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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

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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 chalcones [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, 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.

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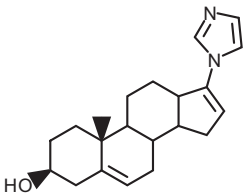
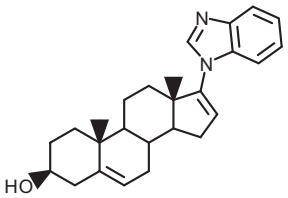
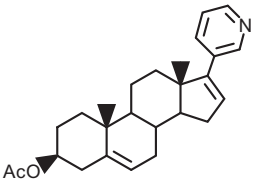
0039-128X/



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), 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), 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,