Available online at www.sciencedirect.com
SCIENCE DIRECT
Steroids 70 (2005) 907–912
Steroids
www.elsevier.com/locate/steroids

Synthesis and antifungal activity of oxygenated cholesterol derivatives

Jean Michel Brunel [a,*], Céline Loncle [a], Nicolas Vidal [a], Michel Dherbomez [b], Yves Letourneux [a,*]

[a] *Laboratoire Synthèse et Etude de Substances Naturelles à Activités Biologiques (SESNAB), IMRN INRA 1111, Faculté des Sciences et Techniques de St Jérôme, Université Paul Cézanne, Aix-Marseille III, Avenue Escadrille Normandie Niémen, 13397 Marseille Cedex 20, France*
[b] *IUT La Rochelle, 15 rue Francois de Vaux de Foletier, F-17026 La Rochelle, France*

Received 7 March 2005; received in revised form 27 June 2005; accepted 30 June 2005
Available online 1 September 2005

Abstract

A series of oxygenated cholesterol derivatives were prepared from new synthetic methods and evaluated for their in vitro antimicrobial properties against human pathogens. The activity was highly dependent on the structure of the different compounds involved. The best results were obtained with hydroxy ketones **2**, **4** and **5** and diketone **7** exhibiting activities against *S. cerevisiae* (ATCC 28383) and *Candida albicans* (CIP 1663-86). For example, compound **2** exhibited high activities against *C. albicans* (CIP 1663-86) and Amphotericine B and miconazole resistant strain *C. albicans* (CIP 1180-79) at a concentration of 1.5 μg/mL.

Keywords: Oxygenated sterols; Antifungal activity; Antimicrobial activity

1. Introduction

Oxygenated sterols, including both autoxidation products and sterol metabolites, have many important biological activities mostly related to the physiological control of cholesterol biosynthesis [1,2]. Several investigators have demonstrated that oxygenated cholesterol such as 7-ketocholesterol and 25-hydroxycholesterol inhibit the activity of β-hydroxy-β-methylglutaryl CoA (HMG CoA) reductase, the rate-limiting enzyme in the biosynthesis of cholesterol, in various in vitro test system [1,3–6]. In 1995, two 4,4-dimethyl-$\Delta^{8,24}$-sterols called FF-MAS (Follicular Fluid-Meiosis Activating Sterol) were found to have a regulatory function in meiosis [7] and since then numerous studies dealing with the design, synthesis and derivation of structure-activity relation ships of FF-MAS related sterol compounds have been reported [8–10]. Nevertheless, few studies have been devoted to the possible antifungal and antimicrobial activities against Gram-positive, Gram-negative bacteria and yeast of such oxygenated sterols. Ourisson et al. were the first to report preliminar results on the cytotoxity of such compounds towards tumor cells [11–13]. In this area, recent results have been reported on the synthesis and antiproliferative, anti-HIV and anti-Asthma properties of various oxygenated sterol derivatives [14–16]. In continuation of our work on biologically active sterol derivatives [17–22], we report herein the synthesis of various new oxygenated cholesterol derivatives and their promising antifungal properties since no biological activities of such derivatives were described to date, even if numerous similar natural compounds possessing interesting biological activities have been isolated.

2. Experimental section

All solvents were purified according to reported procedures, and reagents were used as commercially available. Tetrahydrofuran (THF) was distilled from sodium-benzophenone ketyl immediately prior to use. Ethylacetate and petroleum ether (35–60 °C) were purchased from SDS and used without any further purification. Column

* Corresponding authors. Tel.: +33 491288550; fax: +33 491288440.
E-mail address: bruneljm@yahoo.fr (J.M. Brunel).

0039-128X/$ – see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.steroids.2005.06.007

chromatography was performed on SDS silica gel (70–230 mesh). ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ on a Bruker AC 300 spectrometer working at 300.00 and 75 MHz, respectively (the usual abbreviations are used: s: singulet, d: doublet, t: triplet, q: quadruplet, m: multiplet). Tetramethylsilane was used as internal standard. All chemical shifts are given in ppm.

2.1. Synthesis of 7-keto cholesterol 2

Compound **2** was prepared using a modified procedure previously described by Kinney et al. [23,24]. Cholesterol (50 g, 0.129 mol) and *N*-hydroxyphthalamide (22 g, 0.135 mol) were dissolved in EtOAc-acetone (1.5 L, 1:1 v/v) in a 2 L glass reactor equipped with a condenser and a mechanical stirrer. Benzoyl peroxide (3 g) was added at 50–60 °C. Air was bubbled into the reaction solution and stirring was maintained for 72 h at 50–60 °C. Additional 50/50 EtOAc-acetone was added to the reaction as needed to replenish what was lost due to air flow through the system. The reaction was followed by TLC on silicagel (50% EtOAc in petroleum ether) and judged completed after 72 h. After evaporation of all the solvents in vacuo, petroleum ether was added and the organic phase was washed with sodium carbonate solution until no orange coloration was observed. The organic layers were washed with brine and dried over $MgSO_4$. The solvent was removed and the sterol dissolved in pyridine (200 mL). The pyridine solution was cooled to 0 °C and $CuCl_2$ (1 g) was added. The solution was stirred overnight allowing the solution to warm to room temperature. After addition of water (200 mL), the solution was extracted with EtOAc (3×150 mL). The organic layer was washed with saturated $CuSO_4$ solution until no trace of pyridine was observed. The organic layer was washed with a 0.1 M HCl solution, dried over $MgSO_4$ and concentrated in vacuo. The oily residue was purified by chromatography on a silicagel column using EtOAc/petroleum ether as eluent (50/50) affording the expected hydroxy ketone **2** in 62% yield.

White solid; mp: 116 °C; ^{1}H NMR: $\delta = 5.45$–5.75 (m, 1H), 4.34 (s, 1H), 3.47–3.75 (m, 1H), 0.45–2.12 (m, 41H); ^{13}C: $\delta = 202.81, 165.59, 126.49, 70.89, 55.17, 50.34, 45.80, 43.49, 39.87, 38.67, 36.57, 36.11, 28.40, 26.72, 24.22, 23.22, 22.96, 21.61, 19.26, 17.71, 12.37$. $C_{27}H_{44}O_2$ calcd C 80.9, H 11.1; found C 81.0, H 10.8.

2.2. Synthesis of 7β-hydroxy cholestanol 3

In a 250 mL two necked round flask were placed under argon anhydrous THF (40 mL) at −78 °C and 40 mL of ammonia. Lithium wire (0.7 g, 0.14 mol) was added to the solution with vigorous stirring. Once the lithium was completely dissolved, 7-ketocholesterol **2** (3.5 g, 8.75×10^{-3} mol) was dissolved in 50 mL of anhydrous THF and added to the flask in a steady stream from a 100 mL addition funnel. The reaction was stirred for 3 h at −78 °C before being quenched by the addition of MeOH until no blue coloration was observed. The ammonia was allowed to evaporate overnight at room temperature. The residue was dissolved in 100 mL of a toluene/EtOAc solution. The organic layer was successively washed with a 0.1 N HCl solution, distilled water and brine. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The oily residue was purified by chromatography on a silicagel column using EtOAc/petroleum ether as eluent (1/4–1/1) affording the expected diol 3 in 74% yield.

White solid; mp: 175 °C; ^{1}H NMR: $\delta = 3.95$–4.30 (m, 1H), 0.40–3.45 (m, 47H); ^{13}C: $\delta = 75.21, 71.13, 55.80, 55.31, 52.55, 43.66, 43.48, 42.11, 40.05, 39.56, 38.13, 36.97, 36.26, 35.74, 35.00, 31.65, 31.50, 28.78, 28.07, 26.97, 23.91, 22.88, 22.62, 21.51, 18.86, 14.19, 12.51, 12.23$. $C_{27}H_{48}O_2$ calcd C 80.2, H 11.9; found C 80.4, H 10.9.

2.3. Synthesis of 7β-hydroxy cholestanone 4

A suspension of 7β-hydroxy cholestanol **3** (1 g, 1.9×10^{-3} mol) and silver carbonate on Celite (1 g) in toluene (70 mL) was stirred under argon at reflux overnight. The reaction mixture was filtered through a column of Florisil and the filtrate was concentrated in vacuo. The residue was purified by chromatography on a silicagel column using petroleum ether/ethylacetate (100/0 (100 mL), 50/50 (100 mL), 0/100 (300 mL)) as eluent affording the expected product **4** in 82% yield.

White solid; mp: 180 °C; ^{1}H NMR: $\delta = 3.05$–3.55 (m, 1H), 2.2–2.6 (m, 3H), 0.4–1.92 (m, 42H); ^{13}C: $\delta = 211.55, 74.66, 55.61, 55.24, 51.83, 44.18, 43.94, 43.66, 39.90, 39.54, 38.12, 35.72, 35.16, 28.75, 28.06, 23.89, 22.87, 22.62, 21.80, 18.84, 12.22, 11.63$. $C_{27}H_{46}O_2$ calcd C 80.5, H 11.5; found C 81.6, H 11.3.

2.4. Synthesis of 7α-hydroxycholestanone 5 and 3,7-cholestanedione 6

In a 250 mL two necked round flask were placed under argon at −78 °C 7-ketocholesterol **2** (300 mg, 7.46×10^{-4} mol) dissolved in anhydrous THF (10 mL). L-Selectride (2 equivalents) were slowly added at −78 °C and stirred for 5 h before being quenched by the addition of H_2O_2 and a solution of $NaHCO_3$ (10 mL). The residue was dissolved in 100 mL of ethylacetate, washed with brine and dried over $MgSO_4$. After filtration and evaporation of the solvents, the crude residue of 7α-hydroxy cholestanol (196 mg, 4.85×10^{-4} mol) and silver carbonate on Celite (631 mg) in toluene (70 mL) was stirred under argon at reflux overnight. The reaction mixture was filtered through a column of Florisil and the filtrate was concentrated in vacuo. The residue was purified by chromatography on a silicagel column using petroleum ether/ethylacetate (70/30 → (80/20)) as eluent affording the expected products **5** and **6** in, respectively, 13 and 17% yield.

Compound **5**: white solid; mp: 115 °C; ^{1}H NMR: $\delta = 3.3$–3.85 (m, 1H), 0.60–2.65 (m, 45H); ^{13}C: $\delta = 211.36$,