Synthesis and In Vitro Antiproliferative Activity of Novel Androst-5-ene Triazolyl and Tetrazolyl Derivatives

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Abstract: A straightforward and reliable method for the regioselective synthesis of steroidal 1,4-disubstituted triazoles and 1,5-disubstituted tetrazoles via copper(I)-catalyzed cycloadditions is reported. Heterocycle moieties were efficiently introduced onto the starting azide compound 3β-acetoxy-16β-azidomethylandrost-5-en-17β-ol through use of the “click” chemistry approach. The antiproliferative activities of the newly-synthesized triazoles were determined in vitro on three human gynecological cell lines (HeLa, MCF7 and A2780) using the microculture tetrazolium assay.

Keywords: click chemistry; steroid azides; triazoles; tetrazoles; CuAAC

1. Introduction

In the past few years, the Huisgen 1,3-dipolar cycloaddition of azides and terminal alkynes to form triazoles has received revived attention. Since the independent reports of Sharpless [1] and Meldal [2], this process has become the most extensively studied “click” reaction, as evidenced by a nearly exponential growth in the number of related publications. Compared with the non-catalyzed version
the copper(I)-catalyzed azide-alkyne cycloaddition (CuAAC) has certain advantageous properties, such as regioselectivity, versatility, high conversions and the lack of by-products [3-5]. Moreover, this process performs well in most common laboratory solvents and usually does not require protection from oxygen and water, making it an ideal tool for the synthesis of libraries for initial screening and structure-activity profiling.

In contrast, other 1,3-dipolar cycloadditions between nitriles and organic azides to afford tetrazoles generally requires highly electrophilic nitrile carbon atoms and harsh conditions [6]. Demko and Sharpless recently reported the syntheses of some 1,5-disubstituted tetrazoles [7,8] under solvent-free conditions at 100-120 °C. Furthermore, a series of potential catalysts for these reactions were investigated by Vilarrasa et al. [9], with the aim of achieving milder conditions. The commercially available or easily prepared [10] copper(I) triflate was observed to be the most efficient catalyst.

To the best of our knowledge, only a few examples are to be found in the literature in which 1,3-dipolar cycloadditions have been applied to steroidal azides [11-15]. Thus, in continuation of our program on the synthesis of steroidal heterocycles [16-19], we set out to develop an effective route for the production of novel steroidal triazoles and tetrazoles through use of the “click” chemistry approach. The present paper reports the syntheses of D-ring-substituted androst-5-ene derivatives containing a 1,4-disubstituted triazole (compounds 6a-j, 7a-j) or a 1,5-disubstituted tetrazole moieties (compounds 9a-e, 11a-e).

Five-membered nitrogen heterocycles play an important role in biological systems. Not surprisingly, a number of compounds containing 1,2,3-triazoles are found to exhibit a broad spectrum of biological activities, including antimicrobial [20], anti-HIV [21], antiallergic [22] and antiviral [23] effects. A set of 1,2,3-triazol-1-yl podophyllotoxin derivatives were synthesized and some of them proved to be more potent in inhibiting the growth of human cancer cells than etoposide [24]. Several benzotriazoles proved to be novel and potent antiproliferative agents and some of them exhibited nanomolar IC50 values against human adherent cancer cell lines [25]. A series of substituted tetrazol-5-ones have been synthesized and three of them were found to inhibit leukemia and breast cancer growth in vitro [26]. On the basis of these reports, the triazole and tetrazole ring systems can therefore be regarded as structural blocks suitable for improvement of the anticancer properties of potential pharmacons. Since we reported a set of androstene-fused arylpyrazolines as antiproliferative compounds, it appeared rational to improve the pharmacological profile of the skeleton by means of the introduction of a triazole or tetrazole moiety [19]. Moreover, some 21-triazolyl derivatives of pregnenolone were recently reported as potential anticancer agents by Banday et al. [15]. Thus, the newly-prepared triazolyl derivatives were screened in vitro for their activities against a panel of three human malignant cell lines.

2. Results and Discussion

2.1. Synthesis

To prepare novel steroid triazoles via 1,3-dipolar cycloaddition, 3β-acetoxy-16β-azidomethyl-androst-5-en-17β-ol (5) was chosen as starting compound. The synthetic strategy for the preparation of the starting azide is illustrated in Scheme 1.
Scheme 1. Synthesis of the steroid azide.

Reagents and conditions: (a) Ac₂O, pyridine; (b) KBH₄, MeOH/EtOH (1:1); (c) TsCl, pyridine; (d) NaN₃, DMF, 70 °C, 6 h.

The reaction of 3β-hydroxy-16-hydroxymethylideneandrost-5-en-17-one (2a) [27] with acetic anhydride in pyridine medium afforded the diacetate 2b in excellent yield. According to our earlier observation [28], the reduction of 3β-acetoxy-16-acetoxymethylideneandrost-5-en-17-one (2b) with KBH₄ under pH-controlled conditions leads to three diol isomers. Two of them (compounds 3a, 3b), containing 17β-hydroxy groups with opposite configurations at C-16, were isolated in nearly identical amounts, while the third one, the 16β,17α isomer 3c, was obtained in a significantly smaller quantity (~5%). After separation of the 16β,17β-hydroxymethyl isomer 3a by flash chromatography, the primary hydroxy group in 3a was converted into a good leaving group with p-toluenesulfonyl chloride. Finally, the crude product 4 was used without purification for further nucleophilic substitution with NaN₃ in DMF to provide the desired 3β-acetoxy-16β-azidomethyl-landrost-5-en-17β-ol (5) in good yield.

Several D-ring-substituted androst-5-ene derivatives containing a 1,2,3-triazole ring (compounds 6a-j) were synthesized by the reaction of 5 with various terminal alkynes through use of the “click” chemistry approach (Table 1). Although there are a number of methods for generation of the active catalyst [29], one of the most common techniques was chosen. Thus, the Cu(I) species was generated in situ by the reduction of CuSO₄·5H₂O with sodium ascorbate to minimize the formation of by-products. Furthermore, a mixture of CH₂Cl₂ as solvent and water as co-solvent was employed to eliminate the need for ligands and to simplify the reaction protocol [30].

In all cases, total consumption of the starting compound was observed within 1-4 h at room temperature. The reactions were very selective, and triazole products could be isolated in 78-93% yields. The trace quantities of copper and reagents remaining in the reaction mixtures were removed by flash chromatography. Treatment of 6a-j containing a 3β-acetyl group with KOH in MeOH at 50 °C resulted in the corresponding 3β-hydroxy compounds 7a-j in good yields (Table 1).
Table 1. Synthesis of the 1,4-disubstituted steroidal triazoles and hydrolysis of their 3-acetyl groups.

| Entry | R¹   | Triazoles (6 and 7) | Yield a (%) of 6 | Yield a (%) of 7 |
|-------|------|---------------------|------------------|------------------|
| 1     | a    | a                   | 89               | 82               |
| 2     | b    | b                   | 91               | 81               |
| 3     | c    | c                   | 91               | 88               |
| 4     | d    | d                   | 93               | 85               |
| 5     | e    | e                   | 86               | 86               |
| 6     | f    | f                   | 83               | 87               |
| 7     | g    | g                   | 85               | 91               |
| 8     | h    | h                   | 90               | 82               |
| 9     | i    | i                   | 87               | 90               |
| 10    | j    | j                   | 78               | 83               |

*Yields of purified isolated products.

These outstanding results encouraged us to investigate another example of “click” reactions. The intermolecular [3+2] cycloadditions between the steroid azides 5 and 10 and several nitriles 8a-e containing an electron-withdrawing group (EWG) afforded the desired 1,5-disubstituted steroidal tetrazoles 9a-e and 11a-e. As mentioned earlier, highly electrophilic nitrile carbon atoms are required for successful addition [9]; some commercially available acyl cyanides and cyanoformates were therefore chosen as reagents. In all cases, the reactions were carried out at room temperature, with stirring for 2 days, 10 mol % copper(I) complex Cu₂(OTf)₂·C₆H₆ (OTf = O₃SCF₃) being used as catalyst. The newly-synthesized tetrazolyl compounds could be isolated in 45-72% yields after purification by column chromatography (Table 2).
Table 2. Synthesis of the 1,5-disubstituted steroidal tetrazoles.

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\begin{align*}
\text{Entry} & \quad \text{Reactant} & \text{EWG} & \text{Yield}^a (\%) \text{ of 9a-e} & \text{Yield}^a (\%) \text{ of 11a-e} \\
1 & \text{8a} & \text{MeOCO} & 66 & 59 \\
2 & \text{8b} & \text{EtOCO} & 72 & 64 \\
3 & \text{8c} & \text{BnOCO} & 62 & 53 \\
4 & \text{8d} & \text{MeCO} & 57 & 47 \\
5 & \text{8e} & \text{PhCO} & 54 & 45 \\
\end{align*}
\]

\(^a\) Yields of purified isolated products.

The structures of all synthesized compounds were confirmed by \(^1\)H- and in some cases, \(^13\)C-NMR measurements. The \(^1\)H-NMR spectra of 6a-i and 7a-i revealed the appearance of the new signals of the incorporated aryl groups at 6.9-8.5 ppm as compared with the spectra of the starting azide 5, while the 5'-H singlet of the newly-formed heterocycle was identified at 7.8-8.5 ppm. Compounds 6j and 7j containing a cycloalkyl substituent were exceptions, with a chemical shift of 7.28 ppm (5'-H). As far as the tetrazolyl derivatives are concerned, the newly-formed heterocycle does not contain any protons, but the signal of 5'-C can be identified at 145-149 ppm in the corresponding \(^13\)C-NMR spectra. Furthermore, in the cases of 9c, 11c and 9e, 11e the new signals of the incorporated Ph ring appeared at 7.3-8.3 ppm in the \(^1\)H-NMR spectra.

2.2. Biological Activity

Compounds 7a-j and 11a-e were screened for anticancer activity against a panel of three human cancer cell lines (Table 3). Although there is no generally accepted threshold for efficacy, when the inhibition of cell growth is less than 25% at 30 µM, such a substance may be considered ineffective. No clear structure-activity relationships could be concluded, but triazole-containing androst-5-enes exhibit substantial antiproliferative activity, for which a substituted aromatic group of the triazole ring is preferred. The antiproliferative action of a compound with an unsubstituted phenyl group on the triazole ring (compound 7a) could be maintained or moderately increased by substitution in the para or meta position (compounds 7b-e, 7g), while ortho-OMe (compound 7f) was less effective. Nevertheless, an amino group in the meta position offers no advantage (compound 7h). A pyridyl, but not a cyclopropyl group, instead of phenyl (compounds 7i-j) could be beneficial. Although inactive on HeLa cells at 10 µM, 7c is considered the most effective of the presented compounds. In contrast, tetrazoles substituted on the D-ring of the steroidal skeleton proved to be ineffective with the exception of 11e, which has a moderate effect.
Table 3. Antiproliferative effects of the synthesized compounds.

| Product | µM | HeLa | MCF7 | A2780 |
|---------|----|------|------|-------|
| 7a      | 10 | 64.6 (±1.6) | 35.7 (±0.8) | <25*  |
|         | 30 | 72.6 (±1.9) | 37.2 (±2.2) | 26.9 (±2.7) |
| 7b      | 10 | 77.6 (±0.7) | 40.8 (±1.8) | 35.7 (±2.2) |
|         | 30 | 79.2 (±0.7) | 54.8 (±2.6) | 36.8 (±1.6) |
| 7c      | 10 | <25 | 41.6 (±0.2) | 54.1 (±2.6) |
|         | 30 | 96.9 (±1.7) | 83.7 (±1.3) | 88.5 (±2.1) |
| 7d      | 10 | 78.1 (±0.6) | 47.0 (±2.1) | 45.0 (±2.9) |
|         | 30 | 78.6 (±1.4) | 47.6 (±1.6) | 46.8 (±0.9) |
| 7e      | 10 | 68.7 (±0.3) | 60.2 (±1.9) | 30.9 (±2.2) |
|         | 30 | 74.4 (±0.7) | 62.6 (±0.7) | 32.8 (±1.4) |
| 7f      | 10 | <25 | <25 | 42.3 (±2.7) |
|         | 30 | <25 | 27.1 (±1.3) | 47.8 (±2.1) |
| 7g      | 10 | 61.8 (±0.3) | 54.5 (±1.1) | 31.4 (±2.6) |
|         | 30 | 68.4 (±0.3) | 59.3 (±1.8) | 45.6 (±2.4) |
| 7h      | 10 | 49.2 (±1.3) | <25 | 42.1 (±1.2) |
|         | 30 | 66.2 (±0.8) | 28.6 (±2.4) | 53.2 (±0.9) |
| 7i      | 10 | 55.7 (±2.6) | 46.5 (±2.1) | 31.1 (±1.9) |
|         | 30 | 93.2 (±0.8) | 63.8 (±1.0) | 43.3 (±2.1) |
| 7j      | 10 | 61.7 (±0.4) | 31.3 (±1.1) | 42.3 (±2.6) |
|         | 30 | 63.6 (±0.7) | 50.5 (±1.1) | 47.5 (±1.4) |
| 11a-d   | 10 | <25 | <25 | <25 |
|         | 30 | <25 | <25 | <25 |
| 11e     | 10 | <25 | <25 | <25 |
|         | 30 | <25 | 61.8 (±1.7) | 72.0 (±0.4) |

Cisplatin

| µM | HeLa | MCF7 | A2780 |
|----|------|------|-------|
| 10 | 42.6 (±2.3) | 88.6 (±0.5) | 53.0 (±2.3) |
| 30 | 99.9 (±0.3) | 90.2 (±1.8) | 86.9 (±1.2) |

* Compounds eliciting less than 25% inhibition of proliferation were considered ineffective, and for simplicity the exact results are not given.
3. Experimental

3.1. 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) CH₂Cl₂/EtOAc (95:5 v/v), (B) CH₂Cl₂/EtOAc (80:20 v/v), (C) CH₂Cl₂/EtOAc (50:50 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 \( \mu \)m. 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 and IR spectra were recorded on a BioRad FTS 60A FTIR spectrometer. NMR spectra were obtained at room temperature with a Bruker DRX 500 instrument. Chemical shifts are reported in ppm (\( \delta \) 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 \( \text{N}_2 \), at 35 psi; drying gas \( \text{N}_2 \), 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 \( \mu \)L) with automated needle wash were injected directly into the solvent flow (0.3 mL/min) of CH₃CN/H₂O, 70:30 (v/v) supplemented with 0.1% formic acid. The system was controlled by Agilent LC/MSD Chemstation software.

3.2. 3\( \beta \)-Acetoxy-16-acetoxymethylideneandrost-5-en-17-one (2b)

Compound 2a (19.9 g, 63 mmol) was dissolved in a mixture of pyridine (40 mL) and Ac₂O (40 mL), and the solution was stirred overnight, and then poured onto a mixture of ice and \( \text{H}_2\text{SO}_4 \) (18 mL). The precipitate was collected by filtration, washed to neutrality and dried, resulting in 23.8 g (94%) of 2b, mp 199-202 °C (lit. [28] mp 198-200 °C), \( R_f = 0.68 \) (ss A).

3.3. 3\( \beta \)-Acetoxy-16\( \beta \)-hydroxymethylandrostan-5-en-17\( \beta \)-ol (3a)

Finely powdered 2b (23.8 g, 59.5 mmol) was suspended in a mixture of MeOH and EtOH (1:1, 500 mL), and KBH₄ (8 g, 148 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. After completion of the reaction, the mixture was diluted with water and acidified with dilute HCl. The precipitate that formed was filtered off and washed with water to neutrality. The resulting crude product was purified by column chromatography, with CH₂Cl₂/EtOAc (8:2) as eluent, yielding 3a as a white solid (10.35 g, 48%), mp 197-199 °C (lit. [28] mp 199-201 °C), \( R_f = 0.44 \) (ss C). The spectroscopic data were consistent with those reported in the literature.
3.4. $3\beta$-Acetoxy-16$\beta$-p-toluenesulfonyloxymethylandrost-5-en-17$\beta$-ol (4)

Compound 3a (7.25 g, 20 mmol) was dissolved in pyridine (50 mL), and a solution of $p$-toluene-sulfonyl chloride (7 g, 35 mmol) in pyridine (10 mL) was then added dropwise while cooling in ice. The reaction mixture was allowed to stand overnight, and was then poured into a mixture of ice and H$_2$SO$_4$ (20 mL). The precipitate that formed was filtered off and washed with water to neutrality. This substance was used in the subsequent step without further purification and characterization.

3.5. $3\beta$-Acetoxy-16$\beta$-azidomethylandrost-5-en-17$\beta$-ol (5)

Sodium azide (1.8 g, 28 mmol) was added to a solution of 4 (5.8 g, 11 mmol) in DMF (80 mL). The reaction mixture was stirred at 70 °C for 6 h and then poured into water. The precipitate that formed was allowed to stand overnight, and then filtered off and washed with water. Purification of the resulting crude product by column chromatography with CH$_2$Cl$_2$ as eluent afforded 5 as a white solid (3.75 g, 86%), mp 144-145 °C, $R_f = 0.58$ (ss A); $^1$H-NMR (CDCl$_3$); $\delta$ [ppm] = 0.78 (s, 3H, 18-CH$_3$), 0.95 (m, 1H), 1.03 (s, 3H, 19-CH$_3$), 1.08-1.17 (overlapping m, 3H), 1.45 (m, 1H), 1.51-1.62 (overlapping m, 5H), 1.82-1.90 (overlapping m, 4H), 1.99 (m, 1H), 2.03 (s, 3H, Ac-CH$_3$), 2.32 (m, 2H, 4-H$_2$), 2.38 (m, 1H, 16-H), 3.31 (dd, 1H, $J = 12.0$ Hz, $J = 6.5$ Hz, 16a-H), 3.57 (dd, 1H, $J = 12.0$ Hz, $J = 7.0$ Hz, 16a-H), 3.79 (d, 1H, $J = 10.0$ Hz, 17-H), 4.60 (m, 1H, 3-H), 5.37 (d, 1H, $J = 5.0$ Hz, 6-H); $^{13}$C-NMR (CDCl$_3$); $\delta$ [ppm] = 12.1 (C-18), 19.3 (C-19), 20.5 (C-11), 21.4 (Ac-CH$_3$), 27.7, 30.5, 31.1, 31.6, 36.6, 37.0, 37.4, 38.0, 39.9, 43.6, 49.9, 50.0, 53.3, 73.8 (C-3), 81.3 (C-17), 122.1 (C-6), 139.7 (C-5), 170.5 (Ac-CO); IR (neat, cm$^{-1}$) 3526, 2945, 2909, 2112, 1717, 1439, 1365, 1256, 1032. ESI-MS: 388 (M+H)$^+$; Anal. Calcd for C$_{22}$H$_{33}$N$_3$O$_3$ C, 68.19; H, 8.58; N, 10.84. Found: C, 68.01; H, 8.73; N, 11.04.

3.6. General Procedure for Preparation of $3\beta$-acetoxy-16$\beta$-(4-phenyl-, substituted 4-phenyl- or 4-cycloalkyl-1H-1,2,3-triazol-1-ylmethyl)androstan-5-en-17$\beta$-ols 6a-j

Compound 5 (387 mg, 1 mmol) was dissolved in CH$_2$Cl$_2$ (10 mL), and a solution of CuSO$_4$·5H$_2$O (12.5 mg, 5 mol %) and sodium ascorbate (30 mg, 15 mol %) in water (10 mL) was poured into the organic phase. The appropriate terminal alkyne (1.1 mmol) was added to the reaction mixture, which was then stirred for 1-4 h at ambient temperature. After the disappearance of the starting material (TLC monitoring), the two-phase solution was diluted with water (20 mL) and extracted with CH$_2$Cl$_2$ (2 × 20 mL). The combined organic layers were washed with water, dried over Na$_2$SO$_4$ and evaporated in vacuo. The resulting crude product was purified by flash chromatography with CH$_2$Cl$_2$/EtOAc (90:10), or CH$_2$Cl$_2$/EtOAc (80:20) as eluent.
2.74 (m, 1H, 16-H), 3.88 (d, 1H, J = 10.0 Hz, 17-H), 4.32 (dd, 1H, J = 13.5 Hz, J = 6.5 Hz, 16a-H), 4.58 (m, 1H, 3-H), 4.70 (dd, 1H, J = 13.5 Hz, J = 7.0 Hz, 16a-H), 5.34 (br s, 1H, 6-H), 7.32 (t, 1H, J = 7.0 Hz, 3'-H), 7.53 (br s, 6'-H), 7.82 (m, 3H, 2'-, 6'- and 5'-H); 13C-NMR (CDCl3); δ [ppm] = 12.3 (C-18), 19.3 (C-19), 20.5 (C-11), 21.4 (Ac-CH3), 27.7, 31.0, 31.1, 31.6, 36.6, 37.0, 37.3, 38.0, 41.3, 43.7, 49.9, 50.0, 52.0, 73.8 (C-3), 80.6 (C-17), 120.6 (C-5'), 122.0 (C-6), 125.7 (2C, C-2” and C-6”), 128.2 (C-4”), 128.8 (2C, C-3” and C-5”), 130.2 (C-1”), 139.7 (C-5), 147.2 (C-4’), 170.6 (Ac-CO); IR (neat, cm⁻¹) 3404, 2941, 2910, 1732, 1373, 1242, 1034, 772, 706. ESI-MS: 490 (M+H)⁺; Anal. Calcd for C30H39N3O3: C, 73.59; H, 8.03; N, 8.58. Found: C, 73.70; H, 8.19; N, 8.76.

3β-Acetoxy-16β-[4-(4-ethylphenyl)-1H-1,2,3-triazol-1-ylmethyl]androst-5-en-17β-ol (6b): Alkyne: 4-ethylphenylacetylene (0.15 mL). After purification, 6b was obtained as a white solid (470 mg, 91%), mp 249-250 °C, Rf = 0.36 (ss B); 1H-NMR (CDCl3); δ [ppm] = 0.79 (s, 3H, 18-CH3), 0.94 (m, 1H), 1.02 (s, 3H, 19-CH3), 1.09-1.21 (overlapping m, 3H), 1.25 (t, 3H, J = 7.5 Hz, Et-CH3), 1.41-1.62 (overlapping m, 6H), 1.79-1.92 (overlapping m, 4H), 1.95 (dd, 1H, J = 12.5 Hz, J = 2.5 Hz), 2.03 (s, 3H, Ac-CH3), 2.32 (m, 2H, 4-H2), 2.67 (q, 2H, J = 7.5 Hz, Et-CH2), 2.73 (m, 1H, 16-H), 3.87 (d, 1H, J = 10.0 Hz, 17-H), 4.30 (dd, 1H, J = 13.5 Hz, J = 6.5 Hz, 16a-H), 4.58 (m, 1H, 3-H), 4.69 (dd, 1H, J = 13.5 Hz, J = 7.0 Hz, 16a-H), 5.34 (br s, 1H, 6-H), 7.24 (d, 2H, J = 7.5 Hz, 3’- and 5’-H), 7.72 (d, 2H, J = 7.5 Hz, 2”- and 6”-H), 7.79 (s, 1H, 5’-H); 13C-NMR (CDCl3); δ [ppm] = 12.3 (C-18), 19.3 (C-19), 20.5 (C-11), 21.4 (Ac-CH3), 27.7, 28.6, 31.0, 31.1, 31.6, 36.6, 36.9, 37.0, 37.3, 38.0, 41.3, 43.7, 49.9, 50.0, 52.0, 73.8, 80.6 (C-17), 120.3 (C-5’), 122.1 (C-6), 125.7 (2C, C-2” and C-6”), 127.7 (C-1”), 128.3 (2C, C-3” and C-5”), 139.7 (C-5), 144.4 (C-4”), 147.3 (C-4’), 170.5 (Ac-CO); IR (neat, cm⁻¹) 3383, 2945, 2854, 1730, 1375, 1248, 1032, 976, 833, 613. ESI-MS: 518 (M+H)⁺; Anal. Calcd for C32H43N3O3: C, 74.24; H, 8.37; N, 8.12. Found: C, 74.38; H, 8.24; N, 8.19.

3β-Acetoxy-16β-[4-(3-tolyl)-1H-1,2,3-triazol-1-ylmethyl]androst-5-en-17β-ol (6c): Alkyne: 3-tolyl-acetylene (0.14 mL). After purification, 6c was obtained as a white solid (458 mg, 91%), mp 232-233 °C, Rf = 0.36 (ss B); 1H-NMR (CDCl3); δ [ppm] = 0.79 (s, 3H, 18-CH3), 0.94 (m, 1H), 1.02 (s, 3H, 19-CH3), 1.09-1.21 (overlapping m, 3H), 1.25 (t, 3H, J = 7.5 Hz, Et-CH3), 1.41-1.62 (overlapping m, 6H), 1.79-1.92 (overlapping m, 4H), 1.95 (dd, 1H, J = 12.5 Hz, J = 2.5 Hz), 2.03 (s, 3H, Ac-CH3), 2.32 (m, 2H, 4-H2), 2.38 (s, 3H, 3”-CH3), 2.72 (m, 1H, J = 10.0 Hz, 17-H), 3.87 (d, 1H, J = 13.5 Hz, J = 6.5 Hz, 16a-H), 4.58 (m, 1H, 3-H), 4.69 (dd, 1H, J = 14.0 Hz, J = 6.5 Hz, 16a-H), 5.33 (d, 1H, J = 4.0 Hz, 6-H), 7.13 (d, 1H, J = 7.0 Hz, 5”-H), 7.29 (t, 1H, J = 7.5 Hz, 5”-H), 7.57 (d, 1H, J = 7.5 Hz, 6”-H), 7.65 (s, 1H, 2”-H), 7.81 (s, 1H, 5’-H); 13C-NMR (CDCl3); δ [ppm] = 12.3 (C-18), 19.3 (C-19), 20.4 (C-11), 21.4 (Ac-CH3), 27.7, 29.6, 31.0, 31.1, 31.6, 36.6, 37.0, 37.3, 38.0, 41.3, 43.7, 49.8, 50.0, 52.0, 73.8 (C-3), 80.6 (C-17), 120.6 (C-5”), 122.0 (C-6), 122.7, 126.3, 128.7, 128.9: (4C, C-2”- and C-6”), 130.2 (C-1”), 138.5 (C-3”), 139.7 (C-5), 147.3 (C-4”), 170.6 (Ac-CO); IR (neat, cm⁻¹) 3406, 2922, 2850, 1731, 1364, 1244, 1024, 787, 696. ESI-MS: 518 (M+H)⁺; Anal. Calcd for C32H43N3O3: C, 74.80; H, 8.38; N, 8.49.

3β-Acetoxy-16β-[4-(4-tert-butylphenyl)-1H-1,2,3-triazol-1-ylmethyl]androst-5-en-17β-ol (6d): Alkyne: 4-tert-butylphenylacetylene (0.2 mL). After purification, 6d was obtained as a white solid (507 mg, 93%), mp 318-319 °C, Rf = 0.39 (ss B); 1H-NMR (CDCl3); δ [ppm] = 0.79 (s, 3H, 18-CH3), 0.88
3β-Acetoxy-16β-[4-(4-methoxyphenyl)-1H-1,2,3-triazol-1-ylmethyl]androst-5-en-17β-ol (6e): Alkyne: 4-methoxyphenylacetylene (145 mg). After purification, 6e was obtained as a white solid (447 mg, 86%), mp 241-242 °C, \( R_f = 0.34 \) (ss B); \(^1\)H-NMR (500 MHz, 10% MeOD/CDCl\(_3\)); \( \delta \) [ppm] = 0.81 (s, 3H, 18-CH\(_3\)), 0.93 (m, 1H), 1.03 (s, 3H, 19-CH\(_3\)), 0.93-1.21 (overlapping m, 3H), 1.33-1.62 (overlapping m, 6H), 1.77-1.90 (overlapping m, 4H), 1.97 (dd, 1H, \( J = 12.0 \) Hz, \( J = 2.5 \) Hz), 2.03 (s, 3H, Ac-CH\(_3\)), 2.32 (m, 2H, 4-H\(_2\)), 2.72 (m, 1H, 16-H), 3.82 (d, 1H, \( J = 10.0 \) Hz, 17-H), 3.86 (s, 3H, OCH\(_3\)), 4.28 (dd, 1H, \( J = 13.5 \) Hz, \( J = 8.0 \) Hz, 16a-H), 4.58 (m, 1H, 3-H), 4.70 (dd, 1H, \( J = 13.5 \) Hz, \( J = 6.0 \) Hz, 16a-H), 5.34 (br s, 1H, 6-H), 6.96 (d, 2H, \( J = 8.5 \) Hz, 3"- and 5"-H), 7.21 (d, 2H, \( J = 8.5 \) Hz, 2"- and 6"-H), 7.81 (s, 1H, 5'-H); IR (neat, cm\(^{-1}\)) 3398, 2935, 2902, 1728, 1499, 1373, 1244, 1063, 839, 538. ESI-MS: 520 (M+H\(^+\)); Anal. Calcd for C\(_{31}H\(_{41}\)N\(_3\)O\(_4\)): C, 71.65; H, 7.95; N, 8.09. Found: C, 71.74; H, 7.76; N, 8.22.

3β-Acetoxy-16β-[4-(2-methoxyphenyl)-1H-1,2,3-triazol-1-ylmethyl]androst-5-en-17β-ol (6f): Alkyne: 2-methoxyphenylacetylene (0.14 mL). After purification, 6f was obtained as a white solid (430 mg, 83%), mp 241-242 °C, \( R_f = 0.32 \) (ss B); \(^1\)H-NMR (500 MHz, 10% MeOD/CDCl\(_3\)); \( \delta \) [ppm] = 0.79 (s, 3H, 18-CH\(_3\)), 0.94 (m, 1H), 1.01 (s, 3H, 19-CH\(_3\)), 1.08-1.20 (overlapping m, 3H), 1.33-1.60 (overlapping m, 6H), 1.79-1.96 (overlapping m, 5H), 2.02 (s, 3H, Ac-CH\(_3\)), 2.31 (m, 2H, 4-H\(_2\)), 2.73 (m, 1H, 16-H), 3.86 (s, 3H, OCH\(_3\)), 4.28 (dd, 1H, \( J = 13.5 \) Hz, \( J = 8.0 \) Hz, 16a-H), 4.58 (m, 1H, 3-H), 4.70 (dd, 1H, \( J = 13.5 \) Hz, \( J = 6.0 \) Hz, 16a-H), 5.34 (br s, 1H, 6-H), 6.95 (d, 2H, \( J = 8.5 \) Hz, 3"- and 5"-H), 7.21 (d, 2H, \( J = 8.5 \) Hz, 2"- and 6"-H), 7.81 (s, 1H, 5'-H); IR (neat, cm\(^{-1}\)) 3398, 2935, 2902, 1728, 1499, 1373, 1244, 1063, 839, 538. ESI-MS: 520 (M+H\(^+\)); Anal. Calcd for C\(_{31}H\(_{41}\)N\(_3\)O\(_4\)): C, 71.65; H, 7.95; N, 8.09. Found: C, 71.76; H, 7.76; N, 8.22.

3β-Acetoxy-16β-[4-(4-fluorophenyl)-1H-1,2,3-triazol-1-ylmethyl]androst-5-en-17β-ol (6g): Alkyne: 4-fluorophenylacetylene (0.13 mL). After purification, 6g was obtained as a white solid (430 mg, 85%), mp 263-264 °C, \( R_f = 0.36 \) (ss B); \(^1\)H-NMR (10% MeOD/CDCl\(_3\)); \( \delta \) [ppm] = 0.82 (s, 3H,
18-CH₃), 0.96 (m, 1H), 1.03 (s, 3H, 19-CH₃), 1.10-1.22 (overlapping m, 3H), 1.40-1.63 (overlapping m, 6H), 1.78-1.91 (overlapping m, 4H), 1.97 (dd, 1H, J = 13.0 Hz, J = 2.5 Hz), 2.04 (s, 3H, Ac-CH₃), 2.32 (m, 2H, 4-H₂), 2.72 (m, 1H, 16-H), 3.84 (d, 1H, J = 10.0 Hz, 17-H), 4.30 (dd, 1H, J = 13.5 Hz, J = 8.0 Hz, 16a-H), 4.58 (m, 1H, 5'-H); 13C-NMR (10% MeOD/CDCl₃); δ [ppm] = 12.1 (C-18), 19.1 (C-19), 20.3 (C-11), 21.2 (Ac-CH₃), 27.5, 30.9, 31.0, 31.4, 36.5, 36.8, 37.1, 37.8, 38.0, 40.3, 43.2, 49.1, 49.6, 52.2, 52.4, 73.9 (C-3), 80.3 (C-3'); 115.7 (d, 2C, J = 21.5 Hz, C-3" and C-5"), 120.4 (C-5'), 122.0 (C-6), 126.5 (C-1"), 127.2 (d, 2C, J = 8 Hz, C-2" and C-6"), 139.5 (C-5), 146.4 (C-4'), 162.5 (d, J = 245 Hz, C-4") 170.9 (Ac-CO); IR (neat, cm⁻¹) 3412, 2945, 2912, 1730, 1460, 1243, 1062, 812, 524. ESI-MS: 508 (M+H)⁺; Anal. Calcd for C₃₀H₃₈FN₃O₃: C, 70.98; H, 7.55; N, 8.28. Found: C, 70.86; H, 7.64; N, 8.43.

3\(\beta\)-Acetoxy-16\(\beta\)-[4-(3-aminophenyl)-1H-1,2,3-triazol-1-ylmethyl]androst-5-en-17\(\beta\)-ol (6h): Alkyne: 3-aminophenylacetylene (0.12 mL). After purification, 6h was obtained as a white solid (454 mg, 90%), mp 255-256 °C, Rᵣ = 0.30 (ss C); 1H-NMR (DMSO-d₆); δ [ppm] = 0.76 (s, 3H, 18-CH₃), 0.91 (m, 2H), 0.98 (s, 3H, 19-CH₃), 1.02-1.15 (overlapping m, 3H), 1.38-1.56 (overlapping m, 6H), 1.74-1.90 (overlapping m, 4H), 1.97 (s, 3H, Ac-CH₃), 2.25 (m, 2H, 4-H₂), 2.64 (m, 1H, 16-H), 3.71 (d, 1H, J = 10.0 Hz, 17-H), 4.17 (t, 1H, J = 12.5 Hz, 16a-H), 4.43 (m, 1H, 3-H), 4.57 (dd, 1H, J = 13.5 Hz, J = 5.0 Hz, 16a-H), 5.02 (br s, 1H, OH), 5.30 (br s, 1H, 6-H), 5.66 (br s, 2H, NH₂), 6.56 (d, 1H, J = 7.0 Hz, 4"-H), 6.98 (d, 1H, J = 7.0 Hz, 6"-H), 7.08 (t, 1H, J = 7.5 Hz, 5"-H), 7.14 (s, 1H, 2"-H), 8.41 (s, 1H, 5'-H); 13C-NMR (DMSO-d₆); δ [ppm] = 12.4 (C-18), 19.0 (C-19), 20.1 (Ac-CH₃), 27.3, 30.4, 30.7, 31.0, 31.6, 36.4, 36.8, 37.6, 40.3, 43.2, 49.1, 49.6, 52.2, 73.1 (C-3), 79.4 (C-17), 110.9, 113.7, 113.9, 121.1, 121.9, 129.3, 131.4, 139.4, 146.5, 147.8, 169.7 (Ac-CO); IR (neat, cm⁻¹) 3395, 3228, 2941, 1732, 1454, 1242, 1069, 1034, 795. ESI-MS: 505 (M+H)⁺; Anal. Calcd for C₃₀H₄₀N₄O₃: C, 71.40; H, 7.99; N, 11.10. Found: C, 71.54; H, 7.86; N, 11.23.

3\(\beta\)-Acetoxy-16\(\beta\)-[4-(2-pyridyl)-1H-1,2,3-triazol-1-ylmethyl]androst-5-en-17\(\beta\)-ol (6i): Alkyne: 2-pyridylacetylene (0.11 mL). After purification, 6i was obtained as a white solid (427 mg, 87%), mp 259-260 °C, Rᵣ = 0.23 (ss C); 1H-NMR (CDCl₃); δ [ppm] = 0.80 (s, 3H, 18-CH₃), 0.93 (m, 1H), 1.01 (s, 3H, 19-CH₃), 1.16-1.27 (overlapping m, 3H), 1.39-1.59 (overlapping m, 6H), 1.77-1.87 (overlapping m, 4H), 1.94 (dd, 1H, J = 12.5 Hz, J = 2.5 Hz), 2.01 (s, 3H, Ac-CH₃), 2.30 (m, 2H, 4-H₂), 2.72 (m, 1H, 16-H), 3.87 (d, 1H, J = 10.0 Hz, 17-H), 4.34 (dd, 1H, J = 13.5 Hz, J = 7.5 Hz, 16a-H), 4.57 (m, 1H, 3-H), 4.75 (dd, 1H, J = 13.5 Hz, J = 6.5 Hz, 16a-H), 5.32 (d, 1H, J = 3.0 Hz, 6-H), 7.24 (m, 1H, 4"-H), 7.79 (t, 1H, J = 7.5 Hz, 5"-H), 8.17 (d, 1H, J = 7.5 Hz, 6"-H), 8.30 (s, 1H, 5'-H), 8.54 (d, 1H, J = 3.0 Hz, 3"-H); 13C-NMR (CDCl₃); δ [ppm] = 12.3 (C-19), 19.0 (C-18), 20.4 (C-17), 21.4 (Ac-CH₃), 27.7, 31.0, 31.1, 31.6, 36.6, 37.0, 37.3, 38.0, 41.3, 43.7, 49.8, 50.0, 52.4, 73.8 (C-3), 80.5 (C-17), 120.4 (C-5'), 122.1 (C-6), 122.8, 123.1, 137.4, 139.6 (C-5), 147.3 (C-2"), 148.8 (C-6"), 149.9 (C-4'), 170.5 (Ac-CO); IR (neat, cm⁻¹) 3395, 2932, 2911, 1731, 1435, 1364, 1240, 1032, 789, 540. ESI-MS: 491 (M+H)⁺; Anal. Calcd for C₂₉H₃₈N₄O₃: C, 70.99; H, 7.81; N, 11.42. Found: C, 71.16; H, 7.97; N, 11.61.
3β-Acetoxy-16β-(4-cyclopropyl-1H-1,2,3-triazol-1-ylmethyl)androst-5-en-17β-ol (6j): Alkyne: cyclopropylacetylene (0.09 mL). After purification, 6j was obtained as a white solid (355 mg, 78%), mp 261-263 °C, Rf = 0.30 (ss B); 1H-NMR (CDCl3): δ [ppm] = 0.76 (s, 3H, 18-CH3), 0.90-0.96 (overlapping m, 4H), 1.01 (s, 3H, 19-CH3), 1.08-1.17 (overlapping m, 3H), 1.41-1.61 (overlapping m, 5H), 1.76-1.95 (overlapping m, 5H), 2.02 (s, 3H, Ac-CH3), 2.31 (m, 2H, 4-H2), 2.63 (m, 1H, 16-H), 3.08 (br s, 1H, 1”-H), 3.84 (dd, 1H, J = 10.0 Hz, J = 3.5 Hz, 17-H), 4.19 (dd, 1H, J = 13.5 Hz, J = 6.5 Hz, 16a-H), 4.57-4.61 (overlapping m, 2H, 3- and 16a-H), 5.33 (d, 1H, J = 3.5 Hz, 6-H), 7.28 (s, 1H, 5’-H); 13C-NMR (CDCl3): δ [ppm] = 19.3 (C-19), 20.5 (C-11), 21.4 (Ac-CH3), 27.7, 31.0, 31.1, 31.6, 36.6, 37.0, 37.3, 38.0, 41.4, 43.7, 49.9, 50.0, 51.7, 73.8 (C-3), 80.5 (C-17), 120.5 (C-5’), 122.1 (C-6), 139.7 (C-5), 149.9 (C-4’), 170.5 (Ac-CO); IR (neat, cm−1) 3394, 2943, 1732, 1431, 1372, 1246, 1068, 1034, 814. ESI-MS: 454 (M+H)+; Anal. Calcd for C27H39N3O3: C, 71.49; H, 8.67; N, 9.26. Found: C, 71.61; H, 8.82; N, 9.57.

3.7. General procedure for preparation of 16β-(4-phenyl-, substituted 4-phenyl- or 4-cycloalkyl-1H-1,2,3-triazol-1-ylmethyl)androst-5-ene-3β,17β-diols 7a-j

Compounds 6a-j (0.5 mmol) were deacetylated by dissolving in MeOH (20 mL), adding KOH (150 mg, 2.7 mmol), stirring the mixture for 1 h at 50 °C, and then pouring into water (200 mL) and neutralizing with diluted HCl. The resulting precipitate was filtered off, washed with water and dried. The crude product obtained was purified by flash chromatography (silica gel) to afford 7a-j.

16β-(4-Phenyl-1H-1,2,3-triazol-1-ylmethyl)androst-5-ene-3β,17β-diol (7a): Eluent: CH2Cl2/EtOAc (75:25), yielding 7a as a white solid (184 mg, 82%), mp 264-265 °C, Rf = 0.45 (ss C); 1H-NMR (10% MeOD/CDCl3): δ [ppm] = 0.82 (s, 3H, 18-CH3), 0.94-0.98 (m, 2H), 1.02 (s, 3H, 19-CH3), 1.09-1.25 (overlapping m, 3H), 1.45-1.61 (overlapping m, 5H), 1.81-1.97 (overlapping m, 5H), 2.20-2.28 (m, 2H), 2.73 (m, 1H, 16-H), 3.47 (m, 1H, 3-H), 3.84 (d, 1H, J = 10.0 Hz, 17-H), 4.30 (dd, 1H, J = 13.5 Hz, J = 8.0 Hz, 16a-H), 4.72 (dd, 1H, J = 13.5 Hz, J = 6.0 Hz, 16a-H), 5.31 (br s, 1H, 6-H), 7.34 (t, 1H, J = 7.5 Hz, 4”-H), 7.43 (t, 2H, J = 7.5 Hz, 3”- and 5”-H), 7.79 (d, 2H, J = 7.5 Hz, 2”- and 6”-H), 7.89 (s, 1H, 5’-H); IR (neat, cm−1) 3428, 2944, 2904, 1444, 1236, 1080, 827, 760, 691. ESI-MS: 448 (M+H)+; Anal. Calcd for C28H37N3O2: C, 75.13; H, 8.33; N, 9.39. Found: C, 75.27; H, 8.21; N, 9.56.

16β-[4-(4-Ethylphenyl)-1H-1,2,3-triazol-1-ylmethyl]androst-5-ene-3β,17β-diol (7b): Eluent: CH2Cl2/EtOAc (75:25), yielding 7b as a white solid (192 mg, 81%), mp 261-262 °C, Rf = 0.45 (ss C); 1H-NMR (DMSO-d6): δ [ppm] = 0.75 (s, 3H, 18-CH3), 0.83-0.88 (m, 2H), 0.94 (s, 3H, 19-CH3), 1.03-1.12 (overlapping m, 3H), 1.19 (t, 3H, J = 7.5 Hz, Et-CH3), 1.30-1.44 (overlapping m, 4H), 1.52 (m, 2H), 1.66 (m, 1H), 1.74-1.86 (overlapping m, 3H), 2.05-2.14 (m, 2H), 2.59 (q, 2H, J = 7.5 Hz, Et-CH3), 2.65 (m, 1H, 16-H), 3.24 (m, 1H, 3-H), 3.71 (dd, 1H, J = 9.5 Hz, J = 3.5 Hz, 17-H), 4.18 (m, 1H, 16a-H), 4.58 (overlapping m, 2H, 3-OH and 16a-H), 5.01 (d, 1H, J = 3.5 Hz, 17-OH), 5.21 (br s, 1H, 6-H), 7.26 (d, 2H, J = 7.5 Hz, 3”- and 5”-H), 7.74 (d, 2H, J = 7.5 Hz, 2”- and 6”-H), 8.51 (s, 1H, 5’-H); IR (neat, cm−1) 3383, 2943, 1444, 1240, 1082, 1051, 812, 738, 644. ESI-MS: 476 (M+H)+; Anal. Calcd for C30H41N3O2: C, 75.13; H, 8.33; N, 9.39. Found: C, 75.27; H, 8.21; N, 9.56.
16β-[4-(3-Tolyl)-1H-1,2,3-triazol-1-ylmethyl]androst-5-ene-3β,17β-diol (7c): Eluent: CH₂Cl₂/EtOAc (75:25), yielding 7c as a white solid (203 mg, 88%), mp 237-238 °C, Rf = 0.44 (ss C); ¹H-NMR (DMSO-d₆): δ [ppm] = 0.76 (s, 3H, 18-CH₃), 0.86 (m, 2H), 0.94 (s, 3H, 19-CH₃), 1.04-1.17 (overlapping m, 3H), 1.31-1.43 (overlapping m, 4H), 1.51 (m, 2H), 1.65 (m, 1H), 1.73-1.87 (overlapping m, 3H), 2.07-2.15 (m, 2H), 2.34 (s, 3H, 3''-CH₃), 2.64 (m, 1H, 16-H), 3.24 (m, 1H, 16a-H), 5.00 (d, 1H, J = 4.0 Hz, 17-OH), 7.12 (d, 1H, J = 7.0 Hz, 6'-H), 7.62 (d, 1H, J = 7.0 Hz, 6''-H), 7.66 (s, 1H, 5'-H); IR (neat, cm⁻¹) 3339, 3237, 2931, 1452, 1232, 1049, 787, 696. ESI-MS: 462 (M+H)⁺; Anal. Calcd for C₂₉H₃₉N₃O₂: C, 75.45; H, 8.52; N, 9.10. Found: C, 75.57; H, 8.67; N, 9.32.

16β-[4-(4-Tert-butylphenyl)-1H-1,2,3-triazol-1-ylmethyl]androst-5-ene-3β,17β-diol (7d): Eluent: CH₂Cl₂/EtOAc (75:25), yielding 7d as a white solid (214 mg, 85%), mp 284-285 °C, Rf = 0.49 (ss C); ¹H-NMR (10% MeOD/CDCl₃): δ [ppm] = 0.75 (s, 3H, 18-CH₃), 0.85-0.89 (m, 2H), 0.94 (s, 3H, 19-CH₃), 1.27 (s, 9H, 3 x tBu-CH₃), 0.99-1.13 (overlapping m, 3H), 1.40-1.53 (overlapping m, 5H), 1.70-1.89 (overlapping m, 5H), 2.14-2.21 (m, 2H), 2.66 (m, 1H, 16-H), 3.40 (m, 1H, 3-H), 3.76 (d, 1H, J = 10.0 Hz, 17-H), 4.21 (dd, 1H, J = 14.0 Hz, J = 8.5 Hz, 16a-H), 4.64 (dd, 1H, J = 13.5 Hz, J = 6.0 Hz, 16a-H), 5.24 (br s, 1H, 6-H), 7.38 (d, 2H, J = 8.0 Hz, 3''- and 5''-H), 7.64 (d, 2H, J = 8.0 Hz, 2''- and 6''-H), 7.79 (s, 1H, 5'-H); IR (neat, cm⁻¹) 3477, 2949, 1460, 1215, 1070, 1047, 818, 559. ESI-MS: 504 (M+H)⁺; Anal. Calcd for C₃₂H₄₅N₃O₂: C, 76.30; H, 9.00; N, 8.34. Found: C, 76.17; H, 8.82; N, 8.56.

16β-[4-(4-Methoxyphenyl)-1H-1,2,3-triazol-1-ylmethyl]androst-5-ene-3β,17β-diol (7e): Eluent: CH₂Cl₂/EtOAc (70:30), yielding 7e as a white solid (205 mg, 86%), mp 262-264 °C, Rf = 0.39 (ss C); ¹H-NMR (DMSO-d₆): δ [ppm] = 0.76 (s, 3H, 18-CH₃), 0.86-0.90 (m, 2H), 0.95 (s, 3H, 19-CH₃), 1.03-1.18 (overlapping m, 3H), 1.33-1.43 (overlapping m, 4H), 1.52 (m, 2H), 1.66 (m, 1H), 1.75-1.87 (overlapping m, 3H), 2.08-2.14 (m, 2H), 2.64 (m, 1H, 16-H), 3.24 (m, 1H, 3-H), 3.71 (dd, 1H, J = 9.5 Hz, J = 3.0 Hz, 17-H), 3.79 (s, 3H, 4''-OCH₃), 4.17 (m, 1H, 16a-H), 4.58 (overlapping m, 2H, 3-OH and 16a-H), 4.98 (d, 1H, J = 3.0 Hz, 17-OH), 5.21 (br s, 1H, 6-H), 7.00 (d, 2H, J = 8.5 Hz, 3''- and 5''-H), 7.75 (d, 2H, J = 8.5 Hz, 2''- and 6''-H), 8.44 (s, 1H, 5'-H); IR (neat, cm⁻¹) 3454, 3206, 2930, 1499, 1250, 1068, 1028, 833, 667. ESI-MS: 478 (M+H)⁺; Anal. Calcd for C₂₉H₃₉N₃O₃: C, 72.92; H, 8.23; N, 8.80. Found: C, 73.11; H, 8.05; N, 8.97.

16β-[4-(2-Methoxyphenyl)-1H-1,2,3-triazol-1-ylmethyl]androst-5-ene-3β,17β-diol (7f): Eluent: CH₂Cl₂/EtOAc (70:30), yielding 7f as a white solid (208 mg, 87%), mp 219-220 °C, Rf = 0.46 (ss C); ¹H-NMR (DMSO-d₆): δ [ppm] = 0.76 (s, 3H, 18-CH₃), 0.86 (m, 2H), 0.94 (s, 3H, 19-CH₃), 1.02-1.15 (overlapping m, 3H), 1.32-1.41 (overlapping m, 4H), 1.50 (m, 2H), 1.66 (m, 1H), 1.74-1.85 (overlapping m, 3H), 2.09-2.13 (m, 2H), 2.65 (m, 1H, 16-H), 3.24 (m, 1H, 3-H), 3.70 (dd, 1H, J = 9.5 Hz, J = 3.0 Hz, 17-H), 3.90 (s, 3H, 2''-OCH₃), 4.22 (m, 1H, 16a-H), 4.59 (overlapping m, 2H, 3-OH and 16a-H), 4.97 (d, 1H, J = 3.0 Hz, 17-OH), 5.20 (br s, 1H, 6-H), 7.03 (t, 1H, J = 7.0 Hz, 5''-H), 7.10 (d, 1H, J = 8.0 Hz, 3''-H), 7.31 (t, 1H, J = 7.0 Hz, 4''-H), 8.13 (d, 1H, J = 7.5 Hz, 6''-H), 8.39 (s, 1H, 5'-H); IR (neat, cm⁻¹) 3408, 3252, 2941, 1489, 1246, 1045, 1020, 752. ESI-MS: 478 (M+H)⁺; Anal. Calcd for C₂₉H₃₉N₃O₃: C, 72.92; H, 8.23; N, 8.80. Found: C, 73.10; H, 8.39; N, 8.59.
16β-[4-(4-Fluorophenyl)-1H-1,2,3-triazol-1-ylmethyl]androst-5-ene-3β,17β-diol (7g): Eluent: CH₂Cl₂/EtOAc (70:30), yielding 7g as a white solid (212 mg, 91%), mp 282-283 °C, Rᵣ = 0.43 (ss C); ^1H-NMR (DMSO-ᴅₒ); δ [ppm] = 0.76 (s, 3H, 18-CH₃), 0.87 (m, 2H), 0.95 (s, 3H, 19-CH₃), 1.04-1.14 (overlapping m, 3H), 1.30-1.43 (overlapping m, 4H), 1.53 (m, 2H), 1.64 (m, 1H), 1.75-1.87 (overlapping m, 3H), 2.07-2.14 (m, 2H), 2.63 (m, 1H, 16-H), 3.24 (m, 1H, 3-H), 3.71 (d, 1H, J = 9.5 Hz, 17-H), 4.18 (m, 1H, 16a-H), 4.60 (m, 1H, 16a-H), 5.22 (br s, 1H, 6-H), 7.27 (t, 2H, J = 8.0 Hz, 3"- and 5"-H), 7.86 (t, 2H, J = 8.0 Hz, 2"- and 6"-H), 8.57 (s, 1H, 5'-H); IR (neat, cm⁻¹) 3426, 2941, 1558, 1495, 1231, 1051, 817, 607. ESI-MS: 466 (M+H⁺); Anal. Calcd for C₂₈H₃₆FN₃O₂: C, 72.23; H, 7.79; N, 9.02. Found: C, 72.44; H, 7.66; N, 8.84.

16β-[4-(3-Aminophenyl)-1H-1,2,3-triazol-1-ylmethyl]androst-5-ene-3β,17β-diol (7h): Eluent: CH₂Cl₂/EtOAc (50:50), yielding 7h as a white solid (190 mg, 82%), mp 227-228 °C, Rᵣ = 0.22 (ss C); ^1H-NMR (DMSO-ᴅ₆); δ [ppm] = 0.76 (s, 3H, 18-CH₃), 0.89 (m, 2H), 0.95 (s, 3H, 19-CH₃), 1.05-1.13 (overlapping m, 3H), 1.34-1.52 (overlapping m, 6H), 1.66 (m, 1H), 1.76-1.89 (overlapping m, 3H), 2.07-2.12 (m, 2H), 2.63 (m, 1H, 16-H), 3.24 (m, 1H, 3-H), 3.70 (dd, 1H, J = 9.5 Hz, J = 3.0 Hz, 17-H), 4.18 (m, 1H, 16a-H), 4.57 (overlapping m, 2H, 3-OH and 16a-H), 4.98 (d, 1H, J = 3.0 Hz, 17-OH), 5.13 (br s, 1H, NH₂), 5.21 (br s, 1H, NH₂), 6.51 (d, 1H, J = 7.0 Hz, 4"-H), 6.92 (d, 1H, J = 7.0 Hz, 6"-H), 7.05 (t, 1H, J = 7.0 Hz, 5"-H), 7.08 (s, 1H, 2"-H), 8.39 (s, 1H, 5'-H); IR (neat, cm⁻¹) 3558, 3373, 2936, 1585, 1439, 1066, 790, 586. ESI-MS: 463 (M+H⁺); Anal. Calcd for C₂₈H₃₈N₄O₂: C, 72.69; H, 8.28; N, 12.11. Found: C, 72.86; H, 8.45; N, 12.39.

16β-[4-(2-Pyridyl)-1H-1,2,3-triazol-1-ylmethyl]androst-5-ene-3β,17β-diol (7i): Eluent: CH₂Cl₂/EtOAc (50:50), yielding 7i as a white solid (202 mg, 90%), mp 240-241 °C, Rᵣ = 0.26 (ss C); ^1H-NMR (10% MeOD/CDCl₃); δ [ppm] = 0.76 (s, 3H, 18-CH₃), 0.85 (m, 2H), 0.95 (s, 3H, 19-CH₃), 1.04-1.14 (overlapping m, 3H), 1.37-1.53 (overlapping m, 6H), 1.60 (m, 1H), 1.79-1.91 (overlapping m, 3H), 2.13-2.20 (m, 2H), 2.64 (m, 1H, 16-H), 3.41 (m, 1H, 3-H), 3.76 (d, 1H, J = 10.0 Hz, 17-H), 4.27 (dd, 1H, J = 13.5 Hz, J = 8.0 Hz, 16a-H), 4.67 (dd, 1H, J = 13.5 Hz, J = 6.0 Hz, 16a-H), 5.24 (br s, 1H, 6-H), 7.21 (m, 1H, 4"-H), 7.76 (t, 1H, J = 7.5 Hz, 5"-H), 8.09 (d, 1H, J = 7.5 Hz, 6"-H), 8.22 (s, 1H, 5'-H), 8.46 (d, 1H, J = 3.0 Hz, 3"-H); IR (neat, cm⁻¹) 3331, 2929, 1599, 1460, 1263, 1072, 787, 577. ESI-MS: 449 (M+H⁺); Anal. Calcd for C₂₇H₃₆N₄O₂: C, 72.69; H, 8.28; N, 12.11. Found: C, 72.86; H, 8.45; N, 12.39.

16β-[4-(2-Cyclopropyl-1H-1,2,3-triazol-1-ylmethyl)androst-5-ene-3β,17β-diol (7j): Eluent: CH₂Cl₂/EtOAc (75:25), yielding 7j as a white solid (170 mg, 83%), mp 235-236 °C, Rᵣ = 0.47 (ss C); ^1H-NMR (DMSO-ᴅₒ); δ [ppm] = 0.67 (m, 2H), 0.72 (s, 3H, 18-CH₃), 0.81-0.89 (overlapping m, 4H), 0.94 (s, 3H, 19-CH₃), 1.02-1.13 (overlapping m, 3H), 1.31-1.53 (overlapping m, 6H), 1.66 (m, 1H), 1.77-1.91 (overlapping m, 3H), 2.08-2.14 (m, 2H), 2.57 (m, 1H, 16-H), 3.24 (m, 1H, 3-H), 3.67 (dd, 1H, J = 9.5 Hz, J = 3.5 Hz, 17-H), 4.05 (m, 1H, 16a-H), 4.46 (m, 1H, 16a-H), 4.61 (br s, 1H, 3-OH), 4.95 (d, 1H, J = 3.5 Hz, 17-OH), 5.23 (br s, 1H, 6-H), 7.79 (s, 1H, 5'-H); IR (neat, cm⁻¹) 3401, 3251, 2937, 1433, 1219, 1049, 808, 588. ESI-MS: 412 (M+H⁺); Anal. Calcd for C₂₅H₃₇N₃O₂: C, 72.95; H, 9.06; N, 10.21. Found: C, 73.19; H, 9.27; N, 10.36.
3.8. General procedure for preparation of 3β-acetoxy-16β-(5-substituted-1H-tetrazol-1-ylmethyl)-androst-5-en-17β-ols 9a-e

Compound 5 (387 mg, 1 mmol) was dissolved in CH₂Cl₂ (5 mL), and Cu₂(OTf)₂·C₆H₆ (50 mg, 10 mol %) was added as catalyst. The appropriate nitrile (1.1 mmol) was added to the reaction mixture, which was then stirred for 48 h at ambient temperature. The progress of the reactions was monitored by TLC, and the solvent was then evaporated in vacuo. The resulting crude product was purified by flash chromatography with CH₂Cl₂/EtOAc (95:5) as eluent.

3β-Acetoxy-16β-(5-methoxycarbonyl-1H-tetrazol-1-ylmethyl)androst-5-en-17β-ol (9a): Nitrile: methyl cyanoformate (8a, 0.09 mL) was added to the mixture. After purification, 9a was obtained as a white solid (310 mg, 66%), mp 168-171 °C, Rᶠ = 0.47 (ss B); ¹H-NMR (CDCl₃); δ [ppm] = 0.85 (s, 3H, 18-CH₃), 0.90-0.97 (overlapping m, 2H), 1.02 (s, 3H, 19-CH₃), 1.09-1.22 (overlapping m, 3H), 1.44-1.58 (overlapping m, 5H), 1.71 (m, 1H), 1.83-1.86 (overlapping m, 3H), 1.94 (m, 1H), 2.01 (s, 3H, Ac-CH₃), 2.30 (m, 2H, 4-H₂), 2.86 (m, 1H, 16-H), 3.84 (d, 1H, J = 9.5 Hz, 17-H), 4.57 (m, 1H, 3-H), 4.66 (dd, 1H, J = 13.5 Hz, J = 8.0 Hz, 16a-H), 5.04 (dd, 1H, J = 13.5 Hz, J = 7.0 Hz, 16a-H), 5.34 (d, 1H, J = 4.0 Hz, 6-H); ¹³C-NMR (CDCl₃); δ [ppm] = 11.8 (C-18), 18.9 (C-19), 20.1 (C-11), 21.0 (Ac-CH₃), 27.3, 30.0, 30.8, 31.1, 36.2, 36.6, 36.7, 37.6, 39.8, 43.4, 49.4, 49.6, 51.0, 53.3 (OCH₃), 73.4 (C-3), 80.5 (C-17), 121.6 (C-6), 139.4 (C-5), 145.4 (C-5’), 156.8, 170.2 (Ac-CO); IR (neat, cm⁻¹) 3501, 2934, 1742, 1703, 1427, 1254, 1032, 826, 691. ESI-MS: 473 (M+H)⁺; Anal. Calcd for C₂₅H₃₆N₄O₅: C, 63.54; H, 7.68; N, 11.86. Found: C, 63.75; H, 7.57; N, 12.03.

3β-Acetoxy-16β-(5-ethoxycarbonyl-1H-tetrazol-1-ylmethyl)androst-5-en-17β-ol (9b): Nitrile: ethyl cyanoformate (8b, 0.11 mL) was added to the mixture. After purification, 9b was obtained as a white solid (350 mg, 72%), mp 172-174 °C, Rᶠ = 0.59 (ss B); ¹H-NMR (CDCl₃); δ [ppm] = 0.84 (s, 3H, 18-CH₃), 0.91-0.96 (overlapping m, 2H), 1.01 (s, 3H, 19-CH₃), 1.08-1.15 (overlapping m, 3H), 1.21 (m, 1H), 1.45 (t, 3H, OEt-CH₃), 1.47-1.58 (overlapping m, 5H), 1.70 (m, 1H), 1.82-1.86 (overlapping m, 3H), 1.92 (m, 1H), 2.01 (s, 3H, Ac-CH₃), 2.30 (m, 2H, 4-H₂), 2.85 (m, 1H, 16-H), 3.84 (d, 1H, J = 9.5 Hz, 17-H), 4.51 (q, 2H, OEt-CH₂), 4.56 (m, 1H, 3-H), 4.66 (dd, 1H, J = 13.5 Hz, J = 7.0 Hz, 16a-H), 5.03 (dd, 1H, J = 13.5 Hz, J = 7.0 Hz, 16a-H), 5.33 (d, 1H, J = 4.0 Hz, 6-H); ¹³C-NMR (CDCl₃); δ [ppm] = 12.2 (C-18), 14.0, 19.3 (C-19), 20.4 (C-11), 21.4 (Ac-CH₃), 27.6, 30.3, 31.1, 31.5, 36.6, 36.9, 37.1, 38.0, 40.2, 43.8, 49.7, 50.0, 51.3, 63.4, 73.7 (C-3), 80.8 (C-17), 121.9 (C-6), 139.7 (C-5), 145.9 (C-5’), 156.8, 170.5 (Ac-CO); IR (neat, cm⁻¹) 3428, 3249, 1743, 1721, 1470, 1200, 1000, 854. ESI-MS: 487 (M+H)⁺; Anal. Calcd for C₂₆H₃₈N₄O₅: C, 64.18; H, 7.87; N, 11.51. Found: C, 64.32; H, 7.67; N, 11.66.

3β-Acetoxy-16β-(5-benzyloxycarbonyl-1H-tetrazol-1-ylmethyl)androst-5-en-17β-ol (9c): Nitrile: benzyl cyanoformate (8c, 0.16 mL) was added to the mixture. After purification, 9c was obtained as a white solid (340 mg, 62%), mp 153-156 °C, Rᶠ = 0.21 (ss A); ¹H-NMR (CDCl₃); δ [ppm] = 0.78 (s, 3H, 18-CH₃), 0.88-0.95 (overlapping m, 2H), 1.02 (s, 3H, 19-CH₃), 1.08-1.15 (overlapping m, 3H), 1.43-1.57 (overlapping m, 5H), 1.66 (m, 1H), 1.78-1.86 (overlapping m, 3H), 1.96 (m, 1H), 2.01 (s, 3H, Ac-CH₃), 2.31 (m, 2H, 4-H₂), 2.79 (m, 1H, 16-H), 3.76 (d, 1H, J = 9.5 Hz, 17-H), 4.59-4.63 (overlapping m, 2H, 3- and 16a-H), 5.00 (dd, 1H, J = 13.5 Hz, J = 7.0 Hz, 16a-H), 5.33 (d, 1H, J = 3.0
Hz, 6-H), 5.46 (dd, 2H, J = 21.5 Hz, J = 12.0 Hz, OCH2Ph), 7.37 (m, 3H, 3"-, 4"- and 5"-H), 7.46 (d, 2H, J = 7.0 Hz, 2"- and 6"-H); 13C-NMR (CDCl3); δ [ppm] = 12.1 (C-18), 19.3 (C-19), 20.4 (C-11), 21.4 (Ac-CH3), 27.6, 30.2, 31.1, 31.5, 36.6, 36.9, 37.1, 38.0, 40.2, 43.7, 49.7, 50.0, 51.3, 68.7, 73.7 (C-3), 80.8 (C-17), 122.0 (C-6), 128.7 (2C), 128.9 (2C), 129.0 (C-4"'), 134.0 (C-1"'), 139.7 (C-5), 145.9 (C-5'), 156.6, 170.5 (Ac-CO); IR (neat, cm\(^{-1}\)) 3525, 2945, 1733, 1703, 1454, 1256, 1026, 748, 697. ESI-MS: 549 (M+H)+; Anal. Calcd for C31H40N4O5: C, 67.86; H, 7.35; N, 10.21. Found: C, 67.98; H, 7.52; N, 10.12

3β-Acetoxy-16β-(5-acetyl-1H-tetrazol-1-ylmethyl)androst-5-en-17β-ol (9d): Nitrile: acetyl cyanide (8d, 0.08 mL) was added to the mixture. After purification, 9d was obtained as a white solid (260 mg, 57%), mp 191-193 °C, Rf = 0.33 (ss A); 1H-NMR (CDCl3); δ [ppm] = 0.82 (s, 3H, 18-CH3), 0.91-0.97 (overlapping m, 2H), 1.01 (s, 3H, 19-CH3), 1.09-1.19 (overlapping m, 3H), 1.44-1.59 (overlapping m, 5H), 1.72 (m, 1H), 1.81-1.87 (overlapping m, 3H), 1.95 (m, 1H), 2.01 (s, 3H, Ac-CH3), 2.31 (m, 2H, 4-H2), 2.53 (s, 3H, 5'Ac-CH3), 2.84 (m, 1H, 16-H), 3.81 (d, 1H, J = 9.5 Hz, 17-H), 4.56 (m, 1H, 3-H), 4.64 (dd, 1H, J = 13.5 Hz, J = 7.5 Hz, 16a-H), 5.02 (dd, 1H, J = 13.5 Hz, J = 7.0 Hz, 16a-H), 5.33 (d, 1H, J = 3.5 Hz, 6-H); 13C-NMR (CDCl3); δ [ppm] = 11.9 (C-18), 19.1 (C-19), 20.2 (C-11), 20.6, 21.0 (Ac-CH3), 27.4, 30.2, 30.9, 31.3, 36.4, 36.8, 37.0, 37.8, 39.9, 43.6, 49.5, 49.9, 51.2, 73.5 (C-3), 80.7 (C-17), 121.7 (C-6), 139.6 (C-5), 147.7 (C-5'), 170.4 (Ac-CO), 190.4; IR (neat, cm\(^{-1}\)) 3512, 2931, 1740, 1709, 1486, 1259, 896, 682. ESI-MS: 457 (M+H)+; Anal. Calcd for C25H36N4O4: C, 65.76; H, 7.95; N, 12.27. Found: C, 65.61; H, 8.06; N, 12.51.

3β-Acetoxy-16β-(5-benzoyl-1H-tetrazol-1-ylmethyl)androst-5-en-17β-ol (9e): Nitrile: benzoyl cyanide (8e, 145 mg) was added to the mixture. After purification, 9e was obtained as a white solid (280 mg, 54%), mp 182-185 °C, Rf = 0.30 (ss A); 1H-NMR (CDCl3); δ [ppm] = 0.81 (s, 3H, 18-CH3), 0.88-0.95 (overlapping m, 2H), 1.02 (s, 3H, 19-CH3), 1.09-1.12 (overlapping m, 2H), 1.23 (m, 1H), 1.43-1.61 (overlapping m, 5H), 1.73-1.86 (overlapping m, 4H), 1.93 (m, 1H), 2.01 (s, 3H, Ac-CH3), 2.30 (m, 2H, 4-H2), 2.88 (m, 1H, 16-H), 3.82 (d, 1H, J = 9.5 Hz, 17-H), 4.57 (m, 1H, 3-H), 4.64 (dd, 1H, J = 13.5 Hz, J = 7.5 Hz, 16a-H), 5.03 (dd, 1H, J = 13.5 Hz, J = 7.0 Hz, 16a-H), 5.33 (d, 1H, J = 4.0 Hz, 6-H), 7.54 (t, 2H, J = 7.5 Hz, 3"- and 5"-H), 7.69 (t, 1H, J = 7.5 Hz, 4"-H), 8.33 (d, 2H, J = 7.5 Hz, 2"- and 6"-H); 13C-NMR (CDCl3); δ [ppm] = 11.8 (C-18), 18.9 (C-19), 20.1 (C-11), 21.0 (Ac-CH3), 27.3, 30.0, 30.7, 31.1, 36.2, 36.6, 36.8, 37.6, 39.9, 43.4, 49.4, 49.6, 50.8, 73.4 (C-3), 80.7 (C-17), 121.6 (C-6), 128.4 (2C), 130.6 (2C), 134.5 (C-4"'), 134.7 (C-1¨'), 139.3 (C-5), 149.5 (C-5'), 170.2 (Ac-CO), 181.8; IR (neat, cm\(^{-1}\)) 3533, 2938, 1728, 1702, 1595, 1265, 1026, 921, 692. ESI-MS: 519 (M+H)+; Anal. Calcd for C30H38N4O4: C, 69.63; H, 7.21; N, 10.91.

3.9 16β-Azidomethylandrost-5-ene-3β,17β-diol (10)

Compound 5 (1.94 g, 5 mmol) was dissolved in MeOH (80 mL), and KOH (750 mg, 13.5 mmol) was added. The mixture was stirred for 1 h at room temperature, and then poured into water (800 mL) and neutralized with diluted HCl. The resulting precipitate was filtered off, washed with water and dried. The crude product obtained was purified by flash chromatography to afford 10 as a white solid (1.43 g, 83%), mp 154-157 °C, Rf = 0.19 (ss A); 1H-NMR (CDCl3); δ [ppm] = 0.78 (s, 3H, 18-CH3), 0.93 (m, 1H), 1.02 (s, 3H, 19-CH3), 1.05-1.15 (overlapping m, 3H), 1.42-1.60 (overlapping m, 6H),
1.84-1.89 (overlapping m, 4H), 1.99 (m, 1H), 2.25-2.32 (m, 2H, 4-H2), 2.37 (m, 1H, 16-H), 3.29 (dd, 1H, J = 12.0 Hz, J = 6.5 Hz, 16a-H), 3.52 (m, 1H, 16-H), 3.57 (dd, 1H, J = 12.0 Hz, J = 6.5 Hz, 16a-H), 3.78 (dd, 1H, J = 9.5 Hz, J = 5.0 Hz, 17-H), 5.34 (d, 1H, J = 4.5 Hz, 6-H); 13C-NMR (CDCl3); δ [ppm] = 12.1 (C-18), 19.4 (C-19), 20.6 (C-11), 30.6, 31.2, 31.6, 31.7, 36.6, 37.2, 37.5, 39.9, 42.2, 43.6, 50.0, 50.1, 53.3, 71.6 (C-3), 81.3 (C-17), 121.2 (C-6), 140.9 (C-5); IR (neat, cm−1) 3519, 2941, 2904, 2115, 1452, 1374, 1246, 1028. ESI-MS: 346 (M+H)+; Anal. Calcd for C20H31N3O2: C, 69.53; H, 9.04; N, 12.16. Found: C, 69.38; H, 9.16; N, 12.35.

3.10. General procedure for preparation of 16β-(5-substituted-1H-tetrazol-1-ylmethyl)androst-5-ene-3β,17β-diols 11a-e

Compound 10 (345 mg, 1 mmol) was dissolved in CH2Cl2 (5 mL), and Cu2(OTf)2·C6H6 (50 mg, 10 mol %) was added as catalyst. The appropriate nitrile (1.1 mmol) was added to the reaction mixture, which was then stirred for 48 h at ambient temperature. The progress of the reactions was monitored by TLC, and the solvent was then evaporated in vacuo. The resulting crude product was purified by flash chromatography with CH2Cl2/EtOAc (85:15) as eluent.

16β-(5-Methoxycarbonyl-1H-tetrazol-1-ylmethyl)androst-5-ene-3β,17β-diol (11a): Nitrile: methyl cyanoformate (8a, 0.09 mL) was added to the mixture. After purification, 11a was obtained as a white solid (255 mg, 59%), mp 183-185 °C, Rf = 0.19 (ss B); 1H-NMR (CDCl3); δ [ppm] = 0.86 (s, 3H, 18-CH3), 0.92-0.96 (overlapping m, 2H), 1.02 (s, 3H, 19-CH3), 1.07-1.14 (overlapping m, 3H), 1.46-1.58 (overlapping m, 5H), 1.73 (m, 1H), 1.83-1.87 (overlapping m, 3H), 1.95 (m, 1H), 2.25-2.31 (overlapping m, 2H), 2.87 (m, 1H, 16-H), 3.51 (m, 1H, 3-H), 3.85 (dd, 1H, J = 9.5 Hz, J = 3.5 Hz, 17-H), 4.06 (s, 3H, OCH3), 4.67 (dd, 1H, J = 13.5 Hz, J = 8.0 Hz, 16a-H), 5.04 (dd, 1H, J = 13.5 Hz, J = 7.0 Hz, 16a-H), 5.33 (d, 1H, J = 4.5 Hz, 6-H); ESI-MS: 431 (M+H)+; Anal. Calcd for C23H34N4O4: C, 64.16; H, 7.96; N, 13.01. Found: C, 64.43; H, 7.80; N, 13.19.

16β-(5-Ethoxycarbonyl-1H-tetrazol-1-ylmethyl)androst-5-ene-3β,17β-diol (11b): Nitrile: ethyl cyanoformate (8b, 0.11 mL) was added to the mixture. After purification, 11b was obtained as a white solid (285 mg, 64%), mp 176-179 °C, Rf = 0.27 (ss B); 1H-NMR (CDCl3); δ [ppm] = 0.86 (s, 3H, 18-CH3), 0.92-0.96 (overlapping m, 2H), 1.02 (s, 3H, 19-CH3), 1.07-1.14 (overlapping m, 3H), 1.46-1.58 (overlapping m, 5H), 1.73 (m, 1H), 1.83-1.87 (overlapping m, 3H), 1.95 (m, 1H), 2.25-2.31 (overlapping m, 2H), 2.87 (m, 1H, 16-H), 3.51 (m, 1H, 3-H), 3.85 (dd, 1H, J = 9.5 Hz, J = 3.5 Hz, 17-H), 4.06 (s, 3H, OCH3), 4.67 (dd, 1H, J = 13.5 Hz, J = 8.0 Hz, 16a-H), 5.04 (dd, 1H, J = 13.5 Hz, J = 7.0 Hz, 16a-H), 5.33 (d, 1H, J = 4.5 Hz, 6-H); ESI-MS: 445 (M+H)+; Anal. Calcd for C24H36N4O4: C, 64.16; H, 7.96; N, 13.01. Found: C, 64.43; H, 7.80; N, 13.19.

16β-(5-Benzoxycarbonyl-1H-tetrazol-1-ylmethyl)androst-5-ene-3β,17β-diol (11c): Nitrile: benzyl cyanoformate (8c, 0.16 mL) was added to the mixture. After purification, 11c was obtained as a white solid (268 mg, 53%), mp 178-181 °C, Rf = 0.38 (ss B); 1H-NMR (CDCl3); δ [ppm] = 0.79 (s, 3H, 18-CH3), 0.87-0.94 (overlapping m, 2H), 1.02 (s, 3H, 19-CH3), 1.07-1.15 (overlapping m, 3H), 1.46-1.55 (overlapping m, 5H), 1.67 (m, 1H), 1.78-1.85 (overlapping m, 3H), 1.91 (m, 1H), 2.24-2.32
(overlapping m, 2H), 2.80 (m, 1H, 16-H), 3.51 (m, 1H, 3-H), 3.76 (dd, 1H, \(J=9.5\) Hz, \(J=3.0\) Hz, 17-H), 4.63 (dd, 1H, \(J=13.5\) Hz, \(J=8.0\) Hz, 16a-H), 5.01 (dd, 1H, \(J=13.5\) Hz, \(J=7.5\) Hz, 16a-H), 5.32 (d, 1H, \(J=4.0\) Hz, 6-H), 5.47 (q, 2H, \(J=12.0\) Hz, OCH\(_2\)Ph), 7.38 (m, 3H, 3”-, 4”- and 5”-H), 7.48 (d, 2H, \(J=6.5\) Hz, 2”- and 6”-H); ESI-MS: 507 (M+H)\(^{+}\); Anal. Calcd for C\(_{29}\)H\(_{38}\)N\(_4\)O\(_4\): C, 68.75; H, 7.56; N, 11.06. Found: C, 68.88; H, 7.74; N, 10.89.

16β-(5-Acetyl-1H-tetrazol-1-ylmethyl)androst-5-ene-3β,17β-diol \((11d)\): Nitrile: acetyl cyanide \((8d, 0.08\) mL) was added to the mixture. After purification, \(11d\) was obtained as a white solid (195 mg, 47%), mp 199-202 °C, \(R_f = 0.41\) (ss B); \(^{1}\)H-NMR (CDCl\(_3\)); \(\delta [ppm] = 0.84\) (s, 3H, 18-CH\(_3\)), 0.93-0.97 (overlapping m, 2H), 1.02 (s, 3H, 19-CH\(_3\)), 1.08-1.16 (overlapping m, 3H), 1.47-1.58 (overlapping m, 5H), 1.74 (m, 1H), 1.82-1.87 (overlapping m, 3H), 1.96 (m, 1H), 2.22-2.32 (overlapping m, 2H), 2.55 (s, 3H, 5’Ac-CH\(_3\)), 2.56 (m, 1H, 16-H), 3.51 (m, 1H, 3-H), 3.83 (d, 1H, \(J=9.5\) Hz, 17-H), 4.66 (dd, 1H, \(J=13.5\) Hz, \(J=7.5\) Hz, 16a-H), 5.32 (d, 1H, \(J=3.5\) Hz, 6-H); ESI-MS: 415 (M+H)\(^{+}\); Anal. Calcd for C\(_{23}\)H\(_{34}\)N\(_4\)O\(_3\): C, 66.64; H, 8.27; N, 13.52. Found: C, 66.48; H, 8.38; N, 13.74.

16β-(5-Benzoyl-1H-tetrazol-1-ylmethyl)androst-5-ene-3β,17β-diol \((11e)\): Nitrile: benzoyl cyanide \((8e, 145\) mg) was added to the mixture. After purification, \(11e\) was obtained as a white solid (215 mg, 45%), mp 196-200 °C, \(R_f = 0.34\) (ss B); \(^{1}\)H-NMR (CDCl\(_3\)); \(\delta [ppm] = 0.83\) (s, 3H, 18-CH\(_3\)), 0.91-0.96 (overlapping m, 2H), 1.03 (s, 3H, 19-CH\(_3\)), 1.07-1.14 (overlapping m, 3H), 1.46-1.57 (overlapping m, 5H), 1.71 (m, 1H), 1.81-1.86 (overlapping m, 3H), 1.94 (m, 1H), 2.23-2.31 (overlapping m, 2H), 2.87 (m, 1H, 16-H), 3.51 (m, 1H, 3-H), 3.84 (d, 1H, \(J=9.5\) Hz, 17-H), 4.66 (dd, 1H, \(J=13.5\) Hz, \(J=7.0\) Hz, 16a-H), 5.32 (d, 1H, \(J=4.0\) Hz, 6-H), 7.56 (t, 2H, \(J=7.5\) Hz, 3”- and 5”-H), 7.70 (t, 1H, \(J=7.5\) Hz, 4”-H), 8.34 (d, 2H, \(J=7.5\) Hz, 2”- and 6”-H); ESI-MS: 477 (M+H)\(^{+}\); Anal. Calcd for C\(_{28}\)H\(_{36}\)N\(_4\)O\(_3\): C, 70.56; H, 7.61; N, 11.76. Found: C, 70.77; H, 7.45; N, 11.89.

3.11. Determination of Antiproliferative Activities

Human cancer cell lines were purchased from ECACC (Salisbury, UK). HeLa (cervix adenocarcinoma), A2780 (ovarian carcinoma) and MCF7 (breast adenocarcinoma) cells were cultivated in minimal essential medium 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/well) and attached to the bottom of the well overnight. On the second day, new medium containing the tested compound (at 10 or 30 µM, 200 µL) was added. 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 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 crystals were dissolved in 100 µL DMSO 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 [31]. 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 (0.1% or 0.3%) did not have any significant effect on the cell proliferation.

4. Conclusions

In summary, the efficient syntheses of several D-ring-substituted steroidal triazoles and tetrazoles were achieved by means of 1,3-dipolar cycloadditions. The simple and fast reactions were carried out under mild conditions that furnished the desired compounds in good yields. The novel synthesized compounds were screened for their activities against a panel of three human gynecological cancer cell lines (HeLa, MCF7 and A2780). The application of “click” chemistry to further sterane skeletons was encouraged by these promising results.

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Conflict of Interest

The authors declare no conflict of interest.

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*Sample Availability*: Samples of the compounds 5, 6a-j, 7a-j, 9a-e, 10 and 11a-e are available from the authors.

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