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Exploration of new 3α-pregnenolone ester analogues via Mitsunobu reaction, their anti-HIV activity and molecular modeling study

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ABSTRACT

A new series of (5-pregnen-20-on-3α-yl)-substituted-benzoate analogues (**10**-**13**), (5-pregnene-20-on-3α-yl)-3-(substituted)acrylate derivatives (**17**-**19**) as well as the (17-(2-acetoxyacetyl)pregen-3α-yl)-3,4,5-trihydroxybenzoate (**21**) were synthesized from the β-pregenenolone scaffolds, by applying Mitsunobu reaction. All new compounds were characterized by ^{1}H, ^{13}C and 2D NMR spectroscopy. The inversion in configuration at C-3 during the formation of α-ester analogues was confirmed by NOESY NMR spectroscopy. The new compounds were evaluated for their *in vitro* antiviral activity against the replication of HIV-1 and HIV-2 in MT-4 cells. Compounds **18** showed an EC_{50} value of >1.95 mg/mL. In addition, preliminary structure-activity relationship and molecular modeling of compound **18** has been studied.

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1. Introduction

Steroidal compounds display a variety of biological [1-3] functions and play a very important role in life [4-6], and attracted profound attention for development of potent pharmacological agents for treatments of various diseases [7] including: cardiovascular disease [8], adrenal insufficiencies [9], autoimmune disorders [10], fungal and microbial infections [11,12]. Furthermore, different steroidal derivatives have been considered as potent anti-cancer agents for the treatment of leukemia [7], breast cancer [13-15], prostate cancer [16] and brain tumors [17]. Furthermore, some steroids are promising pharmaceutical targets for important indications like epilepsy, anxiety disorders and dementia [18], while other steroid hormones have long been recognized to have sedative, anesthetic and anti-seizure properties in animals and humans [19-22]. Presence of different functional groups located around the rigid tetracyclic core leads to diversity in the biological actions as these serve as substrates for different targets.

Recently, several steroidal compounds have been synthesized and displayed a key role in a therapeutic strategy for treating advanced prostate cancer (PC) [23-25]. In 1996, Njar *et al.* [20,26] 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 [20-23,26-29]. Later, in 2005, the same group reported the synthesis of galeterone **2** and its Δ^4-3-keto derivative [23-25,29-31], where compound **2** is currently undergoing Phase I/II clinical trials for the treatment of chemotherapy-naive CRPC [26,27,32-33]. However, patients suffering from CRPC can clearly benefit from the newly approved drug abiraterone acetate (Zytiga) **3** [28, 29,34,35]. This pregnenolone derivative was designed as an inhibitor of the enzyme 17α-hydroxylase/$C_{17,20}$-lyase (CYP17A1) [30,36] which catalyzes two key reactions in steroid hormone biosynthesis. Much more recently, we have synthesized new series of pregnenolone having imino-benzothiazoles **4** at C-20, which showed remarkable inhibition of CYP17 hydroxylase activity, Figure 1 [37].

In continuation of our ongoing work on the synthesis of new pregnenolone analogues, we explore here a novel series of α-ester derivatives of pregnenolone with inversion in configuration at C-3, by applying of Mitsunobu reaction [38-40].

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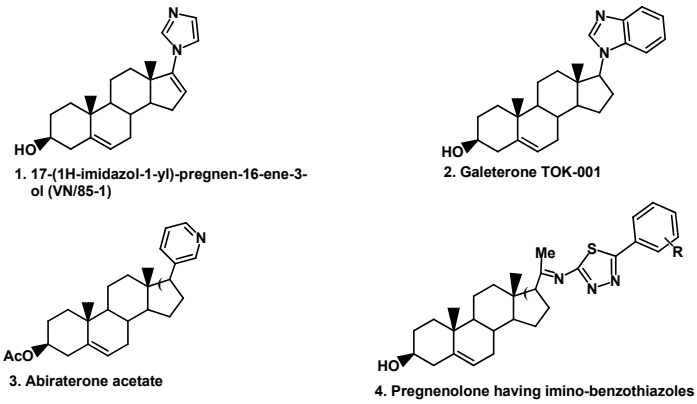


Figure 1. Some inhibitors of CYP 17 hydroxylase-lyase enzyme.

2. Experimental

2.1. Instrumentation

Melting points are uncorrected and were measured on a Büchi melting point apparatus B-545 (Büchi Labortechnik AG, Switzerland). NMR data were obtained on 400 and 600 MHz (^{1}H) and 150.91 MHz (^{13}C) spectrometers (Avance III, Bruker, Germany) with TMS as internal standard and on the δ scale in ppm. Heteronuclear assignments were verified by ^{1}H, ^{13}C HMBC and ^{1}H, ^{13}C HSQC NMR experiments. Microanalytical data were obtained with a Vario, Elemental analyzer (Shimadzu, Japan). Analytical silica gel TLC plates $60F_{254}$ were purchased from Merck. All reagents were obtained from commercial suppliers and were used without further purification.

2.2. Synthesis

2.2.1. General procedure for the synthesis of 3α-substituted aryl ester derivatives of pregnenolone by applying Mitsunobu reaction (10-13)

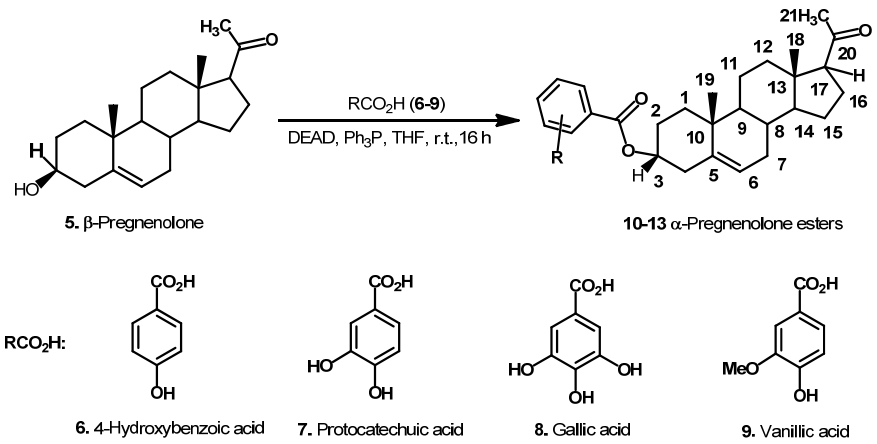
To a solution of 5-pregnene-3β-ol-20-one (**5**) (316 mg 1.00 mmol) in acetonitrile (10 mL) were added substituted benzoic acids **6-9** (1.00 mmol), triphenylphosphine (Ph_3P) (262 mg, 1.00 mmol) and diethylazodicarboxylate (DEAD) (1.00 mmol, 0.13 mL) and the mixture was heated under reflux for 8 h. The reaction was monterited by TLC (*n*-hexane: ethyl acetate, 3:1, *v*:*v*). After cooling, diethyl ether (15 mL) was added and the mixture was partitioned with saturated aqueous solution of $NaHCO_3$ (10 mL), brine solution (10 mL) and finally with water. The organic extract was dried (Na_2SO_4), filtered and the filtrate was evaporated to dryness. The residue was poured onto a short column of silica gel (5 g), using *n*-hexane-ethyl acetate (3:2, *v*:*v*) as eluent to give the desired ester (Scheme 1).

(5-Pregnen-20-on-3α-yl)-4-hydroxybenzoate (**10**): From 4-hydroxybenzoic acid (**6**) (138 mg). Yield: 245 mg (56%). M.p.: 142-144 °C. ^{1}H NMR (600 MHz, DMSO-d_6, δ, ppm): 7.79 (d, 2H, $J_{2',3'}$ = 8.6 Hz, $H_{arom.}$-2' + $H_{arom.}$-6'), 6.83 (d, 2H, $J_{5',6'}$ = =8.6 Hz, $H_{arom.}$-3' + $H_{arom.}$-5'), 5.28 (t, 1H, $J_{6,7}$ = 4.6 Hz, H-6), 4.60 (s, 1H, OH), 3.26 (m, 1H, H-3), 2.58 (m, 1H, H-17), 2.18 (m, 2H, CH_2-4), 2.07 (s, 3H, Me-21), 2.02 (m, 1H, H-16a), 2.00 (m, 1H, H-12a), 1.90 (m, 1H, H-7a), 1.79 (m, 1H, H-1a), 1.72 (m, 1H, H-2a), 1.70 (m, 1H, H-15a), 1.67 (m, 1H, H-11a), 1.61 (m, 1H, H-16b), 1.60 (m, 1H, H-7a), 1.44 (m, 1H, H-11b), 1.41 (m, 1H, H-12b), 1.38 (m, 2H, H-8 + H-2b), 1.20 (m, 2H, H-14 + H-15b), 1.04 (m, 1H, H-1b), 1.00 (m, 1H, H-9), 0.94 (s, 3H, Me-19), 0.54 (s, 3H, Me-18). ^{13}C NMR (150.91 MHz, DMSO-d_6, δ, ppm): 209.1 (C-20), 167.7 (CO_2), 162.1 ($C_{arom.}$-4'), 141.8 (C-5), 131.8 ($C_{arom.}$-2' + $C_{arom.}$-6'), 122.1 ($C_{arom.}$-1'), 120.8 (C-6), 115.6 ($C_{arom.}$-3' + $C_{arom.}$-5'), 70.5 (C-3), 63.1 (C-17), 60.9 (C-14), 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, 31.8 , 31.7 (C-2 + C-7 + C-8 + Me-21), 24.5 (C-15), 22.7 (C-16), 21.1 (C-11), 19.6 (Me-19), 13.4 (Me) 18). Anal. calcd. for $C_{28}H_{36}O_4$: C, 77.03; H, 8.31. Found: C, 76.89; H, 8.19%.

(5-Pregnen-20-on-3α-yl)-3,4-dihydroxybenzoate (**11**): From 2,4-dihyroxybenzoic acid (protoctechunic acid) **7** (154 mg). Yield: 271 mg (60%). M.p.: 129-131 °C. ^{1}H NMR (600 MHz, DMSO-d_6, δ, ppm): 7.62 (m, 2H, $H_{arom.}$-2' + $H_{arom.}$-6'), 7.08 (d, 1H, *J*= 9.0 Hz, $H_{arom.}$-5'), 5.28 (t, 1H, $J_{6,7}$ = 4.1 Hz, H-6), 4.59 (br s, 2H, 2×OH), 3.28 (m, 1H, H-3), 2.57 (m, 1H, H-17), 2.15 (m, 2H, CH_2-4), 2.09 (s, 3H, Me-21), 2.06 (m, 1H, H-16a), 2.00 (m, 1H, H-12a), 1.92 (m, 1H, H-7a), 1.80 (m, 1H, H-1a), 1.71 (m, 1H, H-2a), 1.67 (m,1H, H-15a), 1.60 (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), 1.41 (m, 1H, H-8), 1.38 (m, 1H, H-2b), 1.15 (m, 2H, H-14 + H-15b), 1.04 (m, 1H, H-1b), 1.00 (m, 1H, H-9), 0.95 (s, 3H, Me-19), 0.54 (s, 3H, Me-18). ^{13}C NMR (150.91 MHz, DMSO-d_6, δ, ppm): 208.1 (C-20), 164.30 (CO_2), 149.1 ($C_{arom.}$-4'), 144.1 ($C_{arom.}$-3'), 141.8 (C-5), 129.3 ($C_{arom.}$-1'), 122.8 ($C_{arom.}$-6'), 120.7 (C-6), 117.1 ($C_{arom.}$-2' + $C_{arom.}$-5'), 70.5 (C-3), 63.1 (C-17), 56.6 (C-14), 43.8 (C-9), 42.7 (C-4), 38.5 (C-12), 37.4 (C-1), 36.6 (C-10), 31.9, 31.8, 31.7 (C-2 + C-7 + C-8 + Me-21), 24.5 (C-15), 22.7 (C-16), 21.1 (C-11), 19.6 (Me-19), 13.4 (Me-18). Anal. calcd. for $C_{28}H_{36}O_5$: C, 74.31; H, 8.02. Found: C, 74.07; H, 7.91%.

(5-Pregnen-20-on-3α-yl)-3, 4, 5-trihydroxybenzoate (**12**): From 3,4,5-trihydroxybenzoic acid (gallic acid) **8** (170 mg). Yield: 309 mg (66%). M.p.: 216-218 °C. ^{1}H NMR (600 MHz, DMSO-d_6, δ, ppm): 7.63-7.61 (m, 2H, $H_{arom.}$-2' + $H_{arom.}$-6'), 5.28 (t, 1H, $J_{6,7}$ = 4.3 Hz, H-6), 4.61 (s, 1H, OH), 4.05 (m, 2H, 2×OH), 3.27 (m, 1H, H-3), 2.59 (m, 2H, H-17), 2.15 (m, 2H, CH_2-4), 2.09 (s, 3H, Me-21), 2.03 (m, 1H, H-16a), 2.00 (m, 1H, H-12a), 1.92 (m, 1H, H-7a), 1.80 (m, 1H, H-1a), 1.70 (m, 1H, H-2a), 1.67 (m, 1H, H-15a), 1.61 (m, 1H, H-11a),1.60 (m, 1H, H-16b), 1.54 (m, 1H, H-7b), 1.44 (m, 1H, H-11b), 1.41 (m, 1H, H-12b), 1.38 (m, 1H, H-8), 1.35 (m, 1H, H-2b), 1.16 (m, 2H, H-14+H-15b), 0.99 (m, 1H, H-9), 0.95 (s, 3H, Me-19), 0.54 (s, 3H, Me-18). ^{13}C NMR (150.91 MHz, DMSO-d_6, δ, ppm): 209.0 (C-20), 164.6 (CO_2Ar), 145.4 ($C_{arom.}$-3' + $C_{arom.}$-5'), 141.8 (C-5), 139.1 ($C_{arom.}$-4'), 121.9 ($C_{arom.}$-1'), 120.8 (C-6), 110.3 ($C_{arom.}$-2' + $C_{arom.}$-6'), 70.5 (C-3), 63.1 (C-17), 56.6 (C-14), 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 + 31.8 + 31.7, (C-2 + C-7 + C-8 + Me-21), 24.5 (C-15), 22.7 (C-16), 21.1 (C-11), 19.6 (Me-19), 13.4 (Me-18). Anal. calcd. for $C_{28}H_{36}O_6$: C, 71.77; H, 7.74. Found: C, 71.50; H, 7.68%.

(5-Pregnen-20-on-3α-yl)-4-hydroxy-3-methoxybenzoate (**13**): From 3-methoxyl-4-hydroxylbenzoic acid (vanilic acid) **9** (168 mg). Yield: 280 mg (60%). M.p.: 230-233 °C.



Scheme 1

^{1}H NMR (600 MHz, DMSO-d_6, δ, ppm): 7.59 (d, 2H, $J_{2',6'}$ = 3.4 Hz, $H_{arom.}$-2' + $H_{arom.}$-6'), 7.11 (d, 1H, $J_{5',6'}$ = 7.9 Hz, $H_{arom.}$-5'), 5.27 (t, 1H, $J_{6,7}$ = 2.6 Hz, H-6), 4.60 (br s, 1H, OH), 4.04 (s, 3H ,OMe), 3.32 (m, 1H, H-3), 2.57 (m, 1H, H-17), 2.15 (m, 2H, CH_2-4), 2.07 (s, 3H, Me-21), 2.02 (m, 1H, H-16a), 2.00 (m, 1H, H-12a), 1.91 (m, 1H, H-7a), 1.76 (m, 1H, H-1a), 1.67 (m, 1H, H-2a), 1.61 (m, 1H, H-15a), 1.60 (m, 1H, H-11a), 1.56 (m, 1H, H-16b), 1.54 (m, 1H, H-7b), 1.42 (m, 1H, H-11b), 1.40 (m, 1H, H-12b), 1.38 (m, 1H, H-8), 1.34 (m, 1H, H-2b), 1.35 (m, 1H, H-12b), 1.16 (m, 2H, H-14 + H-15b), 0.97 (m, 1H, H-9), 0.94 (s, 3H, Me-19), 0.54 (m, 3H, Me-18). ^{13}C NMR (150.91 MHz, DMSO-d_6, δ, ppm): 208.1 (C-20), 163.7 (CO_2), 152.8 ($C_{arom.}$-4'), 146.4 ($C_{arom.}$-3'), 141.8 (C-5), 124.5 ($C_{arom.}$-1'), 123.2 ($C_{arom.}$-6'), 120.8 (C-6), 113.6 ($C_{arom.}$-2' + $C_{arom.}$-5'), 70.5 (C-3), 63.1 (C-17), 56.6 (C-14), 55.3 (OMe), 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, 31.8, 31.7 (C-2 + C-7 + C-8 + Me-21), 24.5 (C-15), 22.7 (C-16), 21.1 (C-11), 19.6 (Me-19), 13.4 (Me-18). Anal. calcd. for $C_{29}H_{38}O_5$: C, 74.65; H, 8.21. Found: C, 74.42; H, 8.05%.

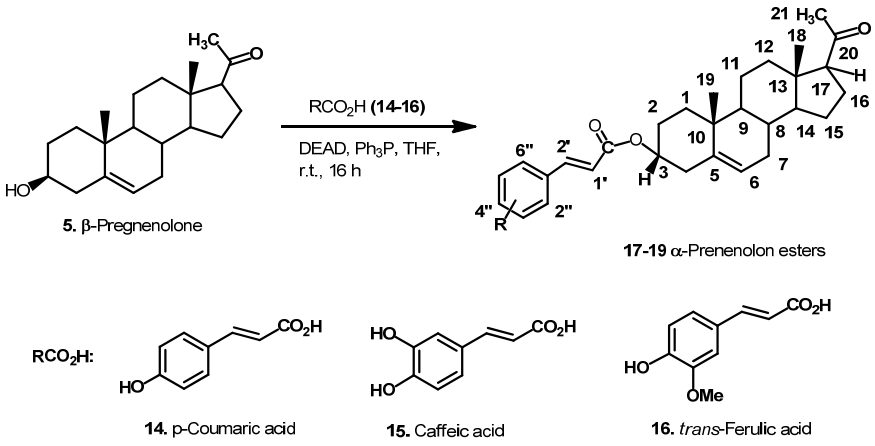
2.2.2. General procedure for the synthesis of (5-pregnen-20-on-3α-yl)-3-substituted-phenyl acrylates (17-19) *by applying Mitsunobu reaction*.

To a solution of 5-pregnene-3β-ol-20-one (**5**) (316 mg, 1.00 mmol) in THF (20 mL), were added substituted acrylic acids **14**-**16** (1.00 mmol), triphenylphosphine (Ph_3P) (262 mg, 1.00 mmol) and diethylazodicarboxylate (DEAD) (0.13 mL, 1.00 mmol). The reaction was monterited by TLC by using mobile phase (*n*-hexane:ethyl acetate, 1:1, *v*:*v*). The mixture was evaporated to dryness and the residue was partitioned between $CHCl_3$ (3×10 mL) and saturated aqueous solution of $NaHCO_3$ (10 mL), brine solution (10 mL) and finally with water. The combined organic extract were dried (Na_2SO_4), filtered and the filtrate was evaporated to dryness. The residue was poured onto a short column of silica gel (5 g), using *n*-hexane:ethyl acetate (1:1, *v*:*v*) as eluent to give the desired ester (Scheme 2).

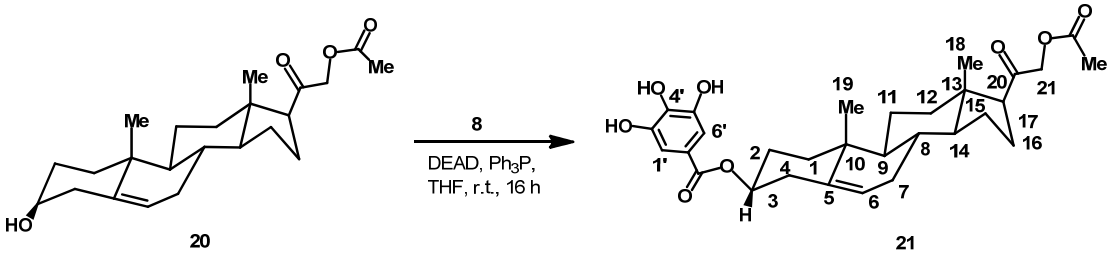
(5-Pregnen-20-on-3α-yl)-3-(4-hydroxyphenyl)acrylate (**17**): From 3-(4-hydroxyphenyl) acrylic acid (*p*-coumaric acid) **14** (164 mg). Yield: 291 mg (63%). M.p.: 322-324 °C. FT-IR (KBr, ν, cm^{-1}) 3505, 3090, 2950, 2865, 1730, 1681, 1651, 1551. ^{1}H NMR (600 MHz, DMSO-d_6, δ, ppm): 7.65 (dd, 2H, $J_{2'',6''}$ = 2.0 Hz, $J_{2'',3''}$ = 8.5 Hz, $H_{arom.}$-2'' + $H_{arom.}$-6''), 7.62 (d, 1H, $J_{1',2'}$ = 10.0 Hz, H_{olefin}-1'), 7.56 (dd, 2H $J_{3'',5''}$ = 2.0 Hz, $J_{5'',6''}$ = 8.5 Hz, $H_{arom.}$-3'' + $H_{arom.}$-5''), 7.53 (d 1H, $J_{1',2'}$ = 10.0 Hz, H_{olefin}-2'), 5.27 (t, 1H, $J_{6,7}$ = 2.6 Hz, H-6), 4.62 (br s, 1H, OH), 3.28 (m, 1H, H-3), 2.56 (m, 1H, H-17), 2.18 (m, 2H, CH_2-4), 2.06 (s, 3H, Me-21), 2.03 (m, 1H, H-16a), 1.99 (m, 1H, H-12a), 1.92 (m, 1H, H-7a), 1.76 (m, 1H, H-1a), 1.68 (m, 1H, H-2a), 1.60 (m, 1H, H-15a), 1.59 (m, 1H, H-11a), 1.57 (m, 1H, H-16b), 1.55 (m, 1H, H-7b), 1.42 (m, 2H, H-11b+ H-12b), 1.40 (m, 1H, H-8), 1.36 (m, 1H, H-2b), 1.19 (m, 2H, H-14 + H-15b), 1.03 (m, 1H, H-1b), 0.99 (m, 1H, H-9), 0.94 (s, 3H, Me-19), 0.54 (s, 3H, Me-18). ^{13}C NMR (150.91 MHz, DMSO-d_6, δ, ppm): 208.9 (C-20), 165.9 (CO_2), 156.6 ($C_{arom.}$-4''), 152.3 (C_{olefin}-2'), 141.8 (C-5), 133.7 ($C_{arom.}$-1''), 129.2 ($C_{arom.}$-2'' + $C_{arom.}$-6''), 120.7 (C-6), 116.1 (C_{olefin}-1'), 114.8 ($C_{arom.}$-3'' + $C_{arom.}$-5''), 70.5 (C-3), 63.1 (C-17), 56.6 (C-14), 49.1 (C-9), 43.7 (C-13), 42.7 (C-4), 38.5 (C-12), 37.4 (C-1), 36.6 (C-10), 31.9 (C-2 + C-7 + C-8 + Me-21), 24.5 (C-15), 22.7 (C-16), 21.1 (C-11), 19.6 (Me-19), 13.4 (Me-18). Anal. calcd. for $C_{30}H_{38}O_4$: C, 77.89; H 8.28. Found: C, 77.63; H, 8.02%.

(5-Pregnen-20-on-3α-yl)-3-(3,4-dihydroxyphenyl)acrylate (**18**): From 3,4-dihydroxycinnamic acid (caffeic acid) **15** (180 mg). Yield: 301 mg (63%). M.p.: 337-339 °C. FT-IR (KBr, ν, cm^{-1}): 3450, 3095, 2950, 2839, 1754, 1681, 1665, 1534, 1492. ^{1}H NMR (600 MHz, DMSO-d_6, δ, ppm): 7.64-7.55 (m. 5H $H_{arom.}$-2'' + $H_{arom.}$-5'' + $H_{arom.}$-6'' + H_{olefin}-1' + H_{olefin}-2'), 5.27 (t, 1H, $J_{6,7}$ = 2.5 Hz, H-6), 5.04 (s, 1H, C3''-OH), 4.61 (br s, 1H, C4'-OH), 3.27 (m, 1H, H-3), 2.56 (m, 1H, H-17), 2.18 (m, 2H, CH_2-4), 2.07 (s, 3H, Me-21), 1.99 (m, 1H, H-16a), 1.95 (m, 1H, H-12a), 1.91 (m, 1H, H-7a), 1.76 (m, 1H, H-1a), 1.71 (m, 1H, H-2a), 1.60 (m, 1H, H-15a), 1.59 (m, 1H, H-11a), 1.57 (m, 1H, H-16b), 1.54 (m, 1H, H-7b), 1.42 (m, 1H, H-11b), 1.40 (m, 1H, H-12b), 1.38 (m, 1H, H-8), 1.35 (m, 1H, H-2b), 1.18 (m, 2H, H-14+H-15b), 1.00 (m, 1H, H-9), 0.97 (m, 1H, H-9), 0.94 (s, 3H, Me-19), 0.54 (s, 3H, Me-18). ^{13}C NMR (150.91 MHz, DMSO-d_6, δ, ppm): 208.9 (C-20), 163.5 (CO_2), 152.3 ($C_{arom.}$-4''), 148.7 ($C_{arom.}$-3''), 145.3 (C_{olefin}-2'), 141.8 (C-5), 129.2 ($C_{arom.}$-1''), 122.1 (C_{olefin}-1'), 120.7 (C-6), 116.2 ($C_{arom.}$-5'' + $C_{arom.}$-6''), 115.6 ($C_{arom.}$-2''), 70.5 (C-3), 63.1 (C-17), 56.6 (C-14), 49.1 (C-9), 43.8 (C-13), 42.7 (C-4), 38.5 (C-12), 37.4 (C-1), 36.6 (C-10), 31.9, 31.8, 31.7 (C-2 + C-7 + C-8 + C-21), 24.5 (C-15), 22.7 (C-16), 21.1 (C-11), 19.6 (Me-19), 13.4 (Me-18). Anal. calc. for $C_{30}H_{38}O_5$: C, 75.28; H, 8.00. Found: C, 75.02; H, 7.81%.

(5-Pregnen-20-on-3α-yl)-3-(4-hydroxy-3-methoxyphenyl) acrylate (**19**): From 3-(3,4-dhydroxyphenyl)acrylic acid (*trans*-ferulic acid) **16** (194 mg). Yield: mg (63%). M.p.: 244-246 °C. FT-IR (KBr, ν, cm^{-1}): 3460, 3090, 2950, 2837, 1751, 1681, 1661, 1531, 1492. ^{1}H NMR (600 MHz, DMSO-d_6, δ, ppm): 7.66-7.55 (m, 5H, $H_{arom.}$-2'' + $H_{arom.}$-5'' + $H_{arom.}$-6'' + H_{olefin}-1' + H_{olefin}-2'), 5.27 (t, 1H, $J_{6,7}$ = 2.9 Hz, H-6), 4.62 (s, 1H, OH), 4.03 (s, 3H, OMe), 3.27 (m, 1H, H-3), 2.54 (m, 1H, H-17), 2.13 (m, 2H, CH_2-4), 2.07 (s, 3H, Me-21), 2.00 (m, 1H, H-16a), 1.95 (m, 1H, H-12a), 1.91 (m, 1H, H-7a), 1.79 (m, 1H, H-1a), 1.68 (m, 1H, H-2a), 1.60 (m. 1H, H-15a), 1.59 (m, 1H, H-11a), 1.57 (m, 1H, H-16b), 1.54 (m, 1H, H-7b), 1.42 (m, 2H, H-11b + H-12b), 1.40



Scheme 2



Scheme 3

(m, 1H, H-8), 1.38 (m, 1H, H-2b), 1.19 (m, 2H, H-14 + H-15b), 1.00 (m, 1H, H-1b), 0.99 (m, 1H, H-9), 0.97 (s, 3H, Me-19), 0.94 (s, 3H, Me-21). ^{13}C NMR (150.91 MHz, DMSO-d_6, δ, ppm): 208.9 (C-20), 164.9 (CO_2), 152.2 (OMe), 147.4 ($C_{arom.}$-4''), 145.0 (C_{olefin}-2'), 141.8 (C-5), 129.2 ($C_{arom.}$-1''), 123.1 (C_{olefin}-1'), 120.74 (C-6), 115.5 ($C_{arom.}$-5'' + $C_{arom.}$-6''), 111.1 ($C_{arom.}$-2''), 70.5 (C-3), 63.1 (C-17), 60.9 (OMe), 56.6 (C-14), 50.0 (C-9), 43.8 (C-13), 42.7 (C-4), 38.5 (C-12), 37.5 (C-1), 36.6 (C-10), 31.9, 31.8, 31.7 (C-2 + C-7 + C-8 + Me-19), 24.5 (C-15), 22.7 (C-16), 21.1 (C-11), 19.6 (Me-19), 13.4 (Me-18). Anal. calcd. for $C_{31}H_{40}O_5$: C, 75.58; H, 8.18. Found: C, 75.32; H, 7.95%.

2.3. (17-(2-Acetoxyacetyl)pregnen-3α-yl)-3,4,5-trihydroxy benzoate (21)

To a solution of 21-acetoxypregnenolone (5-pregnene-3β,21-diol-20-one-21-acetate) (**20**) (375 mg, 1.00 mmol) in THF (10 mL) were added gallic acid **8** (170 mg, 1.00 mmol), triphenylphosphine (Ph_3P) (1.00 mmol, 262 mg) and diethylazodicarboxylate (DEAD) (0.13 mL, 1.00 mmol) and the mixture mixture was stirred at room temperature for 16 h. The mixture was worked up as in experiments **10**-**13** to give compound **21** (369 mg, 70%) (Scheme 3). M.p.: 193-195 °C. ^{1}H NMR (600 MHz, DMSO-d_6, δ, ppm): 7.56 (m, 2H, $H_{arom.}$-2' + $H_{arom.}$6'), 5.82 (s, 2H, CH_2-21), 5.27 (m, 1H, H-6), 4.5 (s, 1H, C4'-OH), 4.03 (m, 2H, C3'-OH + C5'-OH), 3.29 (m 1H H-3), 2.60 (m 1H, H-17) 2.17 (m, 2H, CH_2-4), 1.98 (m, 1H, H-16a), 1.96 (m,1H, H-12a), 1.91 (m, 1H, H-7a), 1.77 (m, 1H, H-1a), 1.71 (m, 1H, H-2a), 1.67 (m, 1H, H-15a), 1.64 (m, 1H, H-11a), 1.63 (m, 1H, H-16b), 1.58 (m, 1H, H-7b), 1.39 (m, 3H, H-11b + H-12b + H-8), 1.35 (m, 1H, H-2b), 1.20-1.16 (m, 5H, OCO*Me* + H-14 + H-15b), 1.00 (m, 1H, H-1b), 0.98 (m, 1H, H-9), 0.95 (s, 3H, Me-19), 0.56 (s, 3H, Me-18). ^{13}C NMR (150.91 MHz, DMSO-d_6, δ, ppm): 204.3 (C-20), 170.2 (O*CO*Me), 164.6 (CO_2), 144.8 ($C_{arom.}$-3' + $C_{arom.}$5'), 141.8 (C-5), 139.3 ($C_{arom.}$4'), 124.5 ($C_{arom.}$1'), 120.7 (C-6), 110.7 ($C_{arom.}$2' + $C_{arom.}$6'), 70.5 (C-70), 69.5 (C-21), 58.5 (C-17), 56.7 (C-14), 49.9 (C-9), 44.4 (C-13), 42.7 (C-4), 38.0 (C-12), 37.4 (C-1), 36.6 (C-10), 31.9, 31.7 (C-2 + C-7 + C-8 + Me-21), 24.6 (C-15), 22.7 (C-16), 21.1 (C-11), 20.8 (OCO*CH*$_3$), 19.6 (Me-19), 13.2 (Me-18). Anal. calcd. for $C_{30}H_{38}O_8$: C, 68.42; H, 7.27. Found: C, 68.17; H, 7.94; N, 7.20%.

3. Results and discussion

3.1. Chemistry

Recently, we prepared in our laboratory the imine-benzothiadiazole analogues of pregnenolone molecule [37], aiming to evaluate their cytochrome CYP17 hydroxylase and HIV inhibition activity. In our present work, we have selected β-pregnenolone a starting material for the synthesis of a new series of α-ester of pregnenolone analogues, with inversion in configuration at C-3, by applying of Mitsunobu reaction. Thus, treatment of **5** with various substituted benzoic acids: *p*-hydroxy-, 3,4-dihydroxy-(protocatechuic acid), 3,4,5-tri hydroxy (gallic acid) and 4-hydroxy-4-methoxy- (vanillic acid) benzoic acids (**6**-**9**), respectively, in THF in the presence of triphenylphosphine (Ph_3P) and diethylazodicarboxylate (DEAD) at room temperature for 16 h, afforded after chromatographic purification, the new derivatives **10**-**13** in 56-66 %, yields (Scheme 1).

Next, our work focused on the synthesis of new pregnenolone analogues having unsaturated carboxylic ester moieties at α-position of C-3, *via* Mitsunobu reaction, from cinnamic acid analogues having the potential phenolic groups. Thus, treatment of 5 with 3-(4-hydroxyphenyl)acrylic acid (*p*-coumaric acid) **14**, 3-(3,4-dihydroxyphenyl)acrylic acid (caffeic acid) **15**, and 3-(4-hydroxy-3-methoxyphenyl)acrylic

acid (*trans*-ferulic acid) **16** in THF and Ph_3P and DEAD as catalysts at room temperature for 16 h afforded, after chromatographic purification, the regioselective new α-ester derivatives **17-19** in 63% yield (Scheme 2).

The structures of compounds **10-13** and **17-19** were identified by ^1H, ^{13}C NMR and mass spectral data, which showed similar patterns of aliphatic proton and carbon atoms, using the NMR spectrum of the starting material pregnenolone **5** as a reference. The doublets at δ 7.79 ($J_{2',3'}$ = 8.6 Hz), and 7.59 ppm ($J_{2',6'}$ = 8.6,3.4 Hz) were assigned to the aromatic protons H-2' and H-6' of compounds **10** and **13**, respectively, while the doublet at δ 6.83 ppm was assigned to H-3' and H-5' of compound **10** ($J_{5',6'}$ = =8.6 Hz). The multiplets at the regions δ 7.66-7.55 ppm were attributed to the aromatic protons H-2' and H-6' of compounds **11**, **12**, **18** and **19**, while the same protons of compound **17** appeared as doublet of doublets at δ 7.65 ppm ($J_{2'',6''}$ = 2.0 Hz, $J_{2'',3''}$ = 8.6 Hz). The doublet of doublets at δ 7.56 ppm ($J_{3'',5''}$ = 2.0 Hz, $J_{5'',6''}$ = 8.5 Hz) was assigned for the aromatic protons H-3'' and H-5'' of **17**, whereas the doublets at δ 7.08 and 7.11 ppm ($J_{5',6'}$ = 9.0 and 7.9 Hz) were attributed to the aromatic protons H-5 of 11 and 13, respectively. The olefinic protons H-1' and H-2' of 17 were resonated at δ 7.62 and 7.53 ppm as two doublets ($J_{1',2'}$ = 10.0 Hz), respectively, while the same protons of compounds **18** and **19** were overlapped with the aromatic protons H-2'', H-5'' and H-6'' at the regions δ 7.66-7.55 ppm. The multiplets at the regions δ 1.80-1.76 ppm and δ 1.04-1.00 ppm were assigned to H-1a and H-1b, respectively, meanwhile the regions δ 1.171-1.68 ppm and δ 1.38-1.35 ppm were attributed to H-2a and H-2b, respectively. The resonances as multiplets at the regions δ 3.32-3.26 ppm were assigned to H-3, whereas multiplets at δ 2.18-2.12 ppm were assigned to CH_2-4. The triplets at δ 5.27 ppm (J = ~4.6-2.5 Hz), was assigned to H-6, while multiplets at the regions δ 1.94-1.91 ppm and 1.55-1.50 ppm were belonged to H-7a and H-7b, respectively. H-8 and H-9 were resonated as multiplets at the regions δ 1.42-1.39 ppm and 1.00-0.97 ppm, respectively, whereas H-11a, H11b and H-12a and H-12b appeared as multiplets at the regions δ 1.60-1.42 ppm and 2.00-1.40 ppm, respectively. The multiplets at the regions δ 1.20-1.16 ppm were assigned for H-14 and H-15a, while H-15b appeared as multiplet at the regions δ 1.67-1.59 ppm. H-16a, H-16b and H-17 were resonated as multiplets at the regions δ 2.06-1.55 and 2.59-2.54 ppm, respectively, whereas Me-18 and M-19 appeared as singlets at the regions δ 0.54 and 0.94 ppm, respectively. The broad singlets or signlets at the region δ 4.62-4.59 ppm were attributed to the hydroxyl groups of benzoic acid moieties of compound **10-19**, except compound **18**, where C3''-OH appeared as a singlet at δ 5.04 ppm. The methoxy groups of compounds **13** and **19** were resonated as singlets at δ 4.04 and 4.03 ppm, respectively. In the ^{13}C NMR spectra of compound **10-19**, the resonances at lower fields at the regions δ 209.1-208.9 ppm were assigned to C-20, while the resonances at the regions δ 167.7-163.5 ppm were attributed to the carbonyl carbon atoms of ester groups. The signals at δ 70.5 and 141.8 ppm were attributed to C-3 and C-3, respectively. C-1 appeared at the regions δ 38.5-37.5 ppm, while C-2, C-7, C-8 and Me-21 were appeared together at the regions δ 31.8, 31.7 and 31.6 ppm. The signals at δ 42.7 and ~120.8 were assigned to C-4 and C-6, respectively. The resonances at δ 50.0-49.1, 36.6 and 21.1 ppm were attributed to C-9, C-10 and C-11, respectively, whereas the signals at δ 38.5, 43.8 and 60.9-56.6 ppm were assigned to C-12, C-13 and C-14, respectively. C-15 – C-17 were resonated at δ 25.5, 22.7 and 63.1 ppm, respectively, while Me-18 and Me-19 appeared at δ 13.4 and 19.6 ppm, respectively. The olefinic carbon atoms C-1' of 17-19 appeared at δ 116.1, 122.1 and 123.1 ppm, meanwhile $C_{olefinic}$-2' were resonated at δ 152.3, 145.3 and 154.0 ppm, respectively. The aromatic carbon atoms were fully analyzed (*c.f.* Experimental section).

Compound **17** has been selected for further NMR experiment. The gradient-selected HMBC spectrum [41] of compound **17** revealed five $^{1,2}J_{C,H}$ couplings: C-20 of the ester group at δ 208.9 ppm coupled with H-17 at δ 2.56 ppm, CO_2 of the ester group at δ 165.9 ppm with $H_{olefinic}$-1' at δ 7.62 ppm, C-3'' aromatic carbon atom at δ 114.8 ppm with H-2'' at δ 7.65 ppm, C-2'' at δ 129.2 ppm with $H_{olefinic}$-2' at δ 7.53 ppm, in addition to a $^{1,2}J_{CH}$ coupling between $C_{olefinic}$-1' at δ 116.1 ppm and $H_{olefinic}$-2' at δ 7.53 ppm. Further, the olefinic protons H-1' at δ 7.62 ppm showed a $^{1,3}J_{CH}$ coupling with CO_2 at δ 165.9 ppm, while two $^{1,3}J_{CH}$ couplings between the aromatic proton H-6'' at δ 7.65 ppm and C-4'' at δ 156.6 ppm as well as $H_{olefinic}$-1' proton at δ 7.62 ppm and C-1'' at δ 133.7 ppm were observed (Figure 2).

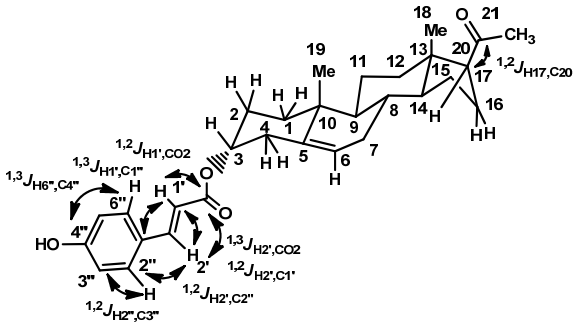


Figure 2. $J_{C,H}$ correlations in the HMBC NMR spectrum of compound **17**.

Treatment of 21-acetoxypregnenolone (5-pregnen-3β,21-diol-20-one 21-acetate) (**20**) with compound **8** under Mitsunobu reaction conditions afforded, after chromatography, the α-ester analogue **21** in 70 % yield (Scheme 3).

The structure of compound **21** was elucidated from its ^1H and ^{13}C NMR spectra. In the ^1H NMR spectra of compound **21**, the aromatic protons H-2' and H-6' appeared as multiplet at δ 7.56 ppm, while the protons of pregnene backbone were fully analyzed (*c.f.* Experimental section). CH2-21 resonated as a singlet at δ 5.82 ppm, while COMe protons appeared as a singlet at δ 2.09 ppm. The three hydroxyl groups of the aromatic ring appeared as δ 4.69 and 4.04 ppm. In the ^{13}C NMR of compound **21**, the resonance at the lower field δ 170.2 ppm was assigned to the carbonyl carbon atom (O*CO*Me), while C-20 (C=O) appeared at δ 208.9 ppm. The methyl carbon atom (OCO*Me*) was resonated at δ 20.8 ppm, whereas the pregnene carbon atoms were fully identified (*cf* Experimental section). All the synthesized were confirmed also from their ^1H, ^{13}C HSQC NMR spectra [42].

The inversion in configuration at C-3 during the formation of the α-ester **10-19** was assigned from their NOESY ^1H, ^1H NMR spectroscopy [43]. Compound **11** has been selected for NOESY NMR correlation. Thus, H-3 at δ= 3.25 ppm was correlated with H-2a at δ = 1.71 ppm, H-1a at δ = 1.80 ppm, as well as H-4a at δ = 2.14 ppm, indicative for existence of H-3 in an β position and the ester in a α position (Figure 3).

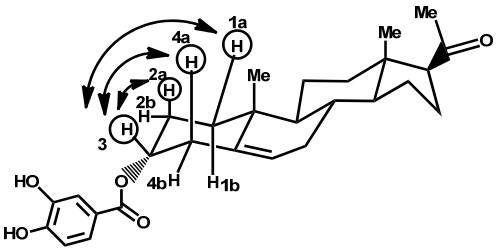


Figure 3. NOESY ^1H,^1H NMR correlation of the analogue **11**.

Table 1. *In-vitro* anti-HIV-1[a] and HIV-2[b] of new pyrimidine analogues **4-13** and **16-27**.

Compound	Virus strain	EC_{50} (µg/mL) [c]	CC_{50} (µg/mL) [d]	SI [e]
10	III_B	> 65.40	65.40	< 1
	ROD	> 65.40	65.40	< 1
11	III_B	> 35.23	35.23	< 1
	ROD	> 35.23	35.23	< 1
12	III_B	> 22.50	22.50	< 1
	ROD	> 22.50	22.50	< 1
13	III_B	> 12.11	12.11	< 1
	ROD	> 12.11	12.11	< 1
17	III_B	> 7.90	7.90	< 1
	ROD	> 7.90	7.90	< 1
18	III_B	> 1.95	1.95	< 1
	ROD	> 1.95	1.95	< 1
19	III_B	> 10.15	10.15	< 1
	ROD	> 10.15	10.15	< 1
AZT	III_B	0.0022	> 25	> 11363
	ROD	0.00094	> 25	> 26596
Nevirapin	III_B	0.050	>4.00	> 80
	ROD	>4.00	>4.00	< 1

[a] Anti-HIV-1 activity measured with strain III_B.
[b] Anti-HIV-2 activity measured with strain ROD.
[c] Compound concentration required to achieve 50% protection of MT-4 cells from the HIV-1 and 2-induced cytopathogenic effect.
[d] Compound concentration that reduces the viability of mock-infected MT-4 cells by 50%.
[e] SI: Selectivity index (CC_{50}/EC_{50}).

3.2. In vitro anti-HIV activity

Compounds **10-14**, **17-19** and **21** were tested for their *in vitro* anti-HIV-1 (strain III_B) and HIV-2 (strain ROD) activity in human T-lymphocyte (MT-4) cells based on an MTT assay [44]. The results are summarized in Table 1, in which the data for nevirapine (BOE/BIRG587) [45] and azidothymidine (DDN/AZT) [46] were included for comparison.

Compounds-induced cytotoxicity was also measured in MT-4 cells parallel with the antiviral activity. All compounds are inactive except compound **18** which showed EC_{50} values of >1.95 µg/mL, respectively, but no selectivity was observed (SI < 1).

From the SAR analysis, we found that the olefin and dihydroxy group substituents at the olefinic benzoate group at C-3 of the pregnenolone ring, *e.g.* compound **18** was well tolerated in the hydrophobic region of HIV RT and then showed higher activity than those of the other benzoate substituents at C-3 of the same ring. This might be explained in term of hydrophobic interaction of the double bond as well as aromatic ring as well as hydrogen bonds of the two hydroxyl groups at the benzoate group with the amino acids of the HIV-1 RT.

4. Molecular modeling analysis

The molecular docking was performed using SYBYL-X 1.1 and the docking results were shown by PyMOL [47]. Our molecular docking analysis of the new analogues based on the modeling study which was performed to understand the binding mode of these analogues with the HIV-RT binding pocket (NNIBP) (PDB code: 3DLG, [48]).

Compound **18** has been selected for the docking modeling study, since its binding energy score -8.2, indicating a selectivity of substituted olefinic benzoate in its binding to the enzyme pocket (Figure 4). As shown in Figure 4, the aromatic ring of compound **18** fitted into an aromatic rich subpocket surrounded by the aromatic side chains of Tyr179 as well as the existence of the hydrogen bond between the hydroxyl group of Tyr179 with those of the benzoate moiety. The pregnenolone backbone was located in the middle of the binding pocket, anchoring the carbonyl substituent at C-20 in a favourable position for hydrogen bonding with the NH_2 group of Lys101, in addition to a hydrogen bond between the oxygen atom of CO_2 group at C-3 with NH2 of Pro132 of the RT enzyme. Overall, the combination of hydrophobic interaction and π-stacking appears to govern the binding of compound **18** with HIV RT.

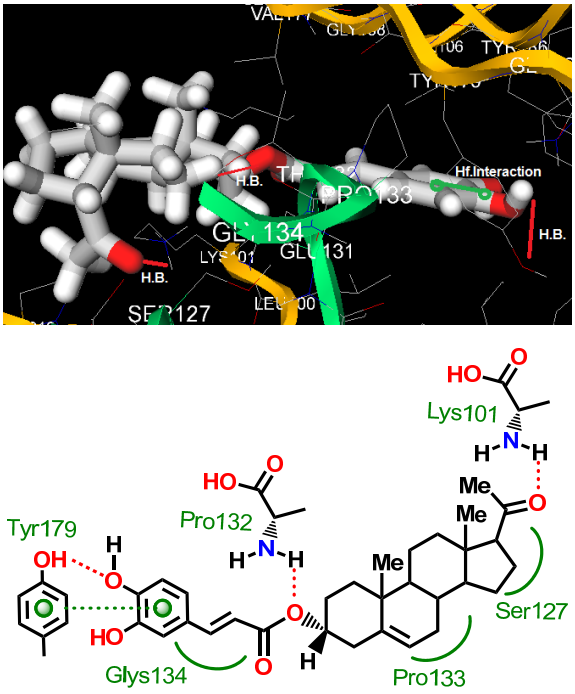


Figure 4. Docked conformation of compound **18** showing three hydrogen bonding: Lys101 with C=O at C-20 and Pro132 with oxygen atom of CO_2 at C-3 of pregnenolone ring, in addition to Tyr179 with hydroxyl group of the benzoate moiety. It exhibited also hydrophobic interaction between phenyl group of the benzoate moiety and Tyr179 of reverse transcriptase (RT) enzyme residues.

5. Conclusion

In conclusion, synthesis of new 3α-substituted aryl ester derivatives of pregnenolone **10-13** and (5-pregnen-20-on-3α-yl)-3-substituted-phenyl acrylates **17-19** by applying Mitsunobu reaction has been described. All the compounds were assigned by their 2D NMR spectroscopy, where NOESY NMR spectra have confirmed the inversion in configuration at C-3 during the formation of α-ester analogues. All the new synthesized compounds have been evaluated for their activity against HIV-1 and 2. Compounds **18** showed potential activity against HIV-1, whereas the others analogues shown moderate to poor activity. Compound **18** have been selected for the

molecular modeling study showing its binding to the reverse transcriptase enzyme pocket through three hydrogen bonding and one hydrophobic interaction.

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References

[1]. Mensah-Nyagan, A. G.; Meyer, L.; Schaeffer, V.; Kibaly, C.; Patte-Mensah, C. *Psychoneuroendocrino.* **2009**, *34*, 169-177.
[2]. Ibrahim-Ouali, M. *Steroids* **2007**, *72*, 475-508.
[3]. Bhatti, H. N.; Khera, R. A. *Steroids* **2012**, *77*, 1267-1290.
[4]. Aggarwal, S.; Thareja, S.; Verma, A.; Bhardwaj, T. R.; Kumar, M. *Steroids* **2010**, *75*, 109-153.
[5]. Li, H. J.; Jiang, Y.; Li, P. *Nat. Prod. Rep.* **2006**, *23*, 735-752.
[6]. Ifere, G. O.; Barr, E.; Equan, A.; Gordon, K.; Singh, Chaudhany, J. J.; Igietseme, J. U.; Ananaba, G. A. *Cancer Detect. Prev*. **2009**, *32*, 319-328.
[7]. Bansal, R.; Guleria, S.; Thota, S.; Bodhankar, S. L.; Patwardhan, M. R.; Zimmer, C. *Steroids* **2012**, *77*, 621-629.
[8]. Dubey, R. K.; Oparil, S.; Imthurn, B.; Jackson, E. K. *Cardiovasc. Res.* **2002**, *53*, 688-708.
[9]. Holst, J. P.; Soldin, S. J.; Tractenberg, R. E.; Guo, T.; Kundra, P.; Verbalis, J. G. *Steroids* **2007**, *72*, 71-84.
[10]. Auci, D. L.; Reading, C. L.; Frincke, J. M. *Autoimmun Rev.* **2009**, *8*, 369-372.
[11]. Jursic, B. S.; Upadhyay, S. K.; Creech, C. C.; Neumann, D. M. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 7372-7375.
[12]. Banday, A. H.; Iqbal, Z. M.; Ganaie, B. A. *Steroids* **2011**, *76*, 1358-1362.
[13]. Billich, A.; Nussbaumer, P.; Lehr, P. *J. Steroid Biochem.* **2000**, *73*, 225-235.
[14]. Saha, P.; Fortin, S.; Leblanc, V.; Parent, S.; Asselin, E.; Berube, G. *Steroids* **2012**, *77*, 1113-1122.
[15]. Bansal, R.; Guleria, S.; Thota, S.; Hartmann, R. W.; Zimmer, C. *Chem. Pharma. Bull.* **2011**, *59*, 327-331.
[16]. Gauthier, S.; Martel, C.; Labrie, F. *J. Steroid Biochem*. **2012**, *132*, 93-104.
[17]. Sheridan, P. J.; Blum, K.; Trachtenberg, M. C. Steroid receptors and disease: cancer, autoimmune, bone, and circulatory disorders, Marcel Dekker Inc., 1988, pp. 289-564.
[18]. Reddz, D. S. *Prog. Brain Res.* **2010**, *186*, 113-137.
[19]. Aird, R. B. *J. Nerv. Mental Dis.* **1944**, *99*, 501-510.
[20]. Aird, R. B.; Gordan, G. S. *J. Am. Med. Assoc*. **1951**, *145*, 715-719.
[21]. Gyermek, L.; Genther, G.; Fleming, N. *Intern. J. Neuropharm*. **1967**, *6*, 191-198.
[22]. Green, C. J.; Halsey, M. J.; Precious, S.; Wardley-Smith, B. *Lab. Animals* **1978**, *12*, 85-89.
[23]. Moffat, L. E.; Kirk, D.; Tolley, D. A.; Smith, M. F.; Beastall, G. *British J. Urology* **1988**, *61*, 439-440.
[24]. Mahler, C.; Verhelst, J.; Denis, L. *Cancer* **1993**, *71*, 1068-1073.
[25]. Lake-Bakaar, G.; Scheuer, P. J.; Sherlock, S. *British Med. J.* **1987**, *294*, 419-442.
[26]. Njar, V. C.; . Klus, G. T.; Brodie, A. M. H. *Bioorg. Med. Chem. Lett*. **1996**, *6*, 2777-2782.
[27]. Njar, V. C.; Kato, K.; Nnane, I. P.; Grigoryev, D. N.; Long, B. J.; Brodie, A. M. *J. Med. Chem.* **1998**, *41*, 902-912.
[28]. Brodie, A.; Njar, V. C. US Patent 6, 200, 965 B1; 2001.
[29]. Handratta, V. D.; Jelovac, D.; Long, B. J.; Kataria, R.; Nnane, I. P.; Njar, V. C.; Brodie, A. M. *J. Steroid Biochem. Mol. Biol.* **2004**, *92*, 155-165.
[30]. Handratta, V. D.; Vasaitis, T. S.; Njar, V. C.; Gediya, L. K.; Kataria, R.; Chopra, P.; Newman, P.; Farquhar, R.; Guo, Z.; Qiu, Y.; Brodie, A. M. *J. Med. Chem.* **2005**, *48*, 2972-2984.
[31]. Brodie, A.; Njar, V. C. WO Patent 2006/093993, 2006.
[32]. Vasaitis, T. S.; Njar, V. C. O. *Future Med. Chem.* **2010**, *2*, 667-680.
[33]. Molina, A.; Belldegrun, A. *J. Urol.* **2011**, *185*, 787-794.
[34]. de Bono, J. S.; Logothetis, C. J.; Molina, A.; Fizazi, K.; North, S.; Chu, L.; Chi, K. N.; Jones, R. J.; *et al. N. Engl. J. Med.* **2011**, *364*, 1995-2005.
[35]. Bryce, A.; Ryan, C. J. *Clin. Pharma. Therap.* **2012**, *91*, 101-108.
[36]. Potter, G. A.; Barrie, S. E.; Jarman, M.; Rowlands, M. G. *J. Med. Chem.* **1995**, *38*, 2463-2471.
[37]. Al-Masoudi, N. A.; Ali, D. S.; Saeed, B.; Hartmann, R. W.; Engel, M.; Rashid, S.; Saeed, A. *Archiv Pharmazie Life Sci.* **2014**, *347*, 896-907.
[38]. Mitsunobu, O.; Yamada, M. *Bull. Chem. Soc. Jpn.* **1967**, *40*, 2380-2382.
[39]. Mitsunobu, O. *Synthesis* **1981**, *1*, 1-28.
[40]. Mitsunobu, O.; Eguchi, M. *Bull. Chem. Soc. Jpn.* **1971**, *44*, 3427-3430.
[41]. Willker, W.; Leibfritz, D.; Kerssebaum, R.; Bermel, W. *Mag. Reson. Chem.* **1993**, *31*, 287-292
[42]. Davis, A. L.; Keeler, J.; Laue, E. D.; Moskau, D. *J. Magn. Reson*. **1992**, *98*, 207-216.
[43]. Anderson, W. A.; Freeman, R. *J. Chem. Phys.* **1962**, *37*, 411-415.
[44]. Pauwels, R.; Balzarini, J.; Baba, M.; Snoeck, R.; Schols, D.; Herdewijn, P.; Desmyter, J.; De Clercq, E. *J. Virol. Methods* **1988**, *20*, 309-321.
[45]. Hargrave, K. D.; Proudfoot, J. R.; Grozinger, K. G.; Cullen, E.; Kapadia, S. R.; Patel, U. R.; Fuchs, V. U.; Mauldin, S. C.; Vitous, J.; Behnke, M. L.; Klunder, J. M.; Pal, K.; Skiles, J. W.; McNeil, D. W.; Rose, J. M.; Chow, G. C.; Skoog, M. T.; Wu, J. C.; Schmidt, G.; Engel, W. W.; Eberlein, W. G.; Saboe, T. D.; Campbell, S. J.; Rosenthal, A. S.; Adams, J. *J. Med. Chem.* **1991**, *34*, 2231-2241.
[46]. Mitsuya, H.; Weinhold, K. J.; Furman, P. A.; Clair, M. H. St.; Lehrmann, S. N.; Gallo, R.; Bolognesi, D.; Barry, D. W.; Broder, S. *Proc. Natl. Acad. Sci. USA* **1985**, *82*, 7096-7100.
[47]. Seeliger, S.; de Groot, B. L. *J. Computer-Aided Mol. Design* **2010**, *24*, 417-422.
[48]. Zhan, P.; Liu, X.; Li, Z.; Fang, Z.; Pannecouque, C.; De Clercq, E. *Chem. Biodivers.* **2010**, *7*, 1717-1727.