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ORIGINAL RESEARCH

Synthesis and CYP17α hydroxylase inhibition activity of new 3α- and 3β-ester derivatives of pregnenolone and related ether analogues

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Abstract A new class of 3α-ester derivatives of pregnenolone, using Mitsunobu reaction conditions, is described. The scheme involved the conversion of pregnenolone (**4**) into 20-(2-hydroxyethyl)imino-pregn-5-en-3β-ol (**8**), followed by tritylation to give the analogue **9**. Treatment of **9** with various aryl carboxylic acids afforded the tritylated 3α-ester pregnenolone analogues **18–23**. Detritylation with AcOH furnished the 3α-substituted aryl ester derivatives **24–29**. Analogously, the 3β-ester analogues **31** and **32** were synthesized from pregnenolone (**4**) and its analogue **30**, using Steglich coupling method, by treatment with rhodamine B in the presence of DCC/DMAP. These derivatives were screened for their CYP17α hydroxylase inhibition activity expressed in *Escherichia coli*. Compound **27** was the most active inhibitor among both series, with IC_{50} value of 1.12 μM and selectivity profile of 88.56 % inhibition of CYP17α hydroxylase enzyme.

Keywords CYP17α hydroxylase · Mitsunobu reaction · Pregnenolone · Prostate cancer · Steglich coupling method

Introduction

Pregnenolone is a known neuroactive steroid (Marx *et al.*, 2011; Ritnner *et al.*, 2014; Marx *et al.*, 2014) and may represent a promising and mechanistically novel agent for cognitive and negative symptoms in schizophrenia and for the treatment of acute or chronic lesions of the nervous system, especially certain neurodegenerative diseases (Flood *et al.*, 1992). Pregnenolone and its sulfated derivative enhance learning and memory in animal models at concentrations that are physiologically relevant and known to be present in human brain (Akwa *et al.*, 2001; Darnaudéry *et al.*, 1998; Vallee *et al.*, 2001; Wong *et al.*, 2015). Further, pregnenolone sulfate (**1**) is known to have antidepressant, anxiogenic, and proconsultant effects (Reddy, 2010). Recently, Baulieu *et al.*, (2011), Bianchi and Baulieu (2012) reported that 3β-methoxypregnenolone (MAP4343) (**2**) is an innovative therapeutic approach for depressive disorders, which associated with neuronal abnoramalties in brain microtubule function, including changes in α-tubulin isoforms.

The presence of different functional groups located around the rigid tetracyclic core of pregnenolone and analogues leads to diversity in the biological actions as these serve as substrates for different targets. Abiraterone acetate (Zytiga) (**3**) (de Bono *et al.*, 2011; Bryce and Ryan, 2012), galeterone and its Δ^4-3-keto derivative (Handratta *et al.*, 2004, 2005; Brodie and Njar, 2006), both

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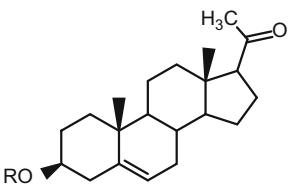
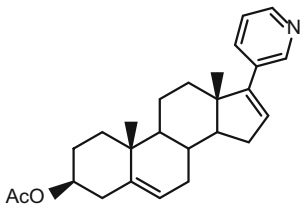
pregnenolone derivatives having pyridine and imidazole moieties at C-17, respectively, were designed for treatment of prostate cancer (PC) by inhibition of the enzyme 17α-hydroxylase/C17,20-lyase (CYP17A1). Hartmann *et al.* (2002; Hu *et al.*, 2010; Haidar *et al.*, 2003) have reported the synthesis of several CYP17 inhibitors as a new strategy for the treatment of prostate carcinoma. Much more recently, we have synthesized new series of pregnenolone having imino-benzothiazoles (Al-Masoudi *et al.*, 2014, 2015b) and biaryl-chalcones (Al-Masoudi *et al.*, 2015a) at C-20, which showed remarkable inhibition of CYP17 hydroxylase activity.

In continuation of our ongoing work on the synthesis of new 3α-pregnenolone analogues (Mahdi *et al.*, 2015), we report here a novel series of 3α-ester derivatives of pregnenolone having 2-hydroxyethyl amine at C-20 with inversion in configuration at C-3, synthesized by applying of Mitsunobu reaction (Mitsunobu and Eguchi, 1971; Mitsunobu, 1981) aiming to develop new steroid analogues for treatment of prostate cancer via the inhibition of CYP17α hydroxylase enzyme.



1. R = SO_3H
2. R = Me



3. Abiraterone acetate

Results and discussion

Chemistry

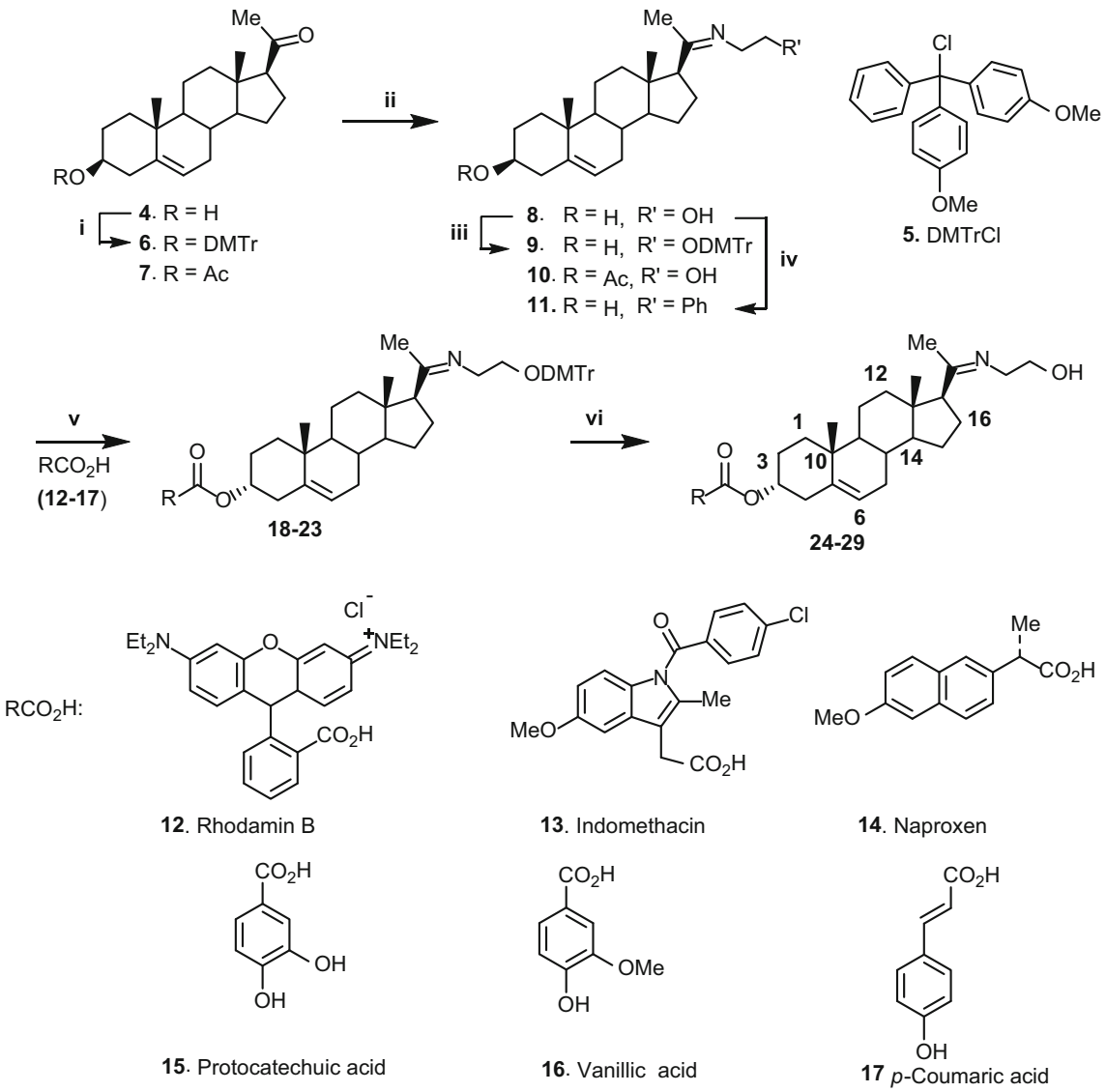
Potential activity of the metabolite 3β-methoxy-pregnenolone (**2**) for treatment of the degenerative diseases prompted us to introduce much more bulky ether group rather than small methoxy residue at C-3 of pregnenolone, aiming to develop a new compound having novel properties and/or affecting novel molecular targets. Thus, treatment of **4** with 4,4′-dimethoxytrityl chloride (DMTrCl) in the presence of pyridine and DMAP at 70 °C afforded after chromatography, the 3β-*O*-tritylated pregnenolone **6** (46 %) (Scheme 1). The structure of **6** was identified by ^{1}H and ^{13}C NMR spectra. In the ^{1}H NMR spectrum, the multiplets in the regions $\delta = 7.75$–6.63 ppm were assigned to thirteen aromatic protons, while the two singlets at δ 3.86 and 3.72 ppm were attributed to the two methoxy groups of trityl moiety. In the ^{13}C NMR spectrum, the resonance at $\delta = 208.5$, 140.2, and 106.7 ppm was assigned to C-20, C-5, and C_{trityl}-*O* carbon atoms, respectively, while the resonance at $\delta = 55.0$ ppm attributed to two methoxy groups. The aromatic and aliphatic carbon atoms were fully analyzed (c.f. "Experimental" section).

Next, compound **4** and its acetate **7** were used in the synthesis of new imine derivatives. Initially, **4** and **7** were reacted with 2-aminoethanol in the presence of glacial AcOH to give the imino-ethanol analogues **8** and **10** in 52 and 61 % yields, respectively. Similarly, treatment of **4** with 2-phenylethylamine afforded the imino derivative **11** (42 %) (Scheme 1). The structures of **8**, **10,** and **11** were identified from their ^{1}H and ^{13}C NMR spectra. In the ^{1}H NMR spectra, CH_2OH and CH_2Ph protons resonated as multiplets at δ 4.04, 4.68, and 2.87 ppm, respectively, while NCH_2 protons appeared as multiplets at $\delta = 1.80$, 1.82, and 2.74 ppm, respectively. The protons of aromatic and pregnene backbones were fully identified (c.f. "Experimental" section).

Mitsunobu reaction is widely used for the inversion of configuration in secondary alcohol derivatives. It has demonstrated a very excellent reactive ability, and efforts have been made toward widening its utilization scope. Some laboratories used Mitsunobu reaction in steroid chemistry for synthesis of potentially active steroids, such as esterification of sterically hindered 17-hydroxy steroids (Tapolcsányiv *et al.*, 2004), conversion of allopregnanolone to isoallopregnanolone (Varasi *et al.*, 1987), as well as isoallopregnanolone to allopregnanolone (Purdy *et al.*, 1990), with inversion of configuration at C-3 and synthesis of some purinyl-steroid derivatives (Cadenas *et al.*, 2005).

In our present work, we have selected 20-(2-hydroxyethyl)imino-pregn-5-en-3β-ol (**8**) as a starting material for the synthesis of new series of α-ester of pregnenolone analogues, with inversion of configuration at C-3, by employing Mitsunobu reaction. The aim of selecting 2-imino-ethanol moiety at C-20 is to facilitate the flexibility of binding of the new synthesized α-ester analogues with the amino acids of CYP17α hydroxylase enzyme. Thus, protection of hydroxyl group of **8** by treatment with DMTrCl in dry pyridine and DMAP for 1 h at room temperature furnished **9** (70 %). Reaction of **9** with various substituted benzoic acids, rhodamine B, indomethacin, naproxen, protocatechuic acid, vanillic acid and *p*-coumaric acid **12–17**, respectively, in dry MeCN in the presence of triphenylphosphine (Ph_3P) and diethylazodicarboxylate (DEAD) as catalysts at room temperature afforded **18–23**. These crude products were directly hydrolyzed, without purification, with 80 % AcOH at room temperature for 30 min to give, after chromatographic purification, the 3α-pregnenolone ester analogues **24–29** in 68–76 % yields (Scheme 1).

The structures of **24–29** were assigned on the basis of their NMR (^{1}H, ^{13}C and 2D), which showed rather similar patterns of the proton and carbon atoms of pregnene



Scheme 1 Reagents and conditions: (i) **5**, DMAP, dry pyridine, 70 °C, overnight; (ii) 2-Ethanolamine, EtOH, reflux, 10 h; (iii) **5**, dry pyridine, 1 h, r,t; (iv) 2-phenylethylamine, EtOH, reflux, 16 h; (v) DEAD, Ph_3P, $ArCO_2H$ (12–17), dry MeCN, RT, 12–14 h; (vi) 80 % aq. AcOH, RT, 30 min

scaffold. In the ^{1}H NMR spectra, the triplets in the range of $\delta = 5.26$–5.29 ppm ($J_{5,6} = 2.1$–4.0 Hz) were attributed to H-6, while the multiplets in the regions $\delta = 3.26$–3.39 and 2.02–2.11 ppm were assigned to H-3 and CH_2-4, respectively. CH_2OH methylene protons appeared as multiplets or quartets in the regions $\delta = 3.75$–4.05 ppm ($J \sim 7.0$ Hz), whereas NCH_2 protons resonated as multiplets in the regions $\delta = 1.80$–1.82 ppm. The aromatic protons of rhodamine B moiety of **24** (H_{rhod}-12′ + $H_{rhod.}$-14′) appeared as multiplets in the region $\delta = 7.62$–7.67 ppm, while the two doublets at $\delta = 6.56$ and 6.71 ppm were assigned to H_{rhod}-15′ and H_{rhod}-1′ ($J = 7.6$ and 7.8 Hz), respectively. The multiplets at δ 6.69 and 6.33 ppm were attributed to $H_{rhod.}$-13′ and $H_{rhod..}$-2′ + $H_{rhod.}$-4′ + $H_{rhod.}$-5′, respectively, whereas the two singlets at δ 6.94 and 5.51 ppm were assigned to $H_{Rhod.}$-5′ and $H_{rhod.}$-9′, respectively. The ^{1}H NMR spectrum of **25** showed doublets at $\delta = 7.88$ and 7.57 ppm that were assigned to $H_{arom.}$-2′ + $H_{arom.}$-6′ and $H_{arom.}$-3′ + $H_{arom.}$-5′ ($J = 8.4$ Hz), respectively, while the doublets at $\delta = 6.94$ and 6.70 ppm were attributed to $H_{indometh.}$-4′ and $H_{indometh.}$-6′ ($J = 7.4$ Hz), respectively. $H_{indometh.}$-7′ appeared as a singlet at δ 6.94 ppm. The aromatic protons of **26–29** and other pregnene protons were fully characterized (c.f. "Experimental" section). In the ^{13}C NMR spectra of **24–29**, the resonances in the low-field region of $\delta = 167.9$–169.7 ppm were assigned to C=N, while the resonances in the region $\delta = 161.6$–172.9 ppm were attributed to the carbonyl carbon atoms of ester groups. The

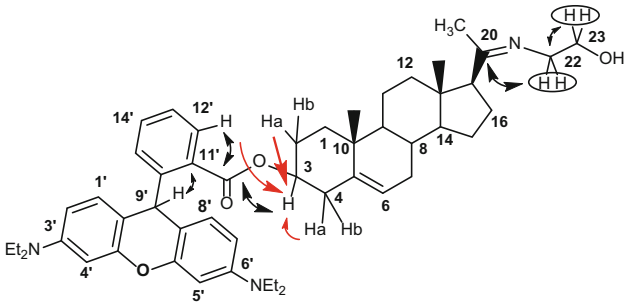


Fig. 1 $J_{C,H}$ correlations in the HMBC (*double-head arrow*), and NOESY (*single-head arrow in red*) correlations of **24**

signals at $\delta = 141.7$–141.8 ppm were attributed to C-5, while C-3 and C-4 resonated in the regions $\delta = 70.5$ and 42.7–42.8, respectively. C-1 appeared at $\delta = 37.4$ ppm, while C-2, C-7, and C-8 were observed together in the region $\delta = 31.6$–31.7 ppm. The signals in the regions $\delta = 60.9$–61.0 and at 63.1 ppm were assigned to CH_2OH and NCH_2 carbon atoms, respectively. The olefinic carbon atoms C-1′ and C-2′ of **29** appeared at $\delta = 116.1$ and 145.2 ppm, respectively. The aromatic carbon atoms were fully identified (c.f. "Experimental" section). Compound **24** selected for further NMR experiments. The gradient-selected HMBC spectrum (Willker *et al.*, 1993) of compound **24** showed a $^{2}J_{C,H}$ coupling between *CH*$_2$–OH protons at δ 3.75 ppm and NCH_2 carbon atom at $\delta = 61.1$ ppm, in addition to a $^{3}J_{C,H}$ between NCH_2 at $\delta = 4.08$ and C=N at $\delta = 168.2$ ppm. A $^{3}J_{C,H}$ coupling between H-3 at $\delta = 3.27$ and CO_2 at $\delta = 163.1$ ppm was also identified. Furthermore, a $^{3}J_{C,H}$ coupling between $H_{rhod.}$-12′ at $\delta = 7.67$ ppm and CO_2 at δ 163.1 ppm, in addition to a $^{3}J_{C,H}$ coupling between $H_{rhod.}$-9′ at δ 5.51 and $C_{rhod.}$-11′ at δ 129.3 ppm, was observed (Fig. 1).

The inversion of configuration at C-3 during the formation of the α-esters **24–29** was assigned from their NOESY ^{1}H, ^{1}H NMR spectroscopy (Anderson and Freeman, 1962). Compound **24** was selected for NOESY NMR correlation. Thus, H-3 at $\delta = 3.27$ ppm showed correlations with $H_{rhod.}$-12′, H-2a, and H-4a at $\delta = 7.67$, 1.69, and 2.10 ppm, respectively, indicative for existence of H-3 in β position and the ester in an α position (Fig. 1).

Further, the configuration of the imine linkage (C=N) of **29** was calculated by the DFT method (GGA) at the level PBE/DNP (Salahub *et al.*, 1991). Figure 2 represents the orbitals of **29**, which revealed that HOMO is located at the imine bond (C=N) of the hydroxyethylimine part, while LUMO is separated on the carbon atom of the imine linkage (C=N). Surprisingly, the total energy of *trans/cis* isomer (−1599.3204728 hartree) is lower than that of the *trans/trans* isomer (−1599.429476 hartree), and such data together with the hydrogen bond between proton of OH group and N atom of the imino-ethanol residue would support that *trans/cis* isomer is the more stable one.

Next, our target was the synthesis of 3β-ester pregnenolone analogues using Steglich coupling method (Neises and Steglich, 1978), with retention of configuration of ester group at C-3 of pregnenolone, in the presence of *N*,*N*′-dicyclohexylcarbodiimide (DCC) as coupling reagents, for biological comparison purposes with our new inverted 3α-ester analogues **24–29**. Thus, treatment of **4** and **30**, prepared previously in our laboratory (Al-Masoudi *et al.*, 2015a), with rhodamine B in the presence of DCC and DMAP as a base at 0 °C and then at room temperature afforded, after chromatography, the 3α-ester analogues **31** and **32** in 40 and 45 % yields, respectively (Scheme 2).

The structures of **31** and **32** were identified by their ^{1}H, ^{13}C, and 2D NMR spectra. The HMBC NMR spectrum of **32** revealed that the olefinic proton H-21 at $\delta = 7.49$ showed two $^{3}J_{C;H}$ couplings: with the aromatic carbon atom (C-1′) at $\delta = 135.7$ ppm and C-17 at $\delta = 60.9$ ppm. A $^{3}J_{C;H}$ coupling between the olefinic proton H-22 at $\delta = 7.97$ ppm and C-20 at δ 202.2 ppm was observed. Additionally, a $^{3}J_{C;H}$ coupling between H-3 at δ 3.13 ppm and CO_2 at δ 169.3 ppm was observed. Three couplings in the NOESY spectrum of **32** supported the existence of the rhodamine ester group in a β position at C-3 of pregnenolone, with retention of configuration.

In addition, all the synthesized compounds were identified by their ^{1}H, ^{13}C HSQC NMR spectra (Davis *et al.*, 1992).

In vitro CYP17 hydroxylase enzyme activity

Inhibition of human CYP17 was determined as previously described by Sergejew and Hartmann (1994) and Hutschenreuter *et al.* (2004). For the source of human CYP17, recombinant *E. coli* coexpressing human CYP17 and NADPH-P450 reductase was used. After homogenization, the 50,000 *g* sediment was incubated with progesterone (25 μM) and the inhibitor. The concentration of inhibitor was 10 μM, using DMSO as a solvent. Separation of the product was performed by HPLC using UV detection. The steroidal CYP17A1 inhibitor abiraterone acetate (**3**) was used as reference compound. The IC_{50} values were determined for compounds **6**, **8**, **9–11**, **24–29**, **31,** and **32;** meanwhile, the inhibition (%) of CYP17 α-hydroxylase was measured for all compounds except **9**, **11**, and **24**, and the results are displayed in Table 1.

The compounds can be divided into two classes according to the ester mimicking moiety at C-3, together with substitution at C-20 by the 2-hydroxyethyl-imino group. From Table 1, the results showed that these derivatives possess no CYP17 inhibition activity, except compound **27** which exhibited a moderate inhibition of

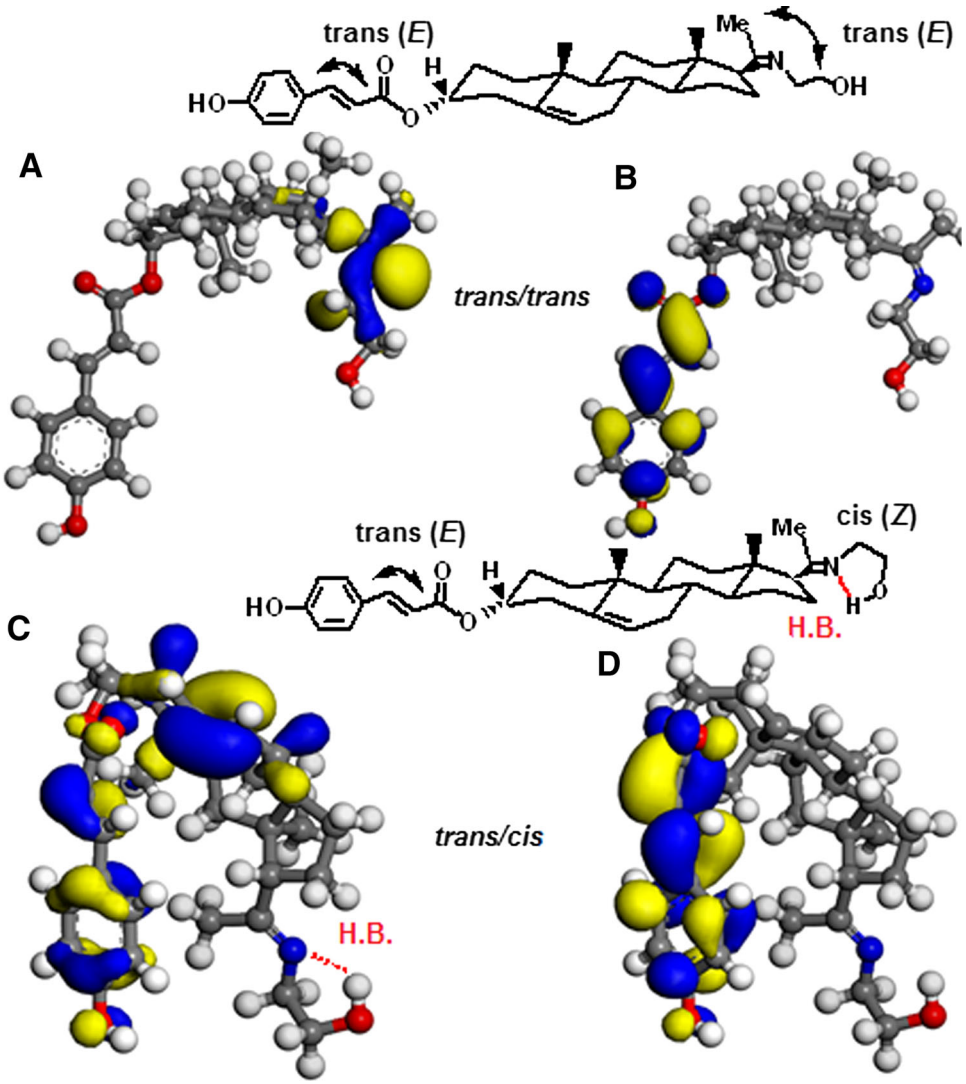
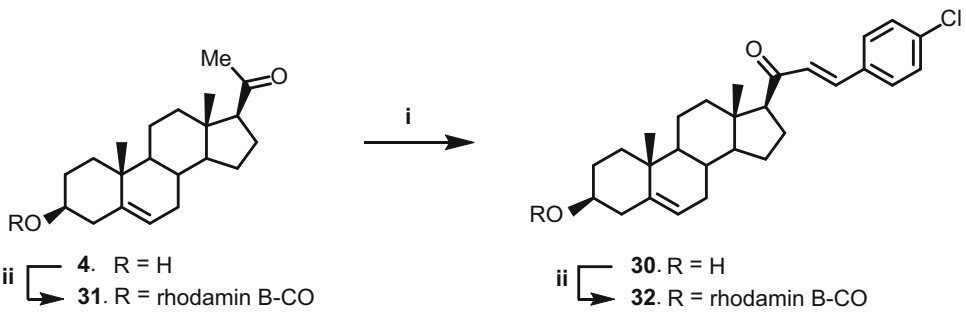


Fig. 2 Energies of compound **29**: **a** *trans* HOMO = −0.18720 hartree; **b** *trans* LUMO = −0.0.08364 hartree; **c** *cis* HOMO = −0.190439 hartree; **d** *cis* LUMO = −0.0.084453 hartree



Scheme 2 Conditions and reagents: (i) 4-Cl–C_6H_4–CHO, EtOH, 2M aq. NaOH, r.t., 24 h; (ii) rhodamine B, DCC, DMAP, dry MeCN, r.t., 24 h

1.12 μM. The activity of **27** might be due to the presence of 3,4-dihydroxyphenyl group as a part of protocatechuic acid, which is reported to possess mixed effects on normal and cancer cells in vitro and in vivo studies (Lin *et al.*, 2007). In addition, the target enzyme CYP/P450 contains a heme in its binding site, which is crucial for the enzymatic reaction and for binding the iron-complexing nitrogen containing inhibitors (Schenkman *et al.*, 1981). Therefore, the activity of **27** can be explained also in terms of the complexation of the Fe^{2+} of the heme with an sp^2 hybridized nitrogen of the C=N group at C-20. Such argument would support the hypothesis that N-ethanol residue at C-20 oriented in a *trans* position and let the lone pair of nitrogen is free for complexation with Fe^{+2}, while the other analogues having a *cis* N-ethanol group leading to a hydrogen bond between OH group and nitrogen atom.

Table 1 Inhibition activity of CYP17 hydroxylase by pregnenolone derivatives

Compd.	Inhibition (%)[a]	IC_{50} (μM)[b]	Compd.	Inhibition (%)[a]	IC_{50} (μM)[b]
6	43.90	23.32	**25**	50.11	26.45
8	64.21	12.21	**26**	61.26	19.73
9	39.43	30.15	**27**	88.56	1.12
10	72.11	9.38	**28**	70.39	4.15
11	51.35	22.12	**29**	58.44	7.79
24	42.25	29.33	**31**	35.22	64.13
ABT[c]	85.38	0.072	**32**	58.54	38.21

[a] Hydroxylase enzyme inhibition measured at 10 μM

[b] Data shown were obtained by performing the tests in duplicates. IC_{50}: concentration of the inhibitor that is required for 50 % inhibition in vitro

[c] *ABT* abiratterone acetate (2.0 μM)

Although none of the test compounds is more active than abiraterone acetate, compound **27** shows a better selectivity profile with 88.56 % inhibition of hydroxylase and is an interesting candidate for further development.

Experiment

Chemistry

Melting points are uncorrected and were measured on a Büchi melting point apparatus B-545 (Büchi Labortechnik AG, Switzerland). Microanalytical data were obtained using a Vario Elemental Analyzer (Shimadzu, Japan). NMR spectra were recorded on 400 and 600 MHz (^{1}H) and on 100 and 150.91 MHz (^{13}C) spectrometers (Bruker, Germany), respectively, with TMS as internal standard and on the δ scale in ppm. Signal assignments for protons were performed by selective proton decoupling or by COSY spectra. Heteronuclear assignments were verified by HSQC and HMBC experiments. IR spectra were measured on a FT-IR spectrophotometer (Shimaduz, Japan) using KBr disks. TLC plates 60 F254 were purchased from Merck. The chromatograms were visualized under UV 254–366 nm and iodine (solvent: hexane-ethyl acetate 3:2).

*3β-O-(4,4′-Dimethoxytrityl)-pregn-5-en-20-one (**6**)* To a solution of pregnenolone **4** (100 mg, 0.32 mmol) in dry pyridine (10 ml) was added 4,4′-dimethoxytrityl chloride (DMTrCl) (**5**) (129 mg, 0.38 mmol), and the mixture stirred for 4.5 h at ambient temperature. Methanol (1.0 ml) was added, and the reaction mixture was stirred for another 10 min. The solvent was removed *in vacuo* and the residual pyridine by co-evaporation with toluene. The product was purified on a short column (5 g) of silica gel, eluting in gradient with MeOH (0–10 %) and $CHCl_3$ as eluent. Fractions showing a single spot on TLC at $R_f = 0.57$ ($CHCl_3$–MeOH 9:1) were collected, and the solvent was evaporated to dryness to give **6** (91 mg, 46 %) as a brown solid; Mp 138–141 °C. FT-IR (KBr, ν, cm^{-1}): 3510 (OH), 1682 (C=C), 1135 (C-O). ^{1}H NMR (DMSO-d_6): $\delta = 7.75$–6.63 (m, 13H, $H_{arom.}$), 5.25 (br s., 1H, H–6), 3.86, 3.72 (2xs, 6H, 2xOMe), 3.26 (m, 1H, H-3), 2.56 (m, 1H, H-17), 2.15 (m, 1H, H-16a), 2.09 (m, 2H, CH_2-4), 2.05 (s, 3H, Me-21), 2.01 (m,1H, H-7a), 1.91 (m, 1H, H-12a), 1.75 (m, 1H, H-1a), 1.68 (m, 1H, H-2a), 1.62 (m, 1H, H-15a), 1.58 (m, 1H, H-16b), 1.57 (m, 1H, H-7b), 1.55 (m, 1H, H-11a), 152 (m, 1H, H-12b), 1.41 (m, 1H, H-8), 1.40 (m, 1H, H-11b), 1.36 (m, 1H, H-2b), 1.13 (m, 2H, H-14 + H-15b), 1.00 (m, 1H, H-1b), 0.98 (m, 1H, H-9), 0.95 (s, 3H, Me-19), 0.52 (s, 3H, Me-18). ^{13}C NMR (DMSO-d_6): $\delta = 208.5$ (C-20), 157.8 (2x$C_{arom.}$-OMe), 141.4 ($C_{arom.}$-1″(A)), 140.2 (C-5), 137.8 ($C_{arom.}$-1″(B) + $C_{arom.}$-1″(C)), 132.0, 129.1, 128.9, 128.4, 127.6, 127.4 ($C_{arom.}$), 120.2 (C-6), 113.9 ($C_{arom.}$-3″(B) + $C_{arom.}$-3″(C)), 106.7 (C_{trityl}-O), 79.9 (C-3), 62.6 (C-17), 56.1 (C-14), 55.0 (2xOMe), 49.5 (C-9), 43.3 (C-13), 42.2 (C-4), 37.9 (C-12), 36.9 (C-1), 36.1 (C-10), 32.0, 31.4 (C-2 + C-7 + C-8 + Me-21), 24.0 (C-15), 22.2 (C-16), 20.6 (C-11), 19.1 (Me-19), 12.9 (Me-18). Anal. Calcd. for $C_{42}H_{50}O_4$ (618.86): C, 81.51; H, 8.14. Found: C, 81.37; H, 8.02.

*20-(2-Hydroxyethyl)imino-pregn-5-en-3β-ol (**8**)* To a stirred solution of **4** (100 mg, 0.32 mmol) in EtOH (10 ml) were added 2-aminoethanol (0.1 ml) and three drops of glacial AcOH, and the mixture heated under reflux for 12 h. After cooling, the mixture was evaporated to dryness and the residue partitioned between $CHCl_3$ (3 × 10 ml) and water (10 ml). The combined organic extracts were dried (Na_2SO_4), filtered and evaporated to dryness. The residue was purified on a short SiO_2 column using the eluent hexane: EtOAc (3:2) to give **8** (60 mg, 52 %) as a brown crystals; Mp 166–168 °C; $R_f = 0.52$. FT-IR (KBr, ν, cm^{-1}): 3379 (OH), 2932 (CH), 1697, 1682 (C=C), 1134 (C-O). ^{1}H NMR (DMSO-d_6): $\delta = 5.26$ (t, 1H, $J_{6,7} = 2.5$ Hz, H-6), 4.04 (m, 2H, CH_2OH), 3.67 (m, 2H,

CH_2OH), 3.27 (m, 1H, H-3), 2.57 (m, 1H, H-17), 2.15 (m, 1H, H-16a), 2.09 (m, 2H, CH_2-4), 2.07 (s, 3H, Me-21), 2.02 (m,1H, H-7a), 1.92 (m, 1H, H-12a), 1.80 (m, 2H, NCH_2), 1.78 (m, 1H, H-1a), 1.68 (m, 1H, H-2a), 1.67 (m, 1H, H-15a), 1.61 (m, 1H, H-16b), 1.59 (m, 1H, H-7b), 1.56 (m, 1H, H-11a), 1.54 (m, 1H, H-12b), 1.44 (8, 1H, H-8), 1.40 (m, 1H, H-11b), 1.37 (m, 1H, 2b), 1.15 (m, 2H, H-14 + H-15b), 1.00 (m, 1H, H-1b), 0.98 (m, 1H, H-9), 0.96 (s, 3H, Me-19), 0.54 (s, 3H, Me-18). ^{13}C NMR (DMSO-d_6): $\delta = 168.5$ (C=N), 141.8 (C-5), 120.7 (C-6), 70.5 (C-3), 63.1 (NCH_2), 60.3 (CH_2OH), 56.6 (C-14), 50.0 (C-9), 43.8 (C-13), 42.7 (C-7 + C-8), 24.5 (C-15), 22.7 (C-16 + C-17), 21.1 (C-11), 19.6 (Me-19), 13.4 (Me-18). Anal. Calcd. for $C_{23}H_{37}NO_2$ (359.55): C, 76.83; H, 10.37; N, 3.90. Found: C, 76.59; H, 10.22, N, 3.63.

*20-(2-((4,4′-Dimethoxytrityloxy)ethyl)imino-pregn-5-en-3β-ol (**9**)* This compound was prepared by following the same procedure as for preparation of **6** from **8** (500 mg, 1.39 mmol) and DMTrCl **5** (339 mg, 1.67 mmol) in dry pyridine (30 ml). Yield: (644 mg, 70 %) as a light yellow solid; Mp 142–145 °C; $R_f = 0.55$. ^{1}H NMR (DMSO-d_6): $\delta = 7.70$–6.69 (m, 13H, $H_{arom.}$), 5.23 (t, 1H, $J_{6,7} = 2.5$ Hz, H-6); 3.85, 3.72 (2xs, 6H, 2xOMe), 3.52 (m, 2H, CH_2OTr), 3.27 (m, 1H, H-3), 2.55 (m, 1H, H-17), 2.16 (m, 1H, H-16a), 2.10 (m, 2H, CH_2-4), 2.04 (s, 3H, Me-21), 2.00 (m,1H, H-7a), 1.93 (m, 1H, H-12a), 1.81 (m, 2H, NCH_2), 1.74 (m, 1H, H-1a), 1.67 (m, 1H, H-2a), 1.60 (m, 1H, H-15a), 1.54 (m, 1H, H-16b), 1.57 (m, 1H, H-7b), 1.55 (m, 1H, H-11a), 152 (m, 1H, H-12b), 1.40 (m, 1H, H-8), 1.38 (m, 1H, H-11b), 1.35 (m, 1H, H-2b), 1.12 (m, 2H, H-14 + H-15b), 1.00 (m, 1H, H-1b), 0.97 (m, 1H, H-9), 0.95 (s, 3H, Me-19), 0.51 (s, 3H, Me-18). ^{13}C NMR (DMSO-d_6): $\delta = 168.2$ (C-20), 157.7 (2x$C_{arom.}$-OMe), 142.0 ($C_{arom.}$-1″(A)), 140.0 (C-5), 137.2, 136.9 ($C_{arom.}$-1″(B) + $C_{arom.}$-1″(C)), 131.9, 129.0, 128.7, 128.2, 127.5, 127.7 ($C_{arom.}$), 120.0 (C-6), 113.3, 113.0 ($C_{arom.}$-3″(B) + $C_{arom.}$-3″(C)), 102.8 (C_{trityl}-O), 79.6 (C-3), 67.1 (CH_2OTr), 63.0 (NCH_2), 70.5 (C-3), 63.1 (NCH_2), 60.3 (CH_2OH), 56.2 (C-14), 49.7 (C-9), 43.6 (C-13), 42.5 (C-7 + C-8), 25.0 (C-15), 24.1 (C-16 + C-17), 20.8 (C-11), 19.5 (Me-19), 13.4 (Me-18). Anal. Calcd. for $C_{44}H_{55}NO_4$ (661.93): C, 79.84; H, 8.38; N, 2.12. Found: C, 79.69; H, 8.29, N, 2.00.

*3β-Acetoxy-20-(2-hydroxyethyl)imino-pregn-5-ene (**10**)* To a solution of **7** (100 mg, 0.28 mmol) in EtOH (10 ml) was added 2-aminoethanol (0.1 ml), and the mixture heated under reflux for 16 h. The reaction mixture was worked up as for compound **8** to give **10** (68 mg, 61 %) as brown crystals; Mp 187–189 °C; $R_f = 0.56$. FT-IR (KBr, ν, cm^{-1}): 3364 (OH), 2939 (C–H), 1651 (C=N), 1751 (*CO*Me), 1018 (C-O). ^{1}H NMR (DMSO-d_6): $\delta = 5.27$ (t, 1H, $J_{6,7} = 3.0$ Hz, H-6), 4.68 (m, 2H, CH_2OH), 3.39 (m, 1H, H-3), 2.59 (m, 1H, H-17), 2.51 (s, 3H, OAc), 2.16 (m, 1H, H-16a), 2.14 (m, 2H, CH_2-4), 2.08 (s, 3H, Me-21), 1.98 (m,1H, H-7a), 1.94 (m, 1H, H-12a), 1.82 (m, 2H, NCH_2), 1.80 (m, 1H, H-1a), 1.69 (m, 1H, H-2a), 1.67 (m, 1H, H-15a), 1.64 (m, 1H, H-16b), 1.58 (m, 1H, H-H-7b), 1.55 (m, 1H, H-11a), 1.52 (m, 1H, H-12b), 1.42 (m, 1H, H-8), 1.39 (m, 1H, H-11b), 1.35 (m, 1H, H-2b), 1.17 (m, 2H, H-14 + H-15b), 1.01 (m, 1H, H-1b), 0.97 (m, 1H, H-9), 0.95 (s, 3H, Me-19), 0.55 (s, 3H, Me-18). ^{13}C NMR (DMSO-d_6) $\delta = 170.2$ (*CO*Me), 168.1 (C=N), 141.8 (C-5), 120.7 (C-6), 70.5 (C-3), 63.1 (NCH_2), 58.5 (CH_2OH), 56.6 (C-14), 49.9 (C-9), 44.4 (C-13), 42.7 (C-4), 38.0 (C-12), 37.4 (C-1), 36.6 (C-10), 31.9 (Me-21), 31.8, 31.7 (C-2 + C-7 + C-8), 24.6 (C-15), 22.7 (C-16 + C-17), 21.1 (C-11), 20.8 (CO*Me*), 19.6 (Me-19), 13.2 (Me-18). Anal. Calcd. for $C_{25}H_{39}NO_3$ (401.58): C, 74.77; H, 9.79; N, 3.49. Found: C, 74.52; H, 9.64; N, 3.22.

*20-(2-Phenylethyl)imino-pregn-5-en-3β-ol (**11**)* To a solution of **4** (100 mg, 0.32 mmol) in EtOH (10 ml) was added 2-phenylethyl amine (0.3 ml) containing few drops of glac. AcOH and the mixture were heated under reflux for 8 h. The reaction mixture was worked up as in experiment for **8** to give **11** (57 mg, 42 %) as a brown powder; Mp 220–223 °C; $R_f = 0.55$. FT-IR (KBr, ν, cm^{-1}): 2947 (C–H), 1682 (C=N), 1574 (C=C), 3510 (OH). ^{1}H NMR (DMSO-d_6): $\delta = 7.31$–7.22 (m, 5H, $H_{arom.}$), 5.27 (br s., 1H, H-6), 3.76 (br s., 1H, OH), 3.27 (m, 1H, H-3), 2.87 (m, 2H, CH_2Ph), 2.74 (m, 2H, NCH_2), 2.57 (m, 1H, H-17), 2.16 (m, 1H, H-16a), 2.11 (m, 2H, CH_2-4), 2.02 (s, 3H, Me-21), 1.94 (m,1H, H-7a), 1.92 (m, 1H, H-12a), 1.79 (m, 1H, H-1a), 1.68 (m, 1H, H-2a), 1.61 (m, 1H, H-15a), 1.59 (m, 1H, H-16b), 1.57 (m, 1H, H-7b), 1.56 (m, 1H, H-11a), 1.53 (m, 1H, H-12b), 1.43 (m, 1H, H-8), 1.39 (m, 1H, H-11b), 1.37 (m, 1H, H-2b), 1.14 (m, 2H, H-14 + H-15b), 1.00 (m, 1H, H-1b), 0.98 (m, 1H, H-9), 0.95 (s, 3H, Me-19), 0.54 (s, 3H, Me-18). ^{13}C NMR (DMSO-d_6): $\delta = 168.9$ (C-20), 141.8 (C-5), 139.7 ($C_{arom.}$-1′), 129.1, 128.7, 126.6 $C_{arom.}$), 120.7 (C-6), 70.5 (C-3), 56.6 (C-14), 52.3 (NCH_2), 50.0 (C-9), 43.8 (C-13), 42.8 (C-4), 38.4 (C-12), 38.0 (CH_2Ph), 37.4 (C-1), 36.6 (C-10), 31.9 (Me-21), 31.7 (C-2 + C-7 + C-8), 24.5 (C-15), 22.7 (C-16 + C-17), 21.1 (C-11), 19.6 (Me-19), 13.4 (Me-18). Anal. Calcd. for $C_{29}H_{41}NO$ (419.65): C, 83.00; H, 9.85; N, 3.34. Found: C, 82.87; H 9.77; N, 3.12.

*General procedure for the synthesis of aryl ester derivatives of 20-(2-hydroxyethyl)imino-pregn-5-en-3α-ol (**18-23**)* To a solution of **9** (331 mg 0.50 mmol) in dry acetonitrile (15 ml) were added substituted benzoic acids (0.50 mmol) in separate experiment (rhodamine B (**12**) (240 mg), indomethacin (**13**) (159 mg), naproxen (**14**) (115 mg), protocatechuic acid (**15**) (77 mg), vanillic acid (**16**) (84 mg), and *p*-coumaric acid (**17**) (84 mg)), followed

by the addition of triphenylphosphine (Ph_3P) (131 mg, 0.50 mmol) and diethylazodicarboxylate (DEAD) (0.50 mmol, 0.065 ml), and the mixture stirred at room temperature for 12–14 h. The reaction was monitored by TLC (*n*-hexane: ethyl acetate, 3:2). After cooling, diethyl ether (20 ml) was added and the mixture was partitioned with saturated aqueous solution of $NaHCO_3$ (20 ml), brine solution (25 ml) and finally water. The organic extract was dried (Na_2SO_4) and filtered, and the filtrate was evaporated to dryness to give a crud products (**18–23**), which used directly in the following step. Compounds **18–23** (0.10 mmol) were treated with 80 % aq. AcOH (10 ml) for 30 min at room temperature. The reaction mixture was evaporated to dryness, and the residue was co-evaporated with SiO_2 (1 g) and poured onto SiO_2 column (10 g). Elution, in gradient, with MeOH (0–10 %) and $CHCl_3$ as eluent afforded the pure desired products.

*20-(2-Hydroxyethyl)iminopregn-5-en-3α-(3,6-diethylamino-9H-xanthen-9-yl)-2-benzoate (**24**)* From **18** (109 mg). Yield: 55 mg (70 %) as a pink solid; Mp 167–170 °C; $R_f = 0.62$. FT-IR (KBr, ν, cm^{-1}): 3000 (OH), 1717 (R*CO*$_2$), 1589 (C=C), 1436 ($NH_{bending}$), 1222 (C–H), 1018 (C-O). ^{1}H NMR (DMSO-d_6): $\delta = 7.67$ (m, 1H, $H_{rhod.}$-12′), 7.62 (m, 1H, $H_{rhod..}$-14′), 7.56 (d, 1H, $J = 7.6$ Hz, $H_{rhod.}$-15′), 6.94 (m, 1H, $H_{rhod.}$-13′), 6.71 (d, 1H, $J = 7.8$ Hz $H_{rhod.}$-1′), 6.69 (s, 1H, $H_{rhod.}$-8′), 6.33 (m, 4H, $H_{rhod.}$-2′ + $H_{rhod.}$-4′ + $H_{rhod.}$-5′), 5.51 (s, 1H, $H_{rhod.}$-9′), 5.27 (t, 1H, $J_{6,7} = 2.3$ Hz, H-6), 4.08 (m, 2H, NCH_2), 4.04 (q, 2H, $J = 7.0$ Hz, *CH*$_2$CH$_3$), 3.75 (m, 4H, *CH*$_2$OH + *CH*$_2$CH$_3$), 3.52 (br s., 1H, OH), 3.27 (m, 1H, H-3), 2.57 (m, 1H, H-17), 2.15 (m, 1H, H-16a), 2.10 (m, 2H, CH_2-4), 2.07 (s, 3H, Me-21), 2.00 (m,1H, H-7a), 1.91 (m, 1H, H-12a), 1.80 (m, 2H, NCH_2), 1.79 (m, 1H, H-1a), 1.69 (m, 1H, H-2a), 1.60 (m, 1H, H-15a), 1.59 (m, 1H, H-16b), 1.57 (m, 1H, H-7b), 1.54 (m, 1H, H-11a), 1.51 (m, 1H, H-12b), 1.51 (m, 1H, H-12b), 1.43 (m, 1H, 1H, H-8), 1.41–1.38 (m, 3H H-11b + *CH*$_2$CH$_3$), 1.35 (m, 1H, H-2b), 1.19–1.14 (m, 5H, H-14 + H-15b + CH$_2$*CH*$_3$), 1.00 (m, 1H, H-1b), 0.97 (m, 1H, H-9), 0.94 (s, 3H, Me-19), 0.54 (s, 3H, Me-18). ^{13}C NMR (DMSO-d_6): $\delta = 168.2$ (C=N), 163.1 (CO_2), 153.1 ($C_{rhod.}$-4a′ + $C_{rhod.}$-5a′), 149.6 ($C_{rhod.}$-3′ + $C_{rhod.}$-6′), 141.8 (C-5), 133.5, 132.8, 132.5, 132.0 ($C_{rhod.}$), 129.3 ($C_{rhod.}$-11′), 129.0 ($C_{rhod.}$-12′), 120.8 (C-6), 113.5 ($C_{rhod.}$-2′), 108.7 ($C_{rhod.}$-7′), 105.7 ($C_{rhod.}$-8a′), 97.4 ($C_{rhod.}$-4′ + $C_{rhod.}$-5′), 70.5 (C-3), 63.1 (NCH_2), 60.9 (CH_2OH), 56.6 (C-14), 49.5 (C-9 + *CH*$_2$CH$_3$), 47.0 (*CH*$_2$CH$_3$), 44.3 (C-13), 42.7 (C-4), 38.4 (C-12), 37.4 (C-1), 36.6 (C-10), 31.9 (Me-21), 31.7 (C-2 + C-7 + C-8), 24.5 (C-15), 22.7 (C-16 + C-17), 21.1 (C-11), 19.6 (Me-19), 15.1 (CH_2-*CH*$_3$), 13.4 (Me-18), 12.8 (CH_2*CH*$_3$). Anal. Calcd. for $C_{51}H_{67}N_3O_4$ (786.11): C, 77.92; H, 8.59; N, 5.35. Found: C, 77.70; H, 8.48; N, 5.11.

*20-(2-Hydroxyethyl)imino-pregn-5-en-3α-(N-(4-chlorobenzoyl)-5-methoxy-2-methylindol-3-yl)acetate (**25**)* From **19** (100 mg). Yield: 57 mg (73 %) as a yellow solid; Mp 155–158 °C; $R_f = 0.57$. FT-IR (KBr, ν, cm^{-1}): 3067 (OH), 1720 (NC=O), 1717 (R*CO*$_2$), 1683 (C=C), 1359, 1222 (C–H), 723 (C-Cl). ^{1}H NMR (DMSO-d_6): $\delta = 7.88$ (d, 2H, $J = 8.4$ Hz, $H_{arom.}$-2′ + $H_{arom.}$-6′), 7.57 (d, 2H, $J = 8.4$ Hz, $H_{arom.}$-3′ + $H_{arom.}$-5′) 7.04 (s, 1H, $H_{indometh.}$-4′), 6.94 (d, 1H, $J = 7.4$ Hz, $H_{indometh.}$-7′), 6.70 (d, 1H, $J = 7.4$ Hz, $H_{indometh.}$-6′), 5.27 (t, 1H, $J_{6,7} = 2.4$ Hz, H-6), 4.05 (m, 2H, *CH*$_2$OH), 3.75 (m, 3H, OMe), 3.52 (br s., 1H, OH), 3.28 (m, 1H, H-3), 3.09 (s, 2H, CO*CH*$_2$), 2.56 (m, 1H, H-17), 2.20 (s, 3H, $C^2_{indometh.}$-Me), 2.15 (m, 1H, H-16a), 2.11 (m, 2H, H-4a), 2.07 (s, 3H, Me-21), 2.02 (m,1H, H-7a), 2.01 (m, 1H, H-4b), 1.96 (m, 1H, H-12a), 1.82 (m, 2H, NCH_2), 1.78 (m, 1H, H-1a), 1.68 (m, 1H, H-2a), 1.61 (m, 1H, H-15a), 1.57 (m, 2H, H-7b + H-16b), 1.53 (m, 1H, H-2b + H-11a), 1.51 (m, 1H, H-12b), 1.40 (m, 2H, H-8 + H-11b), 1.38 (m, 1H, H-2b), 1.18 (m, 2H, H-14 + H-15b), 1.02 (m, 1H, H-1b), 0.98 (m, 1H, H-9), 0.97 (s, 3H, Me-19), 0.54 (s, 3H, Me-18). ^{13}C NMR (DMSO-d_6): δ = 168.3 (C=N + CO_2), 167.5 (N*CO*Ar), 156.0 ($C_{indometh.}$-5′), 141.8 (C-5 + C-Cl), 138.0 ($C_{indometh.}$-3a′), 134.8 ($C_{indometh.}$-2′), 132.5, 129.5, 129.2 ($C_{arom.}$), 120.7 (C-6), 115.0 ($C_{indometh.}$-6′), 113.6 ($C_{indometh.}$-7′), 111.6 ($C_{indometh.}$-4′), 101.6 ($C_{indometh.}$-3′), 70.5 (C-3), 63.1 (NCH_2), 60.9 (CH_2OH), 56.6 (C-14), 55.9 (OMe), 50.0 (C-9), 43.8 (C-13), 42.8 (C-4), 38.4 (C-12), 37.4 (C-1), 36.6 (C-10), 31.9 (Me-21), 31.7 (C-2 + C-7 + C-8 + *CH*$_2$CO), 24.5 (C-15), 22.7 (C-16 + C-17), 21.1 (C-11), 19.6 (Me-19), 15.0 ($C^2_{indometh.}$-*Me*), 13.4 (Me-18). Anal. Calcd. for $C_{42}H_{51}ClN_2O_5$ (699.32): C, 77.92; H, 8.59; N, 5.35. Found: C, 77.70; H, 8.48; N, 5.11.

*20-(2-Hydroxyethyl)imino-pregn-5-en-3α-2-(S)-methyl)-(6-methoxynaphthalen-2-yl-acetate (**26**)* From **20** (87.4 mg). Yield: 39 mg (68 %) as a yellow solid; Mp 181–183 °C; $R_f = 0.55$. FT-IR (KBr, ν, cm^{-1}): 1119 (OH), 1682 (C=C), 1437.65 (C–H), 1192 (C–H). ^{1}H NMR (DMSO-d_6): δ = 7.78–7.28 (m, 6H, $H_{arom.}$), 5.27 (t, 1H, $J_{6,7} = 2.1$ Hz, H-6), 4.05 (m, 2H, *CH*$_2$OH), 3.86 (s, 3H, OMe), 3.46 (m., 2H, *CH*Me), 3.29 (m, 1H, H-3), 2.56 (m, 1H, H-17), 2.16 (m, 1H, H-16a), 2.10 (m, 2H, CH_2-4), 2.02 (s, 3H, Me-21), 1.99 (m,1H, H-7a), 1.93 (m, 1H, H-12a), 1.80 (m, 2H, NCH_2), 1.76 (m, 1H, H-1a + CH*Me*), 1.70 (m, 1H, H-2a), 1.68 (m, 1H, H-15a), 1.60 (m, 1H, H-16b), 1.56 (m, 1H, H-7b), 1.55 (m, 1H, H-11a), 1.52 (m, H, H-12b), 1.44 (m, 1H, H-8), 1.40 (m, 1H, H-11b), 1.37 (m, 1H, H-2b), 1.23 (m, 2H, H-14 + H-15b), 1.06 (m, 1H, H-1b), 1.00 (m, 1H, H-9), 0.94 (s, 3H, Me-19), 0.53 (s, 3H, Me-18). ^{13}C NMR (DMSO-d_6): $\delta = 172.4$ (CO_2), 167.9 (C=N), 157.5 ($C_{naproxin}$-6′), 141.7 (C-5), 132.5 ($C_{naproxin}$-2′), 131. 9 ($C_{naproxin}$-4a′), 132.0, 129.3, 129.2 ($C_{arom.}$), 120.7 (C-6),

119.1 ($C_{naproxin}$-7′), 106.2 (5 ($C_{naproxin}$-5′), 70.5 (C-3), 63.1 (NCH_2), 60.9 (CH_2OH), 56.6 (C-14), 55.6 (OMe), 50.0 (C-9), 43.7 (C-13), 42.7 (C-4), 42.0 (*CH*Me), 38.4 (C-12), 37.4 (C-1), 36.6 (C-10), 31.9 (Me-21), 31.7, 31.6 (C-2 + C-7 + C-8), 24.5 (C-15), 22.7 (C-16 + C-17), 21.1 (C-11), 19.6 (Me-19), 15.0 (CH*Me*), 13.4 (Me-18). Anal. Calcd. for $C_{37}H_{49}NO_4$ (571.37): C, 77.72; H, 8.64; N, 2.45. Found: C, 77.50; H, 8.55; N, 2.23.

*20-(2-Hydroxyethyl)imino-pregn-5-en-3α-(3,4-dihydroxy) benzoate (**27**)* From **21** (80 mg). Yield: 38 mg (76 %) as a light yellow solid; Mp 134–136 °C; $R_f = 0.64$. FT-IR (KBr, ν, cm^{-1}): 3248 (OH), 2932 (CH_2), 1700 (R*CO*$_2$), 169 (C=N), 1535 (C=C). ^{1}H NMR (DMSO-d_6): $\delta = 9.00$ (br s., 2H, 2xOH), 7.56 (d, 1H, $J_{2',6'} = 5.6$ Hz, $H_{arom.}$-2′), 7.12 (d, 1H, $J_{5',6'} = 8.5$ Hz, $H_{arom.}$-6′), 6.52 (d, 1H, $J_{5',6'} = 8.5$ Hz, $H_{arom.}$-5′), 5.27 (t, 1H, $J_{6,7} = 4.0$ Hz, H-6), 4.05 (q, 2H, $J = 6.9$ Hz, *CH*$_2$OH), 3.48 (br s., 1H, OH), 3.26 (m, 1H, H-3), 2.57 (m, 1H, H-17), 2.17 (m, 1H, H-16a), 2.11 (m, 2H, CH_2-4), 2.06 (s, 3H, Me-21), 2.00 (m,1H, H-7a), 1.93 (m, 1H, H-12a), 1.80 (m, 2H, NCH_2), 1.77 (m, 1H, H-1a), 1.69 (m, 1H, H-2a), 1.60 (m, 1H, H-15a), 1.58 (m, 1H, H-16b), 1.56 (m, 1H, H-7b), 1.53 (m, 1H, H-11a), 1.50 (m, 1H, H-12b), 1.44 (m, 1H, H-8), 1.40 (m, 1H, H-11b), 1.37 (m, 1H, H-2b), 1.17 (m, 2H, H-14 + H-15b), 1.02 (m, 1H, H-1b), 0.99 (m, 1H, H-9), 0.97 (s, 3H, Me-19), 0.53 (s, 3H, Me-18). ^{13}C NMR (DMSO-d_6): $\delta = 168.7$ (C=N), 161.6 (CO_2), 148.4 ($C^{4'}_{arom.}$-OH), 146.1 ($C^{3'}_{arom.}$-OH), 141.8 (C-5), 126.6 ($C_{arom.}$-1′), 123.5 ($C_{arom.}$-6′), 120.7 (C-6), 116.2 ($C_{arom.}$-2′ + $C_{arom.}$-5′), 70.5 (C-3), 63.1 (NCH_2), 60.9 (CH_2OH), 56.6 (C-14), 50.0 (C-9), 43.7 (C-13), 42.7 (C-4), 38.5 (C-12), 37.4 (C-1), 36.6 (C-10), 31.9 (Me-21), 31.7 (C-2 + C-7 + C-8), 24.5 (C-15), 22.7 (C-16 + C-17), 21.1 (C-11), 19.6 (Me-19), 13.4 (Me-18). Anal. Calcd. for $C_{30}H_{41}NO_5$ (495.30): C, 72.70; H, 8.34; N, 2.83. Found: C, 72.49; H, 8.26; N, 2.68.

*20-(2-Hydroxyethyl)imino-pregn-5-en-3α-(4-hydroxy-3-methoxy)benzoate (**28**)* From **22** (81 mg). Yield: 37 mg (73 %) as a red semisolid; Mp 202–203 °C; $R_f = 0.60$. FT-IR (KBr, ν, cm^{-1}): 3248 (OH), 2932 (CH_2), 1682 (C=N), 1535 (C=C). ^{1}H NMR (DMSO-d_6): $\delta = 9.78$ (br s., 1H, OH), 7.42 (d, 1H, $J_{5',6'} = 8.0$ Hz, $H_{arom.}$-6′), 7.39 (s, 1H, $H_{arom.}$-2′), 6.69 (d, 1H, $J_{5',6'} = 8.0$ Hz, $H_{arom.}$-5′), 5.26 (t, 1H, $J_{6,7} = 3.5$ Hz, H-6), 4.63 (br s., 1H, OH), 4.04 (q, 2H, $J = 7.0$ Hz, *CH*$_2$OH), 3.84 (s, 3H, OMe), 3.29 (m, 1H, H-3), 2.55 (m, 1H, H-17), 2.16 (m, 1H, H-16a), 2.10 (m, 2H, CH_2-4), 2.06 (s, 3H, Me-21), 1.97 (m,1H, H-7a), 1.92 (m, 1H, H-12a), 1.80 (m, 2H, NCH_2), 1.77 (m, 1H, H-1a), 1.68 (m, 1H, H-2a), 1.61 (m, 1H, H-15a), 1.59 (m, 1H, H-16b), 1.58 (m, 1H, H-7b), 1.55 (m, 1H, H-11a), 1.52 (m, 1H, H-12b), 1.40 (m, 1H, H-8), 1.39 (m, 1H, H-11b), 1.37 (m, 1H, H-2b), 1.18 (m, 2H, H-14 + H-15b), 1.01 (m, 1H, H-1b), 0.99 (m, 1H, H-9), 0.97 (s, 3H, Me-19), 0.53 (s, 3H, Me-18). ^{13}C NMR (DMSO-d_6): $\delta = 169.7$ (C=N), 164.6 (CO_2), 157.0 ($C^{4'}_{arom.}$–OH), 148.7 ($C^{3'}_{arom.}$–OMe), 141.8 (C-5), 126.5 ($C_{arom.}$-1′ + $C_{arom.}$-6′), 120.7 (C-6), 115.9 ($C_{arom.}$-2′), 111.2 ($C_{arom.}$-5′), 70.5 (C-3), 63.1 (NCH_2), 61.0 (CH_2OH), 56.6 (C-14), 56.1 (OMe), 50.0 (C-9), 43.7 (C-13), 42.7 (C-4), 38.5 (C-12), 37.4 (C-1), 36.6 (C-10), 31.9 (Me-21), 31.7 (C-2 + C-7 + C-8), 24.5 (C-15), 22.7 (C-16 + C-17), 21.1 (C-11), 19.6 (Me-19), 13.4 (Me-18). Anal. Calcd. for $C_{31}H_{43}NO_5$ (509.31): C, 73.05; H, 8.50; N, 2.75. Found: C, 72.88; H, 8.41; N, 2.52.

*20-(2-Hydroxyethyl)imino-pregn-5-en-3α-(4-hydroxycinnamate (**29**)* From **23** (81 mg). Yield: 38 mg (75 %) as a light yellow solid; Mp 198–202 °C; $R_f = 0.70$. FT-IR (KBr, ν, cm^{-1}): 3402 (OH), 2924 (CH_2), 1697 (C=N), 1459 (C=C). ^{1}H NMR (DMSO-d_6): $\delta = 8.97$ (s, 1H, OH), 7.51 (d, 2H, $J_{2'',3''} = 8.2$ Hz, $H_{arom.}$-2″ + $H_{arom.}$-6″), 7.47 (d, 1H, $J_{1',2'} = 15.0$ Hz, H_{olefin}-2′), 6.78 51 (d, 2H, $J_{5'',6''} = 8.2$ Hz, $H_{arom.}$-3″ + $H_{arom.}$-5″), 6.28 (d, 1H, $J = 15.0$ Hz, H_{olefin}-1′), 5.26 (t, 1H, $J_{6,7} = 2.6$ Hz, H-6), 4.59 (d, 1H, $J = 3.3$ Hz, OH), 4.03 (q, 2H, $J = 7.0$ Hz, *CH*$_2$OH), 3.27 (m, 1H, H-3), 2.50 (m, 1H, H-17), 2.15 (m, 1H, H-16a), 2.10 (m, 2H, CH_2-4), 2.06 (s, 3H, Me-21), 2.00 (m,1H, H-7a), 1.93 (m, 1H, H-12a), 1.82 (m, 2H, NCH_2), 1.77 (m, 1H, H-1a), 1.68 (m, 1H, H-2a), 1.61 (m, 1H, H-15a), 1.58 (m, 1H, H-16b), 1.56 (m, 1H, H-7b), 1.55 (m, 1H, H-11a), 1.53 (m, 1H, H-12b), 1.42 (m, 1H, H-8), 1.41 (m, 1H, H-11b), 1.37 (m, 1H, H-2b), 1.19 (m, 2H, H-14 + H-15b), 1.01 (m, 1H, H-1b), 0.99 (m, 1H, H-9), 0.96 (s, 3H, Me-19), 0.53 (s, 3H, Me-18). ^{13}C NMR (DMSO-d_6): $\delta = 169.0$ (C=N), 166.2 (CO_2), 157.1 ($C^{4'}_{arom.}$–OH), 145.2 (C_{olefin}-2′), 141.7 (C-5), 131.8 ($C_{arom.}$-2″ + $C_{arom.}$-6″), 129.3 ($C_{arom.}$-1″), 120.7 (C-6), 116.1 (C_{olefin}-1′ + $C_{arom.}$-3″ + $C_{arom.}$-6″), 111.2 ($C_{arom.}$-5′), 70.5 (C-3), 63.1 (NCH_2), 60.9 (CH_2OH), 56.6 (C-14), 50.0 (C-9), 43.7 (C-13), 42.7 (C-4), 38.4 (C-12), 37.4 (C-1), 36.6 (C-10), 31.9 (Me-21), 31.7 (C-2 + C-7 + C-8), 24.5 (C-15), 22.3 (C-16 + C-17), 21.2 (C-11), 19.6 (Me-19), 13.4 (Me-18). Anal. Calcd. for $C_{32}H_{43}NO_4$ (505.70): C, 76.00; H, 8.57; N, 2.77. Found: C, 75.78; H, 8.48; N, 2.56.

*3β-Hydroxy-21-(4-chlorobenzylidene)pregn-5-en-20-one (**30**)* This compound was prepared according to the method previously reported (Al-Masoudi *et al.*, 2015a) from **4** (100 mg, 0.32) and aq. solution of 2 M NaOH (5 ml). Yield: 91 mg (65 %) as a yellow powder; Mp 91–93 °C. The ^{1}H and ^{13}C NMR spectra were similar for the authentic sample prepared previously.

*Pregn-5-en-20-one-3β-(3,6-diethylamino)-9H-xanthen-9-yl)-2-benzoate (**31**)* To a cold stirred solution of **4** (316 mg, 1.00 mmol) in dry MeCN (15 ml) was added rhodamine B (200 mg, 0.42 mol) followed DMAP (40 mg, 0.33 mmol) and DCC (213 mg, 1.10 mmol). The mixture was stirred at

0 °C for 10 min and then at room temperature for 12 h. Dicyclohexylurea (DCU) was filtered, the filtrate was evaporated to dryness, and the residue was partitioned between $CHCl_3$ (3 × 15 ml) and water (15 ml). The combined organic extracts were dried (Na_2SO_4), filtered, and evaporated to dryness. The residue was poured onto a SiO_2 column (5 g) and eluted, in gradient, with MeOH (0–10 %) and $CHCl_3$ as eluent to give **31** (297 mg, 40 %) as a red powder; M.p. 158–161 °C. ^{1}H NMR (DMSO-d_6): δ = 8.21 (d, 1H, J = 6.7 Hz, $H_{rhod.}$-12″), 7.98 (d, 1H, J = 7.5 Hz, $H_{rhod.}$-14″), 7.77 (d, 1H, J = 7.5 Hz, $H_{rhod.}$-15″), 7.71 (d, 1H, J = 7.6 Hz, $H_{rhod.}$-13″), 7.26 (d, 1H, J = 7.4 Hz, $H_{rhod.}$-1″), 6.95 (d, 1H, J = 7.0 Hz, $H_{rhod.}$-8″), 6.45 (m, 4H, $H_{rhod.}$-2″ + $H_{rhod.}$-4″ + $H_{rhod.}$-5″ + $H_{rhod.}$-7″), 5.68 (s, 1H, $H_{rhod.}$-9″), 5.26 (d, 1H, $J_{6,7}$ = 3.4 Hz, H-6), 3.37 (m, 8H, 4x$CH_2$$CH_3$), 3.17 (m, 1H, H-3), 2.57 (m, 1H, H-17), 2.16 (m, 1H, H-16a), 2.09 (m, 2H, CH_2-4), 1.98 (m,1H, H-7a), 1.91 (m, 1H, H-12a), 1.77 (m, 1H, H-1a), 1.66 (m, 1H, H-2a), 1.59 (m, 1H, H-15a), 1.55 (m, 2H, H-16b + H-7b), 1.53 (m, 2H, H-11a + H-12b), 1.41 (, 1H, 1H, H-8), 1.39 (m, 1H, H-11b), 1.37 (m, 1H, H-2b), 1.24 (m, 2H, H-14 + H-15b), 1.17 (m, 1H, H-1b), 1.14 (m, 1H, H-9), 1.10 (t, J = 6.8 Hz, 4x$CH_2$$CH_3$), 0.94 (s, 3H, Me-19), 0.54 (s, 3H, Me-18). ^{13}C NMR (DMSO-d_6): δ = 209.0 (C-20), 169.2 (CO_2), 153.3 ($C_{rhod.}$-4a″ + $C_{rhod.}$-5a″), 149.9 ($C_{rhod.}$-3″ + $C_{rhod.}$-6″), 140.2 (C-5), 135.6 (C-10″), 130.3, 129.2, 125.2, 124.9 ($C_{arom.}$), 120.7 (C-6), 109.1 ($C_{rhod.}$-2″), 107.4 ($C_{rhod.}$-7″ + $C_{rhod.}$-8a″), 97.5 ($C_{rhod.}$-4″ + $C_{rhod.}$-5″), 70.5 (C-3), 60.9 (C-17), 56.8 (C-14), 50.1 (C-9), 48.0 (4x$CH_2$$CH_3$), 44.3 (C-13 + $C_{rhod.}$-9″), 42.7 (C-4), 38.6 (C-12), 37.4 (C-1), 36.6 (C-10), 32.1 (C-2), 31.9 (C-7), 31.7 (C-8), 25.8 (C-15), 24.9 (C-16), 22.8 (C-11), 19.6 (Me-19), 12.8 (4x$CH_2$$CH_3$ + Me-18). Anal. Calcd. for $C_{49}H_{62}N_2O_4$ (742.47): C, 79.21; H, 8.41; N, 3.77. Found: C, 78.95; H, 8.33; N, 3.58.

*21-(4-Chlorobenzylidene)pregn-5-en-20-one-3β-(3,6-diethylamino)-9H-xanthen-9-yl)-2-benzoate (**32**)* This compound was prepared from compound **30** according to the procedure for preparation of **31** (290 mg, 0.66 mmol), rhodamine B (160 mg, 0.34 mmol), DCC (141 mg, 0.73 mmol), and DMAP (27 mg, 0.22 mmol) in dry MeCN (10 ml). Yield: 257 mg (45 %) as a red powder; M.p. 110–112 °C. ^{1}H NMR (DMSO-d_6): δ = 8.20 (br s., 1H, $H_{rhod.}$-12″), 7.97 (d, 1H, J = 15.8 Hz, H-22), 7.74 (d, 2H, J = 7.6 Hz, $H_{arom.}$-2′ + $H_{arom.}$-6′), 7.49 (d, 1H, J = 15.8 Hz, H-21), 7.26 (m, 1H, $H_{rhod.}$-14″), 7.15 (m, 2H, $H_{rhod.}$-13″ + $H_{rhod.}$-15″), 7.00 (d, 2H, J = 7.6 Hz, $H_{arom.}$-3′ + $H_{arom.}$-5′), 6.69 (m, 3H, $H_{rhod.}$-1″ + $H_{rhod.}$-8″), 6.45 (m, 4H, $H_{rhod.}$-2″ + $H_{rhod.}$-4″ + $H_{rhod.}$-5″ + $H_{rhod.}$-7″), 5.69 (s, 1H, $H_{rhod.}$-9″), 5.27 (br s., 1H, H-6), 3.36 (m, 8H, 4x$CH_2$$CH_3$), 3.13 (m, 1H, H-3), 2.52 (m, 1H, H-17), 2.13 (m, 1H, H-16a), 2.05 (m, 2H, CH_2-4), 1.95 (m,1H, H-7a), 1.92 (m, 1H, H-12a), 1.76 (m, 1H, H-1a), 1.64 (m, 1H, H-2a), 1.62 (m, 1H, H-15a), 1.59 (m, 1H, H-16b), 1.56 (m, 1H, H-7b), 1.54 (m, 2H, H-11a), 1.52 (1H, H-12b), 1.41 (, 1H, 1H, H-8), 1.39 (m, 1H, H-11b), 1.37 (m, 1H, H-2b), 1.25 (m, 2H, H-14 + H-15b), 1.16 (m, 1H, H-1b), 1.10 (m, 1H, H-9), 1.09 (t, J = 6.6 Hz, 4x$CH_2$$CH_3$), 0.93 (s, 3H, Me-19), 0.53 (s, 3H, Me-18). ^{13}C NMR (DMSO-d_6): δ = 202.2 (C-20), 169.3 (CO_2), 153.3 ($C_{rhod.}$-4a″ + $C_{rhod.}$-5a″), 149.7 ($C_{rhod.}$-3″ + $C_{rhod.}$-6″), 141.8 (C-5), 140.0 (C-22), 135.7 ($C_{arom.}$-1′ + $C_{arom.}$-4′), 130.6, 129.3 ($C_{arom.}$), 129.1 (C-21), 120.7 (C-6), 112.1 ($C_{rhod.}$-2″), 108.8 ($C_{rhod.}$-7″), 105.8 ($C_{rhod.}$-8a″), 97.5 ($C_{rhod.}$-4″ + $C_{rhod.}$-5″), 70.5 (C-3), 60.9 (C-17), 56.8 (C-14), 50.1 (C-9), 48.0 (4xCH_2-CH_3), 44.3 (C-13 + $C_{rhod.}$-9″), 42.7 (C-4), 38.4 (C-12), 37.3 (C-1), 36.6 (C-10), 32.1 (C-2), 31.9 (C-7), 31.7 C-8), 25.9 (C-15), 24.9 (C-16), 22.8 (C-11), 19.6 (Me-19), 13.7 (Me-18), 12.8 (4x$CH_2$$CH_3$). Anal. Calcd. for $C_{56}H_{65}$-ClN_2O_4 (864.46): C, 77.71; H, 7.57; N, 3.24. Found: C, 77.50; H, 7.49; N, 3.09.

Biological evaluations

CYP17 enzyme preparation

For the source of human CYP17, recombinant *Escherichia coli* system (Ehmer *et al.*, 2000) (coexpressing human CYP17 and NADPH-P450 reductase) was used, and the assay was performed as previously described (Sergejew and Hartmann, 1994; Hutschenreuter *et al.*, 2004) using unlabeled progesterone as substrate and applying HPLC with UV detection for separation.

α-Hydroxylase assay

Determination of the hydroxylase activity of CYP17 was performed by measurement of the conversion of pregnenolone (P5) to 17 α-hydroxylasepregnenolone (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. However, enzyme concentration had to be reduced to keep control conversion in the favorable range of 15–25 % and to prevent dehydroepiandrosterone (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 ethyl acetate and vigorous shaking for 10 min. After a centrifugation

step (5 min, 15,000 *g*), 900 µl of the organic phase was removed and transferred into a fresh tube containing 250 µl phosphate buffer and 50 µl 1 N HCl. Shaking and centrifugation were repeated as described above. Eight hundred microliters of ethyl acetate 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. For the analysis of the hydroxylase assay, an isocratic method, described previously, was used. A 1 + 1 mixture of water and acetonitrile with 0.1 % trifluoroacetic acid was used as eluent. Solvent and scintillator flow was set to 0.9 ml/min.

Conclusion

In conclusion, we applied the esterification of pregnenolone and its analogues the Mitsunobu reaction as well as by Steglich coupling reaction. These methods are versatile and mediation efficient conversion of secondary alcohol to esters with inversion or retention of configuration at C-3 of pregnenolone derivatives. The new 3β-*O*-trityl, 3α-, and 3β-ester pregnenolone derivatives have been synthesized. The synthesized compounds were found not to inhibit CYP17 hydroxylase activity significantly when compared to abiraterone acetate. However, only moderate inhibition of this enzyme was seen with compound **27** ($IC_{50} = 1.12$ µM) with remarkable inhibition selectivity (88.56 %). Therefore, **27** is a promising agent for further structural modification and pharmacological evaluation as a new CYP17 hydroxylase inhibitor.

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