European Journal of Medicinal Chemistry 130 (2017) 1–14

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Research paper

Facile and efficient access to Androsten-17-(1′,3′,4′)-pyrazoles and Androst-17β-(1′,3′,4′)-pyrazoles via Vilsmeier reagents, and their antiproliferative activity evaluation *in vitro*

Jian Li [b, 1], Haibo Huo [c, 1], Rui Guo [b], Biao Liu [a], Longbo Li [d], Wenjia Dan [b], Xinmin Xiao [a], Jiwen Zhang [a, b], Baojun Shi [a, *]

[a] Key Laboratory of Botanical Pesticide R & D in Shaanxi Province, Northwest A&F University, Yangling 712100, Shaanxi, China
[b] Shaanxi Key Laboratory of Natural Products & Chemical Biology, School of Chemistry & Pharmacy, Northwest A&F University, Yangling 712100, China
[c] State Key Laboratory of Crop Stress Biology in Arid Area, College of Life Science Northwest Agriculture and Forestry University, Yangling 712100, China
[d] Department of Chemistry and Biochemistry, University of California, 1156 High Street, Santa Cruz, CA 95064, United States

ARTICLE INFO

Article history:
Received 20 December 2016
Received in revised form
10 February 2017
Accepted 12 February 2017
Available online 20 February 2017

Keywords:
Isopregnanolone
5,16-Pregnadienolone
Vilsmeier reaction
Steroidal phenylpyrazoles
Antiproliferative activity
Apoptosis

ABSTRACT

In this work, twenty-seven novel steroidal pyrazole derivatives were designed and effectively synthesized with two different commercially available staring material, Isopregnanolone **1** and 5,16-Pregnadienolone **7**, via the key intermediates, 17β-(4′-formyl)pyrazolylandrost-3β-yl formate and 17-(4′-formyl)pyrazolylandrost- 5,16-dienes-3β-yl formate, which were obtained from the cyclization of steroidal phenylhydrazone with Vilsmeier reagent catalyzed by phosphorous oxychloride followed by hydrolysis, then Borch reduction to afford the target derivatives under mild conditions. Structures of these compounds were identified by ^{1}H NMR, ^{13}C NMR and high resolution mass spectrometry. Based on our previous work, the cytotoxicity of these derivatives were evaluated by the SRB method against 293T cell lines and three cancer cell lines: A549, Hela and MCF-7. The results indicated that compounds **5b-d**, and **11a-e** exhibited moderate to high cytotoxic activities with IC_{50} values ranging from 0.62 to 7.51 μM. Among the eight hybrids, compound **11b**, with an ethyl amino and a dien-pregn moieties showed the highest potency, with an IC_{50} values of 0.87 μM and 0.53 μM for 293T cell lines and Hela cell lines, respectively. Some structure-activity relationships among the groups of the twenty-seven derivatives are discussed and identify several determinants important for the activity of these compounds. What's more, further molecular mechanism studies suggested that **11b** one of the most potent derivatives caused Hela cell lines apoptosis and arrested the cell cycle at S phase in a concentration dependent manner.

1. Introduction

Cancer, a chronic, uncontrolled, and pathological proliferation of abnormal cells, is the most burdensome disease known for the second death leading cause of death worldwide. About 12 million people are diagnosed with cancer and 7 million patients die of cancer annually. Furthermore, the global burden of cancer is increasing rapidly as the average age of the general population increases [1–3]. Despite advances in diagnosis and therapy, outcomes remain poor on account of the resistance against chemotherapy and targeted drugs which are as widespread as the use of these agents [4]. Another challenge for chemotherapy is lack of selectivity for general anticancer drugs destroy tumor cells as well as normal cells and often cause serious side-effects [5]. As a result, cures of cancer have to be versatile and sophisticated. Consequently, extensive efforts have been devoted to the development of novel anticancer medicines which will reduce limitations on cancer therapy.

In recent years, pyrazoles have attracted significant scientific attention due to their special skeleton and various bioactivities containing anti-inflammatory [6], antimicrobial [7,8], anticonvulsant [9], antidepressant [10], antimycobacterial [11], antioxidant [12], antiviral [13], insecticidal [14] and antitumor [15] activities. Presence of this nucleus in multiple pharmacological agents has made it an indispensible anchor for design and development of new pharmacological agents [16].

* Corresponding author.
E-mail address: shibaojun@nwsuaf.edu.cn (B. Shi).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.ejmech.2017.02.033
0223-5234/

Derivatives of steroids, compounds widely distributed in nature, can be either isolated from nature or synthesized. Steroidal derivatives possess diverse biological activities which play a pivotal role in the treatment of malignancies, and are treated as leading compounds in the discovery of novel anticancer drugs because they reduce systemic toxicity and improve specificity in cancer therapy [17–19]. Steroids bearing heterocycles (pyrazoles, pyrazolines, isoxazoles, isoxazolines, thiazoles) fused to the D-ring of the steroids backbone have been of pharmaceutical interest [20]. Synthetic of derivatives of steroidal pyrazoles have attracted a great deal of attention for the goals of developing leading compounds to withstand diseases [21,22].

Recently, our group [23] and the Eva Franck group [24] have demonstrated that the derivatives 17β-(1-phenyl-4-((ethylamino) methyl)-3-pyrazolyl)androst-5-ene -3β-ol and 17β-(1-phenyl-4-(hydroxymethyl)-3-pyrazolyl)androst-5,16-diene-3β-ol (Fig. 1) bearing a small hydrophilic group attached to 4 position of the pyrazole ring are the most potent compounds among the a series of derivatives. The former exhibited excellent cytotoxicity against A549 cell lines with an IC_{50} value of 0.91 μM. On the basis of our previous test results, we deduced that the hydrophilicity or configuration of the hybrids would dramatically affect their activities.

Base on the aforementioned findings and in the continuation of our ongoing research on 17-(4′-formyl)pyrazolylandrost derivatives related to their anticancer activities, herein we carried out the synthesis of two new series of steroidal pyrazole derivatives (Table 1) and evaluated their cytotoxicity on A549 cells (human alveolar adenocarcinoma cell lines), Hela cells (human cervical cancer cell lines), MCF-7 cells (human breast adenocarcinoma cell line), and 293T cells (human kidney cell line, transformed with large T antigen). Moreover, in order to gain a deeper insight into the mode of action of the title compounds onto the tumor cells some extra experiments, fluorescence microscopy, an annexin V/PI assay, and cell cycle evaluations, were carried out. For the most activate compound **11b**, the effect on the cell cycle and induction of apoptosis were performed against Hela cell lines to investigate its mechanism of action.

2. Results and discussion

2.1. Synthetic studies

The target 17-(4′-formyl)pyrazolylandrost-5,16-dienes-3β-ol and 17-(4′-formyl)pyrazolylandrost-3β-ol hybrids were synthesized from commercially available starting material (Isopregnanolone **1** and 5,16-Pregnadienolone **7**) as illustrated in Scheme 1a and b. The initial key intermediates **3** and **8** were prepared via the Vilsmeier reaction as per previously reported by our group with some modifications [23]. Compound **3**, bearing the formyl group at the 4-position of the pyrazole and formate moiety at the 3-position of the steroid was synthesized from compound **2** which could be easily prepared by condensation of Isopregnaolone **1** and phenylhydrazine in the present of acetic acid via cyclization on treatment with Vilsmeier reagents at 0 °C in 89% yield. Deformylation and reduction of compound 3 using K_2CO_3 or $NaBH_4$ gave 17-(4′-formyl)pyrazolylandrosta-3β-ol **4** and 17-(4′-hydroxymethyl)pyrazolylandrost-3β-ol **6**, respectively, in high yields. Further, the obtained products **4** underwent reaction with different amines in the present of $NaBH(AcO)_3$ in DCE to afford the desired derivatives **5a-5m**. However, following the above standard conditions did not result in the key intermediate **9** effectively, with a poor yield 9.5%. In this situation, we screened the effect of phosphorous oxychloride molar equivalents (3 equiv., 5 equiv.), temperature, and time (Table 2). Consequently, optimal conditions were as follows: 3 equiv. of phosphorus oxychloride, 40 °C, and 24 h; under these conditions, compound **9** could be synthesized efficiently in 91% yield. Subsequent reaction of product **9** through hydrolysis gave 17-(4′-formyl)pyrazolylandrost-5,16-dienes-3β-ol **10** quantitatively. Finally, the aldehyde **10** was subjected to reductive amination to afford the target hybrids **11a-11m**.

Spectra (^1H NMR, ^{13}C NMR and high resolution mass) of the newly synthesized compounds were in full agreement with the proposed structure. In the ^1H NMR spectra of compounds **3** and **9**, the formation of the heterocyclic unit was confirmed by the signal around $\delta = 7.81$ ppm or $\delta = 8.38$ ppm corresponding to the 5′–H on the pyrazole ring. Moreover, the signals of the aldehyde group and the formoxyl group were detected around $\delta = 9.98$ ppm and $\delta = 8.02$ ppm, respectively, and the signals of the phenyl hydrogen atoms appeared in the aromatic region. In addition, the signals of the vinyl hydrogens of pregndiene moiety were observed at $\delta = 6.37$ ppm and $\delta = 5.45$ ppm corresponding to the 6-H and 15-H respectively, and the signals of 3-H, CH_3-18 and CH_3-19 of pregn moiety showed a multiplet at around $\delta = 4.78$ ppm, and two singlets ranging from $\delta = 1.16$ ppm to 0.61 ppm. The above chemical shifts appeared for compounds **3** and **9** indicating the formation of the steroidal pyrazoles.

When Borch reduction was carried out to afford the products ***5a-m***, ***11a-m***, the representative multiplets signals from $\delta = 4.12$ ppm to $\delta = 4.26$ ppm of $-CH_2-$ and the broad peak ranging from $\delta = 3.56$ ppm to $\delta = 4.22$ ppm of $-NH-$ in the ^1H NMR spectra were the vital characteristic peaks of these derivatives. In the ^{13}C NMR of hybrids **3** and **9,** the characteristic peak of the aldehyde group and formoxyl group appeared at about $\delta = 185$ ppm and $\delta = 160$ ppm, respectively. Further, the chemical shift of the aromatic carbons ranging from $\delta = 155$ ppm to $\delta = 119$ ppm showed

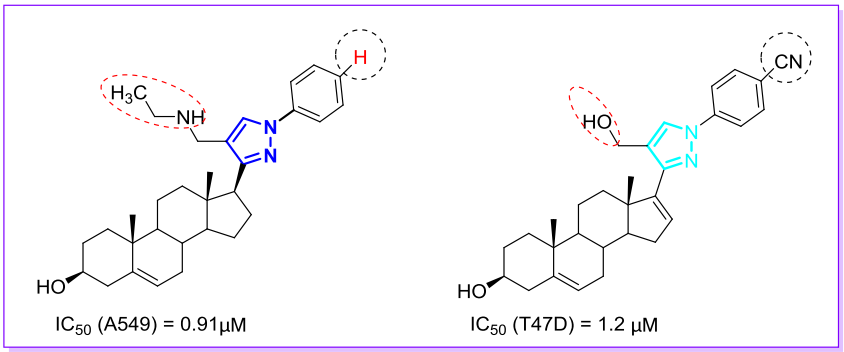
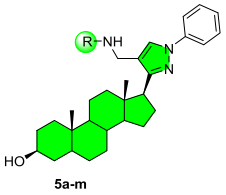
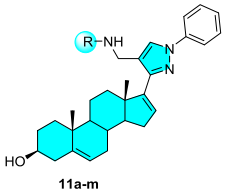


Fig. 1. Structures of previous reported steroidal pyrazole derivatives [23,24].

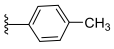
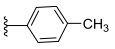
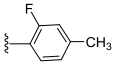
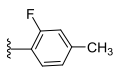
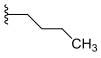
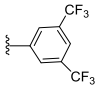
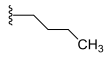
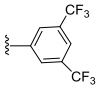
Table 1
Structures of compounds **5a**-**5m** and **11a**-**11m**.



5a-m



11a-m

Compounds	R	Compounds	R	Compounds	R	Compounds	R
5a		**5h**		**11a**		**11h**	
5b		**5i**		**11b**		**11i**	
5c		**5j**		**11c**		**11j**	
5d		**5k**		**11d**		**11k**	
5e		**5l**		**11e**		**11l**	
5f		**5m**		**11f**		**11m**	
5g				**11g**			

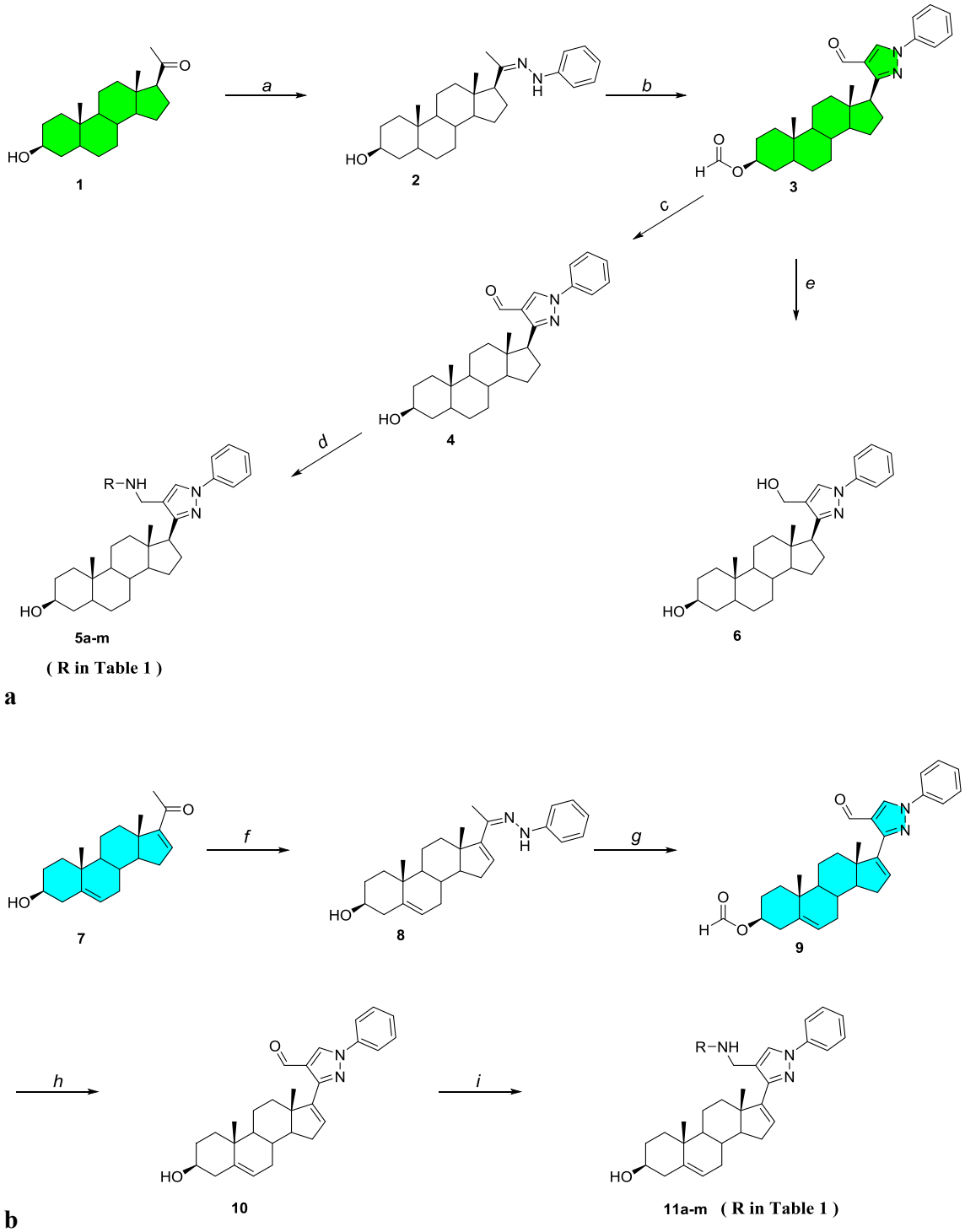
the presence of the benzyl ring and the pyrazole ring. The signals of the formoxyl group disappeared after hydrolysis both in the ^{1}H NMR and ^{13}C NMR spectra; simultaneously, the chemical shift of C-3 in ^{13}C NMR changed from around 73 ppm to 71 ppm.

2.2. Antiproliferative activity

All the synthesized derivatives (**5a**-**5m**, **6**, and **11a**-**11m**) were screened for antiproliferative activities against 293T cell line and three human cancer cell lines: A549, Hella and HepG2 using the RSB assay and were compared with *cis*-platin; preliminary bioassay results are depicted in Table 3.

Compounds **5f**-**5k** and **11f**-**11k**, aromatic amines derivatives which bearing bulky hydrophobic groups, and the diol derivative **6** did not show significant antiproliferative activities (Inhibition values < 20%) for the highest concentration test (10 μM). In contrast, compounds **5a**, **5m** and **11m** displayed modest activities (20% < Inhibition values < 80%), and **5b**-**d** and **11a**-**e** exhibited excellent activities (Inhibition values > 90%) against the four cell lines above. The cell-growth inhibitory potencies of the most active derivatives expressed as IC_{50} are listed in Table 4. These results indicate that these derivatives (**5b**-**d**, **11a**-**e**) show a broad range of growth inhibition effects against four tested cell lines and most of the derivatives display moderate to high activities with IC_{50} ranging from 7.51 to 0.53 μM. In the case of A547 cell lines, the majority of the hybrids (**5b**-**d**, **11a**-**e**) showed acceptable cytotoxic activity (IC_{50} > 2 μM) except compounds **11c** and **11e** with IC_{50} values at 1.57 and 1.93 μM, respectively. With regard to 293T cell lines, compounds **5c**, **5d** and **11b** exhibited significant activity of antiproliferation with IC_{50} values at 0.62, 1.03 and 0.87 μM, respectively. Moreover, the hybrids **5c**, **5d** and **11b** markedly inhibited the proliferation of Hela cell lines with IC_{50} values below 2 μM; the most efficient compound **11b** had an IC_{50} of 0.53 μM which is more effective than compounds 17β-(1-phenyl-4-((ethylamino)methyl)-3-pyrazolyl)androst-5-ene-3β-ol ($IC_{50(A549)}$ = 0.91 μM) from our previous work. The derivatives (**5b**-**d**, **11a**-**e**) displayed a decrease on cell growth inhibitory potency of MCF-7 cell lines as compared to the other three cell lines. Last but not least, the inhibitory properties of these eight compounds were found to be about two to seventeen times better than that of the reference compound *cis*-platin.

Based on the biological results, some correlations could be made between structural changes and compound activities. It is evident from the data that the existence of the double bonds of the pregn moiety influenced the relative activities. The derivatives (**11a**-**e**) with diene-pregn moiety proved to be more favorable which might be attributed to the differences in conformation, the structural rigidity or the molecular volumes (Table 5) which made by using Molinspiration tool [25] of those compounds, and the enlargement of molecular volumes of the derivatives lead to decreasing of their



Scheme 1. a. Synthetic route for 17-(4′-formyl)pyrazolylandrost-3β-ol hybrids. Reagents and conditions: (*a*) Phenylhydrazine, AcOH, rt; (*b*) $POCl_3$, DMF, 0 °C; (*c*) K_2CO_3, THF/H_2O,rt; (*d*) R–NH_2, DCE, $NaBH(AcO)_3$, rt; (*e*) MeOH/THF, $NaBH_4$, rt. b. Synthetic route for 17-(4′-formyl)pyrazolylandrost-5,16-dienes-3β-ol derivatives. Reagents and conditions: (*f*) Phenylhydrazine, AcOH, rt; (*g*) $POCl_3$, DMF, 0 °C–40 °C; (*h*) K_2CO_3, THF/H_2O,rt; (*i*) R–NH_2, DCE, $NaBH(AcO)_3$, rt.

Table 2
Optimization of reaction conditions for preparing compound **9**.

Temperature (°C)	Time (h)	Catalyst (eq.)	Yield (%)
0	24	5	9.5
20	24	5	24
40	24	3	91
80	12	3	35

activities. Moreover, variation of the length or bulkiness of the amino chain at position 4 of the pyrazole ring affects the activities dramatically. In particular, hybrids bearing the aromatic ring or the benzyl group did not give significant activities (Inhibition values ≦ 60%) even for differing functional groups (–Me, –OMe, –F, $-CF_3$) on the aromatic ring. However, derivatives with the shorter methyl, ethyl, propyl, *i*-propyl or the bulkier butyl group were far more

Table 3
Antiproliferative effects of the derivatives valued at 10 μM. SEM: standard error of the mean.

Compounds	Inhibitory ratio (Mean% ± SD%)			
	293T	A549	Hela	MCF-7
5a	79.19 ± 2.33	–	41.33 ± 1.46	45.86 ± 10.92
5b	91.15 ± 1.30	92.39 ± 1.82	88.44 ± 0.91	88.89 ± 0.28
5c	91.90 ± 0.76	91.45 ± 1.13	90.27 ± 0.66	88.64 ± 0.75
5d	87.42 ± 1.08	30.62 ± 3.60	74.12 ± 1.96	70.75 ± 2.38
5e	-[a]	–	29.29 ± 16.54	21.86 ± 7.03
5f	12.50 ± 6.20	–	–	16.95 ± 9.02
5m	60.80 ± 10.77	56.47 ± 6.44	48.30 ± 4.90	44.40 ± 1.32
6	18.47 ± 5.72	–	–	27.82 ± 1.21
11a	91.60 ± 4.48	82.13 ± 1.71	90.52 ± 1.40	81.14 ± 4.02
11b	94.23 ± 1.52	91.33 ± 4.38	92.81 ± 0.25	89.30 ± 2.35
11c	93.67 ± 0.88	91.18 ± 1.87	93.52 ± 0.10	90.71 ± 0.30
11d	94.71 ± 2.02	93.27 ± 0.86	92.38 ± 0.45	92.07 ± 1.02
11e	95.13 ± 2.75	92.92 ± 0.78	92.59 ± 0.15	93.68 ± 0.80
11j	–	–	–	18.15 ± 2.15
11m	50.71 ± 26.71	–	–	25.39 ± 2.10
DMSO	–	–	–	–

[a] Inhibition values < 10% are not detailed and only compounds with inhibition values > 10% are included.

Table 4
IC_{50} of derivatives (**5b-d**, **11a-e**).

Products	IC_{50} ± SD (μM)			
	293T	A549	Hela	MCF-7
5b	5.29 ± 0.70	6.35 ± 0.21	3.28 ± 0.05	3.92 ± 0.88
5c	**0.62 ± 0.07**	6.02 ± 0.80	1.98 ± 0.35	4.84 ± 0.41
5d	1.03 ± 0.13	ND	1.98 ± 0.17	7.45 ± 0.23
11a	2.11 ± 0.11	3.68 ± 0.22	3.86 ± 0.37	6.91 ± 0.89
11b	**0.87 ± 0.04**	7.39 ± 0.48	**0.53 ± 0.07**	2.53 ± 0.15
11c	7.25 ± 0.72	**1.57 ± 0.32**	3.61 ± 0.63	4.29 ± 0.45
11d	7.51 ± 0.92	2.87 ± 0.40	3.49 ± 0.70	6.36 ± 1.3
11e	2.63 ± 0.72	1.93 ± 0.19	3.37 ± 0.22	3.18 ± 0.22
cis-platin	ND	14.3	9.1	15.5

ND: Not determined.

efficient than the aromatic amine derivatives. In addition, these data suggested that the ethyl moiety has the optimal length for a submicromolar activity (compounds **11b**, with the IC_{50} values at 0.87 μM against 293T cell lines and 0.53 μM against Hela cell lines, respectively), while a length reduction to a methyl group or a length increase to a butyl group led to inactive compounds **5a** and **5e**. Furthermore, transformation of the amino chain at position 4 of the pyrazole ring into a propyl (**5c** and **11c**) or an *i*-propyl (**5d** and **11d**) group did not dramatically affect the cytotoxicity activity. It is interesting to note that the highly-polar molecule diol **6** showed no cytotoxicity (inhibition < 10% at 10 μM) against the four cell lines.

2.3. Hoechst 33342 staining

The most active compound **11b** was selected to be further studied its effects on induction of apoptosis in the Hela cell lines. After treated with derivative **11b** at different concentrations (2.5 μM, 5.0 μM and 7.5 μM), the cells were stained with Hoechst 33342. Meanwhile, Hela cell lines that not dealt with the compound **11b** were used as control at for 48 h, and the results were illustrated in Fig. 2. As shown in Fig. 2, the nuclei of the control group (DMSO served as negative control) retained the regular contours and had no obvious morphological changes. On the contrary, the cells treated with the title compound exhibited strong blue florescence and typical apoptotic morphology. Particularly, when treated at a higher concentration of 7.5 μM the contours of many cells became irregular, the nuclei condensed and the apoptotic bodies appeared (Fig. 2d). It suggested the significant cell apoptotic induction of compound **11b** on Hela cell lines in a concentration dependent manner.

2.4. Flow cytometry

Apoptosis, the process of programmed cell death, is an important therapy target for cancer chemotherapy. In order to evaluate the apoptotic assay, flow cytometry using propidium iodide (PI) and annexin-V in Hela cell lines was performed. After incubated with different concentrations (0 μM, 2.5 μM, 5.0 μM and 7.5 μM) of compound **11b** for 24 h, the cells were labeled with the two dyes and the resulting mixture was monitored by flow cytometry. Additionally, the cells untreated with the title compound **11b** were used as the negative control (DMSO served as negative control). It can be observed from Fig. 3 that apoptotic ratios (including the early and late apoptotic ratios) of Hela cells treated with compound **11b** increased gradually in a concentration dependent manner. Moreover, the apoptotic ratios of compound **11b** at various concentrations were found to 0.58% (2.5 μM), 2.08% (5.0 μM) and 7.50% (7.5 μM), respectively, that were higher than that of control group 0.04% (DMSO control). These results above indicated that **11b**

Table 5
Calculated molecular properties of all derivatives.

Compounds	LogP[a]	TPSA[b]	Vol[c]	N_{HA}[d]	N_{HD}[e]	Compounds	LogP	TPSA	Vol	N_{HA}	N_{HD}
5a	5.30	50.08	465.39	4	2	**11a**	5.41	50.08	436.16	4	2
5b	5.68	50.08	482.19	4	2	**11b**	5.78	50.08	452.96	4	2
5c	6.18	50.08	498.99	4	2	**11c**	6.29	50.08	469.70	4	2
5d	5.98	50.08	498.78	4	2	**11d**	6.75	50.08	469.55	4	2
5e	6.74	50.08	515.80	4	2	**11e**	6.85	50.08	486.57	4	2
5f	7.74	50.08	503.44	4	2	**11f**	7.54	50.08	491.01	4	2
5g	8.13	50.08	520.00	4	2	**11g**	7.94	50.08	507.57	4	2
5h	8.15	50.08	520.00	4	2	**11h**	7.97	50.08	507.57	4	2
5i	8.17	50.08	520.00	4	2	**11i**	7.99	50.08	507.57	4	2
5j	7.75	59.31	528.98	5	2	**11j**	7.55	59.31	516.56	5	2
5k	8.24	50.08	524.93	4	2	**11k**	8.08	50.08	512.50	4	2
5l	8.90	50.08	566.03	4	2	**11l**	8.82	50.08	553.61	4	2
5m	6.70	50.08	537.04	4	2	**11m**	6.81	50.08	507.81	4	2
6	5.08	58.28	444.44	4	2						

[a] Octanol-water partition coefficient (LogP).
[b] Topologic polar surface area (TPSA) [$Å^2$].
[c] Molecular volume (Vol.) [$Å^3$].
[d] Sum of O and N H-bond acceptors N_{HA}.
[e] Sum of OH and NH H-bond donors (N_{HD}).

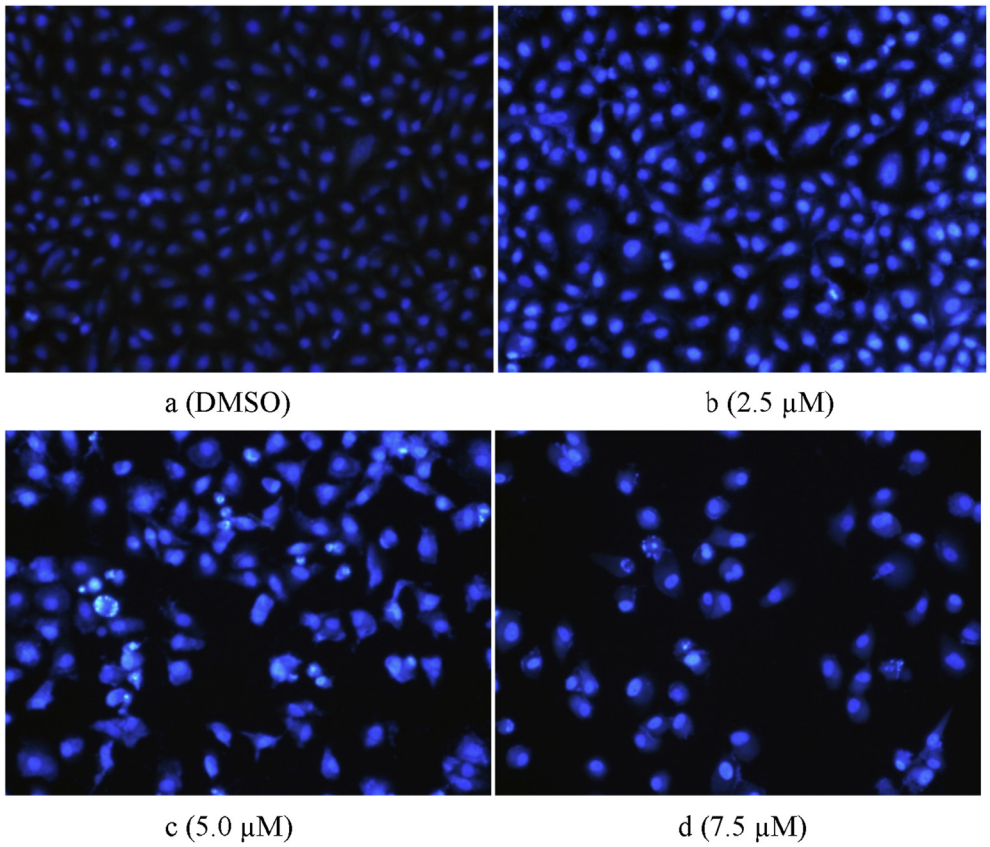


Fig. 2. Hoechst 33342 staining of compound **11b** in Hela cells for 48 h. (a) DMSO used as negative control, (b, c, d) dealt with compound **11b** for 48 h at the concentration of 2.5 μM, 5.0 μM and 7.5 μM, respectively.

caused a slightly increased the cellular apoptotic in a concentration dependent manner.

2.5. Cell cycle analysis

One main feature of the cancer cells is multiply uncontrollable, and then cell cycle arrest become a critical therapy target in oncotherapy [26]. As the above studies have proved that compound **11b** can induce apoptosis, therefore the effect of **11b** on the cell cycle of Hela cells was investigated by flow cytometry as shown in Fig. 4. Treatment with compound **11b** at the concentrations of 2.5 μM, 5.0 μM and 7.5 μM result in accumulation of 19.82%, 24.34% and 24.77% of cells at S phase, respectively, compared to 18.92% in the untreated cells (DMSO served as negative control). Moreover, the population of G1 phase decreased in a certain extent (3.0%, 3.59% and 6.8%) compared to the control cells (56.72%). In addition, for S phase the percentage of the cells altered from 24.36% (control) to 26.45%, 22.53% and 25.31%, respectively. These results suggested that derivative **11b** caused an obvious S phase arrest in a concentration dependent manner.

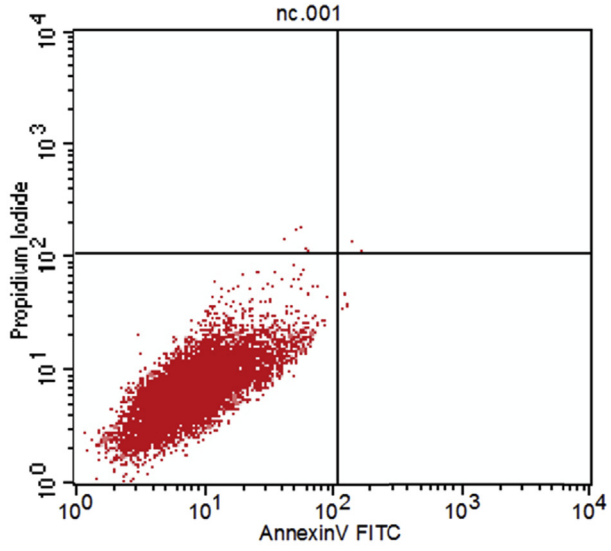
3. Conclusions

In this study, twenty-seven novel steroidal pyrazole hybrids were divided into two categories based on their main skeletons: dien-pregn and pregn moieties; compounds were designed and synthesized via efficient routes under mild conditions. All target molecules were tested for *in vitro* antiproliferative activities against 293T cell lines and three cancer cell lines: A549, Hela and MCF-7 cell lines using the SRB assay. Among them, derivatives **5b-d** and **11a-e** displayed the most promising results at a single-dose concentration of 10 μM against the four cell lines and their IC_{50} values were further determined to screen out the most efficient candidates. We identify compound **11b** to be one of the most active derivatives with an IC_{50} of 0.87 μM against 293T and 0.53 μM against Hela, which was far more efficient than the positive control *cis*-platin whose IC_{50} values ranged from 9.1 to 15.5 μM. Compounds **5b-d, 11a**, and **11c-e** exhibited moderate to high cytotoxic activities with IC_{50} values ranging from 0.62 to 7.51 μM. Additionally, the structure–activity relationship was revealed that different substituents in the pyrazole ring remarkably affected the cytotoxicity. Otherwise, the effects of compound **11b** on cell cycle progression and induction of apoptosis in the Hella cells were studied. Results indicated that compounds **11b** induced cancer cells death and arrested the cell cycle at S phase in a concentration dependent manner. Further structural modifications of this series of compounds in order to improve their potencies are currently in progress.

4. Experimental

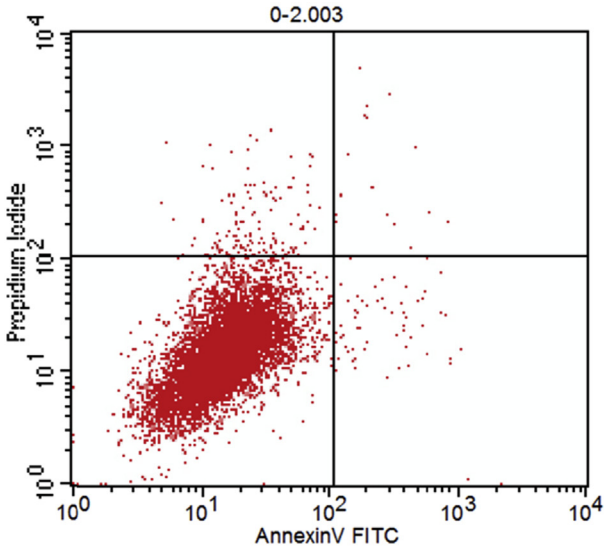
4.1. General

Melting points were determined using an X-4 micromelting point apparatus; high resolution mass spectrometry (HRMS) was performed with a Thermo Scientific LTQ Orbitrap XL. All ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Advance spectrometer at 500 MHz and 125 MHz in $CDCl_3$, CD_3OD or DMSO-d_6 with TMS as the reference. Chemical shift values were given in δ (ppm) and coupling constants were mentioned in Hz. The



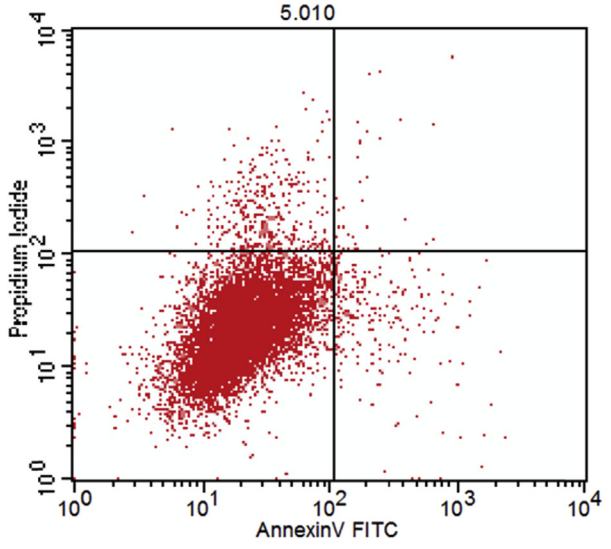
Quad	Events	% Gated	% Total
UL	5	0.05	0.05
UR	3	0.03	0.03
LL	9920	99.88	96.16
LR	4	0.04	0.04

a (DMSO)



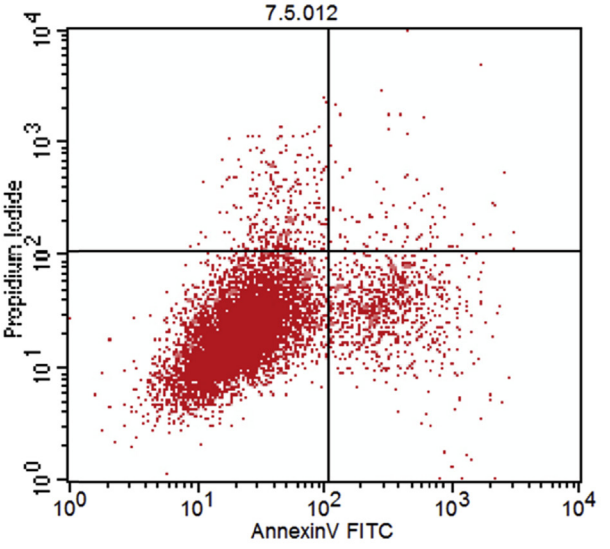
Quad	Events	% Gated	% Total
UL	130	1.31	1.28
UR	16	0.16	0.16
LL	9715	97.93	95.73
LR	59	0.59	0.58

b (2.5 μM)



Quad	Events	% Gated	% Total
UL	363	3.79	3.26
UR	34	0.36	0.31
LL	8941	93.44	80.32
LR	231	2.41	2.08

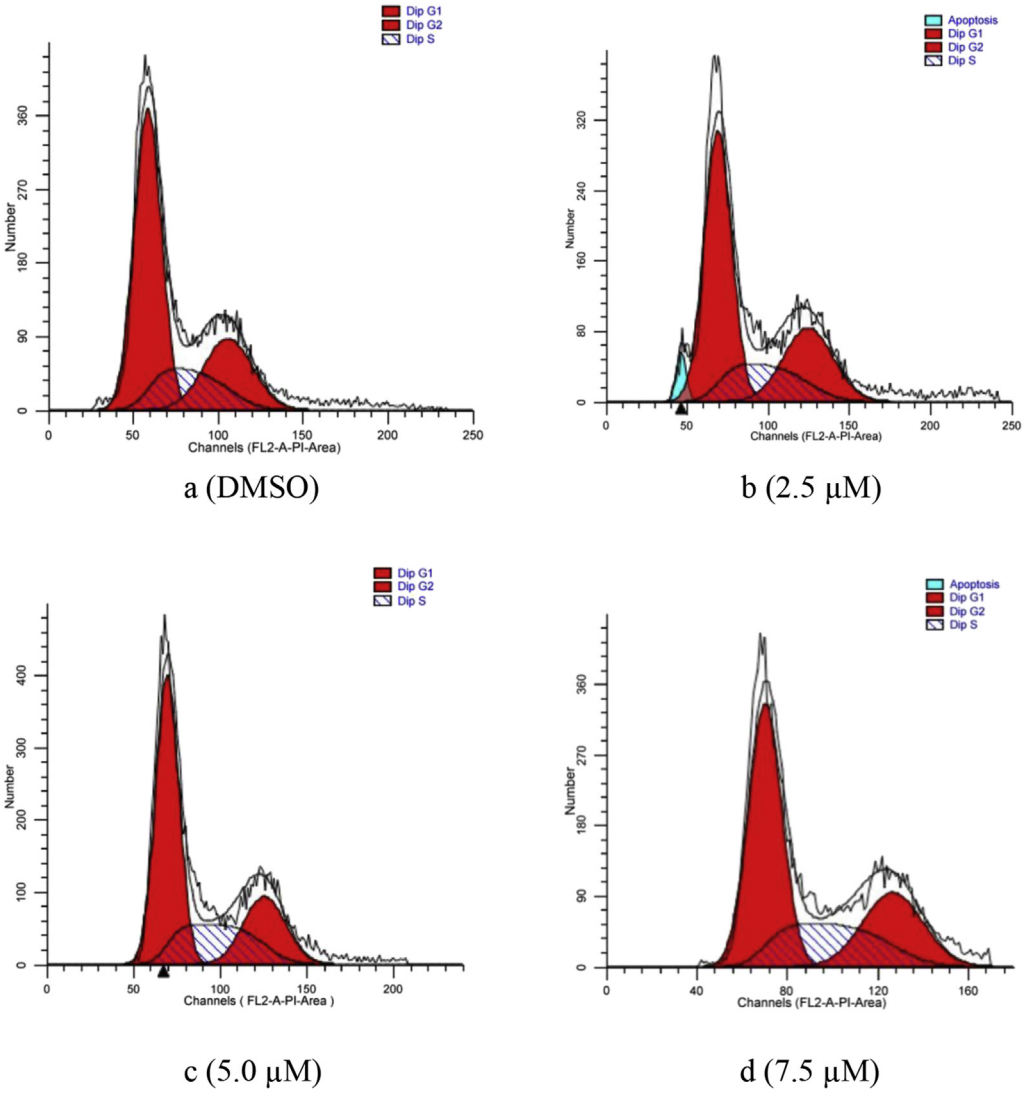
c (5.0 μM)



Quad	Events	% Gated	% Total
UL	303	3.12	2.79
UR	98	1.01	0.90
LL	8494	87.49	78.35
LR	813	8.37	7.50

d (7.5 μM)

Fig. 3. Apoptosis ratio detection by Annexin V/PI assay on the Hela cells for 24 h. (a) DMSO used as negative control, (b, c, d) dealt with compound **11b** for 24 h at the concentration of 2.5 μM, 5.0 μM and 7.5 μM, respectively.



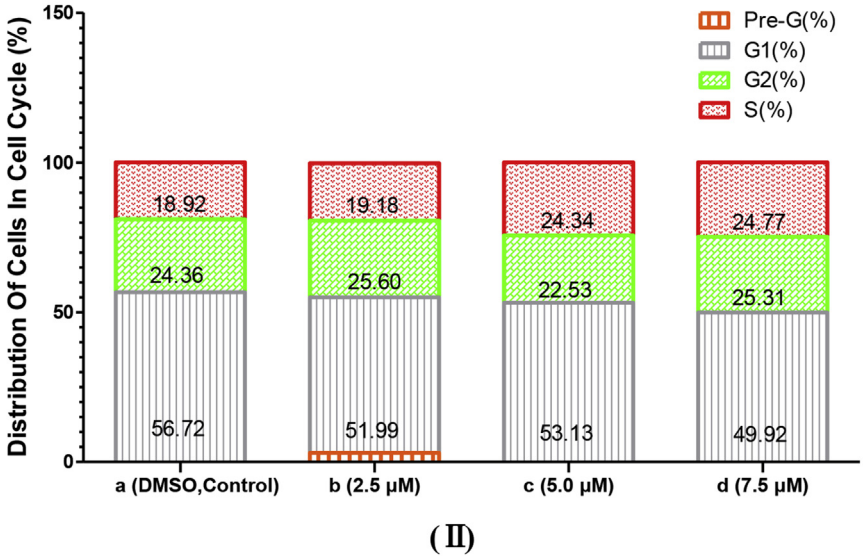


Fig. 4. (I) Effects of compounds **11b** on cell cycle distribution of Hela cells after treatment for 24 h. (a) DMSO used as negative control, (b, c, d) dealt with compound **11b** at the concentration of 2.5 μM, 5.0 μM and 7.5 μM, respectively. (II) Percentage of distribution of cells in cell cycle.

multiplicities of the ^{1}H NMR resonance peaks are reported as a singlet (s), a broad singlet (br), a doublet (d), a double (dd), a triplet (t), a triplet double (td) or a multiplet (m). All solvents and reagents used were obtained from commercial sources without further purification, unless otherwise noted. Column chromatography was performed on silca gel (200–300 mesh). The progress of all reactions were monitored by thin layer chromatography (TLC) with pre-coated GF_{254} silica gel plates and visualisations were achieved with ultraviolet irradiation (254 nm) and iodine. The optical density values (OD) were recorded on a Bio-Tek ELX800 at 540 nm.

4.2. Chemistry

4.2.1. General procedure for steroidal phenylhydrazone **2** and **8**

To a solution of compound **1** (6.36 g, 20 mmol) and **7** (6.28 g, 20 mmol) in acetic acid (50 mL) phenylhydrazine (2.27 g, 21 mmol) was added dropwise; after complete addition, the resulting mixture was stirred at room temperature for 5 h, and full conversion was achieved as monitored by TLC. The precipitate was filtered, washed with acetic acid and dried to give corresponding product as light yellow powder, which was used directly in the next step without further purification.

4.2.2. Procedure for 17β-(4′-formyl)pyrazolylandrost-3β-yl formate **3** and 17-(4′-formyl)pyrazolylandrost-5,16-dienes-3β-yl formate **9**

Phosphorus oxychloride (7.59 g, 5 eq) was added dropwise to anhydrous *N*,*N*-dimethylformamide (30 mL) at 0 °C, and the mixture was stirred at RT for 20 min. Then a solution of **2** (4.08 g, 10 mmol, 1 eq) in anhydrous *N*,*N*-dimethylformamide (40 mL) was added dropwise to the above mixture and stirred at 0 °C for 24 h. After full conversion as monitored by TLC, the reaction mixture was quenched with cooled saturated $NaHCO_3$ solution and the mixture was stirred until bubbling ceased. The precipitate was filtered, dried and purified by column chromatography (CH_2Cl_2: CH_3OH:: 10: 1, v/v) to give the product as white solid.

Phosphorus oxychloride (7.59 g, 5 eq) was added dropwise to anhydrous *N*,*N*-dimethylformamide (30 mL) at 0 °C, and the mixture was stirred at RT for 20 min. Then a solution of **8** (4.04 g, 10 mmol, 1 eq) in anhydrous *N*,*N*-dimethylformamide (40 mL) was added dropwise to the above mixture and stirred at 0 °C for 1 h, and then was heated to 40 °C for another 23 h. After full conversion as monitored by TLC, the reaction mixture was quenched with cooled saturated $NaHCO_3$ solution and the mixture was stirred until bubbling ceased. The precipitate was filtered, dried and purified by column chromatography (CH_2Cl_2: CH_3OH:: 10: 1, v/v) to give the product as white solid.

*4.2.2.1. 17β-(4′-formyl)pyrazolylandrost-3β-yl formate (**3**).* **3**, white solid, Yield: 89%, mp > 250 °C; ^{1}H NMR ($CDCl_3$, 500 MHz): δ (ppm) 9.95 (s, 1H, −CHO), 8.38 (s, 1H, pyrazole 5′-H), 8.02 (s, 1H), 7.72 (d, 2H, *J* = 7.5 Hz), 7.47 (t, 2H, *J* = 7.5 Hz, *J* = 8.5 Hz), 7.34 (t, 1H, *J* = 7.5 Hz), 4.86–4.80 (m, 1H), 3.26 (t, 1H, *J* = 10.0 Hz), 2.57–2.49 (m, 1H), 2.05–1.98 (m, 1H), 1.85–1.78 (m, 4H), 1.65–1.50 (m, 4H), 1.46–1.18 (m, 9H), 1.08–0.95 (m, 2H), 0.83 (s, 3H, CH_3), 0.77–0.73 (m, 1H), 0.61 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 125 MHz): δ (ppm) 184.86 (−CHO), 160.78 (−OCHO), 155.61, 139.35, 130.62, 129.57, 129.57, 127.46, 123.95, 119.58, 119.58, 73.70, 56.30, 54.33, 48.48, 44.88, 44.74, 38.18, 36.73, 36.04, 35.58, 34.01, 32.02, 28.55, 27.48, 25.97, 24.53, 21.04, 13.65 (CH_3), 12.26 (CH_3).

*4.2.2.2. 17-(4′-formyl)pyrazolylandrost-5,16-dienes-3β-yl formate (**9**).* **9**, white solid, Yield: 91%, mp 204–206 °C; ^{1}H NMR ($CDCl_3$, 500 MHz): δ (ppm) 9.98 (s, 1H, −CHO), 8.45 (s, 1H, pyrazole 5′-H), 8.05 (s, 1H), 7.74 (d, 2H, *J* = 9.5 Hz), 7.49 (t, 2H, *J* = 8.5 Hz), 7.36 (t, 1H, *J* = 7.5 Hz), 6.38–6.37 (m, 1H), 5.45–5.44 (m, 1H), 4.78–4.72 (m, 1H), 2.42–2.35 (m, 4H), 2.19–2.07 (m, 2H), 1.93–1.88 (m, 2H), 1.81–1.62 (m, 6H), 1.52–1.46 (m, 1H), 1.20–1.15 (m, 4H), 1.17 (s, 3H, CH_3), 1.12–1.09 (m, 4H), 1.10 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 125 MHz): δ (ppm) 185.21 (−CHO), 160.63 (−OCHO), 151.07, 145.58, 139.75, 139.13, 134.34, 130.12, 129.60, 129.60, 127.57, 123.89, 122.69, 119.32, 119.32, 73.89, 56.77, 50.49, 48.12, 38.11, 36.87, 36.83, 35.02, 32.55, 31.61, 30.40, 27.78, 20.89, 19.26 (CH_3), 16.34 (CH_3).

4.2.3. Procedure for 17β-(4′-formyl)pyrazolylandrost-3β-ol **4** and 17-(4′-formyl)pyrazolylandrost-5,16-dienes-3β-ol **10**

To a solution of **3** (2.37 g, 5 mmol, 1eq) and **9** (2.35 g, 5 mmol, 1eq) in MeOH/THF (20 mL/20 mL), K_2CO_3 (0.76 g, 5.5 mmol, 1.1 eq) was added in one portion at RT and then the resulting mixture was stirred for 1 h. After completion of reaction, the solvent was evaporated in vacuo, and the residue was purified by column chromatography (CH_2Cl_2: MeOH:: 8: 1, v/v) to give the corresponding products as white solid.

*4.2.3.1. 17β-(4′-formyl)pyrazolylandrost-3β-ol (**4**).* **4**, white solid, Yield: 97%, mp 230–232 °C; ^{1}H NMR ($CDCl_3$, 500 MHz): δ (ppm) 9.94 (s, 1H, −CHO), 8.38 (s, 1H, pyrazole 5′-H), 7.71 (d, 2H, *J* = 7.5 Hz), 7.47 (t, 2H, *J* = 7.5 Hz, *J* = 8.5 Hz), 7.34 (t, 1H, *J* = 7.5 Hz), 3.63–3.54 (m, 1H), 3.25 (t, 1H, *J* = 10.0 Hz), 2.56–2.40 (m, 1H), 2.06–1.98 (m, 1H), 1.82–1.68 (m, 4H), 1.59–1.55 (m, 4H), 1.40–1.20 (m, 10H), 1.01–0.95 (m, 2H), 0.80 (s, 3H, CH_3), 0.76–0.70 (m, 1H), 0.61 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 125 MHz): δ (ppm) 184.92 (−CHO), 155.69, 139.36, 130.61, 129.57, 129.57, 127.47, 123.94, 119.59, 119.59, 71.29, 56.40, 54.50, 48.51, 44.95, 44.91, 38.26, 38.23, 37.06, 36.11, 35.61, 32.15, 31.55, 28.73, 25.98, 24.56, 21.10, 13.65 (CH_3), 12.37 (CH_3).

*4.2.3.2. 17-(4′-formyl)pyrazolylandrost-5,16-dienes-3β-ol (**10**).* **10**, white solid, Yield: 96%, mp 182–184 °C; ^{1}H NMR ($CDCl_3$ & CD_3OD, 500 MHz): δ (ppm) 9.85 (s, 1H, −CHO), 8.46 (s, 1H, pyrazole 5′-H), 8.05 (s, 1H), 7.68 (d, 2H, *J* = 8.0 Hz), 7.42 (t, 1H, *J* = 7.5 Hz), 7.36 (t, 2H, *J* = 7.5 Hz), 6.29 (m, 1H), 5.31 (m, 1H), 3.43–3.39 (m, 1H), 3.27 (s, 1H), 2.31–2.00 (m, 5H), 1.77–1.37 (m, 8H), 1.18–1.01 (m, 9H), 1.09 (s, 3H, CH_3), 1.01 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$ & CD_3OD, 125 MHz): δ (ppm) 186.10 (−CHO), 151.55, 145.75, 141.53, 139.25, 134.71, 130.80, 129.77, 129.77, 127.88, 123.84, 121.40, 119.60, 119.60, 71.44, 57.12, 50.87, 48.35, 42.08, 37.40, 36.95, 35.25, 32.71, 31.35, 30.66, 28.49, 21.10, 19.41 (CH_3), 16.42 (CH_3).

4.2.4. General procedure for 17β-(1-phenyl-4-aminomethyl-3-pyrazolyl)androst-3β-ol derivatives **5a-m** and 17-(1-phenyl-4-aminomethyl-3-pyrazolyl)androst-5,16-diene- 3β-ol derivatives **11a-m**

$NaBH(OAc)_3$ (212 mg, 1 mmol, 4 eq) was added to a solution of **4** or **10** (0.25 mmol, 1 eq) and aniline derivatives in anhydrous DCE (15 mL) at RT. After stirring overnight, the solvent was removed under vacuo, and the residue was diluted with saturated aqueous $NaHCO_3$ (15 mL) and extracted with DCM (3 × 20 mL). The combined organic layers were washed with brine (10 mL), dried over Na_2SO_4, filtered and concentrated. The residue was purified by column chromatography (CH_2Cl_2: MeOH: Et_3N:: 50:1: 0.05, v/v/v or 10:1: 0.1, v/v/v) to give products as colored glasslike solid.

*4.2.4.1. 17β-(1-phenyl-4-((methylamino)methyl)-3-pyrazolyl) androst -3β-ol (**5a**).* **5a**, white solid, Yield: 88%, mp 204–206 °C; ^{1}H NMR ($CDCl_3$ & CD_3OD, 500 MHz): δ (ppm) 8.09 (s, 1H), 7.67 (d, 2H, *J* = 8.5 Hz), 7.43 (t, 2H, *J* = 7.0 Hz), 7.26 (t, 1H, *J* = 7.5 Hz), 3.90–3.80 (m, 2H), 3.58–3.53 (m, 1H), 2.74 (t, 1H, *J* = 9.5 Hz), 2.53 (s, 3H, CH_3), 2.47–2.40 (m, 1H), 2.01–1.93 (m, 1H), 1.78–1.70 (m, 4H), 1.56–1.54 (m, 3H), 1.40–1.10 (m, 12H), 1.00–0.93 (m, 2H), 0.81 (s, 3H, CH_3), 0.73–0.67 (m, 1H), 0.64 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$ & CD_3OD,

125 MHz): δ (ppm) 152.48, 140.02, 129.29, 129.29, 127.45, 126.18, 118.91, 118.91, 115.61, 70.81, 56.33, 54.53, 48.39, 44.91, 44.79, 44.33, 38.56, 37.74, 37.02, 35.99, 35.53, 33.17, 32.07, 31.04, 28.65, 26.66, 24.43, 21.07 (CH_3), 13.33 (CH_3), 12.22 (CH_3); HRMS (ESI) *m/z* calcd for $C_{30}H_{44}N_3O^+$ $(M+H)^+$ 462.34789, found 462.34738.

*4.2.4.2. 17β-(1-phenyl-4-((ethylamino)methyl)-3-pyrazolyl)androst -3β-ol (**5b**).* ***5b***, white solid, Yield: 80%, mp 246–248 °C; ^{1}H NMR (DMSO-d_6, 500 MHz): δ (ppm) 8.66 (s, 1H), 7.72–7.70 (m, 2H), 7.50 (t, 2H, *J* = 7.0, 8.5 Hz), 7.30 (t, 1H, *J* = 7.5 Hz), 4.43 (s, br, 1H), 4.01–3.92 (m, 2H), 2.98 (dq, 2H, *J* = 2.0, 7.5 Hz), 2.92 (t, 1H, *J* = 9.5 Hz), 2.40–2.33 (m, 1H), 1.96–1.91 (m, 1H), 1.70–1.60 (m, 4H), 1.53–1.41 (m, 3H), 1.37–1.06 (m, 12H), 1.24 (t, 3H, *J* = 7.5 Hz), 0.96–0.88 (m, 2H), 0.74 (s, 3H, CH_3), 0.71–0.65 (m, 1H), 0.55 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 125 MHz): δ (ppm) 152.71, 139.98, 130.13, 130.13, 129.58, 126.65, 118.52, 118.52, 114.09, 69.79, 56.18, 54.58, 47.58, 44.98, 44.67, 42.06, 40.32, 38.67, 37.95, 37.21, 36.08, 35.69, 32.32, 31.86, 28.88, 26.62, 24.57, 21.16, 13.85 (CH_3), 12.65 (CH_3), 11.57 (CH_3); HRMS (ESI) *m/z* calcd for $C_{31}H_{46}N_3O^+$ $(M+H)^+$ 476.36354, found 476.36313.

*4.2.4.3. 17β-(1-phenyl-4-((propylamino)methyl)-3-pyrazolyl)androst -3β-ol (**5c**).* ***5c***, light brown solid, Yield: 76%, mp 160–162 °C; ^{1}H NMR (DMSO-d_6, 500 MHz): δ (ppm) 8.28 (s, 1H), 7.74–7.73 (m, 2H), 7.45 (t, 2H, *J* = 7.5, 8.0 Hz), 7.30 (t, 1H, *J* = 7.5 Hz), 3.90 (s, br, 1H), 3.63–3.61 (m, 2H), 3.38–3.32 (m, 1H), 2.81 (t, 1H, *J* = 10.0 Hz), 2.56 (t, 2H, *J* = 7.5 Hz), 2.37–2.30 (m, 1H), 1.68–1.58 (m, 4H), 1.55–1.32 (m, 5H), 1.28–1.04 (m, 10H), 0.94–0.90 (m, 2H), 0.87 (t, 3H, *J* = 7.5 Hz), 0.74 (s, 3H, CH_3), 0.70–0.65 (m, 1H), 0.58 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 125 MHz): δ (ppm) 151.54, 139.74, 129.32, 129.32, 126.64, 125.25, 120.35, 117.52, 117.52, 69.23, 55.67, 53.94, 50.41, 47.59, 44.37, 44.07, 42.47, 38.10, 37.83, 36.61, 35.50, 35.10, 31.70, 31.29, 28.32, 26.23, 24.05, 21.87, 21.45, 13.34 (CH_3), 12.06 (CH_3), 11.62 (CH_3); HRMS (ESI) *m/z* calcd for $C_{32}H_{48}N_3O^+$ $(M+H)^+$ 490.37919, found 490.37924.

*4.2.4.4. 17β-(1-phenyl-4-((isopropylamino)methyl)-3-pyrazolyl) androst -3β-ol (**5d**).* ***5d***, white solid, Yield: 83%, mp 216–218 °C; ^{1}H NMR ($CDCl_3$ & CD_3OD, 500 MHz): δ (ppm) 7.94 (s, 1H), 7.67–7.65 (m, 2H), 7.41 (t, 2H, *J* = 7.0, 8.5 Hz), 7.23 (t, 1H, *J* = 7.5 Hz), 3.70 (m, 2H), 3.57–3.53 (m, 1H), 2.92–2.97 (m, 1H), 2.72 (t, 1H, *J* = 10.0 Hz), 2.47–2.39 (m, 1H), 2.00–1.94 (m, 1H), 1.80–1.69 (m, 4H), 1.60–1.54 (m, 3H), 1.45–1.32 (m, 3H), 1.35–1.28 (m, 6H), 1.15 (d, 6H, *J* = 6.5 Hz, $2CH_3$), 1.12–0.86 (m, 4H), 0.81 (s, 3H, CH_3), 0.74–0.69 (m, 1H), 0.66 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$ & CD_3OD, 125 MHz): δ (ppm) 152.13, 140.34, 129.29, 129.29, 126.40, 125.83, 119.70, 118.84, 118.84, 70.99, 56.42, 54.59, 48.66, 44.99, 44.80, 40.62, 38.74, 37.92, 37.10, 36.06, 35.61, 32.15, 31.21, 29.69, 28.74, 26.83, 24.54, 22.12 (CH_3), 21.99 (CH_3), 21.16, 13.49 (CH_3), 12.33 (CH_3); HRMS (ESI) *m/z* calcd for $C_{32}H_{48}N_3O^+$ $(M+H)^+$ 490.37919, found 490.37918.

*4.2.4.5. 17β-(1-phenyl-4-((butylamino)methyl)-3-pyrazolyl)androst -3β-ol (**5e**).* ***5e***, white solid, Yield: 83%, mp 138–140 °C; ^{1}H NMR (DMSO-d_6 & $CDCl_3$, 500 MHz): δ (ppm) 8.35 (s, 1H), 7.73 (d, 2H, *J* = 8.0 Hz), 7.46 (t, 2H, *J* = 7.5, 8.5 Hz), 7.24 (t, 1H, *J* = 7.5 Hz), 3.73 (m, 2H), 3.37–3.33 (m, 1H), 2.84 (t, 1H, *J* = 9.5 Hz), 2.70 (t, 2H, *J* = 7.5 Hz), 2.38–2.32 (m, 1H), 1.69–1.62 (m, 4H), 1.51–1.47 (m, 4H), 1.34–1.11 (m, 12H), 1.08–0.88 (m, 6H), 0.88 (t, 3H, *J* = 7.5 Hz), 0.75 (s, 3H, CH_3), 0.70–0.66 (m, 1H), 0.58 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6 & $CDCl_3$, 125 MHz): δ (ppm) 151.78, 139.71, 129.40, 129.40, 127.30, 125.52, 118.38, 117.72, 117.72, 69.30, 55.74, 54.04, 47.71, 47.48, 44.45, 44.14, 41.98, 38.13, 37.77, 36.69, 35.56, 35.16, 31.77, 31.32, 29.94, 28.38, 26.24, 24.08, 21.17, 20.65, 13.73 (CH_3), 13.36 (CH_3), 12.12 (CH_3); HRMS (ESI) *m/z* calcd for $C_{33}H_{50}N_3O^+$ $(M+H)^+$ 504.39484, found 504.39404.

*4.2.4.6. 17β-(1-phenyl-4-((phenylamino)methyl)-3-pyrazolyl) androst -3β-ol (**5f**).* ***5f***, white solid, Yield: 88%, mp 198–200 °C; ^{1}H NMR ($CDCl_3$, 500 MHz): δ (ppm) 7.81 (s, 1H), 7.64–7.62 (m, 2H), 7.39 (t, 2H, *J* = 7.5 Hz, *J* = 8.5 Hz), 7.21–7.18 (m, 3H), 6.74 (t, 1H, *J* = 7.5 Hz), 6.66 (d, 2H, *J* = 7.5 Hz), 4.21–4.14 (m, 2H), 3.74 (s, br, 1H), 3.60–3.56 (m, 1H), 2.79 (t, 1H, *J* = 9.5 Hz), 2.48–2.40 (m, 1H), 2.01–1.94 (m, 1H), 1.80–1.66 (m, 5H), 1.57–1.54 (m, 2H), 1.42–0.92 (m, 14H), 0.81 (s, 3H, CH_3), 0.70 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 125 MHz): δ (ppm) 152.25, 148.16, 140.36, 129.32, 129.32, 129.29, 129.29, 125.96, 125.71, 120.31, 118.65, 118.65, 117.71, 112.95, 112.95, 71.32, 56.33, 54.52, 48.66, 44.95, 44.81, 38.81, 38.67, 38.26, 37.08, 36.05, 35.60, 32.14, 31.55, 28.73, 26.91, 24.57, 21.19, 13.62 (CH_3), 12.39 (CH_3); HRMS (ESI) *m/z* calcd for $C_{35}H_{46}N_3O^+$ $(M+H)^+$ 524.36354, found 524.36292.

*4.2.4.7. 17β-(1-phenyl-4-((o-tolylamino)methyl)-3-pyrazolyl) androst-3β-ol (**5g**).* ***5g***, glasslike solid, Yield: 92%, mp 186–188 °C; ^{1}H NMR ($CDCl_3$, 500 MHz): δ (ppm) 7.83 (s, 1H), 7.66–7.64 (m, 2H), 7.40 (t, 2H, *J* = 7.5 Hz, *J* = 8.5 Hz), 7.21 (t, 1H, *J* = 7.5 Hz), 7.15 (t, 1H, *J* = 7.5 Hz), 7.07 (d, 1H, *J* = 7.0 Hz), 6.71–6.68 (m, 2H), 4.24–4.17 (m, 2H), 3.61–3.53 (m, 2H), 2.81 (t, 1H, *J* = 9.5 Hz), 2.48–2.40 (m, 1H), 2.12 (s, 3H, CH_3), 2.02–1.94 (m, 1H), 1.79–1.66 (m, 5H), 1.57–1.52 (m, 2H), 1.46–1.08 (m, 12H), 0.99–0.90 (m, 2H), 0.81 (s, 3H, CH_3), 0.72 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 125 MHz): δ (ppm) 152.42, 146.13, 140.35, 130.10, 129.30, 129.30, 127.22, 126.06, 125.74, 122.01, 120.34, 118.67, 118.67, 117.26, 109.89, 71.31, 56.31, 54.49, 48.64, 44.95, 44.77, 38.71, 38.64, 38.25, 37.07, 36.05, 35.60, 32.13, 31.55, 28.73, 27.03, 24.58, 21.14, 17.54 (CH_3), 13.63 (CH_3), 12.38 (CH_3); HRMS (ESI) *m/z* calcd for $C_{36}H_{48}N_3O^+$ $(M+H)^+$ 538.37919, found 538.37925.

*4.2.4.8. 17β-(1-phenyl-4-((m-tolylamino)methyl)-3-pyrazolyl) androst-3β-ol (**5h**).* ***5h***, white solid, Yield: 90%, mp 116–118 °C; ^{1}H NMR ($CDCl_3$, 500 MHz): δ (ppm) 7.81 (s, 1H), 7.63 (d, 2H, *J* = 9.0 Hz), 7.39 (t, 2H, *J* = 7.5 Hz, *J* = 8.5 Hz), 7.22 (t, 1H, *J* = 7.5 Hz), 7.09 (t, 1H, *J* = 7.5 Hz), 6.56 (d, 1H, *J* = 7.5 Hz), 6.48–6.46 (m, 2H), 4.20–4.13 (m, 2H), 3.71–3.55 (m, 2H), 2.79 (t, 1H, *J* = 10.0 Hz), 2.47–2.39 (m, 1H), 2.29 (s, 3H, CH_3), 1.99–1.94 (m, 1H), 1.80–1.66 (m, 5H), 1.56–1.51 (m, 2H), 1.43–1.11 (m, 12H), 1.00–0.91 (m, 2H), 0.81 (s, 3H, CH_3), 0.71 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 125 MHz): δ (ppm) 152.26, 148.20, 140.38, 139.09, 129.29, 129.29, 129.20, 125.97, 125.69, 120.42, 118.70, 118.65, 118.65, 113.77, 110.10, 71.33, 56.33, 54.53, 48.66, 44.96, 44.80, 38.84, 38.65, 38.27, 37.08, 36.06, 35.61, 32.15, 31.56, 28.74, 26.95, 24.58, 21.66, 21.19 (CH_3), 13.62 (CH_3), 12.39 (CH_3); HRMS (ESI) *m/z* calcd for $C_{36}H_{48}N_3O^+$ $(M+H)^+$ 538.37919, found 538.37916.

*4.2.4.9. 17β-(1-phenyl-4-((p-tolylamino)methyl)-3-pyrazolyl) androst-3β-ol (**5i**).* ***5i***, light yellow solid, Yield: 90%, mp 126–128 °C; ^{1}H NMR ($CDCl_3$, 500 MHz): δ (ppm) 7.81 (s, 1H), 7.64–7.62 (m, 2H), 7.39 (t, 2H, *J* = 7.5 Hz, *J* = 8.5 Hz), 7.20 (t, 1H, *J* = 7.5 Hz), 7.01 (d, 2H, *J* = 9.0 Hz), 6.59 (d, 2H, *J* = 9.0 Hz), 4.19–4.12 (m, 2H), 3.84 (s, 1H, NH), 3.60–3.56 (m, 1H), 2.79 (t, 1H, *J* = 9.5 Hz), 2.47–2.40 (m, 1H), 2.25 (s, 3H, CH_3), 2.00–1.95 (m, 1H), 1.80–1.65 (m, 5H), 1.57–1.53 (m, 2H), 1.43–1.10 (m, 12H), 1.00–0.91 (m, 2H), 0.81 (s, 3H, CH_3), 0.70 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 125 MHz): δ (ppm) 152.25, 145.90, 140.39, 129.80, 129.80, 129.28, 129.28, 127.02, 125.96, 125.67, 112.48, 118.65, 118.65, 113.20, 113.20, 71.33, 56.33, 54.53, 48.66, 44.97, 44.81, 39.21, 38.66, 38.27, 37.09, 36.06, 35.61, 32.15, 31.57, 28.74, 26.91, 24.57, 21.19, 20.42 (CH_3), 13.62 (CH_3), 12.39 (CH_3); HRMS (ESI) *m/z* calcd for $C_{36}H_{48}N_3O^+$ $(M+H)^+$ 538.37919, found 538.37921.

*4.2.4.10. 17β-(1-phenyl-4-(((2-methoxyphenyl)amino)methyl)-3-pyrazolyl)androst -3β-ol (**5j**).* ***5j***, light brown solid, Yield: 84%, mp

100–102 °C; ^{1}H NMR ($CDCl_3$, 500 MHz): δ (ppm) 7.84 (s, 1H), 7.65–7.63 (m, 2H), 7.39 (t, 2H, J = 7.5 Hz, J = 8.5 Hz), 7.20 (t, 1H, J = 7.5 Hz), 6.89 (td, 1H, J = 1.5 Hz, J = 7.5 Hz), 6.78 (dd, 1H, J = 1.0 Hz, J = 9.0 Hz), 6.71–6.67 (m, 2H), 4.31 (s, br, 1H), 4.22–4.16 (m, 2H), 3.83 (s, 3H, CH_3), 3.61–3.55 (m, 1H), 2.80 (t, 1H, J = 10.0 Hz), 2.48–2.40 (m, 1H), 2.02–1.94 (m, 1H), 1.80–1.67 (m, 5H), 1.57–1.52 (m, 4H), 1.43–1.19 (m, 10H), 1.00–0.91 (m, 2H), 0.81 (s, 3H, CH_3), 0.70 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 125 MHz): δ (ppm) 152.27, 146.91, 140.43, 138.21, 129.27, 129.27, 126.02, 125.63, 121.35, 120.46, 118.65, 118.65, 116.79, 110.16, 109.47, 71.33, 56.34, 55.42 (CH_3), 54.55, 48.68, 44.97, 44.80, 38.63, 38.61, 38.28, 37.08, 36.07, 35.61, 32.16, 31.57, 28.75, 26.92, 24.58, 21.20, 13.62 (CH_3), 12.39 (CH_3); HRMS (ESI) m/z calcd for $C_{36}H_{48}N_3O_2^+$ $(M+H)^+$ 554.37410, found 554.37329.

*4.2.4.11. 17β-(1-phenyl-4-(((2-fluoro-4-methylphenyl)amino) methyl)-3-pyrazolyl) androst-3β-ol (**5k**).* ***5k***, light yellow solid, Yield: 80%, mp 108–110 °C; ^{1}H NMR ($CDCl_3$, 500 MHz): δ (ppm) 7.81 (s, 1H), 7.63 (d, 2H, J = 7.5 Hz), 7.39 (t, 2H, J = 7.5 Hz, J = 8.5 Hz), 7.20 (t, 1H, J = 7.5 Hz), 6.82–6.80 (m, 2H), 6.66 (t, 1H, J = 8.5 Hz), 4.22–4.15 (m, 2H), 3.84 (s, br, 1H), 3.60–3.56 (m, 1H), 2.78 (t, 1H, J = 9.5 Hz), 2.48–2.40 (m, 1H), 2.24 (s, 3H, CH_3), 2.02–1.94 (m, 1H), 1.80–1.66 (m, 5H), 1.57–1.53 (m, 4H), 1.41–1.10 (m, 10H), 1.00–0.91 (m, 2H), 0.81 (s, 3H, CH_3), 0.70 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 125 MHz): δ (ppm) 152.18, 151.52 (C, J_{CF} = 237.125 Hz), 140.36, 134.15 (C, J_{CF} = 11.875 Hz), 129.28, 129.28, 125.97 (C, J_{CF} = 6.5 Hz), 125.92, 125.71, 124.81 (CH, J_{CF} = 3.0 Hz), 120.12, 118.68, 118.68, 115.27 (CH, J_{CF} = 18.125 Hz), 112.49 (CH, J_{CF} = 3.625 Hz), 71.33, 56.35, 54.54, 48.73, 44.97, 44.80, 38.84, 38.66, 38.26, 37.09, 36.06, 35.61, 32.15, 31.56, 28.74, 26.90, 24.57, 21.19 (CH_3), 20.37 (CH_2), 13.61 (CH_3), 12.38 (CH_3); HRMS (ESI) m/z calcd for $C_{36}H_{47}FN_3O^+$ $(M+H)^+$ 556.36977, found 556.36877.

*4.2.4.12. 17β-(1-phenyl-4-(((3,5-ditrifluoromethyl)phenyl)amino) methyl)-3- pyrazolyl) androst-3β-ol (**5l**).* ***5l***, brown solid, Yield: 74%, mp 124–126 °C; ^{1}H NMR ($CDCl_3$, 500 MHz): δ (ppm) 7.82 (s, 1H), 7.65–7.63 (m, 2H), 7.42 (t, 2H, J = 7.5 Hz,, J = 8.5 Hz), 7.24 (t, 1H, J = 7.0 Hz), 7.18 (s, 1H), 6.97 (s, 2H), 4.26–4.19 (m, 3H), 3.62–3.55 (m, 1H), 2.76 (t, 1H, J = 10.0 Hz), 2.48–2.40 (m, 1H), 2.03–1.95 (m, 1H), 1.79–1.72 (m, 5H), 1.58–1.55 (m, 3H), 1.37–1.27 (m, 4H), 1.23–1.21 (m, 6H), 1.14–1.08 (m, 1H), 1.02–0.91 (m, 2H), 0.81 (s, 3H, CH_3), 0.71 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 125 MHz): δ (ppm) 152.34, 148.47, 140.21, 132.58 (q, C, J_{CF} = 32.375 Hz), 132.58 (q, C, J_{CF} = 32.375 Hz), 129.39, 129.39, 126.06 (d, C, J_{CF} = 2.75 Hz), 123.58 (d, C, J_{CF} = 270.75 Hz), 123.58 (d, C, J_{CF} = 270.75 Hz), 120.33, 118.78, 118.78, 118.66, 111.91 (d, CH, J_{CF} = 2.625 Hz), 111.91 (d, CH, J_{CF} = 2.625 Hz), 110.49 (m, CH, J_{CF} = 3.75 Hz), 71.33, 56.38, 54.50, 48.73, 44.97, 44.85, 38.79, 38.39, 38.25, 37.06, 36.06, 35.62, 32.14, 31.56, 28.72, 27.06, 24.56, 21.16, 13.61 (CH_3), 12.39 (CH_3); HRMS (ESI) m/z calcd for $C_{37}H_{44}F_6N_3O^+$ $(M+H)^+$ 660.33831, found 660.33551.

*4.2.4.13. 17β-(1-phenyl-4-((benzylamino)methyl)-3- pyrazolyl) androst-3β-ol (**5m**).* ***5m***, white solid, Yield: 81%, mp 102–104 °C; ^{1}H NMR ($CDCl_3$ & CD_3OD, 500 MHz): δ (ppm) 7.95 (s, 1H), 7.66–7.65 (m, 2H), 7.43–7.38 (m, 2H), 7.38–7.30 (m, 5H), 7.23 (t, 2H, J = 7.5 Hz), 3.89 (s, 2H), 3.73–3.70 (m, 2H), 3.61–3.57 (m, 1H), 2.59 (t, 1H, J = 9.5 Hz), 2.44–2.37 (m, 1H), 1.94–1.90 (m, 1H), 1.81–1.70 (m, 4H), 1.58–1.55 (m, 1H), 1.47–1.10 (m, 12H), 1.00–0.91 (m, 3H), 0.80 (s, 3H, CH_3), 0.67–0.61 (m, 4H), 0.61 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$ & CD_3OD, 125 MHz): δ (ppm) 152.38, 140.21, 137.34, 129.27, 129.27, 128.71, 128.71, 128.71, 128.71, 127.79, 126.94, 125.94, 118.92, 118.92, 118.43, 70.90, 56.23, 54.52, 52.30, 48.45, 44.94, 44.65, 41.53, 38.40, 37.77, 37.06, 35.98, 35.53, 32.10, 31.07, 28.67, 26.58, 24.40, 21.04, 13.31 (CH_3), 12.23 (CH_3); HRMS (ESI) m/z calcd for $C_{36}H_{48}N_3O^+$ $(M+H)^+$ 538.37919, found 538.37848.

*4.2.4.14. 17β-(1-phenyl-4-((methylamino)methyl)-3-pyrazolyl) androst-5,16-dienes -3β-ol (**11a**).* ***11a***, white solid, Yield: 80%, mp 108–110 °C; ^{1}H NMR ($CDCl_3$ & CD_3OD, 500 MHz): δ (ppm) 8.23 (s, 1H), 7.74–7.72 (m, 2H), 7.45 (t, 2H, J = 7.5 Hz, 8.5 Hz), 7.28 (t, 1H, J = 7.5 Hz), 5.93–5.92 (m, 1H), 5.40–5.39 (m, 1H), 4.00 (m, 2H), 3.52–3.46 (m, 1H), 3.36 (s, 1H), 2.58 (s, 3H), 2.41–2.25 (m, 4H), 2.17–2.06 (m, 1H), 1.88–1.75 (m, 3H), 1.72–1.64 (m, 2H), 1.58–1.26 (m, 5H), 1.15–0.87 (m, 9H), 1.15 (s, 3H, CH_3), 1.08 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$ & CD_3OD, 125 MHz): δ (ppm) 148.34, 147.13, 141.57, 139.99, 129.87, 129.59, 129.59, 127.72, 126.56, 121.39, 118.72, 118.72, 114.74, 71.49, 57.02, 50.88, 48.56, 43.84, 42.12, 37.41, 36.96, 35.47, 33.04, 32.59, 31.82, 31.41, 30.67, 21.15 (CH_3), 19.43 (CH_3), 16.35 (CH_3); HRMS (ESI) m/z calcd for $C_{30}H_{40}N_3O^+$ $(M+H)^+$ 458.31659, found 458.31656.

*4.2.4.15. 17β-(1-phenyl-4-((ethylamino)methyl)-3-pyrazolyl) androst-5,16-dienes -3β-ol(**11b**).* ***11b***, white solid, Yield: 79%, mp 106–108 °C; ^{1}H NMR ($CDCl_3$ & CD_3OD, 500 MHz): δ (ppm) 7.86 (s, 1H), 7.62–7.60 (m, 2H), 7.36–7.32 (m, 2H), 7.16 (t, 1H, J = 7.5 Hz), 5.84 (s, 1H), 5.30 (s, 1H), 3.73–3.67 (m, 3H), 3.42–3.38 (m, 1H), 2.67–2.62 (m, 2H), 2.43–2.40 (m, 2H), 2.27–2.14 (m, 3H), 2.05–1.97 (m, 2H), 1.79–1.67 (m, 3H), 1.63–1.58 (m, 3H), 1.47–1.34 (m, 3H), 1.12–0.80 (m, 11H), 1.07 (t, 3H, CH_3,, J = 7.5 Hz), 1.04 (s, 3H, CH_3), 1.00 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$ & CD_3OD, 125 MHz): δ (ppm) 147.78, 147.67, 141.51, 140.22, 129.43, 129.43, 128.95, 126.11, 125.97, 121.38, 120.18, 118.46, 118.46, 71.43, 56.95, 50.84, 48.40, 43.86, 43.51, 42.09, 37.36, 36.90, 35.59, 32.45, 31.79, 31.37, 30.63, 21.14, 19.38 (CH_3), 16.29 (CH_3), 14.56 (CH_3); HRMS (ESI) m/z calcd for $C_{31}H_{42}N_3O^+$ $(M+H)^+$ 472.33224, found 472.33211.

*4.2.4.16. 17β-(1-phenyl-4-((propylamino)methyl)-3-pyrazolyl) androst-5,16-dienes-3β-ol (**11c**).* ***11c***, white solid, Yield: 62%, mp 130–132 °C; ^{1}H NMR ($CDCl_3$ & CD_3OD, 500 MHz): δ (ppm) 8.06 (s, 1H), 7.72–7.70 (m, 2H), 7.43 (t, 2H, J = 7.5 Hz, 8.5 Hz), 7.25 (t, 1H, J = 7.5 Hz), 5.93–5.92 (m, 1H), 5.39 (d, 1H, J = 5.0 Hz), 3.90–3.83 (m, 2H), 3.52–3.47 (m, 2H), 2.69 (t, 2H, J = 7.5 Hz), 2.48–2.46 (m, 1H), 2.34–2.22 (m, 3H), 2.15–2.06 (m, 2H), 1.87–1.75 (m, 3H), 1.68–1.40 (m, 9H), 1.14–1.04 (m, 8H), 1.14 (s, 3H, CH_3), 1.08 (s, 3H, CH_3), 0.95 (t, 3H, J = 7.5 Hz); ^{13}C NMR ($CDCl_3$ & CD_3OD, 125 MHz): δ (ppm) 147.96, 147.51, 141.51, 140.13, 129.46, 129.46, 129.23, 127.70, 126.12, 121.39, 118.53, 118.53, 118.33, 71.49, 56.98, 50.84, 50.47, 48.43, 43.35, 42.14, 37.35, 36.90, 35.53, 32.49, 31.79, 31.43, 30.62, 21.92, 21.12, 19.41 (CH_3), 16.33 (CH_3), 11.59 (CH_3); HRMS (ESI) m/z calcd for $C_{32}H_{44}N_3O^+$ $(M+H)^+$ 486.34789, found 486.34793.

*4.2.4.17. 17β-(1-phenyl-4-((isopropylamino)methyl)-3-pyrazolyl) androst-5,16-dienes -3β-ol (**11d**).* ***11d***, white solid, Yield: 73%, mp 202–204 °C; ^{1}H NMR ($CDCl_3$ & CD_3OD, 500 MHz): δ (ppm) 8.57 (s, 1H), 7.76–7.74 (m, 2H), 7.43 (t, 2H, J = 7.5 Hz, 8.5 Hz), 7.27 (t, 1H, J = 7.5 Hz), 5.89–5.88 (m, 1H), 5.39 (d, 1H, J = 5.0 Hz), 4.13–4.06 (m, 2H), 3.52–3.46 (m, 1H), 3.36 (s, 1H), 3.27–3.22 (m, 1H), 2.36–2.25 (m, 4H), 2.17–2.05 (m, 2H), 1.86–1.75 (m, 3H), 1.71–1.62 (m, 3H), 1.56–1.49 (m, 3H), 1.37 (d, 3H, J = 6.5 Hz), 1.34 (d, 3H, J = 6.5 Hz), 1.15–1.03 (m, 8H), 1.15 (s, 3H, CH_3), 1.07 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$ & CD_3OD, 125 MHz): δ (ppm) 147.96, 146.53, 141.06, 139.38, 129.72, 129.07, 129.07, 128.19, 126.14, 120.87, 118.30, 118.30, 112.51, 71.04, 56.64, 50.36, 48.60, 48.05, 41.67, 38.41, 36.90, 36.45, 34.82, 32.10, 31.31, 30.96, 30.15, 20.60, 19.25, 18.96 (CH_3), 18.83 (CH_3), 15.91 (CH_3); HRMS (ESI) m/z calcd for $C_{32}H_{44}N_3O^+$ $(M+H)^+$ 486.34789, found 486.34750.

*4.2.4.18. 17β-(1-phenyl-4-((butylamino)methyl)-3-pyrazolyl) androst-5,16-dienes -3β-ol (**11e**).* ***11e***, glasslike solid, Yield: 89%, mp

100–102 °C; ^{1}H NMR ($CDCl_3$ & CD_3OD, 500 MHz): δ (ppm) 7.95 (s, 1H), 7.71–7.69 (m, 2H), 7.43 (t, 2H, *J* = 7.5 Hz, 8.5 Hz), 7.24 (t, 1H, *J* = 7.5 Hz), 5.93–5.94 (m, 1H), 5.40 (d, 1H, *J* = 5.0 Hz), 3.82–3.74 (m, 2H), 3.50–3.48 (m, 1H), 3.37 (s, 1H), 2.67 (t, 2H, 7.5 Hz), 2.51–2.49 (m, 1H), 2.35–2.26 (m, 3H), 2.14–2.07 (m, 2H), 1.89–1.80 (m, 3H), 1.72–1.67 (m, 3H), 1.52–1.26 (m, 8H), 1.15–1.05 (m, 8H), 1.14 (s, 3H, CH_3), 1.05 (s, 3H, CH_3), 0.94 (t, 3H, *J* = 7.5 Hz); ^{13}C NMR ($CDCl_3$ & CD_3OD, 125 MHz): δ (ppm) 147.82, 147.70, 141.50, 140.23, 129.44, 129.44, 128.96, 126.19, 125.97, 121.41, 120.11, 118.48, 118.48, 71.49, 56.97, 50.85, 49.07, 48.40, 44.08, 42.11, 37.35, 36.90, 35.59, 32.46, 31.80, 31.60, 31.40, 30.63, 21.14, 20.52, 19.40 (CH_3), 16.32 (CH_3), 13.95 (CH_3); HRMS (ESI) *m*/*z* calcd for $C_{33}H_{46}N_3O^+$ $(M+H)^+$ 500.36354, found 500.36353.

*4.2.4.19. 17β-(1-phenyl-4-((phenylamino)methyl)-3-pyrazolyl) androst-5,16-dienes -3β-ol (**11f**).* ***11f***, white solid, Yield: 84%, mp 98–100 °C; ^{1}H NMR ($CDCl_3$, 500 MHz): δ (ppm) 7.85 (s, 1H), 7.67 (d, 2H, *J* = 7.5 Hz), 7.40 (t, 2H, *J* = 7.0 Hz), 7.25–7.18 (m, 3H), 7.08 (t, 1H, *J* = 7.5 Hz), 6.74 (t, 1H, *J* = 7.0 Hz), 6.67–6.65 (m, 2H), 6.02 (s, 1H), 5.39 (s, 1H), 4.25 (s, 2H), 3.85 (s, 1H), 3.58–3.47 (m, 1H), 2.63–2.60 (m, 1H), 2.30–2.26 (m, 3H), 2.10–2.04 (m, 2H), 1.85–1.77 (m, 3H), 1.73–1.60 (m, 3H), 1.55–1.48 (m, 3H), 1.15–1.08 (m, 8H), 1.15 (s, 3H, CH_3), 1.08 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 125 MHz): δ (ppm) 147.00, 146.76, 146.07, 140.20, 139.09, 128.42, 128.29, 128.29, 128.29, 128.29, 125.11, 124.77, 120.47, 119.15, 117.28, 117.28, 116.71, 111.93, 111.93, 70.78, 55.73, 49.67, 47.16, 41.36, 38.66, 36.21, 35.75, 34.55, 31.43, 30.68, 30.68, 29.46, 28.68, 18.35 (CH_3), 15.26 (CH_3); HRMS (ESI) *m*/*z* calcd for $C_{35}H_{42}N_3O^+$ $(M+H)^+$ 520.33224, found 520.33213.

*4.2.4.20. 17β-(1-phenyl-4-((o-tolylamino)methyl)-3-pyrazolyl) androst-5,16-dienes -3β-ol (**11g**).* ***11g***, white solid, Yield: 81%, mp 122–124 °C; ^{1}H NMR ($CDCl_3$, 500 MHz): δ (ppm) 7.87 (s, 1H), 7.68 (d, 2H, *J* = 9.5 Hz), 7.42 (t, 2H, *J* = 8.5 Hz), 7.23 (t, 1H, *J* = 7.0 Hz), 7.15 (t, 1H, *J* = 7.5 Hz), 7.08 (d, 1H, *J* = 7.0 Hz), 6.73–6.68 (m, 2H), 6.04–6.03 (m, 1H), 5.40–5.39 (m, 1H), 4.30 (s, 2H), 3.73 (s, 1H), 3.57–3.51 (m, 1H), 2.62 (dt, 1H, *J* = 12.5, 3.5 Hz), 2.34–2.25 (m, 3H), 2.14 (s, 3H), 2.10–2.05 (m, 2H), 1.90–1.75 (m, 3H), 1.70–1.67 (m, 3H), 1.58–1.47 (m, 4H), 1.17–1.06 (m, 8H), 1.17 (s, 3H, CH_3), 1.10 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 125 MHz): δ (ppm) 147.94, 147.08, 145.97, 141.23, 140.12, 130.10, 129.47, 129.32, 129.32, 127.21, 126.27, 125.83, 122.05, 121.49, 120.12, 118.34, 118.34, 117.30, 110.01, 71.81, 56.79, 50.71, 48.21, 42.39, 39.63, 37.24, 36.78, 35.61, 32.45, 31.72, 31.71, 30.49, 21.09, 19.37 (CH_3), 17.52 (CH_3), 16.30 (CH_3); HRMS (ESI) *m*/*z* calcd for $C_{36}H_{44}N_3O^+$ $(M+H)^+$ 534.34789, found 534.34788.

*4.2.4.21. 17β-(1-phenyl-4-((m-tolylamino)methyl)-3-pyrazolyl) androst-5,16-dienes -3β-ol (**11h**).* ***11h***, glasslike solid, Yield: 89%, mp 118–120 °C; ^{1}H NMR ($CDCl_3$, 500 MHz): δ (ppm) 7.85 (s, 1H), 7.67 (d, 2H, *J* = 8.0 Hz), 7.41 (t, 2H, *J* = 7.5 Hz), 7.22 (t, 1H, *J* = 7.0 Hz), 7.08 (t, 1H, *J* = 7.5 Hz), 6.56 (d, 1H, *J* = 7.5 Hz), 6.48–6.47 (m, 2H), 6.03–6.02 (m, 1H), 5.39–5.38 (m, 1H), 4.24 (s, 2H), 3.84 (s, 1H), 3.56–3.51 (m, 1H), 2.61 (dt, 1H, *J* = 12.5, 3.5 Hz), 2.33–2.26 (m, 6H), 2.28 (s, 3H, CH_3), 2.10–2.04 (m, 2H), 1.88–1.76 (m, 3H), 1.70–1.65 (m, 3H), 1.57–1.47 (m, 4H), 1.16–1.06 (m, 8H), 1.16 (s, 3H, CH_3), 1.08 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 125 MHz): δ (ppm) 148.10, 147.82, 147.10, 141.25, 140.15, 139.11, 129.47, 129.31, 129.31, 129.19, 126.14, 125.78, 121.05, 120.31, 118.70, 118.32, 118.32, 113.72, 110.13, 71.82, 56.78, 50.73, 48.19, 42.40, 39.69, 37.25, 36.79, 35.60, 32.46, 31.73, 31.72, 30.51, 21.65, 21.10 (CH_3), 19.38 (CH_3), 16.29 (CH_3); HRMS (ESI) *m*/*z* calcd for $C_{36}H_{44}N_3O^+$ $(M+H)^+$ 534.34789, found 534.34783.

*4.2.4.22. 17β-(1-phenyl-4-((p-tolylamino)methyl)-3-pyrazolyl) androst-5,16-dienes -3β-ol (**11i**).* ***11i***, white solid, Yield: 87%, mp 112–114 °C; ^{1}H NMR ($CDCl_3$ & CD_3OD, 500 MHz): δ (ppm) 7.90 (s, 1H), 7.64 (d, 2H, *J* = 8.5 Hz), 7.39 (t, 2H, *J* = 7.5 Hz), 7.20 (t, 1H, *J* = 7.5 Hz), 6.97 (t, 2H, *J* = 8.0 Hz), 6.60 (d, 2H, *J* = 8.5 Hz), 5.99–5.98 (m, 1H), 5.37–5.34 (m, 1H), 4.20 (s, 2H), 3.49–3.42 (m, 1H), 3.33 (s, 1H), 2.54 (dt, 1H, *J* = 12.5, 3.5 Hz), 2.29–2.20 (m, 6H), 2.21 (s, 3H, CH_3), 2.10–2.02 (m, 2H), 1.88–1.73 (m, 3H), 1.69–1.65 (m, 3H), 1.58–1.45 (m, 4H), 1.13–1.04 (m, 8H), 1.13 (s, 3H, CH_3), 1.08 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$ & CD_3OD, 125 MHz): δ (ppm) 147.78, 147.16, 145.88, 141.29, 140.08, 129.57, 129.57, 129.42, 129.16, 129.16, 126.87, 126.26, 125.68, 121.16, 120.61, 118.22, 118.22, 113.39, 113.39, 71.16, 56.78, 50.75, 48.12, 41.80, 39.86, 37.19, 36.69, 35.49, 32.22, 31.57, 31.06, 30.47, 21.95, 19.92 (CH_3), 19.03 (CH_3), 15.98 (CH_3); HRMS (ESI) *m*/*z* calcd for $C_{36}H_{44}N_3O^+$ $(M+H)^+$ 534.34789, found 534.34790.

*4.2.4.23. 17β-(1-phenyl-4-(((2-methoxyphenyl)amino)methyl)-3-pyrazolyl)androst-5,16-dienes -3β-ol (**11j**).* ***11j***, white solid, Yield: 78%, mp 120–122 °C; ^{1}H NMR ($CDCl_3$, 500 MHz): δ (ppm) 7.86 (s, 1H), 7.67 (d, 2H, *J* = 8.5 Hz), 7.40 (t, 2H, *J* = 7.5 Hz), 7.21 (t, 1H, *J* = 7.5 Hz), 6.87 (td, 1H, *J* = 8.0, 1.5 Hz), 6.80 (dd, 1H, *J* = 7.5, 1.0 Hz), 6.71–6.66 (m, 2H), 6.01–6.00 (m, 1H), 5.40–5.39 (m, 1H), 4.47 (s, 1H), 4.24 (s, 2H), 3.84 (s, 3H), 3.56–3.51 (m, 1H), 2.60 (dt, 1H, *J* = 12.5, 3.5 Hz), 2.31–2.23 (m, 3H), 2.08–2.04 (m, 2H), 1.90–1.74 (m, 3H), 1.70–1.65 (m, 3H), 1.58–1.46 (m, 4H), 1.16–1.05 (m, 8H), 1.16 (s, 3H, CH_3), 1.09 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 125 MHz): δ (ppm) 147.87, 147.09, 146.91, 141.25, 140.20, 138.08, 129.47, 129.29, 129.29, 126.12, 125.73, 121.52, 121.34, 120.44, 118.32, 118.32, 116.81, 110.24, 109.48, 71.83, 56.77, 55.43 (CH_3), 50.75, 48.18, 42.40, 39.51, 37.25, 36.80, 35.57, 32.45, 31.74, 31.74, 30.51, 21.10, 19.38 (CH_3), 16.28 (CH_3); HRMS (ESI) *m*/*z* calcd for $C_{36}H_{44}N_3O_2^+$ $(M+H)^+$ 550.34280, found 550.34321.

*4.2.4.24. 17β-(1-phenyl-4-(((2-fluoro-4-methylphenyl)amino) methyl)-3-pyrazolyl) androst-5,16-dienes -3β-ol (**11k**).* ***11k***, white solid, Yield: 67%, mp 148–150 °C; ^{1}H NMR ($CDCl_3$, 500 MHz): δ (ppm) 7.85 (s, 1H), 7.67 (d, 2H, *J* = 8.5 Hz), 7.40 (t, 2H, *J* = 7.5 Hz), 7.21 (t, 1H, *J* = 7.5 Hz), 6.81 (t, 2H, *J* = 7.0 Hz), 6.60 (t, 1H, *J* = 6.0 Hz), 6.00–5.99 (m, 1H), 5.39–5.38 (m, 1H), 4.27 (s, 2H), 4.01 (s, 1H), 3.56–3.50 (m, 1H), 2.58 (dt, 1H, *J* = 12.5, 3.5 Hz), 2.34–2.24 (m, 6H), 2.24 (s, 3H, CH_3), 2.11–2.03 (m, 2H), 1.88–1.74 (m, 3H), 1.70–1.65 (m, 3H), 1.58–1.45 (m, 4H), 1.16–1.05 (m, 8H), 1.16 (s, 3H, CH_3), 1.08 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 125 MHz): δ (ppm) 151.53 (d, C, J_{CF} = 237.25 Hz), 147.76, 147.19, 141.26, 140.14, 134.02 (d, C, J_{CF} = 12.0 Hz), 129.40, 129.30, 129.30, 127.04 (d, CH, J_{CF} = 6.5 Hz), 126.01, 125.81, 124.81 (d, CH, J_{CF} = 3.125 Hz), 121.49, 120.10, 118.34, 118.34, 115.29 (d, CH, J_{CF} = 18.125 Hz), 112.59 (d, CH, J_{CF} = 3.625 Hz), 71.82, 56.76, 50.72, 48.20, 42.40, 39.71, 37.25, 36.79, 35.56, 32.44, 31.73, 31.71, 30.51, 21.09, 20.36 (d, CH_3, J_{CF} = 1.0 Hz), 19.37 (CH_3), 16.27 (CH_3); HRMS (ESI) *m*/*z* calcd for $C_{36}H_{43}FN_3O^+$ $(M+H)^+$ 552.33847, found 552.33759.

*4.2.4.25. 17β-(1-phenyl-4-(((3,5-ditrifluoromethyl)phenyl)amino) methyl)-3-pyrazolyl) androst-5,16-dienes -3β-ol (**11l**).* ***11l***, white solid, Yield: 59%, mp 90–92 °C; ^{1}H NMR ($CDCl_3$, 500 MHz): δ (ppm) 7.86 (s, 1H), 7.66 (d, 2H, *J* = 8.0 Hz), 7.43 (t, 2H, *J* = 7.5 Hz), 7.25 (t, 1H, *J* = 7.0 Hz), 7.19 (s, 1H), 6.98 (s, 2H), 5.97–5.96 (m, 2H), 5.40–5.39 (m, 1H), 4.30 (s, 3H, NH–CH_2), 3.57–3.55 (m, 1H), 2.57 (dt, 1H, *J* = 12.5, 3.5 Hz), 2.33–2.22 (m, 3H), 2.12–2.04 (m, 2H), 1.88–1.77 (m, 3H), 1.70–1.66 (m, 3H), 1.58–1.39 (m, 3H), 1.16–1.06 (m, 8H), 1.16 (s, 3H, CH_3), 1.09 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 125 MHz): δ (ppm) 148.38, 147.92, 147.08, 141.25, 139.98, 132.44 (q, C, J_{CF} = 32.5 Hz), 132.44 (q, C, J_{CF} = 32.5 Hz), 129.55, 129.42, 129.42, 126.31, 126.16, 123.56 (d, C, *J* = 271.25 Hz), 123.56 (d, C, *J* = 271.25 Hz), 121.46, 118.46, 118.46, 118.34, 111.96 (m, CH), 110.61 (m, CH), 110.61 (m, CH), 71.82, 56.79, 50.70, 48.26, 42.40, 39.20, 37.25, 36.79, 35.51, 32.48, 31.73, 31.69, 30.50, 21.08, 19.38 (CH_3), 16.27 (CH_3); HRMS (ESI) *m*/*z* calcd for $C_{37}H_{40}F_6N_3O^+$ $(M+H)^+$ 656.30701, found 656.30463.

*4.2.4.26. 17β-(1-phenyl-4-((benzylamino)methyl)-3-pyrazolyl) androst-5,16-dienes -3β-ol (**11m**).* ***11m***, white solid, Yield: 76%, mp 82–84 °C; ^{1}H NMR ($CDCl_3$ & CD_3OD, 500 MHz): δ (ppm) 7.85 (s, 1H), 7.61 (m, 2H), 7.34–7.15 (m, 8H), 5.76 (m, 1H), 5.31 (m, 1H), 3.75 (m, 4H), 3.41 (m, 1H), 2.42–2.40 (m, 1H), 2.20–2.17 (m, 3H), 2.20–1.98 (m, 2H), 1.79–1.57 (m, 6H), 1.43–1.36 (m, 3H), 1.03–0.79 (m, 10H), 1.03 (s, 3H, CH_3), 1.00 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$ & CD_3OD, 125 MHz): δ (ppm) 146.86, 146.46, 140.45, 139.17, 138.10, 128.38, 128.38, 128.07, 127.62, 127.62, 127.49, 127.49, 126.41, 125.32, 124.89, 120.35, 119.21, 117.40, 117.40, 70.40, 55.85, 52.27, 49.78, 47.30, 42.29, 41.04, 36.30, 35.84, 34.52, 31.36, 30.74, 30.32, 29.56, 20.07, 18.33 (CH_3), 15.24 (CH_3); HRMS (ESI) *m/z* calcd for $C_{36}H_{44}N_3O^+$ $(M+H)^+$ 534.34789, found 534.34729.

*4.2.5. Procedure for 17β-(1- phenyl -4-methylol-3- pyrazolyl) androst-3β-ol **6***

$NaBH_4$ (15 mg, 0.4 mmol, 2 eq) was added to a solution of ***3*** (0.2 mmol, 1 eq) in 10 mL THF/MeOH:: 1:1, v/v at RT for 0.5 h. After completion the reaction was quenched by AcOH (100 μL) and the solvent was removed in vacuo, and the residue was diluted with saturated aqueous $NaHCO_3$ (10 mL) and extracted with EtOAc (3 × 20 mL). The combined organic layers were washed with brine (10 mL), dried over Na_2SO_4, filtered and concentrated. The residue was purified by column chromatography (CH_2Cl_2: MeOH:: 50:1, v/v) to give products as white solid.

*4.2.5.1. 17β-(1- phenyl -4-methylol-3- pyrazolyl)androst-3β-ol (**6**).* ***6***, white solid, Yield: 96%, mp 200–202 °C; ^{1}H NMR (DMSO-d_6, 500 MHz): δ (ppm) 8.23 (s, 1H), 7.77–7.75 (m, 2H), 7.47 (t, 2H, *J* = 7.0 Hz,, *J* = 8.0 Hz), 7.22 (t, 1H, *J* = 7.5 Hz), 4.44–4.35 (m, 3H), 2.80 (t, 1H, *J* = 10.0 Hz), 2.37–2.29 (m, 1H), 1.92–1.84 (m, 1H), 1.71–1.48 (m, 9H), 1.28–1.11 (m, 9H), 1.05–0.87 (m, 3H), 0.74 (s, 3H, CH_3), 0.70–0.65 (m, 1H), 0.59 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 125 MHz): δ (ppm) 151.74, 140.36, 129.89, 129.89, 127.01, 125.80, 124.07, 118.13, 118.13, 69.82, 56.26, 54.52, 48.41, 44.94, 44.59, 40.28, 38.69, 38.37, 37.18, 36.08, 35.68, 32.28, 31.87, 28.91, 26.73, 24.63, 21.18, 13.94 (CH_3), 12.65 (CH_3); HRMS (ESI) *m/z* calcd for $C_{29}H_{41}N_2O_2^+$ $(M+H)^+$ 449.31625, found 449.31540.

4.3. In vitro cytotoxicity

All derivatives synthesized in this study were evaluated for their cytotoxic bioactivities by the standard RSB method following our previously reported method [23], using 293T cell lines and three cancer cell lines: A549, Hela and MCF-7. *Cis*-platin (Sigma) was used as a reference controls. IC_{50} value of each compound was calculated, given in Table 4. Each assay was done in triplicate.

4.4. Hoechst 33342 staining assay

Hoechst 33342 (Invitrogen, USA) was used as probe to investigate the effect of steroidal derivatives **11b** on nuclear morphological modifications. The Hela cells were seeded at a concentration of 10^6 per well on a sterile cover slip in six-well plates and incubated for 16 h. After that, compound **11b** (2.5 μM, 5.0 μM and 7.5 μM) was added and the cells were incubated for 48 h. DMSO served as negative control. Then, the culture medium containing compound was removed, and cells were fixed in 4% paraformaldehyde for 15 min. After being washed with PBS twice, the cells were stained by 5 μL Hoechst 33342 for 10 min, and then washed with PBS twice. The stained cells were examined immediately by using of IX71SIF-3 fluorescence microscope with 350 nm excitation and 460 nm emission.

4.5. Apoptosis analysis

Apoptosis was discriminated with the annexin V-FICT/PI test. The Hela cells were seeded on six-well at concentration of 10^6 per well and stabilized for 24 h at 37 °C in the humidified atmosphere containing 5% CO_2. Subsequently, the cells were treated with hybrid **11b** at different concentrations, 2.5 μM, 5.0 μM and 7.5 μM, for 24 h. DMSO served as negative control. After that, the Hela cells were washed thrice with cold PBS, and then resuspended in 500 μL cold binding buffer at a concentration of 1 × 10^6 cells/mL. Thereafter, cells were stained with 5 μL AnnexinV-FITC and shaken well. Finally, the cells were mixed with 5 μL PI, incubated for 15 min in the dark and subsequently analyzed using a BD FACS Calibur flow cytometer (BectoneDickinson, San Jose, CA, USA).

4.6. Cell cycle analysis

For flow cytometric analysis of DNA content, 1 × 10^6 Hela cells in exponential growth were treated with compound **11b** at various concentrations, 2.5 μM, 5.0 μM and 7.5 μM, for 24 h. DMSO served as negative control. After that, the cells were collected and fixed with cold 70% ethanol overnight at −20 °C. The cells were then treated with buffer containing RNase A (50 μg/mL) at 37 °C for 30 min, washed with cold PBS thrice, and finally stained by using of 5 μL PI at 4 °C in the dark for 30 min. Samples were analyzed on BD FACS Calibur flow cytometer (BectoneDickinson, San Jose, CA, USA).

Acknowledgments

This study was partially supported with grants from the National Natural Science Foundation of China (31070704, 31300548), the National Key Basic Research Program (973 Program, 2010CB126100) from Science and Technology, Ministry of China, as well as Agricultural Science and Technology Research Foundation of Shaanxi Province (2014k02-10-02).

Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.ejmech.2017.02.033. These data include MOL files and InChiKeys of the most important compounds described in this article.

References

[1] K. Popat, K. McQueen, T.W. Feeley, The global burden of cancer, Best Pract. Res. Clin. Anaesthesiol. 27 (2013) 399–408.
[2] M. Karabacak, M.D. Altıntop, H. Çiftçi, R. Koga, M. Otsuka, M. Fujita and A.Özdemir, Synthesis and evaluation of new pyrazoline derivatives as potential anticancer agents, Molecules 20 (2015) 19066–19084.
[3] P. Ravula, H.B. Vamaraju, M. Paturi, N.S. Chandra, S. Kolli, Design, synthesis, in silico toxicity prediction, molecule docking, and evaluation of novel pyrazole derivatives as potential antiproliferative agents, EXCLI J. 15 (2015) 187–202.
[4] J. Foo, F. Michor, Evolution of acquired resistance to anti-cancer therapy, J. Theor. Biol. 355 (2014) 10–20.
[5] X. Liang, C. Chen, Y. Zhao, P.C. Wang, Circumventing tumor resistance to chemotherapy by nanotechnology, Methods Mol. Biol. 596 (2010) 467–488.
[6] B. Maggio, G. Daidone, D. Raffa, S. Plescia, L. Mantione, V.M. Catena Cutuli, N.G. Mangano, A. Caruso, Synthesis and pharmacological study of ethyl 1-methyl-5-(substituted 3,4-dihydro-4-oxoquinazolin-3-yl)-1H-pyrazole-4-acetates, Eur. J. Med. Chem. 36 (2001) 737–742.
[7] V. Padmavathi, S. Nagi Reddy, K. Mahesh, Synthesis, antimicrobial and antioxidant activities of sulfone linked bis heterocycles—pyrazolyl oxadiazoles and pyrazolyl thiadiazole, Chem. Pharm. Bull. 57 (2009) 1376–1380.
[8] J. Wu, Q. Shi, Z. Chen, M. He, L. Jin, D. Hu, Synthesis and bioactivity of pyrazole acyl thiourea derivatives, Molecules 17 (2012) 5139–5150.
[9] D. Kaushik, S.A. Khan, G. Chawla, S. Kumar, N′-[(5-chloro-3-methyl- 1-phenyl-1H-pyrazol-4-yl) methylene] 2/4-substituted hydrazides: synthesis and anticonvulsant activity, Eur. J. Med. Chem. 45 (2010) 3943–3949.
[10] N. Siddiqui, P. Alam, W. Ahsan, Design, synthesis, and in-vivo pharmacological screening of N,3-(substituted diphenyl)-5-phenyl-1H-pyrazoline-1-

carbothioamide derivatives, Arch. Pharm. Chem. Life Sci. 342 (2009) 173–181.
[11] D. Castagnolo, A. De Logu, M. Radi, B. Bechi, F. Manetti, M. Magnani, S. Supino, R. Meleddu, L. Chisu, M. Botta, Synthesis, biological evaluation and SAR study of novel pyrazole analogues as inhibitors of *Mycobacterium tuberculosis*, Bioorg. Med. Chem. 16 (2008) 8587–8591.
[12] X. Yang, P. Zhang, Y. Zhou, J. Wang, H. Liu, Synthesis and antioxidant activities of novel 4,4′-Arylmethylene-bis(1H-pyrazole-5-ol)s from lignin, Chin. J. Chem. 30 (2012) 670–674.
[13] S.A.F. Rostom, M.A. Shalaby, M.A. El-Demellawy, Polysubstituted pyrazoles, part 5.1 Synthesis of new 1-(4-chlorophenyl)-4-hydroxy-1H-pyrazole-3-carboxylic acid hydrazide analogs and some derived ring systems. A novel class of potential antitumor and anti-HCV agents, Eur. J. Med. Chem. 38 (2003) 959–974.
[14] H. Song, Y. Liu, L. Xiong, Y. Li, N. Yang, Q. Wang, Design, synthesis, and insecticidal activity of novel pyrazole derivatives containing α-hydroxymethyl-N-benzyl carboxamide, α-chloromethyl -N-benzyl carboxamide, and 4,5-dihydrooxazole moieties, J. Agric. Food Chem. 60 (2012) 1470–1479.
[15] B. Insuasty, A. Tigreros, F. Orozco, J. Quiroga, R. Abonia, M. Nogueras, A. Sanchez, J. Cobo, Synthesis of novel pyrazolic analogues of chalcones and their 3-aryl-4-(3-aryl- 4,5-dihydro-1H-pyrazol-5-yl)-1-phenyl-1H-pyrazole derivatives as potential antitumor agents, Bioorg. Med. Chem. 18 (2010) 4965–4974.
[16] M.F. Khan, M.M. Alam, G. Verma, W. Akhtar, M. Akhter, M. Shaquiquzzaman, The therapeutic voyage of pyrazole and its analogs: a review, Eur. J. Med. Chem. 120 (2016) 170–201.
[17] L. Romero-Hern andez, P. Merino-Montiel, S. Montiel-Smith, S. Meza-Reyes, J.L. Vega-Baez, I. Abasolo, S. Schwartz Jr., O. Lopez, J.G. Fernandez-Bolanos, Diosgenin-based thio(seleno)ureas and triazolyl glycoconjugates as hybrid drugs. Antioxidant and antiproliferative profile, Eur. J. Med. Chem. 99 (2015) 67–81.
[18] C. Camoutsis, D. Mourelatos, G. Pairas, E. Mioglou, C. Gasparinatou, Z. IakovidouA, Synthesis and cytogenetic studies of structure–biological activity relationship of esters of Hecogenin and aza-homo-Hecogenin with N,N-bis(2-chloroethyl)aminocinnamic acid isomers, Steroids 70 (2005) 586–593.
[19] F. Alonso, A.M. Cirigliano, M.E. Dávola, G.M. Cabrera, G.E.G. Liñares, C. Labriola, A.A. Barquero, J.A. Ramírez, Multicomponent synthesis of 4,4-dimethyl sterol analogues and their effect on eukaryotic cells, Steroids 84 (2014) 1–6.
[20] Z. Iványia, J. Wölflinga, T. Görbea, M. Szécsib, T. Wittmannb, G. Schneidera, Synthesis of regioisomeric 17β-N-phenylpyrazolyl steroid derivatives and their inhibitory effect on 17α-hydroxylase/$C_{17,20}$-lyase, Steroids 75 (2010) 450–456.
[21] N. Szabó, Z. Iványi, M. Szécsi, J. Julesz, E. Mernyák, J. Huber, J. Wölfling, R. Minorics, I. Zupkó, G. Schneider, Synthesis of methoxycarbonylpyrazolylandrostene derivatives, and their potential inhibitory effect on androgen biosynthesis and cell proliferation, Steroids 98 (2015) 143–152.
[22] A. Romero-López, S. Montiel-Smith, S. Meza-Reyes, P. Merino-Montiel, J.L. Vega-Baez, Synthesis of steroidal derivatives containing substituted, fused and spiro pyrazolines, Steroids 87 (2014) 86–92.
[23] J. Li, X. Zhao, L. Li, Z. Yuan, F. Tan, B. Shi, J. Zhang, Design, synthesis and cytotoxic activity of a novel series of steroidal phenylpyrazoles, Steroids 107 (2016) 45–54.
[24] D. Kovacs, J. Wolfling, N. Szabo, M. Szecsi, Z. Schelz, I. Zupko, E. Frank, Synthesis of novel 17-(40-formyl)pyrazolylandrosta-5,16-dienes and their derivatives as potent 17α-hydroxylase/$C_{17,20}$-lyase inhibitors or antiproliferative agents depending on the substitution pattern of the heteroring, Eur. J. Med. Chem. 120 (2016) 284–295.
[25] Molinspiration Cheminformatics, Molinspiration Property Engine v2014.11. www.molinspiration.com.
[26] M. Mahmoudi, K. Azadmanesh, M.A. Shokrgozar, W.S. Journeay, S. Laurent, Effect of nanoparticles on the cell life cycle, Chem. Rev. 111 (2011) 3407–3432.