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Synthesis and Biological Evaluation of Novel Steroidal 5α,8α-Endoperoxide Derivatives with Aromatic Hydrazone Side Chain as Potential Anticancer Agents

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Abstract—Seven new steroidal 5α,8α-endoperoxide derivatives with C-17 aromatic hydrazone side chain were synthesized. Structures of the synthesized compounds were characterized by IR, ^{1}H NMR, ^{13}C NMR, and mass spectrometry. Anti-proliferative activities of the synthesized compounds were evaluated in vitro by the MTT method. Among the seven compounds, 5α,8α-epidioxy-17-(4-chloro-benzylidene)-hydrazonoandrost-3β-ol showed the strongest anti-proliferative activity against three human cancer cell lines (MCF-7, HepG2, and SK-Hep1).

Keywords: steroid, ergosterol peroxide, dehydroepiandrosterone, dihydrazone, antitumor activity

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INTRODUCTION

Nowadays, natural products have attracted extensive attention in health promotion and disease treatment, including cancer therapy. Natural product-based drug discovery is a major route leading to developing therapeutic drugs for various disease [1]. Ergosterol peroxide (5α,8α-epidioxyergosta-6,22-diene-3β-ol, EP; Fig. 1), is a metabolite of a series of natural sterol endoperoxide derivatives; it has been isolated from many kinds of medicinal fungi [2–4]. Plenty of studies have been reported indicating that ergosterol peroxide can inhibit cancer cells growth through cytotoxicity or anti-angiogenesis [5–11].

Steroidal compounds have always been widely applied in the field of medicine as anti-inflammatory, contraceptive, diuretic, and anticancer agents [12–14]. The special skeleton features of steroidal molecules provide additional fascination for us to design and synthesize new entities with the potential to become promising drugs [15, 16]. On the other hand, hydrazine and its analogues have also been demonstrated to exhibit wide range of activities, including antibacterial, antivirus, and antitumor ones [17–19]. Especially, some novel steroidal hydrazone analogues also presented obviously pharmaceutical activities [20–25].

In the present work, as a part of our search for novel potential anticancer agents related to peroxide steroidal derivatives [26–28], we report the synthesis of novel steroidal endoperoxide derivatives that contain a 5α,8α-peroxy bond and dihydrazone unit attached to C-17 of the *D*-ring system as side-chain. Anti-proliferative activities of the derivatives were evaluated against three human cancer cell lines in vitro.

RESULTS AND DISCUSSION

The general procedure for the synthesis of steroidal 5α,8α-endoperoxide derivatives with C-17 aromatic hydrazone is shown in Scheme 1. Dehydroepiandrosterone (**I**) as starting material was acetylized with acetic anhydride to give compound (**II**), which then underwent bromination and debromination with NBS, *n*-Bu_4NBr, and *n*-Bu_4NF to afford $\Delta^{5,7}$-diene acetate (**III**). Subsequently, compound (**III**) was converted to compound (**IV**) via deacetylation reaction

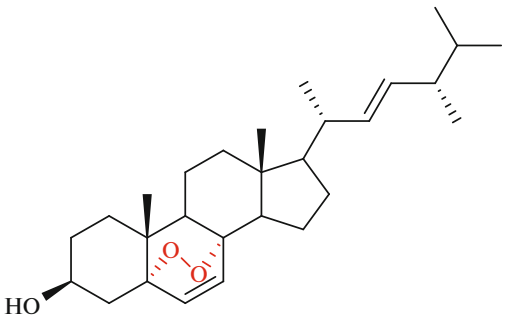

Fig. 1. Ergosterol peroxide (EP).

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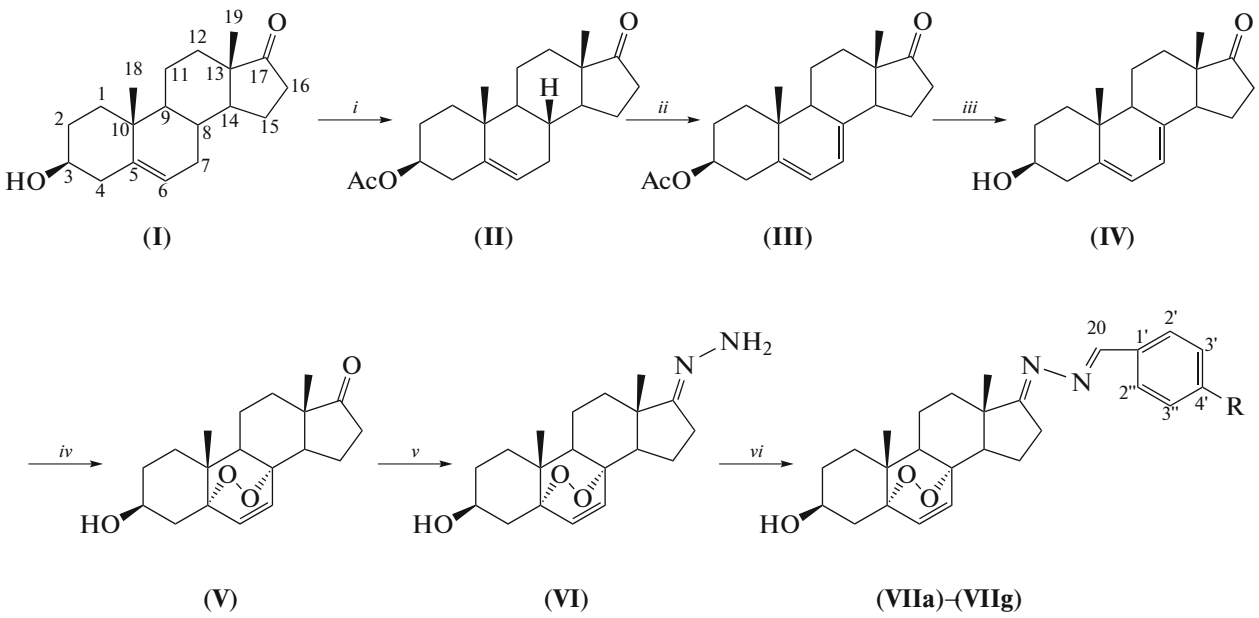
with potassium hydroxide in 30% overall yield from compound (**I**). The key compound (**IV**) was converted to endoperoxide (**V**) through photooxidation reaction with eosin as photosensitizer. Then, the reactions of compound (**V**) with hydrazine hydrate yielded the corresponding steroidal hydrazine (**VI**). At last, the reaction of steroidal hydrazine (**VI**) with different aromatic aldehydes gave our target compounds (**VIIa**–**g**).



(**VII**) R =4-H (**a**), 4-F (**b**), 4-Clr (**c**), 4-Br (**d**), 4-*t*-butyl (**e**), 4-CN (**f**), 4-OCH_3 (**g**)

Scheme 1. Synthesis of novel 5α,8α-peroxide steroid derivatives (**VIIa**–**g**). Reagents and conditions: (*i*) Ac_2O, pyridine, DCM, rt; (*ii*) cyclohexane, NBS, reflux, 1 h; (*iii*) NaOMe, MeOH, reflux, 1 h; Bu_4NF, THF, rt, 12 h; (*iv*) pyridine, O_2, light, 0°C, 0.5 h; (*v*) NH_2NH_2–H_2O, EtOH, 45°C,1 h; (*vi*) EtOH, AcOH, aromatic aldehydes, rt, 1–2 h

The structures of compounds (**VIIa**–**g**) were confirmed by IR, ^{1}H NMR, ^{13}C NMR, and mass spectrometry. Taking compound (**VIIa**) as a typical example, in the ^{1}H NMR spectrum, the downfield chemical shifts of C6-H at 6.54 ppm and C7-H at 6.32 ppm demonstrate a formation of 5α,8α-peroxybond and 6-CH=CH-7 bond. The stereochemistry of the peroxide was assigned by comparison of the ^{1}H NMR and X-ray crystal structure of ergosterol peroxide [26]. In addition, the resonances of C20-H shown at 8.28 ppm demonstrate formation of the –C=N–N=CH– bond in compound (**VIIa**), which could also be demonstrated in the IR spectrum of compound (**VIIa**): the absorption at 1654, 1611, and 1479, corresponding to the –C=N–N=C– bond is observed.

Compounds (**VIIa**–**g**) and EP were evaluated for their anti-proliferative activities against human tumor cell lines (MCF-7, HepG2, SK-Hep1) by MTT assay; cisplatin was used as a positive control. The anti-proliferative activity data of compounds (**VIIa**–**g**) are summarized by IC_{50} values in Table 1.

Compared to the parent EP, most of compounds (**VIIa**–**g**) possess significant anti-proliferative activity against all three tested cancer cell lines. Probably, the electron-withdrawing properties of the substituents in the side chain of the compounds enhanced the anti-proliferative activity. Compound (**VIIc**) with 4-Cl–C_6H_4 at C-17 side chain displayed the strongest anti-proliferative activity against human hepatocellular carcinoma cell lines.

In summary, we have successfully prepared a series of steroidal 5α,8α-endoperoxide derivatives with various aromatic hydrazone substitutes. All the synthesized compounds have been investigated for their in vitro anti-proliferative activities. From the activity studies, compound (**VIIc**) was shown to possess significant anti-proliferative activity against the tested cancer cell lines. The results showed that the substituent changes to the side chain of EP could serve as a feasible launch point for design of new steroidal agents.

EXPERIMENTAL

Reagents were used without further purification. The ^{1}H and ^{13}C NMR spectra (δ, ppm, *J*, Hz) were recorded using the Bruker Avance DRX400 spectrometer (400 and 100 MHz, respectively) in $CDCl_3$ or DMSO-d_6. Tetramethylsilane was used as an internal standard. Melting temperatures were determined on an MP120 auto point apparatus. The mass spectra of

Table 1. In vitro anti-proliferative activity data of compounds (**VIIa**–**g**)

Compd	IC_{50}, μM[1,2]		
	HepG2	SK-Hep1	MCF-7
(**VIIa**)	49.34 ± 0.49	50.43 ± 0.88	>60
(**VIIb**)	11.74 ± 0.32	16.92 ± 0.53	32.45 ± 0.50
(**VIIc**)	9.24 ± 0.11	13.55 ± 0.24	21.32 ± 0.06
(**VIId**)	13.22 ± 0.26	10.14 ± 0.32	20.76 ± 0.32
(**VIIe**)	35.07 ± 0.18	37.20 ± 0.62	57.61 ± 0.88
(**VIIf**)	18.47 ± 0.14	27.17 ± 0.19	39.45 ± 0.44
(**VIIg**)	34.25 ± 0.53	40.20 ± 0.40	>60
EP	15.78 ± 0.30	17.40 ± 0.24	24.43 ± 0.26
Cisplatin	0.65 ± 0.04	2.42 ± 0.06	6.35 ± 0.15

[1] Data are the mean ± SD of three experimental results.
[2] Human liver carcinoma cell line (HepG2, SK-Hep1), human breast adenocarcinoma cell line (MCF-7).

all compounds were tested on an Esquire 6000 mass spectrometer in ESI mode. The IR spectra (ν_{max}, cm^{-1}; KBr) were registered on the Equinox 55 Fourier transform infrared spectrometer (Bruker, Germany). Flash chromatography in the experiments was performed using silica gel (400 mesh).

3β-Acetoxyandrosta-5-en-17-one (II). To a suspension of dehydroepiandrosterone (**I**) (14.4 g, 0.05 mol) in CH_2Cl_2–pyridine, 4 : 1 (80 mL), Ac_2O (6 mL, 0.07 mol) was added over 15 min. The mixture was stirred for 8 h at room temperature and then water (50 mL) was added. The resulting mixture was extracted with EtOAc (2 × 80 mL). The combined organic phase was washed with $NaHCO_3$ saturated aqueous (2 × 50 mL) and brine (2 × 50 mL) and dried over anhydrous Na_2SO_4. Evaporation of solvent gave crude product (**II**) as a white solid (16 g). Yield 97.6%, mp 168–170°C. ^{1}H NMR ($CDCl_3$): 5.40 (1H, d, *J* 5.0, H6), 4.65–4.54 (1H, m, H7), 2.45 (1H, m, H9), 2.34 (2H, d, *J* 7.8, H16), 2.11 (2H, m, 2H, H11), 2.05 (3H, s, OAc), 1.93–1.90 (2H, m, H12), 1.87–1.81 (2H, m, H4), 1.71–1.62 (4H, m, H1, H2), 1.55–1.49 (2H, m, H15), 1.03 (3H, s, H18), 1.00 (1H, d, *J* 3.8, H14), 0.90 (3H, s, H19). Mass spectrum (ESI) *m/z*: 353.9 $[M + Na]^+$.

3β-Acetoxyandrosta-5,7-diene-17-one (III). Solution of intermediate (**II**) (16 g, 0.05 mol) in cyclohexane (70 mL) was heated to 70°C for 20 min and then NBS (12.5 g, 0.07 mol) was added. The mixture was refluxed for 1.5 h. The mixture was diluted with 150 mL water. The precipitate was collected and washed with water. Then the solid was dissolved in DCM, washed with brine, and dried over anhydrous Na_2SO_4. Evaporation of solvent gave a light brown solid (17.5 g, 85%). The mixture of the crude bromide (17.5 g, 0.043 mol) prepared above was added into tetrabutylammonium fluoride (45 mL 1.0 M solution in THF, 0.07 mol, 1.5 equiv.). The resulting brown solution was stirred for 10 h, and then followed by rotary evaporation under 45°C to a yellow solid. The solid was diluted with DCM (100 mL), washed with water (2 × 50 mL), and dried over anhydrous Na_2SO_4. Purification by column chromatography column gave a pale yellow solid as compound (**III**) (5.8 g). Yield 35%, mp 112–115°C. ^{1}H NMR ($CDCl_3$): 5.6 (1H, s, H6), 5.57 (1H, d, *J* 3.2, H7), 4.7 (1H, m, C3-αH), 2.56–2.49 (2H, m, H16), 2.38 (1H, d, *J* 12.5, H9), 2.24–2.17 (2H, m, H11), 2.07 (3H, s, OAc), 1.97–1.92 (2H, m, H12), 1.74 (2H, d, *J* 4.8, H4), 1.70–1.58 (4H, m,H1, H2), 1.40–1.34 (2H, m, H15), 1.27 (1H, s, H14), 0.99 (3H, s, H18), 0.83 (3H, s, H19). MS (ESI) *m/z*: 351.7 $[M + Na]^+$.

3β-Hydroxyandrosta-5,7-diene-17-one (IV). To a suspension of intermediate (**III**) (11 g, 0.034 mol) in MeOH (100 mL) 25% (wt) NaOMe in MeOH (10 mL) was added. The mixture was refluxed for 1 h, then 100 mL of water was added into the mixture over 0.5 h and it was continually stirred for 1 h. The precipitate was collected, washed with water (50 mL), and dried under high vacuum at 40°C to get a brown solid crude product. Purification by column chromatography to give a pale solid as intermediate (**IV**) (9.3 g). Yield 96%, mp 157–159°C. ^{1}H NMR ($CDCl_3$): 6.00 (1H, d, *J* 9.8, H6), 5.70 (1H, d, *J* 9.8, H7), 4.30 (1H, t, *J* 7.9, OH), 3.76–3.60 (1H, m, C3-αH), 1.03 (3H, s, H18), 0.96 (3H, s, H19). MS (ESI) *m/z*: 309.9 $[M + Na]^+$.

3β-Hydroxy-5α,8α-epidioxyandrostan-17-one (V). To a solution of intermediate (**IV**) (140 mg) in pyridine (20 mL) eosin (1 mg) was added in a quartz tube. The mixture was kept in a water-cooled bath and vigorously stirred by bubbling in the oxygen. At the same time, the mixture was irradiated with an iodine tungsten lamp (220 V, 500 W) for 0.5 h. The solution was poured into ice-cold water (20 mL) and extracted with ethyl acetate (2 × 50 mL). The combined organic phase was washed with brine (2 × 50 mL) and dried over anhydrous Na_2SO_4. Evaporation of solvent and then purification by column chromatography (40% EA in PE) gave (**V**) as white needles (101.4 mg, 63%), mp 167.0–168.1°C. ^{1}H NMR ($CDCl_3$): 6.51 (1H, d, *J* 8.5, H6), 6.33 (1H, d, *J* 8.6, H7), 4.00 (1H, s, OH), 3.25–2.12 (1H, m, C3-αH), 2.58–2.47 (1H, m, H9), 2.24–2.12 (2H, m, H16), 2.08–2.00 (2H, m, H11), 1.86–1.81 (2H, m, H12), 1.72–1.62 (1H, m, H4), 1.58–1.54 (4H, m, H1, H2), 1.54–1.49 (1H, m, H14), 1.38–1.22 (2H, m, H15), 1.00 (3H, s, H18), 0.92 (3H, s, H19). MS (ESI) *m/z*: 319.19 $[M + H]^+$.

5α,8α-Epidioxy-17-hydrazonoandrostan-3β-ol (VI). To a solution of intermediate (**V**) (2.0 g, 6.7 mmol) in EtOH (50 mL) 85% hydrazine hydrate (3 mL) was added. The mixture was heated to 45°C and stirred for 2 h. The white solid was collected by filtration. Intermediate (**VI**) was obtained as white solid (2.1 g) after recrystallizing in MeOH. White powder, yield 87%,

mp 182.5–185.6°C. ^{1}H NMR (DMSO-d_6): 6.50 (1H, d, *J* 7.6, H6), 6.32 (1H, d, *J* 7.0, H7), 5.36 (2H, d, *J* 10.4, NH_2), 4.68 (1H, m, OH), 3.16 (1H, m, C3-αH), 0.99 (3H, s, H18), 0.89 (3H, s, H19). MS (ESI) *m/z*: 333.1 $[M + H]^+$.

Synthesis of novel derivatives (VIIa–g). General procedure. To a suspension of intermediate (**VI**) (0.2 mmol) in anhydrous EtOH (30 mL) various aldehydes (0.2 mmol) were added. The mixture was continually stirred under 50°C for 2–3 h until no starting material was left. Then the solvent was evaporated by rotary evaporation. The residue was purified by flash chromatography to afford compounds (**VIIa–g**).

5α,8α-Epidioxy-17-(benzylidene)hydrazonoandrost-3β-ol (VIIa). White powder. Yield 61%, mp 72.5–74.4°C. IR: 1654, 1611, 1479 (C=N–N=C). ^{1}H NMR ($CDCl_3$): 8.28 (1H, s, H20), 7.54 (2H, d, *J* 5.7, H2', H2''), 7.42 (3H, m, H3', H3'', H4'), 6.54 (1H, d, *J* 8.4, H6), 6.32 (1H, d, *J* 8.2, H7), 4.08 (1H, dd, *J* 11.3, 5.6, OH), 3.99–3.96 (1H, m, C3-αH), 2.77–2.65 (2H, m, H16), 2.20–2.10 (1H, m, H9), 2.05–1.80 (4H, m, H11, H12), 1.80–1.62 (4H, m, H4, H2), 1.48–1.43 (2H, m, H1), 1.40–1.25 (2H, m, H15), 1.11 (3H, m, H18), 1.05 (1H, m, H14), 0.93–0.88 (3H, m, H19). ^{13}C NMR ($CDCl_3$): 183.1 (C17), 162.2 (C20), 141.1 (C5), 134.6 (C4'), 130.6 (C2'), 128.8 (C2''), 128.7 (C3'), 128.5 (C3''), 128.1 (C1'), 121.0 (C6), 71.6 (C3), 53.7 (C14), 50.4 (C9), 44.3 (C13), 42.2 (C4), 37.2 (C1), 36.8 (C10), 33.9 (C12), 31.6 (C8), 31.5 (C7), 31.3 (C2), 28.0 (C15), 23.3 (C16), 20.6 (C11), 19.5 (C19), 16.5 (C18). MS (ESI) *m/z*: 421.2 $[M + H]^+$.

5α,8α-Epidioxy-17-(4-fluoro-benzylidene)hydrazonoandrost-3β-ol (VIIb). White powder. Yield 70%, mp 72.3–74.8°C. IR: 1647, 1601, 1508 (C=N–N=C). ^{1}H NMR ($CDCl_3$): 8.27 (1H, s, H20), 7.75–7.73 (2H, m, H2', H2''), 7.09–7.15 (2H, m, H3', H3''), 6.55 (1H, d, *J* 8.2, H6), 6.33 (1H, d, *J* 8.0, H7), 4.01 (1H, m, OH), 3.55 (1H, m, C3-αH), 2.78–2.63 (2H, m, H16), 2.15 (1H, m, H9), 2.05–2.04 (2H, m, H11), 1.96–1.93 (2H, m, H12), 1.89–1.86 (2H, m, H4), 1.77–1.63 (4H, m, H2), 1.63–1.56 (2H, m, H1), 1.36–1.27 (2H, m, H15), 1.13 (3H, m, H18), 1.05 (1H, s, H14), 0.94 (3H, s, H19). ^{13}C NMR ($CDCl_3$): 181.2 (C17), 165.5 (C20), 160.8 (C5), 156.7 (C4'), 136.1 (C2'), 130.1 (C3',C3''), 115.8 (C1'), 115.7 (C6), 82.3 (C3), 66.2 (C14), 51.5 (C9), 49.1 (C13), 45.9 (C4), 37.1 (C1), 36.9 (C10), 34.7 (C12), 33.9 (C8), 30.1 (C7), 29.7 (C2), 27.8 (C15), 23.0 (C16), 20.0 (C11), 18.1 (C19), 16.7 (C18). MS (ESI) *m/z*: 439.2 $[M + H]^+$.

5α,8α-Epidioxy-17-(4-chloro-benzylidene)hydrazonoandrost-3β-ol (VIIc). Yellowish powder. Yield 73%, mp 80.5–81.3°C. IR: 1654, 1608, 1489 (C=N–N=C). ^{1}H NMR ($CDCl_3$): 8.27 (1H, s, H20), 7.71–7.69 (2H, d, *J* 8.0, H2', H2''), 7.42–7.39 (2H, d, *J* 8.4, H3', H3''), 6.55 (1H, d, *J* 8.2, H6), 6.33 (1H, d, *J* 8.2, H7), 4.04–3.96 (1H, m, OH), 3.65 (1H, m, C3-αH), 2.78–2.66 (2H, m, H16), 2.08–2.04 (1H, m, H9), 2.01–1.95 (2H, m, H11), 1.89–1.86 (2H, m, H12), 1.78–1.74 (2H, m, H4), 1.62–1.56 (4H, m, H1, H2), 1.38–1.27 (2H, m, H15), 1.12 (3H, s, H18), 1.06 (1H, s, H14), 0.95 (3H, s, H19). ^{13}C NMR ($CDCl_3$): 181.3 (C17), 156.6 (C20), 136.7 (C5), 136.4 (C4'), 132.9 (C2'), 130.1 (C2''), 129.3 (C3'), 129.0 (C3''), 116.0 (C1'), 115.8 (C6), 82.3 (C3), 66.3 (C14), 51.5 (C9), 49.1 (C13), 46.0 (C4), 37.2 (C1), 36.8 (C10), 34.7 (C12), 33.9 (C8), 30.1 (C7), 29.5 (C2), 27.8 (C15), 23.0 (C16), 20.3 (C11), 18.2(C19), 16.8 (C18). MS (ESI) *m/z*: 455.2 $[M + H]^+$.

5α,8α-Epidioxy-17-(4-bromo-benzylidene)hydrazonoandrost-3β-ol (VIId). White crystals. Yield 60%, mp 76.4–78.7°C. IR: 1654, 1605, 1458 (C=N–N=C). ^{1}H NMR ($CDCl_3$): 8.23 (1H, s, H20), 7.62 (2H, d, *J* 8.0, H2', H2''), 7.54 (2H, d, *J* 7.8, H3', H3''), 6.53 (1H, d, *J* 8.4, H6), 6.31 (1H, d, *J* 8.4, H7), 4.15–4.05 (1H, m, OH), 4.03–3.89 (1H, m, C3-αH), 2.82–2.56 (2H, m, H16), 2.13–2.12 (1H, m, H9), 2.05–1.95 (2H, m, H11), 1.94–1.75 (2H, m, H12), 1.74–1.74 (4H, m, H1, H2), 1.57–1.50 (2H, m, H4), 1.35–1.20 (2H, m, H15), 1.10 (3H, s, H18), 1.05 (1H, s, H14), 0.93 (3H, s, H19). ^{13}C NMR ($CDCl_3$): 181.3 (C17), 156.7 (C20), 136.1 (C5), 131.9 (C4'), 130.9 (C2'), 130.0 (C2''), 129.9 (C3'), 129.5 (C3''), 128.8 (C1'), 115.9 (C6), 82.4 (C3), 66.3 (C14), 51.5 (C9), 49.1 (C13), 46.0 (C4), 37.2 (C1), 36.8 (C10), 34.6 (C12), 33.9 (C8), 30.1 (C7), 29.6 (C2), 27.8 (C15), 23.0 (C16), 20.3 (C11), 19.2 (C19), 18.1 (C18). MS (ESI) *m/z*: 500.2, 502.2 $[M + H]^+$.

5α,8α-Epidioxy-17-(4-(*t*-butyl)-benzylidene)hydrazonoandrost-3β-ol (VIIe). White powder. Yield 87%, mp 91.3–92.2°C. IR: 1651, 1608, 1458 (C=N–N=C). ^{1}H NMR ($CDCl_3$): 8.25 (1H, d, *J* 7.0, H20), 7.69 (2H, dd, *J*8.1, 4.5, H2', H2''), 7.44 (2H, dd, *J* 8.2, 2.9, H3', H3''), 6.55 (1H, d, *J* 8.2,H6), 6.33 (1H, d, *J*8.4,H7), 4.04–3.97 (1H, m, OH), 3.81–3.75 (1H, m, C3-αH), 2.74–2.60 (2H, m, H16), 2.20–2.05 (1H, m, H9), 1.99–1.95 (2H, m, H11), 1.87–1.85 (4H, m, H1, H2), 1.78–1.77 (2H, m, H12), 1.68–1.66 (2H, m, H4), 1.60–1.53 (2H, m, H15), 1.36 (9H, s, 4'-CH_3), 1.12 (1H, s, H14), 1.05 (3H, m, H18), 0.94 (3H, s, H19). ^{13}C NMR ($CDCl_3$): 180.3 (C17), 157.5 (C20), 136.1 (C5), 131.7 (C4'), 130.1 (C2'), 129.9 (C3'), 129.6 (C3''),127.9 (C2''), 125.6 (C1'),116.0 (C6), 82.3 (C3), 66.1 (C14), 51.5 (C9), 49.1 (C13), 45.9 (C4), 37.2 (C1), 36.8 (C10), 34.9 (C12), 34.0 (C8), 31.3 (C-Bu), 30.1 (C7), 27.8 (C15), 23.0 (C16), 20.3 (C11), 18.2 (C19), 18.0 (C18). MS (ESI) *m/z*: 477.3 $[M + H]^+$.

5α,8α-Epidioxy-17-(4-cyano-benzylidene)hydrazonoandrost-3β-ol (VIIf). Yellowish powder. Yield 81%, mp 84.6–85.8°C. IR: 1653, 1617, 1472 (C=N–

N=C). ^{1}H NMR ($CDCl_3$): 8.29 (1H, s, H20), 7.87–7.85 (2H, d, *J* 8.0, H2', H2''), 7.72–7.70 (2H, d, *J* 8.2, H3', H3''), 6.56–6.53 (1H, d, *J* 8.4, H6), 6.35–6.33 (1H, d, *J* 8.4, H7), 4.06–3.99 (1H, m, OH), 3.85–3.80 (1H, m, C3-αH), 2.77–2.64 (2H, m, H16), 2.20–2.15 (1H, m, H9), 2.07–2.04 (1H, m, H11), 2.01–1.94 (2H, m, H1, H2), 1.78–1.74 (2H, m, H12), 1.61–1.56 (2H, m, H4), 1.35–1.26 (2H, m, H15), 1.15 (3H, s, H18), 1.06 (1H, s, H14), 0.95 (3H, s, H19); ^{13}C NMR ($CDCl_3$): 182.0 (C17), 156.7 (C20), 138.5 (C5), 136.2 (C3'), 132.4 (C3''), 129.9 (C2', C2''), 128.4 (C1'), 118.4 (CN), 113.8 (C6), 82.3 (C3), 66.3 (C14), 51.5 (C9), 49.1 (C13), 46.0 (C4), 37.2 (C1), 36.8 (C10), 34.7 (C12), 33.9 (C8), 30.1 (C7), 27.9 (C15), 23.0 (C16), 20.3 (C11), 18.2 (C19), 18.0 (C18). MS (ESI) *m/z*: 446.2 $[M + H]^+$.

5α,8α-Epidioxy-17-(4-methoxy-benzylidene)hydrazonoandrost-3β-ol (VIIg). White powder. Yield 85%, mp 84.2–84.9°C. IR: 1653, 1605, 1512 (C=N–N=C). ^{1}H NMR ($CDCl_3$): 8.37 (1H, s, H20), 7.72–7.70 (2H, d, *J* 8.0, H2', H2''), 6.95–6.93 (2H, d, *J* 8.4, H3', H3''), 6.56–6.54 (1H, d, *J* 8.6, H6), 6.33–6.31 (1H, d, *J* 8.2,H7), 4.04–3.99 (1H, m, OH), 3.86 (3H, m, OCH_3), 3.82–3.80 (1H, m, C3-αH), 2.82–2.65 (2H, m, H16), 2.18–2.15 (1H, m, H9), 2.08–2.04 (1H, m, H11), 2.00–1.91 (2H, m, H1, H2), 1.89–1.86 (2H, m, H12), 1.76–1.67 (4H, m, H12), 1.63–1.59 (2H, m, H4), 1.56–1.52 (2H, m, H15), 1.15 (3H, s, H18), 1.05–1.04 (1H, m, H14), 0.94 (3H, s, H19). ^{13}C NMR ($CDCl_3$): 180.5 (C17), 161.7 (C4'), 157.6 (C20), 136.1 (C5), 130.1 (C3', C3''), 129.7 (C2', C2''), 127.2 (C1'), 114.2 (C6), 82.3 (C3), 66.2 (C14), 55.3 (OCH_3), 51.5 (C9), 49.1 (C13), 45.9 (C4), 37.2 (C1), 36.8 (C10), 34.7 (C12), 34.0 (C8), 30.1 (C7), 27.8 (C15), 23.0 (C16), 20.3 (C11), 18.2 (C19), 18.0 (C18). MS (ESI) *m/z*: 451.2 $[M + H]^+$.

In Vitro Anti-Proliferative Activity

Cytotoxicity activities of all synthesized compounds were tested in the human HepG2, SK-Hep1, and MCF-7 cancer cell lines by MTT assay. Compounds were solubilized in DMSO at gradient concentrations from 5 to 60 μM. Cells were inoculated into 96-well plates for 24 h. The cells were treated with gradient concentrations of compounds for 48 h and then 10 μL of MTT was added onto each well for 2 h. The formazan dye product was measured by the absorbance at 490 nm on a Spectra Max 340 microplate reader. The IC_{50} values of compounds were derived by SPSS nonlinear regression analysis.

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COMPLIANCE WITH ETHICAL STANDARDS

The work has no studies involving humans or animals as subjects of the study.

Conflict of Interests

Authors declare that they have no conflicts of interests.

REFERENCES

1. Bu, M., Yang, B.B., and Hu, L.M., *Curr. Med. Chem.*, 2016, vol. 23, pp. 383–405.
2. Nowak, R., Drozd, M., Mendyk, E., Lemieszek, M., Krakowiak, O., Kisiel, W., Rzeski, W., and Szewczyk, K., *Molecules*, 2016, vol. 21, p. E946.
3. Kim, D.H., Jung, S.J., Chung, I.S., Lee, Y.H., Kim, D.K., Kim, S.H., Kwon, B.M., Jeong, T.S., Park, M.H. Seoung, N.S., and Baek, N.I., *Arch. Pharm. Res.*, 2005, vol. 28, pp. 541–545.
4. Fang, S.T., Liu, X., Kong, N.N., Liu, S.J., and Xia, C.H., *Nat. Prod. Res.*, 2013, vol. 27, pp. 1965–1970.
5. Zhao, S., Ye, G., Fu, G., Cheng, J.X., Yang, B.B., and Peng, C., *Int. J. Oncol.*, 2011, vol. 38, pp. 1319–1327.
6. Han, J., Sohn, E.J., Kim, B., Kim, S., Won, G., Yoon, S., Lee, J., Kim, M.J., Lee, H., Chung, K., and Kim, S.H., *Cancer Cell Int.*, 2014, vol. 14, p. 117.
7. Zhu, R., Zheng, R., Deng, Y., Chen, Y., and Zhang, S., *Phytomedicine*, 2014, vol. 21, pp. 372–378.
8. Rhee, Y.H., Jeong, S.J., Lee, H.J. Lee, H.J., Koh, W., Jung, J.H., Kim, S.H., and Sung-Hoon, K., *BMC Cancer*, 2012, vol. 12, p. 28.
9. Kobori, M., Yoshida, M., Ohnishi-Kameyama, M., and Shinmoto, H., *Br. J. Pharmacol.*, 2007, vol. 150, pp. 209–219.
10. Wu, Q.P., Xie, Y.Z., Deng, Z., Li, X.M., Yang, W., Jiao, C.W., Fang, L., Li, S.Z., Pan, H.H., Yee, A.J., Lee, D.Y., Li, C., Zhang, Z., Guo, J., and Yang, B.B., *PLoS One*, 2012, vol. 7. e44579.
11. Li, X.M., Wu, Q.P., Bu, M., Hu, L.M., Du, W.W., Jiao, C.W., Pan, H.H., Sdiri, M., Wu, N., Xie, Y.Z., and Yang, B.B., *Oncotarget*, 2016, vol. 7, pp. 33948–33959.
12. Hanson, J.R, *Nat. Prod. Rep.*, 2010, vol. 27, pp. 887–899.
13. Shan, L.H., Liu, H.M., Huang, K.X., Dai, G.F., Cao, C., and Dong, R.J., *Bioorg. Med. Chem. Lett.*, 2009, vol. 19, pp. 6637–6639.
14. Yu, B., Zhang, E., Sun, X.N., Ren, J.L., Fang, Y., Zhang, B.L., Yu, D.Q., and Liu, H.M., *Steroids*, 2013, vol. 78, pp. 494–499.
15. Gogoi, S., Shekarrao, K., Duarah, A., Bora, T.C., Gogoi, S., and Boruah, R.C., *Steroids*, 2012, vol. 77, pp. 1438–1445.
16. Haubrich, B.A., *Molecules*, 2018, vol. 23, p. E2768.
17. Słomiak, K., Łazarenkow, A., Chęcińska, L., Kusz, J., Ochocki, J., and Nawrot-Modranka, J., *Molecules*, 2018, vol. 17, E2067.

18. Ke, S.Y., Wei, Y.H., Shi, L.Q., Yang, Q.Y., and Yang, Z.W., *Anticancer Agents Med. Chem.*, 2013, vol. 13, pp. 1291–1298.

19. Cui, J.G., Liu, L., Zhao, D.D., Gan, X., Huang, C.F., Xiao, Q., Qi, B.B., Yang, L., and Huang, Y.M., *Steroids*, 2015, vol. 95, pp. 32–38.

20. Brunel, J.M., Loncle, C., Vidal, N., Dherbomez, M., and Letourneux, Y., *Steroids*, 2005, vol. 70, pp. 907–912.

21. Merlani, M.I., Kemertelidze, E.P., Papadopoulos, K., and Men'shova, N.I., *Bioorg. Khim.*, 2004, vol. 30, pp. 552–557.

22. Mazoir, N., Benharref, A., Bailén, M., Reina, M., and González-Coloma, A., *Phytochemistry*, 2008, vol. 69, pp. 1328–1338.

23. Khan, S.A., Asiri, A.M., and Yusuf, M., *Eur. J. Med. Chem.*, 2009, vol. 44, pp. 2597–2600.

24. Mohamed, N.R., Elmegeed, G.A., Abd-ElMalek, H.A., and Younis, M., *Steroids*, 2005, vol. 70, pp. 131–136.

25. Li, H. and Yang, B.B., *Oncotarget*, 2012, vol. 3, pp. 1653–1668.

26. Bu, M., Cao, T.T., Li, H.X., Guo, M.Z., Yang, B.B., Zhou, Y., Zhang, N., Zeng, C.C., and Hu, L.M., *Steroids*, 2017, vol. 124, pp. 46–53.

27. Bu, M., Cao, T.T., Li, H.X., Guo, M.Z., Yang, B.B., Zeng, C.C., and Hu. L.M., *Chem. Med. Chem.*, 2017, vol. 12, pp. 466–474.

28. Bu, M., Cao, T.T., Li, H.X., Guo, M.Z., Yang, B.B., Zhou, Y., Zhang, N., Zeng, C.C., and Hu, L.M., *Bioorg. Med. Chem. Lett.*, 2017, vol. 27, pp. 3856–3861.