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A new pregnenolone analogues as privileged scaffolds in inhibition of CYP17 hydroxylase enzyme. Synthesis and *in silico* molecular docking study

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ABSTRACT

A new series of 17-(*N*-(arylimino)-5-pregnen-3β-ol derivatives **19–32** as well as carboxylate and acrylate analogues of pregnenolone **37–40** were synthesized and evaluated for their inhibitory activity against human CYP17 hydroxylase expressed in *Escherichia coli*. Compounds **32** and **37** were the most potent analogues in this series, showing inhibition activity with $IC_{50} = 2.11$ and 1.29 μM, respectively. However, the analogue **37** revealed a better selectivity profile (83.21% inhibition of hydroxylase), which is a leading candidate for further development. Molecular docking study of **37** showed binding with the amino acid residues of CYP17 through hydrogen bonds and hydrophobic interaction.

1. Introduction

The enzyme 17α-hydroxylase-C17,20-lyase (P45017, CYP17), a cytochrome P450 monooxygenase, is the key enzyme for androgen and corticoid biosynthesis and has become a valuable target in prostate cancer (PC) treatment [1–6], since the androgens are potent prostate mitogens [7,8] and its elevated levels may be associated with PC risk. A common approach to the synthesis of potent steroidal inhibitors of CYP17 has been the design of substrate-like molecules bearing a heterocycle at the C17 position with privileged heteroatoms (N, S, and O), which can interact as the sixth ligand with the heme iron of the enzyme [9]. In addition, computational studies established that a good inhibitor should possess a sufficiently large hydrophobic core, comparable to a steroid molecule, and bear electronegative groups at its external positions [10,11]. Therefore, recently several nitrogen containing steroidal compounds containing five- or six-membered 17β-exo-heterocycles (preferably nitrogen containing), such as steroidal azoles [12,13] have been developed for the treatment of prostate cancer, including abiraterone acetate (Zytiga) 1 [14,15], VN/124-1 galeterone (2) [16–18], which is currently undergoing phase I/II clinical trials for the treatment of chemotherapy naive castration-resistant prostate cancer (CRPC) [19,20] and VN/85-1 (**3**) [21,22] (Fig. 1), which reduce circulating androgen levels through inhibition of CYP17 [23]. Hartmann and co-workers [24–26] have reported the synthesis of several CYP 17 inhibitors as a new strategy for the treatment of prostate carcinoma. Furthermore, 21-triazolyl [27] and chalcone derivatives [28] of pregnenolone as well as some steroidal isoxazolines and oxazoline residues [29] have been reported with remarkable anticancer and antiproliferative activity against ER+(MCF-7) breast cancer cells, and act as microtubule disruptors.

Numerous modifications to the steroid nucleus have been made in order to study the SAR of bioactive steroids, including substitutions at the 3β-, 11-, 17-, or 21-positions. We are interested in the synthesis and characterization of new steroidal compounds with selective inhibition of CYP17 hydroxylase enzyme properties, for use in the development of more effective treatments of breast and prostate cancer. Building on our previous work [30], in the present study we reported the synthesis of new 17-pregnenolone-imine derivatives with their CYP17 hydroxylase enzyme inhibition activity and the molecular modeling study.

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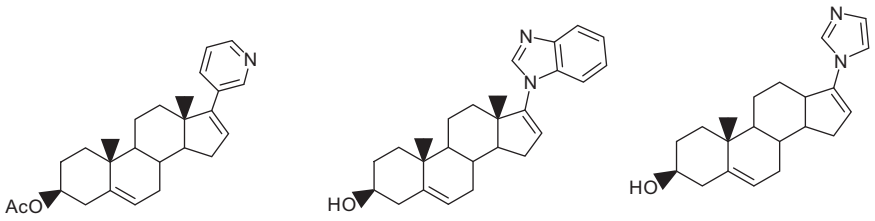

1. Abiraterone acetate **2**. Galeterone TOK-001 **3**. VN/85-1

Fig. 1. CYP17 inhibitors.

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 and HMBC 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. General procedure for the synthesis of 17-(N-(arylimino)-5-pregnen-3β-ol derivatives (**19–32**)

A solution of 5-pregnen-3β-ol-20-one (**4**) (100 mg 0.32 mmol) in EtOH (20 ml) containing substituted anilines **5–18** (0.32 mmol) and glacial acetic acid (1.0 ml) was heated under reflux for 11–16 h. The reaction was monitored by TLC by using *n*-hexane-MeOH (3:1 or 1:1) and *n*-hexane–ethyl acetate (2:1) as eluents. After cooling, the solution was poured onto ice cold water. The solid product was collected by filtration and recrystallized from EtOH to give the desired imine derivatives.

*2.2.1.1. 4-((1-(5-Pregnen-3β-ol-17-yl)ethylidene)amino)-3-methoxybenzoic acid (**19**).* From 4-amino-3-methoxybenzoic acid (**5**) (53 mg). Yield: 89 mg, (60%); M.p.: 199–201 °C. FT-IR (KBr, *ν*, cm^{-1}): 3500, 3200, 3100, 2985, 2856, 1700, 1685; ^{1}H NMR (DMSO-d_6): δ 11.36 (br s, 1H, CO_2H, disappeared on D_2O exchange), 7.74 (m, 2H, $H_{arom.}$-3′ + $H_{arom.}$-5′), 7.50 (m, 1H, $H_{arom.}$-6′), 5.27 (t, 1H, $J_{6,7}$ = 2.7 Hz, H-6), 4.60 (d, 1H, *J* = 4.5 Hz, OH), 3.73 (s, 3H, OMe), 3.26 (m, 1H, H-3), 2.57 (m, 1H, H-17), 2.15 (m, 2H, CH_2-4), 2.09 (s, 3H, Me-21), 2.05 (m, 1H, H-16a), 2.03 (m,1H, H-12a), 1.96 (m, 1H, H-7a), 1.79 (m, 1H, H-1a), 1.70 (m, 1H, H-2a), 1.61 (m, 1H, H-15a), 1.59 (m, 1H, H-11a), 1.57 (m, 1H, H-16b), 1.54 (m, 1H, H-7b), 1.43 (m, 1H, H-11b), 1.42 (m, 1H, H-12b), 1.40 (m, 1H, H-8), 1.37 (m, 1H, H-2b), 1.15 (m, 2H, H-14 + H-15b), 1.14 (m, 1H, H-1b), 1.00 (m, 1H, H-9), 0.96 (s, 3H, Me-19),0.54 (s, 3H, Me-18); ^{13}C NMR (DMSO-d_6): δ 169.3 (CO_2H), 161.5 (C-20), 154.9 (C_2-OMe), 141.8 (C-5 + $C_{arom.}$-1′), 134.3 ($C_{arom.}$-4′), 122.3, 120.7 (C-6 + $C_{arom.}$-6′ + $C_{arom.}$-5′), 106.0 ($C_{arom.}$-3′), 70.5 (C-3), 56.6 (OMe), 56.7 (C-14), 49.1 (C-9), 43.8 (C-13), 42.7 (C-4), 31.9, 31.7, 31.6 (C-2 + C-7 + C-8 + Me-21) 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 $C_{29}H_{39}NO_4$ (465.62): C, 74.81; H, 8.44; N, 3.01. Found: C, 74.60; H, 8.36; N, 2.87.

*2.2.1.2. 4-((1-(5-Pregnen-3β-ol-17-yl)ethylidene)amino)benzoic acid (**20**).* From 4-aminobenzoic acid (**6**) (43 mg). Yield: 90 mg (65%); M.p.: 170–173 °C; FT-IR (KBr, *ν*, cm^{-1}): 3505, 3200, 2965, 1710, 1685, 1570, 1462; ^{1}H NMR (DMSO-d_6): δ 11.80 (s, 1H, CO_2H, disappeared on D_2O exchange), 7.61 (d, 2H, *J* = 7.9 Hz, $H_{arom.}$-3′ + $H_{arom.}$5′), 6.54 (d, 2H, *J* = 7.9 Hz, $H_{arom.}$-2′ + $H_{arom.}$-6′), 5.27 (t, 1H, H-6), 4.58 (br s, 1H, OH), 3.26 (m, 1H, H-3), 2.14 (m, 2H, CH_2-4), 2.06 (s, 3H, Me-21), 2.00 (m, 1H, H-16a), 1.94 (m, 1H, H-12a), 1.91 (m, 1H, H-7a), 1.78 (m, 1H, H-1a), 1.67 (m, 1H, H-2a), 1.57 (m, 4H, H-15a + H-1a + H-16b + H-7b), 1.40 (m, H-11b + H-12b + H-8 + H-7b), 1.14 (m, 2H, H-14 + H-15b), 0.99 (m, 2H, H-1b + H-9), 0.94 (s, 3H, Me-19), 0.54 (s, 3H, Me-18); ^{13}CNMR (DMSO-d_6): δ 188.9 (CO_2H), 165.3 (C-20) 141.8 (C-5 + $C_{arom.}$-1′), 132.0 ($C_{arom.}$-4′), 128.1 ($C_{arom.}$-3′ + $C_{arom.}$-5′), 122.0 ($C_{arom.}$-2′ + $C_{arom.}$-6′), 120.7 (C-6), 70.5 (C-3), 56.6 (C-14), 49.1 (C-9), 43.8 (C-13), 42.7 (C-4), 38.4 (C-12), 37.4 (C-1), 36.6 (C-10), 31.9 31.7, 31.6 (C-2 + C-7 + C-8 + C-21), 24.5 (C-15), 24.5, 23.8 (C-17), 22.7 (C-16), 21.1 (C-11), 19.6 (Me-19), 13.4 (Me-18); Anal. calcd. for $C_{28}H_{37}NO_3$ (435.60): C, 77.20, H, 8.56; N, 3.22. Found: C, 76.97; H, 8.42; N, 3.02.

*2.2.1.3. N-(4-((1-(5-Pregnen-3β-ol-17-yl)ethylidene)amino)phenyl) acetamide (**21**).* From 4-acetamidoaniline (**7**) (48 mg). Yield: 100 mg (70%); M.p.: 115–118 °C; ^{1}H NMR (DMSO-d_6): δ 9.51 (s, 1H, NH), 7.70 (d, 2H, *J* = 7.9 Hz, $H_{arom.}$-3′ + $H_{arom.}$-5′), 6.50 (d, 2H, *J* = 7.9 Hz, $H_{arom.}$-2′ + $H_{arom.}$-6′), 5.59 (br s, 1H, H-6), 4.59 (br s, 1H, OH), 3.35 (m, 1H, H-3), 2.62 (m, 2H, CH_2-4), 2.08 (s, 6H, Me-21 + NHCO*Me*), 2.02 (m, 3H, H-16a + H-12a + H-7a), 1.78 (m, 1H, H-1a), 1.70 (m, 1H, H-2a), 1.59 (m, 4H, H-15b + H-11a + H-16b + H-7b), 1.43 (m, 4H, H-11b + H-12b + H-8 + H-2b), 1.11 (m, 2H, H-14 + H-15b), 0.94 (s, 3H, Me-19), 0.94 (m, 12H, H-1b + H-9), 0.52 (s, 3H, Me-18); ^{13}C NMR (DMSO-d_6): δ 167.8 (NH*CO*Me), 161.4 (C-20), 145.3 ($C_{arom.}$-1′), 141.8 (C-5), 136.7 ($C_{arom.}$-4), 123.6, 120.6 ($C_{arom.}$+C-6), 70.5 (C-3), 56.6 (C-14), 49.1 (C-9), 43.8 (C-13), 42.7 (C-4), 38.4 (C-12), 37.4 (C-1), 36.6 (C-10), 30.9, 31.7, 31.9 (C-2 + C-7 + C-8 + Me-21), 24.5 (C-15), 24.2 (NHCO*Me*), 22.7 (C-16), 21.1 (C-11), 19.6 (Me-19), 13.4 (Me-18); Anal. calcd. for $C_{30}H_{41}N_2O_2$ (447.65): C, 80.49; H, 9.23; N, 3.13. Found: C, 80.24; H, 9.08; N, 2.93.

*2.2.1.4. 2-((1-(5-Pregnen-3β-ol-17-yl)ethylidene)amino)benzamide (**22**).* From 2-aminobenzamide (**8**) (44 mg). Yield: 76 mg (55%); M.p.: 170–173 °C; FT-IR (KBr, *ν*, cm^{-1}): 3505, 3356, 2985, 1685, 1661, 1570, 1492; ^{1}H NMR (DMSO-d_6): δ 7.87 (br s, 2H, NH_2), 7.70–7.52 (m, 4H, $H_{arom.}$), 5.28 (t, $J_{6,7}$ = 2.5 Hz, H-6), 4.59 (d, 1H, $J_{6,7}$ = 4.6 Hz, OH), 3.27 (m, 1H, H-3), 2.57 (t, 1H, H-17), 2.15 (m, 2H, CH_2-4), 2.03 (s, 3H, Me-21), 2.02 (m, 1H, H-16a), 1.96 (m, 1H, H-12a), 1.92 (m, 1H, H-7a), 1.77 (m, 1H, H-1a), 1.70 (m, 1H, H-2a), 1.68 (m, 1H, H-15a), 1.61 (m, 1H, H-11a), 1.58 (m, 1H, H-16b), 1.56 (m, 1H, H-7b), 1.44 (m, 1H, H-11b), 1.43 (m, 1H, H-12b), 1.38 (m, 1H, H-8), 1.37 (m, 1H, H-2b), 1.15 (m, 2H, H-14 + H-15b), 1.00 (m, 2H, H-9 + H-2b), 0.96 (s, 3H, Me-19), 0.54 (s, 3H, Me-18); ^{13}C NMR (DMSO-d_6): δ 165.7 ($CONH_2$), 164.4