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Total synthesis of biologically active 20*S*-hydroxyvitamin D3

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ABSTRACT

A total synthetic strategy of 20*S*-hydroxyvitamin D3 [20*S*-(OH)D3] involving modified synthesis of key intermediates **7** and **12**, Grignard reaction to stereoselectively generate 20*S*-OH and Wittig–Horner coupling to establish D3 framework, was completed in 16 steps with an overall yield of 0.4%. The synthetic 20*S*-(OH)D3 activated vitamin D receptor (VDR) and initiated the expression of downstream genes. In addition, 20*S*-(OH)D3 showed similar inhibitory potency as calcitriol [1,25$(OH)_2$D3] on proliferation of melanoma cells.

1. Introduction

25-Hydroxylase (CYP2R1 or CYP27A1) in the liver and 1α-hydroxylase (CYP27B1) in the kidney are the enzymes responsible for sequential metabolism of vitamin D3 to produce bioactive 1α,25-dihydroxyvitamin D3 [1,25$(OH)_2$D3, calcitriol] [1,2]. 1,25$(OH)_2$D3 activates nuclear vitamin D receptor (VDR), which is found in almost all tissues of the body, to trigger numerous cellular effects including but not limited to stimulation of cell differentiation and/or apoptosis, inhibition of cell proliferation, regulation of secretory and immune factors of many cells, and cell protective functions, in a context dependent fashion [3–7]. Therefore, active forms of vitamin D can be used in therapy of cancer, hyperproliferative, autoimmune and metabolic disorders [8–10]. However, these applications are limited by hypercalcemic (toxic) effect of calcitriol at pharmacological concentrations [11]. This side effect has led to the development of more than 3000 synthetic vitamin D3 analogs showing low calcemic activity [12]. Some of these analogs such as paricalcitol, oxacalcitriol, falecalcitriol, tacalcitol and doxercalciferol were used to treat secondary hyperparathyroidism and psoriasis [8,13]. Besides psoriasis, calcipotriol is also a promising agent for the treatment of pancreatic cancer and is currently undergoing human clinical trial [14] (Fig. 1).

Mammalian cytochrome P450 side-chain cleavage enzyme (P450scc or CYP11A1) not only cleaves the side chain of cholesterol to produce pregnenolone (precursor of all steroids) [15,16] but also hydroxylates vitamin D3 in a sequential fashion [16–20] starting from C20 to form 20*S*-hydroxyvitamin D3 [20*S*-(OH)D3] (**1**, Fig. 1), which is subsequently converted to di- and trihydroxy metabolites [17,19–22]. Functional studies showed that 20*S*-(OH)D3 not only stimulated keratinocyte differentiation program but also inhibited NF-κB activity in human keratinocytes [23]. In addition, it has shown anti-inflammatory activities, strong anti-proliferative effects, anti-leukemia and tumorostatic effects [23–27], protective effects against ultra-violet B (UVB) induced damage [28], as well as antifibrotic activity *in vivo* [29]. These activities are mediated either through activation of the VDR [23,30] or inhibition of RORα and RORγ transcriptional activities [31]. More importantly, while having comparable anti-proliferative potency with 1,25$(OH)_2$D3 which has strong hypercalcemic toxicity at a concentration of 1 μg/kg, 20*S*-(OH)D3 is not hypercalcemic at concentrations as high as 60 μg/kg [24,26,28,32].

Abbreviations: D3, vitamin D3; 20*S*-(OH)D3, 20*S*-hydroxyvitamin D3; 1,25$(OH)_2$D3, 1α,25-dihydroxyvitamin D3; EOM, ethoxymethyl; TBS, *t*-butyldimethylsilyl.

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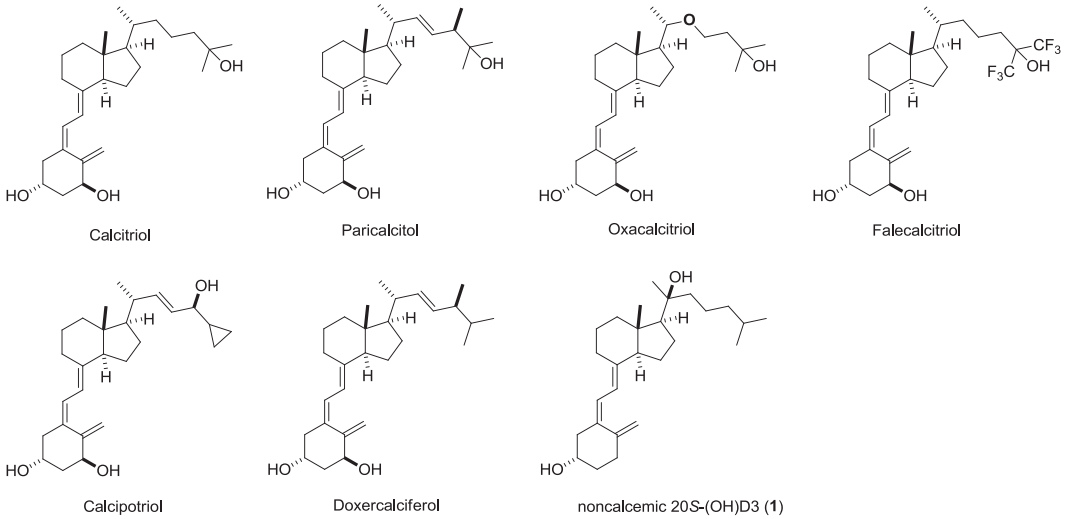

Fig. 1. Marketed vitamin D analogs and noncalcemic 20*S*-(OH)D3.

Unfortunately, in-depth evaluation of the biological activity for 20*S*-(OH)D3 or its analogs was hampered by lack of an efficient synthetic method of its production [33] without using costly enzymatic approaches [20,21,34]. 20*S*-(OH)D3 was firstly chemically synthesized from pregnenolone acetate via Grignard reaction and low yielding UVB irradiation to afford low milligram scale of 20*S*-(OH)D3 stereo-specifically. However, this method also generated structurally similar and physicochemically active by-products including previtamin D3, lumisterol and tachysterol which presented a significant challenge in the purification of 20*S*-(OH)D3 even through preparative HPLC [33]. Due to these disadvantages, scope of synthesis through UVB irradiation of 5,7-dienal precursor, 20*S*-(OH)-7-dehydrocholesterol was confined to very few derivatives, if not to almost exclusive production of 20*S*-(OH)D3. Therefore, an approach that could easily generate large quantity of 20*S*-(OH)D3 with high quality (>98% purity) would facilitate further modifications on the side chain to determine the structure–activity relationships of this promising 20*S*-hydroxyl scaffold. Herein, we report an efficient total synthetic route for stereospecific 20*S*-(OH)D3.

2. Experimental

2.1. General methods

Tetrahydrofuran was distilled from sodium-benzophenone. All other solvents and chemical reagents were obtained from commercial sources and directly used without further purification. Ergocalciferol was purchased from Chem Impex International Inc. Glassware was oven-dried before use. All reactions were performed under an argon atmosphere. TLC was performed on silica gel 60 GF254 and monitored under UV light or visualized using phosphomolybdic acid reagent. Flash chromatography was performed on 230–400 mesh silica gel (Fisher Scientific). Preparative TLC was performed on Analtech TLC Uniplates (250 μm). Melting points were recorded on a MPA100 Automated Melting Point Apparatus. NMR spectra were obtained on a Bruker Ascend 400 (Billerica, MA) spectrometer or a Varian Inova-500 spectrometer (Agilent Technologies, Santa Clara, CA). Chemical shifts are given in ppm with tetramethylsilane (TMS) as an internal reference. All coupling constants (*J*) are given in Hertz (Hz).

2.2. Chemistry

2.2.1. (4R,7aR)-1-((2R,5R,E)-5,6-Dimethylhept-3-en-2-yl)-4-((R,Z)-1-hydroxy-2-((S)-5-hydroxy-2-methylenecyclohexylidene)ethyl)-7a-methyloctahydro-1H-inden-4-ol (**2**)

Following a reported procedure [35], to a solution of ergocalciferol (20 g, 50.5 mmol) in ethanol (2 L) at −45 °C was added dropwise solution of potassium permanganate (9.0 g, 58.7 mmol) in water (300 mL), the mixture was stirred for half an hour and further 1 h at −15 °C and then warmed to room temperature. After 3 h, the precipitate was filtered off and the solution was evaporated to give yellowish crude oil. The crude oil was purified on silica gel column chromatography eluting with hexane/ethyl acetate (3:1) to give pure triol compound **2** as white solid (10.5 g, 65%) and unreacted starting material (5.1 g). Mp: 170–172 °C (MeOH). ^{1}H NMR (400 MHz, CD_3OD): δ 5.52 (dd, J = 9.9, 1.7 Hz, 1H, 6-H), 5.30–5.09 (m, 2H, 22/23-H), 5.02–4.98 (m, 2H, 7/19-H), 4.90 (d, J = 9.9 Hz, 1H, 19-H), 3.65 (m, 1H, 3-H), 2.55 (ddd, J = 12.4, 4.5, 1.9 Hz, 1H), 2.49–2.38 (m, 1H), 2.17–1.90 (m, 5H), 1.90–1.68 (m, 4H), 1.62 (m, 1H), 1.56–1.39 (m, 4H), 1.39–1.03 (m, 5H), 1.00 (d, J = 6.6 Hz, 3H, 21-H), 0.94 (d, J = 6.8 Hz, 3H, 28-H), 0.89–0.81 (t, J = 6.4 Hz, 9H, 18/26/27-H). ^{13}C NMR (101 MHz, CD_3OD): δ 147.35, 140.78, 137.06, 133.12, 126.72, 111.72, 76.25 (C7), 71.42 (C8 or C3), 71.39 (C3 or C8), 61.04, 58.79, 47.60, 45.14, 44.36, 41.70, 41.53, 39.23, 37.21, 34.50, 34.38, 29.13, 22.87, 21.76, 21.29, 20.52, 20.12, 18.27, 13.59. ESI-HRMS: calculated for $C_{28}H_{46}O_3Na$ $[M+Na]^+$ 453.3345, found 453.3352.

2.2.2. (S,Z)-3-(2-Hydroxyethylidene)-4-methylenecyclohexanol (**3**) and (4S,7aR)-1-((2R,5R,E)-5,6-dimethylhept-3-en-2-yl)-7a-methyloctahydro-1H-inden-4-ol (**4**)

To a suspension of **2** (6.0 g, 13.9 mmol) and sodium carbonate (14.6 g, 138 mmol) in dichloromethane (50 mL) was added lead tetraacetate (6.7 g, 15.3 mmol) in portions at 0 °C. After 2 h, the reaction was quenched with ethylene glycol and the mixture was vigorously stirred at 0 °C for 10 min. Water was then added and the mixture was extracted with dichloromethane for five times, washed with brine and dried with Na_2SO_4. The combined extracts were evaporated to give crude oil mixture, which was used for next step without further purification. To a solution of the above crude oily mixture in benzene (100 mL) at 0 °C under argon with stirring, Red-Al (28.7 mmol, 8.6 mL) was added dropwise and the mixture