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A facile 'click' approach to novel 15β-triazolyl-5α-androstane derivatives, and an evaluation of their antiproliferative activities in vitro

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ABSTRACT

Intermolecular Cu(I)-catalyzed azide-alkyne cycloadditions of 15β-azido-17β-hydroxy-5α-androstan-3β-yl acetate with different terminal alkynes under optimized reaction conditions were carried out to furnish 15β-triazolyl derivatives in good yields. Subsequent oxidation of the 'click' products with the Jones reagent afforded the corresponding 17-ketones. All the synthetized compounds were tested on three malignant human cell lines (HeLa, MCF7 and A431) in order to investigate their antiproliferative activities in vitro. Evidence of cell cycle blockade and apoptosis induction was obtained for the most effective five selected compounds by means of flow cytometry and microscopic techniques. The 15β-triazolyl-5α-androstane framework may be considered an appropriate base for the design of steroidal antiproliferative agents.

1. Introduction

Sexual hormones and their derivatives are widely used in the medication of different diseases, though undesirable side-effects may appear because of their primary hormonal activities. Currently the main driving force toward the preparation of steroidal compounds is the development of novel analogs with a biological target other than a hormone receptor, and therefore the reduction or elimination of unwanted hormonal effects. One of the synthetic tools for modifying biological activity is the design of heterocyclic derivatives that are not recognized by the hormone receptor protein in consequence of their specific structure or the fact that their geometry differs from that of the natural hormones. Thus, a variety of steroids with unusual and interesting structures have been synthetized and evaluated for their anti-tumor activities.[1] Experimental results during the past few years have revealed that a number of natural or synthetic steroidal heterocycles play important roles in complex signal transduction mechanisms, and therefore affect the proliferation of human cancer cells without influencing the division of intact cells.[2] Consequently, the application of heterocyclic steroidal compounds in the treatment of cancer offers an excellent possibility for targeted therapy.

The synthetic strategies for the formation of five-membered heterocyclic ring systems include 1,3-dipolar cycloaddition reactions, which have enjoyed undiminished popularity for several decades in view of the large numbers of potential dipoles and dipolarophiles.[3] As one such possibility, Cu(I)-catalyzed azide–alkyne cycloaddition (CuAAC)[4] has received considerable attention in recent years as it meets all of the criteria for 'click' chemistry introduced by Sharpless in 2001 (e.g., regioselectivity, lack of side-reactions, increased reaction rates, high conversions, and tolerability to a variety of common reaction parameters).[5] The 1,4-disubstituted 1,2,3-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 that of an amide bond.[6] Although a number of diverse triazolyl derivatives have been reported to exhibit varied biological activities, including anti-HIV,[7] antibacterial,[8] antihistamine[9] or cytostatic effects,[10] steroids containing this kind of structural moiety have received less attention from both synthetic and pharmacological aspects.[11] Banday et al. recently reported the syntheses of 21-triazolyl derivatives of pregnenolone as potential anticancer agents through use of the 'click' chemistry approach,[12] but without any suggestion as to their mode of action.

The most frequent synthetic modifications of the original sterane framework are performed 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 skeleton (e.g., the introduction of an azido group) has proved to be more difficult,

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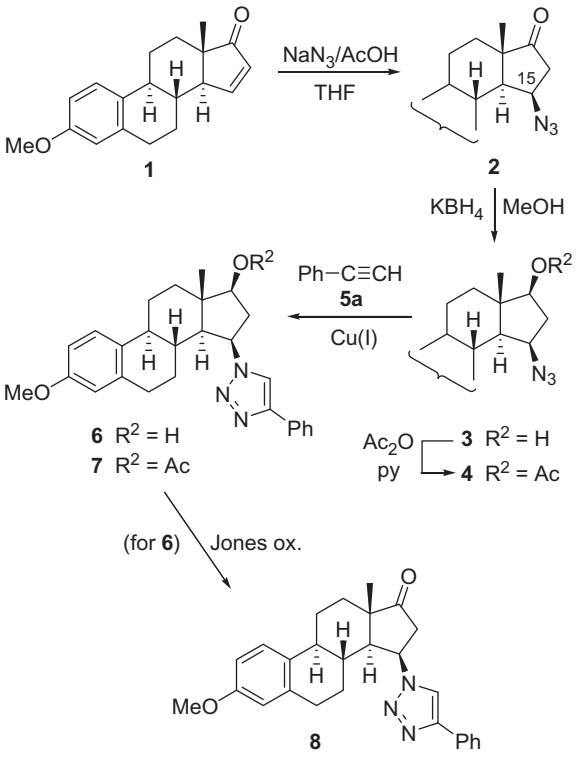
necessitating several reaction steps, and is therefore rarely applied. In this regard, we recently reported the stereoselective synthesis of 1α-azidoandrostanes and their CuAAC with terminal alkynes to furnish novel 1α-triazolyl derivatives with noteworthy in vitro cytostatic effects.[13]

The foregoing results led us to set out to introduce an azido group at the unconventional position 15 of the sterane skeleton, and ultimately to synthetize novel 15-*exo*-triazolyl derivatives via CuAAC. To the best of our knowledge, only a few 15-substituted steroidal derivatives have been synthetized to date; they include 15β-methylated estrone 3-methyl ether, which exerts in vitro cytotoxic activity against human gastric cancer cell line MGC-803.[14] Since some steroid triazoles are also known to exhibit antiproliferative activity,[11c–13] we decided to screen all the synthetized compounds in vitro for their activities against a panel of three human cancer cell lines (HeLa, MCF7 and A431). The most effective compounds were subjected to additional in vitro experiments on HeLa cells, including flow cytometric cell cycle analysis and fluorescence microscopy, in order to characterize the mechanism of their action.

2. Results and discussion

2.1. Synthesis

Preliminary ring-closure experiments on model compound **3**, synthetized from 3-methoxy-1,3,5(10),15-estratetraen-17-one (**1**), were first carried out in order to optimize the reaction conditions (Scheme 1). The azido group was introduced stereoselectively onto position 15β of the sterane skeleton of **1** by the 1,4-Michael addition of azoimide,[15] generated in situ from sodium azide and acetic acid, to afford azidoketone **2** in a yield of 75% after purification by flash chromatography. Since β-substituted ketones such as **2** are often susceptible to elimination and undergo facile transformation to the corresponding enone,[16] **2** was reduced with KBH_4 so as to avoid this adverse side-reaction. The resultant *cis*-azidoalcohol **3** was then reacted with phenylacetylene (**5a**) under various 'click' reaction conditions in order to determine the parameters (catalyst, additives, solvent and temperature) needed for the optimal yields of the desired product **6**. The results are presented in Table 1.



Scheme 1. Synthesis of 15β-triazolylestrones (**6**–**8**).

The most common technique, involving the in situ generation of the Cu(I) species by the reduction of $CuSO_4 \cdot 5H_2O$ with sodium ascorbate, resulted in the corresponding cycloadduct **6** in a yield of only 11%, with a major amount of unreacted starting material **3** (entry 1). A similar result was obtained on the use of CuI catalyst in acetonitrile at room temperature, although both an amine base additive and a complexing ligand were used for formation of the Cu–acetylide complex and to protect the Cu(I) from oxidation (entry 2). Nevertheless, better yields were achieved when toluene as solvent was applied instead of acetonitrile (entries 3 and 4). Triphenylphosphane is presumed to accelerate the rate of the reaction by complexing to Cu(I), since lower conversions were attained without its addition to the reaction mixture. Since CuI-catalyzed cycloadditions are often favored by elevated temperature,[17] the further experiments were carried out under reflux in order to improve the yields of the heterocyclic product **6** and to accelerate the reaction. The best conversion was found on the use of a catalytic amount of CuI with the simultaneous addition of triphenylphospane as stabilizing ligand and excess *N*,*N*-diisopropylethylamine (DIPEA) as amine base; these conditions furnished **6** in a yield of 72% on refluxing in toluene for 4 h (entry 6). The reaction of **5a** with the acetylated azidoalcohol **4** proceeded similarly as for **3**, indicating that the functional group on C-17 does not have a significant effect on the ring-closure (entry 7). However, full conversion of the starting materials **3** or **4** could not be achieved even at elevated temperature; this may be attributed to the OH or OAc group on C-17, which is *cis* and therefore spatially close to the azide dipole, presumably causing a crowded transition state in the Cu(I)-catalyzed process. Similar steric hindrance was observed earlier during the synthesis of 1α-triazolyl derivatives.[13]

After determination of the optimal conditions, an azidoalcohol (**9**) in the 5α-androstane series, readily available from dehydroepiandrosterone in a multistep pathway,[15] was subjected to similar cycloadditions with different aryl-substituted acetylenes (**5a–d**). This resulted in steroidal 15β-*exo*-triazolyl derivatives **10a–d** in yields of ~70%, independently of the substituent on the alkyne dipolarophile (Table 2). Subsequent Jones oxidation of triazolyl alcohols **6** and **10a–d** furnished the corresponding 17-keto analogs **8** and **11a–d** in good yields.

The structures of all synthetized compounds were confirmed by ^1H and ^{13}C NMR measurements. The ^1H NMR spectra of triazoles containing an aromatic moiety connected to the hetero ring (**6–8**, **10a–d**, **11a–d**) revealed the signals of the incorporated aryl groups at 7.1–7.8 ppm as compared with the spectra of the starting azides (**3**, **4** and **9**). The 5′-H singlet of the newly formed heterocycles was identified at 7.7–8.9 ppm.

2.2. Pharmacology

With the exception of the 17-acetate in the estrone series (**7**), the novel triazolyl derivatives (**6**, **8**, **10a–d** and **11a–d**) were subjected to in vitro pharmacological studies of their antiproliferative effects (Table 3). The activities were determined by using three malignant adherent cell lines in the microplate-based MTT colorimetric assay,[18] 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.

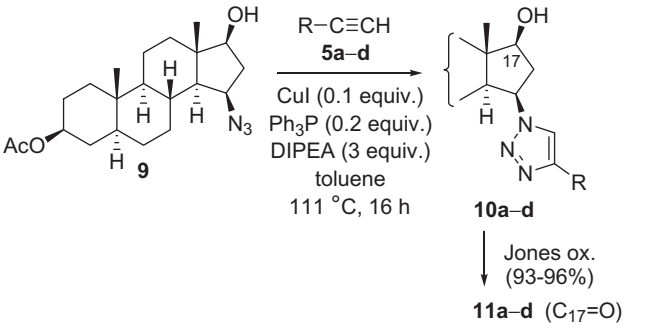
17β-Triazolyl estrones **6** and **8**, similarly to the reference agent cisplatin, exhibited marked antiproliferative effects against A431 cells, while the other two cell lines seemed less sensitive. Since

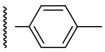
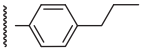
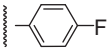
Table 1
CuAAC of model compounds **3** and **4** with phenylacetylene **5a** under different reaction conditions

Entry	Azide	**5a** (equiv)	Catalyst (equiv)	Base (equiv)	Ligand (equiv)	Solvent	T (°C)	t (h)	Yield[a] (%)
1	**3**	(1.0)	$CuSO_4$ (0.02) Na ascorbate (0.1)	—	—	*t*BuOH/H_2O = 1:1	25	96	11
2	**3**	(1.0)	CuI (0.1)	Et_3N (1.0)	Ph_3P (0.2)	CH_3CN	25	96	9
3	**3**	(1.0)	CuI (0.1)	Et_3N (1.0)	—	Toluene	25	96	18
4	**3**	(1.0)	CuI (0.1)	Et_3N (1.0)	Ph_3P (0.2)	Toluene	25	96	40
5	**3**	(1.1)	CuI (0.1)	—	Ph_3P (0.2)	Toluene	111	4	52
6	**3**	(1.1)	CuI (0.1)	DIPEA (3.0)	Ph_3P (0.2)	Toluene	111	4	72
7	**4**	(1.1)	CuI (0.1)	DIPEA (3.0)	Ph_3P (0.2)	Toluene	111	4	70

[a] Yields of purified isolated products.

Table 2
CuAAC of the *cis*-azidoalcohol **9** with terminal alkynes **5a–d** under optimized conditions



Entry	Alkyne	R	Triazole	Yield[a] (%)
1	**5a**		**10a**	73
2	**5b**		**10b**	75
3	**5c**		**10c**	72
4	**5d**		**10d**	72

[a] Yields of purified isolated products.

Table 3
Calculated IC_{50} values of the synthetized triazole derivatives

	IC_{50} values (μM)*		
	HeLa cells	MCF-7 cells	A431 cells
6	>30	>30	1.70
8	14.88	11.35	4.77
10a	7.70	19.24	20.69
10b	9.40	10.28	22.43
10c	6.52	>30	>30
10d	>30	>30	>30
11a	9.16	1.69	9.69
11b	10.27	2.68	10.66
11c	15.01	8.40	>30
11d	10.96	3.39	16.03
Cisplatin	12.43	9.63	2.84

* Mean values from two independent determinations with five parallel wells; standard deviation less than 15%.

substantial differences were not detected between the IC_{50} values of the 5α-androstane counterparts, it could be concluded that the substituent on the triazolyl ring does not have a crucial impact on the anticancer capacities of this skeleton. Although not consequently, the 17-keto function seemed to be favored over the 17-hydroxy group. The results of the MTT assays led to the selection of **10a–c**, **11a** and **11b** for additional experiments in an attempt to elucidate the mechanism of their action.

Treatment of HeLa cells with 3 and 10 μM of the selected agents for 24 or 48 h was followed by flow cytometric cell cycle analysis. The 24-h treatment with each of these compounds resulted in a concentration-dependent decrease in the number of cells in the G1 phase, and also in an accumulation of the G2/M population (Fig. 1). Compound **10c** did not exert any effect on the G2/M phase, but increased the proportion of cells in the synthetic (S) phase. **10a–c** and **11a** also resulted in modest but statistically significant increases in the number of hypodiploid (subG1) cells, which are generally regarded as an apoptotic population.[19] This apoptotic proportion became more pronounced after incubation for 48 h (Fig. 2).

The programmed cell death-inducing capacities of the tested agents were confirmed by detection of the cell morphology and membrane integrity. Separate pictures were taken, illustrating Hoechst 33258 and propidium iodide (PI) fluorescence as morphological markers. After incubation for 24 h, concentration-dependent increases in nuclear condensation and cell membrane permeability were generally detected, indicated by blue and red fluorescence, respectively (Fig. 3). This finding suggests that, after treatment with these 5α-androstane derivatives, the cells can not complete the S-G2/M phases of the cell cycle and this blockade may initiate the apoptotic machinery.

An anticancer agent is generally expected to cause the death of the tumor cells, but the manner of cell death can be crucially relevant. While apoptosis involves a precisely regulated self-decomposition of the cells in question, protecting the surrounding intact cells, necrosis involves the prompt release of the intracellular content, which results in the deterioration of neighboring cells.[20] The biochemical and morphological results indicate that the initiation of apoptosis predominates in the antiproliferative action of the present compounds and their structure can therefore be considered a reasonable skeleton for the design and synthesis of further anticancer lead candidates.

3. Conclusions

In summary, the CuAAC of steroidal 15β,17β-azidoalcohols with different terminal alkynes under optimized reaction conditions gave 15β-*exo*-triazolyl derivatives in good yields. The synthetized 5α-androstane derivatives are of interest from a pharmacological aspect, since several of the analogs proved to exert marked in vitro antiproliferative activity. As apoptosis initiation is involved in their mode of action, these compounds may be considered for the design of further anticancer lead candidates.

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)

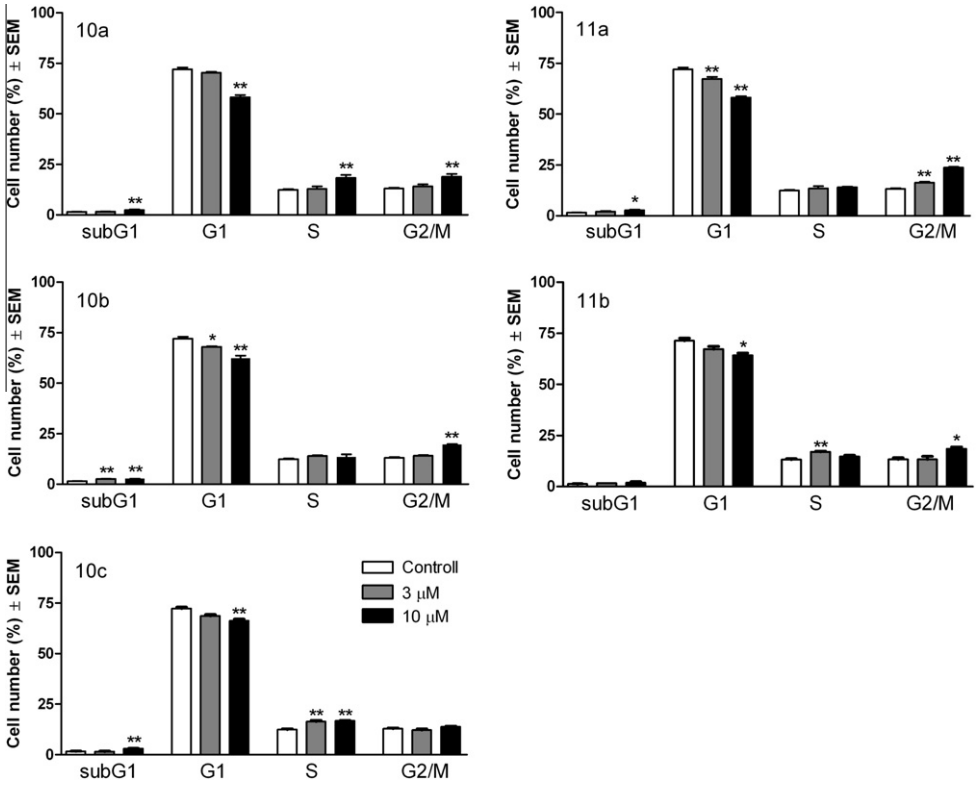


Figure 1. Effects of **10a–c** and **11a–b** on HeLa cell cycle distribution after incubation for 24 h. The data are mean values from three determinations. ∗ and ∗∗ indicate p <0.05 and p <0.01, respectively, as compared with the control cells.

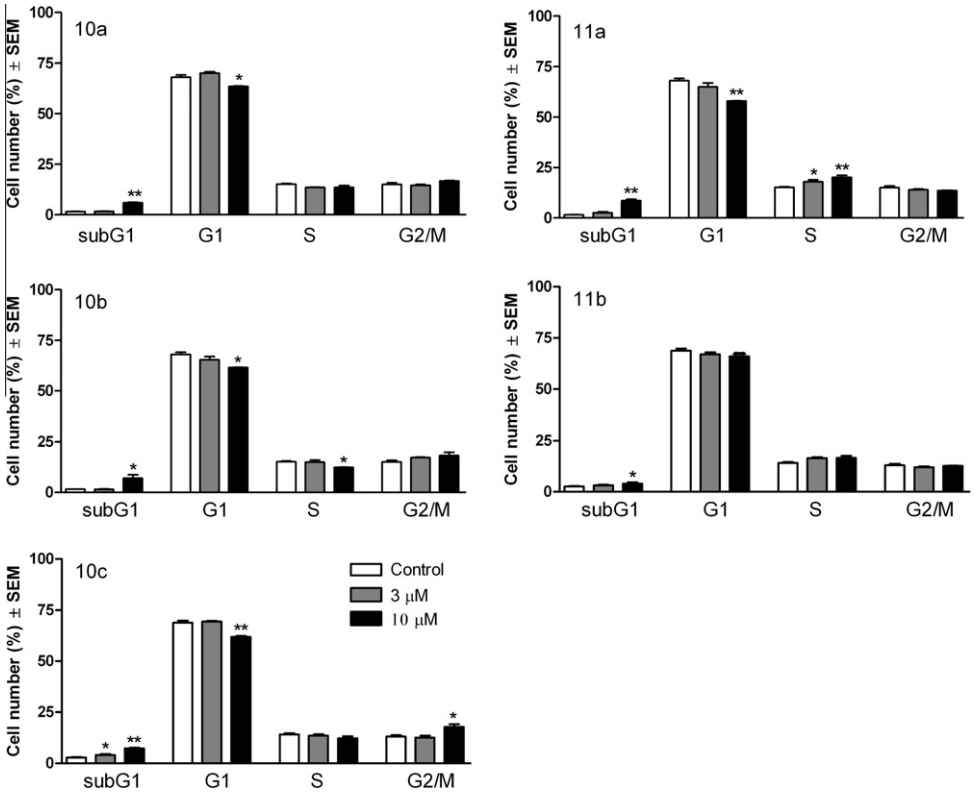


Figure 2. Effects of **10a–c** and **11a–b** on HeLa cell cycle distribution after incubation for 48 h. The data are mean values from three determinations. ∗ and ∗∗ indicate p <0.05 and p <0.01, respectively, as compared with the control cells.

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 (80:20 v/v), (F) CH_2Cl_2/EtOAc (50:50 v/v), (G) CH_2Cl_2/hexane (80:20 v/v). The spots were detected by spraying with 5% phosphomolybdic acid in 50% aqueous phosphoric acid.

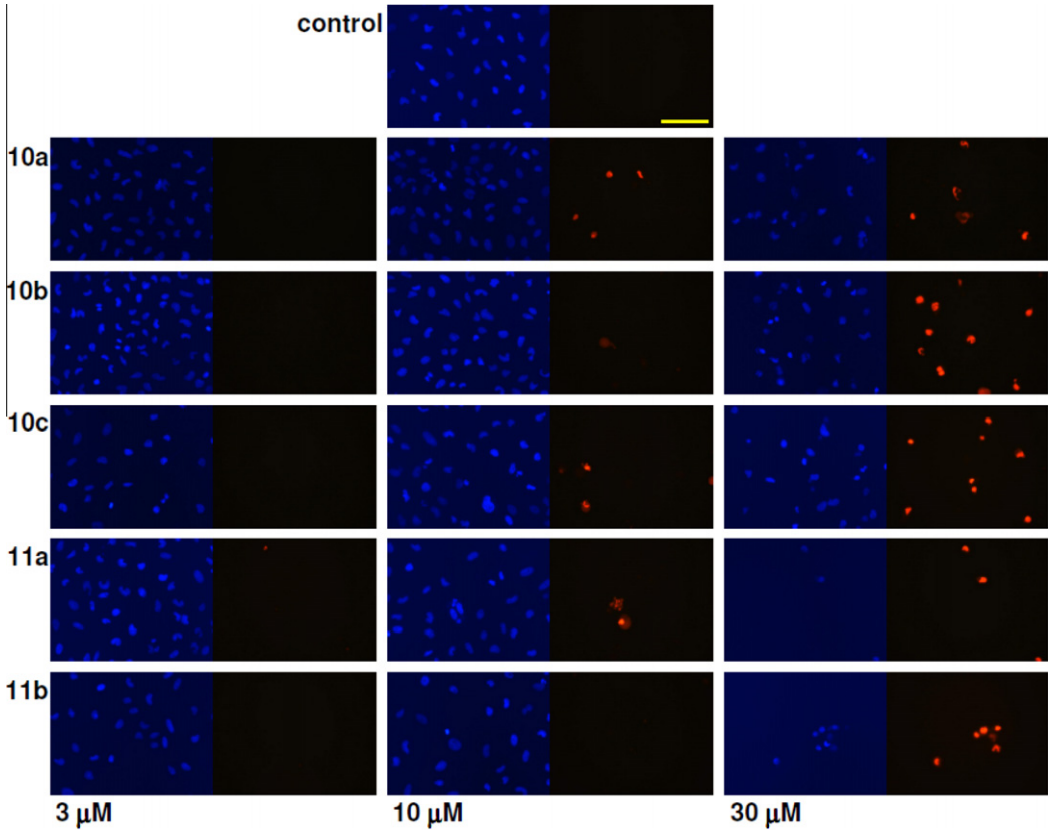


Figure 3. Fluorescence microscopy images of Hoechst 33258—PI double staining. Separate pictures from the same field were recorded for the two markers. HeLa cells were treated with vehicle (control), or with **10a–c** or **11a–b** at 3, 10 and 30 μM. The blue fluorescence indicates Hoeschs 33258, and the red coloration is a result of cellular PI accumulation. The bar in the Hoeschs 33258 control picture indicates 100 μm.

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 PerkinElmer 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; capillary voltage (VCap) 3000 V; fragmentor 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) 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. General procedure for the preparation of triazoles 10a–d

To a solution of 15β-azido-17β-hydroxy-5α-androstan-3β-yl acetate (**9**)[15] (300 mg, 0.80 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 (**5a–d**, 1.1 equiv) was added to the reaction mixture, which was then refluxed for 10 h, allowed to cool and evaporated in vacuo. The resulting crude product was purified by column chromatography.

4.2.1. 3β-Acetoxy-15β-[4′-phenyl-1′*H*-1′,2′,3′-triazol-1′-yl]-5α-androstan-17β-ol (10a)

Alkyne: phenylacetylene (**5a**, 0.09 mL). After purification with CH_2Cl_2/EtOAc (80:20) as eluent, **10a** was obtained as a white solid (279 mg, 73%), mp 243–245 °C, R_f = 0.23 (ss D); ^{1}H NMR (500 MHz, $CDCl_3$): δ_H = 0.81 (s, 3H, 19-H_3), 1.15 (s, 3H, 18-H_3), 1.99 (s, 3H, Ac-H_3), 2.80 (dt, 1H, J = 14.1 Hz, J = 8.5 Hz, 16-H_α), 3.75 (t, 1H, J = 8.6 Hz, 17-H), 4.64 (m, 1H, 3-H), 4.96 (m, 1H, 15-H), 7.32 (t, 1H, J = 7.6 Hz, 4″-H), 7.42 (t, 2H, J = 7.6 Hz, 3″-H and 5″-H), 7.78 (s, 1H, 5′-H), 7.82 (d, 2H, J = 7.6 Hz, 2″-H and 6″-H); ^{13}C NMR (125 MHz, $CDCl_3$): δ_C = 12.3 (C-19), 14.3 (C-18), 20.6 (CH_2), 21.4 (Ac-CH_3), 27.4 (CH_2), 28.1 (CH_2), 30.8 (CH_2), 33.1 (CH), 33.8 (CH_2), 35.7 (C-10), 36.7 (CH_2), 38.9 (CH_2), 40.8 (CH_2), 42.9 (C-13), 44.8 (CH), 55.1 (CH), 55.6 (CH), 58.5 (C-15), 73.4 (C-3), 80.3 (C-17), 120.1 (C-5′), 125.6 (2C, C-2″ and C-6″), 128.1 (C-4″), 128.8 (2C, C-3″ and C-5″), 130.6 (C-1″), 147.0 (C-4′), 170.7 (Ac-C); 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, 73.08; H, 8.41; N, 8.67.

4.3. General procedure for the preparation of triazoles 11a–d

Compound **10a–d** (200 mg) was dissolved in acetone (10 mL) and Jones reagent (0.5 mL) was dropped into the solution, 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.

4.3.1. 3β-Acetoxy-15β-[4′-phenyl-1′*H*-1′,2′,3′-triazol-1′-yl]-5α-androstan-17-one (11a)

Substrate: **10a** (0.42 mmol); product: **11a** (189 mg, 95%), mp 217–218 °C, R_f = 0.33 (ss C); ^{1}H NMR (500 MHz, $CDCl_3$): δ_H = 0.80 (s, 3H, 19-H_3), 0.86 (s, 3H, 18-H_3), 2.01 (s, 3H, Ac-H_3), 2.89 (dd, 1H, J = 19.5 Hz, J = 7.5 Hz, 16-H_β), 3.38 (d, 1H, J = 19.5 Hz, 16-H_α), 4.69 (m, 1H, 3-H), 5.15 (m, 1H, 15-H), 7.34 (t, 1H, J = 7.6 Hz, 4″-H), 7.42 (t, 2H, J = 7.6 Hz, 3″-H and 5″-H), 7.81 (d, 2H, J = 7.6 Hz, 2″-H and 6″-H), 7.83 (s, 1H, 5′-H); ^{13}C NMR (125 MHz, $CDCl_3$): δ_C = 12.2 (C-19), 16.3 (C-18), 20.3 (CH_2), 21.4 (Ac-CH_3), 27.3 (CH_2), 28.0 (CH_2), 31.1 (CH_2), 33.2 (CH), 33.8 (CH_2), 34.3 (CH_2), 35.9 (C-10), 36.6 (CH_2), 43.6 (CH_2), 44.9 (CH), 46.3 (C-13), 55.4 (CH), 56.3 (CH), 56.4 (CH), 73.3 (C-3), 119.8 (C-5′), 125.6 (2C, C-2″ and C-6″), 128.3 (C-4″), 128.9 (2C, C-3″ and C-5″), 130.2 (C-1″), 147.6 (C-4′), 170.7 (Ac-C), 216.7 (C-17); 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, 73.41; H, 7.68; N, 9.09.

4.4. Cell cultures and antiproliferative assays

Human cancer cell lines (HeLa, MCF-7 and A431, isolated from cervical adenocarcinoma, breast adenocarcinoma and skin epidermoid carcinoma, respectively) were maintained in minimal essential medium supplemented with 10% fetal bovine serum, and 1% non-essential amino acids and an antibiotic-antimycotic mixture. All cell lines were purchased from the European Collection of Cell Cultures, Salisbury, UK. The cells were grown in a humidified atmosphere of 5% CO_2 at 37 °C. Cells were seeded onto 96-well plates at a density of 5000 cells/well and allowed to stand overnight, after which the medium containing the tested compound was added. After a 72-h incubation period, viability was determined by the addition of 20 μL of MTT ([3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide]) solution (5 mg/mL). The precipitated formazan crystals were solubilized in DMSO and the absorbance was read at 545 nm with an ELISA reader.[18] Two independent experiments were performed with five parallel wells, and cisplatin was used as positive control. Sigmoidal dose–response curves were fitted to the measured points, and the IC_{50} values were calculated by means of GraphPad Prism 4.0 (GraphPad Software; San Diego, CA, USA).

4.5. Cell cycle analysis by flow cytometry

Flow cytometric analysis was performed in order to characterize the cellular DNA content of treated HeLa cells. After treatment for 24 or 48 h cells (200,000 per condition) were trypsinized (Gibco BRL, Paisley, U.K.), washed with phosphate-buffered saline (PBS) and fixed in 1.0 mL of cold 70% ethanol for 30 min on ice. After two washing steps in cold PBS, DNA was stained with PI (10 μg/mL) in the presence of RNA-ase (50 μg/mL). The samples were then analyzed with a FACStar (Becton-Dickinson; Mountain View, CA, USA). In each analysis, 20,000 events were recorded, and the percentages of the cells in the different cell-cycle phases (subG1, G1, S and G2/M) were calculated by using winMDI2.9. The subG1 fractions were regarded as the apoptotic cell population.[19]

4.6. Hoechst 33258—PI double staining

Near-confluent HeLa cells were seeded into a 96-well plate (5000 cells/well). After incubation for 24 h with the test compound, Hoechst 33258 and PI were added to the culture medium to give final concentrations of 5 and 2 μg/mL, respectively. The cells were incubated with the staining mixture for 1 h at 37 °C and were then photographed by means of a Nikon Eclipse microscope equipped with an epifluorescence attachment containing the appropriate optical blocks and a QCapture CCD camera. The staining allowed the identification of live, early-apoptotic, late-apoptotic and necrotic cells. Hoechst 33258 permeates all the cells and makes the nuclei appear blue. Apoptosis was revealed by nuclear changes such as chromatin condensation and nuclear fragmentation. The necrotic and the late-apoptotic cells were identified as cells which displayed PI uptake, which indicates the loss of membrane integrity, the cell nuclei being stained red.

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Supplementary data

Supplementary data (experimental procedures for the preparation and NMR spectral data of all synthetized compounds) associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2012.01.008.

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