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Synthesis and biological evaluation of novel steroidal[17,16-d][1,2,4]triazolo [1,5-a]pyrimidines

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ABSTRACT

The preparation of steroidal[17,16-d][1,2,4]triazolo[1,5-a]pyrimidines and their biological evaluation as potential anticancer agents are herein reported. These novel heterosteroids (**2**, **4**) were prepared through the condensation reaction of 3-amino-1,2,4-triazole with 16-arylidene-17-ketosteroids (**1**, **3**). All the synthesized compounds were evaluated for their anticancer activity in vitro against PC-3 (human prostatic carcinoma), MCF-7 (human breast carcinoma) and EC9706 (human esophageal carcinoma) cell lines. Among the screened compounds, **2i**, **2n** and **4f** showed significant inhibitory activity against all the three human cell lines.

1. Introduction

During the last decades, steroids bearing heterocycles fused to the A- or D-ring of the steroid skeleton have been of pharmaceutical interest as many of these heterosteroids possess widespread biological activities [1–8]. For example, Cortivazol and similar arylpyrazolo steroids exhibited powerful glucocorticoids and have been extensively investigated as anti-inflammatory agents [2]. Potter et al. reported that N-substituted 1,3,5(10)-estratrien[17,16-c]pyrazole showed potent inhibitory activity of 17β-hydroxysteroid dehydrogenases (17β-HSD) in T47-D human breast cancer cells [8]. Considering the remarkable importance from the pharmacological and synthetic viewpoints, great efforts are being made to annelate steroidal moiety with pyrazole, pyridine, pyran, pyrrole or pyrimidine rings using various synthetic strategies [9–13].

On the other hand, 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 antihypertensive, cardiac stimulant, antimalarial, antifungal, anti-HBV, antimicrobial and anticancer activities [14–21]. In addition, triazolopyrimidines are versatile ligands and their coordination compounds can be considered as model systems for metal–ligand interactions observed in biological systems [20a,22]. The most widely known triazolopyrimidine derivative is the simple molecule of Trapidil, which acts as a platelet-derived growth factor antagonist and as a phosphodiesterase inhibitor [15b]. 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. [21b].

In view of the pharmacological importance of heterosteroids as well as triazolopyrimidines and in continuation of our previous work in developing new bioactive modified steroids [23], recently we reported the synthesis of novel 7′-aryl-androstano[17,16-d][1,2,4]triazolo[1,5-a]pyrimidines derivatives [24]. In order to study the effect on the bioactivity of the aryl at C-7′ of these heterosteroids, several new substituted aryl derivatives were synthesized. Herein, we present the synthesis of these heterosteroids and their biological evaluation for anticancer activity against PC-3, MCF-7 and EC9706 cell lines in vitro.

2. Experimental

2.1. General remarks

All reagents and solvents used were of analytical grade purchased from commercial sources. Thin-layer chromatography (TLC) was carried out on glass plates coated with silica gel (Qingdao Haiyang Chemical Co., G60F-254) and visualized by UV light (254 nm). The products were purified by column chromatography over silica gel (Qingdao Haiyang Chemical Co., 200–300

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mesh). Melting points were determined on a Beijing Keyi XT4A apparatus and are uncorrected. All NMR spectra were recorded with a Bruker DPX 400 MHz spectrometer with TMS as internal standard in $CDCl_3$. Chemical shifts are given as δ ppm values relative to TMS. Mass spectra (MS) were recorded on Q-Tof (Waters) mass spectrometer by electrospray ionization (ESI).

2.2. General procedure for the synthesis of steroidal[17,16-d]triazolopyrimidines **2** and **4**

The 16-arylidene-17-ketosteroids **1** or **3** (1.0 mmol) was dissolved in *n*-BuOH (10 mL). To the solution was added 3-amino-1,2,4-triazole (2.0 mmol) and *t*-BuOK (2.0 mmol). The resulting mixture was refluxed for 30 h. The solvent was removed and CH_2Cl_2 was added. The insoluble *t*-BuOK was filtered and washed thoroughly with CH_2Cl_2. After removal of the solvent, the residue was purified by silica gel chromatography with ethyl acetate/petroleum ether/acetone (4:2:1) and petroleum ether/ethyl acetate (3:2) to give the corresponding steroidal[17,16-d]triazolopyrimidines **2** and **4**, respectively (Scheme 1).

2.2.1. 3-Oxo-4-aza-7′-phenyl-5α-androstano[17,16-d][1,2,4]triazolo[1,5-a]pyrimidine (**2a**)

White solid, yield 58%, mp > 300 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.38 (s, 1H, 2′-H), 7.81 (dd, J = 6.7, 3.0 Hz, 2H, Ar–H), 7.64–7.56 (m, 3H, Ar–H), 6.40 (s, 1H, 4-NH), 3.10 (dd, J = 12.2, 3.8 Hz, 1H, 5α-H), 1.20 (s, 3H, 18-H), 1.00 (s, 3H, 19-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 180.4, 172.4, 155.8, 154.7, 143.0, 131.1, 129.6, 129.1, 128.7, 122.4, 60.6, 55.3, 51.4, 46.7, 36.0, 34.2, 33.2, 32.8, 29.0, 29.0, 28.5, 27.0, 20.7, 17.4, 11.4. HRMS (ESI): *m*/*z* cacld. for $C_{27}H_{32}N_5O$ $(M+H)^+$, 442.2607; found, 442.2610.

2.2.2. 3-Oxo-4-aza-7′-(4-chlorophenyl)-5α-androstano[17,16-d][1,2,4]triazolo[1,5-a]pyrimidine (**2b**)

White solid, yield 67%, mp > 300 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.37 (s, 1H, 2′-H), 7.78 (d, J = 8.2 Hz, 2H, Ar–H), 7.57 (d, J = 8.2 Hz, 2H, Ar–H), 6.56 (s, 1H, 4-NH), 3.09 (d, J = 9.5 Hz, 1H, 5α-H), 1.19 (s, 3H, 18-H), 1.00 (s, 3H, 19-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 180.4, 172.3, 155.9, 154.8, 141.7, 137.3, 131.0, 129.1, 127.5, 122.4, 60.6, 55.3, 51.5, 46.8, 35.9, 34.3, 33.2, 32.8, 29.0, 29.0, 28.6, 27.0, 20.7, 17.4, 11.4. HRMS (ESI): *m*/*z* cacld. for $C_{27}H_{31}ClN_5O$ $(M+H)^+$, 476.2217; found, 476.2223.

2.2.3. 3-Oxo-4-aza-7′-(3-nitrophenyl)-5α-androstano[17,16-d][1,2,4]triazolo[1,5-a]pyrimidine (**2c**)

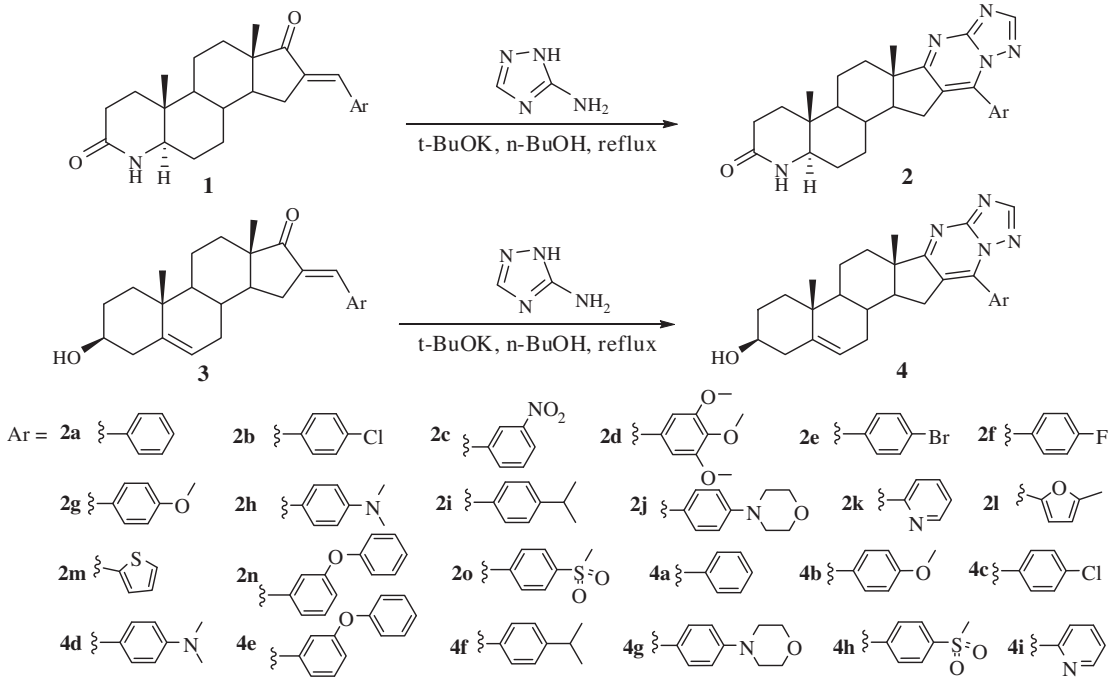
White solid, yield 64%, mp > 300 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.68 (t, J = 1.7 Hz, 1H, Ar–H), 8.44 (dd, J = 8.3, 1.3 Hz, 1H, Ar–H), 8.38 (s, 1H, 2′-H), 8.21 (d, J = 7.8 Hz, 1H, Ar–H), 7.82 (t, J = 8.0 Hz, 1H, Ar–H), 6.49 (s, 1H, 4-NH), 3.09 (dd, J = 12.2, 3.7 Hz, 1H, 5α-H), 1.21 (s, 3H, 18-H), 1.00 (s, 3H, 19-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 180.7, 172.3, 155.8, 155.0, 148.3, 140.1, 135.5, 130.7, 129.9, 125.7, 124.8, 123.0, 60.5, 55.3, 51.5, 46.9, 36.0, 34.3, 33.2, 32.8, 29.0, 28.8, 28.5, 26.9, 20.6, 17.5, 11.4. HRMS (ESI): *m*/*z* cacld. for $C_{27}H_{31}N_6O_3$ $(M+H)^+$, 487.2458; found, 487.2459.

2.2.4. 3-Oxo-4-aza-7′-(3,4,5-trimethoxyphenyl)-5α-androstano[17,16-d][1,2,4]triazolo[1,5-a]pyrimidine (**2d**)

White solid, yield 52%, mp 197–199 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.36 (s, 1H, 2′-H), 7.01 (s, 2H, Ar–H), 6.58 (s, 1H, 4-NH), 3.92 (d, J = 17.1 Hz, 9H, Ar–$(OCH_3)_3$), 3.08 (dd, J = 12.1, 3.3 Hz, 1H, 5α-H), 1.18 (s, 3H, 18-H), 0.98 (s, 3H, 19-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 180.3, 172.3, 156.0, 154.8, 153.3, 142.9, 140.4, 124.0, 122.2, 107.3, 61.0, 60.6, 56.4, 55.3, 51.5, 46.7, 35.9, 34.2, 33.2, 32.8, 29.2, 29.1, 28.5, 26.9, 20.7, 17.4, 11.4. HRMS (ESI): *m*/*z* cacld. for $C_{30}H_{38}N_5O_4$ $(M+H)^+$, 532.2924; found, 532.2921.

2.2.5. 3-Oxo-4-aza-7′-(4-bromophenyl)-5α-androstano[17,16-d][1,2,4]triazolo[1,5-a]pyrimidine (**2e**)

White solid, yield 65%, mp > 300 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.39 (s, 1H, 2′-H), 7.73 (q, J = 8.6 Hz, 4H, Ar–H), 5.98 (s, 1H, 4-NH), 3.12 (dd, J = 11.9, 3.6 Hz, 1H, 5α-H), 1.20 (s, 3H, 18-H), 1.02 (s, 3H, 19-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 180.4, 172.2, 155.9, 154.8, 141.8, 132.0, 131.2, 127.9, 125.8, 122.3, 60.6, 55.3, 51.5, 46.8, 36.0, 34.3, 33.2, 32.8, 29.7, 29.0, 28.5, 27.1, 20.7, 17.4, 11.4. HRMS



Scheme 1. Synthesis of 7′-aryl-androstano[17,16-d][1,2,4]triazolo[1,5-a]pyrimidines.

(ESI): *m*/*z* cacld. for $C_{27}H_{31}BrN_5O$ $(M+H)^+$, 520.1712; found, 520.1710.

*2.2.6. 3-Oxo-4-aza-7′-(4-fluorophenyl)-5α-androstano[17,16-d][1,2,4]triazolo[1,5-a]pyrimidine (**2f**)*

White solid, yield 68%, mp > 300 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.38 (s, 1H, 2′-H), 7.84 (dd, *J* = 8.5, 5.3 Hz, 2H, Ar–H), 7.29 (t, *J* = 8.5 Hz, 2H, Ar–H), 6.34 (s, 1H, 4-NH), 3.14– 3.06 (m, 1H, 5α-H), 1.19 (s, 3H, 18-H), 1.00 (s, 3H, 19-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 180.4, 172.4, 155.9, 154.7, 143.0, 131.1, 129.6, 129.1, 128.7, 122.4, 60.6, 55.3, 51.5, 46.7, 36.0, 34.3, 33.2, 32.8, 29.0, 29.0, 28.5, 27.0, 20.7, 17.4, 11.4. HRMS (ESI): *m*/*z* cacld. for $C_{27}H_{31}FN_5O$ $(M+H)^+$, 460.2513; found, 460.2518.

*2.2.7. 3-Oxo-4-aza-7′-(4-methoxyphenyl)-5α-androstano[17,16-d][1,2,4]triazolo[1,5-a]pyrimidine (**2g**)*

White solid, yield 54%, mp 268–270 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.37 (s, 1H, 2′-H), 7.81 (d, *J* = 8.8 Hz, 2H, Ar–H), 7.10 (d, *J* = 8.8 Hz, 2H, Ar–H), 6.36 (s, 1H, 4-NH), 3.92 (s, 3H, Ar–OCH_3), 3.10 (dd, *J* = 12.1, 3.7 Hz, 1H, 5α-H), 1.19 (s, 3H, 18-H), 1.00 (s, 3H, 19-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 180.2, 172.3, 161.6, 156.0, 154.6, 142.9, 131.4, 121.6, 121.2, 114.1, 60.6, 55.5, 55.4, 51.5, 46.7, 36.0, 34.3, 33.2, 32.8, 29.3, 29.0, 28.6, 27.0, 20.7, 17.4, 11.4. HRMS (ESI): *m*/*z* cacld. for $C_{28}H_{34}N_5O$ $(M+H)^+$, 472.2713; found, 472.2702.

*2.2.8. 3-Oxo-4-aza-7′-(4-dimethylaminophenyl)-5α-androstano[17,16-d][1,2,4]triazolo[1,5-a]pyrimidine (**2h**)*

Yellow solid, yield 55%, mp 257-259 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.35 (s, 1H, 2′-H), 7.79 (d, *J* = 8.9 Hz, 2H, Ar–H), 6.83 (d, *J* = 8.9 Hz, 2H, Ar–H), 6.52 (s, 1H, 4-NH), 3.09 (s, 7H, 5α-H and Ar–$N(CH_3)_2$), 1.18 (s, 3H, 18-H), 1.00 (s, 3H, 19-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 179.9, 172.4, 156.1, 154.4, 151.8, 143.7, 131.2, 125.0, 120.6, 115.6, 111.1, 60.6, 55.5, 51.5, 46.6, 40.1, 35.9, 34.3, 33.2, 32.9, 29.7, 29.1, 28.6, 27.0, 20.7, 17.3, 11.4. HRMS (ESI): *m*/*z* cacld. for $C_{29}H_{37}N_6O$ $(M+H)^+$, 485.3029; found, 485.3033.

*2.2.9. 3-Oxo-4-aza-7′-(4-isopropylphenyl)-5α-androstano[17,16-d][1,2,4]triazolo[1,5-a]pyrimidine (**2i**)*

White solid, yield 60%, mp 234–236 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.37 (s, 1H, 2′-H), 7.75 (d, *J* = 8.0 Hz, 2H, Ar–H), 7.45 (d, *J* = 8.0 Hz, 2H, Ar–H), 6.33 (s, 1H, 4-NH), 3.10 (dd, *J* = 12.0, 3.4 Hz, 1H, 5α-H), 3.08–2.97 (m, 1H, Ar-$CH(CH_3)_2$), 1.33 (d, *J* = 6.8 Hz, 6H, Ar-$CH(CH_3)_2$), 1.19 (s, 3H, 18-H), 1.00 (s, 3H, 19-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 180.3, 172.2, 156.0, 154.7, 152.3, 143.1, 129.6, 126.8, 126.5, 122.0, 60.6, 55.4, 51.5, 46.7, 36.0, 34.3, 34.2, 33.3, 32.9, 29.1, 29.1, 28.6, 27.0, 23.7, 23.7, 20.7, 17.4, 11.4. HRMS (ESI): *m*/*z* cacld. for $C_{30}H_{37}N_5ONa$ $(M+Na)^+$, 506.2896; found, 506.2897.

*2.2.10. 3-Oxo-4-aza-7′-(4-morpholinylphenyl)-5α-androstano[17,16-d][1,2,4]triazolo[1,5-a]pyrimidine (**2j**)*

Yellow solid, yield 51%, mp 298–300 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.37 (s, 1H, 2′-H), 7.81 (d, *J* = 8.7 Hz, 2H, Ar–H), 7.04 (d, *J* = 8.7 Hz, 2H, Ar–H), 6.20 (s, 1H, 4-NH), 3.98–3.82 (m, 4H, protons of morpholine), 3.43–3.27 (m, 4H, protons of morpholine), 3.10 (dd, *J* = 12.0, 3.7 Hz, 1H, 5α-H), 1.19 (s, 3H, 18-H), 1.01 (s, 3H, 19-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 180.0, 172.2, 156.1, 154.6, 152.7, 143.1, 131.2, 121.1, 119.1, 114.0, 66.6, 60.6, 55.5, 51.5, 47.9, 46.6, 36.0, 34.3, 33.3, 32.9, 29.4, 29.1, 28.6, 27.1, 20.7, 17.3, 11.4. HRMS (ESI): *m*/*z* cacld. for $C_{31}H_{38}N_6O_2Na$ $(M+Na)^+$, 549.2954; found, 549.2949.

*2.2.11. 3-Oxo-4-aza-7′-(2-pyridyl)-5α-androstano[17,16-d][1,2,4]triazolo[1,5-a]pyrimidine (**2k**)*

White solid, yield 79%, mp > 300 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.84 (d, *J* = 4.1 Hz, 1H, proton of pyridine), 8.40 (s, 1H, 2′-H), 8.34 (d, *J* = 7.9 Hz, 1H, proton of pyridine), 7.95 (td, *J* = 7.8, 1.2 Hz, 1H, proton of pyridine), 7.48 (dd, *J* = 7.1, 5.1 Hz, 1H, proton of pyridine), 6.47 (s, 1H, 4-NH), 3.09 (dd, *J* = 12.4, 3.7 Hz, 1H, 5α-H), 1.17 (s, 3H, 18-H), 0.99 (s, 3H, 19-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 181.1, 172.3, 156.0, 154.7, 149.9, 148.6, 140.5, 136.5, 126.4, 125.1, 124.3, 60.6, 54.8, 51.6, 46.5, 36.0, 34.3, 33.2, 32.8, 29.2, 29.0, 28.6, 27.0, 20.7, 17.4, 11.4. HRMS (ESI): *m*/*z* cacld. for $C_{26}H_{30}N_6ONa$ $(M+Na)^+$, 465.2379; found, 465.2383.

*2.2.12. 3-Oxo-4-aza-7′-(5-methylfuryl)-5α-androstano[17,16-d][1,2,4]triazolo[1,5-a]pyrimidine (**2l**)*

Yellow solid, yield 43%, mp 289–291 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.44 (s, 1H, 2′-H), 8.16 (d, *J* = 3.5 Hz, 1H, proton of furan), 6.36 (d, *J* = 3.4 Hz, 1H, proton of furan), 6.24 (s, 1H, 4-NH), 3.15 (dd, *J* = 12.1, 3.8 Hz, 1H, 5α-H), 2.50 (s, 3H, protons of furan), 1.12 (s, 3H, 18-H), 1.02 (s, 3H, 19-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 180.0, 172.3, 157.1, 155.7, 154.5, 142.8, 132.8, 122.5, 118.1, 109.6, 60.7, 54.5, 51.6, 46.1, 36.1, 34.2, 33.3, 32.8, 29.7, 29.1, 28.6, 27.2, 20.8, 17.6, 14.2, 11.4. HRMS (ESI): *m*/*z* cacld. for $C_{26}H_{31}N_5O_2Na$ $(M+Na)^+$, 468.2375; found, 468.2379.

*2.2.13. 3-Oxo-4-aza-7′-(2-thienyl)-5α-androstano[17,16-d][1,2,4]triazolo[1,5-a]pyrimidine (**2m**)*

White solid, yield 51%, mp > 300 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.48 (s, 1H, 2′-H), 8.25 (d, *J* = 3.2 Hz, 1H, proton of thiophene), 7.80 (dd, *J* = 5.1, 0.8 Hz, 1H, proton of thiophene), 7.33 (dd, *J* = 5.0, 4.0 Hz, 1H, proton of thiophene), 6.29 (s, 1H, 4-NH), 3.15 (dd, *J* = 9.6, 5.3 Hz, 1H, 5α-H), 1.18 (s, 3H, 18-H), 1.02 (s, 3H, 19-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 180.0, 172.3, 155.8, 154.3, 137.3, 133.8, 132.3, 130.4, 127.6, 119.9, 60.6, 55.0, 51.5, 46.6, 36.0, 34.3, 33.2, 32.9, 30.9, 29.1, 28.6, 27.1, 20.7, 17.6, 14.2, 11.5. HRMS (ESI): *m*/*z* cacld. for $C_{25}H_{30}N_5OS$ $(M+H)^+$, 448.2171; found, 448.2172.

*2.2.14. 3-Oxo-4-aza-7′-(3-phenoxyphenyl)-5α-androstano[17,16-d][1,2,4]triazolo[1,5-a]pyrimidine (**2n**)*

White solid, yield 72%, mp 193–195 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.38 (s, 1H, 2′-H), 7.60–7.53 (m, 2H, Ar–H), 7.45–7.35 (m, 3H, Ar–H), 7.25–7.15 (m, 2H, Ar–H), 7.15–7.07 (m, 2H, Ar–H), 6.25 (s, 1H, 4-NH), 3.11 (dd, *J* = 12.1, 3.7 Hz, 1H, 5α-H) 1.17 (s, 3H, 18-H), 1.01 (s, 3H, 19-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 180.3, 172.2, 157.7, 156.3, 155.9, 154.8, 142.2, 130.6, 130.1, 130.0, 124.1, 124.1, 122.4, 120.9, 119.7, 119.4, 60.6, 55.2, 51.5, 46.7, 36.0, 34.3, 33.2, 32.8, 29.1, 29.0, 28.6, 27.1, 20.7, 17.4, 11.4. HRMS (ESI): *m*/*z* cacld. for $C_{33}H_{36}N_5O_2$ $(M+H)^+$, 534.2869; found, 534.2867.

*2.2.15. 3-Oxo-4-aza-7′-(4-methylsulfonylphenyl)-5α-androstano[17,16-d][1,2,4]triazolo[1,5-a]pyrimidine (**2o**)*

White solid, yield 65%, mp > 300 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.39 (s, 1H, 2′-H), 8.18 (d, *J* = 8.5 Hz, 2H, Ar–H), 8.03 (d, *J* = 8.5 Hz, 2H, Ar–H), 6.32 (s, 1H, 4-NH), 3.16 (s, 3H, Ar–SO_2CH_3), 3.10 (dd, *J* = 12.2, 3.7 Hz, 1H, 5α-H), 1.21 (s, 3H, 18-H), 1.00 (s, 3H, 19-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 180.7, 172.2, 155.8, 155.0, 142.7, 140.6, 134.3, 130.7, 127.8, 123.2, 60.5, 55.3, 51.5, 46.9, 44.4, 36.0, 34.3, 33.2, 32.8, 29.0, 28.8, 28.5, 27.0, 20.7, 17.5, 11.4. HRMS (ESI): *m*/*z* cacld. for $C_{28}H_{34}N_5O_3S$ $(M+H)^+$, 520.2382; found, 520.2386.

*2.2.16. 3β-Hydroxy-5-en-7′-phenyl-androstano[17,16-d][1,2,4]triazolo[1,5-a]pyrimidine (**4a**)*

White solid, yield 67%, mp > 300 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.38 (s, 1H, 2′-H), 7.82 (m, 2H, Ar–H), 7.64–7.57 (m, 3H, Ar–H), 5.37 (d, *J* = 5.0 Hz, 1H, 6-H), 3.63–3.46 (m, 1H, 3α-H), 1.21 (s, 3H, 18-H), 1.19–1.08 (m, 5H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 180.7, 156.0, 154.7, 142.8, 141.4, 131.0, 129.6, 129.2, 128.7, 122.6, 120.6, 71.5, 56.0, 50.3, 46.4, 42.2, 37.1, 36.8, 33.0, 31.6, 31.2, 31.1, 29.2, 20.5, 19.5, 17.1. HRMS (ESI): *m*/*z* cacld. for $C_{28}H_{32}N_4ONa$ $(M+Na)^+$, 463.2474; found, 463.2478.

*2.2.17. 3β-Hydroxy-5-en-7′-(4-methoxyphenyl)-androstano[17,16-d][1,2,4]triazolo[1,5-a]pyrimidine (**4b**)*

White solid, yield 63%, mp 275–277 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.37 (s, 1H, 2′-H), 7.83 (d, *J* = 8.7 Hz, 2H, Ar–H), 7.10 (d, *J* = 8.6 Hz, 2H, Ar–H), 5.37 (d, *J* = 4.5 Hz, 1H, 6-H), 3.92 (s, 3H, Ar–OCH_3), 3.61–3.48 (m, 1H, 3α-H), 1.20 (s, 3H, 18-H), 1.18–1.08 (m, 5H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 180.6, 161.6, 156.0, 154.5, 142.8, 141.4, 131.5, 122.0, 121.3, 120.6, 114.8, 71.5, 56.1, 55.5, 50.3, 46.4, 42.2, 37.1, 36.8, 33.0, 31.6, 31.2, 31.1, 29.4, 20.5, 19.5, 17.1. HRMS (ESI): *m*/*z* cacld. for $C_{29}H_{34}N_4O_2Na$ $(M+Na)^+$, 493.2579; found, 493.2581.

*2.2.18. 3β-Hydroxy-5-en-7′-(4-chlorophenyl)-androstano[17,16-d][1,2,4]triazolo[1,5-a]pyrimidine (**4c**)*

White solid, yield 65%, mp 291–293 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.37 (s, 1H, 2′-H), 7.79 (d, *J* = 8.4 Hz, 2H, Ar–H), 7.58 (d, *J* = 8.4 Hz, 2H, Ar–H), 5.38 (d, *J* = 4.8 Hz, 1H, 6-H), 3.61–3.48 (m, 1H, 3α-H), 1.21 (s, 3H, 18-H), 1.19–1.08 (m, 5H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 180.8, 155.9, 154.7, 141.6, 141.4, 137.3, 131.1, 129.0, 127.6, 122.7, 120.5, 71.5, 56.0, 50.3, 46.5, 42.2, 37.1, 36.8, 33.0, 31.6, 31.2, 31.1, 29.2, 20.5, 19.5, 17.1. HRMS (ESI): *m*/*z* cacld. for $C_{28}H_{32}ClN_4O$ $(M+H)^+$, 475.2265; found, 475.2260.

*2.2.19. 3β-Hydroxy-5-en-7′-(4-dimethylaminophenyl)-androstano[17,16-d][1,2,4]triazolo [1,5-a]pyrimidine (**4d**)*

Yellow solid, yield 61%, mp > 300 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.36 (s, 1H, 2′-H), 7.81 (d, *J* = 8.8 Hz, 2H, Ar–H), 6.84 (d, *J* = 8.8 Hz, 2H, Ar–H), 5.37 (d, *J* = 4.1 Hz, 1H, 6-H), 3.60–3.49 (m, 1H, 3α-H), 3.09 (s, 6H, Ar–$N(CH_3)_2$), 1.20 (s, 3H, 18-H), 1.14 (m, 5H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 180.3, 154.3, 151.8, 143.6, 141.4, 131.2, 125.0, 120.9, 120.7, 115.8, 111.1, 71.5, 56.2, 50.4, 46.3, 42.2, 40.1, 37.1, 36.8, 33.1, 31.6, 31.2, 31.1, 29.8, 20.5, 19.5, 17.1. HRMS (ESI): *m*/*z* cacld. for $C_{30}H_{38}N_5O$ $(M+H)^+$, 484.3076; found, 484.3079.

*2.2.20. 3β-Hydroxy-5-en-7′-(3-phenoxyphenyl)-androstano[17,16-d][1,2,4]triazolo[1,5-a]pyrimidine (**4e**)*

White solid, yield 71%, mp 167–169 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.37 (s, 1H, 2′-H), 7.65–7.51 (m, 2H, Ar–H), 7.48–7.35 (m, 3H, Ar–H), 7.26–7.15 (m, 2H, Ar–H), 7.12 (d, *J* = 7.7 Hz, 2H, Ar–H), 5.39 (d, *J* = 4.9 Hz, 1H, 6-H), 3.60–3.52 (m, 1H, 3α-H), 1.18 (s, 3H, 18-H), 1.17–1.05 (m, 5H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 180.7, 157.7, 156.4, 155.9, 154.7, 142.1, 141.5, 130.7, 130.1, 130.0, 124.2, 124.1, 122.7, 120.8, 120.5, 119.7, 119.4, 71.5, 55.9, 50.3, 46.4, 42.2, 37.1, 36.8, 33.0, 31.6, 31.2, 31.1, 29.1, 20.5, 19.5, 17.1. HRMS (ESI): *m*/*z* cacld. for $C_{34}H_{37}N_4O_2$ $(M+H)^+$, 533.2917; found, 533.2915.

*2.2.21. 3β-Hydroxy-5-en-7′-(4-isopropylphenylphenyl)-androstano[17,16-d][1,2,4]triazolo[1,5-a]pyrimidine (**4f**)*

White solid, yield 66%, mp 248–250 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.38 (s, 1H, 2′-H), 7.77 (d, *J* = 8.0 Hz, 2H, Ar–H), 7.46 (d, *J* = 8.1 Hz, 2H, Ar–H), 5.37 (d, *J* = 4.2 Hz, 1H, 6-H), 3.62–3.49 (m, 1H, 3α-H), 3.08–2.98 (m, 1H, Ar–$CH(CH_3)_2$), 1.34 (d, *J* = 6.8 Hz, 3H, Ar–$CH(CH_3)_2$), 1.33 (d, *J* = 6.8 Hz, 3H, Ar–$CH(CH_3)_2$), 1.21 (s, 3H, 18-H), 1.19–1.08 (m, 5H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 180.7, 156.0, 154.6, 152.2, 143.0, 141.4, 129.7, 126.8, 126.6, 122.4, 120.6, 71.5, 56.1, 50.3, 46.4, 42.2, 37.1, 36.8, 34.3, 33.0, 31.6, 31.2, 31.1, 29.3, 23.8, 23.7, 20.5, 19.5, 17.1. HRMS (ESI): *m*/*z* cacld. for $C_{31}H_{39}N_4O$ $(M+H)^+$, 483.3124; found, 483.3122.

*2.2.22. 3β-Hydroxy-5-en-7′-(4-morpholinylphenyl)-androstano[17,16-d][1,2,4]triazolo[1,5-a]pyrimidine (**4g**)*

Yellow solid, yield 63%, mp > 300 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.37 (s, 1H, 2′-H), 7.82 (d, *J* = 8.9 Hz, 2H, Ar–H), 7.05 (d, *J* = 8.9 Hz, 2H, Ar–H), 5.38 (d, *J* = 4.9 Hz, 1H, 6-H), 3.97–3.83 (m, 4H, protons of morpholine), 3.61–3.49 (m, 1H, 3α-H), 3.42–3.26 (m, 4H, protons of morpholine), 1.21 (s, 3H, 18-H), 1.19–1.07 (m, 5H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 180.4, 156.1, 154.5, 152.6, 143.0, 141.4, 131.2, 121.5, 120.6, 119.2, 114.0, 76.7, 71.6, 66.7, 56.2, 50.3, 47.9, 46.3, 42.2, 37.1, 36.8, 33.0, 31.6, 31.2, 31.1, 29.6, 20.5, 19.5, 17.1. HRMS (ESI): *m*/*z* cacld. for $C_{32}H_{40}N_5O_2$ $(M+H)^+$, 526.3182; found, 526.3185.

*2.2.23. 3β-Hydroxy-5-en-7′-(4-methylsulfonylphenyl)-androstano[17,16-d][1,2,4]triazolo[1,5-a]pyrimidine (**4h**)*

White solid, yield 70%, mp > 300 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.39 (s, 1H, 2′-H), 8.18 (d, *J* = 8.4 Hz, 2H, Ar–H), 8.04 (d, *J* = 8.4 Hz, 2H, Ar–H), 5.38 (d, *J* = 4.9 Hz, 1H, 6-H), 3.62–3.48 (m, 1H, 3α-H), 3.16 (s, 3H, Ar–SO_2CH_3), 1.22 (s, 3H, 18-H), 1.20–1.07 (m, 5H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 181.1, 155.8, 154.9, 142.6, 141.4, 140.5, 134.5, 130.7, 127.8, 123.5, 120.4, 71.5, 56.0, 50.3, 46.6, 44.4, 42.2, 37.1, 36.8, 33.0, 31.6, 31.2, 31.1, 29.0, 20.5, 19.5, 17.1. HRMS (ESI): *m*/*z* cacld. for $C_{29}H_{35}N_4O_3S$ $(M+H)^+$, 519.2430; found, 519.2429.

*2.2.24. 3β-Hydroxy-5-en-7′-(2-pyridyl)-androstano[17,16-d][1,2,4]triazolo[1,5-a]pyrimidine (**4i**)*

White solid, yield 83%, mp 300–301 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.86 (dd, *J* = 4.8, 0.7 Hz, 1H, proton of pyridine), 8.41 (s, 1H, 2′-H), 8.34 (d, *J* = 8.0 Hz, 1H, proton of pyridine), 7.96 (td, *J* = 7.8, 1.8 Hz, 1H, proton of pyridine), 7.49 (ddd, *J* = 7.6, 4.8, 1.0 Hz, 1H, proton of pyridine), 5.38 (d, *J* = 5.1 Hz, 1H, 6-H), 3.61–3.48 (m, 1H, 3α-H), 1.19 (s, 3H, 18-H), 1.17–1.09 (m, 5H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 181.4, 156.0, 154.7, 149.9, 148.7, 141.3, 140.4, 136.5, 126.4, 125.0, 124.6, 120.7, 71.5, 55.5, 50.4, 46.3, 42.2, 37.1, 36.8, 33.0, 31.6, 31.2, 31.1, 29.4, 20.5, 19.5, 17.1. HRMS (ESI): *m*/*z* cacld. for $C_{27}H_{32}N_5O$ $(M+H)^+$, 442.2607; found, 442.2603.

*2.2.25. 3β-Hydroxy-5-en-7′-(4-aminophenyl)-androstano[17,16-d][1,2,4]triazolo[1,5-a]pyrimidine (**4j**)*

Brown solid, yield 41%, mp > 300 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.37 (s, 1H, 2′-H), 7.72 (d, *J* = 8.5 Hz, 2H, Ar–H), 6.83 (d, *J* = 8.5 Hz, 2H, Ar–H), 5.38 (d, *J* = 4.9 Hz, 1H, 6-H), 3.60–3.51 (m, 1H, 3α-H), 1.20 (s, 3H, 18-H), 1.18–1.09 (m, 5H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 180.4, 156.1, 154.4, 149.1, 143.3, 141.4, 131.5, 121.3, 120.7, 118.4, 114.2, 71.6, 56.2, 50.3, 46.3, 42.2, 37.1, 36.8, 33.0, 31.6, 31.2, 31.1, 29.7, 20.5, 19.5, 17.1. HRMS (ESI): *m*/*z* cacld. for $C_{28}H_{34}N_5O$ $(M+H)^+$, 456.2763; found, 456.2761.

2.3. Bioactivity

All the synthesized heterosteroids were subjected to in vitro cytotoxic evaluation against PC-3 (human prostatic carcinoma), MCF-7 (human breast carcinoma) and EC9706 (human esophageal carcinoma) cell lines. The cell lines were cultured in RPMI 1640 medium with 10% fetal bovine serum, 10 U penicillin and 100 μg/mL streptomycin at 37 °C with 5% CO_2 in a humidified incubator. The anticancer potency of test compounds was measured using the 3-(4,5-dimethylthizao1-2-y1)-2,5-diphenyltetrazolium bromide (MTT) assay.

For the test procedure, cells were harvested by trypsinization, washed, counted and distributed to wells of 96-well plates (about 6000 cells per well) in 200 μL of RPMI 1640 culture medium. For each sample, eight serial twofold dilutions were prepared in DMSO. After 24 h of incubation at 37 °C and 5% CO_2 to allow cell attachment, the cells were treated with various concentrations of test samples. Six replicate wells were set up for each experimental condition. The negative and blank control groups were set up at the same time. Plates were returned to the incubator for 72 h under same conditions. Thereafter, 20 μL of the MTT (0.5 mg/mL) solution was added to each well, and the cells were incubated for 4 h. The medium was removed, 150 μL of DMSO per well was added to dissolve the purple formazan crystals formed and plates were gently shaken for 10 min on a mechanical shaker. The optical density (OD) of solubilized formazan was measured at 570 nm with an automatic microplate reader. All the data of the experiment were calculated using the SPSS 16.0 software and were expressed as the IC_{50} (μM) values (Table 1).

3. Results and discussion

3.1. Chemistry

In our previous work, we have described in detail the protocol for the synthesis of 7′-aryl-androstano[17,16-d][1,2,4]triazolo[1,5-a]pyrimidines through the condensation reaction of the intermediates 16-arylidene-17-ketosteroids with 3-amino-1,2,4-triazole (3-AT) in presence of *t*-BuOK [24] (Scheme 1). These intermediates (**1**, **3**) containing the aromatic α,β-unsaturated ketone moiety were prepared by Claisen-Schmidt condensation of 4-aza-androstane-3,17-dione or dehydroepiandrosterone (DHEA) with various aromatic aldehydes catalyzed by KF/Al_2O_3. We then applied the protocol to the preparation of several new substituted aryl derivatives, including four 7′-heteroaryl-substituted ones (**2k**, **2l**, **2m** and **4i**). All the synthesized hetero- steroids were characterized by ^1H, ^{13}C NMR and mass spectra, and the structure of **2b** was further established by X-ray analysis (Fig. 1).

Surprisingly, when the reaction of **3j** and 3-AT was performed under the same reaction conditions, the mass spectrum of the isolated product **4j** (*m*/*z* 455) was not consistent with the expected product **4j′** (*m*/*z* 485). This indicated that the 7′-substituent was *p*-aminophenyl group instead of *p*-nitrophenyl group in the product, which was also corroborated by the ^1H and ^{13}C NMR spectra. A plausible mechanism for the formation of **4j** is shown in Scheme 2. Under basic conditions, **3j** reacted with 3-AT to afford the intermediate **A** via aza-Michael addition and intramolecular cyclization reaction followed by elimination of H_2O. Subsequently, the dihydrotriazolopyrimidine **A** dehydrogenated under the reaction conditions and in presence of the oxidizing agent *p*-nitrophenyl in the molecular structure. Simultaneouly, the nitro group was reduced to amino group. This intramolecular oxidation-reduction was similar to Skraup reaction, in which nitrobenzene served as the oxidizing agent to remove the hydrogen of dihydroquinoline and itself was reduced to aniline [25]. However, the reaction of **1c** and 3-AT afforded the normal product **2c**, which is of interest for further investigation.

Table 1
The in vitro anticancer activity of synthesized compounds **2** and **4**.[a]

Compound	IC_{50} (μM)[b]		
	PC-3	MCF-7	EC9706
2a	>100	>100	>100
2b	35.15	30.16	75.72
2c	>100	>100	>100
2d	>100	>100	>100
2e	>100	>100	>100
2f	>100	>100	>100
2g	>100	>100	>100
2h	>100	87.51	>100
2i	18.02	19.80	60.84
2j	>100	>100	>100
2k	>100	>100	>100
2l	>100	>100	95.44
2m	49.22	32.60	>100
2n	13.95	8.84	14.95
2o	>100	>100	>100
4a	>100	>100	>100
4b	32.59	31.99	>100
4c	17.57	>100	25.50
4d	10.14	20.87	>100
4e	12.01	>100	62.22
4f	7.29	19.67	22.24
4g	>100	>100	>100
4h	>100	>100	>100
4i	>100	>100	>100
4j	25.96	29.45	>100

PC-3: human prostatic carcinoma, MCF-7: human breast carcinoma, EC9706: human esophageal carcinoma.

[a] The results are the mean value of six replicate determinations.

[b] IC_{50} represents the concentration of compound that is required for 50% inhibition using the MTT assay.

3.2. Biology

All the synthesized novel steroidal triazolopyrimidines derivatives were evaluated for their cytotoxic activity in vitro against PC-3 (human prostatic carcinoma), MCF-7 (human breast carcinoma) and EC9706 (human esophageal carcinoma) cell lines. The inhibition of test compounds was determined using the MTT assay. The anticancer activity was indicated in terms of IC_{50} (μM) value calculated by the SPSS 16.0 software and the results were presented in Table 1.

As shown in Table 1, some of the test compounds showed promising anticancer activity for certain cancer cell lines in vitro. It was evident from the data that the changes of substituents on the phenyl ring had a significant influence on the cytotoxicity. When the phenyl rings were replaced by heteroaromatic rings, compound **2k**, **2l**, **2m** and **4i** did not improve the inhibitory activity. For PC-3 cells, the most highly active compound was **4f** with a *p*-isopropyl group on the phenyl ring, which had an IC_{50} value of 7.29 μM. Compounds **2n**, **4d** and **4e** also displayed remarkable inhibition of PC-3 cells with the IC_{50} of 13.95, 10.14 and 12.01 μM, respectively. Compound **2n** with an *o*-phenoxyphenyl group exhibited significant cytotoxic activity (8.84 μM) in comparison with the other test compounds against MCF-7 cells, which was also the most effective one against EC9706 cells (14.95 μM) among these compounds. It was noteworthy that the compounds containing the same substituents in two different series exhibited similar anticancer activity. For example, both of compounds **2i** and **4f** showed potent anticancer activity against the three human cell lines indicating the high degree of selectivity of these *p*-isopropylphenyl compounds. Similar activity was also found for the compounds **2n** and **4e**, both of which contained the *o*-phenoxyphenyl

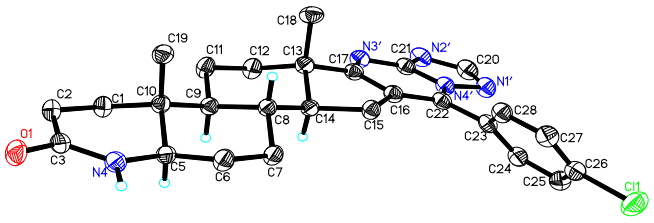
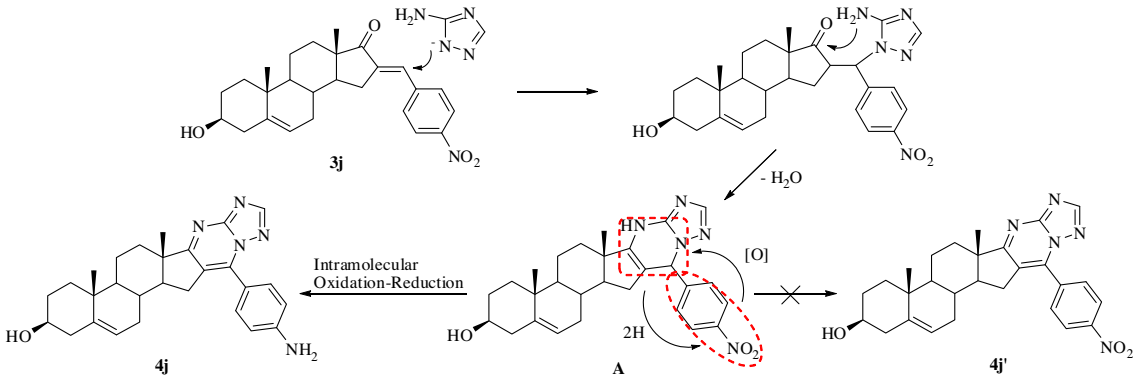


Fig. 1. X-ray crystal structure of **2b**.



Scheme 2. Proposed mechanism for the formation of unexpected product **4j**.

groups. The preliminary results revealed that the *p*-isopropyl and *o*-phenoxy on the phenyl rings were favorable structural moiety to retain the anticancer activity.

4. Conclusion

In summary, we have developed a facile approach for the preparation of novel 7′-aryl-androstano[17,16-d][1,2,4]triazolo[1,5-a]pyrimidines derivatives. All the synthesized novel compounds were evaluated for their anticancer activity in vitro against PC-3, MCF-7 and EC9706 cell lines. The preliminary results exhibited that the substituents on the phenyl ring remarkably influenced the cytotoxicity. The three compounds namely **2i**, **2n** and **4f** were found to possess potent activity against the three human cell lines. Guided by these initial findings, further modifications of these heterosteroids and research on their possible mechanism of inhibiting proliferation of cancer cell lines are underway.

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