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SYNTHESIS OF STEROIDAL [1,2,4]TRIAZOLO[1,5-*a*]PYRIMIDINES AND THEIR ANTIPROLIFERATIVE ACTIVITIES

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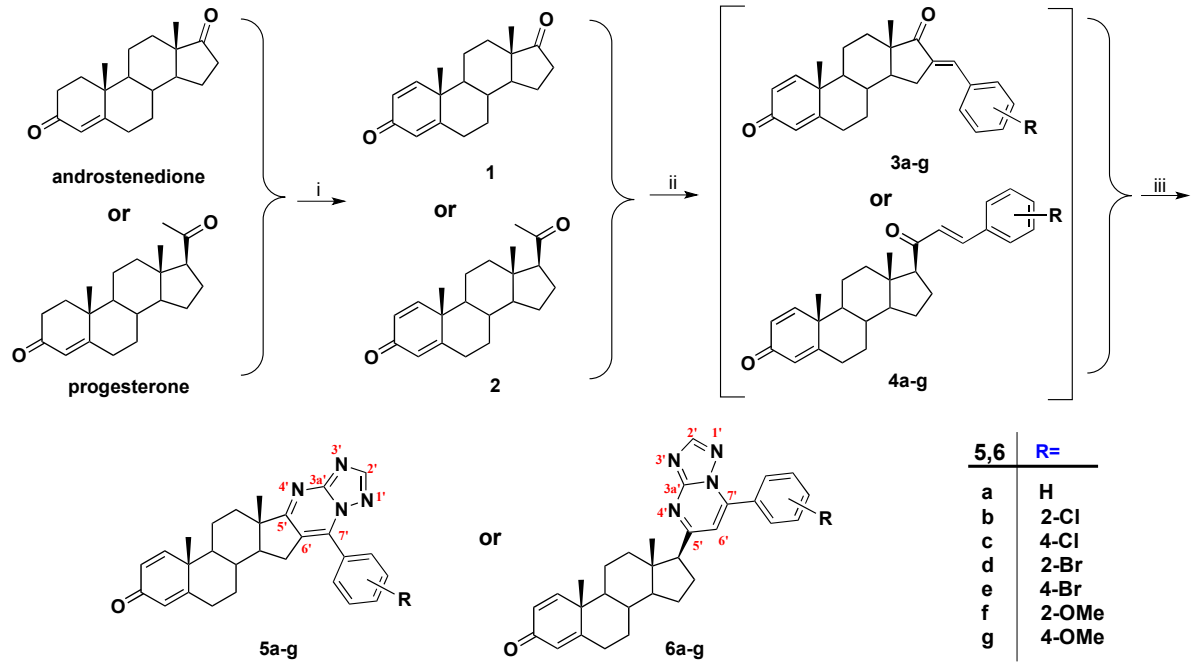
Abstract — A facile strategy for the synthesis of steroidal [1,2,4]triazolo[1,5-*a*]pyrimidines **5a-g** and **6a-g** has been accomplished via a one-pot reaction of steroidal ketones, aromatic aldehydes and 3-amino-1,2,4-triazole in the presence of potassium *tert*-butoxide in refluxing *tert*-butanol. All the synthesized heterosteroids were evaluated for *in vitro* antiproliferative activity against human cancer cells by sulforhodamine B (SRB) assays. The preliminary results showed that compounds **6a** and **6e** possessed potent antiproliferative activities.

Steroids are a class of important multi-cyclic compounds which exhibit diverse biological activities in living organisms, and steroidal derivatives in which D-ring is modified with heterocyclic rings have been of great pharmaceutical interest.[1-7] For example, 17-(3'-pyridyl)androsta-5,16-dien-3β-ol (Abiraterone) blocks testosterone synthesized by inhibiting 17α-hydroxylase-C17,20-lyase ($P450_{17\alpha}$) is successfully applied in the treatment of prostatic carcinoma.[8]

In previous decades, the 1,2,4-triazolo[1,5-*a*]pyrimidines (TPs, a subtype of purine analogs) have been widely investigated and identified to possess multifaceted pharmacological properties, including antimalarial, antifungal and antimicrobial activities.[9-11] Cevipabulin and its analogs, a class of triazolo[1,5-*a*]pyrimidines, were proved to be potent anticancer agents with a unique mechanism of action in promoting tubulin polymerization reported by Beyer et al.[12] In view of the pharmacological importance

of heterosteroids and as well as 1,2,4-triazolo[1,5-*a*]pyrimidine functional group, and in continuation of our commitment to search for novel potential anticancer agents related to steroidal derivatives,[13-18] we have focused our investigation on the synthesis and characterization of novel derivatives having a [1,2,4]triazolo[1,5-*a*]pyrimidine moiety fused or attached to the D-ring of different steroidal skeleton. Besides, we additionally investigated the antiproliferative effects of the new synthesized steroidal derivatives.



Scheme 1. General procedure for the synthesis of steroidal [1,2,4]triazolo[1,5-*a*]pyrimidines. Reagents and conditions: (i) DDQ/TBDMSCl, dioxane, reflux; (ii) *t*-BuOK, *t*-BuOH, benzaldehydes (a-g), reflux; (iii) 3-amino-1,2,4-triazole, reflux

The synthetic procedure of D-ring substituted or fused [1,2,4]triazolo[1,5-*a*]pyrimidine derivatives **5a-g** and **6a-g** is shown in Scheme 1, which commenced with androstenedione or progesterone as the starting materials. The key intermediates, androsta-1,4-diene-3,17-dione **1** and pregna-1,4-diene-3,20-dione **2** were resynthesized following the previous procedure.[19] Treatment of **1** (or **2**) with various aromatic aldehydes **a-g** in presence of potassium *tert*-butoxide in *tert*-butanol, the α,β-unsaturated ketone **3a-g** (or **4a-g**) were obtained through a aldol condensation, and these intermediates were utilized without further purification for the preparation of [1,2,4]triazolo[1,5-*a*]pyrimidine derivatives. Thereafter, a Michael addition between the **3a-g** (or **4a-g**) and 3-amino-1,2,4-triazole provided the targets **5a-g** (or **6a-g**). The reaction mixture was maintained under reflux until disappearance of the starting material affording the desired heterosteroids in moderate yields (50-62%).

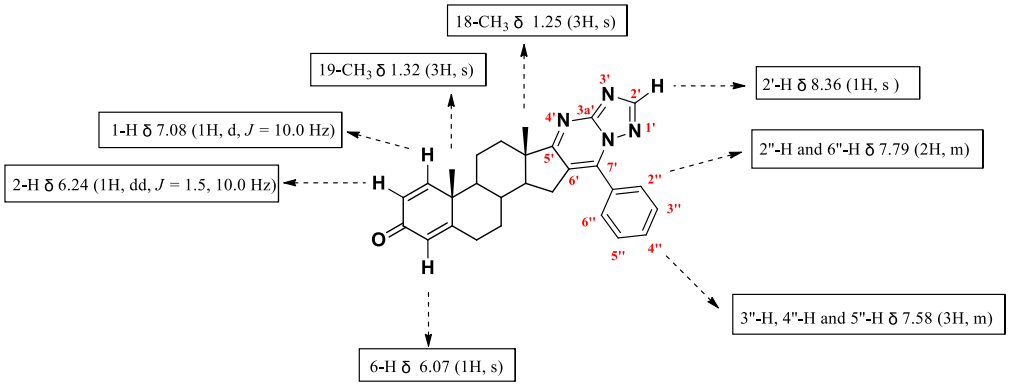
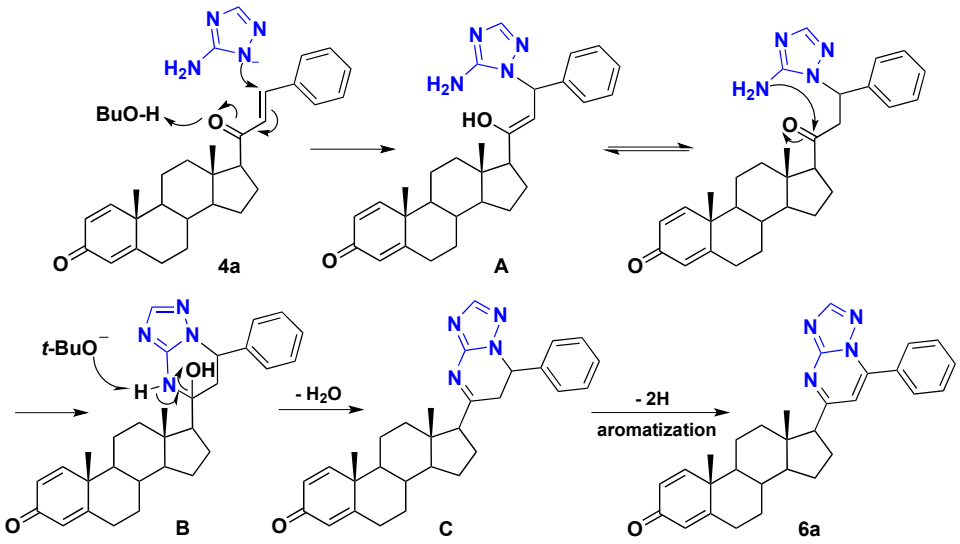


Figure 1. Characteristic chemical shifts of compound **5a**

All the new compounds **5a-g** and **6a-g** were fully characterized by ^{1}H NMR, ^{13}C NMR and high-resolution mass spectra (HRMS) as described for compound **5a**. In the ^{1}H NMR spectrum of compound **5a** (Figure 1), the signals of protons attached to 18-CH_3 and 19-CH_3 appeared at 1.25 and 1.32 ppm, respectively as sharp singlets. Two doublets at 7.08 and 6.24 ppm, respectively had the same coupling constant (J = 10.0 Hz) were assigned to the 1-H and 2-H of steroid nucleus. The signal of 4-H appeared at 6.07 ppm as a singlet. The multiplets at 7.79 and 7.58 ppm, respectively, were assigned to the protons attached to the phenyl group. The proton signal of H-2' (N–CH=N) appeared at 8.36 ppm. In the ^{13}C NMR spectrum, it can be observed characteristic signals at 154.74 (C-2'), 155.87 (C-3a'), 179.88 (C-5'), 122.06 (C-6') and 143.06 (C-7') for the compound **5a**, which evidences the formation of the heterocycle of the [1,2,4]triazolo[1,5-*a*]pyrimidine, and the signals of other carbons existed in phenyl ring and double bond of A-ring were also remarkable. Furthermore, the presence of a molecular ion peak at *m/z* = 437.2320 $(M+H)^+$ in the mass spectrum (calcd for $C_{28}H_{29}N_4O$, 437.2325) confirmed the structure of **5a**.



Scheme 2. Proposed mechanism for the formation of steroidal [1,2,4]triazolo[1,5-*a*]pyrimidine **6a**

Based on the interesting transformations, we also tentatively proposed the possible reaction mechanism for the formation of **6a** from **4a** as shown in Scheme 2. In the presence of potassium *tert*-butoxide, 3-amino-1,2,4-triazole existed as the anion and attacked the β-carbon of aromatic α, β-unsaturated ketone unit of **4a** via aza-Michael addition reaction to afford the intermediate **A**. Subsequently, the amino group attacked the 20-ketone to form the heterocycle **B** via an intramolecular cyclization reaction followed by elimination of H_2O to give the dihydrotriazolopyrimidine **C**. Finally, the target compound **6a** was formed by oxidative aromatization of intermediate **C** under the same condition.

After preliminary screening, the *in vitro* antiproliferative activity of androstenedione, progesterone and these novel [1,2,4]triazolo[1,5-*a*]pyrimidine derivatives against two human cancer cell lines, HepG-2 and HCT116 were evaluated by means of sulforhodamine B colorimetric assay. A typifying example of the triazolopyrimidine class, cevipabulin[12] and etoposide (VP-16) were used as positive compounds. Table 1 showed the inhibitory effects on cell proliferation. As a preliminary study, compared with the starting compounds androstenedione and progesterone, most of the tested compounds showed some inhibition activity, which were inferior to the positive control cevipabulin and etoposide. At the concentrations of 50 μM, compounds **5a-g** (except **5b**, **5d** and **5f**) and **6a-g** displayed a moderate cytotoxic activity (all inhibition rate over 50%, and the highest inhibition rate is 93%) to HCT116 cells, however, only **6a**, **6d** and **6e** were found to have the similar inhibition activity (69%, 75% and 66%, respectively) to HepG-2 cells. The inhibition values at 10 μM were practically always less than 50% on both HepG-2 and HCT116, whereas compound **6a** exhibited selective cytotoxicity toward HepG-2 cell line with the inhibition rate of 52% and the more activity of **6e** on HCT116 cells (the inhibition rate is 63%), which were the most potent of all the screened compounds.

Table 1. *In vitro* inhibitory effects of the compounds against **HepG-2** and **HCT116** cells at 10 μM and 50 μM

Entry	Inhibitory effects (%) [a]			
	HepG-2		HCT116	
	10 μM	50 μM	10 μM	50 μM
androstenedione	N.A. [b]	N.A.	N.A.	N.A.
progesterone	N.A.	N.A.	N.A.	N.A.
5a	N.A.	28%	20%	69%
5b	N.A.	22%	N.A.	N.A.
5c	N.A.	N.A.	31%	89%
5d	N.A.	N.A.	28%	30%
5e	N.A.	47%	32%	78%
5f	N.A.	25%	35%	44%
5g	N.A.	40%	41%	78%
6a	52%	69%	N.A.	69%

6b	N.A.	48%	49%	73%
6c	N.A.	46%	48%	62%
6d	39%	75%	N.A.	93%
6e	47%	66%	63%	80%
6f	N.A.	40%	26%	54%
6g	N.A.	33%	38%	87%
VP-16	74%	-	86%	-
cevipabulin	93%	-	95%	-

[a] All data are the average of four determinations, which were reproducible with deviation less than ± 10%.

[b] N.A. means inhibition values <20%.

In summary, we have accomplished the synthesis of different steroidal heterocycles substituted on the position C-17 and other directly fused to D ring, through the cycloaddition of different α, β-unsaturated ketones and 3-amino-1,2,4-triazole under basic conditions. We also evaluated their cytotoxic activities against two human cancer cell lines and the compounds **6a** and **6e** showed the moderate cytotoxic activity.

EXPERIMENTAL

General methods

The melting points of the products were determined on an X-4 apparatus (Beijing Tech Instrument Co., Beijing, P. R. China) and are uncorrected. Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Advance III 500 instrument in $CDCl_3$ with TMS as internal standard. Chemical shift values (δ) were given in parts per million (ppm). Thin-layer chromatography (TLC) was performed on silica gel GF_{254} (Qingdao Marine Chemical Ltd., P. R. China). Column chromatography (CC) was performed over silica gel (200-300 mesh, Qingdao Marine Chemical Ltd.). The chromatograms were visualized under UV 254-366 nm and iodine. Mass spectra were obtained on Agilent Accurate-Mass-Q-TOF MS 6520 system. All commercially available solvents and reagents were freshly purified.

General procedure for the synthesis of steroidal triazolopyrimidines. A solution of compound **1** (or **2**) (0.4 mmol) and respective aromatic aldehydes **a-g** (0.48 mmol) in *t*-BuOH (3.0 mL) was refluxed for 30 min in the presence of *t*-BuOK (0.8 mmol). Then, 3-amino-1,2,4-triazole (0.44 mmol) was added slowly and the reaction mixture was maintained under reflux until disappearance of the starting material (confirmed by TLC). After completion of the reaction, the solvent was removed and CH_2Cl_2 (6 mL) was added. The insoluble *t*-BuOK was filtered and washed thoroughly with CH_2Cl_2. The residue obtained was dissolved in CH_2Cl_2 and chromatographed on silica gel with CH_2Cl_2/MeOH (40:1, v/v) as eluent to give the corresponding products **5a-g** (or **6a-g**).

(7'-Phenyl-[17,16-*d*][1,2,4]triazolo[1,5-*a*]pyrimidyl)androsta-1,4-dien-3-one (5a): White solid (108 mg, 62%). mp > 300 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 1.13-1.19 (2H, m), 1.25 (3H, s, 18-CH_3),

1.32 (3H, s, 19-CH_3), 1.65-2.82 (11H, m), 6.07 (1H, s, 4-H), 6.24 (1H, dd, *J* = 1.5, 10.0 Hz, 2-H), 7.08 (1H, d, *J* = 10.0 Hz, 1-H), 7.55-7.59 (3H, m, Ar-H), 7.79 (2H, m, Ar-H), 8.36 (1H, s, 2'-H); ^{13}C NMR (125 MHz, $CDCl_3$): δ ppm 17.37, 18.80, 22.39, 29.14, 32.47, 32.72, 32.83, 34.69, 43.48, 46.61, 52.35, 54.71, 122.06, 124.28, 127.81, 128.71, 129.02, 129.58, 131.17, 143.06, 154.74, 155.04, 155.87, 167.78, 179.88, 186.06. HR-MS (ESI): *m/z* 437.2320 $[M+H]^+$ (calcd for $C_{28}H_{29}N_4O$, 437.2325).

(7'-(2''-Chlorophenyl)-[17,16-*d*][1,2,4]triazolo[1,5-*a*]pyrimidyl)androsta-1,4-dien-3-one (5b): White solid (100 mg, 53%). mp > 300 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 1.11-1.17 (2H, m), 1.23 (3H, s, 18-CH_3), 1.32 (3H, s, 19-CH_3), 1.71-2.74 (11H, m), 6.08 (1H, s, 4-H), 6.26 (1H, dd, *J* = 1.5, 10.0 Hz, 2-H), 7.09 (1H, d, *J* = 10.0 Hz, 1-H), 7.44-7.63 (4H, m, Ar-H), 8.34 (1H, s, 2'-H); ^{13}C NMR (125 MHz, $CDCl_3$): δ ppm 17.52, 18.81, 22.45, 28.00, 32.47, 32.67, 32.79, 34.82, 43.49, 46.71, 52.36, 54.46, 123.79, 124.34, 127.37, 127.87, 128.60, 130.51, 130.72, 132.15, 133.19, 140.85, 155.00, 155.08, 155.56, 167.76, 179.58, 186.09. HR-MS (ESI): *m/z* 471.1946 $[M+H]^+$ (calcd for $C_{28}H_{28}ClN_4O$, 471.1932).

(7'-(4''-Chlorophenyl)-[17,16-*d*][1,2,4]triazolo[1,5-*a*]pyrimidyl)androsta-1,4-dien-3-one (5c): White solid (105 mg, 56%). mp > 300 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 1.13-1.19 (2H, m), 1.23 (3H, s, 18-CH_3), 1.30 (3H, s, 19-CH_3), 1.65-2.82 (11H, m), 6.04 (1H, s, 4-H), 6.22 (1H, dd, *J* = 1.5, 10.0 Hz, 2-H), 7.06 (1H, d, *J* = 10.0 Hz, 1-H), 7.54 (2H, d, *J* = 8.0 Hz, Ar-H), 7.75 (2H, d, *J* = 8.0 Hz, Ar-H), 8.33 (1H, s, 2'-H); ^{13}C NMR (125 MHz, $CDCl_3$): δ ppm 17.33, 18.76, 22.34, 29.10, 32.40, 32.68, 32.77, 34.65, 43.43, 46.59, 52.31, 54.67, 122.06, 124.24, 127.34, 127.76, 129.02, 131.01, 137.33, 141.85, 154.76, 154.98, 155.83, 167.70, 179.87, 185.98. HR-MS (ESI): *m/z* 471.1946 $[M+H]^+$ (calcd for $C_{28}H_{28}ClN_4O$, 471.1932).

(7'-(2''-Bromophenyl)-[17,16-*d*][1,2,4]triazolo[1,5-*a*]pyrimidyl)androsta-1,4-dien-3-one (5d): White solid (121 mg, 59%). mp > 300 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 1.11-1.17 (2H, m), 1.22 (3H, s, 18-CH_3), 1.32 (3H, s, 19-CH_3), 1.69-2.74 (11H, m), 6.07 (1H, s, 4-H), 6.26 (1H, dd, *J* = 1.5, 10.0 Hz, 2-H), 7.09 (1H, d, *J* = 10.0 Hz, 1-H), 7.45-7.56 (3H, m, Ar-H), 7.79 (1H, d, *J* = 8.0 Hz, Ar-H), 8.33 (1H, s, 2'-H); ^{13}C NMR (125 MHz, $CDCl_3$): δ ppm 17.40, 18.80, 22.43, 28.02, 32.47, 32.64, 32.85, 34.72, 43.50, 46.80, 52.47, 53.99, 122.64, 123.99, 124.30, 127.84, 127.94, 130.54, 130.70, 132.22, 133.51, 141.98, 155.01, 155.08, 155.59, 167.83, 179.56, 186.09. HR-MS (ESI): *m/z* 515.1424 $[M+H]^+$ (calcd for $C_{28}H_{28}BrN_4O$, 515.1441).

(7'-(4''-Bromophenyl)-[17,16-*d*][1,2,4]triazolo[1,5-*a*]pyrimidyl)androsta-1,4-dien-3-one (5e): White solid (117 mg, 57%). mp > 300 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 1.12-1.19 (2H, m), 1.24 (3H, s, 18-CH_3), 1.32 (3H, s, 19-CH_3), 1.67-2.83 (11H, m), 6.07 (1H, s, 4-H), 6.24 (1H, dd, *J* = 1.5, 10.0 Hz, 2-H), 7.08 (1H, d, *J* = 10.0 Hz, 1-H), 7.68-7.73 (4H, m, Ar-H), 8.35 (1H, s, 2'-H); ^{13}C NMR (125 MHz, $CDCl_3$): δ ppm 17.38, 18.82, 22.40, 29.15, 32.46, 32.73, 32.83, 34.72, 43.48, 46.66, 52.37, 54.74, 122.06, 124.34, 125.82, 127.72, 127.83, 131.18, 132.07, 141.97, 154.85, 155.00, 155.89, 167.70, 179.92, 186.06.

HR-MS (ESI): *m/z* 515.1424 $[M+H]^+$ (calcd for $C_{28}H_{28}BrN_4O$, 515.1441).

(7'-(2''-Methoxyphenyl)-[17,16-*d*][1,2,4]triazolo[1,5-*a*]pyrimidyl)androsta-1,4-dien-3-one (5f): White solid (93 mg, 50%). mp > 300 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 1.11-1.18 (2H, m), 1.23 (3H, s, 18-CH_3), 1.32 (3H, s, 19-CH_3), 1.70-2.64 (11H, m), 3.84 (3H, s, Ar-OCH_3), 6.09 (1H, s, 4-H), 6.26 (1H, dd, *J* = 1.5, 10.0 Hz, 2-H), 7.08 (1H, d, *J* = 10.0 Hz, 1-H), 7.11-7.18 (2H, m, Ar-H), 7.54-7.58 (2H, m, Ar-H), 8.32 (1H, s, 2'-H); ^{13}C NMR (125 MHz, $CDCl_3$): δ ppm 17.48, 18.83, 22.49, 28.40, 32.54, 32.79, 32.88, 34.74, 43.57, 46.67, 52.50, 53.98, 55.75, 111.72, 111.99, 120.79, 120.86, 124.30, 127.84, 130.70, 132.63, 140.74, 154.58, 154.65, 155.18, 157.08, 167.96, 179.14, 186.17. HR-MS (ESI): *m/z* 467.2427 $[M+H]^+$ (calcd for $C_{29}H_{31}N_4O_2$, 467.2441).

(7'-(4''-Methoxyphenyl)-[17,16-*d*][1,2,4]triazolo[1,5-*a*]pyrimidyl)androsta-1,4-dien-3-one (5g): White solid (103 mg, 55%). mp > 300 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 1.13-1.19 (2H, m), 1.25 (3H, s, 18-CH_3), 1.33 (3H, s, 19-CH_3), 1.67-2.82 (11H, m), 3.91 (3H, s, Ar-OCH_3), 6.08 (1H, s, 4-H), 6.26 (1H, dd, *J* = 1.5, 10.0 Hz, 2-H), 7.09 (1H, d, *J* = 10.0 Hz, 1-H), 7.10 (2H, d, *J* = 8.0 Hz, Ar-H), 7.80 (2H, d, *J* = 8.0 Hz, Ar-H), 8.36 (1H, s, 2'-H); ^{13}C NMR (125 MHz, $CDCl_3$): δ ppm 17.37, 18.83, 22.44, 29.44, 32.53, 32.78, 32.88, 34.75, 43.54, 46.59, 52.42, 54.85, 55.55, 114.15, 114.36, 121.14, 121.33, 124.32, 127.84, 131.48, 143.07, 154.73, 155.12, 156.06, 167.87, 179.70, 186.13. HR-MS (ESI): *m/z* 467.2427 $[M+H]^+$ (calcd for $C_{29}H_{31}N_4O_2$, 467.2441).

17β-(7'-Phenyl-[1,2,4]triazolo[1,5-*a*]pyrimid-5'-yl)androsta-1,4-dien-3-one (6a): White solid (110 mg, 59%). mp 121-123 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 0.69 (3H, s, 18-CH_3), 1.13-1.16 (2H, m), 1.23 (3H, s, 19-CH_3), 1.31-3.05 (14H, m), 6.09 (1H, s, 4-H), 6.24 (1H, dd, *J* = 1.5, 10.0 Hz, 2-H), 7.01 (1H, s, 6'-H), 7.05 (1H, d, *J* = 10.0 Hz, 1-H), 7.60-7.62 (3H, m, Ar-H), 8.05-8.07 (2H, m, Ar-H), 8.48 (1H, s, 2'-H); ^{13}C NMR (125 MHz, $CDCl_3$): δ ppm 13.52, 18.76, 22.68, 24.61, 24.73, 32.84, 33.61, 35.82, 38.15, 43.59, 46.05, 52.42, 55.86, 58.59, 110.25, 123.97, 127.62, 128.98, 129.26, 130.02, 131.74, 146.59, 155.50, 155.67, 155.70, 167.93, 169.03, 186.37. HR-MS (ESI): *m/z* 465.2649 $[M+H]^+$ (calcd for $C_{30}H_{33}N_4O$, 465.2644).

17β-(7'-(2''-Chlorophenyl)-[1,2,4]triazolo[1,5-*a*]pyrimid-5'-yl)androsta-1,4-dien-3-one (6b): White solid (116 mg, 58%). mp 144-146 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 0.69 (3H, s, 18-CH_3), 1.14-1.17 (2H, m), 1.24 (3H, s, 19-CH_3), 1.31-3.05 (14H, m), 6.09 (1H, s, 4-H), 6.24 (1H, dd, *J* = 1.5, 10.0 Hz, 2-H), 6.97 (1H, s, 6'-H), 7.04 (1H, d, *J* = 10.0 Hz, 1-H), 7.49-7.50 (1H, m, Ar-H), 7.55-7.61 (3H, m, Ar-H), 8.44 (1H, s, 2'-H); ^{13}C NMR (125 MHz, $CDCl_3$): δ ppm 13.59, 18.76, 22.72, 24.55, 24.74, 32.84, 33.61, 35.83, 38.13, 43.58, 46.24, 52.38, 55.85, 58.63, 112.60, 123.99, 127.22, 127.64, 129.38, 130.51, 131.06, 132.25, 133.36, 144.22, 155.51, 155.64, 155.76, 167.87, 168.95, 186.33. HR-MS (ESI): *m/z* 499.2252 $[M+H]^+$ (calcd for $C_{30}H_{32}ClN_4O$, 499.2259).

17β-(7'-(4''-Chlorophenyl)-[1,2,4]triazolo[1,5-*a*]pyrimid-5'-yl)androsta-1,4-dien-3-one (6c): White

solid (112 mg, 56%). mp 159-161 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 0.68 (3H, s, 18-CH_3), 1.13-1.16 (2H, m), 1.23 (3H, s, 19-CH_3), 1.31-3.05 (14H, m), 6.09 (1H, s, 4-H), 6.24 (1H, dd, *J* = 1.5, 10.0 Hz, 2-H), 6.99 (1H, s, 6'-H), 7.04 (1H, d, *J* = 10.0 Hz, 1-H), 7.57 (2H, d, *J* = 8.0 Hz, Ar-H), 8.04 (2H, d, *J* = 8.0 Hz, Ar-H), 8.48 (1H, s, 2'-H); ^{13}C NMR (125 MHz, $CDCl_3$): δ ppm 13.52, 18.76, 22.68, 24.66, 24.72, 32.83, 33.60, 35.83, 38.17, 43.57, 46.10, 52.42, 55.89, 58.63, 110.07, 124.00, 127.64, 128.33, 129.32, 130.62, 138.12, 145.39, 155.42, 155.61, 155.65, 168.05, 168.93, 186.32. HR-MS (ESI): *m/z* 499.2251 $[M+H]^+$ (calcd for $C_{30}H_{32}ClN_4O$, 499.2259).

17β-(7'-(2''-Bromophenyl)-[1,2,4]triazolo[1,5-*a*]pyrimid-5'-yl)androsta-1,4-dien-3-one (6d): White solid (108 mg, 50%). mp 222-224 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 0.68 (3H, s, 18-CH_3), 1.13-1.16 (2H, m), 1.23 (3H, s, 19-CH_3), 1.30-3.05 (14H, m), 6.08 (1H, s, 4-H), 6.23 (1H, dd, *J* = 1.5, 10.0 Hz, 2-H), 6.94 (1H, s, 6'-H), 7.04 (1H, d, *J* = 10.0 Hz, 1-H), 7.46-7.53 (3H, m, Ar-H), 7.79 (1H, d, *J* = 8.0 Hz, Ar-H), 8.42 (1H, s, 2'-H); ^{13}C NMR (125 MHz, $CDCl_3$): δ ppm 13.61, 18.74, 22.70, 24.49, 24.73, 32.82, 33.58, 35.80, 38.14, 43.55, 46.27, 52.35, 55.81, 58.60, 112.46, 122.61, 123.96, 127.61, 127.79, 130.99, 131.49, 132.29, 133.61, 145.50, 155.44, 155.60, 155.77, 167.92, 168.90, 186.28. HR-MS (ESI): *m/z* 543.1744 $[M+H]^+$ (calcd for $C_{30}H_{32}BrN_4O$, 543.1754).

17β-(7'-(4''-Bromophenyl)-[1,2,4]triazolo[1,5-*a*]pyrimid-5'-yl)androsta-1,4-dien-3-one (6e): White solid (124 mg, 57%). mp 153-155 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 0.67 (3H, s, 18-CH_3), 1.14-1.17 (2H, m), 1.23 (3H, s, 19-CH_3), 1.30-3.03 (14H, m), 6.08 (1H, s, 4-H), 6.23 (1H, dd, *J* = 1.5, 10.0 Hz, 2-H), 6.99 (1H, s, 6'-H), 7.04 (1H, d, *J* = 10.0 Hz, 1-H), 7.73 (2H, d, *J* = 8.0 Hz, Ar-H), 7.96 (2H, d, *J* = 8.0 Hz, Ar-H), 8.47 (1H, s, 2'-H); ^{13}C NMR (125 MHz, $CDCl_3$): δ ppm 13.51, 18.75, 22.66, 24.65, 24.70, 32.82, 33.59, 35.81, 38.15, 43.55, 46.07, 52.41, 55.87, 58.60, 109.99, 123.98, 126.49, 127.63, 128.80, 130.74, 132.27, 145.41, 155.40, 155.59, 155.71, 167.98, 168.90, 186.28. HR-MS (ESI): *m/z* 543.1748 $[M+H]^+$ (calcd for $C_{30}H_{32}BrN_4O$, 543.1754).

17β-(7'-(2''-Methoxyphenyl)-[1,2,4]triazolo[1,5-*a*]pyrimid-5'-yl)androsta-1,4-dien-3-one (6f): White solid (105 mg, 53%). mp 273-275 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 0.69 (3H, s, 18-CH_3), 1.13-1.16 (2H, m), 1.24 (3H, s, 19-CH_3), 1.30-3.03 (14H, m), 3.82 (3H, s, Ar-OCH_3), 6.09 (1H, s, 4-H), 6.24 (1H, dd, *J* = 1.5, 10.0 Hz, 2-H), 6.99 (1H, s, 6'-H), 7.05 (1H, d, *J* = 10.0 Hz, 1-H), 7.10-7.17 (2H, m, Ar-H), 7.55-7.58 (1H, m, Ar-H), 7.63 (1H, d, *J* = 8.0 Hz, Ar-H), 8.41 (1H, s, 2'-H); ^{13}C NMR (125 MHz, $CDCl_3$): δ ppm 13.51, 18.78, 22.73, 24.56, 24.75, 32.87, 33.64, 35.85, 38.07, 43.60, 46.03, 52.46, 55.80, 55.85, 58.54, 111.85, 112.60, 119.18, 120.79, 123.99, 127.64, 130.82, 132.77, 144.55, 155.28, 155.63, 155.67, 157.34, 167.41, 168.99, 186.33. HR-MS (ESI): *m/z* 495.2750 $[M+H]^+$ (calcd for $C_{31}H_{35}N_4O_2$, 495.2755).

17β-(7'-(4''-Methoxyphenyl)-[1,2,4]triazolo[1,5-*a*]pyrimid-5'-yl)androsta-1,4-dien-3-one (6g): White solid (111 mg, 56%). mp 123-125 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 0.67 (3H, s, 18-CH_3),

1.14-1.17 (2H, m), 1.23 (3H, s, 19-CH_3), 1.30-3.03 (14H, m), 3.91 (3H, s, Ar-OCH_3), 6.09 (1H, s, 4-H), 6.24 (1H, dd, *J* = 1.5, 10.0 Hz, 2-H), 6.97 (1H, s, 6'-H), 7.04 (1H, d, *J* = 10.0 Hz, 1-H), 7.10 (2H, d, *J* = 8.0 Hz, Ar-H), 8.10 (2H, d, *J* = 8.0 Hz, Ar-H), 8.47 (1H, s, 2'-H); ^{13}C NMR (125 MHz, $CDCl_3$): δ ppm 13.49, 18.76, 22.69, 24.60, 24.73, 32.85, 33.62, 35.82, 38.16, 43.59, 45.99, 52.45, 55.59, 55.86, 58.55, 109.28, 114.40, 122.09, 123.97, 127.61, 131.07, 146.31, 155.46, 155.67, 156.16, 162.36, 167.66, 169.00, 186.33. HR-MS (ESI): *m/z* 495.2751 $[M+H]^+$ (calcd for $C_{31}H_{35}N_4O_2$, 495.2755).

Antiproliferative activity assay

The HepG-2 and HCT116 cell lines were originally obtained from Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences. The two cells were grown in RPMI-1640 (Sigma) medium containing 10% (v/v) thermally inactivated fetal bovine serum (FBS), penicillin (100 KU/L) and streptomycin (100 KU/L) at 37 °C in a 5% CO_2 humidified incubator. Cells were always used at < 90% of confluence. Antiproliferative activity in vitro was assessed by the SRB colorimetric assay. Briefly, 100 μL exponentially growing cells containing 2.5 × 10^4 cells/mL were added to each well of a 96-well flat-microtiter plate and let cells attach for 24 h. Then the medium was replaced by fresh medium and cells were incubated with various amounts of the test compound for an additional periods. After incubation at 37 °C, culture medium was moved and cells were fixed in situ with trichloroacetic acid (TCA), and plates were washed and dried. Sulforhodamine B solution was added to each well. After the unbound dye is removed and plates were air dried. Bound sulforhodamine B was subsequently solubilized with Tris-base, and the absorbance was read at 540 nm using an Epoch (Bio-Tek) microplate reader. The percentage of cell viability was calculated relative to control wells designated as 100% viable cells.

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