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New biaryl-chalcone derivatives of pregnenolone *via* Suzuki–Miyaura cross-coupling reaction. Synthesis, CYP17 hydroxylase inhibition activity, QSAR, and molecular docking study

Najim A. Al-Masoudi [a,*], Rawaa A. Kadhim [b], Nabeel A. Abdul-Rida [c], Bahjat A. Saeed [d], Mathias Engel [e]

[a] Department of Chemistry, College of Science, University of Basrah, Basrah, Iraq
[b] Department of Chemistry, College of Education, University of Qadisiya, Qadisiya, Iraq
[c] Department of Chemistry, College of Science, University of Qadisiya, Qadisiya, Iraq
[d] Department of Chemistry, College of Education, University of Basrah, Basrah, Iraq
[e] Institut für Pharmazeutische und Medizinische Chemie, Universität des Saarlandes, Saarbrücken, Germany

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ABSTRACT

A new class of steroids is being synthesized for its ability to prevent intratumoral androgen production by inhibiting the activity of CYP17 hydroxylase enzyme. The scheme involved the synthesis of chalcone derivative of pregnenolone **5** which was further modified to the corresponding biaryl-chalcone pregnenolone analogs **16**–**25** using Suzuki–Miyaura cross-coupling reaction. The synthesized compounds were tested for activity using human CYP17α hydroxylase expressed in *Escherichia coli*. Compounds **21** was the most active inhibitor in this series, with IC_{50} values of 0.61 μM and selectivity profile of 88.7% inhibition of hydroxylase enzyme. Molecular docking study of **21** was performed and showed the hydrogen bonds and hydrophobic interaction with the amino acid residues of the active site of CYP17.

1. Introduction

A number of steroids and their derivatives possess divers pharmacological activities as drugs for the treatment of a large number of diseases including cardiovascular [1], autoimmune diseases [2], brain tumors, breast cancer, prostate cancer, osteoarthritis, etc. [3]. Recently, a large number of steroidal derivatives containing five- or six-membered 17β-*exo*-heterocycles (preferably nitrogen containing), such as steroidal azoles [4,5] have been found to cause the inhibition of 17α-hydroxylase/C17-20-lyase (P45017α) which can block adrenal androgen synthesis at an early stage and may therefore be useful in the treatment of prostatic carcinoma [6–11]. In 1996, Njar et al. [12] reported the first steroidal inhibitors of CYP17 bearing a heterocyclic moiety bound to C17 by a nitrogen atom, among which the imidazolyl derivative **1** was found to be the most promising [12–15]. Later, in 2005, the same group reported the synthesis of galeterone **2** and its Δ^4-3-keto derivative [15–17], where **2** is currently undergoing phase I/II clinical trials for the treatment of chemotherapy naive CRPC [18,19]. However, patients suffering from castration-resistant prostate cancer (CRPC) can clearly benefit from the newly approved drug abiraterone acetate (Zytiga) **3** [20,21]. Hartmann and co-workers [22–24] have reported the synthesis of several CYP17 inhibitors as a new strategy for the treatment of prostate carcinoma. In 2014, we have synthesized novel 17-pregnenolone-imine derivatives as well as the 3-*O*-sulfonate and ester analogs at C-3, designed as new CYP17A1 inhibitors [25]. Banday et al. have reported recently some D-ring substituted steroidal chalcone [26] and isoxazolines and oxazolines [27] derivatives with remarkable activity against breast cancer and potential antiproliferative agents against LNCaP, PC-3 and DU-145 cells, respectively.

CYP17 catalyzes two reactions, the 17R-hydroxylation of pregnenolone and progesterone to the corresponding 17R alcohols and the subsequent 17,20-lyase reaction cleaving the C_{17}—C_{20} bond. This yields the 17-keto androgens androstenedione and dehydroepiandrosterone, precursors of all other androgens, including testosterone.

In continuation of our program on the synthesis of D-ring of steroidal inhibitors analog, we investigated the synthesis of biaryl-chalcone pregnenolone derivatives *via* Suzuki cross-coupling reaction together with the CYP17 hydroxylase enzyme inhibition activity, QSAR and the molecular modeling study.

* Corresponding author at: Am Tannenhof 8, 78464 Konstanz, Germany.
E-mail address: najim.al-masoudi@gmx.de (N.A. Al-Masoudi).

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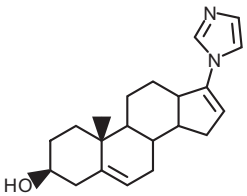
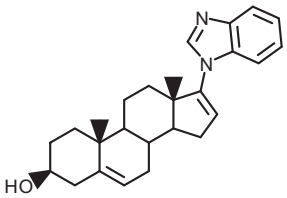
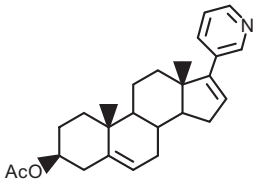
0039-128X/



1. 17-(1H-Imidazol-1-yl)-pregnen-16-ene-3-ol (VN/85-1)



2. Galeterone TOK-001



3. Abiraterone acetate

2. Experimental

2.1. General methods

Melting points are uncorrected and were measured on a Büchi melting point apparatus B-545 (Büchi Labortechnik AG, Switzerland). Microanalytical data were obtained with a Vario Elemental Analyzer (Shimadzu, Japan). NMR spectra were recorded on 400 and 600 MHz (^{1}H) and on 150:91 MHz (^{13}C) spectrometers (Bruker, Germany) with TMS as internal standard and on the δ scale in ppm. Signal assignments for protons were performed by selective proton decoupling or by COSY spectra. Heteronuclear assignments were verified by HSQC, HMBC and DFQ-COSY experiments. Mass spectra (EI, 70 eV, and FAB) were recorded on MAT 8200 spectrometers (Finnegan MAT, USA). TLC plates 60 F254 were purchased from Merck. The chromatograms were visualized under UV 254–366 nm and iodine.

2.2. Chemical synthesis

2.2.1. 17-((1-(4-Chlorophenyl)prop-1-en-2-yl)-5-pregnen-3β-ol (**5**)

To a stirred solution of pregnenolone **3** (100 mg, 0.32 mmol) in EtOH (10 ml) were added 4-chlorobenzaldehyde **4** (50 mg, 0.32 mmol) and aq. solution of 2 M NaOH (5 ml). After stirring at ambient temperature for 24 h, the mixture was neutralized with 1 M HCl and partitioned with EtOAc (3 × 15 ml). The combined organic extracts were washed with brine, dried over Na_2SO_4 and evaporated *in vacuo*. The residue was purified on a short SiO_2 column using the eluent hexane: EtOAc (3:2) as eluent to give **5** (82 mg, 58%) as a yellow powder. M.p.: 98–92 °C; IR (KBr) cm^{-1} 3675 (OH), 2927 (CH_2), 1698, 1508 (C═C), 1049 (C—O); ^{1}H NMR (DMSO-d_6) δ 7.89 (d, 1H, H, $J_{21,22}$ = 16.1 Hz, H-22), 7.75 (d, 2H, $J_{2',3'}$ = 8.3 Hz, $H_{arom.}$-2′ + $H_{arom.}$-6′), 7.61 (d, 1H, $J_{21,22}$ = 16.1 Hz, H-21), 7.49 (d, 2H, $J_{2',3'}$ = 8.3 Hz, $H_{arom.}$-3′ + $H_{arom.}$-5′), 5.27 (t, 1H, $J_{6,7}$ = 2.3 Hz, H-6), 4.61 (br s., 1H, OH), 3.26 (m, 1H, H-3), 2.56 (m, 1H, H-17), 2.16 (m, 1H, H-16a), 2.11 (m, 2H, CH_2-4), 1.94 (m, 1H, H-7a), 1.91 (m, 1H, H-12a), 1.78 (m, 1H, H-1a), 1.68 (m, 1H, H-2a), 1.60 (m, 1H, H-15a), 1.55 (m, 1H, H-16b), 1.53 (m, 1H, H-7b), 1.51 (m, 3H, H-11a + H-12b), 1.43–1.35 (m, 2H, H-2b + H-8 + H-11b), 1.14 (m, 2H, H-14 + H-15b), 1.02 (m, 1H, H-1b), 0.98 (m, 1H, H-9), 0.94 (s, 3H, Me-19), 0.53 (s, 3H, Me-18); ^{13}C NMR (DMSO-d_6,): δ 208.9 (C-20), 141.8 (C-5), 140.0 (C-22), 131.6 ($C_{arom.}$-1′ + $C_{arom.}$-4′), 130.6 ($C_{arom.}$-2′ + $C_{arom.}$-6′), 129.5 ($C_{arom.}$-3′ + $C_{arom.}$-5′), 129.2 (C-21), 120.7 (C-6), 70.5 (C-3), 56.6 (C-14 + C-17), 50.0 (C-9), 43.8 (C-13), 42.7 (C-4), 38.5 (C-12), 37.4 (C-1), 36.6 (C-10), 31.9 (Me-21), 31.8, 31.7 (C-2 + C-7 + C-8), 24.5 (C-15), 22.7 (C-16), 21.1 (C-11), 19.6 (Me-19), 13.4 (Me-18); Anal. calc. for $C_{28}H_{35}ClO_2$ (439.04): C, 76.60; H, 8.04. Found: C, 76.48; H, 7.95.

2.2.2. General procedure for the synthesis of diaryl derivatives of chalconyl pregnenolone via Suzuki cross-coupling reaction (**16**–**25**)

To a solution of **5** (200 mg, 0.40 mmol) in 1-propanol (15 ml) was added arylboronic acid (0.40 mmol) and the mixture was stirred for 15 min at ambient temperature followed by addition of Pd(0)(PPh_3)$_4$ (22 mg, 5% mmol) and aq. solution of 2 M Na_2CO_3 (5 ml). The reaction mixture was heated under reflux for 12–14 h. After cooling, water (5 ml) was added and the mixture was partitioned with EtOAc (3 × 10 ml). The combined organic extracts were washed with aq. solution of 5% Na_2CO_3 (3 × 10 ml), dried (Na_2SO_4) and evaporated *in vacuo*. The residue was purified on a short SiO_2 column using hexane: EtOAc (3:2) as eluent to give the desired product.

2.2.3. (E)-3-(2′,3′-difluoro[1,1′-biphenyl]4-yl)-1-(3β-hydroxy-pregnen-17-yl)prop-2-en-1-one (**16**)

From 2,3-difluorophenylboronic acid **6** (63 mg). Yield: 81 mg (39%) as a colorless powder. M.p.: 170–172 °C; R_f = 0.50; IR (KBr) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 7.89 (d, 1H, $J_{21,22}$ = 16.1 Hz, H-22), 7.75 (d, 2H, $J_{2',3'}$ = 8.3 Hz, $H_{arom.}$-2′ + $H_{arom.}$-6′), 7.61 (d, 1H, $J_{21,22}$ = 16.1 Hz, H-21), 7.49 (d, 2H, $J_{2',3'}$ = 8.3 Hz, $H_{arom.}$-3′ + $H_{arom.}$-5′), 5.27 (t, 1H, $J_{6,7}$ = 2.3 Hz, H-6), 4.61 (br s., 1H, OH), 3.26 (m, 1H, H-3), 2.56 (m, 1H, H-17), 2.16 (m, 1H, H-16a), 2.11 (m, 2H, CH_2-4), 1.94 (m, 1H, H-7a), 1.91 (m, 1H, H-12a), 1.78 (m, 1H, H-1a), 1.68 (m, 1H, H-2a), 1.60 (m, 1H, H-15a), 1.55 (m, 1H, H-16b), 1.53 (m, 1H, H-7b), 1.51 (m, 3H, H-11a + H-12b), 1.43–1.35 (m, 2H, H-2b + H-8 + H-11b), 1.14 (m, 2H, H-14 + H-15b), 1.02 (m, 1H, H-1b), 0.98 (m, 1H, H-9), 0.94 (s, 3H, Me-19), 0.53 (s, 3H, Me-18); ^{13}C NMR (DMSO-d_6,): δ 208.9 (C-20), 141.8 (C-5), 140.0 (C-22 + $C_{arom.}$-4′), 131.6 ($C_{arom.}$-1′ + $C_{arom.}$-4′), 130.6 ($C_{arom.}$-2′ + $C_{arom.}$-6′), 129.5 ($C_{arom.}$-3′ + $C_{arom.}$-5′), 129.2 (C-21), 120.7 (C-6), 70.5 (C-3), 56.6 (C-14 + C-17), 50.0 (C-9), 43.8 (C-13), 42.7 (C-4), 38.5 (C-12), 37.4 (C-1), 36.6 (C-10), 31.9 (Me-21), 31.8, 31.7 (C-2 + C-7 + C-8), 24.5 (C-15), 22.7 (C-16), 21.1 (C-11), 19.6 (Me-19), 13.4 (Me-18); Anal. calc. for $C_{34}H_{28}F_2O_2$ (516.67): C, 79.04; H, 7.41. Found: C, 78.83; H, 7.30.

2.2.4. 4′-(E)-3-hydroxy-pregenen-17-yl)-3-oxyprop-1-en-1-yl)-5-nitro-[1,1′-biphenyl]-2-carboxylic acid (**17**)

From 3-carboxy-5-nitrophenylboronic acid **7** (84 mg). Yield: 102 mg (45%) as a yellow powder. M.p.: 139–141 °C; R_f = 0.57; IR (KBr) cm^{-1} 3402 (OH), 2939 (CH_2), 1651(C═C), 1412 (C—NO_2); ^{1}H NMR (DMSO-d_6) δ 11.89 (s, 1H, CO_2H), 8.68 (d, 2H, J = 3.1 Hz, H-6″), 8.59 (d, 1H, J = 3.1 Hz, H-2″), 8.51 (d, 1H, J = 3.1 Hz, H-4″), 7.75 (d, 2H, $J_{2',3'}$ = 8.5 Hz, $H_{arom.}$-3′ + $H_{arom.}$-5′), 7.63 (d, 1H, $J_{21,22}$ = 16.1 Hz, H-22), 7.49 (d, 2H, $J_{5',6'}$ = 8.5 Hz, $H_{arom.}$-2′ + $H_{arom.}$-6′), 6.93 (d, 1H, $J_{21,22}$ = 16.1 Hz, H-21), 5.29 (t, 1H, $J_{6,7}$ = 2.5 Hz, H-6), 4.68 (br s, 1H, OH), 3.26 (m, 1H, H-3), 2.95 (m, 1H, H-17), 2.18 (m, 1H, H-16a), 2.13 (m, 2H, CH_2-4), 1.96 (m, 1H, H-7a), 1.84 (m, 1H, H-12a), 1.76 (m, 1H, H-1a), 1.68 (m, 1H, H-2a), 1.60 (m, 1H, H-15a), 1.57 (m, 1H, H-16b), 1.55 (m, 1H, H-7b), 1.54 (m, 1H, H-11a + H-12b), 1.43 (m, 1H, H-8), 1.41 (m, 1H, H-11b), 1.36 (m, 1H, H-2b), 1.24 (m, 2H, H-14 + H-15b), 1.01 (m, 1H, H-1b), 0.97 (m, 1H, H-9), 0.93 (s, 3H, Me-19), 0.53 (s, 3H, Me-18); ^{13}C NMR (DMSO-d_6,): δ 200.2 (C-20), 173.9 (CO_2H), 147.8 ($C_{arom.}$—NO_2), 141.8 (C-5), 140.0 (C-22 + $C_{arom.}$-4′), 135.3 ($C_{arom.}$-1″), 134.0 ($C_{arom.}$-1′), 133.6, 132.5, 132.0, 130.6, 129.5 ($C_{arom.}$), 129.2 (C-21), 120.7 (C-6), 70.5 (C-3), 60.9 (C-17),

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56.8 (C-14), 50.1 (C-9), 44.8 (C-13), 42.7 (C-4), 38.6 (C-12), 37.4 (C-1), 36.6 (C-10), 32.1, 31.9, 31.8 (C-2 + C-7 + C-8), 24.7 C-15), 22.8 (C-16), 21.1 (C-11), 19.6 (Me-19), 13.8 (Me-18); Anal. calc. for $C_{35}H_{39}NO_6$ (569.70): C, 73.79; H, 6.90. Found: C, 78.83; H, 7.30.

*2.2.5. (E)-3-(4′-fluoro-[1,1′-biphenyl]-4-yl)-1-(3-hydroxy-prenen-17-yl)prop-2-en-1-one) (**18**)*

From 4-fluorophenylboronic acid **8** (56 mg). Yield: 92 mg (46%) as a semi-solid; R_f = 0.40; IR (KBr) cm^{-1} 3402 (OH), 2939 (CH_2), 1651 (C=C); ^1H NMR (DMSO-d_6) δ 7.69 (d, 2H, $J_{3',4'}$ = 7.8 Hz, $H_{arom.}$-3′ + $H_{arom.}$-5′), 7.65–7.61 (m, 2H, $H_{arom.}$-2″ + $H_{arom.}$-6″), 7.54 (m, 2H, $H_{arom.}$-3″ + $H_{arom.}$-5″), 7.48 (d, 1H, $J_{21,22}$ = 16.0 Hz, H-22), 7.45 (d, 2H, $J_{2',3'}$ = 7.8 Hz, $H_{arom.}$-2′ + $H_{arom.}$-6′), 6.87 (d, 1H, $J_{21,22}$ = 16.0 Hz, H-21), 5.28 (br s, 1H, H-6), 4.29 (br s, 1H, OH), 3.29 (m, 1H, H-3), 2.95 (m, 1H, H-17), 2.18 (m, 1H, H-16a), 2.11 (m, 2H, CH_2-4), 1.96 (m, 1H, H-7a), 1.87 (m, 1H, H-12a), 1.77 (m, 1H, H-1a), 1.70 (m, 1H, H-2a), 1.58 (m, 1H, H-15a), 1.57 (m, 2H, H-7b + H-16b), 1.56 (m, 1H, H-11a), 1.53 (m, 1H, H-12b), 1.45 (m, 1H, H-8), 1.40 (m 1H, H-11b), 1.38 (m, 1H, H-2b), 1.25 (m, 2H, H-14 + H-15b), 1.00 (m, 1H, H-1b), 0.99 (m, 1H, H-9), 0.95 (s, 3H, Me-19), 0.58 (s, 3H, Me-18); ^{13}C NMR (DMSO-d_6,): δ 200.2 (C-20), 159.1 (d, J = 249 Hz, $C_{4''}$-F), 141.8 (C-5), 140.0 (C-22), 135.3 ($C_{arom.}$-1′), 134.1, 133.5, 132.5, 131.0, 129.3 ($C_{arom.}$), 129.2 (C-21), 120.7 (C-6), 110.8 ($C_{arom.}$-3″ + $C_{arom.}$-5″), 70.5 (C-3), 60.9 (C-17), 56.8 (C-14), 50.1 (C-9), 44.9 (C-13), 42.7 (C-4), 38.6 (C-12), 37.4 (C-1), 36.6 (C-10), 32.1, 31.9, 31.8 (C-2 + C-7 + C-8), 24.7 (C-15), 22.8 (C-16), 21.1 (C-11), 19.6 (Me-19), 13.8 (Me-18); ^{19}F NMR (DMSO-d_6,): δ −115.6; Anal. calc. for $C_{34}H_{39}FO_2$ (498.68): C, 81.89; H, 7.88. Found: C, 81.77; H, 7.71.

*2.2.6. (E)-1-(3-hydroxy-pregenen-17-yl)-3-(4′-methylthio)-[1,1′-biphenyl]-4-yl)prop-2-en-1-one (**19**)*

From 4-methylthiophenylboronic acid **9** (67 mg). Yield: 110 mg (52%) as a dark brown solid. M.p.: 84–87 °C; R_f = 0.50; IR (KBr) cm^{-1} 3395 (OH), 2932 (CH_2), 1605 (C=C); ^1H NMR (DMSO-d_6) δ 7.75 (d, 2H, $J_{2'',6''}$ = 8.5 Hz, $H_{arom.}$-2″ + $H_{arom.}$-6″), 7.62 (d, 1H, $J_{21,22}$ = 16.0 Hz, H-22), 7.60 (d, 2H, $J_{2',3'}$ = 8.5 Hz, $H_{arom.}$-3′ + $H_{arom.}$-5′), 7.49 (d, 2H, $J_{2',3'}$ = 8.7 Hz, $H_{arom.}$-2′ + $H_{arom.}$-6′), 7.34 (d, 2H, $J_{2',3'}$ = 8.5 Hz, $H_{arom.}$-3″ + $H_{arom.}$-5″), 6.92 (d, 1H, $J_{21,22}$ = 16.0 Hz, H-21), 5.27 (t, 1H, $J_{6,7}$ = 2.5 Hz, H-6), 3.29 (m, 1H, H-3), 2.98 (m, 1H, H-17), 2.38 (s, 3H, SMe), 2.20 (m, 1H, H-16a), 2.14 (m, 2H, CH_2-4), 1.95 (m, 1H, H-7a), 1.82 (m, 1H, H-12a), 1.75 (m, 1H, H-1a), 1.73 (m, 1H, H-2a), 1.63 (m, 1H, H-15a), 1.57 (m 1H, H-16b), 1.56 (m, 1H, H-7b + H-11a), 1.53 (m, 1H, H-12b), 1.42 (m, 1H, H-8), 1.40 (m, 1H, H-11b), 1.36 (m, 1H, H-2b), 1.23 (m, 2H, H-14 + H-15b), 1.01 (m, 1H, H-1b), 0.98 (m, 1H, H-9), 0.96 (s, 3H, Me-19), 0.53 (s, 3H, Me-18); ^{13}C NMR (DMSO-d_6,): δ 200.1 (C-20), 141.8 (C-5), 140.0 (C-22), 137.7 ($C_{arom.}$-4′ + $C_{arom.}$-SMe), 134.0 ($C_{arom.}$-1″), 132.0 ($C_{arom.}$-1′), 129.3 (C-21), 128.8, 128.5, 127.2, 126.9 ($C_{arom.}$), 120.7 (C-6), 70.5 (C-3), 60.9 (C-17), 56.8 (C-14), 50.1 (C-9), 44.8 (C-13), 42.7 (C-4), 38.6 (C-12), 37.4 (C-1), 36.6 (C-10), 32.1, 31.9, 31.8 (C-2 + C-7 + C-8), 24.7 (C-15), 22.8 (C-16), 21.1 (C-11), 19.6 (Me-19), 15.2 (SMe), 13.8 (Me-18); Anal. calc. for $C_{35}H_{42}O_2S$ (526.29): C, 79.80; H, 8.04. Found: C, 79.39; H, 7.88.

*2.2.7. (E)-1-(3-hydroxy-pregenen-17-yl)-3-(4′-hydroxy)-[1,1′-biphenyl]-4-yl)prop-2-en-1-one (**20**)*

From 4-hydroxyphenylboronic acid **10** (55 mg). Yield: 115 mg (58%) as a colorless powder. M.p.: 150–152 °C; R_f = 0.57; IR (KBr) cm^{-1} 3433 (OH), 2932 (CH_2), 1605 (C=C); ^1H NMR (DMSO-d_6) δ 7.55 (d, 2H, $J_{2',6'}$ = 8.5 Hz, $H_{arom.}$-2′ + $H_{arom.}$-6′), 7.52 (d, 1H, $J_{21,22}$ = 16.1 Hz, H-22), 7.49 (d, 2H, $J_{2',3'}$ = 8.5 Hz, $H_{arom.}$-3′ + $H_{arom.}$-5′), 7.43 (d, 2H, $J_{2',3'}$ = 8.5 Hz, $H_{arom.}$-2″ + $H_{arom.}$-6″), 7.35 $J_{2',3'}$ = 8.5 Hz, $H_{arom.}$-3″ + $H_{arom.}$-5″), 6.92 (d, 1H, $J_{21,22}$ = 16.1 Hz, H-21), 5.28 (t, 1H, $J_{6,7}$ = 2.6 Hz, H-6), 3.26 (m, 1H, H-3), 2.99 (m, 1H, H-17), 2.16 (m, 1H, H-16a), 2.12 (m, 2H, CH_2-4), 1.95 (m, 1H, H-7a), 1.82 (m, 1H, H-12a), 1.75 (m, 1H, H-1a), 1.69 (m, 1H, H-2a), 1.66 (m, 1H, H-15a), 1.63 (m, 1H, H-16b), 1.57 (m, 1H, H-7b), 1.55 (m, 1H, H-11a), 1.53 (m, 1H, H-12b), 1.43 (m, 1H, H-8), 1.39 (m, 1H, H-11b), 1.36 (m, 1H, H-2b), 1.23 (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,): δ 200.0 (C-20), 155.2 ($C_{arom.}$-OH), 141.8 (C-5), 140.0 (C-22), 138.4 ($C_{arom.}$-4′), 135.3 ($C_{arom.}$-1′), 133.6, 132.9, 132.5, 131.9, 129.4 ($C_{arom.}$), 129.2 (C-21), 120.6 (C-6), 70.5 (C-3), 60.9 (C-17), 56.8 (C-14), 50.0 (C-9), 44.9 (C-13), 42.7 (C-4), 38.6 (C-12), 37.4 (C-1), 36.6 (C-10), 32.1, 31.9, 31.7 (C-2 + C-7 + C-8), 25.5 (C-15), 22.6 (C-16), 21.1 (C-11), 19.6 (Me-19), 13.8 (Me-18); Anal. calc. for $C_{34}H_{40}O_3$ (496.69): C, 82.22; H, 8.12. Found: C, 81.97; H, 7.90.

*2.2.8. (E)-3-(2′,4′-dimethoxy-[1,1′-biphenyl]1–4-y)-1-(3-hydroxy-pregenen-17-yl)prop-2-en-1-one (**21**)*

From 3,4-dimethoxyphenylboronic acid **11** (73 mg). Yield: 121 mg (56%) as a dark brown semi-solid; R_f = 0.52; IR (KBr) cm^{-1} 3402 (OH), 2932 (CH_2), 1620 (C=C), 1026 (C—O); ^1H NMR (DMSO-d_6) δ 7.63 (1H, $J_{21,22}$ = 15.6 Hz, H-22), 7.61 (m, 3H, $H_{arom.}$-3′ + $H_{arom.}$-5′ + $H_{arom.}$-6″), 7.55 (d, 2H, $J_{2',3'}$ = 8.5 Hz, $H_{arom.}$-2′ + $H_{arom.}$-6′), 7.17 (d, 1H, $J_{21,22}$ = 15.6 Hz, H-21), 7.14 (s, 1H, $H_{arom.}$-3″), 6.99 (d, 1H, $J_{5'',6''}$ = 8.3 Hz, $H_{arom.}$-5″), 5.26 (t, 1H, $J_{6,7}$ = 2.6 Hz, H-6), 4.62 (br s., 1H, OH), 3.84, 3.77 (2 × s, 6H, 2 × OMe), 3.26 (m, 1H, H-3), 2.99 (m, 1H, H-17), 2.15 (m, 1H, H-16a), 2.10 (m, 2H, CH_2-4), 1.94 (m, 1H, H-7a), 1.81 (m, 1H, H-12a), 1.76 (m, 1H, H-1a), 1.67 (m, 1H, H-2a), 1.65 (m, 1H, H-15a + H-16b), 1.57 (m, 1H, H-7b), 1.56 (m, 1H, H-11a), 1.54 (m, 1H, H-12b), 1.43 (m, 2H + H-2b + H-8), 1.39 (m, 1H, H-11b), 1.15 (m, 2H, H-14 + H-15b), 1.08 (m, 1H, H-1b), 0.98 (m, 1H, H-9), 0.92 (s, 3H, Me-19), 0.51 (s, 3H, Me-18); ^{13}C NMR (DMSO-d_6,): δ 208.9 (C-20), 162.3 ($C_{2''}$-OMe), 160.2 ($C_{4''}$-OMe), 141.7 (C-5), 140.0 (C-22), 133.6 ($C_{arom.}$-4′), 132.5 ($C_{arom.}$-1′), 132.0, 131.9, 129.3 ($C_{arom.}$), 129.2 (C-21), 119.0 (C-6), 112.7 ($C_{arom.}$-5″), 110.9 ($C_{arom.}$-3″), 70.5 (C-3), 63.1 (C-17), 56.6 (C-14), 56.1, (2xOMe), 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, 31.7 (C-2 + C-7 + C-8), 24.5 (C-15), 22.7 (C-16), 21.1 (C-11), 19.6 (Me-19), 13.4 (Me-18); Anal. calc. for $C_{36}H_{44}O_4$ (540.32): C, 79.96; H, 8.20. Found: C, 79.69; H, 8.02.

*2.2.9. (E)-1-(3-hydroxy-pregenen-17-yl)-3-(4′-trimethylsilyl)-[1,1′-biphenyl]-4-yl)prop-2-en-1-one (**22**)*

From 4-trimethylsilylphenylboronic acid **12** (78 mg). Yield: 67 mg (45%) as a gray powder; M.p.: 92–95 °C; R_f = 0.40; IR (KBr) cm^{-1} 3402 (OH), 2932 (C—H), 1622 (C=C), 1026 (C—O); ^1H NMR (DMSO-d_6) δ 7.63–7.45 (m, 9H, $H_{arom.}$ + H-22), 6.92 (d, 1H, $J_{21,22}$ = 16.0 Hz, H-21), 5.26 (t, 1H, $J_{6,7}$ = 2.5 Hz, H-6), 4.62 (br s, 1H, OH), 3.27 (m, 1H, H-3), 2.97 (m, 1H, H-17), 2.17 (m, 1H, H-16a), 2.11 (m, 2H, CH_2-4), 1.93 (m,1H, H-7a), 1.82 (m, 1H, H-12a), 1.74 (m, 1H, H-1a), 1.69 (m, 1H, H-2a), 1.67 (m, 1H, H-15a + H-16b), 1.58 (m, 1H, H-7b + H-11a), 1.56 (m, 1H, H-12b), 1.41 (m, 1H, H-8), 1.39 (m, 1H, H-11b), 1.37 (m, 1H, H-2b), 1.24 (m, 2H, H-14 + H-15b), 1.06 (m, 1H, H-1b), 1.00 (m, 1H, H-9), 0.98 (s, 3H, Me-19), 0.52 (s, 3H, Me-18), 0.26 (s, 9H, $SiMe_3$); 0.52 (s, 3H, Me-18), 0.26 (s, 9H, $SiMe_3$); ^{13}C NMR (DMSO-d_6,): δ 199.5 (C-20), 141.2 (C-5), 140.6 (C-22), 139.5 ($C_{arom.}$-1″), 138.8 ($C_{arom.}$-4′), 134.5 (C-4″), 133.7, 133.0, 131.5, 128.7 ($C_{arom.}$), 128.6 (C-21), 120.2 (C-6), 70.0 (C-3), 62.7 (C-17), 56.3 (C-14), 49.6 (C-9), 44.3 (C-13), 42.2 (C-4), 37.9 (C-12), 36.9 (C-1), 36.1 (C-10), 31.4, 31.1 (C-2 + C-7 + C-8), 25.5 (C-15), 24.0 (C-16), 21.2 (C-11), 19.1 (Me-19), 13.2 (Me-18), −1.2 ($SiMe_3$); Anal. calc. for $C_{37}H_{48}O_2Si_3$ (552.34): C, 80.38; H, 8.75. Found: C, 80.11; H, 8.66.

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*2.2.10. (E)-1-(3-hydroxy-pregenen-17-yl)-3-(4′-(trifluoromethyl)-[1,1′-biphenyl]-4-yl)prop-2-en-1-one (**23**)*

From 2-trifluoromethylphenylboronic acid **13** (76 mg). Yield: 90 mg (41%) as a brown semi-solid; R_f = 0.43; IR (KBr) cm^{-1} 3395 (OH), 2932 (C—H), 1682 (C=$C_{arom.}$); 1026 (C—O); ^{1}H NMR (DMSO-d_6) δ 7.74 (d, H, $J_{5'',6''}$ = 8.0 Hz, $H_{arom.}$-6″), 7.63 (d, 1H, $J_{21,22}$ = 16.1 Hz, H-22), 7.61 (m, 4H, $H_{arom.}$-3′ + $H_{arom.}$-5′ + $H_{arom.}$-3″ + $H_{arom.}$-5″), 7.55 (d, 2H, $J_{2',3'}$ = 8.0 Hz, $H_{arom.}$-2′ + $H_{arom.}$-6′), 7.47 (d, 1H, $J_{4'',5''}$ = 8.0 Hz, $H_{arom.}$-4″), 6.92 (d, 1H, $J_{21,22}$ = 16.1 Hz, H-21), 5.26 (t, 1H, $J_{6,7}$ = 2.6 Hz, H-6), 3.27 (m, 1H, H-3), 2.99 (m, 1H, H-17), 2.16 (m, 1H, H-16a), 2.10 (m, 2H, CH_2-4), 1.94 (m, 1H, H-7a), 1.82 (m, 1H, H-12a), 1.77 (m, 1H, H-1a), 1.74 (m, 1H, H-2a), 1.66 (m, 2H, H-15a + H-16b), 1.57 (m, 1H, H-7b), 1.55 (m, 1H, H-11a), 1.53 (m, 1H, H-12b), 1.40 (m, 1H, H-8), 1.35 (m 1H, H-11b), 1.33 (m, 1H, H-2b), 1.21 (m, 2H, H-14 + H-15b), 1.03 (m, 1H, H-1b), 0.98 (m, 1H, H-9), 0.96 (s, 3H, Me-19), 0.52 (s, 3H, Me-18); ^{13}C NMR (DMSO-d_6,): δ 199.6 (C-20), 141.3 (C-5), 139.5 (C-22), 139.4 ($C_{arom.}$-4′), 136.7 ($C_{arom.}$-1′), 135.3 ($C_{arom.}$-5″), 133.0, 132.5, 132.3, 132.0, 131.5, 0, 131.0 ($C_{arom.}$), 128.7 (C-21), 125.4 (d, $J_{C,F}$ = 249 Hz, CF_3), 120.2 (C-6), 70.0 (C-3), 62.6 (C-17), 56.3 (C-14), 50.0 (C-9), 44.3 (C-13), 42.2 (C-4), 38.1 (C-12), 36.9 (C-1), 36.1 (C-10), 31.5, 31.4, 31.2 (C-2 + C-7 + C-8), 25.5 (C-15), 24.1 (C-16), 22.2 (C-11), 19.1 (Me-19), 13.3 (Me-18); Anal. calc. for $C_{35}H_{39}F_3O_2$ (548.69): C, 76.62; H, 7.16. Found: C, 76.42; H, 7.02.

*2.2.11. 4′-((E)-3-(3-hydroxy-pregenen-17-yl)-3-oxoprop-1-en-1-yl)-[1,1′-biphenyl]3-carbonitrile (**24**)*

From 3-cyanophenylboronic acid **14** (59 mg). Yield: 59 mg (39%) as a brown solid; M.p.: 88–91 °C; R_f = 0.45; IR (KBr) cm^{-1} 3402 (OH); 2932 (C—H), 1358 (C—N), 1682 (C=$C_{arom.}$); ^{1}H NMR (DMSO-d_6) δ 8.29 (s, 1H, $H_{arom.}$-2″), 8.12 (d, 1H, $J_{5'',6''}$ = 7.9 Hz, $H_{arom.}$-6″), 7.89 (d, 1H, $J_{5'',6''}$ = 7.9 Hz, $H_{arom.}$-4″), 7.70 (t, 1H, $J_{4'',5''}$ = 7.9 Hz, $H_{arom.}$-5″), 7.62 (d, 1H, $J_{21,22}$ = 16.1 Hz, H-22), 7.55 (d, 2H, $J_{5',6'}$ = 8.4 Hz, $H_{arom.}$-3′ + $H_{arom.}$-5′), 7.49 (d, 2H, $J_{2',3'}$ = 8.4 Hz, $H_{arom.}$-2′ + $H_{arom.}$-6′), 6.91 (d, 1H, $J_{21,22}$ = 16.1 Hz, H-21), 5.27 (t, 1H, $J_{6,7}$ = 2.5 Hz, H-6), 3.27 (m, 1H, H-3), 4.21 (d, 1H, J = 6.2 Hz, OH), 3.25 (m, 1H, H-3), 2.97 (m, 1H, H-17), 2.13 (m, 1H, H-16a), 2.10 (m, 2H, CH_2-4), 1.93 (m, 1H, H-7a), 1.81 (m, 1H, H-12a), 1.77 (m, 1H, H-1a), 1.70 (m, 1H, H-2a), 1.65 (m, 2H, H-15a + H-16b), 1.56 (m, 1H, 1H-7b), 1.54 (m, 1H, H-11a), 1.52 (m, 1H, H-12b), 1.40 (m, 1H, H-8), 1.34 (m 1H, H-11b), 1.32 (m, 1H, H-2b), 1.23 (m, 2H, H-14 + H-15b), 1.10 (m, 1H, H-1b), 0.98 (m, 1H, H-9), 0.92 (s, 3H, Me-19), 0.54 (s, 3H, Me-18); ^{13}C NMR (DMSO-d_6,): δ 200.2 (C-20), 142.3 ($C_{arom.}$-1″), 141.8 (C-5), 140.0 (C-22), 139.6 ($C_{arom.}$-4′), 135.3 ($C_{arom.}$-1′), 132.5 ($C_{arom.}$-4″ + $C_{arom.}$-6″), 132.0 ($C_{arom.}$-2″), 130.8 ($C_{arom.}$-5″), 129.3 (C-21), 128.3 ($C_{arom.}$-3′ + $C_{arom.}$-5′), 127.4 ($C_{arom.}$-2′ + $C_{arom.}$-6′), 120.7 (C-6), 119.1 (CN), 112.8 ($C_{3''}$-CN), 70.5 (C-3), 63.1 (C-17), 56.8 (C-14), 50.1 (C-9), 44.9 (C-13), 42.7 (C-4), 38.4 (C-12), 37.4 (C-1), 36.6 (C-10), 31.9, 31.7 (C-2 + C-7 + C-8), 25.6 (C-15), 24.3 (C-16), 22.7 (C-11), 19.6 (Me-19), 13.8 (Me-18); Anal. calc. for $C_{35}H_{39}NO_2$ (505.70): C, 83.13; H, 7.77; N, 2.77. Found: C, 82.93; H, 7.65; N, 2.56.

*2.2.12. (E)-3-(4′-ethoxy-[1,1′-biphenyl]-4-yl)-1-(3-hydroxypregenen-17-yl)prop-2-en-1-one (**25**)*

From 4-ethoxyphenylboronic acid **15** (67 mg). Yield: 88 mg (42%) as a brown semi-solid; R_f = 0.40; IR (KBr) cm^{-1} 3339 (OH), 2932 (C—H), 1736 (C=O), 1574 (C=$C_{arom.}$); ^{1}H NMR (DMSO-d_6) δ 7.63–7.60 (m, 3H, H-22 + $H_{arom.}$-3′ + $H_{arom.}$-5′), 7.54 (d, 2H, $J_{2'',6''}$ = 8.6 Hz, $H_{arom.}$-2′ + $H_{arom.}$-6′), 7.48 (d, 2H, $J_{5'',6''}$ = 8.6 Hz, $H_{arom.}$-2″ + $H_{arom.}$-6″), 6.95 (d, 2H, $J_{2'',3''}$ = 8.6 Hz, $H_{arom.}$-3″ + $H_{arom.}$-5″), 6.85 (d, 1H, $J_{21,22}$ = 16.0 Hz, H-21), 5.25 (t, 1H, $J_{6,7}$ = 2.5 Hz, H-6), 4.05 (q, 2H, J = 6.9 Hz, O$CH_2$$CH_3$), 3.28 (m, 1H, H-3), 3.02 (m, 1H, H-17), 2.18 (m, 1H, H-16a), 2.07 (m, 2H, CH_2-4), 1.96 (m, 1H, H-7a), 1.84 (m, 1H, H-12a), 1.75 (m, 1H, H-1a), 1.68 (m, 1H, H-2a), 1.65 (m, 1H, H-16b), 1.60 (m, 1H, H-15a), 1.56 (m, 1H, H-7b + H-11a), 1.54 (m, 1H, H-12b), 1.42 (m, 1H, H-8), 1.34 (m, 1H, H-11b), 1.32 (t, 3H, O$CH_2$$CH_3$), 1.30 (m, 1H, H-2b), 1.25 (m, 2H, H-14 + H-15b), 1.00 (m, 1H, H-1b), 0.97 (m, 1H, H-9), 0.96 (s, 3H, Me-19), 0.56 (s, 3H, Me-18); ^{13}C NMR (DMSO-d_6,): δ 200.0 (C-20), 158.2 ($C_{arom.}$-OEt), 141.6 ($C_{arom.}$-4′), 141.5 (C-5), 140.0 (C-22), 135.3 ($C_{arom.}$-1′), 132.0 ($C_{arom.}$-1″), 129.1 (C-21), 129.1, 127.7, 126.0 ($C_{arom.}$), 120.5 (C-6), 115.6 ($C_{arom.}$-3″ + $C_{arom.}$-5″), 70.8 (C-3), 60.9 (C-17), 64.6 (O$CH_2$$CH_3$), 61.3 (C-17), 56.1 (C-14), 50.2 (C-9), 44.2 (C-13), 42.4 (C-4), 38.6 (C-12), 37.3 (C-1), 36.2 (C-10), 32.0, 31.7, 31.4 (C-2 + C-7 + C-8), 25.7 (C-15), 24.8 (C-16), 22.2 (C-11), 19.2 (Me-19), 14.5 (O$CH_2$$CH_3$), 13.2 (Me-18); Anal. calc. for $C_{36}H_{44}O_3$ (524.33): C, 82.40; H, 8.45. Found: C, 82.21; H, 8.36.

2.3. Biological evaluations

2.3.1. 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 co-workers [28]. Membrane fractions were diluted to a concentration that gives a 15–25% conversion in the controls for the different assays.

2.3.2. 17α-Hydroxylase enzyme assay

Determination of the hydroxylase activity of CYP17 was performed by measurement of the conversion of P_5 to 17OHP_5. An assay mixture consisting of 140 μl phosphate buffer (0.05 M, pH 7.4, 1 μM $MgCl_2$, 0.1 μM EDTA, and 0.1 μM dithiothreitol), 50 μl NADPH generating system (in phosphate buffer with 50 μM glucose-6-phosphate, 5.75 μM $NADP^+$, and 27.5 U/ml 5 glucose-6-phosphate dehydrogenase) and 5 μl substrate solution (25 μM [3H]-P_5) 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 1N 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 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 ethyl acetate was evaporated to dryness in a fresh tube and re-dissolved in 40 μl acetonitrile/water (1:1) for HPLC analysis.

2.3.3. 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 [29].

3. Results and discussion

3.1. Chemistry

Treatment of pregnenolone **3** with *p*-chlorobenzaldehyde (**4**) gave the chalcone derivative **5** (58%). The Suzuki–Miyaura cross-coupling reaction [30] has been employed in the preparation of new pregnenolone analogs. Thus, treatment of **5** with the appropriate arylboronic acids (*e.g.*: 2-fluorophenyl-, 3-fluorophenyl-, 4-fluor

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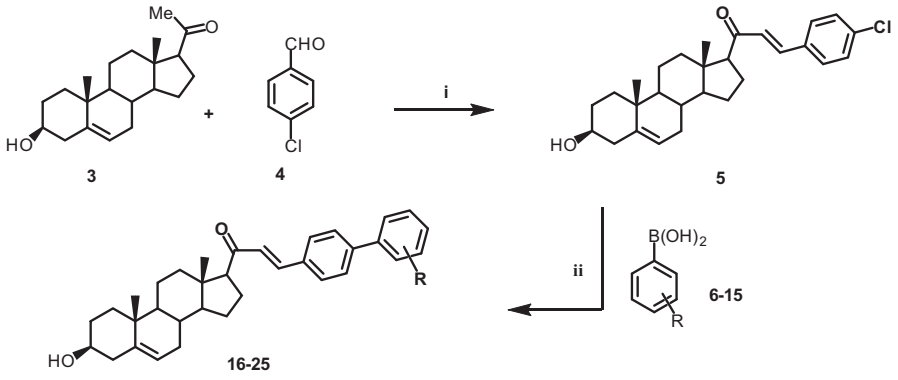

Entry	R	R	Entry
6, 16	2,3-F_2	2,4-OMe_2	11, 21
7, 17	3-NO_2,5-CO_2H	4-$SiMe_3$	12, 22
8, 18	4-F	4-CF_3	13, 23
9, 19	4-SMe	3-CN	14, 24
10, 20	4-OH	4-OEt	15, 25

Scheme 1. Reagents and conditions: (i) EtOH, 2 M aq. NaOH, 24 h, r.t.; (ii) $Pd(Ph_3P)_4$, K_2CO_3,1-PrOH, reflux, 12–14 h.

ophenyl-, 3,4-difluorophenyl-, 3,4-dimethoxy phenyl-, 2-methylthiophenyl-, 4-nitrophenyl-, 4-ethoxycarbonylphenyl-, 4-hydroxyphenyl-, 3-cyanophenyl-, and 4-triflouromethylphenyl boronic acids 6–15, using palladium(0) tetrakis-triphenylphosphine ($Pd(0)Ph_3P)_4$) and sodium bicarbonate as catalysts in 1-propanol afforded **16–25** in 72–78% yield (Scheme 1).

The structures of **5** and **17–28** were assigned on the basis of their NMR (^1H, ^{13}C and 2D), which showed rather similar patterns of the proton and carbon atoms of pregnen scaffold. The ^1H NMR spectrum of **5** showed beside the expected signals for pregnenolone moiety two doublets at δ 7.61 and 7.89 ppm with a large J coupling (~16.0 Hz) were assigned for the olefin protons H-21 and H-22 of chalcone moiety. The ^1H NMR spectra of **17–28** were characterized by the presence of additional aromatic protons and carbon atoms, indicative for arylation of the chalconyl pregnenolone analogs. The aromatic protons protons appeared as doublets or multiplets at the regions δ 8.59–6.95 ppm (J = 8.5–7.9 or 3.1 Hz). The triplets at δ 5.29–5.25 ppm ($J \sim 2.6$ Hz) were assigned to H-6, while H-3 and CH_2-4 appeared as multiplets at the regions δ 2.16–2.07 and 1.58–1.53 ppm, respectively. The other aliphatic protons were fully analyzed (*c.f.* Experimental section). H-21 and H-22 were resonated as two doublets at the regions δ 7.17–6.85 and 7.63–7.50 ppm, respectively with a large $J \sim 16.0$ Hz, indicative for the *trans*-configuration of the chalcone protons (H-21 and H-22). In the ^{13}C NMR spectra of **5** and **17–28**, the resonances at δ 208.9–199.5 ppm were assigned for C-20, whereas the olefinic carbon atoms (C-21, C-22) were appeared at the regions δ 129.3–128.6 and 140.0.139.5 ppm, respectively. The aromatic, other pregnen aliphatic carbon atoms and the substituents have been fully identified (*c.f.* Experimental section). Compound **19** was selected for further NMR studies, since its gradient HMBC spectrum [31] allowed the identification of H-21 at δ_H 6.92 ppm from the $^2J_{C,H}$ couplings to the chalconyl atom (C-22) at δ_C 135.2 ppm as well as the carbonyl carbon atom (C-20) at δ_C 200.1 ppm. A $^2J_{C,H}$ coupling between H-22 at δ_H 7.62 ppm and the aromatic carbon atom (C-1′) at δ_C 132.0 ppm was observed. Furthermore, SMe protons at δ_H 2.38 ppm showed a $^3J_{C,H}$ coupling to the aromatic carbon atom (C-4″) at δ 137.7 ppm. Additionally, $^3J_{C,H}$ couplings between C-20 and H-16a and H-16b at δ_H 2.20 and 1.57 ppm, respectively, were distinguished (Fig. 1). The configuration of the chalcone protons (H-21 and H-22) was further confirmed by the ^1H,^1H NOESY NMR spectrum [32]. Thus, the spectrum of **19** revealed coupling

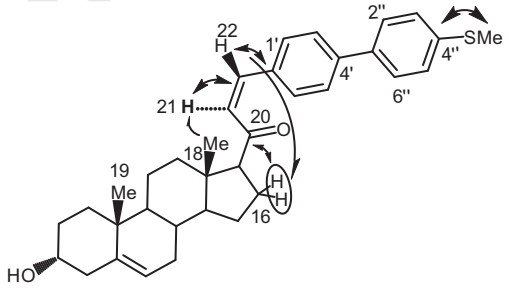

Fig. 1. $J_{C,H}$ correlations in the HMBC (double head arrow), and NOESY (single head arrow) correlations of (**19**).

between H-21 at δ_H 6.92 ppm and Me-18 at δ_H 0.53 ppm in addition for the coupling between H-22 at δ_H 7.62 ppm H-16a at δ_H 2.20 ppm. These data confirmed that both H-21 and H-22 are in a *trans*-configuration (Fig. 1). All the compounds have been identified by their ^1H,^{13}C HSQC NMR spectra [33].

Further, the configuration of the olefinic protons (C_{21}=C_{22}) of **16–25** has been calculated by the DFT method (GGA) at the level PBE/DNP [34]. The results in Table 1 demonstrated that the *trans* configuration of these analogs is the more stable. Fig. 2 represents the orbitals of **16**, which revealed that the HOMO in both *trans* and *cis* isomers is located at the olefinic bond (C_5=C_6) of the pregnen part, while the LUMO is separated on the carbon atoms of the olefinic linkage (C_{21}=C_{22}) and the oxygen atom of the carbonyl group.

3.2. 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 **5** and **16–25** in different concentrations dissolved in DMSO (final concentration usually between 0 and 10 μM). The results are presented in Table 2. The steroidal CYP17A1 inhibitor abiraterone acetate (**3**) was used as a reference compound. Aiming at the development of new drugs for the treatment of

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Table 1
Energies of the analogs **16–25** calculated at the theory level PBE/DN.

Compd.	*cis*-isomer	*trans*-isomer	Compd.	*cis*-isomer	*trans*-isomer
16	−1667.103153	−1667.109300	**21**	−1697.5689582	−1697.5768487
17	−1667.103153	−1861.5775018	**22**	−1877.1550518	−1877.1669258
18	−1567.8899860	−1567.8972197	**23**	−1560.8606047	−1560.8679660
19	−1906.0519105	−1906.0661973	**24**	−1805.5785310	−1805.5854794
20	−1543.8767651	−1543.8839474	**25**	−1622.3943784	−1622.4105373

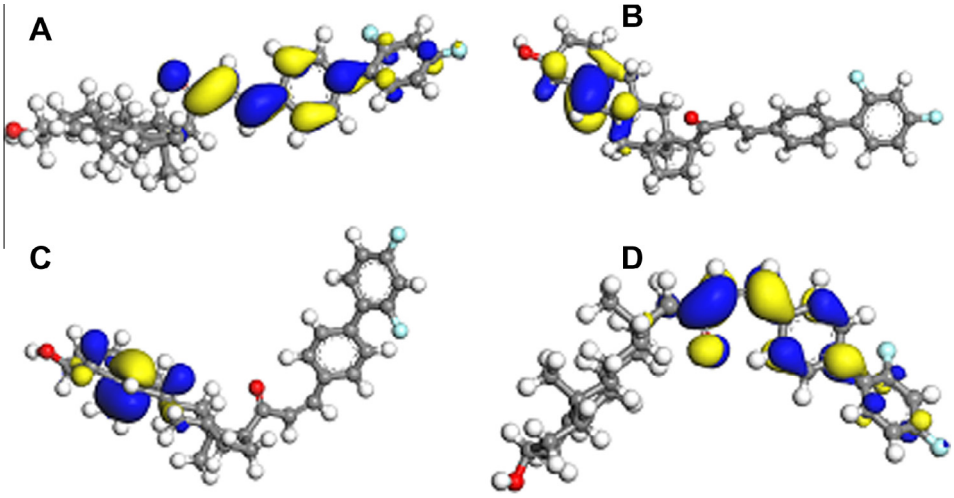

Fig. 2. Represents the energies of compound **16**: (A) *trans* HOMO = −0.106368 hartree; (B) *trans* LUMO = −0.106368 hartree, (C) *cis* HOMO = −0.186773 hartree; (D) *cis* LUMO = −0.104875 hartree.

Table 2
Inhibition of CYP17 hydroxylase enzyme by pregnenolone analogs.

Compd.	Inhibition %[a]	IC_{50} (μM)[b]	Compd.	Inhibition %[a]	IC_{50} (μM)[b]
5	78.2	3.10 ± 0.8	**20**	68.4	nd
16	78.7	1.53 ± 0.2	**21**	88.7	0.61 ± 0.0
17	76.2	2.80 ± 0.5	**22**	60.0	nd
18	83.8	1.10 ± 0.1	**23**	75.6	1.25 ± 0.6
19	70.1	2.85 ± 0.4	**24**	80.1	1.91 ± 0.3
ABT[c]	92.93	0.072	**25**	58.9	2.97 ± 0.7

[a] Data shown were obtained by performing the tests in duplicate. IC_{50}: the concentration of the inhibitor that is required for 50% inhibition *in vitro*.
[b] Hydroxylase enzyme inhibition measured at 10 μM.
[c] ABT: abiraterone acetate; nd: not determined.

prostate cancer, the effects of steroidal compounds on androgen biosynthesis were evaluated *in vitro*.

Derivatization of the pregnenolone scaffold by a *p*-chlorophenyl-chalcone group is readily tolerated in 17-position (compound **5**) followed by introduction of substituted aryl groups *via* Suzuki cross-coupling reaction as accomplished in compounds **16–25**. The inhibitory profile (Table 2) showed that **5**, **16–19**, and **23–25** exhibited inhibition of CYP17 with IC_{50} values of 0.10–3.10 μM. Interestingly, compound **21** having 2,4-dimethoxyphenyl substituents, pronounced a higher inhibitory activity with IC_{50} = 0.61 μM and inhibition of 88.7% in comparison for those carrying other substituents. Introduction of nitro and carboxy residues in *meta* positions, 2,3-difluoro, 4-fluoro 4-trifluomethyl or 3-cyano groups as accomplished in compounds **16–18**, **23** and **24** decreased the inhibitory potency, which might be due to its electron withdrawing effects reducing the electron density of the sp^2 hybrid of the C_{21}=C_{22} and phenyl π bonds, which in turn decrease the hydrophobicity interactions with the CYP17 amino residues. However, the two methoxy group would enhance such the hydrophobicity interactions of both the phenyl and C_{21}=C_{22} through π–π interactions. Such argument is in agreement with the results reported previously [35].

3.3. Molecular docking study

The structural common features of compounds **5** and **16–25** to abiraterone acetate (**3**) (pregnenolone scaffold) with some inhibitory activity against CYP17, prompted us to study their interactions 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: 3ruk) was obtained from Protein Data Bank (http://www.rcsb.org) [36].

AutoDock4.2 software [37] and AutoDock Tools were used for docking experiments following Lamarckian Genetic algorithm (hybrid of genetic and local search algorithm) [38]. Best docked conformation of each ligand based on docking parameters and binding site residues interactions and selected among top ten docked conformations generated during docking study. The results were visualized with AutoDock Tools software, whereas the automated docking experiments have been reported previously in details [25].

Compound **21** has been selected for the docking modeling study, since it is the most active candidate of the series, meanwhile its binding energy score −13.02 indicating a selectivity and potency profile of dimethoxy-biphenylchalcone-pregnenolone to bind the active site of HIV-RT pocket (Fig. 3). Detailed analysis of the binding mode showed an interesting complexation of heme iron (Fe^{+2}) with the lone pair of oxygen atom OH group at C-3. Beside the complexation, the phenyl ring B point toward the aromatic ring of Phe300 residue apparently developing π–π stacking

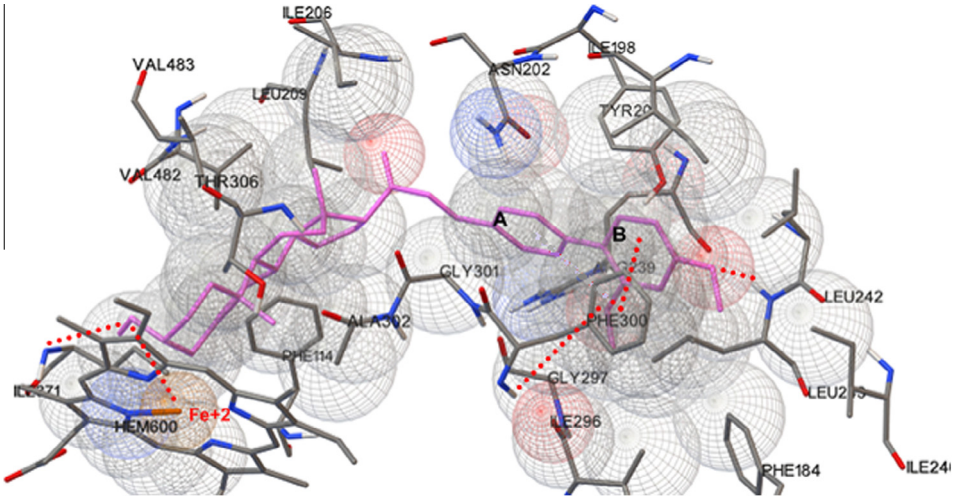

Fig. 3. Computer model of human CYP17 (pdbid 3ruk) with **21**. Complexation of heme-Fe^{+2} (*brown*) with lone pair of oxygen atom of OH group at C-3. Three hydrogen bonds are shown: Ile371 with OH at C-3; Gly297 and Leu242 with 2″ and 4″-OMe groups of phenyl moiety (B). In addition, a hydrophobic interaction between phenyl group B and Phe300 of the CYP17 enzyme amino acid residues is observed. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

interactions with the phenyl ring B. In addition, the chalcone–pregnenolone backbone locate in the middle of the binding pocket, anchoring the dimethoxy substituents at C-3″ and 4″ of the phenyl ring B in a favorable position for hydrogen bonding with the amino group of Gly297 and Leu242 and other hydrogen bond of OH group at C-3 with the amino group of Ile371 of the hydroxylase enzyme. Overall, the combination of hydrophobic interactions of Phe114, Gly301, Ala302, Thr306, Ile371 and π-stacking appears to govern the binding of compound **21** with hydroxylase enzyme.

In summary, the affinity of these compounds towards CYP17 hydroxylase enzyme is governed by their hydrophobic character and the strength of the bond between oxygen lone pair of OH group at C-3 and heme iron (Fig. 3) [39].

4. Conclusion

It has been established that the growth of most prostate carcinomas depends on androgen stimulation. The inhibition of cytochrome P450-17 (CYP17) to block androgen biosynthesis is therefore regarded as a promising approach to therapy. A series of biaryl chalcone pregnenolone analogs were synthesized, and evaluated as CYP17 inhibitors. It was found that the dimethoxy substitutions in the 2,4-positions at the aromatic ring as accomplished in **21** proved to be favorable in terms of both inhibitory potency and selectivity. Docking study performed with human CYP17 homology model suggested the presence of multipolar interactions: complexation of heme iron with OH group at C-3, in addition to the hydrogen bonds and hydrophobic interaction. This analog could be a promising candidate which may be a useful lead in CYP17 inhibition studies.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.steroids.2015.05.011.

References

[1] Dubey RK, Oparil S, Imthurn B, Jackson EK. Sex hormones and hypertension. Cardiovasc Res 2002;53:688–708.
[2] Latham KA, Zamora A, Drought H, Subramanian S, Matejuk A, Offner H, et al. Estradiol treatment redirects the isotype of the autoantibody response and prevents the development of autoimmune arthritis. J Immunol 2003;171(11):5820–7.
[3] (a) Sheridan PJ, Blum K, Trachtenberg M. Steroid receptors and disease. New York: Marcel Dekker; 1988. p. 289–564(b) Moudgil VK. Steroid receptors in health and.
[4] Njar VCO. High-yield synthesis of novel imidazoles and triazoles from alcohols and phenols. Synthesis 2000;14:2019–28.
[5] Banday AH, Shameem SA, Gupta BD, Kumar HMS. D-ring substituted 1,2,3-triazolyl 20-keto pregnenanes as potential anticancer agents: synthesis and biological evaluation. Steroids 2010;75:801–4.
[6] Moreira VM, Salvador JAR, Vasaitis TS, Njar VC. CYP17 Inhibitors for prostatecancer treatment – an update. Curr Med Chem 2008;15:868–99.
[7] Moreira VMA, Vasaitis TS, Guo Z, Njar VCO, Salvador JAR. Synthesis of novel C17 steroidal carbamates. Studies on CYP17 action, androgen receptor binding and function, and prostate cancer cell growth. Steroids 2008;73:1217–27.
[8] Moreira VMA, Vasaitis TS, Njar VCO, Salvador JAR. Synthesis and evaluation of novel 17-indazole androstene derivatives designed as CYP17 inhibitors. Steroids 2007;72:939–48.
[9] Owen CP. 17α-Hydroxylase/17,20-Lyase (P45017α) inhibitors in the treatment of prostate cancer: A review. Anti-Cancer Agents Med Chem 2009;9:613–26.
[10] Pezaro CJ, Mukherji D, De Bono JS. Abiraterone acetate: redefining hormone treatment for advanced prostate cancer. Drug Discov Today 2012;17:221–6.
[11] Vasaitis TS, Bruno RD, Njar VCO. CYP17 inhibitors for prostate cancer therapy. J Steroid Biochem Mol Biol 2011;125:23–31.
[12] Njar VC, Klus GT, Brodie AMH. Nucleophilic vinylic "addition-elimination" substitution reaction of 3β-acetoxy-17-chloro-16-formylandrosta-5,16-diene: A novel and general route to 17-substituted steroids. Part 1 – Synthesis of novel 17-azolyl- Δ16-steroids; inhibitors of 17α-hydroxylase/17,20-Lyase (17α-Lyase). Bioorg Med Chem Lett 1996;6:2777–82.
[13] Njar VC, Kato K, Nnane IP, Grigoryev DN, Long BJ, Brodie AM. Novel 17-Azolyl steroids, potent inhibitors of human cytochrome 17α-hydroxylase-C17,20-Lyase (P45017α): Potential agents for the treatment of prostate cancer. J Med Chem 1998;41:902–12.
[14] Brodie A, Njar VC. 17-Azolyl steroids useful as androgen synthesis inhibitors. United States Patent 6200965 B1; 2001.
[15] Handratta VD, Jelovac D, Long BJ, Kataria R, Nnane IP, Njar VC, Brodie AM. Potent CYP17 inhibitors: Improved syntheses, pharmacokinetics and anti-tumor activity in the LNCaP human prostate cancer model. J Steroid Biochem Mol Biol 2004;92:155–65.
[16] Handratta VD, Vasaitis TS, Njar VC, Gediya LK, Kataria R, Chopra P, Newman D, Farquhar R, Guo Z, Qiu Y, Brodie AM. Novel C-17-heteroaryl steroidal CYP17 inhibitors/antiandrogens: synthesis, in vitro biological activity, pharmacokinetics, and antitumor activity in the LAPC4 human prostate cancer xenograft model. J Med Chem 2005;48:2972–84.
[17] Brodie A, Njar VC, Novel C-17-heteroaryl steroidal CYP17 inhibitors/Antiandrogens: synthesis, in vitro biological activities, pharmacokinetics and antitumor activity. WO Patent 093993; 2006.
[18] Vasaitis TS, Njar VCO. Novel, potent anti-androgens of therapeutic potential: recent advances and promising developments. Future Med Chem 2010;2:667–80.

[19] Molina A, Belldegrun A. Novel therapeutic strategies for castration resistant prostate cancer: inhibition of persistent androgen production and androgen receptor mediated signalling. J Urol 2011;185:787–94.
[20] de Bono JS, Logothetis CJ, Molina A, Fizazi K, North S, Chu L, Chi KN, Jones RJ, Goodman OB, Saad F, Staffurth JN, Mainwaring P, Harland S, Flaig TW, Hutson TE, Cheng T, Patterson H, Hainsworth JD, Ryan CJ, Sternberg CN, Ellard SL, Fléchon A, Saleh M, Scholz M, Efstathiou E, Zivi A, Bianchini D, Loriot Y, Chieffo N, Kheoh T, Haqq CM, Scher HI. Abiraterone and increased survival in metastatic prostate cancer. N. Engl. J. Med. 2011;364:1995–2005.
[21] Bryce A, Ryan CJ. Development and clinical utility of abiraterone acetate as an androgen synthesis inhibitor. Clin Pharmacol Ther 2012;91:101–8.
[22] Hu Q, Negri M, Olgen S, Hartmann RW. The role of fluorine substitution in biphenyl methylene imidazole-type CYP17 inhibitors for the treatment of prostate carcinoma. ChemMedChem 2010;5:899–910.
[23] Hartmann RW, Ehmer RW, Haidar S, Hector M, Jose J, Klein CD, Seidel SB, Sergejew TF, Wachall BG, Wächter GA, Zhuang Y. Inhibition of CYP 17, a new strategy for the treatment of prostate cancer. Arch Pharm Chem Life Sci 2002;335:119–28.
[24] Haidar S, Ehmer PB, Barassin S, Batzl-Hartmann C, Hartmann R. Effects of novel 17α-hydroxylase/C17, 20-lyase (P450 17, CYP 17) inhibitors on androgen biosynthesis in vitro and *in vivo*. J Steroid Biochem Mol Biol 2003;84:555–62.
[25] Al-Masoudi NA, Ali DS, Saeed B, Hartmann RW, Engel M, Rashid S, Saeed A. New CYP17 hydroxylase inhibitors: synthesis, biological evaluation, QSAR, and molecular docking study of new pregnenolone analogs. Arch Pharm Chem Life Sci 2014;347:896–907.
[26] Akram SMM, Peerzadah GM, Banday AH. Scope of non-estrogenic steroidal congeners against breast cancer. J Adv Chem 2013;5:626–32.
[27] Banday AH, Giri AK, Parveen R, Bashir N. Design and synthesis of D-ring steroidal isoxazolines and oxazolines as potential antiproliferative agents against LNCaP, PC-3 and DU-145. Steroids 2014;83:93–8.
[28] Hutschenreuter TU, Ehmer PB, Hartmann RW. Synthesis of hydroxy derivatives of highly potent non-steroidal CYP 17 inhibitors as potential metabolites and evaluation of their activity by a non cellular assay using recombinant human enzyme. J Enzyme Inhib Med Chem 2004;18:17–32.
[29] Krug SJ, Hu Q, Hartmann RW. Hits identified in library screening demonstrate selective CYP17A1 lyase inhibition. J Steroid Biochem Mol Biol 2013;134:75–9.
[30] Miyaura N, Suzuki A. Palladium-catalyzed cross-coupling reactions of organoboron compounds. Chem Rev 1995;95:2457–83.
[31] Willker W, Leibfritz D, Kerssebaum R, Bermel W. Gradient selection in inverse heteronuclear correlation spectroscopy. Magn Reson Chem 1993;31:287–92.
[32] Anderson WA, Freeman R. Influence of a second radiofrequency field on high-resolution nuclear magnetic resonance spectra. J Chem Phys 1962;37:411–5.
[33] Davis AL, Keeler J, Laue ED, Moskau D. Experiments for recording pure-absorption heteronuclear correlation spectra using pulsed field gradients. J Magn Reson 1992;98:207–16.
[34] Salahub DR, Fournier R, Młynarski P, Papai I, St-Amant A, Ushio J. In: Labanowski J, Andzelm J, editors. Density functional methods in chemistry. New York: Springer-Verlag; 1991. p. 77–100.
[35] Pinto-Bazurco Mendieta MAE, Negri M, Jagusch C, Müller-Vieira U, Lauterbach T, Hartmann RW. Synthesis biological evaluation, and molecular modeling of abiraterone analogues: Novel CYP17 inhibitors for the treatment of prostate cancer. J Med Chem 2008;51:5009–18.
[36] DeVore NM, Scott EE. Structures of cytochrome P450 17A1 with prostate cancer drugs abiraterone and TOK-001. Nature 2012;282:116–9.
[37] Morris GM, Huey R, Lindstrom W, Sanner MF, Belew RK, Goodsell DS, Olson AJ. Autodock4 and AutoDockTools4: automated docking with selective receptor flexibility. J Comput Chem 2009;16:2785–91.
[38] Morris GM, Goodsell DS, Halliday RS, Huey R, Hart WE, Belew RK, Oslon AJ. Automated Docking Using a Lamarckian Genetic Algorithm and and Empirical Binding Free Energy Function. J Comput Chem 1998;19:1639–62.
[39] Correia MA, Ortiz de Monteflano PR. Inhibition of Cytochrome P450 Enzymes. In: Paul R, de Motellano O, editors. Cytochrome P450: structure, mechanism, and biochemistry. New York: Kluwer Academic/Plenum Publishers; 2005. p. 247–322.

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