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Synthesis, In Vitro Anti-HIV Activity, Cytotoxicity, and Computational Studies of Some New Steroids and Their Pyrazoline and Oxime Analogues

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Abstract—There is an urgent need for the design and development of new and safer drugs for the treatment of HIV infection, active against the currently resistant viral strains by development of new non-nucleoside reverse transcriptase inhibitors (NNRTIs). A series of pregnenolone analogues, 3-((aryl)-1-(5-pregnen-3β-ol-17-yl)prop-2-en-1-ones, were synthesized. Further, treatment of 3-((4-bromo-, 4-trifluoromethyl, or 4-methylphenyl)-1-(preg-5-en-3β-ol-17-yl)prop-2-en-1-ones with thiosemicarbazide in ethanolic KOH or hydrazine hydrate in HOAc gave 5-(4-bromo-, 4-trifluoromethyl, or 4-methylphenyl)-3-(preg-5-en-3β-ol-17-yl)-4,5-dihydro-1*H*-pyrazoline-1-carbothioamides and 1-*O*-acetyl-(5-(4-bromophenyl)-3-(preg-5-en-3β-ol-17-yl)-4,5-dihydro-1*H*-pyrazoline, respectively. Analogously, treatment of 3-((4-bromophenyl)-1-(preg-5-en-3β-ol-17-yl)prop-2-en-1-one with hydroxylamine afforded the *Z*/*E* isomers of 3-(4-bromophenyl)-1-(preg-5-en-3β-ol-17-yl)prop-2-en-1-one oxime. The new compounds were assayed against HIV-1 and HIV-2 in MT-4 cells. Compounds 3-(thiophene-2-yl)-1-(preg-5-en-3β-ol-17-yl)prop-2-en-1-one and 1-*O*-acetyl-(5-(4-bromophenyl)-3-(preg-5-en-3β-ol-17-yl)-4,5-dihydro-1*H*-pyrazoline were the most active in inhibiting HIV-1 and HIV-2 with IC_{50} = 60.5 μM (SI > 2, against HIV-2 and SI < I against HIV-1), and > 0.29 μM (SI < I), respectively, suggesting to be new leads in the development of antiviral agents. QSAR of 3-((aryl)-1-(5-pregnen-3β-ol-17-yl)prop-2-en-1-ones and 5-(substituted phenyl)-3-(5-preg-5-3β-ol-17-yl)-4,5-dihydro-1*H*-pyrazole-1-carbothioamides has been studied. The conformational analysis of 5-(4-trifluoromethylphenyl)-3-(preg-5-en-3β-ol-17-yl)-4,5-dihydro-1*H*-pyrazoline-1-carbothioamide and 1-*O*-acetyl-(5-(4-bromophenyl)-3-(preg-5-en-3β-ol-17-yl)-4,5-dihydro-1*H*-pyrazoline as well as the molecular docking study of the latter compound have been investigated.

Keywords: anti-HIV activity, α-unsaturated ketones, cytotoxicity, molecular docking study, QSAR, pregnenolone

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INTRODUCTION

A number of steroids and their derivatives possess diverse pharmacological activities as drugs for the treatment of a large number of diseases including cardiovascular [1] or autoimmune diseases [2], brain tumors [3], breast cancer, prostate cancer [4], osteoarthritis [5], etc. Recently several nitrogen-containing steroidal compounds containing five or six-membered 17β-*exo*-heterocycles (preferably nitrogen containing), such as steroidal azoles [6, 7] have been developed for the treatment of prostate cancer, including abiraterone acetate (Zytiga), [8, 9] galeterone and its Δ^4-3-keto derivative [10]. Several steroids having arylated prop-2-en-1-ones [11–14], pyrazoles, and pyrazolines [15] and oxime analogue [16] have been reported as potential 5α-reductase inhibitors or anticancer agents. Both pregnenolone derivatives having pyridine or imidazole moieties at C-17, respectively, were designed for treatment of prostate cancer (PC) by inhibition of the enzyme 17α hydroxylase/C17,20-lyase (CYP17A1). Hartmann et al. [17], Silvador et al., [18] and Haidar et al. [19] have reported the synthesis of several CYP17 steroidal inhibitors as a new strategy for the treatment of prostate carcinoma. Recently, we have synthesized a new series of 17-*N*-imino-benzo-

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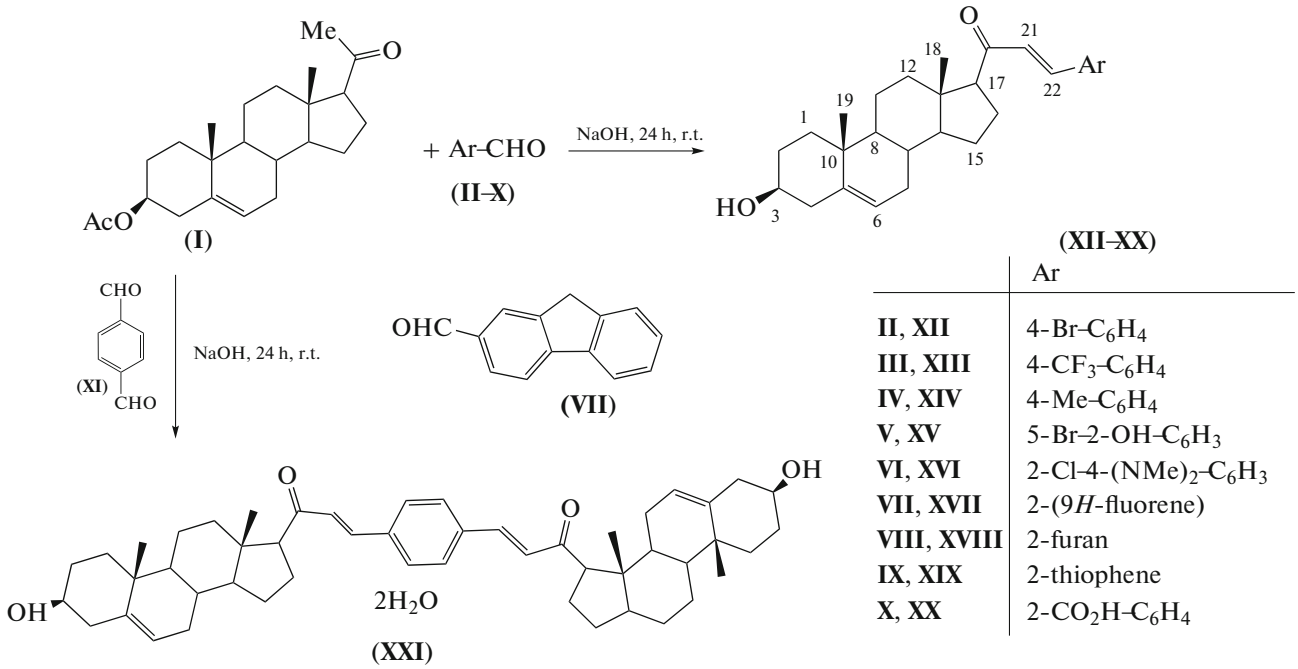
thiazole [20] as well as 17-*N*-(iminoalkyl) derivatives of β-pregnenolone [21]. Furthermore, we prepared a series of biaryl-chalconyl pregnenolone analogues [22] to evaluate their abilities to prevent the intratumoral androgen production by inhibiting the activity of the CYP17 hydroxylase enzyme. In addition, Mitsunobu reaction has been employed in synthesis of 3α-*O*-ester analogues [23] from β-pregnenolone *via* the inversion of configuration at C-3. Numerous modifications to the steroid nucleus have been made in order to study the SAR of bioactive substituents at the 3β-, 11-, 17-, or 21-positions. Compounds having arylated-propenone moieties are very versatile as physiologically active compounds and substrates for the evaluation of various organic syntheses. These compounds have been reported to possess several biological activities such as anticancer [24], antimalarial [25], anti-inflammatory [26], anti-HIV [27, 28] and antifungal [29] agents. Therefore, we are interested in the chemistry of the steroids bearing potent substituents at C-17.

In view of varied pharmacological activities of steroids and arylated-propenone derivatives, herein we report the synthesis of some pregnenolones having arylated-prop-2-en-1-ones and their pyrazoline and oxime analogues with evaluation of their anti-HIV activity and cytotoxicity. The new analogues have been theoretically investigated by applying density functional theory (DFT) to understand the structure activity relationship.

RESULTS AND DISCUSSION

Chemistry

In our present work, we have selected pregnenolone (**I**) as starting material for the synthesis of a new series of pregnenolone having arylated prop-2-en-1-ones along with modification of the prop-2-en-1-one moiety. Thus, subsequent treatment of **(I)** with the desired aryl aldehydes such as: 4-bromo-, 4-trifluoromethyl-, 4-methyl-, 5-bromo-2-hydroxy- and 2-chloro-4-(dimethylmino)benzaldehyde (**II**)–(**VI**), as well as 9*H*-fluorene-, furan-, or thiophene-2-carboaldehyde (**VII**)–(**IX**), or 2-formylbenzoic acid (**X**) in EtOH in the presence of 2M NaOH at room temperature for 24 h, proceeded smoothly to give the new pregnenolone derivatives (**XII**)–(**XX**) in 68 to 84% yield (Scheme 1).



	Ar
II, **XII**	4-Br-C_6H_4
III, **XIII**	4-CF_3-C_6H_4
IV, **XIV**	4-Me-C_6H_4
V, **XV**	5-Br-2-OH-C_6H_3
VI, **XVI**	2-Cl-4-$(NMe)_2$-C_6H_3
VII, **XVII**	2-(9*H*-fluorene)
VIII, **XVIII**	2-furan
IX, **XIX**	2-thiophene
X, **XX**	2-CO_2H-C_6H_4

Scheme 1. Synthesis of some arylated-prop-2-en-1-one analogues of pregnenolone.

The structures of compounds (**XII**)–(**XXI**) were assigned on the basis of their NMR spectra (^{1}H-, ^{13}C and 2D), which showed rather similar patterns of the proton and carbon atoms of pregnen scaffold. In the ^{1}H NMR of (**XII**)–(**XXI**), H-21 and H-22 resonated as two doublets in the regions δ 7.06–6.57 and 7.91–7.33 ppm, respectively, with a large $J_{21,22}$ of 16.2–15.8 Hz, indicative for the *trans*-configuration of the prop-2-en-1-one protons (H-21 and H-22). The aromatic protons H-2' + H-6' and H-3' + H-5' of (**XII**)–(**XIV**) appeared as two doublets at the regions δ 7.52–7.25 and 7.41–7.17 ppm (J = 8.4–7.4 Hz), respectively.

The other aromatic protons of (**XII**)–(**XXI**) were all assigned (*c.f.* Experimental section). The triplets or doublets at δ 5.36–5.26 ppm (J = 5.2–3.8 Hz) were assigned to 6-H, while 3-H resonated as multiplets in the regions δ 3.57–3.28 ppm. The multiplets in the regions δ = 2.57–2.33 ppm assigned to the CH_2-4 pro-

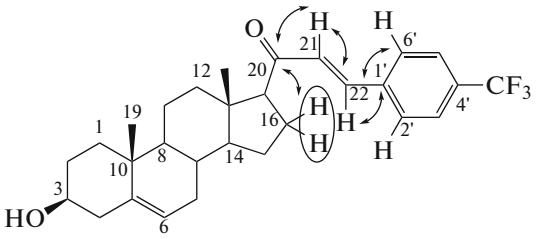
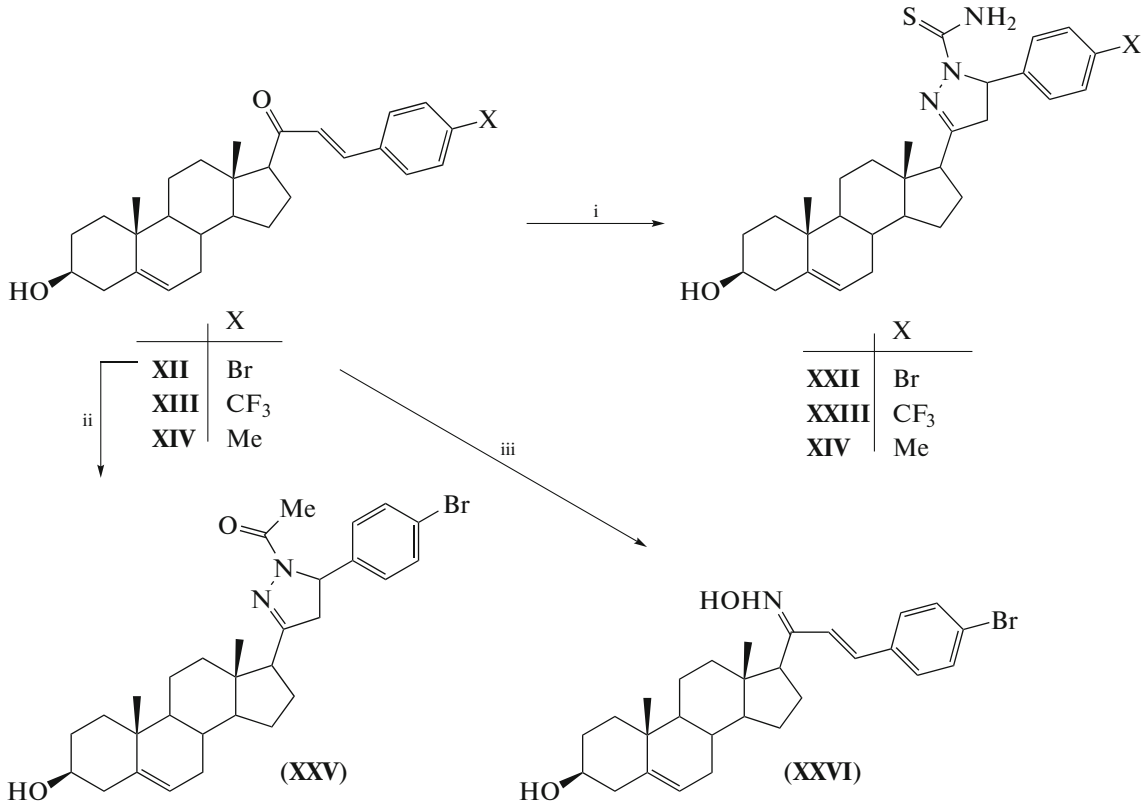


Fig. 1. $J_{C,H}$ correlations in the NMR HMBC correlations of (**XIII**).

tons. The other aliphatic protons were fully identified (*c.f.* Experimental section). In the ^{13}C-NMR spectra of (**XII**)–(**XXI**), the resonances in the regions δ 209.5–198.9 ppm were assigned to C-20, whereas the olefinic carbon atoms (C-21, C-22) appeared at δ 128.1–125.7 and 141.8–140.8 ppm, respectively, except for compounds (**XVIII**) and (**XIX**) where C-21 resonated at δ 128.1 and 133.7 ppm, and C-22 at δ 124.2 and 132.4 ppm, respectively. The resonances of the other aryl and pregnen aliphatic carbon atoms and of the substituents were all identified and fully assigned (*c.f.* Experimental section).

Compound (**XIII**) was selected for further NMR studies, since its gradient HMBC spectrum [30] allowed the identification of H-21 at δ_H 6.85 ppm from its $^2J_{C,H}$ couplings to carbon atom (C-22) at δ_C 140.8 ppm as well as the carbonyl carbon atom (C-20) at δ_C 200.0 ppm. A $^2J_{C,H}$ coupling between H-22 at δ_H = 7.55 ppm and the aromatic carbon atom (C-1') at δ_C 138.3 ppm was observed. Furthermore, H-16a and H-16b protons at δ_H 2.17 and 1.61 ppm showed $^3J_{C,H}$ couplings to the carbonyl carbon atom (C-20) at δ_C 200.0 ppm. Additionally, $^3J_{C,H}$ couplings between C-22 and the aromatic protons H-2' and H-6' at δ_H 7.25 ppm were observed (Fig. 1).

Next, steroids (**XII**)–(**XIV**) were treated with thiosemicarbazide in boiling ethanolic KOH to give the pyrazoline-*N*^{1}-carbothioamide analogues (**XXII**)–(**XXIV**) in 68, 71 and 67% yield, respectively. Analogously, treatment of (**XIII**) with hydrated hydrazine in the presence of HOAc at ambient temperature for 20 h afforded the *N*^{3}-acetylpyrazoline derivative (**XXV**) in 63% yield. Treatment of (**XV**) with hydroxylamine in dry pyridine at ambient temperature furnished the oxime analogue (**XXVI**) in 67% yield (Scheme 2).



Scheme 2. Reagents and conditions: (i) thiosemicarbazide, EtOH, K_2CO_3, reflux, 4 h; (ii) $NH_2NH_2 \cdot H_2O$, HOAc, ambient temperature, 20 h; (iii) NH_2OH, dry pyridine, ambient temperature, 22 h.

The structures of compounds (**XXII**)–(**XXIV**) were assigned from their ^{1}H-, ^{13}C and 2D NMR spectra, which showed similar patterns of the proton and carbon atoms of pregnen backbone. In the ^{1}H NMR spectra, CH_2-4 and H-5 of the pyrazoline ring appeared as multiplets in the regions δ 3.16–2.91 and

4.58–4.56 ppm, respectively, while the downfield shifted doublets at the regions δ = 7.67–7.35 and 7.28–7.02 ppm were assigned to the aromatic protons H-3' + H-5' and H-2' + H-6', respectively. The multiplets at δ 3.21, 2.91 and 2.98 ppm were assigned to H-17, merged with those of CH_2-4 of the pyrazoline ring. The other aliphatic protons of pregnen scaffold were all identified and assigned (*c.f.* Experimental section).

The ^{13}C NMR spectra of compounds (**XXII**)–(**XXIV**) showed signals at δ 175.0, 174.6 and 174.7 ppm, which was attributed to the C=S carbon atom of the thioamide group, while the carbonyl carbon atom of the acetyl group of (**XXV**) appeared at δ 167.8 ppm. The resonances at δ 161.1, 166.6, 159.9 and 158.9 ppm were assigned to C-3 of the pyrazoline residue of compounds (**XXII**)–(**XXV**). Carbon atom C-4 of the pyrazoline ring resonated at δ 36.8–36.2 ppm, while C-5 of the same ring appeared at δ 69.7 and 69.9, 69.6 and 64.8 ppm, respectively. C-17 of the pregnen core resonated at the regions δ 62.7–62.5 ppm. The aromatic and pregnen carbon atoms have been fully assigned (*cf.* Experimental section).

The structure of (**XXVI**) was determined from its IR, ^1H and ^{13}C NMR spectra. In the ^1H NMR, the two broad singlets at δ 11.01 and 10.32 ppm assigned for the *Z* and *E* isomers of the N–OH group, respectively, which are exchangeable with D_2O. The aromatic and aliphatic protons were fully distinguished (Experimental section). In the ^{13}C NMR spectrum, the higher field resonances of the C_{20}-NOH carbon atom (δ 154.9, 152.5 ppm, *Z*/*E* isomers, respectively) is significantly shifted to higher field in comparison to that of starting material (**XV**) (δ 200.1 ppm). This is indicative for oxime formation. The resonance signals of carbon atoms C-20. C-21 and C-22 appeared at δ 128.1, 127.9 and 141.2, 140.0 ppm for the *Z* and *E* isomers, respectively. The other carbon atoms have also been identified.

In Vitro Anti-HIV Activity

Compounds (**XII**)–(**XXVI**) were evaluated for their inhibitory activity against HIV-1 (strain III_B) and HIV-2 (strain ROD), which were monitored by the inhibition of the virus-induced cytopathic effect in the human T-lymphocyte (MT-4) cells, using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) method [31]. The results are summarized in Table 1, in which the data for nevirapine [32], and 3'-azido-3'-deoxythymidine (AZT) [33], are included for comparison purposes. The cytotoxicity of the compounds was determined in parallel. The compounds are largely devoid of antiretroviral activity against both HIV-1 and HIV-2, although they showed cytotoxicity against MT-4 cells at micromolar concentrations. Of the title compounds tested, only compound (**XIX**) show some activity against HIV-2 replication in cell culture (IC_{50} = 60.5 μM, CC_{50} > 125.0 μM) with SI > 2. Interestingly, compound (**XXV**) exhibited significant cytotoxicity of > 0.29 μM against the human T-lymphocytes, and can be a promising antiproliferative agent against cancer cell lines, since Ducki et al. [34] have discovered that some chalcone derivatives exhibited cytotoxic activity against the K562 human leukaemia cell line.

From the structure-activity relationship analysis, we found that the thiophene moiety at substituent of the prop-2-en-1-one residue, *e.g.* compounds (**XIX**) was well tolerated in the hydrophobic binding pocket of HIV-2 reverse transcriptase (RT) and then showed higher activity than those of other substituents at C-22 of the prop-2-en-1-one residue. Therefore, (**XIX**) proposed to act as a nonnucleoside reverse transcriptase inhibitor (NNRTI), although its activity spectrum is limited to HIV-2.

Computational Study Geometry Optimization

The geometry optimization process is carried out using an repeated process, until the total energy of a structure is minimized, i.e., it corresponds to a local minimum in the potential energy surface. The minimum-energy molecular structures of our selected compounds were completely optimized by using density-functional theory (DFT) calculations employing Becke's three-parameter exchange potential and the Lee–Yang–Parr correlation functional (B3LYP) [35] in conjunction with the three split valence basis sets 6-311+G(d,p). The frequency simulations were performed at the same level, to confirm the optimized geometries as a true minimum (no imaginary frequency). All optimizations and vibrational frequency calculations were done using Gaussian 16 suite of programs [36].

Compounds (**XVII**), (**XIX**), (**XXIII**) and (**XXV**) have been selected to calculate their potential energy surface and relative stability. Harmonic vibrational frequency calculations were undertaken for the optimized geometries. The vibration frequency calculations showed no imaginary frequencies (except for the structures (**XXIII**) (C) and (**XXV**) (C)) which ascertain that the structures were minima on the potential energy surface. The relaxed potential energy surface (PES) scan was done involving the H17-C17-$C3_{pyrazoline}$-$N2_{pyrazoline}$ dihedral angle which was stepped 38 times by increments of 10° using the B3LYP/6-31G level of theory. The located energy minima and transition point at the PES were then fully optimized at the B3LYP/6-311+G(d,p) level of theory for a better description of the energy barrier (Table 2).

The configuration of the olefinic protons (C_{21}=C_{22}) of (**XVII**) and (**XIX**) has been calculated by the DFT method (GGA) at the B3LYP/6-311+G(d,p) level of theory. Figure 2S (Supplementary materials) represents the orbitals of (**XVII**) and (**XIX**)which revealed that the LUMO is separated on the carbon

atoms of the olefinic linkage (C_{21}=C_{22}) and the oxygen atom of the carbonyl group. In addition, Table 2 and Fig. 2 demonstrated that the *trans* configuration of these analogues is more stable. Furthermore, Fig. 2 represents energies of *trans*,*cis* HOMO and *trans*,*cis* LUMO of (**XVII**) and (**XIX**) as well as (**XXIII**) and (**XXV**), meanwhile Table 2 showed that compound (**XXV**) has ΔE_{A-B} value (−0.00255 ha) lower than those of (**XXIII**) (−0.00186 ha), suggesting that the pyrazole analogue (**XXV**) is more stable.

Conformational Analysis

Compounds (**XXIII**) and (**XXV**) have been selected to study their conformations by calculation of the potential energy surface (PES) around H17-C17-$C3_{pyrazoline}$-$N2_{pyrazoline}$ torsion angle. The PES of both compounds are almost identical, but look asymmetrical as shown in Figs. 3 and 4.

Compound **25** revealed two minimum energy points at 145° and 355° which represent the global and local minimum points (conforms **a** and **b**, respectively), since **b** showed relatively higher energy than **a** by 1.3 kcal mol^{-1}. At 195°, the molecule showed a higher energy conform (**c**) than the others by 2.2 kcal mol^{-1}. In addition, at 265° the diagram is characterized by appearence of transition state with a higher energy by 5.8 kcal mol^{-1} (conform **d**), separated the other two conforms of lower energy (Figs. 4 and 5).

From the above data, we concluded that there is influence for aromatic or amide groups on the shape of PES, however, the main effect might arised from repulsion of hydrogen atoms around the torsional angle between C-17, $C3_{pyrazoline}$ and hydrogen atoms at carbon atoms 16 (2H), 17 (1H), and $C4_{pyrazoline}$ (2H). The conform (**XXVc**) has been excluded from our calculations since it included unexpected (imaginary) frequencies, then it is unstable conform. Furthermore, the energy differences between the conforms (**a**) and (**b**) as well as (**d**) (5 kcal mol^{-1}) indicates that both (**a**) and (**b**) may be existing in the crystal phase. Table 3 represents the energies of the conforms (**XXIIIa–d**) and (**XXVa–d**).

Molecular Modeling Analysis

The molecular docking was performed using the Molecular Operating Environment 2016 (MOE 2016) software and the docking results were also shown by MOE [37]. Molecular docking analysis of the new analogues is based on the modeling study, which was performed to understand the binding mode of these analogues with HIV RT binding pocket (NNIBP) (PDB ID: 3DLG) [38]. The molecular binding simulation results of the most active compound in this study, (**XXV**) with HIV-1 RT are displayed in Fig. 6, panels (a) and (b).

Table 1. In vitro anti-HIV-1[a] and HIV-2[b] activity of pregnenolone derivatives

Compd.	Virus strain	av. IC_{50}, μM[c]	av. CC_{50}, μM[d]	SI[e]
(XII)	III_B	>13.30	13.30	<1
	ROD	>13.30	13.30	<1
(XIII)	III_B	>55.38	55.38	<1
	ROD	>55.38	55.38	<1
(XIV)	III_B	>51.70	51.70	<1
	ROD	>51.70	51.70	<1
(XV)	III_B	>12.05	12.05	<1
	ROD	>12.05	12.05	<1
(XVI)	III_B	>12.95	12.95	<1
	ROD	>12.95	12.95	<1
(XVII)	III_B	>7.62	7.62	<1
	ROD	>7.62	7.62	<1
(XVIII)	III_B	>12.00	12.00	<1
	ROD	>12.00	12.00	<1
(XIX)	III_B	>125.00	>125.00	×1
	ROD	60.50	>125.00	>2
(XX)	III_B	>13.98	13.98	<1
	ROD	>13.98	13.98	<1
(XXI)	III_B	>47.75	47.75	<1
	ROD	>47.75	47.75	<1
(XXII)	III_B	>11.78	11.78	<1
	ROD	>11.78	11.78	<1
(XXIII)	III_B	>7.92	7.92	<1
	ROD	>7.92	7.92	<1
(XXIV)	III_B	>7.49	7.49	<1
	ROD	>7.49	7.49	<1
(XXV)	III_B	>0.29	0.29	<1
	ROD	>0.029	0.029	<1
(XXVI)	III_B	>10.28	10.28	<1
	ROD	>10.28	10.28	<1
Nevirapine	III	0.05	>4	>80
	ROD	>4	>4	<1
AZT	III_B	0.0019	>25	>13144
	ROD	0.0018	>25	>14245

[a]Anti-HIV-1 activity measured against strain III_B; [b]anti-HIV-2 activity measured against strain ROD; [c]compound concentration required to achieve 50% protection of MT-4 cells from the HIV-1 and 2-induced cytopathogenic effect; [d]Average CC_{50}: compound concentration that reduces the viability of mock-infected MT-4 cells by 50%; [e]SI: selectivity index (CC_{50}/IC_{50}). SI value: ×1 stand for 1 or <1. All data represents the mean values of at least two separate experiments.

Table 2. Free energies and population of the *trans* and *cis* conformers of the steriods (**XVII**) and (**XIX**) and pyrazoles (**XXIII**) and (**XXV**)

Compd.	Free energy corrected for ZPE, ha	ΔG, kcal mol^{-1}	Population, %
(**XVII**) *trans*	–1507.449438	0	~100
(**XVII**) *cis*	–1507.439538	6.212	~0
(**XIX**) *trans*	–1559.208099	0	99.976282
(**XIX**) *cis*	–1559.200218	4.945	0.00023718
(**XXIIIa**)	–2104.04479	0	86.3
(**XXIIIb**)	–2104.04293	0.00174	13.7
(**XXVa**)	–4013.30722	0	93.7
(**XXVb**)	–4013.30467	0.002549	6.3

ZPE: zero point energy dependent on the PES curvature difference between reactant state and transition state (i.e. difference in second derivative matrices at the two stationary points).

Table 3. Energies of the conforms (**XXIIIa**–**d**) and (**XXVa**–**d**)

Entry	Relative energy of the global minimum (A)*	Energy of local minimum (B)*	Energy of local minimum (C)*	Energy of transition state (D)*
23	0.00	1.20	2.2	5.8
25	0.00	1.32	2.2	5.8

* kcal mol^{-1}.

According to the HIV-1 RT docking results, it was observed that compound (**XXV**) binds RT *via* only one H-bond between OH group of (**XXV**) at C-3 and Glu224. Furthermore, an interaction between the aromatic ring of the steroid and Val106. As shown in Fig. 3, the aromatic ring of (**XXV**) fitted into an arene-rich subpocket surrounded by the aromatic side chains of Tyr188, Tyr318 and Phe227 residues. Overall, the combination of hydrophobic interaction and π–alkyl and H-bond appear to govern the binding of compound (**XXV**) with HIV RT (S = –6.3718, RMSD = 1.196).

Singh et al. [39] reported that the hydrophobic interactions play a crucial role in ligand–protein binding. In these, the valine amino acid is the second top most hydrophobic amino acid (first one is isoleucine) and is responsible for hydrophobic interaction with R-substituents of the compounds. Accordingly, our results are in accordance with Singh and co-workers report by observation of a π–alkyl hydrophobic interaction between the aromatic ring of (**XXV**) and Val106.

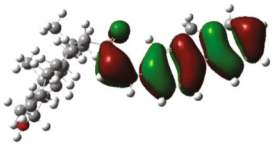
Therefore, theoretical calculations were used to examine the influence of π–CH-interaction in determining the conformational flexibility of folding models.

Quantum-Structure Activity Relationships (QSAR)

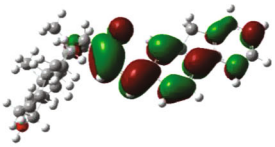
To obtain the QSAR models, we have correlated the quantum parameters with each other and the biological activity (IC_{50}), since some parameters lead to the biological activities of the inhibitors. Therefore, it is important in QSAR study to establish the relationship between IC_{50} and numerous parameters by regression models. In this study, we investigated the structural features and conformational behaviour and the optimized geometries of building blocks of chalconyl steroids and their analogues, at the PWC/DNP level of theory using the software Dmol [40] in Materials Studio package. The QSAR study was done with the Materials Studio package using genetic function approximation (GFA) technique [41]. The quantum chemical indices E_{HOMO}, E_{LUMO}, Dipole X-axis and molecular surface area were calculated with VAMP software. This will serve as a basis for future theoretical and experimental work on more complex aromatic steroid structures related to their biological activity.

The QSAR models were developed using different statistical methods like stepwise multiple linear regression, genetic function approximation and genetic partial least squares with descriptors of different categories (quantum chemical, physicochemical, spatial and substituent constants). In this study, the QSAR models were built by means of Multiple Linear Regression (MLR)) technique embedded in Material Studio, a modeling and simulation software using the experimentally obtained biological activities as the dependent variables and the computed molecular descriptors as independent variables.

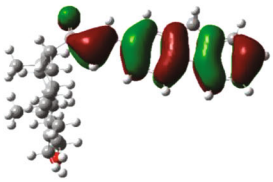
The activity data [IC_{50} (μM)] was converted to the logarithmic scale pIC_{50} [–logIC_{50} (M)] and then used for the subsequent quantitative structure activity relationship analyses as the response variables. With the



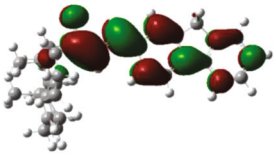
(**XVII**) *cis* HOMO, $E = -0.34431$ ha



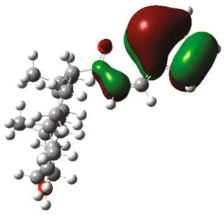
(**XVII**) *cis* LUMO, $E = -0.24721$ ha



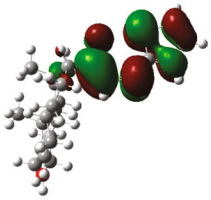
(**XVII**) *trans* HOMO, $E = -0.34351$ ha



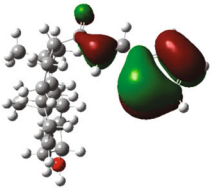
(**XVII**) *trans* LUMO, $E = -0.24526$ ha



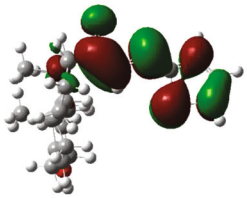
(**XIX**) *cis* HOMO, $E = -0.31723$ ha



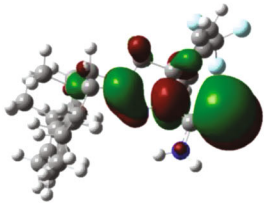
(**XIX**) *cis* LUMO, $E = -0.23195$ ha



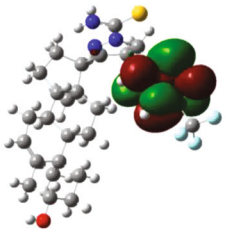
(**XIX**) *trans* HOMO, $E = -0.31855$ ha



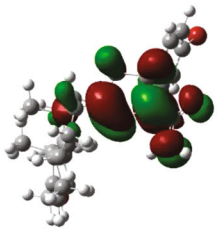
(**XIX**) *trans* LUMO, $E = -0.23248$ ha



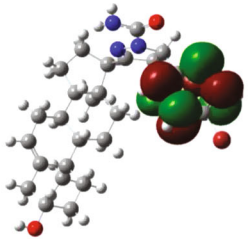
(**XXIII**) HOMO (A), $E = -0.20225$ ha



(**XXIII**) LUMO (B), $E = -0.06003$ ha



(**XXV**) HOMO (A), $E = -0.20399$ ha



(**XXV**) LUMO (B), $E = -0.05215$ ha

Fig. 2. Represents energies of *trans*,*cis* HOMO and *trans*,*cis* LUMO of (**XVII**) and (**XIX**) as well as (**XXIII**) and (**XXV**).

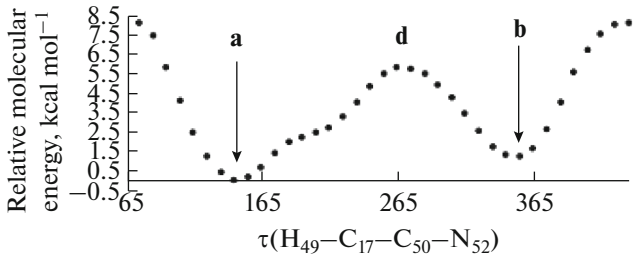


Fig. 3. Conformational analysis (relaxed scan) of (**XXIIIa–d**) calculated at B3LYP/6-31G level around H17-C17-$C3_{pyrazoline}$-$N2_{pyrazoline}$ torsional angle.

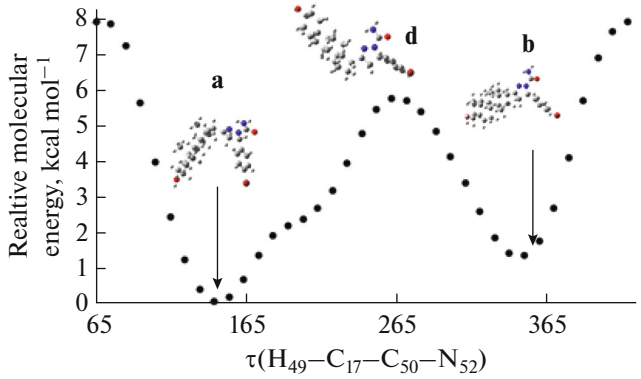


Fig. 4. Conformational analysis (relaxed scan) of (**XXVa–d**) calculated at B3LYP/6-31G level around H17-C17-$C3_{pyrazoline}$-$N2_{pyrazoline}$ torsion angle.

selected descriptors, we have selected two models to study the QSAR of these molecules as in Eqs. (1) and (2).

Model 1:

$\log 1/C_{50}$ = −0.226396628 Hydrogen bond acceptor − 1.857303675 E_{LUMO} + 0.529057861 (E_{HOMO} × Dipole X) − 1.307999224 (E_{LUMO} × Dipole X) + 0.007634617 (Molecular surface area × Dipole X) + 0.473736392. (1)

R^2 = 0.969; adjusted R^2 = 0.950; cross validated R^2 = 0.933; significant regression = yes; Friedman LOF = 0.24744900; critical SOR *F* value = 50.8.

Model 2:

log 1/C = −0.5488 ramp(Alog P − 7.713) −58.473 ramp(E_{LUMO} + 0.0878) + 7.311 ramp(Total dipole − 2.610) − 7.753 ramp(Total dipole − 2.708) + 0.353 ramp (Total dipole − 4.033340227) + 1.074. (2)

R^2 = 0.998; adjusted R^2 = 0.997; cross validated R^2 = 0.762; significant regression = yes; Friedman LOF = 0.01127600; critical SOR *F* value = 771.8.

In conclusion, the binding score for the tested compounds were congruent with their anti-HIV activity. A good correlation between the predicted and the experimentally observed inhibitory activities (pIC_{50}) (Tables 4 and 5) of the most steroid analogues suggested that the identified binding conformations of these inhibitors are reliable. The results of docking study provided an insight into the pharmacophoric structural requirements (compound **XXV**) for HIV RT inhibitory activity of this class of molecules.

EXPERIMENTAL

Melting points are uncorrected and were measured on a Büchi melting point apparatus B-545 (Büchi-Labortechnik AG, Switzerland). Microanalytical data were obtained with a Vario Elemental Analyzer (Shimadzu, Japan). NMR spectra were recorded on 400 and 600 MHz (^{1}H) and on 150 : 91 MHz (^{13}C) spectrometers (Bruker, 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. Mass spectra (EI, 70 eV, and FAB) were recorded on MAT 8200 spectrometers (Finnegan MAT, USA). TLC plates 60 F254 were purchased from Merck. The chromatograms were visualized under UV 254–366 nm and iodine.

General Procedure for the Synthesis of 3-((Aryl)-1-(5-Pregnen-3β-ol-17-yl)prop-2-en-1-ones (XII)–(XXI)

To a stirred solution of pregnenolone acetate (**I**) (194 mg, 0.54 mmol) in EtOH (10 mL) were added the substituted aldehydes (**II**)–(**XI**) (0.54 mmol), followed by the addition of an aq. solution of 2 M NaOH (10 mL). After stirring at ambient temperature for 24 h, the mixture was neutralized with 1 M HCl and partitioned with EtOAc (3 × 15 mL). The combined organic extracts were washed with brine, dried (Na_2SO_4) and evaporated to dryness. The residue was purified on a short SiO_2 column using the eluent hexane: EtOAc (3 : 2) to afford the desired steroid. For preparation of steroid **21**, 1.08 mmol of compound **1** has been used.

For atom numbering refer to Schemes 1–3.

3-((4-Bromophenyl)-1-(preg-5-en-3β-ol-17-yl)-prop-2-en-1-one (XII). From 4-bromo-benzaldehyde (**II**) (100 mg). Yield: 198 mg (76%) as a colorless powder, mp 144–147°C. IR spectrum, film, ν, cm^{-1}: 3655 (OH), 2929 (CH_2), 1694, 1510 (C=C), 1052 (C–O). ^{1}H NMR spectrum DMSO-d_6, δ, ppm: 7.52 (d, 2H, $J_{2',3'}$ = 8.4 Hz, H_{arom}-2' + H_{arom}-6'), 7.47 (d, 1H, $J_{21,22}$ = 16.0 Hz, H-22), 7.41 (d, 2H, $J_{2',3'}$ = 8.4 Hz, H_{arom}-3' + H_{arom}-5'), 6.75 (d, 1H, $J_{21,22}$ = 16.0 Hz, H-21), 5.36 (t, 1H, $J_{6,7}$ = 4.0 Hz, H-6), 5.30 (bs, 1H, OH), 3.53 (m, 1H, H-3), 2.83 (m, 1H, H-17), 2.17 (m, 1H, H-16a), 2.36 (m, 2H, CH_2-4), 2.12 (m, 1H, CH_2-7), 2.01 (m, 1H, H-12a), 1.85 (m, 1H, H-1a), 1.71 (m, 1H, H-2a), 1.69 (m, 1H, H-15a), 1.62 (m, 1H, H-16b), 1.54 (m, 2H, H-11a + H-12b), 1.49-1.42 (m, 3H, H-2b + H-8 + H-11b), 1.31–1.27 (m, 2H, H-14 + H-15b), 1.14 (m, 1H, H-1b), 1.10 (m, 1H, H-9), 1.00

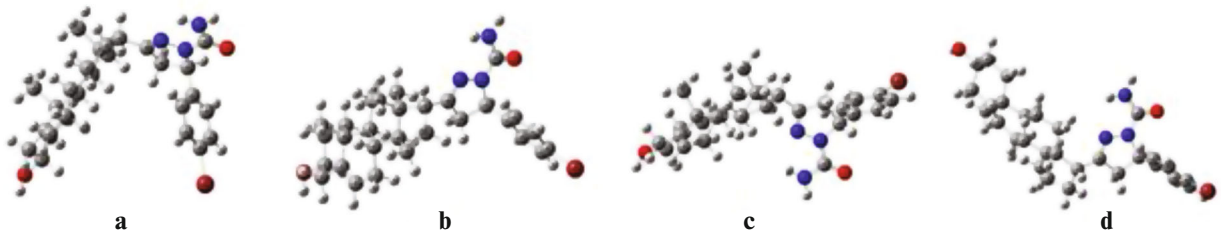


Fig. 5. Conforms (**XXVa**–**d**) calculated at B3LYP/6-31G level around H17-C17-C3$_{pyrazoline}$-N2$_{pyrazoline}$ torsion angle.

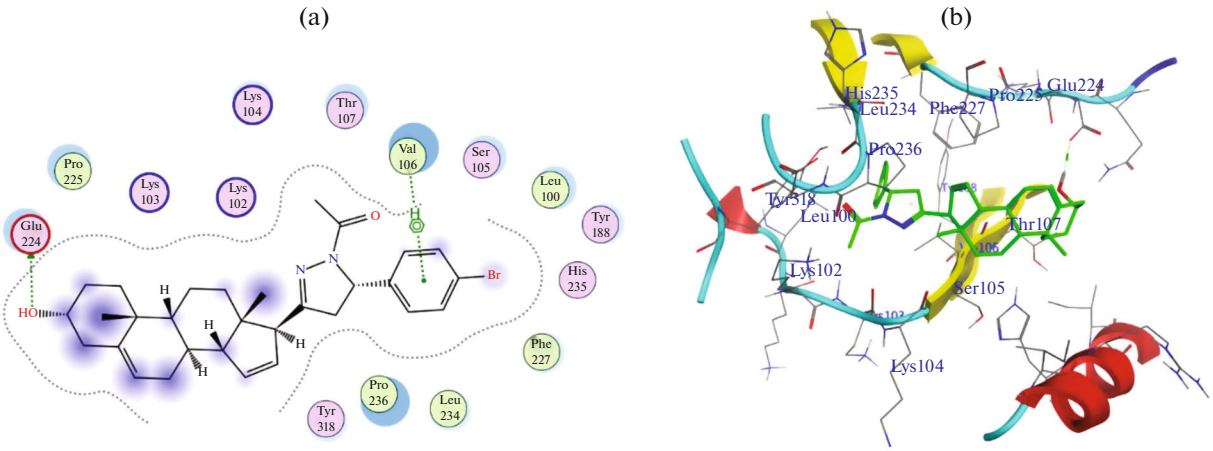


Fig. 6. Docked conformation of (**XXV**) ((a) 2D; (b) 3D) with HIV-RT (PDB ID: 3DLG), showing one hydrogen bond: Glu224 with OH at C-3 of the steroid. It also exhibits hydrophobic interactions involving the π–CH-interaction between aromatic ring of the steroid and Val106 residue of HIV RT.

(s, 3H, Me-19), 0.64 (s, 3H, Me-18). ^{13}C NMR spectrum, DMSO-d_6, δC, ppm: 200.1 (C-20), 140.8 (C-22), 140.0 (C-5), 133.8 (C_{arom}-1'), 132.1 (C_{arom}-3' + C_{arom}-5'), 129.6 (C_{arom}-2' + C_{arom}-6'), 127.3 (C-21), 124.5 (C_{arom}-4'), 121.4 (C-6), 71.7 (C-3), 57.1 (C-14), 56.9 (C-17), 50.1 (C-9), 45.0 (C-13), 42.3 (C-4), 39.2 (C-12), 37.3 (C-1), 36.5 (C-10), 32.0, 31.8, 31.6 (C-2 + C-7 + C-8), 24.7 (C-15), 22.8 (C-16), 21.1 (C-11), 19.4 (Me-19), 13.5 (Me-18). Found, %: C 69.57; H 7.43. $C_{28}H_{35}BrO_2$. Calculated, %: 69.56, H 7.30. M 483.49.

3-((4-Trifluoromethylphenyl)-1-(preg-5-en-3β-ol-17-yl)prop-2-en-1-one (**XIII**). From 4-trifluoromethylbenzaldehyde (**III**) (94 mg). Yield: 191 mg (72%) as a colorless powder, mp 138–140°C. ^{1}H NMR spectrum DMSO-d_6, δ, ppm: 7.55 (d, 1H, $J_{21,22}$ = 16.0 Hz, H-22), 7.25 (d, 2H, $J_{2',3'}$ = 7.4 Hz, H_{arom}-2' + H_{arom}-6'), 7.17 (d, 2H, $J_{5',6'}$ = 7.2 Hz, H_{arom}-3' + H_{arom}-5'), 6.85 (d, 1H, $J_{21,22}$ = 16.0 Hz, H-21), 5.36 (t, 1H, $J_{6,7}$ = 4.1 Hz, H-6), 5.30 (bs, 1H, OH), 3.53 (m, 1H, H-3), 2.86 (m, 1H, H-17), 2.17 (m, 1H, H-16a), 2.36 (m, 2H, CH_2-4), 2.12 (m, 2H, CH_2-7), 2.00 (m, 1H, H-12a), 1.85 (m, 1H, H-1a), 1.74 (m, 1H, H-2a), 1.64 (m, 1H, H-15a), 1.61 (m, 1H, H-16b), 1.57 (m, 1H, H-11a), 1.55 (m, 1H, H-12b), 1.51–1.44 (m, 3H, H-2b + H-8 + H-11b), 1.28 (m, 2H, H-14 + H-15b), 1.13 (m, 1H, H-1b), 1.10 (m, 1H, H-9), 1.00 (s, 3H, Me-19), 0.65 (s, 3H, Me-18). ^{13}C NMR spectrum, DMSO-d_6, δC, ppm: 200.0 (C-20), 140.8 (C-22), 139.5 (C-5), 138.3 (C_{arom}-1'), 129.0 (C-4'), 128.3 (C_{arom}-3' + C_{arom}-5'), 125.9 (C-21), 125.3 (C_{arom}-2' + C_{arom}-6'), 124.7 (d, $J_{C,F}$ = 235.5 Hz, CF_3), 121.4 (C-6), 71.7 (C-3), 57.2 (C-14), 56.2 (C-17), 50.1 (C-9), 45.1 (C-13), 42.3 (C-4), 39.2 (C-12), 37.3 (C-1), 36.5 (C-10), 32.0, 31.8, 31.6 (C-2 + C-7 + C-8), 24.7 (C-15), 22.7 (C-16), 21.1 (C-11), 19.4 (Me-19), 13.5 (Me-18). Found, %: C 71.19; H 7.51. $C_{29}H_{35}F_3O_2 \cdot H_2O$. Calculated, %: C 71.00; H 7.60. M 490.61.

3-((4-Methylphenyl)-1-(preg-5-en-3β-ol-17-yl)-prop-2-en-1-one (**XIV**). From 4-methylbenza-ldehyde (**IV**) (65 mg). Yield: 198 mg (84%) as a colorless powder, mp 242–245°C [Lit. [11] mp 243–245°C]. IR spectrum, film, ν, cm^{-1}: 3645 (OH), 2932 (CH_2), 1690, 1515 (C=C), 1055 (C-O. ^{1}H NMR spectrum DMSO-d_6, δ, ppm: 7.52 (d, 1H, $J_{21,22}$ = 15.9 Hz, H-22), 7.45 (d, 2H, $J_{2',3'}$ = 8.0 Hz, H_{arom}-2' + H_{arom}-6'), 7.19 (d, 2H, $J_{5',6'}$ = 8.0 Hz, H_{arom}-3' + H_{arom}-5'), 6.74 (d, 1H, $J_{21,22}$ = 15.9 Hz, H-21), 5.35 (t, 1H, $J_{6,7}$ = 4.1 Hz, H-6), 5.29 (bs, 1H, OH), 3.53 (m, 1H, H-3), 2.84 (m,

1H, H-17), 2.17 (m, 1H, H-16a), 2.37 (m, 2H, CH_2-4), 2.12 (s, 3H, C4'-Me), 2.04 (m, 2H, CH_2-7), 2.00 (m, 1H, H-12a), 1.84 (m, 1H, H-1a), 1.72 (m, 1H, H-2a), 1.64 (m, 1H, H-15a), 1.62 (m, 1H, H-16b), 1.53 (m, 2H, H-11a + H-12b), 1.51–1.49 (m, 3H, H-2b + H-8 + H-11b), 1.28 (m, 2H, H-14 + H-15b), 1.13 (m, 1H, H-1b), 1.00 (m, 1H, H-9), 1.01 (s, 3H, Me-19), 0.67 (s, 3H, Me-18). ^{13}C NMR spectrum, DMSO-d_6, δC, ppm: 209.5 (C-20), 141.5 (C-22), 140.8 (C-5), 140.7 (C_{arom}-1'), 132.1 (C4'-*Me*), 129.6 (C_{arom}-3' + C_{arom}-5'), 128.3 (C_{arom}-2' + C_{arom}-6'), 127.0 (C-21), 129.6 (C_{arom}-4'), 121.4 (C-6), 71.7 (C-3), 57.2 (C-14), 56.9 (C-17), 50.0 (C-9), 44.9 (C-13), 42.3 (C-4), 38.9 (C-12), 37.3 (C-1), 36.5 (C-10), 31.8, 31.8, 31.6 (C-2 + C-7 + C-8), 24.5 (C-15), 22.8 (C-16), 21.4 (C-11), 21.1 (C4'-Me), 19.4 (Me-19), 13.2 (Me-18). Found, %: C 79.71; H, 8.98. $C_{29}H_{38}O_2 \cdot H_2O$. Calculated, %: C 79.77; H 9.23. M 436.64.

3-((2-Bromo-2-hydroxylphenyl)-1-(preg-5-en-3β-ol-17-yl)prop-2-en-1-one (**XV**). From 5-bromo-2-hydroxybenzaldehyde (**V**) (108 mg). Yield: 183 mg (68%) as a colorless powder, mp 158–160°C. IR spectrum, film, ν, cm^{-1}: 3645 (OH), 2958 (CH_2), 1687, 1540 (C=C), 1052 (C-O). ^{1}H NMR spectrum DMSO-d_6, δ, ppm: 7.94 (bs, 1H, H_{arom}-6'), 7.78 (d, 1H, $J_{3',4'}$ = 7.7 Hz, H_{arom}-4'), 7.58 (d, 1H, $J_{21,22}$ = 16.1 Hz, H-22), 7.03 (d, 1H, $J_{21,22}$ = 16.1 Hz, H-21), 6.82 (d, 1H, $J_{3',4'}$ = 7.7 Hz, H_{arom}3'), 5.28 (d, 1H, $J_{6,7}$ = 3.8 Hz, H-6), 4.63 (bs, 1H, OH), 3.57 (m, 1H, H-3), 3.04 (m, 1H, H-17), 2.38 (m, 1H, H-16a), 2.36 (m, 2H, CH_2-4), 2.15 (m, 2H, CH_2-7), 1.82 (m, 1H, H-12a), 1.76 (m, 1H, H-2a), 1.66 (m, 1H, H-15a), 1.59 (m, 1H, H-16b), 1.57 (m, 1H, H-11a), 1.54 (m, 1H, H-12b), 1.51 (m, 1H, H-8b), 1.47 (m, 1H, H-11b), 1.44 (m, 1H, H-2b), 1.26 (m, 1H, H-14), 1.24 (m, 1H, H-15b), 1.14 (m, 1H, H-1b), 1.00 (m, 1H, H-9), 0.92 (m, 3H, Me-19), 0.53 (m, 3H, Me-18). ^{13}C NMR spectrum, DMSO-d_6, δC, ppm: 199.8 (C-20), 155.1 (C2'-OH), 141.1 (C-22), 139.0 (C-5), 129.5 (C_{arom}-4'), 128.9 (C_{arom}-6'), 126.7 (C-21), 120.4 (C-6), 118.5 (C_{arom}-1'), 116.7 (C_{arom}-5'), 69.9 (C-3), 56.3 (C-14), 56.0 (C-7), 49.6 (C-9), 44.4 (C-13), 42.1 (C-4), 38.0 (C-12), 36.8 (C-1), 36.1 (C-10), 31.5, 31.4, 31.3 (C-2 + C-7 + C-8), 24.2 (C-15), 22.2 (C-16), 20.5 (C-11), 19.1 (Me-19), 12.1 (Me-18). Found, %: C 67.26; H 7.11. $C_{28}H_{35}BrO_3$. Calculated, %: C 67.33; H 7.00. M 499.47.

3-((2-Chloro-4-)dimethylamino)phenyl)-1-(preg-5-en-3β-ol-17-yl)prop-2-en-1-one (**XVI**). From 2-chloro-4-(*N,N*-dimethylamino)benzaldehyde (**VI**) (99 mg). Yield: 193 mg (74%) as a yellow powder, mp 153–155°C. IR spectrum, film, ν, cm^{-1}: 3664 (OH), 2961 (CH_2), 1679, 1547 (C=C), 1052 (C–O). ^{1}H NMR spectrum DMSO-d_6, δ, ppm: 7.68 (d, 1H, $J_{21,22}$ = 16.0 Hz, H-22), 7.13 (d, 1H, $J_{5',6'}$ = 7.9 Hz, H_{arom}-6'), 6.93 (bs, 1H, H_{arom}-3'), 6.74 (m, 2H, H-21 + H_{arom}-5'), 5.26 (d, 1H, $J_{6,7}$ = 4.1 Hz, H-6), 4.61 (bs, 1H, OH), 3.45 (m, 1H, H-3), 3.03 (m, 1H, H-17), 2.57 (m, 2H, CH_2-4), 2.18 (m, 1H, H-16a), 2.14 (m, 2H, CH_2-7), 2.02 (m, 1H, H-12a), 1.72 (m, 1H, H-2a), 1.67 (m, 1H, H-15a), 1.61 (m, 1H, H-16b), 1.57 (m, 1H, H-11a), 1.54 (m, 1H, H-12b), 1.54 (m, 1H, H-8b), 1.50 (m, 1H, H-11b), 1.47 (m, 1H, H-2b), 1.37 (m, 1H, H-14), 1.34 (m, 1H, H-15b), 1.16 (m, 1H, H-1b), 1.13 (m, 1H, H-15b), 1.03 (m, 1H, H-9), 0.95 (s, 3H, Me-19), 0.54 (s, 3H, Me-18). ^{13}C NMR spectrum, DMSO-d_6, δC, ppm: 203.1 (C-20), 154.4 (*C*4'-NMe_2), 151.1 (C2'-OH), 141.1 (C-22), 139.7 (C-5), 132.8 (C-Cl), 130.5 (C-6'), 125.7 (C-21), 120.9 (C-1'), 119.5 (C-6), 111.1 (C-3'), 110.4 (C-5'), 69.9 (C-3), 56.0 (C-14), 55.0 (C-7), 49.4 (C-9), 43.1 (C-13), 42.1 (C-4), 40.9 (NMe_2), 39.0 (C-12), 36.8 (C-1), 36.0 (C-10), 31.4, 31.2, 31.1 (C-2 + C-7 + C-8), 24.1 (C-15), 22.0 (C-16), 20.9 (C-11), 19.3 (Me-19), 12.9 (Me-18). Found, %: C 75.01; H 8.60; N 2.69. $C_{30}H_{40}ClNO_2$. Calculated, %: C 74.74; H 8.36; N 2.90. M 482.09.

3-(9*H*-Fluoren-2-yl)-1-(preg-5-en-3β-ol-17-yl)-prop-2-en-1-one (**XVII**). From 9*H*-fluorene-2-carboaldehyde (**VII**) (105 mg). Yield: 221 mg (83%) as a yellow crystals, mp 137–140°C. IR spectrum, film, ν, cm^{-1}: 3653 (OH), 2940 (CH_2), 1684, 1520 (C=C), 1057 (C–O). ^{1}H NMR spectrum DMSO-d_6, δ, ppm: 8.13 (d, 1H, $J_{5',6'}$ = 8.0 Hz, H-5'), 8.05 (d, 1H, $J_{4',3'}$ = 7.7 Hz, H-4'), 7.67 (d, 1H, $J_{21,22}$ = 16.0 Hz, H-22), 7.60 (d, 1H, H-1'), 7.53 (d, 1H, $J_{7',8'}$ = 8.0 Hz, H-8'), 7.37 (m, 2H, H-3' + H-7'), 6.76 (d, 1H, $J_{21,22}$ = 16.0 Hz, H-21), 5.34 (t, 1H, $J_{6,7}$ = 4.1 Hz, H-6), 4.44 (m, 2H, OH + H-9'), 3.44 (m, 1H, H-3), 2.85 (m, 1H, H-17), 2.37 (m, 2H, CH_2-4), 2.16 (m, 1H, H-16a), 2.12 (m, 2H, CH_2-7), 2.02 (m, 1H, H-12a), 1.87 (m, 1H, H-1a), 1.77 (m, 1H, H-2a), 1.65 (m, 1H, H-15a), 1.60 (m, 1H, H-16a), 1.59 (m, 1H, H-11a), 1.57 (m, 1H, H-12b), 1.52 (m, 1H, H-8), 1.43 (m, 1H, H-11b), 1.41 (m, 1H, H-2b), 1.28 (m, 1H, H-14), 1.27 (m, 1H, H-15b), 1.16 (m, 1H, H-1b), 1.09 (m, 1H, H-9), 0.97 (s, 3H, Me-19), 0.54 (s, 3H, Me-18). ^{13}C NMR spectrum, DMSO-d_6, δC, ppm: 208.4 (C=O), 143.5 (C-8a'), 143.1 (C-9a'), 141.4 (C-22), 140.7 (C-5a'), 139.4 (C-4a'), 133.5 (C-2'), 128.1 (C-4'), 127.3 (C-7'), 126.0 (C-6'), 125.7 (C-21+C-8'), 125.3 (C-1'), 123.2 (C-3'), 121.8 (C-5'), 73.1 (C-3), 55.9 (C-14), 55.0 (C-7), 49.2 (C-9), 43.1 (C-13), 40.6 (C-4), 38.8 (C-12), 37.5 (C-9'), 36.4 (C-1), 36.0 (C-10), 31.2, 31.1 (C-2 + C-7+C-8), 23.9 (C-15), 22.1 (C-16), 20.9 (C-11), 18.8 (Me-19), 12.8 (Me-18). (Me-18). Found, %: C 84.86; H 7.91. $C_{35}H_{40}O_2$. Calculated, %: C 85.01; H 8.02. M 492.69.

3-(Furan-2-yl)-1-(preg-5-en-3β-ol-17-yl)prop-2-en-1-one (**XVIII**). From furan-2-carboaldehyde (**VIII**) (52 mg). Yield: 165 mg (74%) as a brown powder, mp 112–114°C [Lit. [11] mp 111–113°C]. IR spectrum, film, ν, cm^{-1}: 3645 (OH), 2955 (CH_2), 1679, 1530 (C=C), 1062 (C–O). ^{1}H NMR spectrum

DMSO-d_6, δ, ppm: 7.85 (d, 1H, J = 1.8 Hz, H_{furan}-5'), 7.33 (d, 1H, $J_{21,22}$ = 15.8 Hz, H-22), 6.96 (d, 1H, J = 3.4 Hz, H_{furan}-3'), 6.64 (dd, 1H, $J_{4',3'}$ = 1.8, 3.4 Hz, H_{furan}-4'), 6.59 (d,1H, $J_{21,22}$ = 15.8 Hz, H-21), 5.27 (t, 1H, $J_{6,7}$ = 5.0 Hz, H-6), 4.62 (d, 1H, OH), 3.53 (m, 1H, H-3), 2.92 (m, 1H, H-17), 2.38 (m, 2H, CH_2-4), 2.18 (m, 1H, H-16a), 2.16 (m, 1H, H-7a), 2.10 (m, 1H, H-17b), 1.95 (m, 1H, H-12a), 1.83 (m, 1H, H-1a), 1.76 (m, 1H, H-2a), 1.68 (m, 1H, H-15a), 1.60 (m, 1H, H-16b), 1.57 (m, 1H, H-11a), 1.56 (m, 1H, H-12b), 1.54-1.51 (m, 2H, H-8 + H-11b), 1.42 (m, 1H, H-2b), 1.29-1.28 (m, 2H, H-14 + H-15b), 1.15 (m, 1H, H-1b), 1.10 (m, 1H, H-9), 0.97 (s, 3H, Me-19), 0.51 (s, 3H, Me-18). ^{13}C NMR spectrum, DMSO-d_6, δC, ppm: 209.0 (C=O), 151.2 (C_{furan}-2'), 146.4 (C_{furan}-5'), 141.5 (C-5), 128.1 (C-21), 124.2 (C-22), 120.8 (C-6), 117.1 (C_{furan}-3'), 113.4 (C_{furan}-4'), 70.5 (C-3), 56.7 (C-14), 56.0 (C-7), 50.1 (C-9), 44.8 (C-13), 42.7 (C-4), 38.6 (C-12), 37.4 (C-1), 36.6 (C-10), 32.0, 31.9, 31.7 (C-2 + C-7 + C-8), 24.5 (C-15), 22.7 (C-16), 21.0 (C-11), 19.6 (Me-19), 13.8 (Me-18). Found, %: 75.46; H 8.99. $C_{26}H_{34}O_3 \cdot H_2O$. Calculated, %: C 75.69; H 8.80. M 412.57.

3-(Thiophene-2-yl)-1-(preg-5-en-3β-ol-17-yl)-prop-2-en-1-one (**XIX**). From thiophen-2-carboaldehyde (**IX**) (61 mg). Yield: 175 mg (79%) as a colorless powder, mp 121–123°C. IR spectrum, film, ν, cm^{-1}: 3652 (OH), 2958 (CH_2), 1675, 1534 (C=C), 1066 (C–O). ^{1}H NMR spectrum DMSO-d_6, δ, ppm: 7.71 (d, 1H, J = 5.2 Hz, $H_{thiophen}$-5'), 7.67 (d, 1H, $J_{21,22}$ = 15.8 Hz, H-22), 7.54 (d, 1H, J = 3.6 Hz, $H_{thiophen}$-3'), 7.15 (dd, 1H, $J_{4',3'}$ = 3.6, 5.2 Hz, $H_{thiophen}$-4'), 6.57 (d, 1H, $J_{21,22}$ = 15.8 Hz, 21-H), 5.27 (t, 1H, $J_{6,7}$ = 5.2 Hz, 6-H), 4.58 (d, 1H, OH), 3.44 (m, 1H, 3-H), 2.95 (m, 1H, H-17), 2.33 (m, 2H, CH_2-4), 2.18 (m, 1H, H-16a), 2.13 (m, 1H, H-7a), 2.08 (m, 1H, H-7b), 1.93 (m, 1H, H-12a), 1.83 (m, 1H, H-1a), 1.76 (m, 1H, H-2a), 1.68 (m, 1H, H-15a), 1.59 (m, 1H, H-16b), 1.56 (m, 2H, H-11a + H-12b), 1.54–1.52 (m, 2H, H-8 + H-11b), 1.41 (m, 1H, H-2b), 1.30 (m, 1H, H-14), 1.27 (m, 1H, H-15b), 1.16 (m, 1H, H-1b), 1.10 (m, 1H, H-9), 0.99 (s, 3H, Me-19), 0.52 (s, 3H, Me-18). ^{13}C NMR spectrum, DMSO-d_6, δC, ppm: 198.9 (C=O), 141.2 (C-5), 139.4 ($C_{thiophen}$-2'), 133.7 (C-21), 132.4 (C-22), 120.1 (C-6), 125.5 ($C_{thiophen}$-5'), 128.5 ($C_{thiophen}$-3'), 125.5 ($C_{thiophen}$-4'), 69.8 (C-3), 56.7 (C-14), 56.1 (C-7), 49.5 (C-9), 44.2 (C-13), 42.1 (C-4), 37.9 (C-12), 36.8 (C-1), 36.0 (C-10), 31.5 (C-2), 31.3 (C-7), 31.2 (C-8), 24.1 (C-15), 22.1 (C-16), 21.5 (C-11), 19.0 (Me-19), 13.1 (Me-18). Found, %: C 75.82, H 8.20. $C_{26}H_{34}O_2S$. Calculated, %: C 76.05; H 8.35. M 410.61.

17-((1-(2-Carboxyphenyl)prop-1-en-2-yl)-preg-5-en-3β-ol (**XX**). From 2-formylbenzoic acid (**X**) (81 mg). Yield: 196 mg (81%) as a colorless powder, mp 145–147°C. IR spectrum, film, ν, cm^{-1}: 3652 (OH), 2938 (CH_2), 1691, 1535 (C=C), 1061 (C–O). ^{1}H NMR spectrum DMSO-d_6, δ, ppm: 10.60 (bs, 1H, CO_2H), 8.12 (d, 1H, J = 7.8 Hz, H_{arom}-3'), 7.91 (d, 1H, $J_{21,22}$ = 16.0 Hz, H-22), 7.65 (m, 2H, H_{arom}-4' + H_{arom}-6'), 7.83 (m, 1H, 5'-H_{arom}-5'), 6.79 (d, 1H, $J_{21,22}$ = 16.0 Hz, H-21), 5.28 (t, 1H, $J_{6,7}$ = 4.1 Hz, H-6), 4.59 (bs, 1H, OH), 3.44 (m, 1H, H-3), 3.25 (m, 1H, H-17), 2.56 (m, 2H, CH_2-4), 2.17 (m, 1H, H-16a), 2.06 (m, 2H, CH_2-7), 2.00 (m, 1H, H-12a), 1.91 (m, 1H, H-1a), 1.77 (m, 1H, H-2a), 1.66 (m, 1H, H-15a), 1.62 (m, 1H, H-16b), 1.59 (m, 1H, H-11a), 1.56 (m, 1H, H-12b), 1.53-1.40 (m, 3H, H-2b + H-8 + H-11b), 1.37–1.34 (m, 2H, H-14 + H-15b), 1.15 (m, 1H, H-1b), 1.03 (m, 1H, H-9), 1.00 (s, 3H, Me-19), 0.53 (s, 3H, Me-18). ^{13}C NMR spectrum, DMSO-d_6, δC, ppm: 208.4 (C-20), 167.0 (CO_2H), 141.8 (C-22), 141.2 (C-5), 137.8 (C_{arom}-1'), 132.3 (C_{arom}-5'), 128.3 (C_{arom}-3' + C_{arom}-6'), 127.7 (C_{arom}-4'), 120.1 (C-6), 128.3 (C_{arom}-2' + C_{arom}-6'), 125.9 (C-21), 125.3 (C_{arom}-4'), 120.1 (C-6), 69.9 (C-3), 56.4 (C-14), 56.0 (C-7), 49.4 (C-9), 43.1 (C-13), 42.1 (C-4), 39.2 (C-12), 38.8 (C-1), 36.8 (C-10), 31.1, 31.3, 31.4 (C-2 + C-7 + C-8), 23.9 (C-15), 22.1 (C-16), 20.5 (C-11), 19.0 (Me-19), 12.8 (Me-18). Found, %: C 69.57; H 7.43. $C_{29}H_{36}O_4$. Calculated, %: C 77.65, H 8.08. M 448.59.

3,3'-(1,4-Phenylene)-bis(preg-5-en-3β-ol-17-yl)-prop-2-en-1-one (**XXI**). From terephthal-aldehyde (**XI**) (73 mg) and **1** (387 mg, 1.08 mmol, 2.0 equiv). Yield: 299 mg (76%) as a colorless powder, mp 147–149°C. IR spectrum, film, ν, cm^{-1}: 3657 (OH), 2974 (CH_2), 1674, 1535 (C=C), 1069 (C–O). ^{1}H NMR spectrum DMSO-d_6, δ, ppm: 7.94 (bs, 4H, H_{arom}), 7.58 (d, 2H, $J_{21,22}$ = 16.2 Hz, 2 × H-22), 7.06 (d, 2H, $J_{21,22}$ = 16.1 Hz, 2 H-21), 5.27 (t, 2H, $J_{6,7}$ = 4.0 Hz, 2 × H-6), 4.57 (d, 2H, 2 × OH), 3.28 (m, 2H, 2 × H-3), 3.21 (m, 2H, 2 × H-17), 2.33 (m, 4H, 2 × CH_2-4), 2.19 (m, 2H, 2 × H-16a), 2.11 (m, 2H, 2 × H-7a), 2.08 (m, 2H, 2 × H-7b), 1.99 (m, 2H, 2 × H-12a), 1.85 (m, 2H, 2 × H-1a), 1.78 (m, 2H, 2 × H-2a), 1.68 (m, 2H, 2 × H-15a), 1.60 (m, 2H, 2 × H-16b), 1.57 (m, 4H, 2 × H-11a + 2 × H-12b), 1.56–1.54 (m, 4H, 2 × H-8 + 2 × H-11b), 1.42 (m, 2H, 2 × H-2b), 1.30 (m, 2H, 2 × H-14), 1.27 (m, 2H, 2 × H-15b), 1.18 (m, 2H, 2 × H-1b), 1.11 (m, 2H, 2 × H-9), 0.93 (s, 6H, 2 × Me-19), 0.54 (s, 6H, 2 × Me-18). ^{13}C NMR spectrum, DMSO-d_6, δC, ppm: 202.4 (C=O), 141.8 (C-22 + C-22'), 140.9 (C-5 + C-5'), 133.8 (C_{arom}-1'' + C_{arom}-4''), 129.3 (C_{arom}-3''), 129.2 (C_{arom}-2'' + C_{arom}-6''), 126.9 (C-21 + C-21'), 120.3 (C-6 + C-6'), 70.7 (2 × C-3), 56.9 (C-14 + C-14'), 56.7 (C-7 + C-7'), 50.1 (C-9 + C-9'), 44.4 (C-13 + C-13'), 42.0 (C-4 + C-4'), 38.2 (C-12 + 12'), 37.2 (C-1 + C-1'), 36.0 (C-10 + 10'), 31.5 (C-2 + C-2'), 31.2 (C-7 + C-7'), 30.6 (C-8 + C-8'), 24.6 (C-15 + 15'), 22.6 (C-16 + C-16'), 21.7 (C-11 + 11'), 19.3 (Me-19 + Me-19'), 13.3 (Me-18 + Me-18'). Found, %: C 78.55; H 8.98. $C_{50}H_{64}O_4 \cdot 2H_2O$. Calculated, %: C 78.29; H, 9.20. M 729.04.

General procedure for preparation of 5-(substituted phenyl)-3-(5-preg-5-3β-ol-17-yl)-4,5-dihydro-1*H*-pyrazole-1-carbothioamides (XXII)-(XXIV). To a stirred solution of (**XII**)–(**XIV**) (1.00 mmol) in EtOH (10 mL) was added thiosemicarbazide (127 mg, 1.40 mmol) and K_2CO_3 (100 mg) and the reaction mixture was heated under reflux for 5 h. After reaction completion (tlc), the solution was evaporated to dryness and the residue was partitioned between $CHCl_3$ (3 × 20 mL) and brine solution (30 mL). The combined organic layers were dried (Na_2SO_4), filtered and evaporated to dryness. The residue was purified on a short column of SiO_2 (5 g) using EtOAc-hexane (3 : 2) as eluent to give the desired pyrazoline derivatives.

5-(4-Bromophenyl)-3-(preg-5-en-3β-ol-17-yl)-4,5-dihydro-1*H*-pyrazoline-1-carbothio-amide (XXII). From (**XII**) (484 mg). Yield: 378 mg (68%) as orange crystals, mp 149–151°C. IR spectrum, film, ν, cm^{-1}: 3655 (OH), 3357 (NH_2), 2968 (CH_2), 1670, 1541 (C=C), 1058 (C–O). ^{1}H NMR spectrum DMSO-d_6, δ, ppm: 9.78 (bs, 2H, NH_2), 7.35 (d, 2H, J = 8.0 Hz, H_{arom}-3' + H_{arom}-5'), 7.02 (d, 2H, J = 8.0 Hz, 2'-H_{arom}-2' + H_{arom}-6'), 5.27 (t, 1H, J = 5.4 Hz, H-6), 4.57 (m, 1H, 5-$H_{pyrazoline}$-5), 3.59 (m, 1H, H-3), 3.21 (m, 1H, H-17), 3.16 (m, 2H, CH_2-4$_{pyrazoline}$), 2.33 (m, 1H, CH_2-4), 2.19 (m, 1H, H-16a), 2.13 (m, 2H, CH_2-7), 2.07 (m, 1H, H-12a), 1.92 (m, 1H, H-1a), 1.77 (m, 1H, H-2a), 1.69 (m, 1H, H-15a), 1.65 (m, 1H, H-16b), 1.61 (m, 1H, H-11a), 1.58 (m, 1H, H-12b), 1.53 (m, 1H, H-8), 1.49 (m, 1H, H-11b), 1.37 (m, 1H, H-2b), 1.25 (m, 2H, H-14 + 15b-H), 1.22 (m, 1H, H-9), 1.14 (m, 1H, H-1b), 0.94 (s, 3H, Me-19), 0.53 (s, 3H, Me-18). ^{13}C NMR spectrum, DMSO-d_6, δ*C*, ppm: 175.0 (C=S), 161.6 ($C_{pyrazoline}$-3), 141.4 (C_{arom}-1'), 131.1 (C_{arom}-3' + C_{arom}-5'), 127.7 (C_{arom}-2' + C_{arom}-6'), 125.3 (C-6), 119.4 (C-Br), 71.8 (C-3), 69.7 ($C_{pyrazoline}$-5), 62.6 (C-17), 56.7 (C-14), 49.9 (C-9), 45.1 (C-13), 42.3 (C-4), 38.8 (C-12), 36.8 (C-1), 37.8 (C-10), 36.8 ($C_{pyrazoline}$-4 + C-1), 32.4 (C-7), 31.8 (C-2), 31.6 (C-8), 23.9 (C-15), 21.0 (C-11), 19.0 (Me-19), 12.8 (Me-18). Found, %: C 62.39; H 6.71; N 7.32. $C_{29}H_{38}BrN_3OS$. Calculated, %: C 62.58; H 6.88; N 7.55. M 555.61.

5-(4-Trifluoromethylphenyl)-3-(preg-5-en-3β-ol-17-yl)-4,5-dihydro-1*H*-pyrazoline-1-carbo-thioamide (XXIII). From (**XII**) (491 mg). Yield: 387 mg (71%) as a yellow powder, mp 137–141°C. IR spectrum, film, ν, cm^{-1}: 3663 (OH), 3367 (NH_2), 2962 (CH_2), 1665, 1537 (C=C), 1059 (C–O). ^{1}H NMR spectrum DMSO-d_6, δ, ppm: 8.10 (bs, 2H, NH_2), 7.35 (d, 2H, J = 8.0 Hz, H_{arom}3' + H_{arom}-5'), 7.28 (d, 2H, J = 8.0 Hz, H_{arom}-2' + H_{arom}-6'), 5.82 (s, 1H, OH), 5.26 (t, 1H, J = 4.6 Hz, H-6), 4.58 (m, 1H, $H_{pyrazoline}$-5), 3.22 (m, 1H, H-3), 2.91 (m, 3H, CH_2-4$_{pyrazoline}$ + H-17), 2.33 (m, 2H, CH_2-4), 2.17 (m, 2H, H-16a), 2.13 (m, 2H, CH_2-7), 2.08 (m, 1H, H-12a), 1.86 (m, 1H, H-1a), 1.76 (m, 1H, H-2a), 1.69 (m, 1H, H-15a), 1.66 (m, 1H, H-16b), 1.60 (m, 1H, H-11a), 1.56 (m, 1H, H-12b), 1.54 (m, 1H, H-8), 1.49 (m, 1H, H-11b), 1.43 (m, 1H, H-2b), 1.31 (m, 1H, H-14), 1.26 (m, 1H, H-15b), 1.18 (m, 1H, H-1b), 1.13 (m, 1H, H-9), 0.93 (s, 3H, Me-19), 0.53 (s, 3H, Me-18). ^{13}C NMR spectrum, DMSO-d_6, δC, ppm: 174.6 (C=S), 166.6 ($C_{pyrazoline}$-3), 141.2 (C_{arom}-1'), 127.6 (C_{arom}-4'), 125.6 (d, $J_{C,F}$ = 240 Hz, CF_3), 125.3 (C_{arom}-3' + C_{arom}-5'), 123.8 (C_{arom}-2' + C_{arom}-6'), 120.1 (C-6), 71.0 (C-3), 71.0 (C-3), 69.9 ($C_{pyrazoline}$-5), 62.5 (C-17), 56.0 (C-14), 49.4 (C-9), 43.1 (C-13), 42.1 (C-4), 38.6 (C-12), 36.8 ($C_{pyrazoline}$-4 + C-1), 36.0 (C-10), 31.3–31.1 (C-2 + C-7 + C-8), 23.9 (C-15), 22.1 (C-16), 20.9 (C-11), 19.1 (Me-19), 12.8 (Me-18). Found, %: C 65.84, H 6.89, N 7.52. $C_{29}H_{38}F_3N_3OS$. Calculated, %: C 66.03, H 7.02, N 7.70. M 545.71.

5-(4-Methylphenyl)-3-(preg-5-en-3β-ol-17-yl)-4,5-dihydro-1*H*-pyrazoline-1-carbo-thioamide (XXIV). From (**XIV**) (437 mg). Yield: 329 mg (67%) as a yellow powder, mp 189–191°C. IR spectrum, film, ν, cm^{-1}: 3655 (OH), 3361 (NH_2), 2957 (CH_2), 1660, 1542 (C=C), 1052 (C–O). ^{1}H NMR spectrum DMSO-d_6, δ, ppm: 8.04 (bs, 2H, NH_2), 7.67 (d, 2H, J = 8.3 Hz, H_{arom}-3' + H_{arom}-5'), 7.21 (d, 2H, J = 8.2 Hz, H_{arom}-2' + H_{arom}-6'), 5.67 (s, 1H, OH), 5.27 (t, 1H, J = 4.5 Hz, H-6), 4.56 (m, 1H, $H_{pyrazoline}$-5), 3.23 (m, 1H, H-3), 2.98 (m, 3H, CH_2-4$_{pyrazoline}$ + H-17), 2.32 (m, 2H, CH_2-4), 2.17 (m, 1H, H-16a), 2.13 (m, 2H, CH_2-7), 2.07 (m, 1H, H-14), 2.05 (s, 3H, Ar–*CH_3*), 1.99 (m, 1H, H-12a), 1.90 (m, 1H, H-1a), 1.77 (m, 1H, H-2a), 1.69 (m, 1H, H-15a), 1.66 (m, 1H, H-15b), 1.60 (m, 1H, H-11a), 1.56 (m, 1H, H-12b), 1.54 (m, 1H, H-8), 1.49 (m, 1H, H-11b), 1.42 (m, 1H, H-2b), 1.29 (m, 1H, H-14), 1.25 (m, 1H, H-15b), 1.17 (m, 1H, H-1b), 1.13 (m, 1H, H-9), 0.94 (s, 3H, Me-19), 0.54 (s, 3H, Me-18). ^{13}C NMR spectrum, DMSO-d_6, δ*C*, ppm: 174.7 (C=S), 159.9 ($C_{pyrazoline}$-3), 141.2 (C_{arom}-1'), 135.7 (C_{arom}-4'), 129.1 (C_{arom}-2' + C_{arom}-6'), 127.1 (C_{arom}-3' + C_{arom}-5'), 120.1 (C-6), 71.4 (C-3), 69.6 ($C_{pyrazoline}$-5), 62.6 (C-17), 55.7 (C-14), 49.6 (C-9), 43.4 (C-13), 42.1 (C-4), 38.1 (C-12), 36.8 ($C_{pyrazoline}$-4 + C-1), 36.0 (C-10), 31.6, 31.3, 31.1 (C-2 + C-7 + C-8), 23.7 (C-15), 22.1 (C-16), 20.9 (C-11), 20.6 (Ar–*CH_3*), 19.0 (Me-19), 12.8 (Me-18). Found, %: C 73.01; H 8.30; N 8.39. $C_{30}H_{41}N_3OS$. Calculated, %: C 73.28; H 8.40; N 8.55. M 491.73.

1-*O*-Acetyl-(5-(4-bromophenyl)-3-(preg-5-en-3β-ol-17-yl)-4,5-dihydro-1H-pyrazoline (XXV). A mixture of **12** (**XII**) (101 mg, 0.21 mmol) and hydrazine hydrate (4 equiv, 1.20 mmol) containing HOAc (0.1 mL) was stirred at room temperature for 20 h. After reaction completion (tlc), the mixture was poured into ice water, and the product was extracted with CH_2Cl_2 (3 × 15 mL). The combined organic layer was evaporated to dryness. The residue was purified by recrystallization from EtOH to give (**XXV**) (71 mg, 63%)

as a pale yellow powder, mp 294–297°C (dec.). IR spectrum, film, ν, cm^{-1}: 3640 (OH), 2965 (CH_2), 1723, 1653 (C=O), 1540 (C=C). ^1H NMR spectrum DMSO-d_6, δ, ppm: 7.86 (d, 2H, J = 8.5 Hz, H_{arom}-3' + H_{arom}-5'), 7.70 (d, 2H, J = 8.5 Hz, H_{arom}-2' + H_{arom}-6'), 5.34 (s, 1H, OH), 5.27 (d, 1H, J = 3.0 Hz, H-6), 4.57 (m, 1H, $H_{pyrazoline}$-5), 3.19 (m, 1H, H-3), 2.93 (m, 3H, $H_{pyrazoline}$-4 + H-17), 2.27 (m, 2H, CH_2-4), 2.17 (m, 1H, H-16a), 2.17 (m, 1H, H-7a), 2.14 (m, 1H, H-7b), 2.03 (m, 1H, H-12a), 1.90 (m, 1H, H-1a), 1.83 (s, 3H, $COCH_3$), 1.78 (m, 1H, H-2a), 1.69 (m, 1H, H-15a), 1.67 (m, 1H, H-16b), 1.60 (m, 1H, H-11a), 1.56 (m, 1H, H-12b), 1.54 (m, 1H, H-8), 1.49 (m, 1H, H-11b), 1.42 (m, 1H, H-2b), 1.31 (m, 1H, H-14), 1.23 (m, 1H, H-15b), 1.17 (m, 1H, H-1b), 1.14 (m, 1H, H-9), 0.94 (s, 3H, Me-19), 0.55 (s, 3H, Me-18). ^{13}C NMR spectrum, DMSO-d_6, δ*C*, ppm: 167.8 (*CO*Me), 158.9 ($C_{pyrazoline}$-3), 141.2 (C_{arom}-1'), 131.5 (C_{arom}-3' + C_{arom}-5'), 126.7 (C_{arom}-2' + C_{arom}-6'), 121.5 (C–Br), 120.2 (C-6), 70.7 (C-3), 64.8 ($C_{pyrazoline}$-5), 62.5 (C-17), 55.5 (C-14), 49.6 (C-9), 43.2 (C-13), 42.1 (C-4), 38.2 (C-12), 36.8 ($C_{pyrazoline}$-4 + C-1), 36.0 (C-10), 31.5, 31.3, 31.1 (C-2 + C-7 + C-8), 23.7 (C-15), 22.9 (C-16), 22.1 ($CO\mathit{CH_3}$), 20.9 (C-11), 19.0 (Me-19), 12.8 (Me-18). Found, %: C 66.52; H 7.15; N 6.08. $C_{30}H_{38}O_2N_2Br$. Calculated, %: C 66.78; H 7.29; N 5.19. M 538.53.

3-(4-Bromophenyl)-1-(preg-5-en-3β-ol-17-yl)-prop-2-en-1-one oxime (XXVI). To a solution of (**XII**) (101 mg, 0.21 mmol) in dry pyridine (5 mL) was added NH_2OH (48 mg, 0.70 mmol) and the mixture was stirred at room temperature for 22 h. The reaction mixture was partitioned between 10% eq. HCl (10 mL) and CH_2Cl_2 (3 × 15 mL). The combined organic layers were washed with brine (3 × 10 mL), then dried (Na_2SO_4) and filtered. The organic extract was evaporated to dryness and the residue was recrystallized from EtOH to give (**XXVI**) (70 mg, 67%) as a yellow powder, mp 127–130°C. IR spectrum, film, ν, cm^{-1}: 3574 (OH), 2972 (CH_2), 1722, 1635 (C=O), 1543 (C=C), 1064 (C-O). ^1H NMR spectrum DMSO-d_6, δ, ppm: 11.01 (bs, 1H, N–OH (Z)), 10.32 (bs, 1H, N–OH (E)), 7.55 (d, 2H, J = 8.5 Hz, H_{arom}-3' + H_{arom}-5'), 7.50 (d, 2H, J = 8.1 Hz, H_{arom}-2' + H_{arom}-6'), 7.41 (d, 1H, J = 17.0 Hz, H-22), 7.03 (d, 1H, J = 17.0 Hz, H-21), 5.68 (s, 1H, OH), 5.27 (d, 1H, H-6), 3.23 (m, 1H, H-3), 2.93 (m, 1H, H-17), 2.32 (m, 2H, CH_2-4), 2.17 (m, 1H, H-16a), 2.14 (m, 1H, H-7a), 2.11 (m, 2H, H-7b), 1.96 (m, 1H, H-12a), 1.91 (m, 1H, H-1a), 1.76 (m, 1H, H-2a), 1.69 (m, 1H, H-15a), 1.66 (m, 1H, H-16b), 1.59 (m, 1H, H-11a), 1.56 (m, 1H, H-12b), 1.53 (m, 1H, H-8), 1.49 (m, 1H, H-11b), 1.44 (m, 1H, H-2b), 1.27 (m, 1H, H-14), 1.24 (m, 1H, H-15b), 1.18 (m, 1H, H-1b), 1.14 (m, 1H, H-9), 0.93, 0.92 (s, 3H, Me-19, *Z*/*E*), 0.59, 0.55 (s, 3H, Me-18, *Z*/*E*). ^{13}C NMR spectrum, DMSO-d_6, δC, ppm: 154.9, 152.5 (*C*=N–OH, *Z*/*E*), 141.2, 140.0 (C-22, *Z*/*E*), 140.3 (C-5), 136.0, 135.8 (C_{arom}-1', *Z*/*E*), 131.5 (C_{arom}-3' + C_{arom}-5'), 128.7 (C_{arom}-2' + C_{arom}-6'), 128.1, 127.9 (C-21, *Z*/*E*), 123.8 (C–Br), 120.2, 120.1 (C-6, *Z*/*E*)), 70.7 (C-3), 56.0 (C-14), 55.4 (C-7), 49.6 (C-9), 45.8 (C-13), 42.1 (C-4), 38.3 (C-12), 36.8 (C-1), 36.0 (C-10), 31.5, 31.3, 31.2 (C-2 + C-7 + C-8), 23.7 (C-15), 22.5 (C-16), 22.6 (C-11), 19.0 (Me-19, *Z*/*E*), 13.3, 12.9 (Me-18, *Z*/*E*). Found, %: C 67.01; H 7.39; N 2.57. $C_{28}H_{37}BrNO_2$. Calculated, %: C 67.33; H 7.47; N 2.80. M 499.51.

Biology

In vitro anti-HIV assay. Evaluation of the antiviral activity of **12-26** (**XII**)–(**XXVI**) against the HIV-1 strain (III_B) and the HIV-2 strain (ROD) in MT-4 cells was performed using an MTT assay as described previously [31]. In brief, stock solutions (10-times final concentration) of test compounds were added in 25-μL volumes to two series of triplicate wells to allow simultaneous evaluation of their effects on mock and HIV-infected cells at the beginning of each experiment. Serial five-fold dilutions of tested compounds (0.0002–125.0 μM) were made directly in flat-bottomed 96-well microtiter trays using a Biomek 3000 robot (Beckman instruments). Untreated control, HIV- and mock-infected cell samples were included for each sample. HIV-1 (III_B) [42] or HIV-2 (ROD) [43] stock (50 μL) at 100–300 CCID50 (50% cell culture infectious dose) or culture medium was added to either of the infected or mock-infected wells of the microtiter tray. Mock-infected cells were used to evaluate the effect of test compound on uninfected cells in order to assess the cytotoxicity of the test compounds. Exponentially growing MT-4 cells [44] were centrifuged for 5 min at 1000 rpm (Minifuge T, rotor 2250; Heraeus, Germany), and the supernatant was discarded. The MT-4 cells were resuspended at 6 × 10^5 cells per mL, and volumes of 50 μL were transferred to the microtiter tray wells. Five days after infection, the viability of the mock- and HIV-infected cells was examined spectrophotometrically.

CONCLUSION

A novel series of pregnenolone derivatives (**XII**)–(**XXVI**) derivatives have been successfully synthesized, and assayed for their inhibitory activity against HIV-1 and HIV-2 in MT-4 cells. One of these analogues (steroid (**XIX**)) exhibited activity against HIV-2 (IC_{50} value of 60.50 μM, SI = 2), which suggested to be a new candidate as nonnucleoside reverse transcriptase inhibitor. Furthermore, compound (**XXV**) showed a cytotoxicity against MT-4 with CC_{50} > 0.29 μM (SI < 1). The computational study of compounds (**XVII**), (**XIX**), (**XXIII**) and (**XXV**) has been performed and the results indicated that *trans* configuration of these analogues are the more stable. Modeling calculations of

(**XII**)–(**XXVI**) have given significant information during the QSAR study to build a strategy for improving the biological activity of the new synthesized steroids. In addition, the docking study of compound (**XXV**) exhibited a hydrogen bond of OH at C-3 with Glu224, in addition to a CH- hydrophobic interaction of aromatic ring of (**XXV**) and Val106 residue of HIV RT.

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COMPLIANCE WITH ETHICAL STANDARDS

This article does not contain any studies involving human participants performed by any of the authors and does not contain any studies involving animals performed by any of the authors.

Conflict of Interests

The authors declare that they have no conflicts of interest.

SUPPLEMENTARY MATERIALS

Supplementary Material is available electronically from https://al-masoudi.de/wp-content/uploads/2020/05/Suppl. Mat_.Russ_.J.Bioorg.Chem_.pdf or from the corresponding author on request.

Supplementary materials are available for this article at https://doi.org/10.1134/S1068162020050039 and are accessible for authorized users.

REFERENCES

1. Ambrosy, A.P., Butler, J., Ahmed, A., Vaduganathan, M., van Veldhuisen, D.J., Colucci,W.S., and Gheorghiade, M., *J. Am. Coll. Cardiol.*, 2014, vol. 63, pp. 1823–1832. https://doi.org/10.1016/j.jacc.2014.01.051
2. Latham, K.A., Zamora, A., Drought, H., Subramanian, S., Matejuk, A., Offner, H., and Rosloniec, E.F., *J. Immunol.*, 2003, vol. 171, pp. 5820–5827. https://doi.org/10.4049/jimmunol.171.11.5820
3. Dietrich, J., Rao, K., S. Pastorino, and Kesari, S., *Expert. Rev. Clin. Pharmacol.*, 2011, vol. 4, pp. 233–242. https://doi.org/10.1586/ecp.11.1
4. Rassokhina, I.V., Volkova, Y.A., Kozlov, A.S., Scherbakov, A.M., Andreeva, O.M., Shirinian, V.Z., and Zavarzin, I.V., *Steroids*, 2016, vol. 113, pp. 29–37. https://doi.org/10.1016/j.steroids. 2016.06.001
5. Spadari, A., Romagnoli, N., Predieri, P.G., Borghetti, P., Cantoni, A.M., Corradi, A., *Res. Veter. Sci.*, 2013, vol. 94, pp. 379–387. https://doi.org/10.1016/j.rvsc.2012.11.020
6. Moreira, V.M., Vasaitis, T.S., Njar, V., and Salvador, J.A.R., *Steroids*, 2007, vol. 72, pp. 939–948. https://doi.org/10.1016/j.steroids.2007.08.004
7. Banday, A.H., Shameem, S.A., Gupta, B.D., and Kumar, H.M.S., *Steroids*, 2010, vol. 75, pp. 801–804. https://doi.org/10.1016/j.steroids.2010.02.015
8. de Bono, J.S., Logothetis, C.J., Molina, A., Fizazi, K., North, S., Chu, L., Chi, K.N., Jones, R.J., and Goodman, O.B., Saad, F., Staffurth, J.N., Mainwaring, P., Harland, S., Flaig, T.W., Huston, T.E., Cheg, T., Patterson, H., Hainsworth, J.D., Ryan, C.J., Sternberg, C.N., Ellard, S.L., Fléchon, A., Saleh, M., Scholz, M., Efstathiou, E., Zivi, A., Bianchini, D., Loriot, Y., Chieffo, N., Kheoh, N., Haqq, C.M., and Scher, H., *N. Engl. J. Med.*, 2001, vol. 364, pp. 1995–2005. https://doi.org/10.1056/NEJMoa1014618
9. Bryce, A. and Ryan, C.J., *Clin. Pharmacol. Ther.*, 2012, vol. 91, pp. 101–108. https://doi.org/10.1038/clpt.2011.275
10. Handratta, V.D., Vasaitis, T.S., Njar, V.C., Gediya, L.K., Kataria, R., Chopra, P., Newman, D., Farquhar, R., Guo, Z., Qiu, Y., and Brodie, A.M., *J. Med. Chem.*, 2005, vol. 48, pp. 2972–2984. https://doi.org/10.1021/jm040202w
11. Banday, A.H., Zargar, and M.I., Ganaie, B.A., *Steroids*, 2011, vol. 76, pp. 1358–1362. https://doi.org/10.1016/j. steroids.2011.07.001
12. Lone, I.H., Khan, K.Z., Fozdar, B.I., and Hussain, F., *Steroids*, 2013, vol. 78, pp. 945–950. https://doi.org/10.1016/j.steroids.2013.05.015
13. Banday, A.H., Akram, S.S.M., and Shameem, S.A., *Steroids*, 2014, vol. 84, pp. 64–69. https://doi.org/10.1016/ j.steroids.2014.03.010
14. Sisodia, B.S., Negi, S.A., Darokar, M.P., Dwivedi, U.N., and Khanuja, S.P., *Chem. Biol. Drug Des.*, 2012, vol. 79, pp. 610–615. https://doi.org/10.1111/j.1747-0285.2012.01323.x
15. Banday, A.H., Shameem, S.A., and Jeelani, S., *Steroids*, 2014, vol. 92, pp. 13–19. https://doi.org/10.1016/j.steroids.2014.09.004
16. Choudhary, M.I., Alam, M.S., Atta-ur-Rahman, Yousuf, S., Wu, Y.-C., Lin, A.-S., and Shaheen, F., *Steroids*, 2011, vol. 76, pp. 1554–1559. https://doi.org/10.1016/j.steroids. 2011.09.006
17. Pinto-Bazurco M.M.A., Negri, M., Jagusch, C., Müller-Vieira, U., Lauterbach, T., and Hartmann, R.W., *J. Med. Chem.*, 2018, vol. 51, pp. 5009–5018. https://doi.org/10.1021/ jm800355c
18. Salvador, J.A.R., Moreira, V.M., and Silvestre, S.M., Steroidal CYP17 inhibitors for prostate cancer treatment: from concept to clinic, in *Advances in Prostate Cancer*, Hamilton, G., Ed., Rijeka, Croatia: InTech, 2013, chapter 12, pp. 275–304. https://doi.org/10.5772/45948
19. Haidar, S., Ehmer, P.B., Barassin, S., Batzl-Hartmann, C., Hartmann, R.W., *J. Steroid. Biochem. Mol. Biol.*, 2003, vol. 84, pp. 555–562. https://doi.org/10.1016/S0960-0760(03)00070-0
20. Al-Masoudi, N.A., Ali, D.S., Saeed, B., Hartmann, R.W., Engel, M., and Rashid, S., *Arch. Pharm. Chem. Life Sci.*, 2014, vol. 374, pp. 896–907. https://doi.org/10.1002/ardp.201400255
21. Al-Masoudi, N.A., Mahdi, K.M., Abdul-Rida, N.A., Saeed, B.A., and Engel, M., *Steroids*, 2015, vol.

pp. 52–59.
https://doi.org/10.1016/j.steroids.2015.05.002

22. Al-Masoudi, N.A., Kadhim, R.A., Abdul-Rida, N.A., Saeed, B.A., and Engel, M., *Steroids*, 2015, vol. 101, pp. 43–50.
https://doi.org/10.1016/j.steroids.2015.05.011
23. Mahdi, K.M., N Abdul-Rida, N.A., and Al-Masoudi, N.A., *Eur. J. Chem.*, 2015, vol. 6, pp. 1–7.
https://doi.org/10.5155/eurjchem.6.1.1-7.1139
24. Karthikeyan, C., Moorthy, N.S.H.N., Ramasamy, S., Vanam, U., Manivannan, E., Karunagaran, D., and Trivedi, P., *Recent Pat. Anticancer Drug Discov.*, 2015, vol. 10, pp. 97–115.
https://doi.org/10.2174/1574892809666140819153902
25. Dominguez, J.N., León, C., Rodrigues, J., de Dominguez, N.G., Gut, J., and Rosenthal, P.J., *J. Med. Chem.*, 2005, vol. 48, pp. 3654–3658.
https://doi.org/10.1021/jm058208o
26. Lee, S.H., Seo, G.S., Kim, J.Y., Jin, X.Y., Kim, H.D., and Sohn, D.H., *Eur. J. Pharmacol.*, 2006, vol. 532, pp. 178–186.
https://doi.org/10.1016/j.ejphar.2006.01.005
27. Al-Hazam, H.A., Al-Shamkani, Z.A., Al-Masoudi, N.A., Saeed, B.A., and Pannecouque, C., *Z. Naturforsch.*, 2017, vol. 72, pp. 249–256.
https://doi.org/10.1016/j.ejphar.2006.01.005
28. Rizvi, S.U.F., Siddiqui, H.L., Johns, M., Detorio, M., and Schinazi, R.F., *Med. Chem. Res.*, 2012, vol. 21, pp. 3741–3749.
https://doi.org/10.1007/s00044-011-9912-x
29. Svetaz, L., Tapia, A., Lopez, S.N., Furlan, R.L.E., Petenatti, E., Pioli, R., Schmeda-Hirschmann, G., and Zacchino, S.A., *J. Agric. Food Chem.*, 2004, vol. 52, pp. 3297–3300.
https://doi.org/10.1021/jf0352 13x
30. Willker, W., Leibfritz, D., Kerssebaum, R., and Bermel, W., *Magn. Reson. Chem.*, 1993, vol. 31, pp. 287–292.
https://doi.org/10.1002/mrc.1260310315
31. Pannecouque, C., Daelemans, D., and De Clercq, E., *Nat. Protoc.*, 2008, vol. 3, pp. 427–434.
https://doi.org/10.1038/ nprot.2007.517
32. Hargrave, K.D., Proudfoot, J.R., Grozinger, K.G., Cullen, E., Kapadia, S.R., Patel, U.R., Fuchs, V.U., Mauldin, S.C., Vitous, J., Behnke, M.L., Klunder, J.M., Pal, K., Skiles, J.W., McNieil, D.W., Rose, J.M., Chow, G.C., Skoog, M.T., Wu, J.C., Schmidt, G., Engel, W.E., Eberlein, W.G., Saboe, T.D., Rosenthal, A.S., and Adams, J., *J. Med. Chem.*, 1991, vol. 34, pp. 2231–2241.
https://doi.org/10.1021/jm00111a045
33. Mitsuya, H., Weinhold, K.J., Furman, P.A., St. Clair, M.H., Lehrmann, H.N., Gallo, R.C., Bolognesi, D., Barry, D.W., and Broder, S., *Proc. Natl. Acad. Sci. U. S. A.*, 1985, vol. 82, pp. 7096–7100.
https://doi.org/10.1073/pnas.82.20.7096
34. Ducki, S., Forrest, R., Hadfield, J.A., Kendall, A., Lawrence, N.J., McGown, A.T., David, D., and Rennison, D., *Bioorg. Med. Chem. Lett.*, 1998, vol. 8, pp. 1051–1056.
https://doi.org/10.1016/S0960-894X (98)00162-0
35. Becke, A.D., *J. Chem. Phys.*, 1993, vol. 98, pp. 5648–5652.
https://doi.org/10.1063/1.464913
36. Frisch, M.J., Trucks, G.W., Schlegel, H.B., Scuseria, G.E., Robb, M.A., Cheeseman, J.R., Scalmani, G., Barone, V., et al., Gaussian, Inc., Wallingford CT, 2016.
37. Molecular Operating Enviroment (MOE), 2013.08, Chemical Computing Group ULC, 1010 Sherbooks St. West, Suit #910, Montreal, QC, Canada, H3A 2R7, 2018.
38. Ren, J., Chamberlain, P.P., Stamp, A., Short, S.A., Weaver, K.L., Romines, K.R., Hazen, R., Freeman, A., Ferris, R.G., Andrews, C.W., Boone, L., Chan, H.H., and Stammers, D.K., *J. Med. Chem.*, 2008, vol. 51, pp. 5000–50008.
https://doi.org/10.1021/jm8004493
39. Sahu, V.K., Khan, A.K.R., Singh, R.K., and Singh, P.P., *Am. J. Immunol.*, 2008, vol. 4, pp. 33–42.
https://doi.org/10.3844/ajisp.2008.33.42
40. *DMOL^3 User Guide*, San Diego, CA, USA: Accelrys, Inc., 2003.
41. Rogers, D., and Hopfinger, A.J., *J. Chem. Inf. Comput. Sci.*, 1994, vol. 34, pp. 854–866.
https://doi.org/10.1021/ ci00020a020
42. Popovic, M., Sarngadharan, M.G., Read, E., and Gallo, R.C., *Science*, 1984, vol. 224, pp. 497–500.
https://doi.org/10.1126/science.6200935
43. Barré-Sinoussi, F., Chermann, J.C., Rey, F., Nugeyre. M.T., Chamaret, S., Gruest, J., Dauguet, C., Axler-Blin, C., Vezinet-Brun, F., Rouzioux, C., Rozenbaum, W., and Montagnier, L., *Science*, 1983, vol. 220, pp. 868–871.
https://doi.org/10.1126/science.6189183
44. Miyoshi, I., Taguchi, H., Kobonishi, I., Yoshimoto, S., Ohtsuki, Y., Shiraishi, Y., and Akagi, T., *Gann. Monogr. Cancer Res.*, 1982, vol. 28, pp. 219–228.