available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/steroids

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

Vânia M.A. Moreira[a], *Tadas S. Vasaitis*[b], *Zhiyong Guo*[b], *Vincent C.O. Njar*[b,**], *Jorge A.R. Salvador*[a,*]

[a] *Laboratório de Química Farmacêutica, Faculdade de Farmácia, Universidade de Coimbra, Rua do Norte, 3000-295 Coimbra, Portugal*
[b] *Department of Pharmacology & Experimental Therapeutics, University of Maryland School of Medicine, Baltimore, 655W Baltimore Street, Baltimore, MD 21201-1559, USA*

ARTICLE INFO

Article history:
Received 30 January 2008
Received in revised form
13 May 2008
Accepted 27 May 2008
Published on line 6 June 2008

Keywords:
1,1′-Carbonylbis(2-methylimidazole)
CYP17
Prostate cancer
Androgen receptor
PC cell lines
Steroids

ABSTRACT

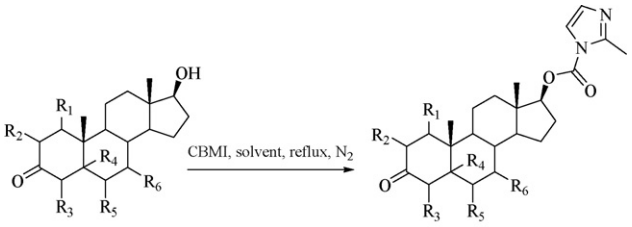
We have exploited the reaction of 1,1′-carbonylbis(2-methylimidazole) (CBMI) with several 17β-hydroxy androstanes to synthesize a series of novel C17 steroidal carbamates. Structural elucidation features have been provided for the final compounds based on 1D and 2D NMR techniques, IR spectroscopy, and 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-

* *Corresponding author.* Tel.: +351 239 859950; fax: +351 239 827126.
** *Corresponding author.* Tel.: +1 410 706 5885; fax: +1 410 706 0032.
E-mail addresses: vnjar001@umaryland.edu (V.C.O. Njar), salvador@ci.uc.pt (J.A.R. Salvador).
0039-128X/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.steroids.2008.05.010



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),