Organic & Biomolecular Chemistry

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Cite this: *Org. Biomol. Chem.*, 2011, **9**, 8051

www.rsc.org/obc

PAPER

Efficient approach to novel 1α-triazolyl-5α-androstane derivatives as potent antiproliferative agents†‡

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Received 5th July 2011, Accepted 31st August 2011
DOI: 10.1039/c1ob06086d

Stereoselective 1,4-Michael addition of azoimide to 17β-acetoxy-5α-adrost-1-en-3-one was carried out to furnish a 1α-azido-3-ketone, which was reduced to give the 3β- and 3α-hydroxy epimers in a ratio of 5 : 2. The Cu(I)-catalyzed 1,3-dipolar cycloaddition of the major isomer to terminal alkynes afforded 1α-triazolyl derivatives, which were deacetylated to the corresponding 3β,17β-diols or oxidized to the analogous 3-ketones. However, the ability of the minor 1α,3α-azidoalcohol to undergo similar cyclization was found to be affected significantly by the steric bulk of the substituents on the alkyne reaction partner. All triazolyl compounds were tested *in vitro* on three malignant gynecological cell lines (HeLa, MCF7 and A2780).

1. Introduction

The Huisgen 1,3-dipolar cycloaddition of organic azides to terminal alkynes has received considerable attention in recent years following the independent introduction of Cu(I) catalysis in 2002 by the research groups of Sharpless[1] and Meldal.[2] 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,[3] and eliminating the need for elevated temperature and a prolonged reaction time. The catalytic version has further advantageous benefits, such as high yields of the desired products, tolerability to a variety of common parameters (functional groups, solvents, pH and temperature), the lack of side-reactions and insensitivity to the steric and electronic properties of the reactants. Consequently, such Cu(I)-catalyzed azide–alkyne cycloaddition (CuAAC) commonly meets all the criteria for click chemistry[4] and has gained general application across a wide variety of disciplines, including preparative organic synthesis,[5] polymer and material science,[6] dendrimer design,[7] chemical ligation[8] and combinatorial drug discovery.[9]

The triazole formed during the reaction can mimic the atom placement and electronic properties of a peptide bond; however, it is essentially chemically inert against oxidation, reduction and hydrolytic conditions, and possesses a much stronger dipole moment than an amide bond.[10] Perhaps due in part to the structural mimicry, a number of diverse 1,2,3-triazoles have been reported to exhibit varied biological activity, including anti-HIV,[11] antibacterial,[12] antihistamine[13] or cytostatic effects.[14]

The main driving force toward the preparation of steroidal compounds at present is the development of novel derivatives with a biological target other than a hormone receptor and therefore the reduction or elimination of the undesirable hormonal activity. The synthetic tools for achievement of this goal are (i) the synthesis of molecules lacking the functionalities necessary for effective binding to the hormone receptors;[15] (ii) modification of the binding ability by chemical transformation of the extant functional groups;[16] (iii) steric hindrance of the substrate–receptor interaction by chemical substitution near the original groups;[17] (iv) altering the primary stereostructure or the number of ring members;[18] and (v) the design of heterocyclic derivatives that are not recognized by the receptor protein in consequence of their specific structure or the fact that their geometry differs from that of the natural hormones.[19] The most frequent synthetic modifications are introduced at the positions adjacent to the existing C-3, C-17 or C-20 functional groups, where substitution is facilitated. Substitution at other positions of the sterane skeleton has proved to be more difficult, necessitating several reaction steps, and is therefore rarely applied. To the best of our knowledge, only a few 1-substituted derivatives have been synthesized to date, among them 1-methylated androstanes (methenolone and mesterolone) exerting anabolic rather than androgenic activity[20] (Fig. 1).

The foregoing results led us to set out to introduce an azido group at the unconventional position 1 of the sterane skeleton and thereby to synthesize a mesterolone analog (Fig. 1). The intermolecular ring closure of the steroidal azide with different terminal alkynes *via* CuAAC was also planned with a view to

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† Dedicated to Professor Gy. Schneider on the occasion of his 80th birthday.

‡ Electronic supplementary information (ESI) available: Experimental procedures for the preparation and NMR spectral data of all synthesized compounds. See DOI: 10.1039/c1ob06086d

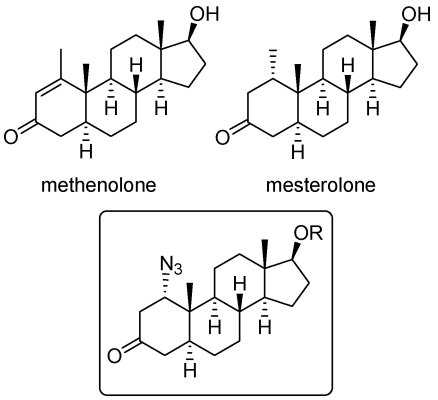


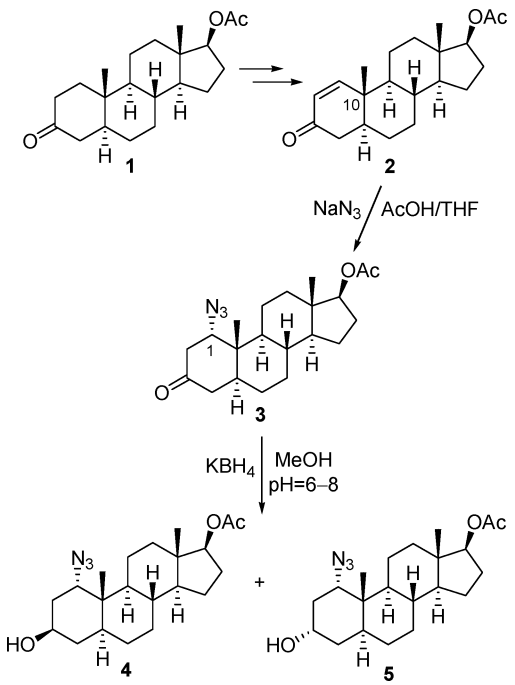
Fig. 1 1-Substituted steroids in the 5α-androstane series.

preparing novel 1-*exo*-triazolyl derivatives. Since some steroid triazoles are known to exert antiproliferative activity,[21] it was decided to screen all these compounds *in vitro* for their activities against a panel of three human cancer cell lines (HeLa, MCF7 and A2780). Although determination of their affinities for the androgen receptor did not fall within the scope of the present work, the steric bulk of the heterocyclic moiety at position 1 may interfere with the receptor binding.

2. Results and discussion

2.1. Synthetic studies

For the introduction of an azido group onto position 1 of the sterane skeleton, the starting material applied was 17β-acetoxy-5α-adrost-1-en-3-one (**2**), which is readily available from stanolone acetate (**1**) in a two step-pathway, by bromination at C-2 and subsequent dehydrohalogenation[22] (Scheme 1). The 1,4-Michael addition of azoimide,[23] generated *in situ* from sodium azide and acetic acid, afforded 17β-acetoxy-1α-azido-5α-androstan-3-one

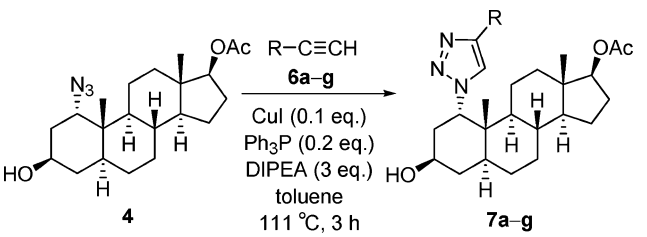


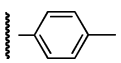
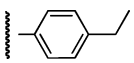
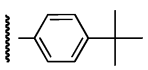
Scheme 1 Synthesis of 1α-azides in the 5α-androstane series.

(**3**) in a yield of 67% after purification by flash chromatography. The stereoselective formation of the 1α-azido derivative (**3**) is not surprising considering the steric bulk of the adjacent angular β-methyl group on C-10. Since β-substituted ketones such as **3** are often susceptible to elimination and undergo facile transformation to the corresponding enone,[24] azidoketone **3** was reduced with KBH_4 under pH-controlled conditions so as to avoid this adverse side-reaction. The ^1H NMR spectrum of the reaction mixture indicated that the epimeric diols **4** and **5** were formed in a ratio of 5 : 2. After separation by column chromatography, the isomeric azidoalcohols were subjected to CuAAC with different terminal alkynes. Although CuAAC is generally not affected by the steric features of the alkyne and azide components, the *trans* and *cis* azidoalcohols **4** and **5** displayed considerably different behavior under similar reaction conditions. Accordingly, the opposite or same spatial orientation of the hydroxy group on C-3 with that of the azido function on C-1 was expected to have an influence on the intermolecular ring closures.

During optimization of the reaction conditions for the CuAAC of **4** with phenylacetylene (**6a**), the best conversion was found to occur on the use of a catalytic amount of CuI as Cu(I) source, triphenylphosphane[15] as stabilizing ligand to protect the Cu(I) from oxidation, and excess *N*,*N*-diisopropyl ethylamine as base to facilitate formation of the active copper acetylide complex, and to minimize side-reactions.[25] Ring closure in refluxing toluene for 3 h furnished the corresponding phenyltriazolyl derivative **7a** in excellent yield after purification (Table 1, entry 1). After determi-

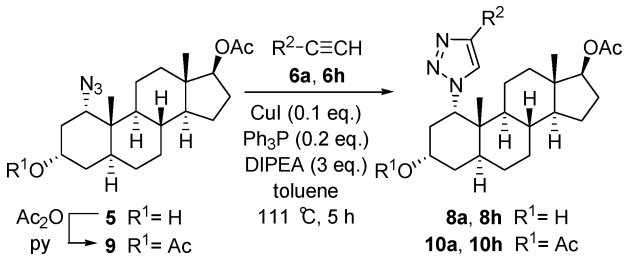
Table 1 CuAAC of steroidal *trans* azidoalcohol **4** with terminal alkynes

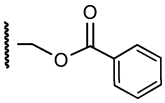
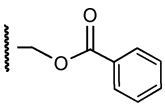


Entry	Alkyne	R	Triazole	Yield[a] (%)
1	**6a**		**7a**	93
2	**6b**		**7b**	93
3	**6c**		**7c**	92
4	**6d**		**7d**	93
5	**6e**		**7e**	96
6	**6f**		**7f**	93
7	**6g**		**7g**	97

[a] Yields of purified isolated products.

Table 2 CuAAC of steroidal *cis* azidoalcohol **5** and its acetate **9** with terminal alkynes



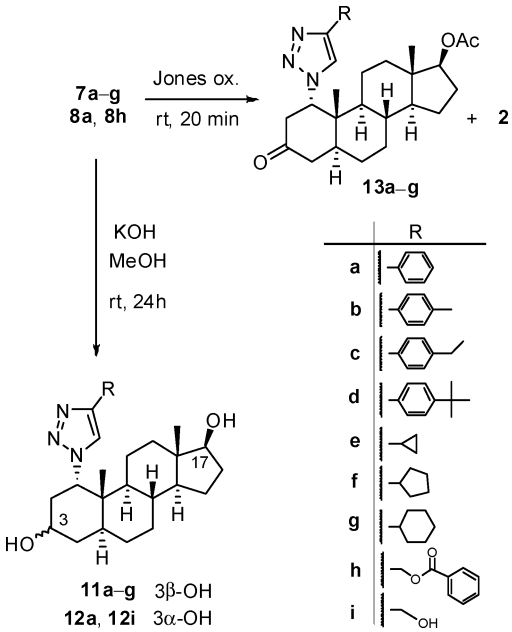
Entry	Azide/alkyne	R^2	Triazole	Yield[a] (%)
1	**5**/**6a**		**8a**	61
2	**5**/**6h**		**8h**	83
3	**9**/**6a**		**10a**	30
4	**9**/**6h**		**10h**	86

[a] Yields of purified isolated products.

nation of the optimal conditions, similar cycloadditions of **4** with different aryl- and cycloalkyl-substituted acetylenes (**6b–g**) were performed, which resulted in steroidal 1α-*exo*-triazolyl derivatives (**7b–g**) in yields exceeding 90%, independently of the substituents on the alkyne dipolarophile (Table 1, entries 2–7). However, the reaction of the *cis* azidoalcohol **5** with phenylacetylene **6a** was not complete even within 5 h, and the purified product **8a** was obtained in a yield of only 61% (Table 2, entry 1). Treatment of substrate **5** with benzoic acid propargyl ester **6h**, containing the aromatic ring farther from the reaction center than in phenylacetylene **6a**, resulted in the triazolyl derivative **8h** in a higher isolated yield (83%) (Table 2, entry 2). The reaction of **6h** with the acetylated azidoalcohol **9** proceeded similarly as for **8h**, but an even lower conversion was observed on the use of phenylacetylene **6a** (Table 2, entries 3 and 4). These results suggest that the intermolecular ring closure is significantly influenced by the OH group on C-3, spatially close to the azide dipole, and especially by the steric bulk of the alkyne substituent, which presumably causes a crowded transition state of the Cu(I)-catalyzed process.

The heterocyclic products (**7a–g**, **8a** and **8h**) were deacetylated in alkaline methanol to the corresponding 3β,17β-diols (**11a–g**) and 3α,17β-diols (**12a** and **12i**), respectively (Scheme 2). In the course of the reaction of **8h**, the side-chain of the triazolyl moiety was also hydrolyzed to give the rather polar hydroxymethyl-substituted derivative **12i**. The 3-keto analogs (**13a–g**) were also obtained by Jones oxidation, during which a slight formation of enone **2** was observed.

The structures of all synthesized compounds were confirmed by ^{1}H and ^{13}C NMR measurements. The ^{1}H NMR spectra of triazoles containing an aromatic moiety connected directly (**7a–d**, **8a**, **10a**, **11a–d**, **12a**, **13a–d**) or indirectly (**8h**, **10h**) to the hetero ring revealed the signals of the incorporated aryl groups at 7.2–8.0 ppm as compared with the spectra of the starting azides (**4**, **5** and **9**). The 5′-H singlet of the newly formed heterocycles was identified at 7.5–8.3 ppm for the aryl-substituted derivatives, and at 7.1–7.3 ppm for those containing cycloalkyl substituents (**7e–g**, **11e–g**, **13e–g**). The most characteristic difference between the ^{1}H spectra of the 3β- (**7a–g**, **11a–g**) and 3α-OH compounds (**8a**, **8h**, **12a**, **12i**) was the upfield shift of 3-H in the former group of derivatives, due to the magnetic anisotropic effect caused by the aromatic triazole ring *cis* to this proton. The influence of the heteroaromatic ring was also manifested in the higher chemical shift of the OH proton in **7a–g** and **11a–g**, which even in $CDCl_3$ appeared as a doublet with a coupling constant of around 10.3 Hz. This assignment was confirmed by HSQC and HMBC measurements.



Scheme 2 Synthesis of 3,17-diols and 3-keto derivatives of steroidal triazoles.

2.2. Pharmacological studies

With the exceptions of the 3,17-diacetates (**10a** and **10h**), the novel triazolyl derivatives (**7a–g**, **8a**, **8h**, **11a–g**, **12a**, **12i** and **13a–g**) were subjected to *in vitro* pharmacological studies of their antiproliferative effects (Table 3). The activities were determined by using three malignant gynecological cell lines in the microplate-based MTT colorimetric assay,[26] in comparison with cisplatin as positive control. Although there is no generally accepted threshold for efficacy, a substance exhibiting less than 50% inhibition of cell growth at 30 μM can not be considered a promising lead compound. Final concentrations not exceeding 30 μM were therefore used in the *in vitro* assays. The structural diversity of the tested compounds suggested certain structure–activity relationships. With the only exception of **7g**, the *trans*-hydroxytriazoles bearing an acetate at C-17 (**7a–g**) did not exhibit substantial activity. Interestingly, the phenyl-substituted *cis*-hydroxytriazole **8a**, bearing the 3-OH group and the hetero ring in the same spatial orientation, proved to be more potent than its *trans*

Table 3 Calculated IC_{50} values of synthesized triazole derivatives

Compound	IC_{50} (μM)		
	HeLa	MCF7	A2780
7a	> 30	> 30	> 30
7b	> 30	> 30	> 30
7c	> 30	15.33	11.30
7d	5.87	> 30	7.40
7e	> 30	> 30	> 30
7f	> 30	> 30	> 30
7g	6.77	12.04	7.01
8a	13.55	20.51	11.83
8h	10.31	8.96	17.17
11a	> 30	> 30	> 30
11b	27.37	> 30	15.33
11c	> 30	> 30	22.96
11d	> 30	> 30	13.79
11e	> 30	> 30	> 30
11f	13.89	15.87	16.48
11g	16.82	18.97	11.98
12a	27.83	> 30	> 30
12i	> 30	> 30	> 30
13a	1.22	26.24	11.22
13b	1.12	21.22	11.79
13c	1.13	21.33	12.32
13d	1.16	12.68	9.20
13e	1.64	15.96	11.68
13f	1.55	25.74	10.85
13g	1.40	20.33	11.81
cisplatin	12.43	9.63	1.30

counterpart **7a**. The effect was similar for the triazole containing an ester side-chain (**8h**) instead of a phenyl group. Deacetylation of the 17-acetates **7a–g** did not cause any noteworthy effect for the aryl-substituted compounds (**11a–d**), but appeared favorable for the cyclopentyl-substituted derivative **11f**. Hydrolysis of 17-acetates **8a** and **8h** resulted in less potent derivatives **12a** and **12i**. The compounds obtained by Jones oxidation (**13a–g**) exerted outstanding cytotoxic activity on HeLa cells, characterized by IC_{50} values between 1 and 2 μM, *i.e.* lower than that of the reference cisplatin. On the other hand, the other two cell lines (and especially MCF7) seemed to be less sensitive to these structures. The selective, rather than the generally toxic, behavior of this latter group of 1α-triazolyl-5α-androstane derivatives could be regarded as a valuable feature, and this skeleton is therefore suitable for further lead-finding research.

3. Conclusions

In summary, a novel A-ring-modified steroidal 1α,3-azidoketone was prepared from stanolone acetate *via* a multi-step stereoselective synthesis, and reduced to give a diastereomeric mixture of azidoalcohols. CuAAC of the *trans* and *cis* isomers with different terminal alkynes was achieved under optimized reaction conditions to give 1α-*exo*-triazolyl derivatives in good to excellent yields. The reactions were affected significantly by the stereostructure of the steroidal azide component and especially by the steric bulk of the substituent in the acetylene dipolarophile. The synthesized 5α-androstane derivatives are of interest from a pharmacological aspect, since several analogs proved to exert marked *in vitro* cytotoxic activity. A certain selectivity against HeLa cells was also confirmed.

4. Experimental

4.1. General

Melting points (mp) were determined on a Kofler block and are uncorrected. 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/EtOAc (98 : 2 v/v), (C) CH_2Cl_2/EtOAc (95 : 5 v/v), (D) CH_2Cl_2/EtOAc (90 : 10 v/v), (E) CH_2Cl_2/EtOAc (80 : 20 v/v), (F) CH_2Cl_2/EtOAc (70 : 30 v/v), (G) CH_2Cl_2/EtOAc (50 : 50 v/v), (H) CH_2Cl_2/EtOAc (40 : 60 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: Merck silica gel 60, 40–63 μm. All solvents were distilled prior to use. Reagents and materials were obtained from commercial suppliers and were used without purification. Elementary analysis data were determined with a Perkin-Elmer CHN analyzer model 2400. NMR spectra were obtained at room temperature with a Bruker DRX 500 instrument. 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. Automated flow injection analyses were performed by using an HPLC/MSD system. The system comprised an Agilent 1100 micro vacuum degasser, a quaternary pump, a micro-well plate autoinjector and a 1946A MSD equipped with an electrospray ion source (ESI) operated in positive ion mode. The ESI parameters were: nebulizing gas N_2, at 35 psi; drying gas N_2, at 350 °C and 12 L min^{-1}; capillary voltage (VCap) 3000 V; fragmenter voltage 70 V. The MSD was operated in scan mode with a mass range of m/z 60–620. Samples (0.2 μL) with automated needle wash were injected directly into the solvent flow (0.3 mL min^{-1}) of CH_3CN/H_2O 70 : 30 (v/v) supplemented with 0.1% formic acid. The system was controlled by Agilent LC/MSD Chemstation software.

4.2. 17β-Acetoxy-1α-azido-5α-androstan-3-one (3)

Compound **2** (10.0 g, 30.2 mmol) was dissolved in a mixture of THF (100 mL) and acetic acid (100 mL), and a solution of NaN_3 (15.5 g, 0.20 mol) in water (40 mL) was poured into the organic phase. The reaction mixture was stirred at ambient temperature for 24 h, and then poured into saturated $NaHCO_3$ solution and extracted with CH_2Cl_2 (3 × 50 mL). The combined organic layers were dried over Na_2SO_4 and evaporated *in vacuo*. Purification of the resulting crude product by flash chromatography with CH_2Cl_2 as eluent afforded **3** as a white solid (7.6 g, 67%), mp 127–129 °C, R_f 0.38 (ss A); ^{1}H NMR (500 MHz, $CDCl_3$): δ_H = 0.80 (s, 3H, 18-H_3), 0.86–0.96 (m, 2H), 1.05 (s, 3H, 19-H_3), 1.12 (m, 1H), 1.25–1.53 (m, 9H), 1.61–1.69 (m, 2H), 1.76 (m, 1H), 2.03 (s, 3H, Ac-CH_3), 2.13–2.22 (m, 3H), 2.58 (d, 1H, J = 18.8 Hz, 2-H_α), 2.69 (dd, 1H, J = 18.8 Hz, J = 3.8 Hz, 2-H_β), 3.97 (bs, 1H, 1-H), 4.60 (t, 1H, J = 8.5 Hz, 17-H); ^{13}C NMR (125 MHz, $CDCl_3$): δ_C = 12.1 (C-18), 12.7 (C-19), 20.3 (CH_2), 21.1 (Ac-CH_3), 23.5 (CH_2), 27.5 (CH_2), 28.3 (CH_2), 30.7 (CH_2), 35.2 (CH), 36.5 (CH_2), 39.2 (CH), 39.5 (C-10), 42.5 (CH_2), 42.6 (C-13), 44.3 (CH_2), 47.4 (CH), 50.4 (CH), 66.5 (C-1), 82.6 (C-17), 171.1 (Ac-CO), 207.8 (C-3); Anal. Calcd for $C_{21}H_{31}N_3O_3$ C, 67.53; H, 8.37; N, 11.25. Found: C, 67.35; H, 8.52; N, 11.45%.

4.3. 17β-Acetoxy-1α-azido-5α-androstan-3β-ol (4) and -3α-ol (5)

Compound **3** (7.0 g, 18.7 mmol) was dissolved in MeOH (100 mL), and KBH_4 (5.0 g, 89.1 mmol) was added in small portions. To maintain pH 6–8, the solution was repeatedly acidified as needed with MeOH/AcOH (1 : 1), using bromothymol blue as indicator. The mixture was stirred for 3 h, and after completion of the reaction, diluted with water and acidified with dilute HCl. The precipitate that formed was filtered off, washed with water and dried. The resulting epimeric azidoalcohols could be separated by column chromatography with 1% EtOAc/CH_2Cl_2 as eluent, yielding **4** (4.53 g, 68%) and **5** (2.14 g, 27%). (**4**): mp 145–147 °C, R_f 0.55 (ss C); ^{1}H NMR (500 MHz, $CDCl_3$): δ_H = 0.80 (s, 3H, 18-H_3), 0.83 (s, 3H, 19-H_3), 0.88-0.96 (m, 1H), 1.09–1.50 (m, 11H), 1.61–1.75 (m, 4H), 1.88–1.93 (m, 1H), 2.02 (s, 3H, Ac-CH_3), 2.08 (m, 1H), 2.12–2.19 (m, 1H), 2.90 (d, 1H, J = 9.4 Hz), 3.67 (bs, 1H, 1-H), 3.94 (m, 1H, 3-H), 4.60 (t, 1H, J = 8.4 Hz, 17-H); ^{13}C NMR (125 MHz, $CDCl_3$): δ_C = 12.2 (C-18), 12.5 (C-19), 19.9 (CH_2), 21.1 (Ac-CH_3), 23.4 (CH_2), 27.5 (CH_2), 28.1 (CH_2), 31.0 (CH_2), 32.1 (CH_2), 32.5 (CH), 35.3 (CH), 35.8 (CH_2), 36.5 (CH_2), 40.1 (C-10), 42.6 (C-13), 48.1 (CH), 50.7 (CH), 65.0 and 66.5 (C-1 and C-3), 82.6 (C-17), 171.1 (Ac-CO); Anal. Calcd for $C_{21}H_{33}N_3O_3$ C, 67.17; H, 8.86; N, 11.19. Found: C, 67.05; H, 9.05; N, 11.36%. (**5**): mp 141–143 °C, R_f 0.35 (ss C); ^{1}H NMR (500 MHz, $CDCl_3$): δ_H = 0.77 (s, 3H, 18-H_3), 0.85 (s, 3H, 19-H_3), 0.87–0.92 (m, 1H), 1.06–1.39 (m, 8H), 1.42–1.50 (m, 3H), 1.58–1.64 (m, 3H), 1.67–1.74 (m, 3H), 2.02 (s, 3H, Ac-CH_3), 2.10–2.19 (m, 2H), 3.70 (bs, 1H, 1-H), 3.91 (m, 1H, 3-H), 4.58 (t, 1H, J = 8.4 Hz, 17-H); ^{13}C NMR (125 MHz, $CDCl_3$): δ_C = 12.1 (C-18), 13.2 (C-19), 20.1 (CH_2), 21.1 (Ac-CH_3), 23.5 (CH_2), 27.5 (CH_2), 28.3 (CH_2), 31.0 (CH_2), 34.6 (CH_2), 35.2 (CH), 36.5 (CH_2), 37.7 (CH_2), 37.8 (CH), 39.2 (C-10), 42.6 (C-13), 47.7 (CH), 50.5 (CH), 65.6 and 66.2 (C-1 and C-3), 82.7 (C-17), 171.1 (Ac-CO); Anal. Calcd for $C_{21}H_{33}N_3O_3$ C, 67.17; H, 8.86; N, 11.19. Found: C, 67.03; H, 9.02; N, 11.40%.

4.4. General procedure for the preparation of triazoles 7a–g

To a solution of 17β-acetoxy-1α-azido-5α-androstan-3β-ol (**4**) (300 mg, 0.79 mmol) in toluene (10 mL) were added Ph_3P (41 mg, 0.16 mmol), CuI (15 mg, 0.08 mmol) and DIPEA (0.40 mL, 2.4 mmol). Finally the appropriate terminal alkyne (**6a–g**, 1.1 eq) was added to the reaction mixture, which was then refluxed for 3 h, allowed to cool and evaporated *in vacuo*. The resulting crude product was purified by column chromatography.

17β-Acetoxy-1α-[4′-phenyl-1′*H*-1′,2′,3′-triazol-1′-yl]-5α-androstan-3β-ol (7a). Alkyne: phenylacetylene (**6a**, 0.09 mL). After purification with CH_2Cl_2/EtOAc (90 : 10) as eluent, **7a** was obtained as a white solid (355 mg, 93%), mp 266–268 °C, R_f 0.32 (ss D); ^{1}H NMR (500 MHz, $CDCl_3$): δ_H = 0.22 (m, 1H), 0.76 (s, 3H, 18-H_3), 0.82–0.89 (m, 3H), 1.06 (s, 3H, 19-H_3), 1.19–1.29 (m, 2H), 1.34–1.44 (m, 4H), 1.49–1.80 (m, 6H), 1.98 (s, 3H, Ac-CH_3), 2.05 (m, 2H), 2.34–2.42 (m, 2H), 4.01 (bs, 1H, 3-H), 4.43 (t, 1H, J = 8.4 Hz, 17-H), 4.58 (d, 1H, J = 5.2 Hz, 1-H), 5.19 (bs, 1H, 3-OH), 7.34 (t, 1H, J = 7.3 Hz, 4″-H), 7.43 (t, 2H, J = 7.3 Hz, 3″-H and 5″-H), 7.80 (d, 2H, J = 7.3 Hz, 2″-H and 6″-H), 7.91 (s, 1H, 5′-H); ^{13}C NMR (125 MHz, $CDCl_3$): δ_C = 12.4 (C-18), 13.4 (C-19), 21.1 (Ac-CH_3), 21.3 (CH_2), 23.3 (CH_2), 27.4 (CH_2), 28.7 (CH_2), 30.4 (CH_2), 32.9 (CH), 33.2 (CH_2), 35.8 (CH), 36.3 (2C, 2 × CH_2), 40.4 (C-10), 42.6 (C-13), 47.5 (CH), 50.5 (CH), 63.8 and 64.0 (C-1 and C-3), 82.4 (C-17), 122.3 (C-5′), 125.8 (2C, C-2″ and C-6″), 128.3 (C-4″), 128.9 (2C, C-3″ and C-5″), 130.2 (C-1″), 146.7 (C-4′), 170.9 (Ac-CO); ESI-MS: 478 $[M+H]^+$; Anal. Calcd for $C_{29}H_{39}N_3O_3$ C, 72.92; H, 8.23; N, 8.80. Found: C, 73.03; H, 8.39; N, 8.98%.

4.5. 3α,17β-Diacetoxy-1α-azido-5α-androstane (9)

Compound **5** (1.0 g, 2.7 mmol) was dissolved in a mixture of pyridine (30 mL) and Ac_2O (15 mL), and the solution was stirred at room temperature for 4 h. It was then poured onto a mixture of ice and H_2SO_4 (25 mL), diluted with water and extracted with CH_2Cl_2 (3 × 50 mL). The combined organic layers were washed with water, dried over Na_2SO_4 and evaporated *in vacuo*. The resulting crude product was purified by flash chromatography with CH_2Cl_2 as eluent to give **9** (978 mg, 88%), mp 88–91 °C, R_f 0.23 (ss A); ^{1}H NMR (500 MHz, $CDCl_3$): δ_H = 0.77 (s, 3H, 18-H_3), 0.87 (s, 3H, 19-H_3), 0.88–0.93 (m, 1H), 1.06–1.12 (m, 1H), 1.17–1.69 (m, 14H), 1.73 (m, 1H), 1.78–1.83 (m, 1H), 2.01 (s, 3H, Ac-CH_3), 2.02 (s, 3H, Ac-CH_3), 2.11–2.20 (m, 2H), 3.72 (bs, 1H, 1-H), 4.59 (t, 1H, J = 8.4 Hz, 17-H), 4.96 (m, 1H, 3-H); ^{13}C NMR (125 MHz, $CDCl_3$): δ_C = 12.1 (C-18), 13.2 (C-19), 20.1 (CH_2), 21.1 (Ac-CH_3), 21.3 (Ac-CH_3), 23.5 (CH_2), 27.5 (CH_2), 28.2 (CH_2), 30.9 (CH_2), 31.2 (CH_2), 33.5 (CH_2), 35.2 (CH), 36.5 (CH_2), 37.5 (CH), 39.3 (C-10), 42.6 (C-13), 47.6 (CH), 50.5 (CH), 65.4 and 69.1 (C-1 and C-3), 82.7 (C-17), 170.3 (Ac-CO), 171.1 (Ac-CO); Anal. Calcd for $C_{23}H_{35}N_3O_4$ C, 66.16; H, 8.45; N, 10.06. Found: C, 66.37; H, 8.29; N, 10.19%.

4.6. General procedure for the preparation of triazoles 8a, 8h, 10a and 10h

To a solution of 17β-acetoxy-1α-azido-5α-androstan-3α-ol (**5**) (300 mg, 0.79 mmol) or 3α,17β-diacetoxy-1α-azido-5α-androstane (**9**) (300 mg, 0.72 mmol) in toluene (10 mL) were added Ph_3P (41 mg, 0.16 mmol), CuI (15 mg, 0.08 mmol) and DIPEA (0.40 mL, 2.4 mmol). Finally, the appropriate terminal alkyne (**6a** or **6h**, 1.1 eq) was added to the reaction mixture, which was then refluxed for 5 h, allowed to cool and evaporated *in vacuo*. The resulting crude product was purified by column chromatography.

17β-Acetoxy-1α-[4′-phenyl-1′*H*-1′,2′,3′-triazol-1′-yl]-5α-androstan-3α-ol (8a). Substrate: **5**, alkyne: phenylacetylene (**6a**, 0.09 mL). After purification with CH_2Cl_2/EtOAc (80 : 20) as eluent, **8a** was obtained as a white solid (233 mg, 61%), mp 182–184 °C, R_f 0.29 (ss E); ^{1}H NMR (500 MHz, $CDCl_3$): δ_H = 0.37 (m, 1H), 0.75 (s, 3H, 18-H_3), 0.80–0.91 (m, 3H), 1.11 (s, 3H, 19-H_3), 1.18–1.33 (m, 2H), 1.34–1.47 (m, 5H), 1.49–1.62 (m, 4H), 1.94 (m, 1H), 1.97 (s, 3H, Ac-CH_3), 1.99–2.01 (m, 3H), 2.47 (m, 1H), 4.42 (t, 1H, J = 8.3 Hz, 17-H), 4.60-4.65 (m, 2H, 1-H and 3-H), 7.32 (t, 1H, J = 7.3 Hz, 4″-H), 7.41 (t, 2H, J = 7.3 Hz, 3″-H and 5″-H), 7.71 (s, 1H, 5′-H), 7.78 (d, 2H, J = 7.3 Hz, 2″-H and 6″-H); ^{13}C NMR (125 MHz, $CDCl_3$): δ_C = 12.3 (C-18), 13.9 (C-19), 21.1 (Ac-CH_3), 21.3 (CH_2), 23.3 (CH_2), 27.4 (CH_2), 28.8 (CH_2), 30.6 (CH_2), 35.7 (CH), 36.4 (2C, 2 × CH_2), 37.8 (CH_2), 38.0 (CH), 40.1 (C-10), 42.6 (C-13), 47.4 (CH), 50.3 (CH), 65.5 and 65.7 (C-1 and C-3), 82.4 (C-17), 121.4 (C-5′), 125.5 (2C, C-2″ and C-6″), 128.2 (C-4″), 128.9 (2C, C-3″ and C-5″), 130.2 (C-1″), 146.0 (C-4′), 171.0 (Ac-CO); ESI-MS: 478 $[M+H]^+$; Anal. Calcd for $C_{29}H_{39}N_3O_3$ C, 72.92; H, 8.23; N, 8.80. Found: C, 73.06; H, 8.10; N, 8.93%.

4.7. General procedure for the preparation of 11a–g, 12a and 12i

Compound **7a–g**, **8a**, or **8h** (120 mg) was dissolved in MeOH (10 mL), and KOH (50 mg, 0.89 mmol) was added. The solution was stirred at room temperature for 24 h, diluted with water and acidified with dilute HCl. The precipitate that formed was filtered off, washed with water and dried.

1α-[4′-Phenyl-1′*H*-1′,2′,3′-triazol-1′-yl]-5α-androstane-3β,17β-diol (11a). Substrate: **7a** (0.25 mmol). **11a** was obtained as a white solid (105 mg, 96%), mp 149-151 °C, R_f 0.22 (ss F); ^{1}H NMR (500 MHz, $CDCl_3$): δ_H = 0.18 (m, 1H), 0.73 (s, 3H, 18-H_3), 0.76–0.84 (m, 3H), 1.08 (s, 3H, 19-H_3), 1.13–1.28 (m, 2H), 1.31–1.81 (m, 10H), 1.97 (m, 1H), 2.06 (m, 1H), 2.34–2.45 (m, 2H), 3.48 (t, 1H, J = 8.4 Hz, 17-H), 4.04 (bs, 1H, 3-H), 4.59 (d, 1H, J = 5.6 Hz, 1-H), 7.36 (t, 1H, J = 7.5 Hz, 4″-H), 7.45 (t, 2H, J = 7.6 Hz, 3″-H and 5″-H), 7.82 (d, 2H, J = 7.6 Hz, 2″-H and 6″-H), 7.94 (s, 1H, 5′-H); ESI-MS: 436 $[M+H]^+$; Anal. Calcd for $C_{27}H_{37}N_3O_2$ C, 74.45; H, 8.56; N, 9.65. Found: C, 74.59; H, 8.44; N, 9.82%.

4.8. General procedure for the preparation of triazoles 13a–g

Compound **7a–g** (200 mg) was dissolved in acetone (10 mL) and Jones reagent (0.5 mL) was dropped into the reaction mixture, which was then stirred at room temperature for 20 min., and diluted with water. The precipitate that formed was filtered off and dried, and the crude product was purified by column chromatography. The by-product (**2**) could generally be isolated, in yields of 19–28%.

17β-Acetoxy-1α-[4′-phenyl-1′*H*-1′,2′,3′-triazol-1′-yl]-5α-androstan-3-one (13a). Eluent: CH_2Cl_2/EtOAc (98 : 2), yielding **2** (32 mg, 23%) and **13a** as a white solid (144 mg, 72%), mp 195–197 °C, R_f 0.44 (ss D); ^{1}H NMR (500 MHz, $CDCl_3$): δ_H = 0.40 (m, 1H), 0.71 (m, 1H), 0.82 (s, 3H, 18-H_3), 0.94 (m, 1H), 1.12 (m, 1H), 1.26 (s, 3H, 19-H_3), 1.30 (m, 3H), 1.43–1.61 (m, 5H), 1.80 (m, 1H), 1.95 (m, 1H), 2.02 (s, 3H, Ac-CH_3), 2.05–2.19 (m, 2H), 2.34 (m, 1H), 2.52 (m, 1H), 2.69 (m, 1H), 2.97 (m, 1H), 4.53 (t, 1H, J = 8.2 Hz, 17-H), 5.02 (d, 1H, J = 4.5 Hz, 1-H), 7.34 (t, 1H, J = 7.2 Hz, 4″-H), 7.43 (t, 2H, J = 7.1 Hz, 3″-H and 5″-H), 7.63 (s, 1H, 5′-H), 7.82 (d, 2H, J = 7.1 Hz, 2″-H and 6″-H); ^{13}C NMR (125 MHz, $CDCl_3$): δ_C = 12.4 (C-18), 13.8 (C-19), 21.1 (Ac-CH_3), 21.2 (CH_2), 23.3 (CH_2), 27.4 (CH_2), 28.3 (CH_2), 30.1 (CH_2), 35.6 (CH), 36.4 (CH_2), 38.2 (CH), 40.1 (C-10), 42.8 (C-13), 43.1 (CH_2), 44.0 (CH_2), 47.5 (CH), 50.3 (CH), 64.6 (C-1), 82.3 (C-17), 119.8 (C-5′), 125.7 (2C, C-2″ and C-6″), 128.3 (C-4″), 128.9 (2C, C-3″ and C-5″), 130.2 (C-1″), 147.1 (C-4′), 171.0 (Ac-CO), 206.6 (C-3); ESI-MS: 476 $[M+H]^+$; Anal. Calcd for $C_{29}H_{37}N_3O_3$ C, 73.23; H, 7.84; N, 8.83. Found: C, 73.44; H, 7.73; N, 8.99%.

4.9. Determination of antiproliferative activities

Cytotoxic effects were measured *in vitro* on three human cell lines of gynecological origin (ECACC, Salisbury, UK): HeLa (cervix adenocarcinoma), A2780 (ovarian carcinoma) and MCF7 (breast adenocarcinoma). The cells were cultivated in minimal essential medium (Sigma-Aldrich) supplemented with 10% fetal bovine serum, 1% non-essential amino acids and an antibiotic-antimycotic mixture. 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 compound was added. The highest concentration was 30 μM. After incubation for 72 h at 37 °C in humidified air containing 5% CO_2, the living cells were assayed by the 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 reduced MTT was assayed at 545 nm, using a microplate reader; wells with untreated cells were utilized as controls.[26] 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 DMSO content of the medium did not have any significant effect on the cell proliferation.

Acknowledgements

This work was supported by the New Hungary Development Plan (TÁMOP 4.2.1/B-09/1/KONV-2010-0005) and the Hungarian Scientific Research Fund (OTKA PD72403 and K101659). 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 Mrs Irén Forgó (University of Szeged, Hungary) for technical support.

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