Full Paper

New CYP17 Hydroxylase Inhibitors: Synthesis, Biological Evaluation, QSAR, and Molecular Docking Study of New Pregnenolone Analogs

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A new series of pregnenonlone analogs were synthesized and evaluated for their inhibitory activity against cytochrome P450 (CYP17 hydroxylase enzyme). In general, the 5-aryl-1,3,4-thiadiazol-2-yl)-imino-pregnenolone derivatives **11**–**15** were more active than the sulfonate **24**–**31** and the ester **37**–**41** analogs. Derivative **12** showed optimal activity in this series, with IC_{50} values of 2.5 μM compared with the standard abiraterone ($IC_{50} = 0.07$ μM). However, the analogs **11** and **25** showed a better selectivity profile (81.5 and 82.7% inhibition of hydroxylase, respectively), which may be a useful lead in CYP17 inhibition studies. Molecular docking studies demonstrated quite similar binding patterns of all new pregnenolone derivatives at the active site of CYP17 through hydrogen bonding and hydrophobic interaction.

Keywords: Anti-HIV activity / CYP17 hydroxylase enzyme / Molecular docking study / Pregnenolone / QSAR

Received: June 27, 2014; Revised: August 19, 2014; Accepted: August 22, 2014

DOI 10.1002/ardp.201400255

Introduction

Cytochrome P450's are enzymes, which catalyze a large number of biological reactions, for example, hydroxylation, *N*-, *O*-, *S*-dealkylation, epoxidation, or desamination. Their substrates include fatty acids, steroids, or prostaglandins. In addition, a high number of various xenobiotics are metabolized by these enzymes [1]. The enzyme 17α-hydroxylase-C17,20-lyase (P45017, CYP17, and androgen synthase), a cytochrome P450 monooxygenase, is the key enzyme for androgen and corticoid biosynthesis and has become a valuable target in prostate cancer (PC) treatment [2–7], since the androgens are potent prostate mitogens [8, 9] and its elevated levels may be associated with PC risk. Several non-steroidal compounds have been synthesized, which displayed better inhibitory properties and afforded very potent CYP17 inhibitors [1, 2, 7, 10–15]. Out of these compounds, ketoconazole **1** (Fig. 1), an imidazole fungicide that has inhibitory activity toward CYP17 [16, 17], has been used clinically in high dose for the treatment of advanced PC [4]. However, the fact that it concomitantly inhibits other steroidal P450 enzymes causing significant side effects [18] has limited its use. A common approach to the synthesis of potent steroidal inhibitors of CYP17 has been the design of substrate-like molecules bearing a heterocycle at the C17 position with privileged heteroatoms (N, S, and O), which can interact as the sixth ligand with the heme iron of the enzyme. In addition, computational studies established that a good inhibitor should possess a sufficiently large hydrophobic core, comparable to a steroid molecule, and bear electronegative groups at its external positions [19].

In 1996, Njar et al. [20] reported the first steroidal inhibitors of CYP17 bearing a heterocyclic moiety bound to C17 by a nitrogen atom, among which the imidazolyl derivative **2** was found to be the most promising [20–23]. Later, in 2005, the

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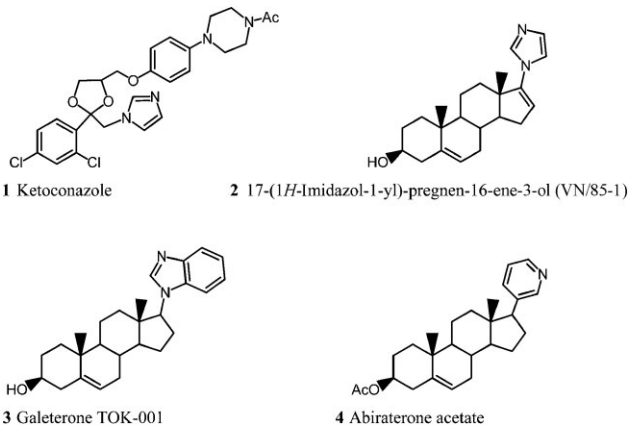


Figure 1. Some inhibitors of CYP 17 hydroxylase-lyase enzyme.

same group reported the synthesis of galeterone **3** and its Δ^4-3-keto derivative [23–25], where **3** is currently undergoing phase I/II clinical trials for the treatment of chemotherapy-naive CRPC [26, 27]. However, patients suffering from CRPC can clearly benefit from the newly approved drug abiraterone acetate (Zytiga) **4** [28, 29]. This pregnenolone derivative was designed as an inhibitor of the enzyme 17α-hydroxylase/$C_{17,20}$-lyase (CYP17A1) [30], which catalyzes two key reactions in steroid hormone biosynthesis.

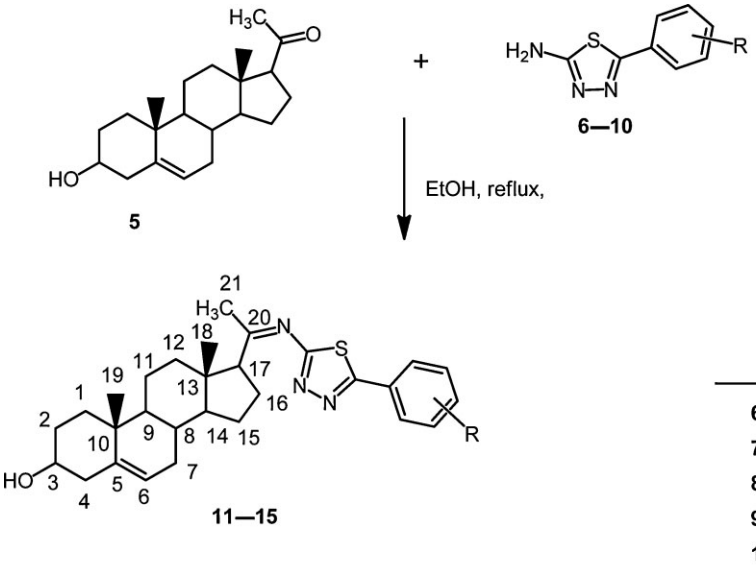
Herein, we report the synthesis and biological evaluation of novel 17-pregnenolone-imine derivatives as well as the 3-*O*-sulfonate and ester analogs at C-3, designed as new CYP17A1 inhibitors.

Results and discussion

Chemistry

Treatment of pregnenolone **5** with various 2-amino-5-aryl-2,3,4-thiadiazoles (e.g., 5-phenyl-, 5-(4-methoxyphenyl)-, 5-(4-bromophenyl)-, 5-(4-chlorophenyl)-, and 5-(4-nitrophenyl)-2-amino-3,4-thiadiazoles **6**–**10** in EtOH, using HOAc as a catalyst afforded, after purification by recrystallization, the imino derivatives **11**–**15** in 75, 74, 80, 78, and 75% yield, respectively (Scheme 1).

The structures of **11**–**15** were assigned on the basis of their ^{1}H, and ^{13}C NMR spectra, which showed similar patterns of aliphatic proton and carbon atoms, using the NMR spectrum of the starting material pregnenolone **5** as a reference. The multiplet or doublets at the regions δ 8.30–7.42 ppm ($J \sim 8.9$ Hz) were assigned to the aromatic protons. The triplets at δ 5.30 ppm ($J \sim 2.5$ Hz, for the analogs **11**–**13**) and doublet of doublets ($J = 2.2$, 3.1 Hz, for the analogs **14** and **15**) were assigned to H-6, respectively, while the broad singlets at the region $\delta = 4.60$–3.60 ppm were attributed to the hydroxyl group at C-3. The multiplets at the regions δ 3.40–3.32 ppm were assigned to H-3. H-17 appeared as triplets at δ 2.57 ppm ($J \sim 9.0$ Hz), whereas the multiplets at the regions δ 2.22–2.10 ppm were assigned to CH_2-4. In the ^{13}C NMR spectra of **11**–**15**, the resonances of C-20 were shifted from $\delta \sim 200$ ppm (chemical shift of C-20 of prognenolone **5**) into chemical shifts δ 164.6–163.8 ppm, indicative for the formation of the imino group and attributed to the azomethine carbon atoms. The signals at δ 175.5–169.9 ppm and δ 161.8–154.0 ppm were assigned to the C-5′ and C-2′ of the thiadiazole ring,



	R
6, 11	H
7, 12	4-OMe
8, 13	4-Br
9, 14	4-Cl
10, 15	4-NO_2

Scheme 1. Synthesis of 17-((5-aryl-1,3,4-thiadiazol-2-yl)imino)-5-pregnen-3β-ol derivatives.

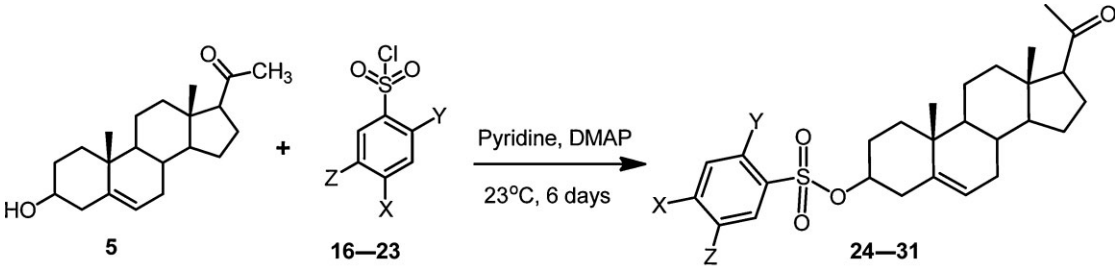
respectively. C-3 and C-6 appeared at the regions δ 71.7–69.6 and 121.4–120.2 ppm, respectively. The other protons and carbon atoms of pregnenolone backbone were fully analyzed (*cf.* Experimental). Compound **12** has been selected for further NMR studies, since its HMBC spectrum [31] revealed several $^{3}J_{C;H}$ and $^{2}J_{C;H}$ couplings. A $^{2}J_{C;H}$ between H-17 at δ 2.58 ppm and C-20 at δ 164.1 ppm, as well as a $^{3}J_{C;H}$ with C-15 at δ 25.1 ppm were observed.

Next, pregnenolone **5** was treated with various arylsulfonyl chlorides (aryl: 4-fluorophenyl- **16**, 2-trifluoromethoxy- **17**, 4-nitrophenyl- **18**, 3-cyanophenyl- **19**, 4-bromophenyl- **20**, 4-bromo-2-trifluoromethyphenyl- **21**, 3-fluoro-4-methoxyphenyl- **22**, and 4-bromo-2,4-difluorophenyl- **23**) in the presence of pyridine and DMAP to give, after purification by column chromatography, the sulfonate analogs **24–31** in 71–61% yield (Scheme 2).

The structures of **24–31** were assigned from their ^{1}H NMR and ^{13}C NMR as well as from the comparison with the spectra of **11–15**. In the ^{1}H NMR spectra of **24–31**, broad singlets, doublets, and multiplet at the regions δ 8.15–7.21 ppm were assigned to the aromatic protons ($J \sim 9.0$ Hz for the doublets). The aliphatic protons of the pregnenolone scaffold were fully analyzed as following: H-6 of the pregnane ring appeared as triplets at the regions δ 5.38–5.34 ppm ($J \sim 2.6$ Hz), while H-3 of the same ring resonated as multiplets at the regions δ 4.48–3.73 ppm. The ^{13}C NMR spectra of **24–31** contained similar resonance signals of the pregnenolone carbons rings C-1 to C-21. The higher-field signals between δ 219.4 and 209.0 ppm were assigned to the carbonyl group (C-20), while the resonances at the regions of δ 141.8–137.3 ppm were assigned to C-5. The chemical shifts at the regions δ 82.9–67.0 and 64.0–63.2 ppm were attributed to C-3 and C-17, respectively. The resonances at the regions δ 60.1–57.0 and δ 56.9–48.2 ppm were assigned to the pregnane carbon atoms C-14 and C-9, respectively. The other carbon atoms were fully analyzed (*cf.* Experimental).

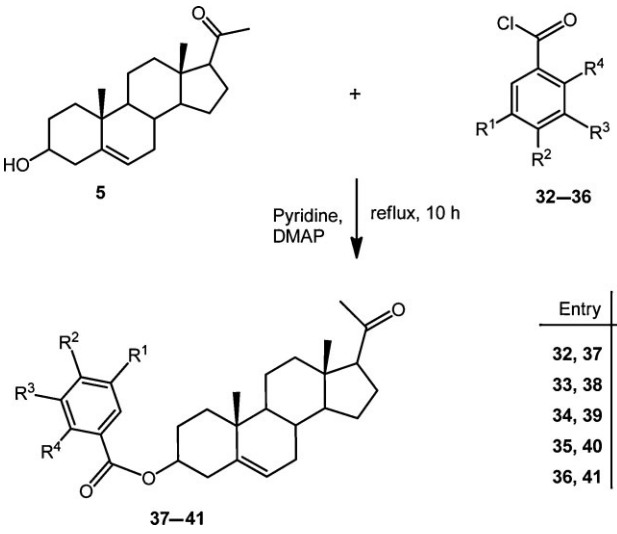
Our work was modified by synthesis of new pregnenolone derivatives bearing ester moieties at C-3, aiming for evaluation of their inhibitory activity against the CYP17 hydroxylase enzyme, which might leading for the treatment of breast cancer. Thus, treatment of **5** with substituted acyl chlorides (acyl: 4-chloro-3-nitrobenzoyl- **32**, 2,4,5-trifluoro-3-methoxybenzoyl- **33**, 3,5-dinitrobenzoyl- **34**, 3-methoxybenzoyl- **35**, and benzoyl- **36** groups) in pyridine and DMAP afforded, after purification by column chromatography, the ester analogs **37–41** in 73–62% yield (Scheme 3).

The structures of **37–41** were determined from their NMR (^{1}H, ^{13}C), which showed a similar pattern of the pregnenolone scaffold. The structures were deduced as well from the comparison with those of the analogs **11–15** and **24–31** (*cf.* Experimental). The aromatic protons resonated as multiplet, doublets, triplets, or doublet of doublets at the regions δ 9.20–7.06 ppm ($J \sim 8.8$–2.2 Hz). In the ^{13}C NMR spectra of **37–41**, the higher field resonances at δ 209.5 ppm and δ 166.0–161.9 ppm were assigned to the carbonyl carbon atoms (C_{20}=O) and CO_2 at C-3, respectively. Resonances at the regions δ 36.7 and 27.8 ppm were assigned to C-1 and C-2, respectively, while the signals at the regions δ 76.7–74.4, 38.8 and 139.9–138.9 ppm were attributed to C-3 to C-5, respectively. The other protons and carbon atoms of pregnenolone backbone and aromatic ring were fully analyzed (*cf.* Experimental). Additionally, all the structures of the new synthesized analogs have been identified by ^{1}H, ^{13}C HSQC NMR spectroscopy [32].



	16,24	17,25	18,26	19,27	20,28	21,29	22,30	23,31
X	F	H	NO_2	H	Br	Br	OCH_3	Br
Y	H	OCF_3	H	H	H	OCF_3	OCF_3	H
Z	H	H	H	CN	H	H	F	F

Scheme 2. Synthesis of 5-pregnen-3β-arylsulfonyl-20-one derivatives.



Entry	R^1	R^2	R^3	R^4
32, 37	NO_2	Cl	H	H
33, 38	F	OCH_3	F	F
34, 39	NO_2	H	NO_2	H
35, 40	H	H	OCH_3	H
36, 41	H	H	H	H

Scheme 3. Synthesis of 5-pregnen-3β-yl-(substituted-benzoyl)-20-one derivatives.

Bioactivities

In vitro CYP17 hydroxylase enzyme activity

The hydroxylase substrate, 17-hydroxypregnenolone is formed *in situ* by the cytochrome P450 17α-hydroxylase enzyme during a 10 min preincubation. At this time point, almost all pregnenolone substrate has been transformed into 17α-hydroxypregnenolone (>95%). The incubation of an aliquot is stopped using HCl. At this time point, the hydroxylase assay is initiated by addition of our test compounds **11**–**15**, **24**–**31**, and **37**–**41** in different concentrations dissolved in DMSO (final concentration usually between 0 and 10 μM). The results are presented in Table 1. The steroidal CYP17A1 inhibitor abiraterone acetate **4** was used as a reference compound.

The compounds have been divided into three classes according to pregnenolone ring mimicking moiety bearing the residual groups: substituted thiadiazole groups at C-20, arylsulfonyl, and aryl ester groups at C-3.

When the compound was substituted by hydrogen forming groups such as oxo or hydroxyl, the resulting compounds exhibited variation of activity profiles compared to corresponding pregnenolone molecule. The results of Table 1 showed that **11**–**15** exhibited remarkable inhibition of CYP17 with IC_{50} values of 4.7, 2.5, 3.4, 3.0, and 3.2 μM, respectively, whereas blocking of the hydroxyl group at C-2 by the sulfonate or ester groups (analogs **24**–**31**, **24**–**31**, and **37**–**41**) showed a significant decrease in the inhibitory activity. Hence, it is notable that compounds that furnished a hydrogen bond

Table 1. Inhibition of CYP17 hydroxylase enzyme by pregnenolone analogs.

Compd.	IC_{50} (μM)[a)]	Inhibition %[b)]	Compd.	IC_{50} (μM)[a)]	Inhibition %[b)]
11	4.7 ± 0.7	81.5 ± 2.0	**28**	8.1 ± 0.4	77.3 ± 4.4
12	2.5 ± 0.0	78.6 ± 3.0	**29**	14.6. ± 8.2	72.1 ± 4.1
13	3.4 ± 0.9	74.7 ± 4.2	**30**	nd	67.3 ± 6.9
14	3.0 ± 0.4	76.3 ± 7.5	**31**	nd	67.3 ± 6.9
15	3.2 ± 0.6	11.15	**37**	67.3 ± 6.9	61.9 ± 12.7
24	nd	72.0 ± 7.8	**38**	nd	5.4 ± 6.5
25	9.1 ± 1.4	82.7 ± 3.3	**39**	nd	12.8 ± 3.3
26	15.2 ± 1.4	65.7 ± 6.0	**40**	nd	3.0 ± 2.2
27	11.2	78.7 ± 0.0	**41**	nd	6.1 ± 7.4
ABT[c)]	0.07				

[a)] Data shown were obtained by performing the tests in duplicate, IC_{50}: the concentration of a inhibitor that is required for 50% inhibition *in vitro*.
[b)] Hydroxylase enzyme inhibition measured at 50 μM.
[c)] ABT, abiraterone; KTZ, ketoconazole; nd, not determined.

formation are always more potent and selective, which is probably caused by some interactions between hydrogen bond forming groups and certain polar amino acid residues of the CYP17 enzyme active site [19]. Interestingly, compound **12** having a 4-methoxyphenyl substituent showed a higher inhibitory activity in comparison for those carrying other substituents (e.g., H, Br, Cl, and NO_2). These data illustrated that electron-donating substituents (like methoxy group) would increase the heme iron-complexing imine of the thiadiazole nitrogen atoms interaction. In general, the common feature of the analogs **11–15** is a nitrogen atom, which be able to interact with the heme iron (Fe^{+2}) located in the active center of CYP enzyme. Such argument is in agreement with the results reported previously [19, 33]. Although none of the tested compounds is more active than abiraterone or ketoconazole, compounds **11** and **25** showed a better selectivity profile (81.5 and 82.7% inhibition of hydroxylase, respectively) than those of some other reported steroidal inhibitors [34]. Therefore, these analogs are interesting candidates for further investigation.

Molecular docking study

The fact that molecules **11–15** having structural common features similar to abiraterone **4** (pregnenolone scaffold) did show some inhibitory activity against CYP17A1, which prompted us to study their interaction with the active sites of the enzyme via the molecular computational studies. In the docking study, the X-ray crystal structure of human microsomal cytochrome protein P450 (CYP17; PDBID: 1RUK) was obtained from Protein Data Bank (http://www.rcsb.org) [19]. The structure geometries were subsequently optimized using the Steepest Descent method followed by Conjugate Gradient in 2000 steps using Accelrys Discovery Studio (Version2.1, Accelrys Software, http://accelrys.com/). Optimization was carried out by applying the CHARMm force field. Automated dockings were performed to locate the appropriate binding orientations and conformations of these inhibitors CYP17 protein pocket using AutoDock4.2 tool according to the specified instructions [35]. In brief, polar hydrogen atoms and Kollman charges were assigned to the receptor protein. For small molecules preparation, Gasteiger partial charges were designated and non-polar hydrogen atoms were merged. All torsions for ligands were allowed to rotate during docking procedure. The program AutoGrid was used to generate the grid maps. Each grid was centered at the structure of the corresponding receptor. The grid dimensions were $80 \times 80 \times 80\,Å^3$ with points separated by 0.375 Å. In general, random starting positions, random orientations, and torsions were used. The translation, quaternion, and torsion steps were taken from default values indicated in AutoDock. The Lamarckian genetic algorithm (LGA) method was used for minimization using default parameters. The standard docking protocol for rigid and flexible ligand docking consisted of 50 runs, using an initial population of 150 randomly placed individuals, with 2.5×106 energy evaluations, a maximum number of 27,000 iterations, mutation rate of 0.02, crossover rate of 0.80, and an elitism value of 1. Cluster analysis was performed on the docked results using an RMS tolerance of 1.0 Å. The binding energy of each cluster is the mean binding energy of all the conformations present within the cluster. Subsequently, cluster with the lowest binding energy and higher number of conformations was selected as the docked pose of that particular ligand. Table 2 shows the docking parameters, whereas Table 3 illustrates the binding energies, docking energies, and inhibition constant values of the pregnenolone analogs **11–40**.

Docking results

Our molecular docking analysis of the new analogs was based on the human CYP17 homology model [36–38], which exhibited quite similar binding patterns at the active site of CYP17. The prospective ligands were ranked according to the highest binding energy as the best conformers. Thus, the binding energy score for **11–15** series ranged from −7 to −11 (Table 3), while for **24–32** series, it ranged from −10 to −11 kcal/mol, indicating a selectivity of thiadiazole functional groups in binding mode alterations. Further, due to the larger binding pocket of CYP17, bulkier compounds like **11–15** derivatives easily placed themselves inside the binding gorge. This also depicts a broad specificity of these compounds to bind the same position of CYP17.

The best selected dock pose of the analog **11**, used as standard inhibitor, exhibited three hydrogen bond interactions with the triad residue Arg212 with $N^3_{\text{thiazdiazole}}$ at 3.57 Å, Phe215 and Gly491 with the OH group at C-3, in addition to a hydrophobic interaction, and thus participate in π–π interaction between the phenyl group binding the thiazole moiety and Phe302 of CYP17 residues (Fig. 2).

From the analysis data of Table 1, we conducted that the analogs **24–31** and **37–41** having bulky sulfonate or acylated groups at C-3 would not be able to productively engage with the enzyme, in comparison to the standard abiraterone acetate **4**, the outcome was well correlated with experimental

Table 2. Docking parameters.

Grid parameters		Docking parameters	
Spacing	0.375 Å	Energy evaluations	2.5×106
Grid center	80X Å	Iterations	27,000
	80Y Å	Mutation rate	0.02
	80Z Å	Crossover rate	0.80
		Elitism value	1
		RMS tolerance	1.0 Å

Table 3. Binding energies, docking energies, and inhibition constant values of **11–40**.

Ligand/properties	11	12	13	14	15	24	25	26
Binding energy (kcal/mol)	−8.94	−7.89	−7.95	−11.29	−8.37	−9.89	−10.04	−12.10
K_i (nM)	282.16	1.66	5.5	5.34	727.19	56.74	43.88	1.36
Intermolecular energy (kcal/mol)	−10.13	−9.38	−9.14	−12.48	−9.87	−11.08	−11.83	−13.59
vdW + H-bond + desolv. energy (kcal/mol)	−9.74	−9.03	−9.05	−12.38	−8.85	−10.94	−11.83	−12.46
Electrostatic energy (kcal/mol)	−0.39	−0.35	−0.09	−0.10	−1.02	−0.14	0.0	−1.13
Final total internal energy (kcal/mol)	−0.13	−0.92	−0.7	0.34	−1.15	−0.44	0.02	−0.75
Torsional free energy (kcal/mol)	1.19	1.49	1.19	1.19	1.49	1.19	1.79	1.49
Unbound system's energy (kcal/mol)	−0.13	−0.92	−0.7	0.34	−1.15	−0.44	0.02	−0.75
Temperature (K)	298.5	298.5	298.5	298.5	298.5	298.5	298.5	298.5

Ligand/properties	27	28	29	30	31	37	38	39	40
Binding energy (kcal/mol)	−11.01	−10.64	−7.73	−9.92	−10.46	−12.91	−9.41	−12.31	−9.38
K_i (nM)	8.55	15.81	2.15	55.81	21.19	346	125.91	944.48	61.12
Intermolecular energy (kcal/mol)	−12.2	−11.84	−9.52	−11.39	−11.66	−14.4	−10.9	−14.1	−11.33
vdW + Hbond + desolv energy (kcal/mol)	−12.0	−11.74	−9.12	−11.4	−11.6	−12.98	−10.92	−12.35	−11.36
Electrostatic energy (kcal/mol)	−0.2	−0.14	−0.4	0.01	−0.03	−1.42	0.02	−1.75	0.03
Final total internal energy (kcal/mol)	−0.61	−0.66	−0.38	−0.54	−0.49	−0.31	−0.46	−0.38	−0.67
Torsional free energy (kcal/mol)	1.19	1.19	1.79	1.49	1.19	1.49	1.49	1.79	1.49
Unbound system's energy (kcal/mol)	−0.61	−0.66	−0.38	−0.54	−0.49	−0.31	−0.46	−0.38	−0.67
Temperature (K)	298.5	298.5	298.5	298.5	298.5	298.5	298.5	298.5	298.5

results, except few analogs, which showed one hydrogen bond interaction.

By docking experiment, Ala365, Ala370, and Arg212, were identified as key residues, located within the binding pocket of compounds **11–15**.

In summary, our study gives an idea about the interaction between the active site residues and the substrate, which is explained on the basis of size, hydrophobicity of the binding pocket, and the position of the substituent (i.e., at C-3 or C-20 of the pregnenolone moiety).

Quantum-structure–activity relationship (QSAR)

QSAR has been used widely to predict the hazard of untested chemicals with already tested chemicals by developing statistical relationships between molecular structure linear descriptors and biological activity [39]. The Hansch linear free-energy model is the most popular mathematical approach to QSAR. It explains: (i) the physicochemical aspects of drug transport and distribution from point of application to the point of effect and (ii) the drug–receptor interaction. Semi-empirical self-consistent-field molecular orbital (SCF-MO) method at Austin Model 1 (AM1) [40] level within restricted Hartree–Kock (RHF) [41] formalism has been considered to optimize fully the geometry of the pregnenolone molecule in its ground state. Geometry optimization was carried out by using a conjugate gradient method (Polak–Ribiere algorithm) [42]. The self-consistent-field SCF convergence was set at 0.001 kcal/mol and the RMS gradient was set to 0.001 kcal/(Å mol) in the calculation. Geometry optimizations were carried out by PM3 method as implemented in the Hyperchem 7.5 package. We performed all the calculations using Hyperchem-7.52 program. In addition, the correlation analysis and the regression analysis for quantum parameters were performed by using Minitab program release 11.11. All calculations were performed on a window XP workstation in Pentium IV PC.

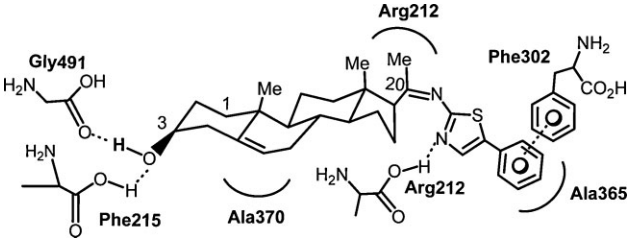


Figure 2. Compound **11** shows three hydrogen bondings: Arg212 with $N^3_{thiazdiazole}$, Phe212 and Gly491 with OH group at C-3. In addition, hydrophobic interaction between phenyl group binding the thiazole moiety and Phe302 of CYP17 residues was observed.

Consequently, hydrophobicity is the vital character affecting the biological activity of pregnenolone derivatives, such as log *P* (the hydrophobic parameter), dipole moment (μ), polarizability (*P*), etc.

Geometry parameters

Geometry parameters are another kind of steric parameters that have some relations with molecular conformation like molecular volume (*V*), molecular surface (*S*), etc. It is important to select appropriate molecular descriptors for

QSAR, such as electronic, hydrophobic, and the geometry [43] parameters. The selected quantum parameters of the pregnenolone inhibitors are listed in Table 4.

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 [43]. By the multiple regression analysis, we have selected three models since r shows an improved values as in Eqs. (1–3).

Two variables:

$$IC_{50} = -1.489(\pm 1.35)\mathrm{Mu} - 10.87(\pm 10.5)E_{\mathrm{LUMO}} - 5.137(\pm 8.81) \quad (1)$$

$r = 0.959$, $s = 0.33$, $F = 11.329$

Three variables:

$$\text{(a) } IC_{50} = -1.616(\pm 0.68)\mathrm{Mu} + 2.011(\pm 2.98)E_{\mathrm{HOMO}} - 13.82(\pm 6.71)E_{\mathrm{LUMO}} + 10.16(\pm 23.0) \quad (2)$$

$r = 0.999$, $s = 0.054$, $F = 306.7$

$$\text{(b) } IC_{50} = -1.466(\pm 3.48)\log p - 0.0776(\pm 0.083)\mathrm{Area} + 0.0368(\pm 0.73)\mathrm{Mass} + 54.72(\pm 53.4)$$

$r = 0.998$, $s = 0.105$, $F = 81.0$

One variable:

$$IC_{50} = -0.0395(\pm 0.034)\mathrm{Area} + 33.13(\pm 25.3) \quad (3)$$

$r = 0.908$, $s = 0.397$, $F = 14.0$

- Values for V (A^3) used for calculation of coefficients of the $V^{1/3} - \log P$ linear equation.
- F: the overlap function between the inhibitor and environment electron density.
- S: surface area (A^2).

However, regarding the above interpretation we found the calculated IC_{50} of compounds **11–15** are almost in accordance with their observed IC_{50} values (Table 5) as inhibitors for the CYP17 enzyme.

The regression equation is

$$IC_{50} = -61.016869254 \times (\text{Balaban index JX}) - 4.780334024(\text{Kappa} - 3) + 0.049160570(\text{Kappa} - 1) + 85.403822251$$

$R^2 = 0.99999900$; adjusted $R^2 = 0.99999600$;
cross validated $R^2 = 0.99979100$

Significant regression = yes; significance-of-regression F-value = 3.612021e + 005; critical SOR F value (95%) = 216.84349500; computed experimental error = 0.00000000; replicated points = 0.

Experimental

General

Melting points were recorded on melting point apparatus (VEEGO), or a Büchi melting point apparatus B-545 (BÜCHI Labortechnik AG, Switzerland) and are uncorrected. ^{1}H and ^{13}C NMR spectra was recorded on a Bruker (Avance III, Germany) spectrospin-400 and 600 MHz (^{1}H) and 150.91 MHz (^{13}C) spectrometers using ($CDCl_3$) containing tetramethylsilane or (DMSO-d_6) as internal standard (chemical shifts in δ, ppm). Heteronuclear assignments were verified by HSQC, ^{1}H, ^{13}C COSY, ROESY, and HMBC experiments. The IR spectra were measured as KBr disc by using F.T. IR-8400 (Shimadzu, Japan). Microanalytical data were obtained with a Vario elemental apparatus (Shimadzu, Japan). The purity of final products (≥95%) was confirmed by analytical high performance liquid chromatography (HPLC). HPLC/MS

Table 4. The IC_{50} and electronic and molecular descriptors calculations used in the QSAR study, the dipole moment μ (in Debye), binding energy (kcal/mol) and heat of formation (kcal/mol).

Compd.	log *p*	*A*	*V*	Ref.	μ	Mass (*S*)	E_{HOMO}	E_{LUMO}	IC_{50}
11	7.88	722.09	1305.73	140.80	1.383	475.69	−9.0892	−1.0765	4.7
12	7.62	770.02	1382.06	147.27	2.201	505.72	−8.85739	−0.99148	2.5
13	8.67	761.62	1370.10	148.42	3.113	554.59	−9.340458	−1.231013	3.4
14	8.39	749.95	1349.97	145.61	3.371	510.14	−9.343598	−1.238171	3.0
15	7.83	762.62	1367.72	148.13	6.401	520.79	−9.513567	−1.628791	3.2
26	7.18	723.18	1322.82	134.05	7.883	558.63	−9.519161	−1.171255	9.1
27	5.45	719.12	1295.32	133.81	3.057	519.63	−9.744872	−1.827834	15.2
28	5.53	712.34	1287.25	132.22	4.013	499.64	−9.695738	−1.260856	11.2
29	6.29	711.14	1294.18	134.10	5.178	553.53	−9.485376	−1.140302	8.1
30	7.97	760.80	1385.75	141.68	5.18	637.52	−9.586634	−1.463645	14.6
31	5.81	673.08	1223.85	126.66	6.353		−9.365929	−0.7808181	67.3

Table 5. The observed and calculated IC_{50} of pregnenolone derivatives.

Compd.	IC_{50} obs.	IC_{50} calcd.	Residual
11	4.7	4.699778	−0.0002
12	2.5	2.4997	−0.0003
13	3.4	3.36496	−0.035
14	3.0	3.001	0.001
15	3.2	3.2002	0.0002

was performed on a MQS electrospray mass spectrometer (Thermo Fisher) or 70 eV EI and FAB MAT 8200 spectrometers (Finnigana MAT, USA). UV chamber was used for detection of spots in TLC. Eluent solvent of TLC were stated, while development reagent was iodine. Column chromatography was carried out with silica gel powder (230–400 mesh size) by using appropriate solvents.

*General procedure for the preparation of 17-((5-aryl-1,3,4-thiadiazol-2-yl)imino)-5-pregnen-3β-ol derivatives (**11**–**15**)*

To a solution of pregnen-3β-ol-20-one (**5**) (316 mg, 1.00 mmol) in EtOH (20 mL) and glacial acetic acid (1 mL) were added 5-aryl-2-amino-1,3,4-thiadiazoles **6**–**10** (1.10 mmol) and the mixture was heated under reflux for 6–8 h. After cooling, the solution was poured onto ice-cold water. The solid product was collected by filtration, and recrystallized from EtOH to give the desired products.

*17-((5-Phenyl-1,3,4-thiadiazol-2-yl)imino)-5-pregnen-3β-ol (**11**)*

From 2-amino-5-phenyl-1,3,4-thiadiazole (**6**) (195 mg). Yield: 357 mg (75%), m.p.: 166–168°C, $R_f = 0.61$. ^{1}H NMR ($CDCl_3$): δ 7.79–7.42 (m, 5H, $H_{arom.}$), 5.29 (t, 1H, $J = 5.1$ Hz, H-6), 3.62 (br s, 1H, OH), 3.38 (m, 1H, H-3), 2.58 (t, 1H, $J = 9.1$ Hz, H-17), 2.22 (m, 2H, CH_2-4); 2.06 (m, 2H, H-12a + H-16a), 2.00 (s, 3H, Me-21), 1.84 (m, 1H, H-7a), 1.76 (m, 1H, H-1a), 1.66 (m, 1H, H-2a), 1.61–153 (m, 4H, H-7b + H-11a + H-15a + H-16b), 1.43–1.35 (m, 4H, H-2b + H-8 + H-11b + H-12b), 1.18 (m, 2H, H-14 + H-15b), 1.03 (m, 2H, H-1b + H-9), 0.98 (s, 3H, Me-19), 0.90 (s, 3H, Me-18). ^{13}C NMR ($CDCl_3$): δ 173.7 (C5′-thiodiazole), 164.5 (C-20), 158.5 (C2′-thiodiazole), 142.4 (C-5), 132.5, 130.4, 129.9, 127.4 ($C_{arom.}$), 121.4 (C-6), 71.7 (C-3), 57.6 (C-14), 51.1 (C-9), 44.3 (C-13), 43.3 (C-4), 39.5 (C-12), 38.2 (C-1), 37.3 (C-10), 32.7 (Me-21), 32.5 (C-8), 31.5 (C-7), 29.4 (C-2), 25.1 (C-15), 23.4 (C-16 + C-17), 21.8 (C-11), 19.8 (Me-19), 13.5 (Me-18). EI: *m/z*: 475 $[M]^+$. Anal. calcd. for $C_{24}H_{37}N_3OS$ (475.69): C, 73.22; H, 7.84; N, 8.83. Found: C, 73.01; H, 7.77; N 8.69.

*17-((5-(4-Methoxyphenyl-1,3,4-thiadiazol-2-yl)imino)-5-pregnen-3β-ol (**12**)*

From 2-amino-5-(4-methoxyphenyl-1,3,4-thiadiazole (**7**) (228 mg). Yield: 374 mg (74%), m.p.: 162–164°C, $R_f = 0.30$. ^{1}H NMR ($CDCl_3$): δ 7.71 (d, 2H, $J = 8.9$ Hz, $H_{arom.}$), 6.99 (d, 2H, $J = 8.9$ Hz, $H_{arom.}$), 5.29 (t, 1H, $J = 5.0$ Hz, H-6), 3.83 (s, 3H, OMe), 3.62 (br s, 1H, OH), 3.36 (m, 1H, H-3), 2.58 (t, 1H, $J = 9.0$ Hz, H-17), 2.20 (m, 2H, CH_2-4), 2.05 (m, 2H, H-12a + H-16a), 1.94 (s, 3H, Me-21), 1.83 (m, 1H, H-7a), 1.76 (m, 1H, H-1a), 1.64 (m, 1H, H-2a), 1.60–1.56 (m, 4H, H-7b + H-11a + H-15a + H-16b), 1.48–1.37 (m, 4H, H-2b + H-8 + H-11b + H-12b), 1.12 (m, 2H, H-14 + H-15b), 1.02 (m, 2H, H-1b + H-9), 0.97 (s, 3H, Me-19), 0.89 (s, 3H, Me-18). ^{13}C NMR ($CDCl_3$): δ 175.5 (C5′-thiodiazole), 164.1 (C-20), 161.8 (C2′-thiodiazole + *C*-OMe), 142.4 (C-5), 128.9, 125.1, 121.1, 115.2 ($C_{arom.}$ + C-6), 71.7 (C-3), 57.6 (OMe), 56.7 (C-14), 51.0 (C-9); 44.4 (C-13), 43.5 (C-4), 39.7 (C-12), 38.2 (C-1), 37.7 (C-10), 32.7 (Me-21), 32.4 (C-8), 31.6 (C-7), 29.5 (C-2), 25.4 (C-15), 23.5 (C-16 + C-17), 21.8 (C-11), 19.8 (Me-19), 13.6 (Me-18). EI: *m/z*: 505 $[M]^+$. Anal. calcd. for $C_{30}H_{39}N_3OS$ (505.71): C, 71.25; H, 7.77; N, 8.31. Found: C, 71.13; H, 7.70; N, 8.22.

*17-((5-(4-Bromophenyl-1,3,4-thiadiazol-2-yl)imino)-5-pregnen-3β-ol (**13**)*

From 2-amino-5-(4-bromophenyl-1,3,4-thiadiazole (**8**) (232 mg). Yield 443 mg (80%), m.p.: 172–174°C, $R_f = 0.33$. IR (ν_{max}, cm^{-1}): 3256, 3063, 2966, 1636, 1510, 1464, 1290, 1069, 925 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 7.70 (d, 2H, $J = 8.9$ Hz, $H_{arom.}$), 7.66 (d, 2H, $J = 8.9$ Hz, $H_{arom.}$), 5.26 (t, 1H, $J = 5.0$ Hz, H-6), 4.60 (br s, 1H, OH), 3.32 (m, 1H, H-3), 2.56 (t, 1H, $J = 8.9$ Hz, H-17), 2.21 (m, 2H, CH_2-4), 2.03 (m, 2H, H-12a + H-16a), 1.98 (s, 3H, Me-21), 1.81 (m, 1H, H-7a), 1.77 (m, 2H, H-1a), 1.62 (m, 1H, H-2a), 1.60–1.54 (m, 4H, H-7b + H-11a + H-15a + H-16b), 1.46–1.38 (m, 4H, H-2b + H-8 + H-11b + H-12b), 1.14 (m, 2H, H-14 + H-15b), 1.03 (m, 2H, H-1b + H-9), 0.98 (s, 3H, Me-19), 0.88 (s, 3H, Me-18). ^{13}C NMR ($CDCl_3$): δ 174.8 (C5′-thiodiazole), 164.6 (C-20), 156.1 (C2′-thiodiazole), 141.2 (C-5), 132.0, 130.2, 128.1, 122.6 ($C_{arom.}$), 120.5 (C-6), 69.6 (C-3), 56.0 (C-14), 49.4 (C-9), 43.2 (C-13), 42.2 (C-4), 38.9 (C-12), 38.0 (C-1), 36.8 (C-10), 31.4 (Me-21), 31.8 (C-8), 31.5 (C-7), 29.2 (C-2), 24.0 (C-15), 22.2 (C-16 + C-17), 20.6 (C-11), 19.1 (Me-19), 12.9 (Me-18). EI: *m/z*: 554 $[M]^+$. Anal. calcd. for $C_{29}H_{36}ClN_3OS$ (554.58): C, 62.81; H, 6.54; N, 7.58. Found: C, 62.70; H, 6.39; N, 7.40.

*17-((5-(4-Chlorophenyl-1,3,4-thiadiazol-2-yl)imino)-5-pregnen-3β-ol (**14**)*

From 2-amino-5-(4-chlorophenyl-1,3,4-thiadiazole (**9**) (211 mg). Yield: 398 mg (78%), m.p.: 176–178°C, $R_f = 0.36$. ^{1}H NMR ($CDCl_3$): δ 7.77 (d, 2H, $J = 8.8$ Hz, $H_{arom.}$), 7.52 (d, 2H, $J = 8.8$ Hz, $H_{arom.}$), 5.26 (dd, 1H, $J = 3.1$, 2.2 Hz, H-6), 4.60 (d, 1H, $J = 4.6$ Hz, OH), 3.32 (m, 1H, H-3), 2.58 (t, $J = 9.1$ Hz, H-17), 2.19 (m, 2H, CH_2-4), 2.04 (m, 2H, H-12a + H-16a), 1.97 (s, 3H, Me-21), 1.89 (m, 1H, H-7a), 1.78 (m, 1H, H-1a), 1.69 (m, 1H, H-2a), 1.58–1.56 (m, 4H, H-7b + H-11a + H-15a + H-16b), 1.41–1.37 (m, 4H, H-2b + H-8 + H-11b + H-12b), 1.13 (m, 2H, H-14 + H-15b), 1.04 (m, 2H, H-1b + H-9), 0.93 (s, 3H, Me-19), 0.85 (s, 3H, Me-18). ^{13}C NMR $CDCl_3$): δ 174.5 (C5′-thiodiazole), 164.6 (C-20), 156.1 (C2′-thiodiazole), 141.2 (C-5), 133.9, 129.8, 129.1, 127.9 ($C_{arom.}$), 120.4 (C-6), 69.9 (C-3), 56.0 (C-14), 49.4 (C-9), 43.2 (C-4 + C-13), 42.2 (C-12), 37.9 (C-1), 36.9 (C-10), 31.4 (s, 3H, Me-21), 31.2 (C-8), 31.0 (C-7), 29.2 (C-2), 24.0 (C-15), 22.2 (C16 + C-17), 20.6 (C-11), 19.1 (Me-19), 12.9 (Me-18). EI: *m/z*: 510 $[M]^+$. Anal. calcd. for $C_{29}H_{36}ClN_3OS$ (510.13): C, 68.28; H, 7.11; N, 8.24. Found: C, 68.09; H, 7.05; N, 8.12.

*17-((5-(4-Nitrophenyl-1,3,4-thiadiazol-2-yl)imino)-5-pregnen-3β-ol (**15**)*

From 2-amino-5-(4-nitrophenyl-1,3,4-thiadiazole (**10**) (244 mg). Yield: 391 mg (75%), m.p.: 188–190°C, $R_f = 0.29$. IR (ν_{max}, cm^{-1}): 3405, 3070, 2929, 1682, 1341, 1051, 853. ^{1}H NMR ($CDCl_3$): δ 8.30 (d, 2H, $J = 9.0$ Hz, $H_{arom.}$), 8.02 (d, 2H, $J = 9.0$ Hz, $H_{arom.}$), 5.26 (dd, 1H, $J = 3.3$, 2.1 Hz, H-6), 4.60 (d, 1H, $J = 4.8$ Hz, OH), 3.32 (m, 1H, H-3), 2.58 (t, 1H, $J = 9.0$ Hz, H-17), 2.10 (m, 2H, CH_2-4), 2.06 (m, 2H, H-12a + H-16a), 1.91 (m, 3H, Me-21), 1.78 (m, 1H, H-7a), 1.75 (m, 1H,

H-1a), 1.60 (m, 1H, H-2a), 1.56–1.52 (m, 4H, H-7b + H-11a + H-15a + H-16b), 1.45–1.33 (m, 4H, H-2b + H-8 + H-11b + H-12b), 1.13 (m, 2H, H.14 + H-15b), 1.04 (m, 2H, H-1b + H-9), 0.93 (s, 3H, Me-19), 0.85 (s, 3H, Me-18). ^{13}C NMR ($CDCl_3$): δ 169.9 (C5′-thiodiazole), 163.8 (C-20), 154.0 (C2′-thiodiazole), 147.3 (C-NO_2), 141.2 (C-5), 136.8, 127.0, 124.4 ($C_{arom.}$), 120.2 (C-6), 69.9 (C-3), 56.0 (C-14), 49.4 (C-9), 43.2 (C-4 + C-13), 42.2 (C-12), 37.9 (C-1), 36.9 (C-1), 36.1 (C-10), 31.4 (Me-21), 31.2 (C-8), 31.0 (C-7), 29.1 (C-2), 24.0 (C-15), 22.2 (C-16 + C-17), 20.6 (C-11), 19.1 (Me-19), 12.9 (Me-18). EI: *m/z*: 520 [M]. Anal. calcd. for $C_{29}H_{36}N_4O_3S$ (520.69): C, 66.89; H, 6.97; N, 10.76. Found: C, 66.72; H, 6.88; N, 10.58.

*General procedure for the preparation of pregnenolone sulfonates (**24**–**31**)*

To a stirred solution of **5** (316 mg, 1.00 mmol) in abs. pyridine (20 mL) arylsulfonyl chlorides **16**–**23** (1.5 mmol) were added. The reaction mixture was stirred for 6 days at 23°C, and then quenched by 2 N HCl (10 mL), followed by extraction with ethyl acetate. The organic layers were washed with saturated $NaHCO_3$, brine, dried ($MgSO_4$), and filtered. The filtrate was evaporated to dryness and the residue was purified on a SiO_2 column (5 g) (eluent: *n*-hexane/ethyl acetate 3:2) to give the desired product.

*3-β-(4-Fluorophenylsulfonyl)-5-pregnen-20-one (**24**)*

From 4-fluorophenylsulfonyl chloride (**16**) (291 mg). Yield: 299 mg (63%), m.p.: 118–119°C, R_f = 0.57. IR (ν_{max}, cm^{-1}): 2940, 1728, 1631, 1508, 1447, 1370, 1256, 1160, 910. ^{1}H NMR ($CDCl_3$): δ 7.91 (dd, 2H, $J_{2',3'} = J_{5',6'} = 9.0$ Hz, $J_{2',F} = J_{6',F} = 3.0$ Hz, $H_{arom.}$-2′ + $H_{arom.}$-6′), 7.21 (dd, 2H, $J_{3',F} = J_{5',F} = 5.0$ Hz, $H_{arom.}$-3′ + $H_{arom.}$-5′), 5.30 (t, 1H, J = 2.8 Hz, H-6), 4.34 (m, 1H, H-3), 2. 49 (t, J = 9.0 Hz, H-17), 2.15 (m, 2H, CH_2-4), 2.09 (m, 2H, H-12a + H-16a), 2.02 (s, 3H, Me-21), 1.83 (m, 1H, H-7a), 1.80 (m, 1H, H-1a), 1.64 (m, 1H, H-2a), 1.61 (m, 4H, H-7b + H-11a + H-15a + H-16b), 1.41–1.38 (m, 4H, H-2b + H-8 + H-11b + H-12b), 1.22 (m, 2H, H-14 + H-15b), 1.14 (m, 2H, H-1b + H-9), 1.03 (s, 3H, Me-19), 0.70 (s, 3H, M-18). ^{13}C NMR ($CDCl_3$): δ 209.7 (C_{20}=O), 166.8 (d, $J_{C,F}$ = 256 Hz, C-F), 138.9 (C-5), 134.0, 130.8, 130.6, 123.7 ($C_{arom.}$), 116.8 (C-6), 82.9 (C-3), 63.8 (C-17), 57.0 (C-14), 50.0 (C-9), 44.1 (C-13), 39.0 (C-4), 37.1 (C-12), 36.6 (C-10), 31.9 (C-1 + C-7), 30.3 (C-8 + Me-21), 28.9 (C-2), 24.7 (C-15), 23.1 (C-16), 21.2 (C-11), 19.3 (Me-19), 13.4 (Me-18). EI: *m/z*: 474 $[M]^+$. Anal. calcd. for $C_{27}H_{35}FO_4S$ (474.63): C, 68.32; H, 7.43. Found: C, 68.01; H, 7.31.

*3-β-(2-Trifluoromethoxybenzenesulfonyl)-5-pregnen-20-one (**25**)*

From 2-trifluoromethoxyphenylsulfonyl chloride (**17**) (389 mg). Yield: 362 mg (67%), m.p.: 88–90°C, R_f = 0.74. ^{1}H NMR ($CDCl_3$): δ 7.24 (m, 5H, $H_{arom.}$), 5.36 (t, 1H, J = 2.5 Hz, H-6), 3.79 (m, 1H, H-3), 2.57 (t, 1H, J = 8.8 Hz, H-17), 2.19 (m, 2H, CH_2-4), 2.10 (s, 3H, Me-21), 2.03 (m, 2H, H-12a + H-16a), 1.89 (m, 1H, H-7a), 1.84 (m, 1H, H-1a), 1.66 (m, 1H, H-2a), 1.58 (m, 4H, H-7b + H-11a + H-15a + H-16b), 1.47–1.44 (m, 4H, H-2b + H-8 + H-11b + H-12b), 1.19 (m, 2H, H-14 + H-15b), 1.12 (m, 2H, H-1b + H-9), 1.00 (s, 3H, Me-19), 0.61 (s, 3H, Me-18). ^{13}C NMR ($CDCl_3$): δ 209.5 (C_{20}=O), 160.3 (*C*-OCF_3), 140.8 (C-5), 132.1, 126.8, 125.1, 123.2, 122.7, 122.2 ($C_{arom.}$ + C-6), 80.5 (C-3), 63.7 (C-17), 56.9 (C-14), 50.0 (C-9), 43.3 (C-13), 39.1 (C-4), 36.8 (C-10 + C-12), 33.3 (C-1 + C-7), 30.5 (C-8 + Me-21), 28.3 (C-2), 24.5 (C-15), 22.8 (C-16), 21.0 (C-11), 19.3 (Me-19), 13.2 (Me-18). EI: *m/z*: 540 $[M]^+$. Anal. calcd. for $C_{28}H_{35}F_3O_5S$ (540.63): C, 62.20; H, 6.53. Found: C, 61.98; H, 6.48.

*3β-(4-Nitrophenylsulfonyl)-5-pregnen-20-one (**26**)*

From 4-nitrophenylsulfonyl chloride (**18**) (330 mg). Yield: 341 mg (68%), m.p.: 84–86°C, R_f = 0.72. ^{1}H NMR ($CDCl_3$): δ 8.15, 7.98 (2 × d, 4H, J = 8.9 Hz, $H_{arom.}$), 5.38 (t, 1H, J = 2.6 Hz, H-6), 4.10 (m, 1H, H-3), 2.47 (t, 1H, J = 8.9 Hz, H-17), 2.11 (m, 2H, CH_2-4), 2.06 (s, 3H, Me-21), 1.97 (m, 2H, H-12a + H-16a), 1.82 (m, 1H, H-7a), 1.79 (m, 1H, H-1a), 1.64 (m, H, H-2a), 1.57 (m, 4H, H-7b + H-11a + H-15a + H-16b), 1.41–1.34 (m, 4H, H-2b + H-8 + H-11b + H-12b), 1.18 (m, 2H, H-14 + H-15b), 0.93 (s, 3H, Me-19), 0.70 (s, 3H, Me-18). ^{13}C NMR ($CDCl_3$): δ 209.0 (C_{20}=O), 152.0 (C_{arom}-1), 148.1 (C_{arom}-4), 137.3 (C-5), 127.2, 123.4, 122.9, 120.8 ($C_{arom.}$ + C-6), 71.4 (C-3), 63.2 (C-17), 56.8 (C-14), 50.0 (C-9), 44.0 (C-13), 38.8 (C-4 + C-12), 36.8 (C-10), 33.0 (C-1), 33.3 (C-7), 31.4 (C-8), 30.4 (Me-21), 28.2 (C-2), 24.2 (C-15), 23.6 (C-16), 21.0 (C-11), 20.0 (Me-19), 13.5 (Me-18). EI: *m/z*: 501 $[M]^+$. Anal. calcd. for $C_{27}H_{35}NO_6S$ (501.63): C, 64.65; H, 7.03; N, 2.79. Found: C, 64.48; H, 6.96; N, 2.59.

*3β-(3-Cyanophenylsulfonyl)-5-pregnen-20-one (**27**)*

From 3-cyanophenylsulfonyl chloride (**19**) (302 mg). Yield: 293 mg (61%), m.p.: 108–110°C, R_f = 0.77. ^{1}H NMR ($CDCl_3$): δ 7.40 (d, 2H, J = 8.9 Hz, $H_{arom.}$), 7.24 (br s, 1H, $H_{arom.}$), 5.36 (t, 1H, J = 3.0 Hz, H-6), 4.48 (m, 1H, H-3), 2.52 (t, 1H, J = 9.0 Hz, H-17), 2.18 (m, 2H, CH_2-4), 2.11 (s, 3H, Me-21), 2.04 (m, 2H, H-12a + H-16a), 1.88 (m, 1H, H-7a), 1.80 (m, 1H, H-1a), 1.67 (m, H, H-2a), 1.64 (m, 4H, H-7b + H-11a + H-15a + H-16b), 1.42 (m, 4H, H-2b + H-8 + H-11b + H-12b), 1.18 (m, 2H, H-14 + H-15b), 1.14 (m, 2H, H-1b + H-9), 0.98 (s, 3H, Me-19), 0.64 (s, 3H, Me-18). ^{13}C NMR ($CDCl_3$): δ 210.0 (C_{20}=O), 141.1 (C_{arom}-1 + C-5), 128.9, 125.2, 123.3, 122.8 ($C_{arom.}$ + C-6), 114.4 (*C*-CN), 113. (CN), 72.4 (C-3), 64.0 (C-17), 57.0 (C-14), 48.2 (C-9), 44.1 (C-13), 40.3 (C-4 + C-12), 35.2 (C-10), 32.0 (C-1 + C-7), 30.6 (C-8 + Me-21), 28.1 (C-2), 24.5 (C-15), 23.0 (C-16), 21.0 (C-11), 19.3 (Me-19), 13.4 (Me-18). EI: *m/z*: 481 $[M]^+$. Anal. calcd. for $C_{28}H_{35}NO_4S$ (481.65): C, 69.82; H, 7.32; N, 2.91. Found: C, 69.68; H, 7.29; N, 2.80.

*3β-(4-Bromophenylsulfonyl)-5-pregnen-20-one (**28**)*

From 4-bromophenylsulfonyl chloride (**20**) (284 mg). Yield: 342 mg (64%), m.p.: 168–170°C, R_f = 0.54. ^{1}H NMR ($CDCl_3$): δ 7.68, 7.51 (2 × d, 4H, J = 8.8 Hz, $H_{arom.}$), 5.36 (t, 1H, J = 2.8 Hz, H-6), 4.47 (m, 1H, H-3), 2.53 (t, 1H, J = 8.8 Hz, H-17), 2.19 (m, 2H, CH_2-4), 2.12 (s, 3H, Me-21); 2.03 (m, 2H, H-12a + H-16a), 1.87 (m, 1H, H-7a), 1.83 (m, 1H, H-1a), 1.68 (m, 1H, H-2a), 1.63 (m, 4H, H-7b + H-11a + H-15a + H-16b), 1.43 (m, 4H, H-2b + H-8 + H-11b + H-12b), 1.20 (m, 2H, H-14 + H-15b), 1.16 (m, 2H, H-1b + H-9), 0.93 (s, 3H, Me-19); 0.65 (s, 3H, Me-18). ^{13}C NMR ($CDCl_3$): δ 210.0 (C_{20}=O), 141.6 (C-5), 129.1, 125.4, 123.5, 123.0, 122.4 ($C_{arom.}$ + C-6), 68.4 (C-3), 64.0 (C-17), 57.3 (C-14), 50.1 (C-9), 43.6 (C-13), 39.2 (C-4 + C-12), 36.4 (C-10), 34.0 (C-1), 32.0 (C-7), 30.1 (C-8 + Me-21), 29.1 (C-2), 24.7 (C-15), 23.2 (C-16), 21.2 (C-11), 19.0 (Me-19), 13.6 (Me-18). EI: *m/z*: 535 $[M]^+$. Anal. calcd. for $C_{27}H_{35}BrO_4S$ (535.53): C, 60.55; H, 6.59. Found: C, 60.36; H, 6.38.

*3β-(4-Bromo-2-trifluoromethylphenylsulfonyl)-5-pregnen-20-one (**29**)*

From 4-bromo-2-trifluoromethylphenylsulfonyl chloride (**21**) (510 mg). Yield: 378 mg (61%), m.p.: 82–84°C, R_f = 0.75. ^{1}H NMR ($CDCl_3$): δ 7.68 (d, 2H, J = 8.9 Hz, $H_{arom.}$), 7.51 (br s, 1H, $H_{arom.}$), 5.36 (t, 1H, J = 2.4 Hz, H-6), 4.48 (m, 1H, H-3), 2.56 (t, 1H, J = 9.0 Hz, H-17), 2.18 (m, 2H, CH_2-4), 2.11 (s, 3H, Me-21), 2.02 (m, 2H, H-12a + H-16a), 1.86 (m, 1H, H-7a), 1.81 (m, 1H, H-1a), 1.64 (m, 1H, H-2a), 1.61 (m, 4H, H-7b + H-11a + H-15a + H-16b), 1.44 (m, 4H, H-2b + H-

8 + H-11b + H-12b), 1.23 (m, 2H, H-14 + H-15b), 1.13 (m, 2H, H-1b + H-9), 0.88 (s, 3H, Me-19), 0.60 (s, 3H, Me-18). ^{13}C NMR ($CDCl_3$): δ 210.0 (C_{20}=O), 168.0 (*C*-OCF_3), 141.0 (C-5), 132.4, 129.1, 127.0, 123.0, 122.4 ($C_{arom.}$ + C-6), 68.5 (C-3), 64.2 (C-17), 57.1 (C-14), 50.2 (C-9), 43.6 (C-13), 39.4 (C-4), 39.0 (C-12), 36.6 (C-10), 33.6 (C-1), 31.8 (C-7), 30.0 (C-8 + Me-21), 29.2 (C-2), 24.8 (C-15), 23.3 (C-16), 21.2 (C-11), 19.5 (Me-19), 13.5 (Me-18). EI: *m/z*: 619 $[M]^+$. Anal. calcd. for $C_{28}H_{34}BrF_3O_5S$ (619.53): C, 54.28; H, 5.53. Found: C, 54.11; H, 5.46.

3β-(3-Fluoro-4-methoxyphenylsulfonyl)-5-pregnen-20-one (30)

From 3-fluoro-4-methoxyphenylsulfonyl chloride (**22**) (336 mg). Yield: 333 mg (66%), m.p.: 112–114°C, R_f = 0.75. ^{1}H NMR ($CDCl_3$): δ 7.45 (d, 2H, $J_{H,F}$ = 9.0 Hz, $H_{arom.}$), 7.03 (d, 2H, J = 8.9 Hz, $H_{arom.}$-5 + $H_{arom.}$-6), 5.34 (d, 1H, J = 2.8 Hz, H-6), 4.50 (m, 1H, H-3), 3.90 (s, 3H, OMe), 2.54 (t, 1H, J = 9.0 Hz, H-17), 2.19 (m, 2H, CH_2-4), 2.10 (s, 3H, Me-21), 2.03 (m, 2H, H-12a + H-16a), 1.86 (m, 1H, H-7a), 1.82 (m, 1H, H-1a), 1.69 (m, H, H-2a), 1.58 (m, 4H, H-7b + H-11a + H-15a + H-16b), 1.45 (m, 4H, H-2b + H-8 + H-11b + H-12b), 1.20 (m, 2H, H-14 + H-15b), 1.10 (m, 2H, H-1b + H-9), 1.01 (s, 3H, Me-19), 0.61 (s, 3H, Me-18). ^{13}C NMR ($CDCl_3$): δ 209.4 (C_{20}=O), 166.4 (*C*-OMe), 151.1 (C-F, J = 249 Hz), 140.8 (C-5), 137.3, 132.1, 128.9, 126.8, 125.1, 122.7 ($C_{arom.}$ + C-6), 67.0 (C-3), 63.7 (C-17), 57.1 (C-14), 56.3 (OMe), 50.0 (C-9), 44.0 (C-13), 39.1 (C-4), 38.8 (C-12), 36.4 (C-10), 33.3 (C-1), 31.7 (C-7), 31.5 (C-8 + Me-21), 29.5 (C-2), 24.4 (C-15), 22.8 (C-16), 21.0 (C-11), 19.3 (Me-19), 13.2 (Me-18). EI: *m/z*: 504 $[M]^+$. Anal. calcd. for $C_{28}H_{37}FO_5S$ (504.65): C, 66.64; H, 7.39. Found: C, 66.49; H, 7.30.

3β-(4-Bromo-2,4-difluorophenylsulfonyl)-5-pregnen-20-one (31)

From 4-bromo-2,4-difluorophenylsulfonyl chloride (**23**) (437 mg). Yield: 406 mg (71%), m.p.: 104–106°C, R_f = 0.80. ^{1}H NMR ($CDCl_3$): δ 7.68 (m, 1H, $H_{arom.}$), 7.51 (m, 1H, $H_{arom.}$), 5.35 (d, 1H, J = 2.7 Hz, H-6), 4.20 (m, 1H, H-3), 2.50 (t, 1H, J = 9.0 Hz, H-17), 2.20 (m, 2H, CH_2-4), 2.10 (s, 3H, Me-21), 2.02 (m, 2H, H-12a + H-16a), 1.90 (m, 1H, H-7a), 1.79 (m, 1H, H-2a), 1.64 (m, 4H, H-7b + H-11a + H-15a + H-16b), 1.45 (m, 4H, H-2b + H-8 + H-11b + H-12b), 1.23 (m, 2H, H-14 + H-15b), 1.15 (m, 2H, H-14 + H-15b), 1.10 (m, 2H, m, 2H, H-1b + H-9), 0.93 (s, 3H, Me-19), 0.61 (s, 3H, Me-18). ^{13}C NMR ($CDCl_3$): δ 219.4 (C_{20}=O), 163.0 (C5′-F, $J_{C5',F}$ = 259 Hz), 155.0 (C2′-F, $J_{C2',F}$ = 258 Hz), 141.8 (C-5), 126.8, 125.1, 122.7, 122.5, 122.1 ($C_{arom.}$ + C-6), 117.8 (C-Br), 68.1 (C-3), 63.9 (C-17), 57.3 (C-14), 50.2 (C-9), 44.1 (C-13), 39.1 (C-4), 38.6 (C-12), 36.5 (C-10), 32.5 (C-1), 31.8 (C-7), 31.3 (C-8 + C-21), 29.1 (C-2), 24.5 (C-15), 23.0 (C-16), 21.2 (C-11), 19.5 (Me-19), 13.3 (Me-18). EI: *m/z*: 571 $[M]^+$. Anal. calcd. for $C_{27}H_{33}BrF_2O_4S$ (571.51): C, 56.74; H, 5.82. Found: C, 56.59; H, 5.77.

General procedure for the preparation of acylated pregnenolone derivatives (37–41)

To a stirred solution of **5** (1.00 mmol) in abs. pyridine (20 mL) and DMAP (mg) were added acyl chlorides **32**–**36** (1.5 mmol), and the mixture was heated under reflux for 10 h. The reaction was quenched by adding 2 N HCl (20 mL) and extracted with ethyl acetate (20 mL). The mixture organic layer was washed with saturated $NaHCO_3$ and brine, dried over ($MgSO_4$), filtered, and evaporated to dryness. The product was purified on SiO_2 column using ethyl acetate/*n*-hexane as an eluent to give the desired product.

3β-(4-Chloro-3-nitrobenzoyl)-5-pregnen-20-one (37)

From 4-chloro-3-nitrobenzoyl chloride (**32**) (330 mg). Yield: 345 mg (69%), m.p.: 140–142°C, R_f = 0.33. ^{1}H NMR ($CDCl_3$): δ 8.77 (d, 1H, $J_{2',6'}$ = 2.2 Hz, $H_{arom.}$-2), 8.22 (dd, 2H, $J_{5',6'}$ = 8.8 Hz, $H_{arom.}$-5 + $H_{arom.}$-6), 5.40 (t, 1H, J = 5.5 Hz, H-6), 4.84 (m, 1H, H-3), 2.52 (t, 1H, J = 9.0 Hz, H-17), 2.20 (m, 2H, CH_2-7), 2.11 (s, 3H, Me-21), 2.00 (m, 2H, H-12a + H-16a), 1.91 (m, 1H, H-7a), 1.80 (m, 1H, H-1a), 1.70 (m, 1H, H-2a), 1.60 (m, 4H, H-7b + H-11a + H-15a + H-16b), 1.51 (m, 4H, H-2b + H-8 + H-11b + H-12b), 1.26 (m, 2H, H-14 + H-15b), 1.17 (m, 2H, H-1b + H-9), 1.06 (s, 3H, Me-19), 0.62 (s, 3H, Me-18). ^{13}C NMR ($CDCl_3$): δ 209.5 (C_{20}=O), 163.7 (C=O), 158.0 (*C*-NO_2), 138.0 (C-5), 133.2, 127.2 ($C_{arom.}$), 123.4 (C-6), 75.3 (C-3), 63.7 (C-17), 56.9 (C-14), 50.0 (C-9), 44.0 (C-13), 38.8 (C-4), 38.1 (C-12), 37.0 (C-10), 36.7 (C-1), 31.8 (C-7 + C-8 + Me-21), 27.8 (C-2), 24.5 (C-15), 22.9 (C-16), 21.0 (C-11), 19.3 (Me-19), 13.2 (Me-18). EI: *m/z*: 500 $[M]^+$. Anal. calcd. for $C_{28}H_{34}ClNO_5$ (500.03): C, 67.26; H, 6.85; N, 2.80. Found: C, 67.11; H, 6.75; N, 2.68.

3β-(2,4,5-Trifluoro-3-methoxybenzoyl)-5-pregnen-20-one (38)

From 2,4,5-trifluoro-3-methoxybenzoyl chloride (**33**) (291 mg). Yield: 294 mg (62%), m.p.: 150–152°C, R_f = 0.66. ^{1}H NMR ($CDCl_3$): δ 7.43 (m, 1H, $H_{arom.}$); 5.40 (t, 1H, J = 5.1 Hz, H-6), 4.83 (m, 1H, H-3), 4.02 (s, 3H, O-Me), 2.54 (t, 1H, J = 9.0 Hz, H-17), 2.45 (m, 2H, CH_2-4), 2.20 (m, 2H, CH_2-4), 2.11 (s, 3H, Me-21), 2.03 (m, 2H, H-12a + H-16a), 1.98 (m, 1H, H-7a), 1.86 (m, H, H-1a), 1.70 (m, 1H, H-2a), 1.62 (m, 4H, H-7b + H-11a + H-15a + H-16b), 1.58–1.44 (m, 4H, H-2b + H-8 + H-11b + H-12b), 1.23 (m, 2H, H.14 + H-15b), 1.20 (m, 2H, H-14 + H-15b), 1.18 (m, 2H, H-1b + H-9), 1.04 (s, 3H, Me-19), 0.62 (s, 3H, Me-18). ^{13}C NMR ($CDCl_3$): δ 209.4 (C_{20}=O), 162.0 (C=O), 148.6, 145.2, 143.2 ($C_{arom.}$), 138.9 (C-5), 134.5, 129.4 ($C_{arom.}$), 123.2 (C-6), 110.0 ($C_{arom.}$), 75.6 (C-3), 63.7 (C-17 + OMe), 57.0 (C-14), 51.0 (C-9), 44.1 (C-13), 38.8 (C-4), 38.3 (C-12), 37.2 (C-10), 36.5 (C-1), 32.0 (C-7 + C-8 + Me-21), 28.0 (C-2), 24.7 (C-15), 22.9 (C-16), 21.1 (C-11), 19.3 (Me-19), 13.3 (Me-18). EI: *m/z*: 474 [M]. Anal. calcd. for $C_{29}H_{35}F_3O_4$ (504.58): C, 69.03; H, 6.99. Found: C, 70.19; H, 6.92.

3β-(3,5-Dinitrobenzoyl)-5-pregnen-20-one (39)

From 3,5-dinitrobenzoyl chloride (**34**) (345 mg). Yield: 342 mg (67%), m.p.: 206–208°C, R_f = 0.59. ^{1}H NMR ($CDCl_3$): δ 9.20 (t, 2H, J = 2.2 Hz, $H_{arom.}$-2 + $H_{arom.}$-6), 9.13 (d, 1H, J = 2.2 Hz, $H_{arom.}$-4), 5.44 (t, 1H, J = 5.0 Hz, H-6), 5.00 (m, 1H, H-3), 2.53 (t, 1H, J = 9.0 Hz, H-17), 2.20 (m, 2H, CH_2-4), 2.13 (s, 3H, Me-21), 2.03 (m, 2H, H-12a + H-16a), 2.00 (m, 1H, H-7a), 1.82 (m, 1H, H-1a), 1.70 (m, 1H, H-2a), 1.64 (m, 4H, H-7b + H-11a + H-15a + H-16b), 1.58–1.45 (m, 4H, H-2b + H-8 + H-11b + H-12b), 1.23 (m, 2H, H-14 + H-15b), 1.20 (m, 2H, H.14 + H-15b), 1.18 (m, 2H, H-1b + H-9), 1.08 (s, 3H, Me-19), 0.63 (s, 3H, Me-18). ^{13}C NMR ($CDCl_3$): δ 209.4 (C_{20}=O), 161.9 (C=O), 148.7 ($C_{arom.}$-3 + $C_{arom.}$-5), 139.0 (C-5), 134.7 ($C_{arom.}$-1), 129.4 ($C_{arom.}$-2 + $C_{arom.}$-6), 123.6 (C-6), 122.2 ($C_{arom.}$-4), 76.7 (C-3), 63.9 (C-17), 56.8 (C-14), 50.0 (C-9), 44.2 (C-13), 38.8 (C-4), 38.4 (C-12), 37.1 (C-10), 36.6 (C-1), 31.6 (C-7 + C-8 + Me-21), 27.7 (C-2), 24.5 (C-15), 23.2 (C-16), 21.0 (C-11), 19.4 (Me-19), 13.3 (Me-18). EI: *m/z*: 510 $[M]^+$. Anal. calcd. for $C_{28}H_{34}N_2O_7$ (510.58): C, 65.87; H, 6.71; N, 5.49. Found: C, 65.68; H, 6.62; N, 5.29.

3β-(3-Methoxybenzoyl)-5-pregnen-20-one (40)

From 3-methoxybenzoyl chloride (**35**) (278 mg). Yield 320 mg (71%), m.p.: 156–158°C. R_f = 0.68. ^{1}H NMR ($CDCl_3$): δ 7.62 (dd, 1H, J = 7.7, 2.6 Hz, $H_{arom.}$-5), 7.60 (d, 1H, J = 7.7 Hz, $H_{arom.}$-6), 7.54 (d,

1H, $J = 2.6$ Hz, $H_{arom.}$-2), 7.06 (dd, 1H, $J = 7.7$, 2.5 Hz, $H_{arom.}$-4), 5.40 (t, $J = 5.1$ Hz, H-6); 4.83 (m, 1H, H-3), 3.83 (s, 3H, OMe), 2.54 (t, $J = 9.1$ Hz, H-17), 2.50 (m, 2H, CH_2-4), 2.20 (m, 2H, CH_2-7), 2.11 (s, 3H, Me-21), 2.01 (m, 2H, H-12a + H-16a), 1.93 (m, 1H, H-7a), 1.85 (m, 1H, H-1a), 1.70 (m, 1H, H-2a), 1.62 (m, 4H, H-7b + H-11a + H-15a + H-16b), 1.57–1.44 (m, 4H, H-2b + H-8 + H-11b + H-12b), 1.24 (m, 2H, H-14 + H-15b), 1.18 (m, 2H, H-1b + H-9), 1.06 (s, 3H, Me-19), 0.62 (s, 3H, Me-18). ^{13}C NMR ($CDCl_3$): δ 209.5 (C_{20}=O), 165.9 (C=O), 159.5 ($C_{3'}$-OMe), 139.7 (C-5), 132.1 ($C_{arom.}$-1), 129.2 ($C_{arom.}$-5), 122.5 (C-6), 119.2 ($C_{arom.}$-4), 114.1 ($C_{arom.}$-2), 74.5 (C-3), 63.7 (C-17), 56.9 (C-14), 55.4 (O-Me), 49.9 (C-9), 44.0 (C-13), 38.8 (C-4), 38.1 (C-12), 37.0 (C-10), 36.7 (C-1), 31.8 (C-7 + C-8 + Me-21), 27.8 (C-2), 24.5 (C-15), 22.8 (C-16), 21.0 (C-11), 19.3 (Me-19), 13.2 (Me-18). EI: *m/z*: 450 [M]. Anal. calcd. for $C_{29}H_{38}O_4$ (450.61): C, 77.30; H, 8.50. Found: C, 77.14; H, 8.41.

3β-(3-O-Benzoyl)-5-pregnen-20-one (41)

From benzoyl chloride (**36**) (211 mg). Yield: 307 mg (73%), m.p.: 182–184°C, $R_f = 0.67$. IR (ν_{max}, cm^{-1}): 2940, 1712, 1450, 1355, 1271, 1112, 1026, 945. ^{1}H NMR ($CDCl_3$): $\delta = 8.04$ (m, 5H, $H_{arom.}$), 5.42 (t, $J = 5.0$ Hz, H-6), 4.90 (m, 1H, H-3), 2.60 (t, 1H, $J = 9.0$ Hz, H-17), 2.50 (m, 2H, CH_2-4), 2.20 (m, 2H, CH_2-7), 2,13 (s, 3H, Me-21), 2.07 (m, 2H, H-12a + H-16a), 2.00 (m, 1H, H-7a), 1.80 (m, 1H, H-1a), 1.70 (m, 1H, H-2a), 1.64 (m, 4H, H-7b + H-11a + H-15a + H-16b), 1.56–1.44 (m, 4H, H-2b + H-8 + H-11b + H-12b), 1.25 (m, 2H, H-14 + H-15b), 1.22 (m, 2H, H-1b + H-9), 1.07 (s, 3H, Me-19), 0.64 (s, 3H, Me-18). ^{13}C NMR ($CDCl_3$): δ 209.5 (C_{20}=O), 166.0 (C=O), 139.9 (C-5), 132.7, 130.8, 129.5, 128.3 ($C_{arom.}$), 122.5 (C-6), 74.4 (C-3), 63.7 (C-17), 56.8 (C-14), 50.1 (C-9), 44.2 (C-13), 38.7 (C-4), 38.2 (C-12), 37.2 (C-10), 36.9 (C-1), 31.7 (C-7 + C-8 + Me-21), 28.0 (C-2), 24.7 (C-15), 22.9 (C-16), 21.2 (C-11), 19.5 (Me-19), 13.3 (Me-18). EI: *m/z*: 420 [M]. Anal. calcd. for $C_{28}H_{36}O_3$ (420.58): C, 79.96; H, 8.63. Found: C, 79.96; H, 8.52.

Biological evaluations

CYP17 enzyme preparation

The coexpression of human CYP17 and rat NADPH-P450-reductase in *Escherichia coli* and the isolation of the membrane fractions were performed as described by Hartmann and coworkers [44]. Membrane fractions were diluted to a concentration that gives a 15–25% conversion in the controls for the different assays.

17α-Hydroxylase enzyme assay

Determination of the hydroxylase activity of CYP17 was performed by measurement of the conversion of P5 to 17OH-P5. An assay mixture consisting of 140 μL phosphate buffer (0.05 M, pH 7.4, 1 mM $MgCl_2$, 0.1 mM EDTA, and 0.1 mM dithiothreitol), 50 μL NADPH generating system (in phosphate buffer with 50 mM glucose-6-phosphate, 5.75 mM $NADP^+$, and 27.5 U/mL 5 glucose-6-phosphate dehydrogenase) and 5 μL substrate solution (25 μM [3H]-P5) was preincubated at 37°C for 5 min. The reaction was started by addition of 50 μL enzyme preparation. Compared to the lyase assay, however, enzyme concentration had to be reduced to keep control conversion in the favorable range of 15–25% and to prevent DHEA formation. After a 30 min incubation at 37°C, the enzyme reaction was stopped by addition of 50 μL 1 N HCl. Extraction of the steroids was performed by addition of 1000 μL ethylacetate and vigorous shaking for 10 min. After a centrifugation step (5 min, 15,000g), 900 μL of the organic phase were removed and transferred into a fresh tube containing 250 μL phosphate buffer and 50 μL 1 N HCl. Shaking and centrifugation was repeated as described above. 800 μL ethylacetate solution was evaporated to dryness in a fresh tube and redissolved in 40 μL acetonitrile/water (1:1) for HPLC analysis.

HPLC methods

HPLC separation of the steroids was performed using an Agilent 1100 HPLC system with PDA detector (Böblingen, Germany), a CC 125/3 Nucleodur 100-3 C-18 ec column (Macherey-Nagel, Düren, Germany) and a Berthold radioflow detector LB509 with scintillator pump (Bad Wildbad, Germany). Quickszint Flow 302 LSC Cocktail (Zinsser Analytic, Frankfurt/Main, Germany) was used as scintillator fluid. More details are reported in [45].

We thank Basrah University (Iraq) for a scholarship to D. S. Ali. Mr. U. Haunz and Miss A. Friemel of the Chemistry Department, University of Konstanz (Germany) are gratefully acknowledged for the 2D-NMR experiments.

The authors have declared no conflict of interest.

References

[1] E. Baston, F. R. Leroux, *Recent Patents Anti-Cancer Drug Discov.* **2007**, *2*, 31–58.

[2] V. M. A. Moreira, J. A. R. Salvador, T. S. Vasaitis, V. C. Njar, *Curr. Med. Chem.* **2008**, *15*, 868–899.

[3] V. M. A. Moreira, T. S. Vasaitis, Z. Guo, V. C. O. Njar, J. A. R. Salvador, *Steroids* **2008**, *73*, 1217–1227.

[4] V. M. A. Moreira, T. S. Vasaitis, V. C. O. Njar, J. A. R. Salvador, *Steroids* **2007**, *72*, 939–948.

[5] C. P. Owen, *Anti-Cancer Agents Med. Chem.* **2009**, *9*, 613–626.

[6] C. J. Pezaro, D. Mukherji, J. S. De Bono, *Drug Discov. Today* **2012**, *17*, 221–226.

[7] T. S. Vasaitis, R. D. Bruno, V. C. O. Njar, *J. Steroid Biochem. Mol. Biol.* **2011**, *125*, 23–31.

[8] C. Lee, *Br. J. Urol.* **1997**, *79*, 21–27.

[9] R. M. Lunn, D. A. Bell, J. L. Mohler, J. A. Taylor, *Carcinogenesis* **1999**, *20*, 1727–1731.

[10] S. E. Barrie, M. Jarman, *Endocr. Relat. Cancer* **1996**, *3*, 25–39.

[11] M. Jarman, H. J. Smith, P. J. Nicholls, C. Simons, *Nat. Prod. Rep.* **1998**, *15*, 495–512.

[12] V. C. Njar, A. M. Brodie, *Curr. Pharm. Des.* **1999**, *5*, 163–180.

[13] R. W. Hartmann, P. B. Ehmer, S. Haidar, M. Hector, J. Jose, C. D. Klein, S. B. Seidel, T. Sergejew, B. G. Wachall, G. A. Wachter, *Arch. Pharm. Pharm. Med. Chem.* **2002**, *335*, 119–128.

[14] F. Leroux, *Curr. Med. Chem.* **2005**, *12*, 1623–1629.

[15] T. Hakki, R. Bernhardt, *Pharmacol. Ther.* **2006**, *111*, 27–52.

[16] A. Pont, P. L. Williams, S. Azhar, R. E. Reitz, C. Bochra, E. R. Smith, D. A. Stevens, *Arch. Intern. Med.* **1982**, *142*, 2137–2140.

[17] R. J. Santen, H. Van den Bossche, J. Symoens, J. Brugmans, R. De Coster, *J. Clin. Endocrinol. Metab.* **1983**, *57*, 732–736.

[18] G. Lake-Bakaar, P. J. Scheuer, S. Sherlock, *Br. Med. J.* **1987**, *294*, 419–422.

[19] A. Schappach, H. D. Höltje, *Pharmazie* **2001**, *56*, 835–884.

[20] V. C. Njar, G. T. Klus, A. M. H. Brodie, *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2777–2782.

[21] V. C. Njar, K. Kato, I. P. Nnane, D. N. Grigoryev, B. J. Long, A. M. Brodie, *J. Med. Chem.* **1998**, *41*, 902–912.

[22] A. Brodie, V. C. Njar, *US Patent 6,200,965 B1*, **2001**.

[23] V. D. Handratta, D. Jelovac, B. J. Long, R. Kataria, I. P. Nnane, V. C. Njar, A. M. Brodie, *J. Steroid Biochem. Mol. Biol.* **2004**, *92*, 155–165.

[24] V. D. Handratta, T. S. Vasaitis, V. C. Njar, L. K. Gediya, R. Kataria, P. Chopra, D. Newman, R. Farquhar, Z. Guo, Y. Qiu, A. M. Brodie, *J. Med. Chem.* **2005**, *48*, 2972–2984.

[25] A. Brodie, V. C. Njar, WO Patent 2006/093993, **2006**.

[26] T. S. Vasaitis, V. C. O. Njar, *Future Med. Chem.* **2010**, *2*, 667–680.

[27] A. Molina, A. Belldegrun, *J. Urol.* **2011**, *185*, 787–794.

[28] J. S. de Bono, C. J. Logothetis, A. Molina, K. Fizazi, S. North, L. Chu, K. N. Chi, R. J. Jones, O. B. Goodman, F. Saad, J. N. Staffurth, P. Mainwaring, S. Harland, T. W. Flaig, T. E. Hutson, T. Cheng, H. Patterson, J. D. Hainsworth, C. J. Ryan, C. N. Sternberg, S. L. Ellard, A. Fléchon, M. Saleh, M. Scholz, E. Efstathiou, A. Zivi, D. Bianchini, Y. Loriot, N. Chieffo, T. Kheoh, C. M. Haqq, H. I. Scher, *N. Engl. J. Med.* **2011**, *364*, 1995–2005.

[29] A. Bryce, C. J. Ryan, *Clin. Pharmacol. Ther.* **2012**, *91*, 101–108.

[30] G. A. Potter, S. E. Barrie, M. Jarman, M. G. Rowlands, *J. Med. Chem.* **1995**, *38*, 2463–2471.

[31] W. Willker, D. Leibfritz, R. Kerssebaum, W. Bermel, *Magn. Reson. Chem.* **1993**, *31*, 287–292.

[32] A. L. Davis, J. Keeler, E. D. Laue, D. Moskau, *J. Magn. Reson.* **1992**, *98*, 207–216.

[33] N. M. DeVore, E. E. Scott, *Nature* **2012**, *282*, 116–119.

[34] J. A. R. Salvador, V. M. Moreira, S. M. Silvestre, in *Advances in Prostate Cancer* (Ed.: G. Hamilton), Licensee InTech, **2013**, Chap. 12.

[35] J. K. Yano, M. R. Wester, G. A. Schoch, K. J. Griffin, C. D. Stout, E. F. Johnson, *J. Biol. Chem.* **2004**, *279*, 38091–38094.

[36] G. M. Morris, D. S. Goodsell, R. S. Halliday, R. Huey, W. E. Hart, R. K. Belew, A. J. Olson, *J. Comput. Chem.* **1994**, *19*, 1639–1662.

[37] S. Ulmschneider, U. Müller-Vieira, M. Mitrenga, R. W. Hartmann, S. Oberwinkler-Marchais, C. D. Klein, M. Bureik, R. Bernhardt, I. Antes, T. J. Lengauer, *J. Med. Chem.* **2005**, *48*, 1796–1805.

[38] S. Ulmschneider, U. Müller-Vieira, C. D. Klein, I. Antes, T. Lengauer, R. W. Hartmann, *J. Med. Chem.* **2005**, *48*, 1563–1575.

[39] M. Voets, I. Antes, C. Scherer, U. Müller-Vieira, K. Biemel, C. Barassin, S. Oberwinkler-Marchais, R. W. Hartmann, *J. Med. Chem.* **2005**, *48*, 6632–6642.

[40] M. J. S. Dewar, E. G. Zoebisch, E. F. Healy, J. P. P. Stewart, *J. Am. Chem. Soc.* **1985**, *107*, 3902–3909.

[41] C. C. J. Roothaan, *Rev. Mod. Phys.* **1951**, *23*, 69–89.

[42] P. Fletscher, *Practical Methods of Optimization*, Wiley, New York **1990**.

[43] C. D. Selassie, in *Burger's Medicinal Chemistry and Drug Discovery*, Vol. *1*, 6th edition (Ed.: D. J. Abraham), Wiley, New York **2003**.

[44] T. U. Hutschenreuter, P. B. Ehmer, R. W. Hartmann, *J. Enzyme Inhib. Med. Chem.* **2004**, *19*, 17–32.

[45] S. J. Krug, Q. Hu, R. W. Hartmann, *J. Steroid Biochem. Mol. Biol.* **2013**, *134*, 75–79.

