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Synthesis of novel C17 steroidal carbamates Studies on CYP17 action, androgen receptor binding and function, and prostate cancer cell growth

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ABSTRACT

We have exploited the reaction of 1,1′-carbonylbis(2-methylimidazole) (CBMI) with several 17β-hydroxy androstanes to synthesize a series of novel C17 steroidal carbamates. Structural elucidation features have been provided for the final compounds based on 1D and 2D NMR techniques, IR spectroscopy, and related literature. The new compounds were tested for inhibition of human cytochrome 17α-hydroxylase-$C_{17,20}$-lyase (CYP17) and androgen receptor (AR) binding and function effects. Their inhibitory potential against PC-3 cell proliferation was also evaluated. Compounds **11** and **23** were found to inhibit CYP17 with IC_{50} values of 17.1 and 11.5 μM, respectively. The carbamate moiety at C17 allowed tight binding of the synthesized compounds to both wild-type (wt–) and mutated AR. When bound to the mutated AR, the compounds were found to have a dual effect, stimulating transcription at low concentrations while almost fully blocking it at the higher concentrations tested, in the presence of the natural androgen dihydrotestosterone (DHT). Compounds **8** and **12** were the most active against PC-3 cell proliferation with EC_{50} values of 2.2 and 0.2 μM, respectively.

1. Introduction

Androgen biosynthesis in the body is mediated by CYP17, a key enzyme which converts C21 precursors (pregnenolone and progesterone) to the related C19 steroids, dehydroepiandrosterone (DHEA) and androstenedione, in the testes and adrenals [1–5]. These C19 steroids are androgen precursors and can be further metabolized in steroidogenic tissues to more potent androgens such as testosterone and dihydrotestosterone (DHT). Androgens then bind to the androgen receptor (AR) and initiate a series of events that will eventually lead to AR-mediated responses such as the synthesis of specific proteins like prostate-specific antigen (PSA) and triggering of cell proliferation [6,7]. Thus, effective inhibitors of this enzyme could be useful in the treatment of prostate cancer (PC) [8–14], for which androgen deprivation therapy has been standard treatment since the Nobel Prize winner work of Huggins et al. [15,16].

Although around 80% of the human PC show favorable response to androgen deprivation therapy [6,17], relapses are seen invariably when tumors emerge as androgen-independent and apoptosis-resistant [18]. Several mecha-

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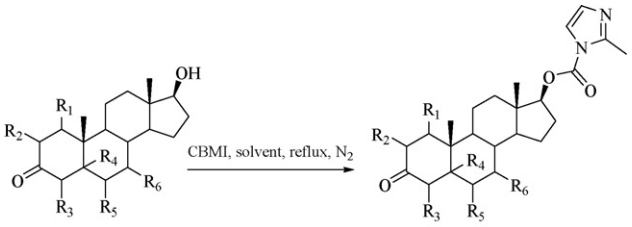


1.....$R_1 = R_2 = R_5 = R_6 = H$; R_3,R_4 = double bond.....7
2.....$R_1,R_2 = R_3,R_4 = R_5,R_6$ = double bond.....8
3.....$R_1 = R_2 = H$; $R_3,R_4 = R_5,R_6$ = double bond.....9
4.....$R_1 = R_2 = R_3 = R_4 = R_5 = R_6 = H$.....10
5.....$R_1,R_2 = R_3,R_4$ = double bond; $R_5 = R_6 = H$.....11
6.....R_1,R_2 = double bond; $R_3 = R_4 = R_5 = R_6 = H$.....12

Scheme 1 – Reaction of 17β-hydroxysteroids with CBMI.

nisms have been proposed for this finding which include AR amplification, AR mutations, alterations in the balance between transcriptional coactivators and corepressors, activation of signal transduction pathways that by-pass the AR [7,19], and enhanced intracellular conversion of adrenal androgens to testosterone and DHT [20].

C17 steroidal carbamates have been reported to inhibit PC related enzymes such as 5α-reductase [21] and CYP17 [22], and to possess antiproliferative effects against PC cell lines [23]. We have exploited the reaction of the commercially available CBMI with several 17β-hydroxy steroidal substrates, in appropriate solvent, at reflux [24], in order to prepare a series of novel 17β-(2′-methylimidazole) carboxylates bearing the androstane backbone (**7–12**, **15**, **18**, and **23**), herein referred to as steroidal C17 steroidal carbamates (Schemes 1 and 2). The reaction of *N*,*N*′-carbonyldiimidazole reagents with alcohols and phenols has been reported to afford either imidazole-*N*-carboxylic esters [24–29] or *N*-alkylimidazoles [26,28,30–34], depending on the nature of the starting alcohol and on the reaction conditions used. Indeed, transfer of imidazole from 1,1′-carbonyldiimidazole (CDI) and 1,1′-(thiocarbonyl)diimidazole (TCDI) was found to occur exclusively with benzylic or vinylogous benzylic alcohols [26]. Only carbamates were obtained from the reaction of non-benzylic primary and secondary aliphatic alcohols with CDI [28]. For benzylic primary alcohols, formation of *N*-alkylimidazoles was reported to proceed reasonably well at 170 °C in several solvents and by way of the initially formed carbamate [28]. However, elimination was found to occur as a significant side reaction for benzylic secondary alcohols with β-hydrogen atoms. With one exception, reactions of *N*,*N*-disubstituted β-aminoalcohols with CDI afforded *N*-alkylation products under relatively mild conditions [28,34]. In our case, the reaction proceeded smoothly to afford the C17 steroidal carbamates, **7–12**, **15**, **18**, and **23**, in good yields. We tested our new compounds for CYP17 inhibition. In addition, due to the multifactorial nature of PC as a disease and following the observation that several compounds designed as CYP17 inhibitors have been shown to bind to the AR and interfere with its function [35–39], we decided to evaluate their effects on AR binding and androgen-mediated transcription. Their ability to inhibit the proliferation of PC-3 cells, which derive from bone metastases and do not express the AR [40,41], has also been determined.

2. Experimental

2.1. Chemistry

2.1.1. General

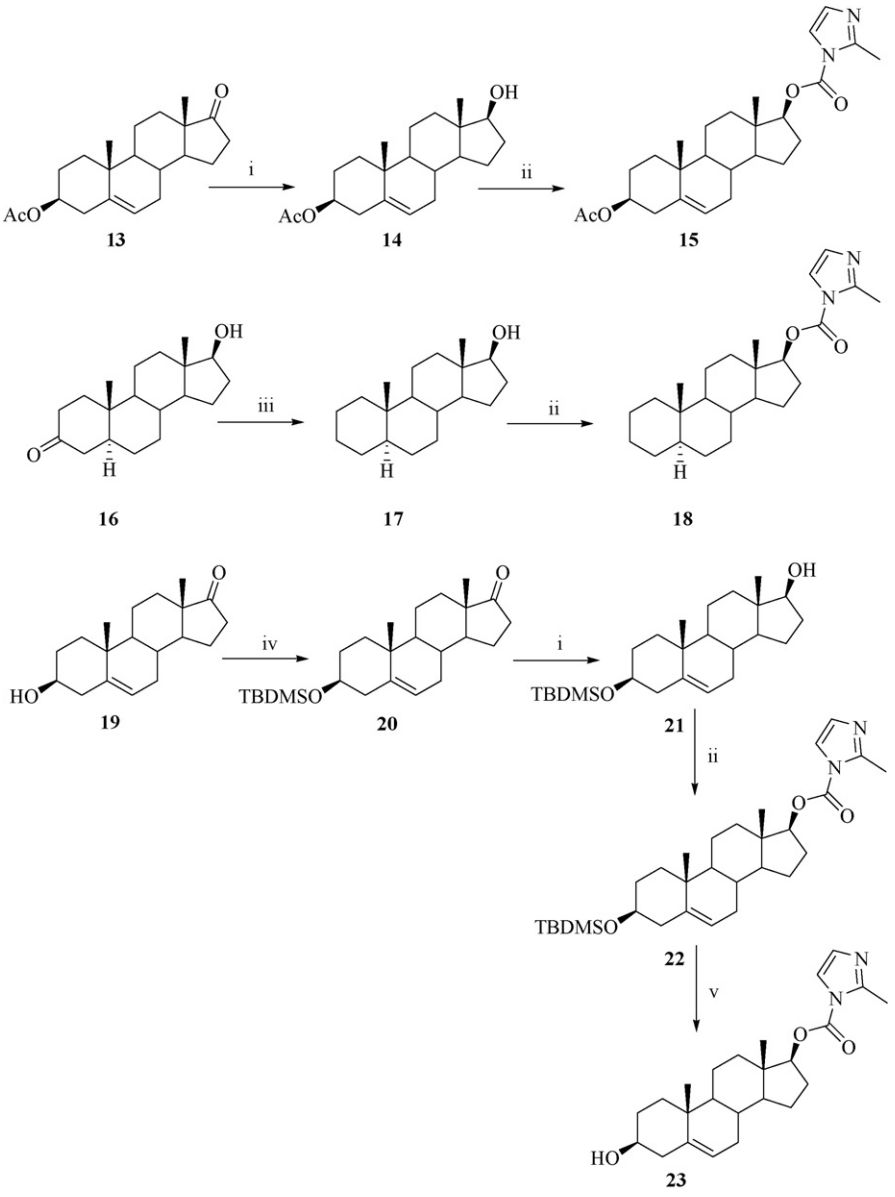
Steroid compounds were purchased from Sigma–Aldrich Co and Steraloids Inc. All reagents were obtained from Sigma–Aldrich Co. All solvents used were previously dried and purified according to standard procedures. For thin layer chromatography (TLC) analysis, Kieselgel 60HF_{254}/Kieselgel 60G were used. Melting points were determined using a BUCHI Melting Point B-540 apparatus and are uncorrected. IR spectra were obtained using a JASCO FT/IR-420 spectrophotometer (FTIR-ATR). NMR spectra were obtained using a Brucker Digital NMR—Avance 300 apparatus or a Varian 600 MHz spectrometer, in $CDCl_3$ with Me_4Si as the internal standard. Mass spectra were recorded on a Finnigan Polaris Q GC/MS Benchtop Ion Trap mass spectrometer. Elemental analysis was carried out on a Fisons Instruments EA 1108 CHNS-O elemental analyser.

2.1.2. 3-Oxoandrost-4-en-17β-yl-2′-methyl-1H-imidazole-1-carboxylate (7)

A solution of 17β-hydroxyandrost-4-en-3-one (**1**) (200 mg, 0.69 mmol) and 1,1′-carbonylbis(2-methylimidazole) (CBMI) (211 mg, 1.11 mmol) in anhydrous acetonitrile (6.6 ml) was refluxed for 24 h (the reaction was monitored by TLC and stopped after complete consumption of the substrate). Water (30 ml) was added to the mixture and the resulting precipitate was dissolved in diethyl ether (80 ml). The aqueous phase was extracted twice with diethyl ether (2× 30 ml). The organic phase was then washed with water (10 ml), brine (10 ml), dried with anhydrous Na_2SO_4, filtered, and the solvent was removed under reduced pressure to afford compound **7** as a white solid (225.3 mg, 82%), which was recrystallized from a mixture of ethyl acetate and *n*-hexane: mp 200–203 °C; IR 1142, 1300, 1613, 1663, 1757 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) δ 0.91 (s, 3H, 18-H_3), 1.17 (s, 3H, 19-H_3), 2.61 (s, 3H, 2′-CH_3), 4.78 (m, 1H, 17α-H), 5.70 (brs, 1H, 4-H), 6.83 (brs, 1H, 4′-H), 7.32 (brs, 1H, 5′-H); ^{13}C NMR ($CDCl_3$, 150.8 MHz) δ 12.3 (C18), 16.7 (2′-CH_3), 17.3 (C19), 20.4, 23.3, 27.3, 31.3, 32.5, 33.8, 35.3, 35.6, 36.5, 38.5 (C10), 42.7 (C13), 49.9, 53.5, 86.4 (C17), 118.0 (C5′), 123.9 (C4), 127.5 (C4′), 147.8 (C2′), 149.3 (OCO), 170.4 (C5), 199.2 (C3). EI-MS *m*/*z* (%): 396 (32) M^+, 271 (52), 253 (100), 157 (34), 147 (60), 119 (34), 105 (44), 91 (38); Anal. calcd. for $C_{24}H_{32}N_2O_3$: C 72.70, H 8.47, N 7.06, found: C 72.60, H 8.47, N 7.24.

2.1.3. 3-Oxoandrosta-1,4,6-trien-17β-yl-2′-methyl-1H-imidazole-1-carboxylate (8)

The method followed that described for compound **7** but using 17β-hydroxyandrosta-1,4,6-trien-3-one (**2**) (40 mg; 0.14 mmol) and CBMI (42.8 mg; 0.22 mmol) in anhydrous acetonitrile (1.3 ml) at reflux. After 15 h, more CBMI (24.1 mg; 0.13 mmol) was added and 5 h later the reaction was complete. Compound **8** (48.6 mg; 88%): mp (acetone) 199–201 °C; IR 1143, 1301, 1600, 1646, 1758 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.04 (s, 3H, 18-H_3),



Scheme 2 – Synthesis of compounds 15, 18 and 23. Reagents and conditions: (i) NaBH4, THF/MeOH 1:1; (ii) CBMI, solvent, reflux; (iii) Zn, glacial CH_3COOH; (iv) TBDMSCl, DMAP, NEt_3, DMF, reflux; (v) $TMSCl/H_2O$, EtOH.

1.23 (s, 3H, 19-H_3), 2.77 (s, 3H, 2′-CH_3), 4.87 (m, 1H, 17α-H), 6.01 (m, 1H, 2-H), 6.02 (brs, 1H, 4-H), 6.28 (m, 2H, 6-H and 7-H), 6.70 (brs, 1H, 4′-H), 7.06 (m, 1H, 1-H), 7.41 (brs, 1H, 5′-H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 12.3, 16.8, 20.6, 21.2, 22.9, 27.2, 36.3, 37.6, 41.0, 43.2, 47.9, 48.0, 85.8 (C17), 118.0 (C5′), 124.0 (C4), 127.8, 128.1 and 128.2 (C2, C6 and C4′), 136.6 (C7), 147.8 (C2′), 149.3 (OCO), 152.6 (C1), 161.9 (C5), 186.2 (C3). EI-MS *m/z* (%): 392 (34) M^+, 267 (34), 171 (100), 159 (51), 145 (46), 128 (35), 121 (10), 83 (25); Anal. calcd. for $C_{24}H_{28}N_2O_3$: C 73.44, H 7.19, N 7.14, found: C 73.80, H 7.56, N 6.80.

2.1.4. 3-Oxoandrosta-4,6-dien-17β-yl-2′-methyl-1H-imidazole-1-carboxylate (9)

The method followed that described for compound **7** but using 17β-hydroxyandrosta-4,6-dien-3-one (**3**) (34 mg; 0.12 mmol) and CBMI (36.1 mg; 0.19 mmol) in anhydrous acetonitrile (1.3 ml) at reflux. After 15 and 22 h, more CBMI (20.3 mg; 0.11 mmol) and (22.6 mg; 0.12 mmol), respectively, was added and 15 h later the reaction was complete. Compound **9** (38.4 mg; 92%): mp (acetone) 185–186 °C; IR 1143, 1302, 1617, 1659, 1753 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.01 (s, 3H, 18-H_3), 1.14 (s, 3H, 19-H_3), 2.78 (s, 3H, 2′-CH_3), 4.89 (m, 1H, 17α-H), 5.70 (brs, 1H, 4-H), 6.30 (m, 2H, 6-H and 7-H), 7.00 (brs, 1H, 4′-H), 7.41 (brs, 1H, 5′-H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 12.2, 13.5, 16.2, 16.8, 20.0, 22.9, 27.3, 33.7, 35.9, 36.4, 37.2, 43.6, 47.7, 50.4, 86.0 (C17), 118.0 (C5′), 123.9 (C4), 127.7 and 128.4 (C6 and C4′), 139.3 (C7), 147.8 (C2′), 149.3 (OCO), 163.2 (C5), 199.5 (C3). EI-MS *m/z* (%): 394 (48) M^+, 269 (100), 251 (35), 213 (32), 173 (89), 145 (64), 117 (28), 81 (36); Anal. calcd. for $C_{24}H_{30}N_2O_3$: C 73.07, H 7.66, N 7.10, found: C 72.90, H 7.96, N 7.24.

2.1.5. 3-Oxo-5α-androst-17β-yl-2′-methyl-1H-imidazole-1-carboxylate (10)

The method followed that described for compound **7** but using 17β-hydroxy-5α-androstan-3-one (**4**) (100 mg; 0.34 mmol) and CBMI (104.7 mg; 0.55 mmol) in anhydrous acetonitrile (5 ml) at reflux. After 29 h, more CBMI (26.6 mg; 0.14 mmol) was added and 31 h later the reaction was complete. Compound **10** (96.6 mg; 94%): mp (acetonitrile) 195–196 °C; IR 1152, 1282, 1710, 1744 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 0.92 (s, 3H, 18-H_3), 1.04 (s, 3H, 19-H_3), 2.66 (s, 3H, 2′-CH_3), 4.80 (m, 1H, 17α-H), 6.88 (brs, 1H, 4′-H), 7.36 (brs, 1H, 5′-H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 11.4, 12.4, 16.8, 20.7, 23.4, 27.3, 28.6, 31.0, 35.0, 35.6, 36.7, 38.0, 38.4, 42.9, 44.5, 46.5, 50.2, 53.5, 86.8 (C17), 118.1 (C5′), 127.5 (C4′), 147.8 (C2′), 149.4 (OCO), 211.7 (C3). EI-MS *m*/*z* (%): 398 (24) M^+, 273 (36), 255 (100), 159 (37), 123 (46), 107 (42), 81 (52), 79 (43); Anal. calcd. for $C_{24}H_{34}N_2O_3$: C 72.33, H 8.60, N 7.03, found: C 72.16, H 8.74, N 7.31.

2.1.6. 3-Oxoandrosta-1,4-dien-17β-yl-2′-methyl-1H-imidazole-1-carboxylate (11)

The method followed that described for compound **7** but using 17β-hydroxyandrosta-1,4-dien-3-one (**5**) (400 mg; 1.4 mmol) and CBMI (531.3 mg; 2.79 mmol) in anhydrous dichloromethane (14 ml) at reflux for 28 h. Compound **11** (402.2 mg; 73%): mp (ethanol) 165–167 °C; IR 1149, 1280, 1625, 1664, 1761 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 0.98 (s, 3H, 18-H_3), 1.26 (s, 3H, 19-H_3), 2.66 (s, 3H, 2′-CH_3), 4.79 (m, 1H, 17α-H), 6.09 (brs, 1H, 4-H), 6.24 (m, 1H, 2-H), 6.88 (brs, 1H, 4′-H), 7.05 (m, 1H, 1-H), 7.35 (brs, 1H, 5′-H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 12.4, 16.8, 18.6, 22.2, 23.5, 27.2, 32.5, 32.9, 35.2, 36.4, 42.9, 43.3, 49.5, 51,9, 86.2 (C17), 118.0 (C5′), 124.0 (C4), 127.6 and 127.8 (C2 and C4′), 147.8 (C2′), 149.4 (OCO), 155.4 (C1), 168.5 (C5), 186.2 (C3). EI-MS *m*/*z* (%): 394 (36) M^+, 229 (10), 173 (37); 147 (80), 122 (73), 91 (76), 83 (100), 81 (22); Anal. calcd. for $C_{24}H_{30}N_2O_3$: C 73.07, H 7.66, N 7.10, found: C 73.26, H 7.60, N 7.25.

2.1.7. 3-Oxo-5α-androst-1-en-17β-yl-2′-methyl-1H-imidazole-1-carboxylate (12)

The method followed that described for compound **7** but using 17β-hydroxy-5α-androst-1-en-3-one (**6**) (80 mg; 0.28 mmol) and CBMI (150.5 mg; 0.79 mmol) in anhydrous dichloromethane (2.6 ml) at reflux for 5 h. Compound **12** (92.4 mg; 84%): mp (ethanol) 212–214 °C; IR 1152, 1283, 1674, 1745 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) δ 0.92 (s, 3H, 18-H_3), 1.02 (s, 3H, 19-H_3), 2.65 (s, 3H, 2′-CH_3), 4.80 (m, 1H, 17α-H), 5.85 (m, 1H, 2-H), 6.86 (brs, 1H, 4′-H), 7.11 (m, 1H, 1-H), 7.34 (brs, 1H, 5′-H); ^{13}C NMR ($CDCl_3$, 150.8 MHz) δ 12.6, 13.0, 16.7, 20.6, 23.3, 27.3, 27.4, 30.6, 35.3, 36.7, 38.9, 40.8, 43.0, 44.1, 49.7, 50.3, 86.8 (C17), 118.3 (C5′), 127.7 and 127.8 (C2 and C4′), 147.1 (C2′), 149.6 (OCO), 158.0 (C1), 200.1 (C3). EI-MS *m*/*z* (%): 396 (16) M^+, 271 (14), 161 (26), 121 (31), 107 (100), 95 (54), 81 (31), 79 (26); Anal. calcd. for $C_{24}H_{32}N_2O_3$: C 72.70, H 8.13, N 7.06, found: C 72.50, H 8.51, N 7.20.

2.1.8. 17β-Hydroxyandrost-5-en-3β-yl acetate (14)

DHEA acetate (**13**) (100 mg; 0.30 mmol) was dissolved in a mixture of THF/MeOH 1:1 (2 ml) at room temperature. The mixture was then cooled in an ice bath while $NaBH_4$ (30 mg; 0.79 mmol) was carefully added. After 1 h at room temperature, the reaction was quenched by adding acetone dropwise. The mixture was then concentrated under reduced pressure. Water (6 ml) was added along with ethyl acetate (20 ml). The mixture was left under magnetic stirring for a couple of hours and the resulting aqueous phase was extracted with ethyl acetate (2× 30 ml) again. The organic phase was washed with aqueous saturated $NaHCO_3$ solution (10 ml) and water (10 ml), dried over anhydrous Na_2SO_4, filtered, and the solvent was removed under reduced pressure to afford compound **14** (83 mg; 82%), which was recrystallized from a mixture of MeOH and water: mp 144–147 °C; IR 1716, 3423 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 0.76 (s, 3H, 18-H_3), 1.04 (s, 3H, 19-H_3), 2.04 (s, 3H, 3β-OAc), 3.65 (m, 1H, 17α-H), 4.60 (m, 1H, 3α-H), 5.37 (m, 1H, 6-H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 13.5, 19.4, 20.4, 21.3, 21.9, 27.6, 30.8, 31.3, 31.7, 35.8, 36.5, 36.8, 37.9, 47.4, 50.0, 51,6, 73.7 (C3); 81.4 (C17), 122.1 (C6), 139.5 (C5), 170.4 (CH_3CO); EI-MS *m*/*z* (%): 332 (2) M^+, 272 (100), 239 (56), 183 (26), 143 (45), 105 (44), 91 (54), 79 (40); Anal. calcd. for $C_{21}H_{32}O_3$: C 75.86, H 9.70, found: C 75.50, H 9.70.

2.1.9. 3β-Acetoxyandrost-5-en-17β-yl-2′-methyl-1H-imidazole-1-carboxylate (15)

The method followed that described for compound **7** but using compound **14** (596 mg; 1.79 mmol) and CBMI (657 mg; 3.45 mmol) in anhydrous acetonitrile (15 ml) at reflux for 29 h. Compound **15** (611 mg; 77%): mp (ethyl acetate/*n*-hexane) 151–152 °C; IR 1147, 1302, 1725, 1750 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 0.92 (s, 3H, 18-H_3), 1.05 (s, 3H, 19-H_3), 2.04 (s, 3H, 3β-OAc), 2.67 (s, 3H, 2′-CH_3), 4.61 (m, 1H, 3α-H), 4.80 (m, 1H, 17α-H), 5.39 (m, 1H, 6-H), 6.87 (brs, 1H, 4′-H), 7.36 (brs, 1H, 5′-H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 12.3, 16.8, 19.3, 20.3, 21.4, 23.4, 27.4, 27.6, 31.3, 31.6, 36.5, 36.6, 36.9, 38.0, 42.6, 49.7, 50.6, 73.7 (C3), 86.8 (C17), 118.1 (C5′), 122.0 (C6), 127.5 (C4′), 139.7 (C5), 147.8 (C2′), 149.4 (OCO), 170.5 (CH_3CO). EI-MS *m*/*z* (%): 440 (8) M^+, 380 (100), 255 (78), 145 (65), 129 (89), 91 (56), 81 (72), 67 (46); Anal. calcd. for $C_{26}H_{36}N_2O_4$: C 70.88, H 8.24, N 6.36, found: C 70.58, H 8.42, N 6.75.

2.1.10. 5α-Androstan-17β-ol (17)

Details of the synthesis of this compound were reported previously [42].

2.1.11. 5α-Androstane-17β-yl-2′-methyl-1H-imidazole-1-carboxylate (18)

The method followed that described for compound **7** but using compound **17** (379.2 mg; 1.37 mmol) and CBMI (521.1 mg; 2.74 mmol) in anhydrous dichloromethane (14 ml) at reflux. After 27 h more CBMI (100.8 mg; 0.53 mmol) was added and 29 h later the reaction was complete. The resulting white solid was purified by flash chromatography (ethyl acetate:petroleum ether 40–60 °C 2:1) to afford compound **18** (385.1 mg; 73%): mp (acetone) 112–115 °C; IR 1149, 1273, 1751 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 0.80 (s, 3H, 18-H_3), 0.89 (s, 3H, 19-H_3), 2.66 (s, 3H, 2′-CH_3), 4.78 (m, 1H, 17α-H), 6.87 (brs, 1H, 4′-H), 7.35 (brs, 1H, 5′-H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 12.1, 12.5, 16.8, 20.1, 22.0, 23.4, 26.7, 27.4, 28.7, 28.9, 31.5, 35.2, 36.2, 36.9, 38.6, 42.9, 46.9, 50.5, 54.5, 87.1 (C17), 118.1 (C5′), 127.3 (C4′), 147.8 (C2′), 149.4 (OCO). EI-MS *m*/*z* (%): 384.1 (14) M^+, 259 (73), 163 (69), 149 (100), 135 (52), 121 (47), 81 (71), 67 (56); Anal. calcd. for $C_{24}H_{36}N_2O_2$: C 74.96, H 9.44, N 7.28, found: C 74.66, H 9.82, N 7.00.

2.1.12. 3β-[[(t-Butyl)dimethylsilyl]oxy]androst-5-en-17-one (20)

Details of the synthesis of this compound were reported previously [43].

2.1.13. 3β-[[(t-Butyl)dimethylsilyl]oxy]androst-5-en-17β-ol (21)

The method followed that described for compound **14** but using compound **20** (839.1 mg; 2.1 mmol) in THF/MeOH 1:1 (16.8 ml) and $NaBH_4$ (208.2 mg; 5.50 mmol). Compound **21** (808.7 mg; 96%): mp (ethyl acetate/*n*-hexane) 165–167 °C; IR 3277 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 0.06 [s, 6H, $(CH_3)_2Si$], 0.76 (s, 3H, 18-H_3), 0.89 [s, 9H, $(CH_3)_3C$], 1.01 (s, 3H, 19-H_3), 3.48 (m, 1H, 3α-H), 3.65 (m, 1H, 17α-H), 5.32 (m, 1H, 6-H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ −4.6, 10.9, 18.2, 19.4, 20.6, 23.4, 25.9, 30.4, 31.5, 31.9, 32.0, 36.5, 36.6, 37.3, 42.7, 42.8, 50.2, 51.3, 72.5 (C3), 81.9 (C17), 120.8 (C6), 141.6 (C5). EI-MS *m*/*z* (%): 404 (2) M^+, 347 (100), 329 (38), 253 (41), 199 (21), 159 (34), 105 (30), 75 (40); Anal. calcd. for $C_{25}H_{44}O_2S_i$: C 74.19, H 10.96, found: C 74.30, H 10.70.

2.1.14. 3β-[[(t-Butyl)dimethylsilyl]oxy]androst-5-en-17β-yl-2′-methyl-1H-imidazole-1-carboxylate (22)

The method followed that described for compound **7** but using compound **21** (116 mg; 0.29 mmol) and CBMI (87.2 mg; 0.46 mmol) in a mixture of anhydrous acetonitrile (3.8 ml) and 1,2-dichloroethane (2 ml) at reflux. After 63 and 69 h, more CBMI, (21.8 mg; 0.11 mmol) and (54.5 mg; 0.29 mmol) respectively, was added and 3 h later the reaction was complete. Compound **22** (116.1 mg; 79%): mp (ethyl acetate/*n*-hexane) 146–147 °C; IR 1148, 1308, 1761 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 0.06 [s, 6H, $(CH_3)_2Si$], 0.89 [s, 9H, $(CH_3)_3C$], 0.91 (s, 3H, 18-H_3), 1.02 (s, 3H, 19-H_3), 2.78 (s, 3H, 2′-CH_3), 3.49 (m, 3α-H), 4.83 (m, 1H, 17α-H), 5.33 (m, 1H, 6-H), 6.99 (brs, 1H, 4′-H), 7.40 (brs, 1H, 5′-H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ −4.6, 12.3, 16.7, 18.2, 19.4, 20.4, 23.5, 25.9, 27.4, 31.3, 31.6, 31.9, 36.6, 36.7, 37.3, 42.7, 49.9, 50.7, 72.4 (C3), 86.9 (C17), 118.1 (C5′), 120.5 (C6), 127.3 (C4′), 141.6 (C5), 147.8 (C2′), 149.4 (OCO). EI-MS *m*/*z* (%): 513 (2) M^+, 455 (51), 329 (26), 253 (32), 157 (51), 145 (31), 105 (26), 91 (46); Anal. calcd. for $C_{30}H_{48}N_2O_3S_i$: C 70.27, H 9.43, N 5.46 found: C 70.00, H 9.42, N 5.60.

2.1.15. 3β-Hydroxyandrost-5-en-17β-yl-2′-methyl-1H-imidazole-1-carboxylate (23)

To a solution of compound **22** (1 g; 1.95 mmol) in ethanol (25 ml), water (45 μl) was added followed by TMSCl (60 μl; 0.5 mmol). The resulting pH was 5. Every 30 min, water (22.5 μl) and TMSCl (30 μl; 0.25 mmol) were added in a total of 7 additions until a pH of 1 was reached. After 3.5 h the reaction was quenched by neutralization with aqueous saturated $NaHCO_3$ solution. The final mixture was concentrated under reduced pressure and water (50 ml) was added. The resulting precipitate was resuspended in ethyl acetate (250 ml). The aqueous phase was further extracted with ethyl acetate (2× 50 ml). The final organic phase was washed with water (35 ml), dried over anhydrous $MgSO_4$, filtered, and the solvent was removed under reduced pressure to give a white solid which was purified by flash chromatography (ethyl acetate:petroleum ether 40–60 °C 2:1). Compound **23** was obtained (350.3 mg; 30%) and recrystallized from THF: mp 181–183 °C; IR 1146, 1300, 1757, 3262 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 0.92 (s, 3H, 18-H_3), 1.04 (s, 3H, 19-H_3), 2.67 (s, 3H, 2′-CH_3), 3.54 (m, 1H, 3α-H), 4.81 (m, 1H, 17α-H), 5.36 (m, 1H, 6-H), 6.88 (brs, 1H, 4′-H) 7.36 (brs, 1H, 5′-H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 12.3, 16.6, 19.3, 20.4, 23.4, 27.3, 31.3, 31.5, 31.6, 36.4, 36.7, 37.1, 42.1, 42.6, 49.8, 50.6, 71.5 (C3), 87.0 (C17), 118.1 (C5′), 121.0 (C6), 127.1 (C4′), 140.9 (C5), 147.6 (C2′), 149.3 (OCO). EI-MS *m*/*z* (%): 398 (4) M^+, 272 (76), 239 (100), 205 (86), 145 (70), 131 (58), 105 (86), 91 (72), Anal. calcd. for $C_{24}H_{34}N_2O_3$: C 72.33, H 8.60, N 7.03, found: C 72.60, H 8.93, N 6.80.

2.2. Biology

2.2.1. General

The human PC cell lines LNCaP and PC-3 were obtained from the American Type Culture Collection (Rockville, MD). 293T cells were the gift of Dr. Yun Qiu (UMB, Maryland), and LAPC4 cells were provided by Dr. Charles L. Sawyers (UCLA School of Medicine). RPMI 1640 medium, Dulbecco's Modified Eagle Medium (DMEM), Opti-MEM medium, Dulbecco's Phosphate Buffered Saline (DPBS), trypsin–versene, and penicillin/streptomycin (P/S) were obtained from Gibco-BRL. Fetal Bovine Serum (FBS) and Charcoal-stripped Serum (CSS) were obtained from Biofluids Inc. Poly-L-lysine, triamcinolone acetonide, ketoconazole, DHT, and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) powder were obtained from Sigma–Aldrich Co. Casodex was kindly provided by Astra-Zeneca Inc. VN/85-1 was prepared as previously reported [36]. Scintiverse BD Cocktail (Scint-analyzed) fluid was obtained from Fisher Scientific. The synthetic androgen methyltrienolone [^3H]R1881, with a specific activity of 72 Ci/mmol, was purchased from PerkinElmer. [21-$^3H_3$]-17α-hydroxypregnenolone, with a specific activity of 13.61 μCi/μmol, was prepared as described by Akhtar et al. [44]. The calcium phosphate transfection kit (Promega Profection Mammalian Transfection System), the Dual Luciferase kit and the Lipofectamine 2000 Reagent were purchased from Promega. The *pCDNA3Hmod17(His)*$_4$ construct was designed as previously reported [45]. The Probasin luciferase reporter construct ARR_2-Luc was generated by insertion of the minimal probasin promoter ARR_2 into the polyclonal linker region of pGL3-enhancer vector (Promega) [46]. The pRL-null (Promega) was used as the internal control. Radioactivity measurements were performed in a Tri-carb 2100 TR liquid scintillation analyzer. Absorbance and luminescence measurements were made using a Victor 1420 Multilabel counter.

293T and 293T-CYP17 cells were routinely maintained in DMEM supplemented with 10% FBS and 1% P/S solution. LNCaP and PC-3 cells were grown in RPMI 1640 medium supplemented with 10% FBS and 1% P/S solution. LAPC4 cells were grown in RPMI supplemented with 15% FBS, 1% P/S solution, and 10 nM DHT.

2.2.2. In vitro CYP17 assay

For this assay, 100 mm plates were coated with poly-L-lysine (0.01 mg/ml) for 30 min, rinsed twice with sterilized distilled water, and allowed to dry for 2 h. 293T Cells were then plated in DMEM at a density sufficient for achieving approximately 60% confluency on the following day, for transfection. Three hours prior to transfection the DMEM was renewed on the plates. The cells were transfected with the *pCDNA3Hmod17(His)*$_4$

construct using the calcium phosphate method, with the Promega Profection Mammalian Transfection System, according to the manufacturer's protocol. Briefly, the construct was combined with a $CaCl_2$ solution, added drop-wise with light vortex to a *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES) solution, and incubated at room temperature for 30 min. The final mixture was vortexed and dripped into the 100 mm plates. The medium was changed 18 h later and enzyme activity was assayed as described below 48 h after transfection.

293T-CYP17 cells were divided evenly in 6-well plates after reaching 80% confluency on the 100 mm plates. On the following day, cells were washed with Dulbecco's Phosphate Buffered Saline (DPBS) and incubated with phenol red-free DMEM (with 5% Charcoal-stripped Serum [CSS] and 1% penicillin/streptomycin [P/S]) containing a saturating concentration of [21-$^3H_3$]17α-hydroxypregnenolone (7 μM) and the test compounds in the desired concentrations. The plates were left to incubate for 18 h at 37 °C. The steroids were extracted with 2 ml of chloroform at 4 °C. After 2 h, the aqueous phase was collected and charcoal suspension was added to a 2.5% final concentration. Following a 30 min incubation at 4 °C, an aliquot of the aqueous supernatant was removed and radioactivity measured by liquid scintillation counting. The compounds showing inhibitory activity were further evaluated for IC_{50} values. IC_{50} values were obtained directly from plots relating percentage inhibition versus inhibitor concentration over appropriate ranges, using Graphpad Prism software (GraphPad Software Inc., San Diego, CA). Each compound was tested at a minimum of seven different concentrations.

2.2.3. *Competitive AR-binding assay*

LNCaP or LAPC4 cells were transferred to clear RPMI medium (with 5% CSS and 1% P/S) 3 days before the start of the experiment. 24-Well plates were coated with poly-L-lysine (0.01 mg/ml) for 30 min, rinsed with sterilized distilled water, and dried for 2 h. The cells were then plated ($2–3 \times 10^5$ cells/well) and allowed to attach. The following day the medium was replaced by phenol red-free RPMI (with 1% P/S added) containing a saturation concentration (5 nM) of [^{3}H]R1881, triamcinolone acetonide (1 μM), and the desired concentrations of the test compounds. Following a 2 h incubation period at 37 °C, cells were washed twice with ice-cold DPBS, and solubilized in DPBS containing 0.5% Sodium Dodecyl Sulphate (SDS) and 20% glycerol. Extracts were removed and cell associated radioactivity counted in a scintillation counter. All results represent an average of a minimum of three wells. To determine the EC_{50} values of the test compounds, a minimum of eight concentrations of each test compound was used. EC_{50} values were determined by non-linear regression with Graphpad Prism software.

2.2.4. *Luciferase transactivation assay*

LNCaP cells were transferred to phenol red-free RPMI medium (with 5% CSS and 1% P/S) 3 days before the start of the experiment. 24-Well plates were coated with poly-L-lysine (0.01 mg/ml) for 30 min, rinsed with sterilized distilled water, and dried for 2 h. The cells were plated at 1×10^5 cells/well in 24-well plates. On the following day the cells were dually transfected with ARR_2-Luc and the *Renilla* luciferase reporting vector pRL-null using the Lipofectamine Reagent (Lipofectamine 2000 Reagent, Promega), according to the manufacturer's protocol. Briefly, both plasmids were diluted in Opti-MEM medium and mixed gently. The Lipofectamine reagent was also diluted in Opti-MEM medium and allowed to stand at room temperature for 5 min before being combined with the plasmid mixture and allowed to stand for another 30 min at room temperature. The final mixture was then added to the cells. After a 24 h incubation period at 37 °C, the cells were incubated with fresh medium and treated with DHT (5 nM) and/or the selected compounds. After 18 h, the cells were washed twice with ice-cold DPBS and assayed using the Dual Luciferase kit (Promega) according to the manufacturer's protocol. Briefly, cells were lysed with 100 μl of luciferase lysing buffer, collected in a microcentrifuge tube, and pelleted by centrifugation. Supernatants (20 μl aliquots) were transferred to corresponding wells of opaque 96-well plates. Luciferin (78 μl) was added to each well and the light produced during the luciferase reaction was measured in a Victor 1420 Multilabel counter. After this measurement, a Stop and Glo reagent (78 μl) was added to quench the firefly luciferase signal and initiate the *Renilla* luciferase luminescence. *Renilla* luciferase luminescence was also measured in the Victor 1420 Multilabel counter. The results were presented as the fold induction, that is, the relative luciferase activity of the treated cells divided by that of the control, normalized to *Renilla*. All results represent an average of a minimum of three wells.

2.2.5. *Cell culture and viability assay*

To determine the effect of our novel compounds on PC-3 cell proliferation, 1.5×10^4 cells/well were plated in 24-well plates in RPMI medium (with 10% FBS and 1% P/S). After a 24 h attachment period, the cells were treated with the novel compounds (0.1–20 μM). The medium was changed every 3 days and the number of viable cells was compared by MTT assay on the seventh day. Briefly, MTT (0.5 mg/ml) in phenol red-free RPMI medium (with 5% CSS and 1% P/S) was added to each well and incubated at 37 °C for 4 h. Following incubation, the medium was aspirated completely with care taken not to disturb the formazan crystals. DMSO (400 μl) was used to solubilize these crystals. After slight shaking, the plates were immediately read at 570 nm on a Victor 1420 Multilabel counter. All results represent an average of a minimum of three wells. To determine the EC_{50} values of the test compounds, PC-3 cells were incubated with a minimum of eight concentrations of each test compound. EC_{50} values were determined by non-linear regression with Graphpad Prism software.

3. Results and discussion

3.1. *Chemistry*

The general procedure for the synthesis of the novel steroidal C17 carbamates **7–10** and **15** (Schemes 1 and 2) involved dissolution of the 17β-hydroxysteroids and CBMI, in acetonitrile at reflux, under N_2 (Scheme 1). For compounds **11**, **12** (Scheme 1), and **18** (Scheme 2) dichloromethane was used

whereas for compound **22** (Scheme 2) a mixture of acetonitrile and 1,2-dichloroethane was needed for total dissolution of the substrate. Yields obtained with this reaction ranged from 73 to 94%. In general, the reaction was clean affording only one product, with no additional purification steps needed other than aqueous washing.

For the synthesis of compound **15**, previous to the introduction of the carbamate moiety at C17, the commercially available DHEA acetate (**13**) was reduced with $NaBH_4$ in THF/MeOH to the corresponding 17β-hydroxy derivative **14**, in 82% yield (Scheme 2). The synthesis of compound **18** began with the Clemmensen-type reduction of 17β-hydroxy-5α-androstan-3-one (**16**) with zinc dust in glacial acetic acid, according to the literature [42]. Compound **17** was then treated with CBMI in dichloromethane, at reflux, to afford **18** in 73% yield (Scheme 2). For the synthesis of compound **23**, treatment of DHEA (**19**) with *t*-butyldimethylsilyl chloride (TBDMSCl) afforded the 3β-*O*-protected steroid **20** which was reduced with $NaBH_4$ in THF/MeOH to give the desired 17β-hydroxy substrate **21** (Scheme 2). Compound **22** was prepared according to the general procedure with CBMI. Alkyl *t*-butyldimethylsilyl ethers have been reported to be efficiently cleaved with *in situ* generated HCl, from trimethylsilyl chloride (TMSCl) and water, in acetonitrile, at room temperature [47]. Thus, we used a similar approach to obtain compound **23**. Small amounts of TMSCl and water were added to a solution of **22** in ethanol, which was used to replace acetonitrile due to poor solubility of the substrate in this solvent. The desired compound **23** was obtained in 30% yield after purification by flash chromatography.

The final reaction products were characterized by IR and 1D and 2D NMR spectroscopy, and assignments were performed based on our analysis and related literature [29,48,49]. The IR pattern of the C17 carbamate moiety can be easily seen for compound **18**. A strong band was visible on the IR spectrum at 1751 cm^{-1} for the C=O stretching vibration. A pair of bands was also seen at 1149 and 1273 cm^{-1} due to C—O stretching vibrations.

The assignment of some of the ^1H and ^{13}C resonances for compound **7** was made using a 2D Heteronuclear Multiple Bond Correlation (HMBC) experiment. The aromatic region consisted of two ^1H peaks: 6.83 (brs, 1H) and 7.32 (brs, 1H). The signal at 7.32 ppm correlated with the aromatic carbon signal at 127.5 ppm, which was assigned as C4′ according to related literature [29]. Thus, the signal at 7.23 ppm was assigned as 5′-H and the signal at 6.83 ppm as 4′-H. The two aromatic ^1H signals also correlated with the quaternary ^{13}C signal at 147.8 ppm. This signal correlated with the ring methyl protons (2′-CH_3) found at 2.61 ppm. We therefore assigned this value to the quaternary carbon C2′ of the methylimidazole ring. The quaternary ^{13}C signal at 149.3 ppm correlated exclusively to the 17α-H multiplet at 4.78 ppm and was assigned as the carbamate carbonyl carbon (OCO).

3.2. *Biology*

3.2.1. *CYP17 inhibition*

The CYP17 inhibition assay was performed as reported previously [36,37,39,45,50–57]. Thus, $C_{17,20}$-lyase activity was monitored by determination of the amount of radiolabelled acetic acid that originated from the breakdown of the C21 side chain of [21-^3H]-17α-hydroxypregnenolone. IC_{50} values were determined from dose–response curves and are shown in Table 1. Compounds **11** and **23** showed inhibition of the enzyme with IC_{50} values of 17.1 and 11.5 μM, respectively (Table 1, entries 5 and 9). All other compounds tested were less than 30% effective at 10 μM. Two CYP17 inhibitors, ketoconazole, currently used in clinical practice for PC treatment [58–61], and VN/85-1 [36] were chosen for comparison and displayed better inhibition of the enzyme with IC_{50} values in the nM range (Table 1, entries 10 and 11).

There are common features currently known to be important for optimum binding of an inhibitor to the enzyme's active site [62]. These include a sufficiently large hydrophobic skeleton with an overall size comparable to a steroid, substitution by electronegative groups only at the external positions of the hydrophobic skeleton, one heteroatom-containing group which can form a stable coordination with the heme iron of the enzyme at one of the external positions, and another group that can accept and/or donate hydrogen bonds at the opposite side [62]. Although our compounds meet these general requirements, they were unable to account for a better inhibition profile than the chosen standards. One of the reasons that may contribute to this finding is the fact that no double bond is present at C16 which has also been reported as an important feature for CYP17 inhibition [63]. In addition, the presence of a carboxylate spacer group between the methylimidazole ring and the C17 of the steroid nucleus may not be able to allow the molecules to accommodate the enzyme's active site properly.

3.2.2. *AR binding*

Competitive binding of the compounds to the AR was evaluated using a saturating concentration of the radiolabelled androgen [^3H]R1881 in the presence of different concentrations of test compound, essentially as previously described [57,64,65]. LAPC4 and LNCaP cells were chosen which express the wild-type (wt–) and mutated receptor, respectively.

Compounds **7** and **10** showed very high affinity towards the mutated receptor with EC_{50} values of 0.4 and 0.3 μM (Table 1, entries 1 and 4). Compound **10** also bound very tightly to the wt-receptor with an EC_{50} of 0.2 μM, being about 24 times more effective than Casodex (Table 1, entries 4 and 12). In fact, Casodex, an antiandrogen currently used in PC therapy, showed lower affinity towards both types of AR than all the tested compounds that were selected for EC_{50} determination, with the exception of compound **9** which showed similar affinity towards the mutated AR (Table 1, entries 3 and 12). Thus, the synthesized compounds proved to be suitable for binding to the AR. The best interaction was achieved with compound **10** (Table 1, entry 4). The fact that compound **18** shows no binding ability suggests that the presence of a H-bond acceptor/donor at the C3 position is fundamental for binding (Table 1, entry 8). Moreover, the presence of a 3β-acetoxy group seems to be specific of wt-AR binding. Compound **15** only binds to the wt-AR whereas compound **23**, bearing a 3β-hydroxy group binds to both receptors (Table 1, entries 7 and 9). Thus, a more detailed study using this set of compounds could provide an interesting contribution to the affinity labelling of the AR.

Table 1 – Effect of the synthesized compounds on CYP17 action, AR binding and PC-3 cell proliferation

Entry	Compound	CYP17 inhibition, 293T IC_{50}[a] (μM)	AR binding		Cell proliferation, PC-3 EC_{50}[c] (μM)
			LNCaP EC_{50}[b] (μM)	LAPC4 EC_{50}[b] (μM)	
1	7	NA[d]	0.4	0.8	NA
2	8	NA	NA	1.6	2.2
3	9	NA	1.1	0.8	NA
4	10	NA	0.3	0.2	NA
5	11	17.1	0.6	0.7	NA
6	12	NA	0.6	1.4	0.2
7	15	NA	NA	0.3	NA
8	18	NA	NA	NA	NA
9	23	11.5	0.7	2.3	NA
10	Ketoconazole	47 nM	NT	NT	NT
11	VN/85-1	1.29 nM	NT	NT	NT
12	Casodex	NT[e]	1.0	4.5	NT

[a] IC_{50} is the concentration of compound required to inhibit the enzyme activity by 50%.
[b] EC_{50} is the concentration of compound that inhibits 50% of the binding.
[c] EC_{50} is the concentration of compound that inhibits 50% of cell growth.
[d] NA = not active.
[e] NT = not tested.

3.2.3. *Mutated AR transcription effects*

To determine whether compounds **7**, **9–12**, and **23**, which bound to the mutated AR (Table 1, entries 1, 3–6 and 9), could block transcription, a study was performed on LNCaP cells transiently transfected with the probasin luciferase reporter construct ARR_2-Luc (luciferase activity assay) [46,66]. The effect of the tested compounds is depicted in Fig. 1 for compound **12** chosen as an example. All the other tested compounds displayed similar behaviour.

Compound **12** alone at the lower concentration of 0.1 μM was able to stimulate transcription on the LNCaP-ARR_2-Luc cells. However, at 10 μM, no transcriptional activity was observed with compound **12** (close to control level). This compound seems to have a dual effect showing some androgenic activity at lower concentrations that is not observed at higher concentrations. Luciferase expression was increased about 113-fold when compared to control, following treatment with DHT (5 nM), after 18 h. Compound **12** was only able to effectively block DHT-mediated transcriptional activity at 5 μM providing full blockage at the higher concentration of 10 μM.

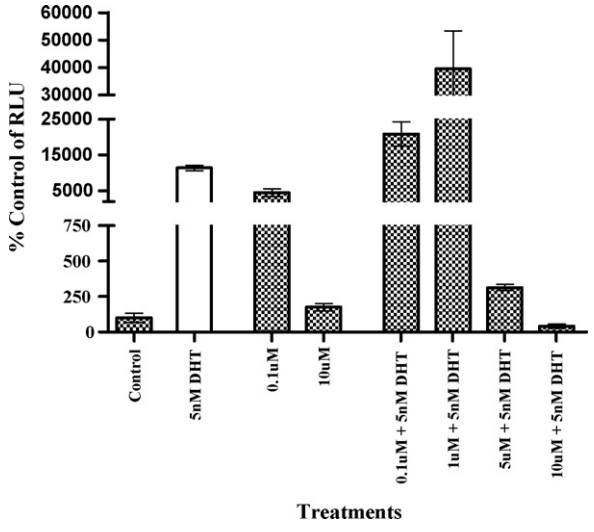


Fig. 1 – Effect of compound 12 on mutant-AR mediated transcription (LNCaP-ARR2-Luc cells). One-way ANOVA with a Dunnett post-test was used to analyze the data: *$p < 0.01$ vs. control.

The reasons for this dual behaviour are currently unknown. An agonistic effect on the LNCaP AR has been reported before for other compounds designed as CYP17 inhibitors as well as for 4-hydroxyflutamide [35,38]. Ketoconazole has also been shown to significantly stimulate AR-mediated transcription on LNCaP cells, at 5 μM [38]. Antagonist and agonist activities have been suggested to be determined by receptor binding affinity, ligand concentration, and the presence or absence of competing high affinity natural ligands [65]. In order to explain the concentration-dependent transcription blockage of AR mediated transcription provided by the two metabolites of the fungicide vinclozolin, the authors raised the possibility that mixed ligand dimmers, *i.e.* agonist (natural androgen) and antagonist bound in the same dimer, were required for antagonism, whereas same ligand dimers of sufficiently high affinity would promote receptor activation [65]. Thus, the dual effect demonstrated by our synthesized compounds on LNCaP-AR mediated transcription might be due to the presence of mixed ligands of compound and a metabolite. However, further studies which are beyond the scope of the present work, would be required to confirm this hypothesis. In addition, toxicity of the higher concentrations of compound for the biological system could also account for the observed effect.

3.2.4. *PC-3 cell proliferation*

The potential of the synthesized compounds to inhibit cell proliferation was studied on PC-3 cells. All compounds were screened at 0.1, 1, 10, and 20 μM and the best ones were selected for EC_{50} determination (Fig. 2, Table 1).

The compounds were inhibitory towards PC-3 cells mostly at 10 and 20 μM (Fig. 2). Compound **23** was not active. Com-

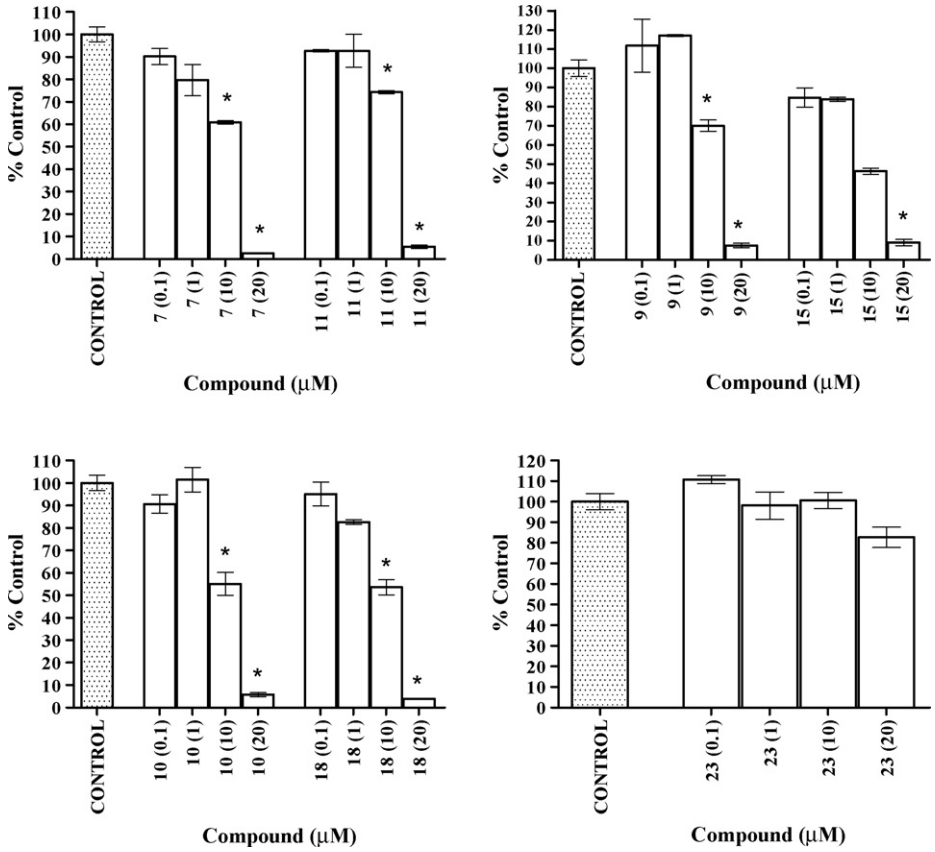


Fig. 2 – Effect of compounds 7, 9, 10, 11, 15, 18 and 23 on PC-3 cell proliferation. One-way ANOVA with a Dunnett post-test was used to analyze the data: *$p < 0.01$ vs. control.

pounds **8** and **12** showed EC_{50} values of 2.2 and 0.2 μM, respectively (Table 1, entries 2 and 6). PC-3 cells are human PC cells derived from bone metastases and do not express the AR. Thus, inhibition of proliferation of these cells suggests that cell death occurs through non AR-mediated processes such as apoptosis or cell cycle arrest.

Assays performed using 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-2*H*-tetrazolium-5-carboxyanilide inner salt (XTT) on LNCaP and LAPC4 cells with the compounds, showed cell proliferation inhibition only at high concentrations of 10 and 20 μM (data not shown).

4. Conclusions

A series of novel C17 steroidal carbamates **7**–**12**, **15**, **18** and **23** have been synthesized and their structural elucidation provided by use of IR, 1D and 2D NMR techniques, and related literature. Studies were performed on their CYP17 inhibition ability, AR binding affinity and mediated transcription effects. The ability to inhibit PC-3 cell proliferation was also studied.

Compounds **11** and **23** were found to inhibit CYP17 with IC_{50} values of 17.1 and 11.5 μM, respectively, however less potently than both ketoconazole and VN/85-1. The lack of a C16 double bond and the presence of a carboxylate spacer group at C17 could account for their low inhibitory effects. The carbamate moiety at C17 allowed tight binding of the synthesized compounds to both wt- and mutated-AR. Binding of the synthesized C17 steroidal carbamates to both the wt- and mutated-AR was more effective than binding with Casodex. When bound to the mutated AR, the compounds were found to have a dual effect, stimulating transcription at low concentrations while almost fully blocking it at the higher concentrations tested, in the presence of the natural androgen DHT.

The synthesized compounds were found to inhibit the proliferation of PC-3 cells, which lack the AR and thus represent a study model for advanced metastatic PC. Compounds **8** and **12** were the most active with EC_{50} values of 2.2 and 0.2 μM, respectively. Their action on PC-3 cells suggests that other mechanisms non-AR related may account for the observed effect, such as apoptosis or cell cycle arrest.

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