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ORIGINAL ARTICLE

A novel pregnene analogs: synthesis, cytotoxicity on prostate cancer of PC-3 and LNCPa-AI cells and in silico molecular docking study

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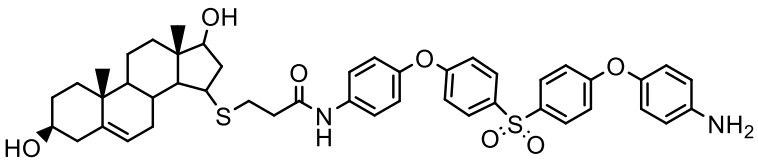
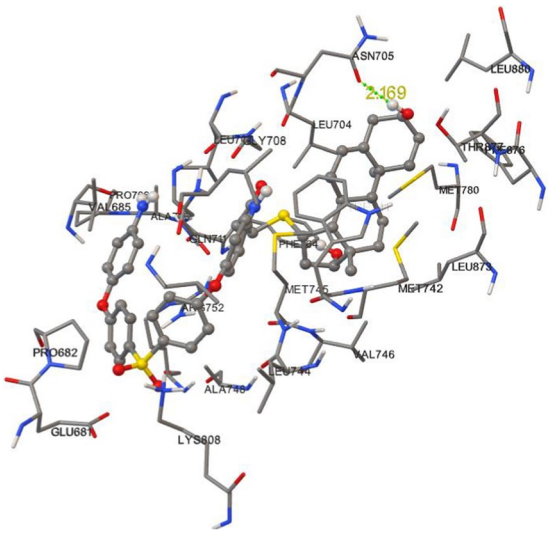
Abstract

New pregnene analogs of *N*-hydroxamic acid **6**, imino-propane hydrazides **7** and **8** as well as the aryl amides **9**–**11**, oxadiazole, pyrazole and sulfinyl analogs **13**–**15**, via the hydrazide analog **5** of methyl ((5-pregnen-3β,17β-diol-15α-yl)thio) propanoate (**4**) were synthesized. The in vitro cytotoxic activities of selected synthesized steroids against two human prostate cancer cell lines (PC-3, and LNCaP-AI) were evaluated by MTT assay. Compound **10** was the most active cytotoxic agent among these steroids against PC-3 and LNCaP-AI cell lines with inhibition of 96.2%, and 93.6% at concentration levels of 10.0 µM and 91.8%, and of 79.8% at concentration of 1.0 µM, respectively. Molecular docking study of **10** showed a hydrogen bonding with the amino acid Asn705 residue of the receptor 1E3G, together with hydrophobic interactions. Therefore, compound **10** can be considered as a promising anticancer agent due to its potent cytotoxic activity.

Graphic abstract



Cytotoxicity against PC-3 and LNCaP cell lines = 96.2%, and 93.6%, respectively



Keywords Amides · Anticancer activity · Molecular docking study · Oxadiazole · Pregnene analogs · Pyrazole

Introduction

Prostate cancer (PCa) is the second common invasive cancer in male worldwide [1]. Because PCa is initially androgen sensitive, it will respond to anti-hormonal therapy. Later it becomes androgen-refractory and continues to progress

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Extended author information available on the last page of the article

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in the absence of androgens [2]. This form of the disease is known as castrate resistance PCa (CRPC), which has no effective therapeutic strategies at this time [3]. Several pharmaceutically active ingredients have been approved for the treatment of CRPC in patients; however, progression is often inevitable [4]. Among the approved compounds are steroids, which form an essential class of biologically active compounds [5–7]. Steroids and their derivatives are promising drugs for the treatment of several diseases including autoimmune diseases [8], cardiovascular diseases [9], brain tumors [10], breast cancer [11], prostate cancer [12], or osteoarthritis [13]. Moreover, they act as antiviral agents [14–16].

Inhibition of the vital androgen biosynthesis enzyme CYP17 17α-hydroxy/17,20-lyase prevents androgen production, which has been shown to be an effective treatment option for prostate carcinoma [17, 18]. The synthesis of several CYP17 inhibitors for the treatment of PCa has since become an emerging field [19–26]. Recently, our laboratory reported chalconyl steroid **1** (Fig. 1) as a CYP17 inhibitor with an IC_{50} value of 0.61 µM [27]. Furthermore, Njar et al. [28] reported that novel steroids with azole moieties functioned as potent inhibitors of cytochrome P-450 dependent ATRA (all-trans-retinoic acid) 4-hydroxylase enzyme. Since then, several steroids containing nitrogen substituents have been prepared as potential agents for the treatment of PCa [29–31]. Abiraterone acetate (Zytiga) (**2**, Fig. 1) [32, 33], galeterone (**3**, Fig. 1) and its D^4-3-keto derivative [34–36] are examples of such antiandrogen compounds used for treatment of PCa. Ligr et al. [37] showed that mifepristone inhibits GRβ-coupled LNCaP-ARA70b cell proliferation. Additionally, finasteride (PROSCAR®), a type II-selective 5α-reductase inhibitor, was the first 5α-reductase (5AR) inhibitor approved in the USA for the treatment of benign prostatic hyperplasia (BPH) and PCa. Importantly, finasteride reduces the proliferation rate of DU145 and PC-3 prostate cancer cells in vitro [38], although several reports classified these cell lines as hormone-independent [39].

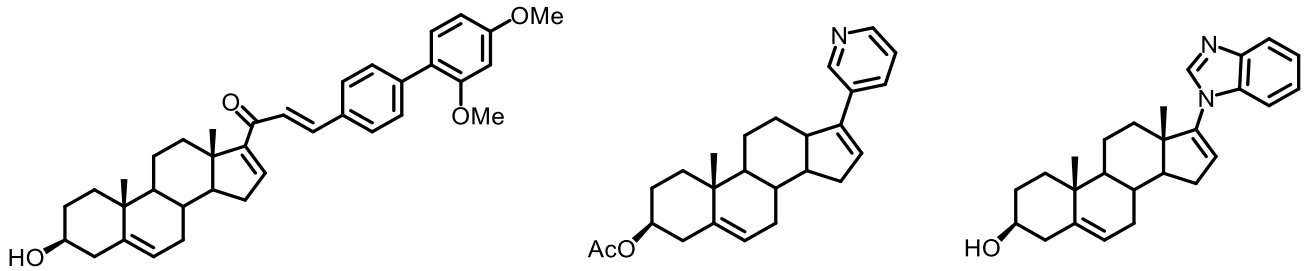
In view of the varied pharmacological activities of steroids and chalcones and in continuation of our ongoing work on the synthesis of new steroidal inhibitors for CYP17α hydroxylase, as well as analysis of 17β-hydroxydehydrogenase (17β-HSD) [40] and anti-HIV agents [41], we report the synthesis of some new pregnene steroids **5**–**12** as well as their cytotoxicity on PC-3 and LNCaP-AI prostate cancer cells, and an in silico molecular docking study.

Results and discussion

Chemistry

((5-Pregnen-3β,17β-diol-15α-yl)thio)propanehydrazide (**5**), prepared from the ester analog **4** in 70% yield, was the key intermediate for the synthesis of the new substituted hydrazide and amide derivatives. Thus, treatment of **5** with $NH_2OH.HCl$ in the presence of NaOMe/MeOH furnished the *N*-hydroxy-propanamide analog **6** (60% yield). Analogously, the reaction of **5** with 4-bromo-benzaldehyde or 4-hydroxy-3-methoxy-acetophenone (acetovanillone) in acidic medium led to the formation of the imine derivatives **7** and **8** in 57 and 62% yield, respectively. Boiling of a solution of **5** with 2-amino-5-nitrothiazole, 4-(4-(4-(4-aminophenoxy) phenylsulfinyl)phenoxy)benzenamine, 2-amino-4-nitrophenol, and 2,6-diamino-acridine in the presence of NaOMe in DMF gave, after a chromatographic purification, the corresponding amide derivatives **9**–**12** in 42–66% yield (Scheme 1).

The structures of **5**–**12** are based on their NMR (^1H, ^{13}C, and 2D), which showed somewhat similar patterns of the protons and carbon atoms of pregnene moiety. The ^1H NMR spectra of **5**–**12** showed two multiplets at the regions δ 2.90–2.65 and 2.74–2.45 ppm assigned for the methylene protons CH_2-21 and CH_2-22, respectively. The lower field singlet at δ 10.02 ppm was attributed to the imine proton (*H*C=N) of **7**, while the resonances of the aromatic protons H-2′ + H-6′ and H-3′ + H-5′ appeared as a doublet of doublets at δ 7.67 and 7.75 ppm (J = 7.8 Hz), respectively.



1. (E)-3-(20,40-dimethoxy-[1,10-biphenyl]1-4-yl)-1-(3-hydroxypregenen-17-yl)prop-2-en-1-one

2. Abiraterone acetate (Zytiga)

3. Galeterone TOK-001

Fig. 1 Examples of some steroids-based anticancer agents

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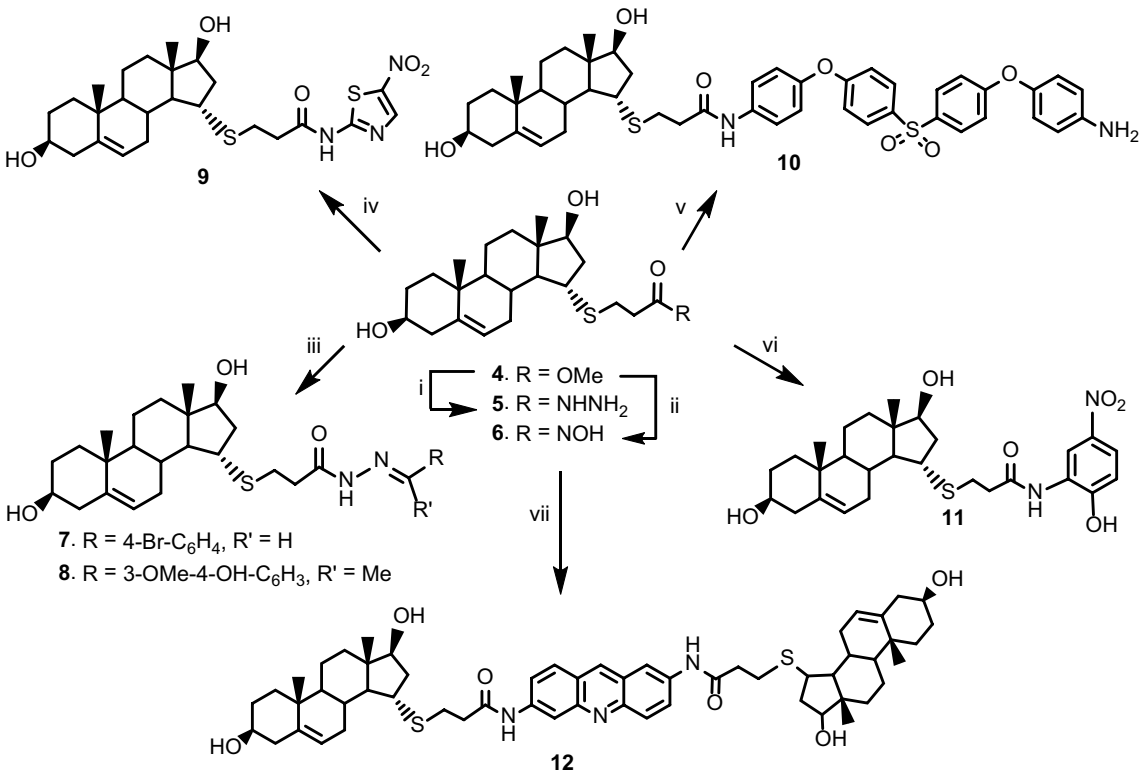

Scheme 1 Conditions and reagents: (i) $NH_2NH_2{\cdot}H_2O$, DMF, reflux, 10 h; (ii) $NH_2OH{\cdot}HCl$, NaOMe-MeOH, reflux 12 h; (iii) 4-Br-C_6H_4-CHO or 3-OMe-4-OH-C_6H_3-COMe, EtOH, HOAc, reflux, 12–13 h; (iv) 2-amino-5-nitrothiazole, DMF, NaOMe, reflux, 12 h; (v) 4,4′-((sulphonyl-bis(4,1-phenylene)bis(oxy))dianiline, DMF, NaOMe, reflux, 16 h; (vi) 2-amino-4-nitrophenol, DMF, NaOMe, reflux, 12 h; (vii) 2,6-diamino-acridine, DMF, NaOMe, reflux, 24 h

The two doublets at δ 7.45 and 7.53 ppm were assigned for H-5 and H-6 of **8** ($J_{5,6}=7.7$ Hz), respectively, while H-2 appeared as a singlet at δ 6.89 ppm. The singlet at δ 8.15 ppm was assigned to H-4 of the thiophene ring of **9**, whereas the broad singlets at δ 8.81 and 7.76 ppm were attributed to the aromatic protons H-6 and H-3 together with H-4 of **11**. In the ^{13}C NMR spectra, the lower field resonances at the regions δ 175.5–167.7 ppm were assigned for the carbonyl carbon atoms of amide group of **5**–**12**. Resonances at δ 80.5 ppm were assigned for C-17, whereas the resonance signals of the C=N carbon atoms of **7** and **8** appeared at δ 144.3 and 148.6 ppm, respectively. Compounds **5**–**12** showed signals at the regions δ 29.9–25.5 and 36.8–34.8 ppm, which were assigned to the methylene carbon atoms CH_2-21 and CH_2-22, respectively. In addition, the carbon atoms C-2, C-4 and C-5 of the thiazole moiety of **9** appeared at δ 147.6, 141.6 and 130.8 ppm, respectively. The other carbon atoms of the aromatic, pregnene backbone and the substituents were fully analyzed (cf. Experimental section). Compound **8** was selected for more detailed NMR studies. In the gradient-selected HMBC spectrum [42] of **8**, the Me*C*=N carbon atom at δ 148.6 ppm showed two $^3J_{C,H}$ correlations with aromatic protons H-2′ and H-6′ at δ_H 7.67 ppm, while the protons of the methoxy group at C-5 of the aromatic ring at δ_H 3.84 ppm showed a $^3J_{C,H}$ a correlation with C-5 of the same ring at δ_C 152.1 ppm. In addition, a $^2J_{C,H}$ coupling between methylene protons (CH_2 at C-22) at δ_H 2.58 ppm and the carbonyl carbon atom of the amide group at δ_C 167.7 ppm was observed. Furthermore, H-15 of the pregnene scaffold at δ_H 3.14 ppm showed a $^3J_{C,H}$ coupling to methylene protons (CH_2 at C-21) at δ_C 29.6 ppm (Fig. 2).

Next, **5** was treated with 2-chlorobenzoic acid in the presence of $POCl_3$ to furnish the oxadiazole analog **13** in 56% yield. Further, treatment of **5** with acetylacetone in acidic EtOH afforded the 3,5-dimethyl-1*H*-pyrazol analog **14** in

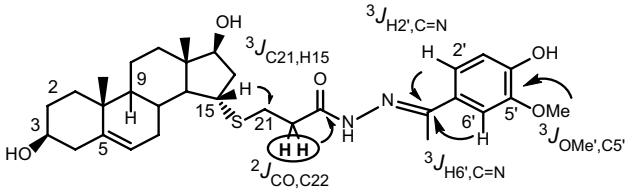

Fig. 2 $J_{C;H}$ correlations in the HMBC NMR spectrum of **8**

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64% yield. Selective oxidation of **14** with 1.0 mol of *m*-chloroperbenzoic acid (*m*-CBPA) at 0–5 °C for 5 h proceeded smoothly and gave, after chromatographic purification, the sulfoxide **15** (60%). Interestingly, there was no indication for the formation of a 5,6-epoxide ring resulting from possible oxidation of the 5,6-olefinic bond as indicated by the ^{1}H, ^{13}C NMR spectra and elemental analysis (Scheme 2).

Structures of **13**–**15** were established by ^{1}H, ^{13}C NMR and 2D NMR spectra. In ^{1}H NMR spectra of **14** and **15**, proton H-4 of the pyrazole ring resonated as a singlet at δ 7.99 and 8.01 ppm, respectively, whereas methylene protons CH_2-21 appeared as multiplets at δ 2.80 and 2.84. Methylene protons CH_2-22 resonated at δ 2.64 and 2.65 ppm, respectively. In the ^{13}C NMR spectrum of **13**, C-2 and C-4 of the oxadiazole ring appeared at δ 149.9 and 162.5 ppm, respectively. In addition, the ^{13}C resonance for C-3 of the pyrazole rings of **14** and **15** is shifted to δ 141.6, 142.0 ppm, while C-4 of the same ring appeared at δ 120.4 and 122.4 ppm, as well as C-5 at δ 142.0 ppm, respectively. The methylene protons CH_2-21 and CH_2-22 of **13**–**15** were found in the regions of δ 29.4–28.4 and at 36.7 ppm, respectively. All other aromatic and aliphatic protons and carbon atoms of pregnene scaffold and substituents were identified and correspondingly assigned (cf. Experimental Section).

In vitro cytotoxic activity

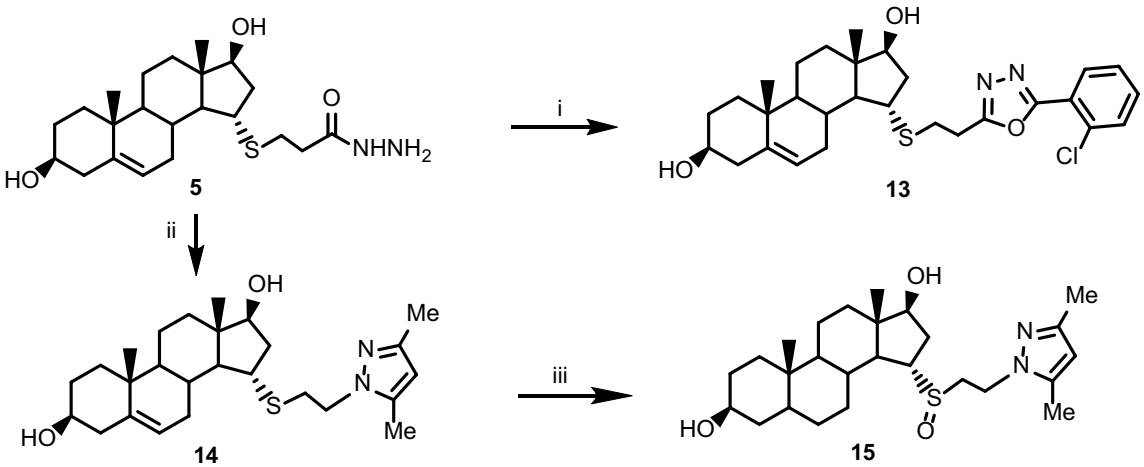
The synthesized compounds **4, 7**–**10, 13**, and **15** were selected for evaluation of their cytotoxic activity in vitro against two human PCa cell lines (PC-3, and LNCaP-AI), using an MTT assay [43]. Results are summarized in Table 1. Some of the compounds exhibited moderate to strong cytotoxic activity against the tested PCa cell lines.

Table 1 In vitro inhibition of novel steroids

Compd.	% Cytotoxicity	
	PC-3[a]	LNCaP-AI[a]
4	56.1 ± 2.76	40.7 ± 1.56
7	52.2 ± 2.02	37.9 ± 0.98
8	77.0 ± 3.00	19.1 ± 0.64
9	55.9 ± 1.97	79.7 ± 3.40
10	96.2 ± 5.77	93.6 ± 5.23
13	58.6 ± 2.20	5.4 ± 0.13
15	< 1.0 ± 0.01	25.0 ± 1.11

[a]% Inhibition is taken from three experiments at a concentration of 10 μM; PC-3, and LNCaP-AI human PCa cell lines

In particular, compound **10** displayed strong cytotoxic activity at a concentration of 10.0 μM against both PC-3 and LNCaP-AI cell lines. This molecule inhibited PC-3 and LNCaP-AI cell growth at about 96.2%, and 93.6% (Table 1), respectively, and 91.8%, and 79.8% at concentration of 1.0 μM (data not shown), respectively. Compound **8** exhibited reduced cell proliferation of PC-3 cells by 77%, while it was not as effective in LNCaP-AI cells. Meanwhile, compound **9** showed reduced cell proliferation of LNCaP-AI by 79.7% but could only reduce cell viability by 55.9% in PC-3 cells. It is clear from these data that substitution of the ester group of the steroid molecules by hydrazide or amide moieties enhances the cytotoxic effect of these steroids on the PCa cells, while the nature of the substituent of the hydrazide or amide, e.g., aromatic rings (compound **10**) influence the relative cytotoxicity. This can be attributed to their disparity in either protein binding properties or bioavailability.



Scheme 2 Conditions and reagents: (i) 2-chlorobenzoic acid, $POCl_3$, reflux, 10 h; (ii) acetylacetone, EtOH, HOAc, reflux, 7 h; (iii) mCPBA, DCM, Stirr at 0–5 °C, 5 h

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Molecular docking study

The human androgen receptor (AR) (1E3G) has been exploited as a main therapeutic target for PCa. The three-dimensional structure of human AR was obtained from the Brookhaven Protein Data Bank (PDB ID: 1E3G (http://www.rcsb.org). The crystal structure was refined by removing water molecules and the cofactor, phosphate ion. Hydrogen atoms were added and electronic charges were assigned to the protein atoms using the kollman united atoms force field by using AutoDock Vina 1.1.2-4, 2011 [44].

In our search for new lead compounds as human AR inhibitors that are structurally related to galeterone (**3**) as an AR antagonist, we identified that **10** constitutes the most active candidate in the above series. The binding energy score for **10** is—9.5 kcal mol^{-1}, indicating a good selectivity and potency of this analog to bind to the active site of the protein receptor pocket (1E3G). As suggested by the model and visualized in Fig. 3, the location of the pregnene backbone in the middle of the binding pocket anchors the hydroxyl group at C-3 of the pregnene scaffold in a favorable position for a hydrogen bond with the lone pair of oxygen atom (C=O) of the carboxylic group of amino acid

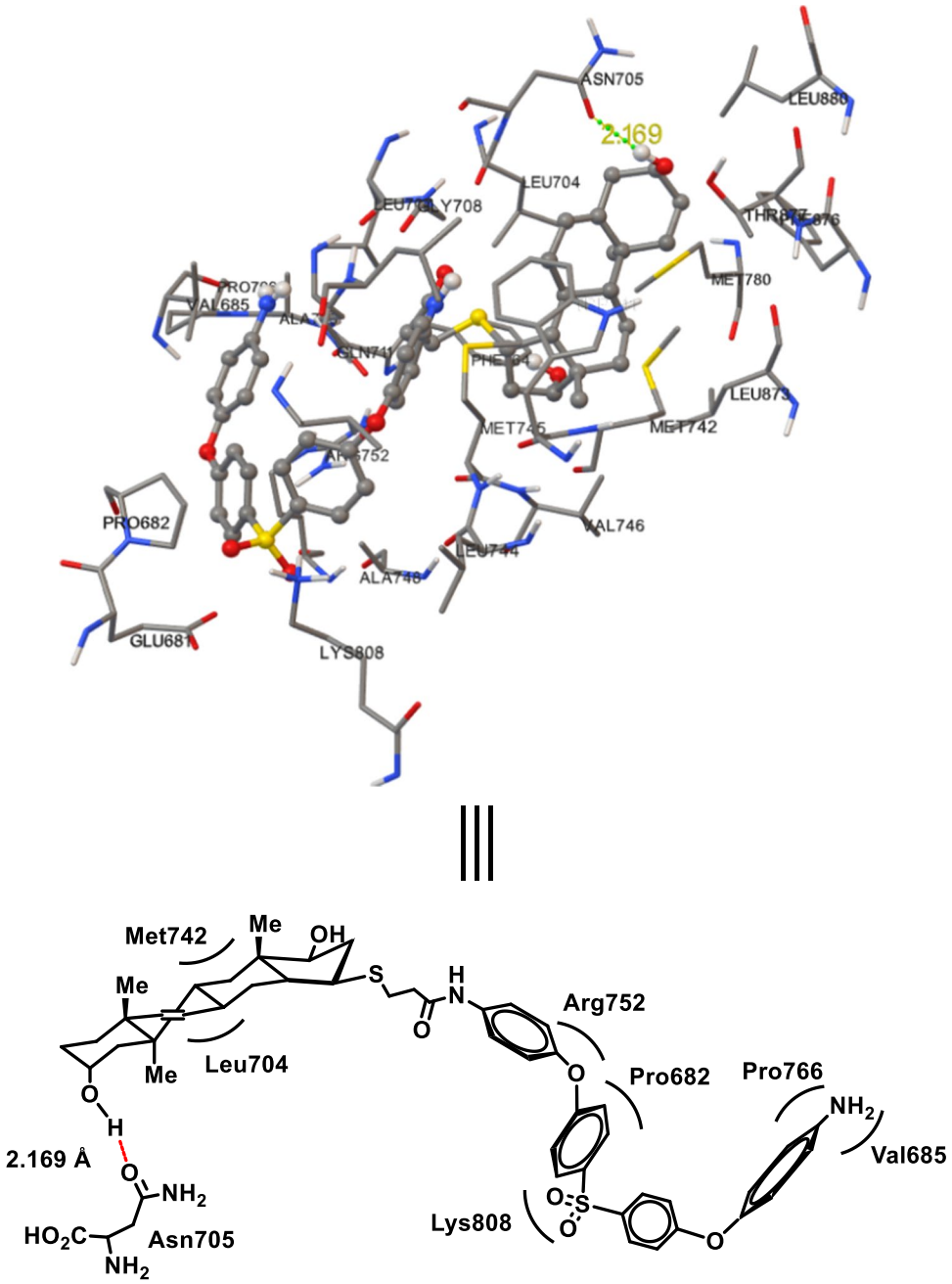

Fig. 3 Computer model of human AR (pdb id 1e3g) with **10** shows a hydrogen bond between the OH group at C-3 of pregnene scaffold and the lone pair of oxygen atom (C=O) of the carboxylic group of amino acid Asn705 (2.167 Å) residue. In addition, non-bonded amino acid residues as Leu704, Met742, Arg752, Lys808, Pro682, Pro766, and Val685 of the receptor surrounding the steroid **10** were observed

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Asn705 (2.167 Å) residue. Besides this binding, there are non-bonded amino acid residues such as Leu704, Met742, Arg752, Lys808, Pro682, Pro766 and Val685 surrounded by **10**, which further would enhance its inhibitory potency.

Experimental

Materials and methods

Melting points are uncorrected and were measured on a Buchi melting point apparatus B-545 (Buchi Labortechnik AG, Switzerland). NMR spectra were recorded on 400 MHz (^{1}H) and on 75 MHz (^{13}C) spectrometers (Bruker DPX-400, Germany) with TMS as internal standard and on the δ scale in ppm. Signal assignments for protons were performed by selective proton decoupling or by COSY spectra. Heteronuclear assignments were verified by HSQC and HMBC experiments. IR spectra were recorded on a Shimadzu. TLC plates 60 F_{254} were purchased from Merck. Chromatograms were visualized under UV 254–366 nm and iodine. MTT method [43] was used for evaluation of cytotoxic activity after 72 h of treatment with 10 µM concentrations of these steroids.

((5-Pregnen-3β,17β-diol-15α-yl)thio)propanehydrazide (5)

A mixture of methyl ((5-pregnen-3β,17β-diol-15α-yl)thio) propanoate (**4**) (500 mg, 1.23 mol) and excess of hydrazine hydrate 80% (5 mL) in DMF (15 mL) was refluxed for 10 h. Progress of reaction was monitored by TLC (*n*-hexane–ethyl acetate) (3:2). The reaction mixture was evaporated and allowed to cool. The resulting solid was filtered, dried and recrystallized from EtOH to give **5** 350 mg (70%) as a yellow powder; m.p.: 152–153 °C; $R_f = 0.32$; FTIR (ν_{max}, cm^{-1}): 3442 (OH), 3421, 3284 (NH_2), 2964, 2933 ($CH_{aliph.}$) 1660 ($C{=}O_{amide}$), 1633 (NH), 1633 (C=C). ^{1}H NMR (DMSO): δ 9.06 (s, 1H, NH_{amide}), 6.88 (br s, 2H, NH_2), 5.28 (t, 1H, $J_{6,7} = 2.3$ Hz, H-6), 4.53, (br s, 1H, 3-OH), 3.72 (br s, 1H, 17-OH), 3.44 (m, 1H, H-17), 3.27 (br s, 1H, H-3), 3.06 (m, 1H, H-15), 2.64 (m, 2H, 2.67 (m, 2H, CH_2-21), 2.45 (m, 2H, CH_2-22), 2.36 (m, 1H, H-16a), 2.31 (m, 1H, H-7a), 2.14 (m, 2H, CH_2-4), 1.81 (m, 1H, H-1a), 1.76 (m, 1H, H-12a), 1.68 (m, 1H, H-2a), 1.55 (m, 2H, H-8 + H-16b), 1.55 (m, 1H, H-7b), 1.49 (m, 1H, H-11a), 1.39 (m, 1H, H-2b), 1.35 (m, 1H, H-11b), 1.25 (m, 1H, H-14), 0.96 (m, 4H, Me-19 + H-12b), 0.83 (m, 2H, H-1b + H-9), 0.71 (s, 3H, Me-18). ^{13}C NMR (DMSO): δ 170.5 ($C{=}O_{amide}$), 141.6 (C-5), 120.9 (C-6), 80.5 (C-17), 70.5 (C-3), 54.4 (C-14), 50.5 (C-9), 43.6 (C-13 + C-16), 42.7 (C-4 + C-15), 37.7 (C-12) 37.5 (C-1), 36.7 (C-10 + CH_2-22), 31.8 (C-2), 31.3 (C-7), 29.8 (C-8), 28.7 (CH_2-21), 20.5 (C-11), 19.5 (Me-19), 14.2 (Me-18). Anal. calc. for $C_{22}H_{36}N_2O_2S$ (408.60): C, 64.67; H, 8.88; N, 6.86. Found: C, 64.59; H, 8.80; N, 6.93.

N-Hydroxy-((5-pregnen-3β,17β-diol-15α-yl)thio) propanamide (6)

A solution of hydroxylamine hydrochloride (100 mg, 1.47 mmol) and sodium methoxide (79.3 mg, 1.47 mmol) in 15 mL EtOH was stirred for 2 h to produce a white precipitate; then a solution (300 mg, 0.73 mmol) of steroid analog in (10 mL) was added, The mixture was heated under reflux for 12 h, after completion of reaction by TLC (*n*-hexane–ethyl acetate) (4:1) the solution was concentrated under reduced pressure. The residue was recrystallized from ethanol to afford the target compound as a light-yellow powder 180 mg (60%); m.p.: 208–210 °C; $R_f = 0.30$; FTIR (ν_{max}, cm^{-1}): 3385 (OH + NH_{amide}), 2933, 2850 ($CH_{aliph.}$) 1724 ($C{=}O_{amide}$), 1660 (C=C). ^{1}H NMR (DMSO): δ 9.03 (s, 1H, NH_{amide}), 8.01 (s, 1H, NOH), 5.28 (t, 1H, $J_{6,7} = 2.23$ Hz, H-6), 4.36, (br s, 1H, 3-OH), 3.62 (br s, 1H, 17-OH), 3.48 (m, 1H, H-17), 3.25 (m, 1H, H-3), 3.09 (m, 1H, H-15), 2.67 (m, 2H, CH_2-21), 2.45 (m, 2H, CH_2-22), 2.31 (m, 1H, H-16a), 2.29 (m, 1H, H-7a), 2.14 (m, 2H, CH_2-4), 1.81 (m, 1H, H-1a), 1.76 (m, 1H, H-12a), 1.68 (m, 1H, H-2a), 1.64 (m, 2H, H-8 + H-16b), 1.50 (m, 2H, H-7b + H-11a), 1.39 (m, 1H, H-2b), 1.35 (m, 1H, H-11b), 1.25 (m, 1H, H-14), 0.96 (s, 4H, Me-19 + H-12b), 0.83 (m, 2H, H-1b + H-9), 0.71 (s, 3H, Me-18). ^{13}C NMR (DMSO): δ 172.8 ($C{=}O_{hydroxamic}$), 141.6 (C-5), 120.9 (C-6), 80.5 (C-17), 70.5 (C-3), 54.4 (C-14), 50.5 (C-9), 43.6 (C-13 + C-16), 42.7 (C-4 + C-15), 37.7 (C-12), 37.5 (C-1), 36.8 (C-10 + CH_2-22), 31.9 (C-2), 31.3 (C-7), 29.9 (C-8 + CH_2-21), 20.5 (C-11), 19.6 (Me-19), 14.2 (Me-18). Anal. calc. for $C_{22}H_{36}N_2O_2S$ (409.58): C, 64.51; H, 8.61; N, 3.42. Found: C, 64.62; H, 8.53; N, 3.33.

General procedure for the synthesis of hydrzide Schiff bases 7 and 8

To a solution of **5** (100 mg, 0.24 mmol) in EtOH (20 mL) was added the desired aldehyde or ketone (0.24 mmol) followed by HOAc (1 mL) and the mixture was heated under reflux for 12–13 h. The reaction was monitored by TLC by using *n*-hexane–ethyl acetate (3:2) as eluents. After cooling, the solution was poured onto ice cold water. The solid product was collected, filtered, dried and recrystallized from EtOH to give the desired product.

N′-(4-(Bromobenzylidene)-3-((5-pregnen-3β,17β-diol-15α -yl)thio)propanehydrazide (7)

From 4-Bromo-benzaldehyde (44 mg). Yield: 78 mg (57%) as a yellow powder; m.p.: 191–193 °C; $R_f = 0.37$; FTIR (ν_{max}, cm^{-1}): 3441 (OH), 2966, 2939 ($CH_{aliph.}$),

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1664 ($C{=}O_{amide}$), 1625 (C=N), 1587 ($C{=}C_{arom.}$). ^{1}H NMR (DMSO): δ 10.02 (s, 1H, *H*C=N), 8.72 (s, 1H, NH_{amide}), 7.75 (dd, 2H, $J = 7.8$ Hz, $H_{arom.}$-3 + $H_{arom.}$-5), 7.67 (dd, 2H, $J = 7.8$ Hz, $H_{arom.}$-2 + $H_{arom.}$-6), 5.28 (t, 1H, $J_{6,7} = 2.3$ Hz, H-6), 4.60 (br s, 1H, OH-3), 3.72 (s, 1H, OH-17), 3.47 (m, 1H, H-17), 3.27 (m, 1H, H-3), 3.08 (m, 1H, H-15), 2.65 (m, 2H, CH_2-21), 2.49 (m, 2H, CH_2-22), 2.37 (m, 1H, H-16a), 2.32 (m, 1H, H-7a), 2.14 (m, 2H, CH_2-4), 1.80 (m, 1H, H-1a), 1.75 (m, 1H, H-12a), 1.71 (m, 1H, H-2a), 1.63 (m, 2H, H-8 + H-16b), 1.49 (m, 2H, H-7b + H-11a), 1.39 (m, 1H, H-2b), 1.35 (m, 1H, H-11b), 1.24 (m, 1H, H-14), 0.95 (m, 4H, Me-19 + H-12b), 0.83 (m, 2H, H-1b + H-9), 0.70 (s, 3H, Me-18). ^{13}C NMR (DMSO): δ 165.5 ($C{=}O_{amide}$), 144.3 (C=N), 141.6 (C-5), 132.3 ($C_{arom.}$-1 + $C_{arom.}$-3 + $C_{arom.}$-5), 129.1 ($C_{arom.}$-2 + $C_{arom.}$-6), 123.6 (C–Br), 120.9 (C-6), 80.5 (C-17), 70.5 (C_3-OH), 54.4 (C-14), 50.5 (C-9), 43.7 (C-13 + C-16), 42.7 (C-4 + C-15), 37.7 (C-1), 37.5 (C-12), 36.7 (CH_2-22 + C-10), 31.9 (C-2), 31.3 (C-7), 29.8 (C-7), 28.5 (CH_2-21), 20.5 (C-11), 19.5 (Me-19), 14.2 (Me-18). Anal. calc. for $C_{29}H_{39}BrN_2O_3S$ (575.6): C, 60.51; H, 6.83; N, 4.87. Found: C, 60.45; H, 6.75; N, 4.82.

N′-(1-((4-Hydroxy-3-methoxyphenyl)ethylidene)-3-((5-pregnen-3β,17β-diol-15α-yl)thio)propane hydrazide (**8**)

From 4-hydroxy-3-methoxy-acetophenone (acetovanillone) (39 mg). Yield: 83 mg (62%) as a yellow powder; m.p.: 189-199 °C; $R_f = 0.34$; FTIR (ν_{max}, cm^{-1}): 3402 (OH), 2931, 2854 ($CH_{aliph.}$), 1674 ($C{=}O_{amide}$), 1658 (C=N), 1589 ($C{=}C_{arom.}$), 1126 (C_{phenol}–OH). ^{1}H NMR (DMSO): δ 9.36 (s, 1H, Ar-*OH*), 8.00 (s, 1H, NH_{amide}), 7.53 (d, 1H, $J_{5,6} = 7.7$ Hz, $H_{arom.}$-6), 7.45 (d, 1H, $H_{arom.}$-5), 6.89 (s, 1H, $H_{arom.}$-2), 5.28 (t, 1H, $J_{6,7} = 2.3$, H-6), 4.62 (br s, 1H, 3-OH), 3.84 (s, 3H, OMe), 3.81 (s, 1H, 17-OH), 3.39 (m, 1H, H-17), 3.27 (m, 1H, H-3), 3.14 (m, 1H, H-15), 2.88 (m, 2H, CH_2-21), 2.58 (m, 5H, *Me*-C=N+CH_2-22), 2.35 (m, 1H, H-16a), 2.29 (m, 1H, H-7a), 2.10 (m, 2H, CH_2-4), 1.91 (m, 1H, H-1a), 1.76 (m, 1H, H-12a), 1.72 (m, 1H, H-2a), 1.57 (m, 4H, H-8 + H-16b), 1.51 (m, 2H, H-7b + H-11a), 1.39 (m, 1H, H-2b), 1.35 (m, 1H, H-11b), 1.25 (m, 1H, H-14), 0.96 (m, 4H, H-12b + Me-19), 0.83 (m, 2H, H-1b + H-9), 0.71 (s, 3H, Me-18). ^{13}C NMR (DMSO): δ 167.7 ($C{=}O_{amide}$), 152.1 (C–OH + *C*-OMe), 148.0 (Me*C*=N), 141.6 (C-5), 129.3 ($C_{arom.}$-1), 123.8 ($C_{arom.}$-6), 120.3 (C-6), 115.4 ($C_{arom.}$-2), 111.6 ($C_{arom.}$-5), 80.6 (C-17), 70.5 (C-3). 56.1 (OMe), 54.4 (C-14), 50.5 (C-9), 42.7 (C-13 + C-16), 42.1 (C-4 + C-15), 37.7 (C-12), 37.5 (C-1), 36.7 (C-10 + CH_2-22), 31.9 (C-2), 31.3 (C-7), 29.8 (C-8), 29.6 (CH_2-21), 26.7 (*Me*C=N), 20.5 (C-11), 19.5 (Me-19), 14.2 (Me-18). Anal. calc. for $C_{29}H_{39}BrN_2O_3S$ (575.6): C, 60.51; H, 6.83; N, 4.87. Found: C, 60.45; H, 6.75; N, 4.82.

General procedure for the synthesis of aryl amide derivatives **9–11**

To a solution of **4** (100 mg, 0.24 mmol) in DMF (25 mL) were added substituted amine (0.36 mmol) and NaOMe (190 mg, 0.36 mmol) and the reaction mixture was heated under reflux for 16–24 h. Progress of the reaction was monitored by TLC (*n*-hexane–ethyl acetate) (4:1). After completion of the reaction, the mixture was cooled and added to ice water then extracted with DCM (3 × 20 mL). The combined organic extracts were dried (Na_2SO_4), filtered and, evaporated to dryness. The residue was purified on a SiO_2 column (5 g), using *n*-hexane–ethyl acetate (4:1) as eluent to give the desired amide derivative.

N-(5-Nitrothiazol-2-yl)((5-pregnen-3β,17β-diol-15α-yl)thio) propanamide (**9**)

From 2-amino-5-nitrothiazole (52 mg). Yield: 55 mg (44%) as a yellow powder; m.p.: 136–137 °C; $R_f = 0.31$; FTIR (ν_{max}, cm^{-1}): 3410 (OH), 2933, 2854 ($CH_{aliph.}$),1664 ($C{=}O_{amide}$), 1548 ($C{=}N_{thiazole}$), 1461 ($C{=}C_{thiazole}$). ^{1}H NMR (DMSO): δ 9.39 (br s, 1H, NH_{amide}), 8.15 (s, 1H, $H_{thiazole}$-4), 5.29 (br s, 1H, H-6), 4.63, (br s, 1H, 3-OH), 3.64 (s, 1H, 17-OH), 3.36 (m, 1H, H-17), 3.20 (m, 1H, H-3), 3.15 (m, 1H, H-15), 2.81 (m, 2H, CH_2-21), 2.74 (m, 2H, CH_2-22), 2.30 (m, 1H, H-16a), 2.20 (m,1H, H-7a), 2.11 (m, 2H, CH_2-4), 1.92 (m, 1H, H-1a), 1.79 (m, 1H, H-12a), 1.73 (m 1H, H-2a), 1.68 (m, 2H, H-8 + H-16b), 1.52 (m, 2H, H-7b + H-11a), 1.40 (m, 1H, H-2b), 1.35 (m, 1H, H-11b), 1.25 (m, 1H, H-14), 1.01 (m, 4H, H-12b + Me-19), 0.87 (m, 2H, H-1b + H-9), 0.70 (s, 3H, Me-18). ^{13}C NMR (DMSO): δ 172.4 ($C{=}O_{amide}$), 147.6 ($C_{thiazole}$-2), 141.6 ($C_{thiazole}$-4), 139.8 (C-5), 130.8 ($C_{thiazole}$-5), 120.6 (C-6), 80.5 (C-17), 70.5 (C-3), 54.4 (C-14), 50.5 (C-9), 43.6 (C-13 + C-16) 42.7 (C-4 + C-15), 37.7 (C-12), 37.5 (C-1), 36.7 (C-10 + CH_2-22), 31.9 (C-2), 31.2 (C-7), 29.8 (C-8), 29.5 (CH_2-21), 20.5 (C-11), 19.5 (Me-19), 14.2 (Me-19). Anal. calc. for $C_{25}H_{35}N_3O_3S_2$ (521.69): C, 57.56; H, 6.76; N, 8.05. Found: C, 57.42; H, 6.55; N, 7.89.

N-(4-(4-(4-(4-Aminophenoxy)phenylsulfonyl)phenoxy) phenyl)((5-pregnen-3β,17β-diol-15α-yl thio)propanamide (**10**)

From 4-(4-(4-(4-aminophenoxy)phenylsulfinyl)phenoxy) benzenamine (151 mg). Yield: 120 mg (62%) as a brown powder; m.p.: 120–122 °C; $R_f = 0.24$; FTIR (ν_{max}, cm^{-1}): 3458 (OH), 3375, 3230 (NH_2), 3091 ($C{-}H_{arom.}$), 2933, 2904 ($CH_{aliph.}$), 1662 ($C{=}O_{amide}$), 1579, 1487 ($C{=}C_{arom.}$), 1149 (SO_2), 1072 (C–O–C). ^{1}H NMR (DMSO): δ 8.52 (s,1H, NH_{amide}), 7.97 (br s, 2H, NH_2), 7.92 (dd, 6H, $J = 7.9$ Hz, $H_{arom.}$-3″ + $H_{arom.}$-5″ + $H_{arom.}$-2‴ + $H_{arom.}$-6‴),

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7.13 (dd, 6H, $J = 7.9$ Hz, $H_{arom.}$-2′ + $H_{arom.}$-6′ + $H_{arom.}$-2″ + $H_{arom.}$-6″$H_{arom.}$-3‴ + $H_{arom.}$-5‴), 6.48 (dd, 2H, $J = 7.9$ Hz, $H_{arom.}$-3′ + $H_{arom.}$-3′), 6.28 (dd, 2H, $J = 7.9$ Hz, $H_{arom.}$-2‴ + $H_{arom.}$-6‴), 6.23 (dd, 2H, $J = 7.9$ Hz, $H_{arom.}$-3‴ + $H_{arom.}$-5‴), 5.36 (br s,1H, H-6), 4.51 (br s, 1H, 3-OH), 3.62 (s, 1H, 17-OH), 3.49 (m, 1H, H-17), 3.29 (m, 1H, H-3), 3.07 (m, 1H, H-15), 2.90 (m, 2H, CH_2-21), 2.74 (m, 2H, CH_2-22), 2.30 (m, 2H, H-7a + H-16a), 2.12 (m, 2H, CH_2-4), 1.80 (m, 1H, H-1a), 1.76 (m, 1H, H-12a), 1.72 (m 1H, H-2a), 1.69 (m, 4H, H-8 + H-16b), 1.49 (m, 2H, H-7b + H-11a), 1.40 (m, 1H, H-2b), 1.35 (m, 1H, H-11b), 1.24 (m, 1H, H-14), 0.96 (m, 4H, H-12b + Me-19), 0.84 (m, 2H, H-1b + H-9), 0.72 (s, 3H, Me-18). ^{13}C NMR (DMSO): δ 172.4 ($C{=}O_{amide}$), 162.1 ($C_{arom.}$-1″ + $C_{arom.}$-4″), 155.9 ($C_{arom.}$-4′), 151.3 ($C_{arom.}$-1‴ + $C_{arom.}$-NH_2), 141.6 (C-5), 135.3 ($C_{arom.}$-4″ + $C_{arom.}$-1‴), 130. 9 ($C_{arom.}$-1′), 130.2 ($C_{arom.}$-3″ + $C_{arom.}$-5″ + $C_{arom.}$-2‴ + $C_{arom.}$-6‴), 120.9 (C-6 + $C_{arom.}$-3‴ + $C_{arom.}$-5‴), 118.2 ($C_{arom.}$-2′ + $C_{arom.}$-6′ + $C_{arom.}$-3‴ + $C_{arom.}$-5‴), 111.3 ($C_{arom.}$-2‴ + $C_{arom.}$-6‴), 107.4 ($C_{arom.}$-3′ + $C_{arom.}$-5′), 80.5 (C-17), 70.5 (C-3), 54.4 (C-14), 51.9 (C-9), 43.6 (C-13 + C-16), 42.7 (C-4 + C-15), 37.5 (C-12), 36.7 (C-1), 36.3 (C-10 + CH_2-22), 31.9 (C-7 + C-8), 31.3 (C-2), 29.8 (C-7), 28.4 (C-8), 25.8 (CH_2-21), 20.5 (C-11), 19.5 (Me-19), 14.2 (Me-18). Anal. calc. for $C_{46}H_{52}N_2O_7S_2$ (809.05): C, 68.29; H, 6.48; N, 3.46. Found: C, 68.13; H, 6.35; N, 3.58.

N-(2-Hydroxy-5-nitrophenyl) ((5-pregnen-3β,17β-diol-15α-yl)thio)propanamide (11)

From 2-amino-4-nitrophenol (55 mg). Yield:70 mg (66%) as a red powder; m.p.: 114–116 °C; $R_f = 0.27$; FTIR (ν_{max}, cm^{-1}): 3444 (OH), 2937, 2902 ($CH_{aliph.}$), 1625 ($C{=}O_{amide}$), 1525, 1436 ($C{=}C_{arom.}$). ^{1}H NMR (DMSO) δ 9.76 (s, 1H, NH_{amide}), 8.81 (br s, 1H, $H_{arom.}$-6), 7.76 (br s, 2H, $H_{arom.}$-3 + $H_{arom.}$-4), 5.03 (br s, 1H, H-6), 4.40, (br s, 1H, 3-OH), 3.40 (m, 2H, 17-OH-17 + H-17), 3.25 (m, 1H, H-3 + H-15), 2.85 (m, 2H, CH_2-21), 2.55 (m, 2H, CH_2-22), 2.41 (m, 2H, H-7a + H-16a), 2.07 (m, 2H, CH_2-4), 1.89 (m, 1H, H-1a), 1.81 (m, 1H, H-12a), 1.78 (m 1H, H-2a), 1.56 (m, 2H, H-8 + H-16b), 1.51 (m, 2H, H-7b + H-11a), 1.43 (m, 1H, H-2b), 1.30 (m, 1H, H-11b), 1.25 (m, 1H, H-14), 1.10 (m, 4H, H-12b + Me-19), 0.71 (m, 2H, H-1b + H-9), 0.58 (s, 3H, Me-18). ^{13}C NMR (DMSO): δ 174.1 ($C{=}O_{amide}$), 148.7 ($C_{arom.}$–OH), 146.5 ($C_{arom.}$–NO_2), 142.0 (C-5), 130.6 ($C_{arom.}$-1′), 129.0 ($C_{arom.}$-3′), 123.5 ($C_{arom.}$-3′), 120.3 (C-6), 80.5 (C-17), 70.6 (C-3), 54.6 (C-14), 50.4 (C-9), 43.7 (C-13 + C-16), 42.7 (C-4 + C-15), 37.7 (C-12), 37.5 (C-1), 36.7 (C-10 + CH_2-22), 31.9 (C-2), 29.8 (C-7), 29.5 (C-8), 25.5 (CH_2-21), 20.8 (C-11), 19.5 (Me-19), 14.4 (Me-18). Anal. calc. for $C_{28}H_{38}N_2O_6S$ (530.68): C, 63.37; H, 7.22; N, 5.28. Found: C, 63.25; H, 7.01; N, 5.21.

2,6-Bis-((5-pregnen-3β,17β-diol-15α-yl) thio)-*N*,*N*′-acridine-propenamide (12)

From 2,6-diamino-acridine (94 mg). Yield: 100 mg (42%) as a red powder; m.p.: 197–199 °C; $R_f = 0.18$; FTIR (ν_{max}, cm^{-1}): 414 (OH), 3232 (NH_{amide}), 2935, 2864 ($CH_{aliph.}$), 1722 ($C{=}O_{amide}$), 1660 (C=N), 1602 ($C{=}C_{arom.}$). ^{1}H NMR (DMSO): δ 8.71 (s, 1H, NH_{amide}), 7.97 (br s, 2H, $H_{arom.}$-3′ + $H_{arom.}$-3′), 7.86 (m, 3H, $H_{arom.}$-4′ + $H_{arom.}$-7′ + $H_{arom.}$-8′), 7.74 (m, 2H, $H_{arom.}$-1′ + $H_{arom.}$-9′), 5.27 (br s, 1H, H-6), 4.64 (br s, 1H, 3-OH), 3.95 (s, 1H, 17-OH), 3.61 (m, 1H, H-17), 3.35 (m, 1H, H-3), 3.07 (m, 1H, H-15), 2.75 (m, 2H, CH_2-21), 2.55 (m, 2H, CH_2-22), 2.32 (m, 2H, H-7a + H-16a), 2.14 (m, 2H, CH_2-4), 1.79 (m, 1H, H-1a), 1.72 (m, 1H, H-12a), 1.67 (m, 1H, H-2a), 1.62 (m, 2H, H-8 + H-16b), 1.53 (m, 2H, H-8 + H-16b), 1.48 (m, 2H, H-7b + H-11a), 1.38 (m, 1H, H-2b), 1.30 (m, 1H, H-11b), 1.24 (m, 1H, H-14), 0.95 (m, 4H, H-12b + Me-19), 0.82 (m, 2H, H-1b + H-9), 0.70 (s, 3H, Me-18). ^{13}C NMR (DMSO): δ 172.4 ($C{=}O_{amide}$), 150.2 ($C_{arom.}$-9′), 144.4 ($C_{arom.}$-4a′ + $C_{arom.}$-6′ + $C_{arom.}$-10a′), 141.6 (C-5), 134.0 ($C_{arom.}$-2′ + $C_{arom.}$-8′), 132.5 ($C_{arom.}$-3′), 130.7 ($C_{arom.}$-8a′), 129.1 ($C_{arom.}$-7′), 120.8 (C-6 + $C_{arom.}$-1a′ + $C_{arom.}$-4′), 115.0 ($C_{arom.}$-9a′), 113.5 (C-5′), 80.5 (C-17), 70.5 (C-3), 54.4 (C-14), 50.5 (C-9), 43.6 (C-13 + C-16), 42.7 (C-4 + C-15), 37.6 (C-12), 36.7 (C-1), 34.8 (C-10 + CH_2-22), 31.3 (C-2), 29.8 (C-7), 28.4 (C-8), 27.8 (CH_2-21), 20.5 (C-11), 19.5 (Me-19), 14.2 ((Me-18). Anal. calc. for $C_{57}H_{75}N_3O_6S_2$ (962.36): C, 71.14; H, 7.86; N, 4.37. Found: C, 71.24; H, 8.11; N, 4.17.

15α-(2-(5-(2-Chlorophenyl)-1,3,4-oxadiazol-2-yl)ethylthio)-5-pregnen-3β,17β-diol (13)

To a solution of **5** (130 mg, 0.31 mmol) in $POCl_3$ (8 mL) was added 2-chlorobenzoic acid (50 mg, 0.32 mmol) and the reaction mixture was heated under reflux with stirring for 10 h. The reaction mixture was monitored by TLC, using *n*-hexane–ethyl acetate (3:2) as eluents. After cooling, the mixture was poured onto crushed ice. The solid product was washed with a saturated solution $NaHCO_3$ and the organic layer extracted with DCM (3 × 20 mL). The combined organic layers were evaporated to dryness and then recrystallized from EtOH to give **13** (93 mg, 56%) as a brown powder; m.p.: 118–119 °C; $R_f = 0.3$; FTIR (ν_{max}, cm^{-1}): 3417 (OH), 2939 and 2862 ($CH_{aliph.}$) 1689 ($C{=}N_{oxadiazole}$), 1658, 1566 ($C{=}C_{arom.}$). ^{1}H NMR (DMSO): δ 7.97 (dd, 1H, $J = 2.0$, 7.8 Hz, $H_{arom.}$-6′), 7.56 (dd, 1H, $J = 2.0$, 7.8 Hz, $H_{arom.}$-3′), 7.18 (m, 2H, $H_{arom.}$-4′ + $H_{arom.}$-4′), 5.12 (br s, 1H, H-6), 4.29 (br s, 1H, 3-OH), 3.84 (s, 1H, 17-OH), 3.39 (m, 1H, H-17), 3.20 (m, 1H, H-3), 3.08 (m, 1H, H-15), 2.65 (m, 2H, CH_2-21), 2.60 (m, 2H, CH_2-22), 2.27 (m, 2H, H-7a + H-16a), 2.05 (m, 2H, CH_2-4), 1.79 (m, 1H, H-1a), 1.75 (m, 1H, H-12a), 1.68 (m, 1H, H-2a), 1.58 (m,

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2H, H-8 + H-16b), 1.53 (m, 2H, H-7b + H-11a), 1.41 (m, 1H, H-2b), 1.30 (m, 1H, H-11b), 1.25 (m, 1H, H-14), 0.97 ((m, 4H, H-12b + Me-19), 0.75 (m, 2H, H-1b + H-9), 0.70 (s, 3H, Me-18). ^{13}C NMR (DMSO): δ 162.3 ($C_{oxadiazole}$-5′), 149.9 ($C_{oxadiazole}$-2′), 139.9 (C-5), 136.9 ($C_{arom.}$-1″), 128.4 ($C_{arom.}$-2″), 127.2 ($C_{arom.}$-3″ + $C_{arom.}$-4″ + $C_{arom.}$-6″), 123.0 ($C_{arom.}$-5″), 121.8 (C-6), 82.0 (C-17), 73.6 (C-3), 53.7 (C-14), 49.9 (C-9), 42.4 (C-13 + C-16), 41.7 (C-4 + C-15), 38.1 (C-12), 37.5 (C-1), 36.7 (C-10 + CH_2-22), 31.0 (C-2), 29.6 (C-7), 29.2 (C-8), 28.4 (CH_2-21), 20.3 (C-11), 19.3 (Me-19), 14.86 (Me-18). Anal. calc. for $C_{29}H_{37}ClN_2O_3S$ (529.14): C, 65.83; H, 7.05; N, 5.09. Found: C, 65.72; H, 7.22; N, 5.07.

15α-((2-(3,5-Dimethyl-1*H*-pyrazol-1-yl)ethyl) thio)-5-pregnen-3β,17β-diol (**14**)

A solution of acetylacetone (0.03 mL, 0.24 mmol) in EtOH (10 mL) containing AcOH (1 mL) was stirred at room temperature for 1 h; then a solution of **5** (100 mg, 0.24 mmol) was added and the reaction mixture was refluxed for 7 h. The reaction was monitored by TLC using *n*-hexane: ethyl acetate (3:2) as eluent. After cooling at room temperature, the reaction mixture, the obtained crystalline product was filtered, dried and purified by recrystallization from EtOH to give **14** (73 mg 64%) as a light-yellow powder; m.p.: 203–204 °C; R_f = 37; FTIR (ν_{max}, cm^{-1}): 3394 (OH), 3031 ($CH_{arom.}$), 2931, 2846 ($CH_{aliph.}$), 1691 ($C{=}O_{amide}$), 1666 (C=N), 1542 ($C{=}C_{arom.}$). ^{1}H NMR (DMSO): δ 7.99 (s, 1H-$H_{pyrazole}$-4), 5.28 (br s, 1H, Hz, H-6), 4.64 (m, 1H, 3-OH), 3.60 (br s, 1H, 17-OH), 3.44 (m, 1H, H-17), 3.27 (m, 1H, H-3), 3.09 (m, 1H, H-15), 2.80 (m, 2H, CH_2-21), 2.64 (m, 2H, CH_2-22), 2.31 (s, 3H, C3′$_{pyrazole}$-*Me*), 2.29 (s, 3H, C5′$_{pyrazole}$-*Me*), 2.14 (m, 2H, H-7a + H-16a), 2.09 (m, 2H, CH_2-4), 1.80 (m, 1H, H-1a), 1.76 (m, 1H, H-12a), 1.71 (m, 1H, H-2a), 1.57 (m, 2H, H-8 + H-16b), 1.49 (m, 2H, H-7b + H-11a), 1.35 (m, 1H, H-2b), 1.30 (m, 1H, H-11b), 1.24 (m, 1H, H-14), 0.96 (s, 4H, Me-19 + H-12b), 0.82 (m, 2H, H-1b + H-9), 0.70 (s, 3H, Me-18); ^{13}C NMR (DMSO): δ 142.0 ($C_{pyrazole}$-5′), 141.6 (C-5 + $C_{pyrazole}$-3′), 120.9 (C-6), 120.4 ($C_{pyrazole}$-4′), 80.5 (C-17), 70.5 (C-3), 54.4 (C-14), 50.5 (C-9), 43.6 (C-13 + C-16), 42.7 (C-4 + 15), 37.7 (C-12), 37.5 (C-1), 36.7 (C-10 + CH_2-22), 31.9 (C-2), 31.3 (C-7), 29.8 (C-8), 29.1 (CH_2-21), 20.5 (C-11), 19.5 (Me-19), 14.2 (Me-18), 12.2 (2x$C_{pyrazole}$-Me). Anal. calc. for $C_{26}H_{40}N_2O_2S.1/5H_2O$ (453.66): C, 68.82; H, 9.10; N, 6.17. Found: C, 68.71; H, 8.53; N, 5.99.

15α-((2-(3,5-Dimethyl-1*H*-pyrazol-1-yl)ethyl)sulfinyl)-5α,6β -epoxy-pregnan-3β,17β-diol (**15**)

To a solution of *m*-chloroperoxybenzoic acid (76 mg, 0.44 mmol) in DCM (15 mL) was added dropwise a solution of **15** (120 mg, 0.29 mmol) in DCM (10 mL). The reaction mixture was stirred at 0–5 °C for 5 h. The reaction was monitored by TLC using (*n*-hexane: ethyl acetate) (3:2) until the reaction was completed. The mixture was partitioned between DCM (3 × 20 mL) and a solution of $NaHCO_3$, and the combined organic layer was dried (Na_2SO_4), filtered and evaporated to dryness. The residue was purified on a SiO_2 column (5 g) using *n*-hexane: ethyl acetate (4:1) as eluent to afford **15** as a white solid (85 mg, 60%); m.p.: 186–188 °C; R_f = 0.34. ^{1}H NMR (DMSO): δ 8.01 (s, 1H-$H_{pyrazole}$-4), 5.28 (br s, 1H, H-6), 4.63 (br s, 1H, 3-OH), 3.55 (br s, 1H, 17-OH), 3.37 (m, 1H, H-17), 3.25 (m, 1H, H-3), 3.15 (m, 1H, H-15), 2.84 (m, 2H, CH_2-21), 2.65 (m, 2H, CH_2-22), 2.15 (m, 6H, C3′$_{pyrazole}$-Me + C5′$_{pyrazole}$-Me), 2.11 (m, 4H, CH_2-4 + 7a + 16a), 1.81 (m, 1H, H-1a), 1.76 (m, 1H, H-12a), 1.72 (m, 1H, H-2a), 1.52 (m, 2H, H-8 + H-16b), 1.49 (m, 2H, H-7b + H-11a), 1.39 (m, 1H, H-11b), 1.26 (m, 1H, H-14), 0.98 (s, 4H, Me-19 + H-12b), 0.87 (m, 2H, H-1b + H-9), 0.71 (s, 3H, Me-18). ^{13}C NMR (DMSO): δ 144.5 ($C_{pyrazole}$-3′), 142.0 (C-5 + $C_{pyrazole}$-5′), 122.4 ($C_{pyrazole}$-4′), 120.4 (C-6), 80.0 (C-17), 70.5 (C-3), 54.5 (C-9), 50.9 (C-14), 47.3 (C-9), 42.6 (C-13 + C-16), 42.1 (C-4 + C-15), 37.5 (C-1 + C-12), 36.7 (C-10 + CH_2-22), 33.6 (C-2), 31.9 (C-7), 31.6 (C-8), 29.4 (CH_2-21), 20.5 (C-11), 19.5 (C-19), 14.2 (C-18), 12.2 (2x$C_{pyrazole}$-Me). Anal. calc. for $C_{26}H_{40}N_2O_3S.1/2\ H_2O$ (469.66): C, 66.48; H, 8.79; N, 5.96. Found: C, 66.20; H, 8.37; N, 5.62.

In vitro cytotoxic assay

Evaluation of the cytotoxic activity in vitro of **4**, **7**–**10, 13**, and **15** against two human PCa cell lines (PC-3, and LNCaP-AI) was described previously [45]. Briefly, human PCa cell lines PC-3 (androgen receptor negative) and LNCaP-AI (androgen receptor positive) were obtained from the American Type Culture Collection (Rockville, MD). LNCaP-AI and PC-3 cells were grown in RPMI medium containing 5% fetal bovine serum (FBS, Gibco) supplemented with 1% penicillin–streptomycin. LNCaP-AI and PC-3 cells were plated in 96 well cell plates at 1×10^4 per cell for 24 h. Cells were treated with 10.0 µM concentration of sample (5 µL/well) for 48 h. Each experiment was performed three times in quadruplicate. After 72 h, 100 µL/well of MTT (2 mg/mL in PBS) was added for 4 h in the dark. After removal of MTT solution, 100 µL/well DMSO was added to dissolve the formazan purple crystal for 10 min. The absorbance at 540 nm was then measured by using a Molecular Devices SpectaMax 190.

Conclusion

A novel series of *N*-hydroxamic acid **6**, imino-propane hydrazides derivatives **7** and **8** as well as the aryl amide analogs **9**–**11** of methyl ((5-pregnen-3β,17β-diol-15α-yl)

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thio)propanoate (**4**) were synthesized. Several analogs were assayed for anticancer activity against two human PCa cell lines, PC-3 and LCNaP-AI. It was found that compound **10** was the most active molecule and showed a significant cytotoxic effect against both PCa cell lines (> 90%). Docking studies performed with the human AR homology model (PDB: 1E3G) suggested the presence of hydrogen bonding between hydrogen atom of OH group on our synthesized steroids at C-3 and the lone pair of oxygen atom (C=O) of amino acid Asn705 of AR, in addition to the hydrophobic interactions. Therefore, **10** could be a promising lead as anticancer agent against PCa cells due to its potent cytotoxic activity.

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Compliance with ethical standards

Conflict of interest No potential conflict of interest was reported by the authors.

References

1. Jemal A, Siegel R, Ward E, Hao Y, Xu J, Murray T, Thun MJ (2008) Cancer statistics 2008. Cancer J Clin 58:71–96. https://doi.org/10.3322/CA.2007.0010
2. Suzuki H, Ueda T, Ichikawa T, Ito H (2003) Androgen receptor involvement in the progression of prostate cancer. Endocr Relat Cancer 10:209–216
3. Harris WP, Mostaghel EA, Nelson PS, Montgomery B (2009) Androgen deprivation therapy: progress in understanding mechanisms of resistance and optimizing androgen depletion. Nat Clin Pract Urol 6:76–85. https://doi.org/10.1038/ncpuro1296
4. Nevedomskaya E, Baumgart SJ, Haendler B (2018) Recent advances in prostate cancer treatment and drug discovery. Int J Mol Sci 19:1359–1384. https://doi.org/10.3390/ijms19051359
5. Edmondson RJ, Monaghan JM (2001) The epidemiology of ovarian cancer. Int J Gynecol Cancer 11:423–429. https://doi.org/10.1046/j.1525-1438.2001.01053.x
6. Maria J, Ghini AA, Gerardo B (2003) 6,19-Carbon-bridged steroids. Synthesis of 6,19-methano progesterone. Org Biomol Chem 1:939–943. https://doi.org/10.1039/b211974a
7. Leng TD, Zhang JX, Xie J, Zhou SJ, Huang YJ, Zhou YH, Zhu WB, Yan GM (2010) Synthesis and anti-glioma activity of 25(R)-spirostan-3β-5α,6β,19-tetrol. Steroids 75:224–229. https://doi.org/10.1016/j.steroids.2009.12.005
8. Latham KA, Zamora A, Drought H, Subramanian S, Matejuk A, Offiner H et al (2003) Estradiol treatment redirects the isotope of the autoantibody response and prevents the development of autoimmune arthritis. J Immunnol 171:5820–5827. https://doi.org/10.4049/jimmunol.171.11.5820
9. Dubey RK, Oparil S, Imthum B, Jackson EK (2002) Sex hormones and hypertension. Cardiovasc Res 53:688–708. https://doi.org/10.1016/s0008-6363(01)00527-2
10. Kostaras X, Cusano F, Kline GA, Roa W, Easaw J (2014) Use of dexamethasone in patients with high-grade glioma: a clinical practice guideline. Curr Oncol 21:e493–e503. https://doi.org/10.3747/co.21.1769
11. Stanway SJ, Delavault P, Purohit A, Woo LWL, Thurieau C, Potter BVL, Reed MJ (2007) Steroid sulfatase: a new target for the endocrine therapy of breast cancer. Oncologist 12:370–374. https://doi.org/10.1634/theoncologist.12-4-370
12. Ndibe C, Wang CG, Sonpavde G (2015) Corticosteroids in the management of prostate cancer: a critical review. Curr Treat Options Oncol 16:6. https://doi.org/10.1007/s11864-014-0320-6
13. Cho H, Walker A, Williams J, Hasty KA (2015) Study of osteoarthritis treatment with anti-inflammatory drugs: cyclooxygenase-2 inhibitor and steroids. Biomed Res Int 2015:595273. https://doi.org/10.1155/2015/595273
14. Cushman M, Golebiewski WM, Pommier Y, Mazumder A, Reymen D, De Clercq E, Graham L, Rice WG (1995) Cosalane analogues with enhanced potencies as inhibitors of HIV-1 protease and integrase. J Med Chem 38:443–452. https://doi.org/10.1021/jm00003a007
15. Casimiro-Garcia A, De Clercq E, Pannecouque C, Witvrouw M (2000) Synthesis and anti-HIV activity of cosalane analogues incorporating nitrogen in the linker chain. Bioorg Med Chem 8:191–200. https://doi.org/10.1016/S0968-0896(99)00269-2
16. Galabov AS, Nikolaeva L, Todorovab D, Milkovab T (1998) Antiviral activity of cholesteryl esters of cinnamic acid derivatives. Z Naturforsch C 53:883–887
17. Vasaitis TS, Bruno RD, Njar VCO (2011) CYP17 inhibitors for prostate cancer therapy. J Steroid Biochem Mol Biol 125:23–31. https://doi.org/10.1016/j.jsbmb.2010.11.005
18. Njar VCO (2000) High-yield synthesis of novel imidazoles and triazoles from alcohols and phenols. Synthesis 14:2019–2028
19. Moreira VM, Vasaitis TS, Njar VC, Salvador JAR (2007) Synthesis and evaluation of novel 17-indazole androstane derivatives designed as CYP17 inhibitors. Steroids 72:939–948. https://doi.org/10.1016/j.steroids.2007.08.004
20. Moreira VMA, Salvador JAR, Vasaitis TS, Njar VCO (2008) Synthesis inhibitors for prostate cancer treatment—an update. Curr Med Chem 15:868–899. https://doi.org/10.2174/092986708783955428
21. Moreira VMA, Vasaitis TS, Guo Z, Njar VCO, Salvador JAR (2008) Synthesis of novel C17 steroidal carbamates, studies on CYP17 action, androgen receptor binding and function, and prostate cancer cell growth. Steroids 73:1217–1227. https://doi.org/10.1016/j.steroids.2008.05.010
22. Owen CP (2009) 17α-hydroxylase/17,20-lyase (p450(17α)) inhibitors in the treatment of prostate cancer: a review. Anticancer Agents Med Chem 9:613–626. https://doi.org/10.2174/187152009788680046
23. Hartmann RW, Ehmer RW, Haidar S, Hector M, Jose J, Klein CD, Seidel SB, Sergejew TF, Wachall BG, Wächter GA, Zhuang Y (2002) Review: inhibition of CYP 17, a new strategy for the treatment of prostate cancer. Arch Pharm Pharm Med Chem 335:119–128. https://doi.org/10.1002/1521-4184(200204)335:4%3c119:AID-ARDP119%3e3.0.CO;2
24. Hu Q, Negri M, Olgen S, Hartmann RW (2010) The role of fluorine Substitution in biphenyl methylene imidazole type CYP17 inhibitors for the treatment of prostate carcinoma. ChemMedChem 5:899–910. https://doi.org/10.1002/cmdc.201000065
25. Haidar S, Ehmer PB, Barassin S, Batzl-Hartmann C, Hartmann RW (2003) Effects of novel 17 α-hydroxylase/C17, 20-lyase (P450 17, CYP 17) inhibitors on androgen biosynthesis in vitro and in vivo. J Steroid Biochem Mol Biol 84:555–562. https://doi.org/10.1016/s0960-0760(03)00070-0
26. Al-Masoudi NA, Abdul-Rida NA, Kadhim RA, Krugs SJ, Engels M, Saeed BA (2016) Synthesis and CYP17a hydroxylase inhibition activity of new 3α- and 3β-ester derivatives of pregnenolone and related ether analogues. Med Chem Res 25:310–321. https://doi.org/10.1007/s00044-015-1480-z

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27. Al-Masoudi NA, Kadhim RA, Abdul-Rida NA, Saeed BA, Engel M (2015) New biaryl-chalcone derivatives of pregnenolone via Suzuki–Miyaura cross-coupling reaction. Synthesis, CYP17 hydroxylase inhibition activity, QSAR, and molecular docking study. Steroids 101:43–50. https://doi.org/10.1016/j.steroids.2015.05.011
28. Njar VC, Brodie AM (1999) Inhibitors of 17alpha-hydroxylase/17,20-lyase (CYP17): potential agents for the treatment of prostate cancer. Curr Pharm Des 5:163–180
29. Banday AH, Shameem SA, Gupta BD, Kumar HMS (2010) D-ring substituted 1,2,3-triazolyl 20-keto pregnenanes as potential anticancer agents: synthesis and biological evaluation. Steroids 75:801–804. https://doi.org/10.1016/j.steroids.2010.02.015
30. Al-Masoudi NA, Mahdi KM, Abdul-Rida NA, Saeed BA, Engel M (2015) A new pregnenolone analogues as privileged scaffolds in inhibition of CYP17 hydroxylase enzyme. Synthesis and in silico molecular docking study. Steroids 100:52–59. https://doi.org/10.1016/j.steroids.2015.05.002
31. Al-Masoudi NA, Ali DS, Saeed B, Hartmann RW, Engel M, Rashid S, Saeed A (2014) New CYP17 hydroxylase inhibitors: synthesis, biological evaluation, QSAR, and molecular docking study of new pregnenolone analogs. Arch Pharm Chem Life Sci 347:896–907. https://doi.org/10.1002/ardp.201400255
32. de Bono JS, Logothetis CJ, Molina A, Fizazi K, North S, Chu L et al (2011) Abiraterone and increased survival in metastatic prostate cancer. Engl J Med 364:1995–2005. https://doi.org/10.1056/NEJMoa1014618
33. Bryce A, Ryan CJ (2012) Development and clinical utility of abiraterone acetate as an androgen synthesis inhibitor. Clin Pharmacol Ther 91:101–108. https://doi.org/10.1038/clpt.2011.275
34. Handratta VD, Jelovac D, Long BJ, Kataria R, Nnane IP, Njar VC, Brodie AM (2004) Potent CYP17 inhibitors: Improved syntheses, pharmacokinetics and antitumor activity in the LNCaP human prostate cancer model. J Steroid Biochem Mol Biol 92:155–165. https://doi.org/10.1016/j.jsbmb.2004.07.006
35. Handratta VD, Vasaitis TS, Njar VC, Gediya LK, Kataria R, Chopra P et al (2005) Novel C-17-heteroaryl steroidal CYP17 inhibitors/antiandrogens: synthesis, in vitro biological activity, pharmacokinetics, and antitumor activity in the LAPC4 human prostate cancer xenograft model. J Med Chem 48:2972–2984. https://doi.org/10.1021/jm040202w
36. Brodie A, Njar VC (2006). Novel C-17-heteroaryl steroidal CYP17 inhibitors/antian-drogens: synthesis, in vitro biological activities, pharmacokinetics and antitumor activity. WO Patent 093993
37. Ligr M, Li Y, Logan SK, Taneja S, Melamed J, Lepor H, Garabedian MJ, Lee P (2012) Mifepristone inhibits GRβ-coupled prostate cancer cell proliferation. J Urol 188:981–988. https://doi.org/10.1016/j.juro.2012.04.102
38. Wilt TJ, Macdonald R, Hagerty K, Schellhammer P, Tacklind J, Somerfield MR, Kramer BS (2010) 5-α-Reductase inhibitors for prostate cancer chemoprevention: an updated Cochrane systematic review. BJU Int 106:1444–1451
39. Bologna M, Muzi P, Biordi L, Festuccia C, Vicentini C (1995) Finasteride dose-dependently reduces the proliferation rate of the LNCaP human prostatic cancer cell line *in vitro*. Urology 45:282–290. https://doi.org/10.1016/0090-4295(95)80019-0
40. Al-Masoudi NA, Sami A, Abdul-Rida NA, Fortscher M (2018) New cholic acid analogs: synthesis and 17β-hydroxydehydrogenase (17β-HSD) inhibition activity. Z Naturforsch B 73:211–223. https://doi.org/10.1515/znb-2018-0192
41. Mahdi KM, Abdul-Rida NA, Al-Masoudi NA (2015) Exploration of new 3α-pregnenolone ester analogues via Mitsunobu reaction, their anti-HIV activity and molecular modeling study. Euro J Chem 6:1–7. https://doi.org/10.5155/eurjchem.6.1.1-7.1139
42. Willker W, Leibfritz D, Kerssebaum R, Bermel W (1993) Gradient selection in inverse heteronuclear correlation spectroscopy. Magn Reson Chem 31:287–292. https://doi.org/10.1002/mrc.1260310315
43. Mosmann T (1983) Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 65:55–63. https://doi.org/10.1016/0022-1759(83)90303-4
44. Trott O, Olson AJ (2010) AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading. J Comput Chem 31:455–461. https://doi.org/10.1002/jcc.21334
45. Miller DR, Tzeng C-C, Farmer T, Keller ET, Caplan S, Chen Y-S, Chen T-L, Lin M-F (2018) Novel CIL-102 derivatives as potential therapeutic agents for docetaxel-resistant prostate cancer. Cancer Lett 436:96–108. https://doi.org/10.1016/j.canlet.2018.07.039

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