2 Hz, 2-H), 7.18 (d, 1H, J = 8.6 Hz, 1-H), 7.28 (d, 2H, J = 7.9 Hz, 3′- and 5′-H), 7.83 (d, 2H, J = 7.9 Hz, 2′- and 6′-H). ^{13}C NMR (δ, ppm): 13.0 (C-18), 21.3 (tolyl-CH_3), 23.3, 26.3, 27.2, 27.4, 29.8, 38.3, 38.8, 43.7, 46.1, 52.5, 55.2 (3-OCH_3), 70.1 (C-17), 78.3 (C = CI), 111.5 (C-2), 113.8 (C-4), 126.2 (C-1), 127.7 and 129.1 (2x2C: C-2′, -3′, -5′, -6′), 128.2 (C-1′), 132.1 (C-10), 137.8 and 138.3 (C-5 and C-4′), 149.3 (C = CI), 157.5 (C-3).

Continued elution with CH_2Cl_2 yielded pure **10b** (285 mg, 67%) as a white solid. Mp 310–312 °C; R_f = 0.60 (ss C); $[\alpha]_D^{20}$ + 17 (c 1 in $CHCl_3$). (Found C, 78.53; H, 7.89. $C_{28}H_{33}N_3O$ requires C, 78.65; H, 7.78%.)

^{1}H NMR (δ, ppm, C_6D_6): 0.31 (s, 3H, 18-H_3), 2.47 (s, 3H, tolyl-CH_3), 2.72 (m, 2H, 6-H_2), 3.41 (s, 3H, 3-OCH_3), 3.99 (t, 1H, J = 9.6 Hz, 17-H), 6.72 (d, 1H, J = 2.2 Hz, 4-H), 6.80 (dd, 1H, J = 8.6 Hz, J = 2.2 Hz, 2-H), 7.26 (d, 1H, J = 8.6 Hz, 1-H), 7.25 (overlapping multiplets, 3H, 3′-, 4′- and 5′-H), 7.31 (s, 1H, C = CH), 8.03 (d, 2H, J = 7.5 Hz, 2′- and 6′-H). ^{13}C NMR (δ, ppm): 16.5 (C-18), 21.5 (tolyl-CH_3), 21.7, 22.9, 28.0, 29.7, 30.3, 32.7, 39.6, 42.1, 46.9, 50.0, 55.5 (3-OCH_3), 70.6 (C-17), 111.9 (C-2), 114.5 (C-4), 120.5 (C = CH), 127.8 and 129.9 (2x2C: C-2′, -3′, -5′, -6′), 129.3 (C-1), 132.0 (C-1′), 134.3 (C-10), 138.8 (2C: C-5 and C-4′), 148.6 (C = CH), 158.3 (C-3).

2.14. 3-Methoxy-17β-(4′-benzoyloxymethyl-1H-1,2,3-triazol-1-yl)estra-1,3,5(10)-triene (**10c**) and 3-methoxy-17β-(4′-benzoyloxymethyl-5′-iodo-1H-1,2,3-triazol-1-yl)estra-1,3,5(10)-triene (**11c**)

Compound **5** and propargyl benzoate (**7c**, 0.20 ml) were used for the synthesis as described in Section 2.4. The crude product was chromatographed on silica gel with CH_2Cl_2/hexane (1:1 v/v) to yield pure **11c** (40 mg, 6.7%) as a white solid. Mp 155–156 °C; R_f = 0.60 (ss C); $[\alpha]_D^{20}$ -73 (c 1 in $CHCl_3$). (Found C, 58.16; H, 5.57. $C_{29}H_{32}IN_3O_3$ requires C, 58.30; H, 5.40%.) ^{1}H NMR (δ, ppm): 0.80 (s, 3H, 18-H_3), 2.88 (m, 2H, 6-H_2), 3.78 (s, 3H, 3-OCH_3), 4.57 (m, 1H, 17-H), 5.45 (d, 2H, J = 4.8 Hz, OCH_2), 6.65 (d, 1H, J = 2.2 Hz, 4-H), 6.70 (dd, 1H, J = 8.6 Hz, J = 2.2 Hz, 2-H), 7.17 (d, 1H, J = 8.6 Hz, 1-H), 7.43 (t, 2H, J = 7.3 Hz, 3′- and 5′-H), 7.56 (t, 1H,

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J = 7.3 Hz, 4′-H), 8.07 (d, 2H, J = 7.3 Hz, 2′- and 6′-H). ^{13}C NMR (δ, ppm):13.0 (C-18), 23.3, 26.3, 27.1, 27.4, 29.7, 38.3, 38.8, 43.7, 46.1, 52.5, 55.2 (3-OCH_3), 58.3 (OCH_2), 70.5 (C-17), 82.9 (C = C<u>I</u>), 111.6 (C-2), 113.8 (C-4), 126.2 (C-1), 128.3 and 129.8 (2x2C: C-2′, -3′, -5′, -6′), 129.7 (C-1′), 132.0 (C-10), 133.1 (C-4′), 137.8 (C-5), 146.0 (C = CI), 157.6 (C-3), 166.2 (OCO).

Continued elution with CH_2Cl_2/hexane (2:1 v/v) yielded pure **10c** (292 mg, 62%) as a white solid. Mp 139–141 °C; R_f = 0.55 (ss C); $[\alpha]_D^{20+}$20 (c 1 in $CHCl_3$). (Found C, 73.62; H, 6.91. $C_{29}H_{33}N_3O_3$ requires C, 73.86; H, 7.05%.) ^{1}H NMR (δ, ppm): 0.59 (s, 3H, 18-H_3), 2.88 (m, 2H, 6-H_2), 3.77 (s, 3H, 3-OCH_3), 4.50 (t, 1H, J = 9.6 Hz, 17-H), 5.49 (s, 2H, OCH_2), 6.64 (d, 1H, J = 2.2 Hz, 4-H), 6.70 (dd, 1H, J = 8.6 Hz, J = 2.2 Hz, 2-H), 7.18 (d, 1H, J = 8.6 Hz, 1-H), 7.43 (t, 2H, J = 7.3 Hz, 3′- and 5′-H), 7.56 (t, 1H, J = 7.3 Hz, 4′-H), 7.71 (s, 1H, C = CH), 8.05 (d, 2H, J = 7.3 Hz, 2′- and 6′-H). ^{13}C NMR (δ, ppm): 12.2 (C-18), 23.1, 26.0, 26.2, 27.3, 29.7, 37.0, 38.8, 43.8, 44.8, 51.9, 55.2 (3-OCH_3), 58.2 (OCH_2), 70.7 (C-17), 111.5 (C-2), 113.8 (C-4), 123.5 (C = C<u>H</u>), 126.3 (C-1), 128.3 and 129.7 (2 × 2C: C-2′, -3′, -5′, -6′), 129.6 (C-1′), 132.1 (C-10), 133.1 (C-4′), 137.7 (C-5), 142.1 (C = CH), 157.5 (C-3), 166.5 (OCO).

*2.15. 3-Methoxy-17β-[4′-(4‴-toluoyloxymethyl)-1H-1,2,3-triazol-1-yl]estra-1,3,5(10)-triene (**10d**) and 3-methoxy-17β-[4′-(4‴-toluoyloxymethyl)-5′-iodo-1H-1,2,3,-triazol-1-yl]estra-1,3,5(10)-triene (**11d**)*

Compound **5** and propargyl 4-methylbenzoate (**7d**, 0.20 ml) were used for the synthesis as described in Section 2.4. The crude product was chromatographed on silica gel with CH_2Cl_2/hexane (1:1 v/v) to yield pure **11d** (46 mg, 7.5%) as a white solid. Mp 180–182 °C; R_f = 0.65 (ss C); $[\alpha]_D^{20}$ -74 (c 1 in $CHCl_3$). (Found C, 58.83; H, 5.46. $C_{30}H_{34}IN_3O_3$ requires C, 58.92; H, 5.60%). ^{1}H NMR (δ, ppm): 0.80 (s, 3H, 18-H_3), 2.40 (s, 3H, tolyl-CH_3), 2.89 (m, 2H, 6-H_2), 3.78 (s, 3H, 3-OCH_3), 4.57 (t, 1H, J = 9.5 Hz, 17-H), 5.43 (d, 2H, J = 4.8 Hz, OCH_2), 6.65 (d, 1H, J = 2.2 Hz, 4-H), 6.70 (dd, 1H, J = 8.6 Hz, J = 2.2 Hz, 2-H), 7.18 (d, 1H, J = 8.6 Hz, 1-H), 7.22 (d, 2H, J = 7.3 Hz, 3′- and 5′-H), 7.96 (d, 2H, J = 7.3 Hz, 2′- and 6′-H). ^{13}C NMR (δ, ppm): 13.0 (C-18), 22.0 (tolyl-CH_3), 23.3, 26.3, 27.1, 27.3, 29.7, 38.3, 38.8, 43.7, 46.1, 52.5, 55.2 (3-OCH_3), 58.2 (OCH_2), 70.5 (C-17), 82.8 (C = C<u>I</u>), 111.5 (C-2), 113.8 (C-4), 126.2 (C-1), 127.0 (C-1′), 129.0 and 129.9 (2x2C: C-2′, -3′, -5′, -6′), 132.0 (C-10), 137.8 (C-5), 143.8 (C-4′), 146.0 (C = CI), 157.5 (C-3), 166.3 (OCO).

Continued elution with CH_2Cl_2/hexane (2:1 v/v) yielded pure **10d** (282 mg, 58%) as a white solid. Mp 172–174 °C; R_f = 0.60 (ss C); $[\alpha]_D^{20}$ + 24 (c 1 in $CHCl_3$). (Found C, 74.05; H, 7.38. $C_{30}H_{35}N_3O_3$ requires C, 74.20; H, 7.26%.) ^{1}H NMR (δ, ppm): 0.58 (s, 3H, 18-H_3), 2.40 (s, 3H, tolyl-CH_3), 2.88 (m, 2H, 6-H_2), 3.77 (s, 3H, 3-OCH_3), 4.50 (t, 1H, J = 9.5 Hz, 17-H), 5.47 (s, 2H, OCH_2), 6.64 (d, 1H, J = 2.2 Hz, 4-H), 6.70 (dd, 1H, J = 8.6 Hz, J = 2.2 Hz, 2-H), 7.18 (d, 1H, J = 8.6 Hz, 1-H), 7.22 (d, 2H, J = 7.3 Hz, 3′- and 5′-H), 7.71 (s, 1H, C = CH), 7.94 (d, 2H, J = 7.3 Hz, 2′- and 6′-H). ^{13}C NMR (δ, ppm): 12.2 (C-18), 21.6 (tolyl-CH_3), 23.1, 26.0, 26.2, 27.4, 29.7, 36.9, 38.8, 43.8, 44.7, 51.9, 55.2 (3-OCH_3), 58.1 (OCH_2), 70.7 (C-17), 111.5 (C-2), 113.8 (C-4), 123.5 (C = C<u>H</u>), 126.3 (C-1), 127.1 (C-1′), 129.0 and 129.8 (2 × 2C: C-2′, -3′, -5′, -6′), 132.0 (C-10), 137.7 (C-5), 142.2 and 143.8 (C = CH and C-4′), 157.5 (C-3), 166.6 (OCO).

*2.16. General procedure for the synthesis of 5′-iodotriazoles (**9a-9d** and **11a-11d**)*

3-Methoxyestra-1,3,5(10)-trien-17α-azide (**3**) (312 mg, 1.00 mmol) or 3-methoxyestra-1,3,5(10)-trien-17β-azide (**5**) (312 mg, 1 mmol) was dissolved in CH_2Cl_2 (15 ml), and Et_3N (0.2 ml, 2 mmol), substituted acetylene derivative (**7a–d**, 2 mmol), ICl (1.5 mmol) and finally CuI (190 mg, 10 mmol) were added. The heterogenous reaction mixture was stirred under N_2 for 24 h, and then diluted with 1% $Na_2S_2O_3$ solution (30 ml), and extracted with CH_2Cl_2 (2 × 30 ml). The combined organic phases were dried over Na_2SO_4, and evaporated *in vacuo*. The crude product was purified by flash chromatography, using CH_2Cl_2/hexane (1:3 v/v), which eluted the starting material (**3** or **5**) and then CH_2Cl_2/hexane (1:1) to obtain the steroidal 5′-iodotriazoles (**9a-d** and **11a-d**).

*2.17. 3-Methoxy-17α-(4′-phenyl-5′-iodo-1H-1,2,3-triazol-1-yl)estra-1,3,5(10)-triene (**9a**)*

Compound **3** and phenylacetylene (**7a**) were used for the synthesis as described in Section 2.15. After purification, **9a** was obtained as a white solid (485 mg, 89%). The physical data were the same as described in Section 2.8.

*2.18. 3-Methoxy-17α-[4′-(4‴-tolyl)-5′-iodo-1H-1,2,3-triazol-1-yl]estra-1,3,5(10)-triene (**9b**)*

Compound **3** and 4-tolylacetylene (**7b**) were used for the synthesis as described in Section 2.15. After purification, **9b** was obtained as a white solid (510 mg, 92%). The physical data were the same as described in Section 2.9.

*2.19. 3-Methoxy-17α-(4′-benzoyloxymethyl-5′-iodo-1H-1,2,3-triazol-1-yl)estra-1,3,5(10)-triene (**9c**)*

Compound **3** and propargyl benzoate (**7c**) were used for the synthesis as described in Section 2.15. After purification, **9c** was obtained as a white solid (512 mg, 85%). The physical data were the same as described in Section 2.10.

*2.20. 3-Methoxy-17α-(4′-toluoyloxymethyl-5′-iodo-1H-1,2,3-triazol-1-yl)estra-1,3,5(10)-triene (**9d**)*

Compound **3** and propargyl 4-methylbenzoate (**7d**) were used for the synthesis as described in Section 2.15. After purification, **9d** was obtained as a white solid (503 mg, 82%). The physical data were the same as described in Section 2.11.

*2.21. 3-Methoxy-17β-(4′-phenyl-5′-iodo-1H-1,2,3-triazol-1-yl)estra-1,3,5(10)-triene (**11a**)*

Compound **5** and phenylacetylene (**7a**) were used for the synthesis as described in Section 2.15. After purification, **11a** was obtained as a white solid (492 mg, 91%). The physical data were the same as described in Section 2.12.

*2.22. 3-Methoxy-17β-[4′-(4‴-tolyl)-5′-iodo-1H-1,2,3-triazol-1-yl]estra-1,3,5(10)-triene (**11b**)*

Compound **5** and 4-tolylacetylene (**7b**) were used for the synthesis as described in Section 2.15. After purification, **11b** was obtained as a white solid (515 mg, 93%). The physical data were the same as described in Section 2.13.

*2.23. 3-Methoxy-17β-(4′-benzoyloxymethyl-5′-iodo-1H-1,2,3-triazol-1-yl)estra-1,3,5(10)-triene (**11c**)*

Compound **5** and propargyl benzoate (**7c**) were used for the synthesis as described in Section 2.15. After purification, **11c** was obtained as a white solid (498 mg, 83%). The physical data were the same as described in Section 2.14.

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2.24. 3-Methoxy-17β-[4′-(4‴-toluoyloxymethyl)-5′-iodo-1H-1,2,3-triazol-1-yl]estra-1,3,5(10)-triene (**11d**)

Compound **5** and propargyl 4-methylbenzoate (**7d**) were used for the synthesis as described in Section 2.15. After purification **11d** was obtained as a white solid (528 mg, 86%). The physical data were the same as described in Section 2.15.

2.25. Single-crystal X-ray diffraction of **9b**

Crystal data: $C_{28}H_{32}IN_3O$, Fwt.: 553.47, colorless, prism, size: 0.75 x 0.25 x 0.10 mm, monoclinic, space group $P\ 2_1$, $a = 10.1759(2)$ Å, $b = 7.11640(10)$ Å, $c = 18.2809(3)$ Å, $\alpha = 90°$, $\beta = 100.9050(10)°$, $\gamma = 90°$, $V = 1299.92(4)$ Å^3, $T = 295(2)$ K, $Z = 2$, $D_x = 1.414$ Mg/m^3. A crystal of **9b** was mounted on a loop. Cell parameters were determined by least-squares using 12629 ($6.69 \leqslant \theta \leqslant 66.54°$) reflections. Intensity data were collected on an R-AXIS-RAPID diffractometer (graphite monochromator Cu-$K\alpha$ radiation, $\lambda = 1.54187$ Å) at 293(2) K in the range $6.69 \leqslant \theta \leqslant 66.54°$; CrystalClear SM 1.4.0 Programs for data collection and reduction (Rigaku/MSC Inc., 2008). A total of 13464 reflections were collected, of which 4157 were unique [R(int) = 0.0512, $R(\sigma) = 0.0631$]; intensities of 3906 reflections were greater than $2\sigma(I)$. Completeness to $\theta = 0.950$. A numerical absorption correction was applied to the data (the minimum and maximum transmission factors were 0.051 and 0.443). The structure was solved by direct methods [24] and subsequent difference syntheses. Anisotropic full-matrix least-squares refinement on F^2 for all non-hydrogen atoms yielded R_1=0.0388 and $wR^2 = 0.0922$ for 1332 [$I > 2\sigma(I)$] and $R_1 = 0.0421$ and $wR^2 = 0.0943$ for all (4157) intensity data, number of parameters = 301, goodness-of-fit = 1.08, absolute structure parameter $x = 0.045(8)$ [25]. The maximum and minimum residual electron density in the final difference map were 0.47 and −1.41 e Å^{-3}. Hydrogen atomic positions were calculated from assumed geometries. Hydrogen atoms were included in structure factor calculations, but were not refined. The isotropic displacement parameters of the hydrogen atoms were approximated from the U(eq) value of the atom they were bonded to. Comparison of the experimental powder X-ray diffractogram with a calculated one from the final single-crystal structure confirmed the identity of the crystal with that of the bulk material [26]. All pertinent further calculations, molecular graphics, etc. were performed by using program PLATON and Mercury.

2.26. Determination of antiproliferative activities

The antiproliferative properties of the prepared triazolylestranes were determined on a panel of human adherent cancer cell lines of gynecological origin. MCF7, MDA-MB-36, MDA-MB-231 and T47D cells were isolated from breast cancer, while A2780 and HeLa cells were from ovarian and cervical cancer, respectively. All cell lines were purchased from European Collection of Cell Cultures (ECCAC, Salisbury, UK). Cells were cultivated in minimal essential medium supplemented with 10% fetal bovine serum, 1% non-essential amino acids and an antibiotic–antimycotic mixture. All media and supplements were obtained from PAA Laboratories GmbH, Pasching, Austria. Near-confluent cancer cells were seeded onto a 96-well microplate (5000 cells/well) and, after overnight standing, new medium (200 μl) containing the tested compounds was added. After incubation for 72 h at 37 °C in humidified air containing 5% CO_2, the living cells were assayed by addition of 5 mg ml^{-1} MTT solution (20 μl). MTT was converted by intact mitochondrial reductase and precipitated as blue crystals during a 4-h contact period. The medium was then removed and the precipitated formazan crystals were dissolved in DMSO (100 μl) during a 60-min period of shaking at 25 °C. Finally, the reducted MTT was assayed at 545 nm, using a microplate reader; wells with untreated cells were utilized as controls [27]. Sigmoidal dose–response curves were fitted to the determined data and the IC_{50} values (the concentration at which the extent of cell proliferation was half that of the untreated control) were calculated by means of GraphPad Prism 4.0 (GraphPad Software, San Diego, CA, USA). All *in vitro* experiments were carried out on two microplates with at least five parallel wells. Cisplatin was used as positive control. Stock solutions of the tested substances (10 mM) were prepared with DMSO. The highest DMSO content of the medium (0.3%) did not have any substantial effect on the cell proliferation.

3. Results and discussion

3.1. Synthetic studies

To prepare novel steroid triazoles via 1,3-dipolar cycloaddition, 17α- and 17β-azido-estrone-3-methyl ether (**3** and **5**) were chosen as starting compounds. The synthetic strategy for the preparation of the starting azides is illustrated in Scheme 1, and the synthesis of steroidal 1,2,3-triazoles by CuAAC is outlined in Scheme 2.

Stereoselective reduction of 3-methoxyestrone (**1**) leading to 3-methoxyestra-17β-ol (**2a**) was followed by tosylation to give **2b**, which then underwent facile S_N2 substitution with NaN_3 in *N,N*-dimethylformamide to furnish the corresponding 17α-azido compound **3** [19]. The iodination of **2a** by the Appel reaction [28] proceeded with Walden inversion to yield 3-methoxy-17α-iodoestrane (**4**). The further nucleophilic exchange reaction with NaN_3 in *N,N*-dimethylformamide furnished the 17β-azido compound **5** in moderate yield. The exchange reaction was accompanied by elimination to give 3-methoxyestra-16-ene (**6**) too.

The CuAAC of **3** with phenylacetylene (**7a**) was carried out in refluxing CH_2Cl_2 with CuI as catalyst in the presence of PhP_3 (0.2 equivalent) at room temperature to obtain the required 1, 4-disubstituted triazole (**8**) in moderate yield after 24 h (Table 1, entry 1).

Ph_3P is assumed to accelerate the rate of the reaction and to improve the solubility of the catalyst by complexing to CuI, since no appreciable transformation was noted without its addition to the reaction mixture. The application of CuI in such reactions is known to require high temperature or at least an amine base additive such as Et_3N or *N,N*-diisopropylethylamine for adequate formation of the Cu-acetylide complex. Moreover, certain complexing ligands are often employed in order to enhance the activity of the catalyst and to protect the CuI from oxidation [29].

To accelerate the CuAAC process and increase the yield of the required 1,4-substituted triazoles, Ph_3P was replaced by Et_3N. In the presence of Et_3N, the TLC chromatogram showed not only the 1,4-triazoles (**8a–d** and **10a–d**), but also new compounds (**9a–d** and **11a–d**) in very low concentrations (Table 1, entries 2–9).

In the case of the reaction described in Section 2.8, analytical determination after chromatographic separation indicated that the compound present in very low concentration was the 5′-iodo-1,4-disubstituted triazole (**9b**).

X-ray structure analysis of one representative compound (**9b**) demonstrated the presence of iodine in the molecule (Fig. 1).

Large displacement parameters at C(6) indicate puckering in ring B. As it was not feasible to split this site for alternative positions, we adopted a single atom final model. Accordingly, ring B has a distorted twist chair form, while ring C is an almost regular chair form and the puckering of ring D involves twisting on C(13)–C(14). The other rings are planar, as expected. Apart from shape and constitutions, the crystal structure reveals a few non-trivial intermolecular short contacts. The rather short $I1'\cdots N3'_{[x,y-1,z]}$ distance of 2.912(8) Å demands mention. This is

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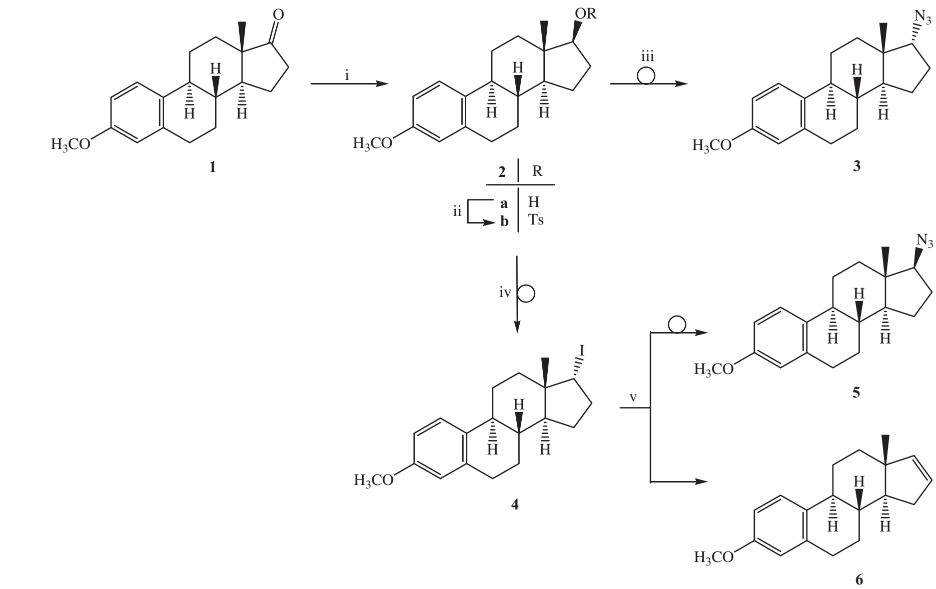
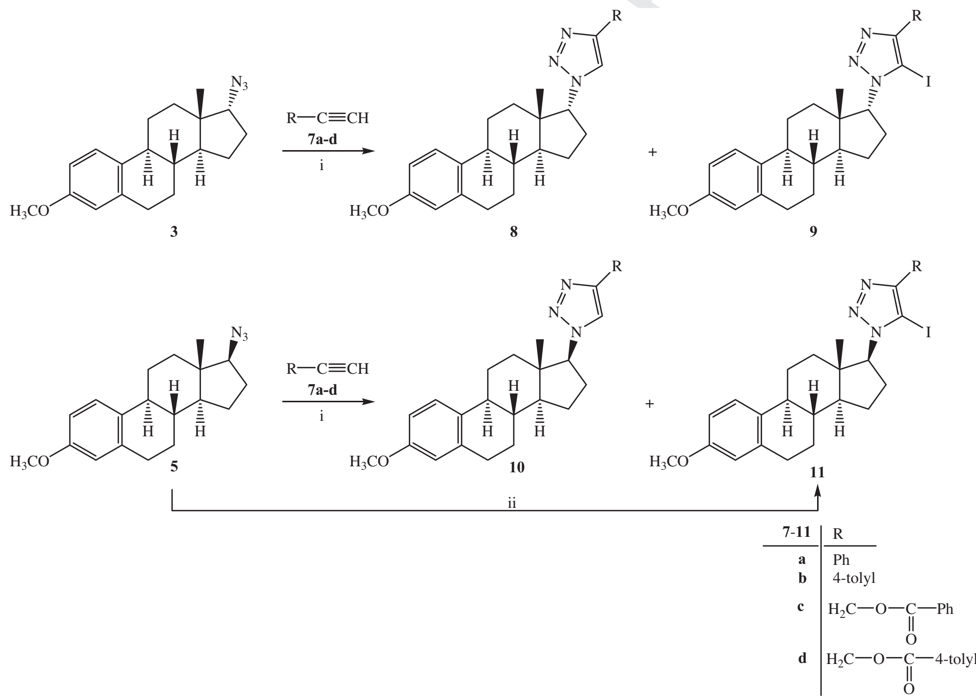


Scheme 1. Reagents and conditions: (i) KBH_4, MeOH; (ii); TsCl, pyridine, rt, 24 h; (iii) NaN_3, DMF, 100 °C, 24 h; (iv) Ph_3P, imidazole, I_2, toluene, 80 °C, 2 h; (v) NaN_3, DMF, 60 °C, 24 h.



Scheme 2. Reagents and conditions: (i) Ph_3P or Et_3N, CuI, CH_2Cl_2, rt, 24 h; (ii) Et_3N, CuI, ICl, CH_2Cl_2, rt, 24 h.

shorter by 0.62 Å than the sum of the van der Waals radii, and is also interesting relative to the I1′ – C5′ bond length of 2.075(10) Å. A further interesting feature of this short approach is the $N3'_{[x,y-1,z]}$ ··· I1′ – C5′ angle of 161.8(2)°. This indicates a co-linear approach mimicking an S_N attack on the largely asymmetric electron cloud of the halogen atom [30]. This may make an essential contribution to the formation of this crystal, as it represents an infinite one-dimensional chain with a [0 1 0] base vector. In accord with this idea, another electrophilic C7″ – H7C′···I1′ short contact develops (Table 2) at 94° to the nucleophilic contact. This short contact from a virtually labile methyl terminus is supported by a second C7″ – H7B′···O1 short contact, also with nearly ideal H-bridge conditions.

The geometry of these interactions that may be responsible for the formation of the **9b** crystal is illustrated in Fig. 2. This shows an additional possibility of a C – H ··· ϕ interaction between adjacent *p*-toluene rings involving C3′ – H as donor atoms.

Although iodo-containing triazoles are sometimes reported as minor products in various CuAAC reactions in the presence of DMAP (4-dimethylaminopyridine), there have been no reports of the ability of any organic base to control the product distribution in alkyne-azide cycloadditions. During some Cu-promoted

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Table 1
Syntheses of triazoles (**8**–**11**).

Entry	Substrate	Alkyne	Conditions	Product	Yield[a] (%)
1	**3**	**7a**	[b]	**8a**	52
2	**3**	**7a**	[c]	**8a + 9a**	61 + 6.5
3	**3**	**7b**	[c]	**8b + 9b**	45 + 7.6
4	**3**	**7c**	[c]	**8c + 9c**	61 + 5.5
5	**3**	**7d**	[c]	**8d + 9d**	63 + 7.8
6	**5**	**7a**	[c]	**10a + 11a**	57 + 7.7
7	**5**	**7b**	[c]	**10b + 11b**	67 + 6.2
8	**5**	**7c**	[c]	**10c + 11c**	62 + 6.7
9	**5**	**7d**	[c]	**10d + 11d**	58 + 7.5
10	**3**	**7a**	[d]	**8a + 9a**	42 + 21
11	**3**	**7a**	[e]	**9a**	89
12	**3**	**7b**	[e]	**9b**	92
13	**3**	**7c**	[e]	**9c**	85
14	**3**	**7d**	[e]	**9d**	82
15	**5**	**7a**	[e]	**11a**	91
16	**5**	**7b**	[e]	**11b**	93
17	**5**	**7c**	[e]	**11c**	83
18	**5**	**7d**	[e]	**11d**	86

[a] After purification by column chromatography.
[b] Ratios: azide/alkyne/base (Ph_3P)/CuI – 1.0/2.0/2.0/0.1.
[c] Ratios: azide/alkyne/base (Et_3N)/CuI – 1.0/ 2.0/2.0/0.1.
[d] Ratios: azide/alkyne/base (Et_3N)/CuI – 1.0/2.0/2.0/1.0.
[e] Ratios: azide/alkyne/base (Et_3N)/nucleophile (ICl)/CuI – 1.0/2.0/2.0/1.5/1.0.

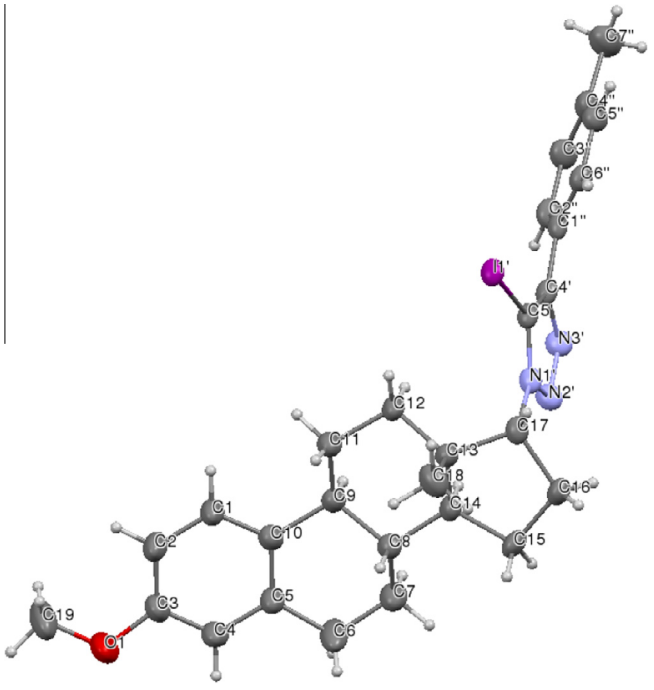

Fig. 1. Molecular structure of **9b** in a 30% probability anisotropic displacement plot with atomic labeling of non-H atoms.

Table 2
Non-classical C – H···X (O,I)-type intermolecular short contacts in the crystal structure of **9b**. Standard uncertainties are given only for the non-H parameters.

Donor – H···acceptor	D – H (Å)	H···A (Å)	D···A (Å)	D - H···A (°)
C7″ –H7B′···O1$_{[x,y,1+z]}$	0.96	2.45	3.395 (16)	170
C7″ –H7C′···I1′$_{[1-x,1/2+y,1-z]}$	0.96	3.02	3.947 (10)	163

alkyne-azide cycloadditions in the presence of DMAP, Dzyuba et al. observed the formation of ca. 20% of 5-iodo-1,4-triazole [31]. Such compounds are considered to be produced from the trapping of a Cu-triazolyl intermediate with electrophilic halogen. The incorporation of iodine onto the triazole moiety usually requires the presence of electrophilic iodine (I^+) to produce 5-iodotriazoles, but this aerobic oxidative halogenation in CuAAC reactions is very sluggish [32]. Fokin et al. recently reported that 1-iodoalkynes, which are stable and readily accessible internal acetylenes, exhibit exceptional reactivity in Cu-catalyzed annulation reactions with organic azides to produce 5-iodo-1,2,3-triazoles [33]. Although several syntheses of iodotriazoles are known, the reaction requires stoichiometric amounts of Cu catalysts and employs reactive electrophilic halogenating reagents, e.g. I_2 or ICl [34], *N*-bromosuccinimide or *N*-iodosuccinimide [35]. Moreover, some procedures require extended reaction times and generate mixtures of 5-*H* and 5-iodotriazoles [36]. An effective synthetic protocol for 5-halotriazoles involving novel *tert*-butyldimethylsilylchloride-activated aerobic oxidative halogenations has been introduced [37], and an improved method has been developed for the preparation of 5-iodotriazoles, in which coupling of an organic azide with a terminal alkyne in the presence of $Cu(ClO_4)_2$ and an alkali metal iodide under mild conditions gives the required 5-iodo compound [38].

Since triazolyl derivatives attached to the sterane skeleton have been reported to exhibit antiproliferative activity [19–21], it appeared interesting to investigate the formation of 5-iodotriazoles. On the basis of the observations of Wu et al. [34] relating to the production of 5-iodotriazoles, we increased the quantity of Cu catalyst to one equivalent, but the quantity of 5′-iodotriazole did not increase proportionally (Table 1, entry 10). When 1.5 equivalent of ICl was used as an electrophile in the presence of 10 equivalents of CuI and Et_3N, the yield of 5′-iodotriazole increased dramatically (Table 1 and 11–18 entries).

The structures of the new synthetized compounds (**1**–**11**) were confirmed by ^1H and ^{13}C NMR measurements. In the ^1H NMR spectra of the 17β-triazoles (**10a**,**b** and **11a**,**b**), the signals of 17-H were seen as triplets at around 4.7 ppm, but in the cases of **10c**,**d** and **11c**,**d** the corresponding signals were present at lower chemical shift (4.5 ppm), indicating the difference in the 4′ substituent. In the spectra of the 17α compounds (**8** and **9**), the signal of 17-H was a doublet or a double doublet and followed the same order as mentioned above. All the ^{13}C NMR spectra revealed the C-17 signal at around 70 ppm, but with great differences in the spectra of the 5′-iodo (**9** and **11**: C-5′ at around 80 ppm) or 5′-H (**8** and **10**: C-5′at around 120 ppm) derivatives as concerns the chemical shift of C-5′. This downfield shift of C-5′ clearly indicates the presence of iodine on the triazole ring.

The optical rotations of the compounds, measured in $CHCl_3$, presented a characteristic picture. The $[\alpha]_D^{20}$ values of the 17α compounds (**3** and **8a-d**) were between +14 and +32, while those of the compounds bearing iodine on position 5′ (**9a-c**) were between +106 and +153. For the 17β compounds (**5** and **10a–d**), the values were between +17 and +42, while for the iodine-bearing compounds, the corresponding values were less negative: between -71 and −85 for **11a–d**.

*3.2. Determination of the antiproliferative properties of the newly synthetized azides (**3** and **5**) and triazoles (**8**–**11**)*

We recently reported the synthesis and CuAAC reactions of steroidal azidoalcohols with terminal alkynes [19–21]. The 1,2,3-triazoles were evaluated for their *in vitro* antiproliferative activities against human adherent cancer cell lines. Some derivatives exhibited substantial activities on the proliferation of the cells, comparable to those of the reference agent cisplatin. In view of these results, the aim of our present study included the testing of the newly synthetized 17-triazoles (**8**–**11**) of estrone-3-methyl ether and their precursors (**3** and **5**) on a panel of human adherent

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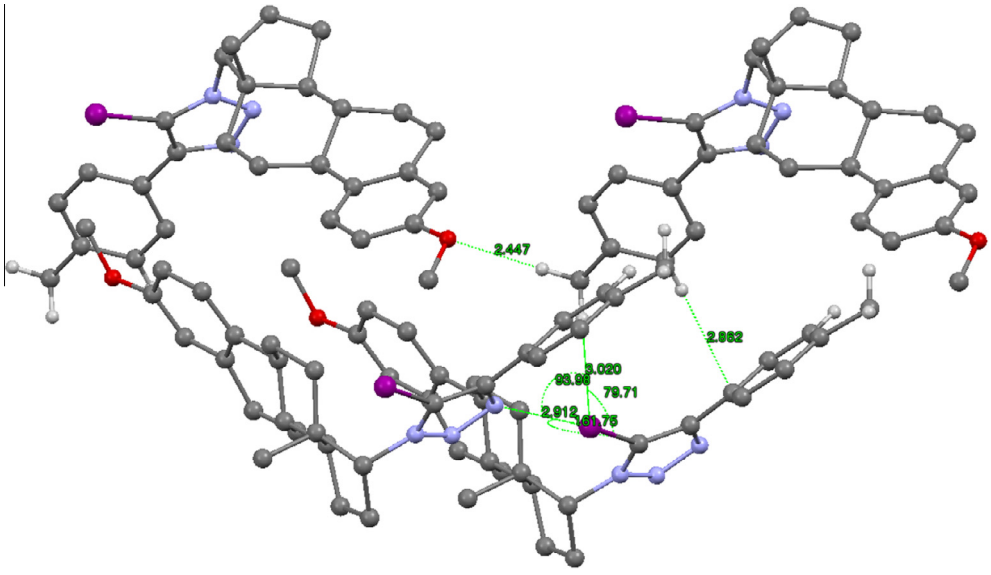

Fig. 2. Characteristic short contacts in the **9b** crystal, indicated by broken lines, as printed from program Mercury. For the sake of clarity, only three relevant H atoms are shown.

Table 3
Experimentally determined growth inhibitory data for the synthetized azides (**3** and **5**) and triazoles (**8**–**11**).

Comp.	Concn. (μM)	Growth inhibition (%) ± SEM					
		HeLa	A2780	MCF-7	MDA-MB-231	MDA-MB-361	T47D
3	10	–*	–	–	–	–	–
	30	–	–	–	–	23.7 ± 1.8	–
5	10	–	37.1 ± 2.0	–	–	–	–
	30	28.8 ± 1.3	38.2 ± 2.3	–	–	–	–
8a	10	–	–	–	–	–	–
	30	25.9 ± 2.7	–	–	–	21.8 ± 1.6	26.0 ± 1.3
8b	10	–	–	–	–	–	–
	30	28.5 ± 0.9	–	35.8 ± 1.5	37.1 ± 0.8	43.9 ± 2.1	47.5 ± 1.4
8c	10	20.9 ± 0.8	–	–	–	–	–
	30	32.5 ± 2.2	28.1 ± 2.8	38.8 ± 0.9	25.9 ± 2.9	52.5 ± 3.0	39. 6 ± 1.4
8d	10	28.9 ± 2.1	25.1 ± 1.4	21.7 ± 1.7	–	–	39.7 ± 2.4
	30	38.3 ± 2.0	25.5 ± 1.2	48.1 ± 1.1	20.0 ± 1.2	35.3 ± 1.8	51.2 ± 1.3
9a	10	–	–	–	–	–	–
	30	–	20.5 ± 2.6	22.9 ± 1.7	21.0 ± 1.2	39.7 ± 2.00	–
9b	10	–	–	–	–	–	–
	30	–	–	–	–	–	–
9c	10	31.4 ± 1.9	–	38.0 ± 1.5	–	25.7 ± 2.9	39.4 ± 2.0
	30	31.9 ± 1.4	22.3 ± 1. 7	38.7 ± 0.9	–	33.9 ± 1.3	47.4 ± 1.2
9d	10	–	–	40.1 ± 1.8	–	–	33.8 ± 2.3
	30	26.7 ± 2.2	–	41.2 ± 1.1	–	21.3 ± 2.2	36.0 ± 1.9
10a	10	45.3 ± 1.3	26.5 ± 2.8	42.0 ± 0.5	23.3 ± 1.5	56.6 ± 2.9	52.6 ± 2.6
	30	47.8 ± 1.8	30.2 ± 2.0	39.4 ± 1.1	26.1 ± 1.8	57.4 ± 1.0	57.8 ± 1.5
10b	10	–	–	38.5 ± 1.7	–	–	26.2 ± 2.0
	30	–	–	51.3 ± 1.8	26.1 ± 1.3	20.3 ± 2.7	37.8 ± 0.7
10c	10	–	–	–	–	–	–
	30	28.2 ± 1.7	35.4 ± 1.3	43.6 ± 1.5	–	33.1 ± 1.7	45.4 ± 1.0
10d	10	–	–	–	–	20.5 ± 2.5	32.8 ± 2.6
	30	32.3 ± 2.2	22.7 ± 0.9	59.0 ± 2.0	–	48.5 ± 1.2	57.3 ± 0.9
11a	10	–	23.0 ± 2.0	–	–	–	–
	30	–	32.7 ± 2.5	20.9 ± 0.4	–	–	–
11b	10	27.3 ± 0.8	34.9 ± 1.4	20.8 ± 2.9	26.4 ± 1.5	41.1 ± 2.7	34.3 ± 1.2
	30	60.1 ± 0.6	39.9 ± 0.8	62.1 ± 0.8	35.9 ± 2.4	63.6 ± 1.3	50.7 ± 1.9
11c	10	29.5 ± 1.7	–	–	–	–	–
	30	39.6 ± 1.9	22.9 ± 1.3	–	23.8 ± 2.0	25.2 ± 1.3	–
11d	10	–	–	–	–	–	–
	30	–	–	–	–	24.8 ± 2.8	_
Cisplatin	10	42.6 ± 2.3	53.0 ± 2.3	66.9 ± 1.8	20.8 ± 0.8	67.5 ± 1.0	51.0 ± 2.0
	30	99.9 ± 0.3	86.9 ± 1.2	96.8 ± 0.4	71.7 ± 1.2	87.7 ± 1.1	57.9 ± 1.4

* Growth inhibitory values lower than 20% are considered not substantial and are therefore not given in the table.

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cancer cell lines (HeLa, A2780, MCF7, MDA-MB-231, MDA-MB-361 and T47D) by means of MTT assays. We wished to determine the impact of the following structural modifications on the *in vitro* antiproliferative activity: the inversion of the configuration at C-17, the replacement of 5′-H on the triazole ring by iodine and the nature of the 4′ substituent. The epimeric 17-azides (**3** and **5**) were found to exert moderate cell-growth inhibition (< 50%) at 10 or 30 μM (Table 3). The triazoles (**8**–**11**) proved to be more potent, depending on their structure. The17β derivatives (**10** and **11**) displayed slightly higher activities than their epimeric 17α counterparts (**8** and **9**). Compounds **10a** and **11b** approached or exceeded 50% growth inhibition on some cell lines. Higher activities were generally detected against the breast cancer cell lines which express the estrogen receptor (MDA-MB-361, T47D or MCF7) than against the triple negative MDA-MB-231. Compound **11b**, bearing an iodine instead of 5′-H on the triazole ring, inhibited the proliferation of the cells most efficiently, but did not exhibit selectivity toward the different tumor cells. In the 17α-triazole series (**8** and **9**), agents with more bulky substituents on the triazole ring (**c** and **d**) exhibited higher activities than those with a phenyl or 4-tolyl group (**a** and **b**). This trend was valid for the 17β-triazole analogs (**10** and **11**), **11b** proved to exhibiting the highest overall action among the presented agents. The presence of iodine did not have a substantial impact on the activities. Since the cytostatic activities of these estranetriazoles less than those of cisplatin, it is evident that these compounds are not potential lead molecules for additional investigations. It can be concluded that the nature of the functional group on C-4′ is not a crucial determining structural moiety.

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