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Synthesis and biological activities of vitamin D-like inhibitors of CYP24 hydroxylase

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ABSTRACT

Selective inhibitors of CYP24A1 represent an important synthetic target in a search for novel vitamin D compounds of therapeutic value. In the present work, we show the synthesis and biological properties of two novel side chain modified 2-methylene-19-nor-1,25$(OH)_2D_3$ analogs, the 22-imidazole-1-yl derivative **2** (VIMI) and the 25-*N*-cyclopropylamine compound **3** (CPA1), which were efficiently prepared in convergent syntheses utilizing the Lythgoe type Horner–Wittig olefination reaction. When tested in a cell-free assay, both compounds were found to be potent competitive inhibitors of CYP24A1, with the cyclopropylamine analog **3** exhibiting an 80–1 selective inhibition of CYP24A1 over CYP27B1. Addition of **3** to a mouse osteoblast culture sustained the level of 1,25$(OH)_2D_3$, further demonstrating its effectiveness in CYP24A1 inhibition. Importantly, the *in vitro* effects on human promyeloid leukemia (HL-60) cell differentiation by **3** were nearly identical to those of 1,25$(OH)_2D_3$ and *in vivo* the compound showed low calcemic activity. Finally, the results of preliminary theoretical studies provide useful insights to rationalize the ability of analog **3** to selectively inhibit the cytochrome P450 isoform CYP24A1.

1. Introduction

The hormonal form of vitamin D_3, 1α,25-dihydroxyvitamin D_3 (1,25$(OH)_2D_3$, **1**) (Fig. 1) has long been known for its central role in controlling calcium and phosphate homeostasis. In addition, a growing body of investigations is continuously unveiling the significance of vitamin D in a diverse array of physiological functions, including cell proliferation, differentiation and apoptosis, and immune response. Consequently, vitamin D deficiency has been associated with a number of diseases such as cancers, autoimmune dysfunctions, cardiovascular diseases, and infections [1–3]. As the therapeutic activity of exogenously administered 1,25$(OH)_2D_3$ is limited by its capacity to induce hypercalcemia, numerous efforts have been directed to develop therapeutically useful 1,25$(OH)_2D_3$ analogs. Although many of them have resulted in useful therapies, in particular for the treatment of kidney, bone and skin diseases [4,5], their use in other areas remains nonexistent primarily due to the difficulty of achieving an effective concentration without also raising serum calcium.

Vitamin D_3, the natural form of vitamin D produced in the skin through UV exposure, is biologically inert. Activation of vitamin D_3 is initiated in the liver to produce 25-hydroxyvitamin D_3 (25$(OH)D_3$), which is further converted to 1α,25-dihydroxyvitamin D_3 (1,25$(OH)_2D_3$), the hormonally active secosteroid, by 25$(OH)D_3$ 1α-hydroxylase (CYP27B1) mainly in the kidney. Metabolic inactivation of 1,25$(OH)_2D_3$ in its target cells is initiated by side chain hydroxylations at the C-23, C-24, and C-26 positions. Of these hydroxylation sites, it is now accepted that the sequential oxidation and cleavage of the side chain by mitochondrial 1,25$(OH)_2D_3$ 24-hydroxylase (CYP24A1) (Fig. 2) is the major pathway by which the hormone is inactivated [6]. CYP24A1, as the main catabolizing enzyme of 1,25$(OH)_2D_3$, can also break down vitamin D analogs in a similar catabolic process. Elevated CYP24A1 levels have been seen in many types of human cancers [7], immune dysfunctions, and secondary hyperparathyroidism. In addition, intervention with vitamin D analogs often stimulates the expression of CYP24A1, resulting in rapid elimination of the active metabolites, that drastically reduces the effectiveness of the treatment. Targeting CYP24 provides the opportunity to increase endogenous levels of 1,25$(OH)_2D_3$, or reduce the effective dose of exogenous 1,25$(OH)_2D_3$. Conventional drug design approaches have led to the development of a range of P450 inhibitors that are currently used in the clinic, primarily as antifungal agents. Effective inhibitors can be found in a class of bifunctional lipophilic compounds (entitled azoles) that directly link to the heme iron by the azole nitrogen and, additionally, interact with

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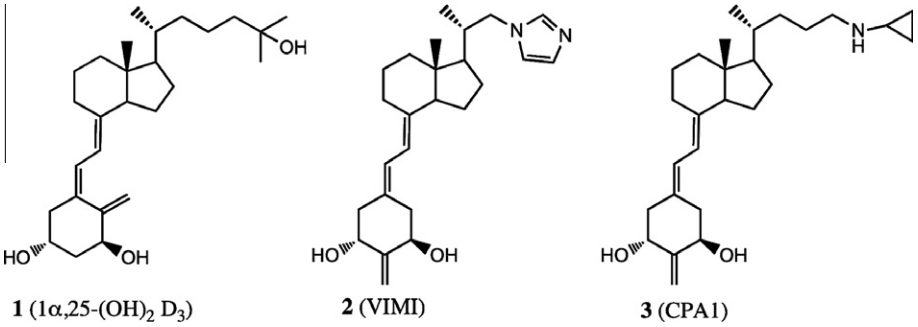

Fig. 1. Chemical structures of 1α,25-dihydroxyvitamin-D_3 and new designed CYP24 inhibitors.

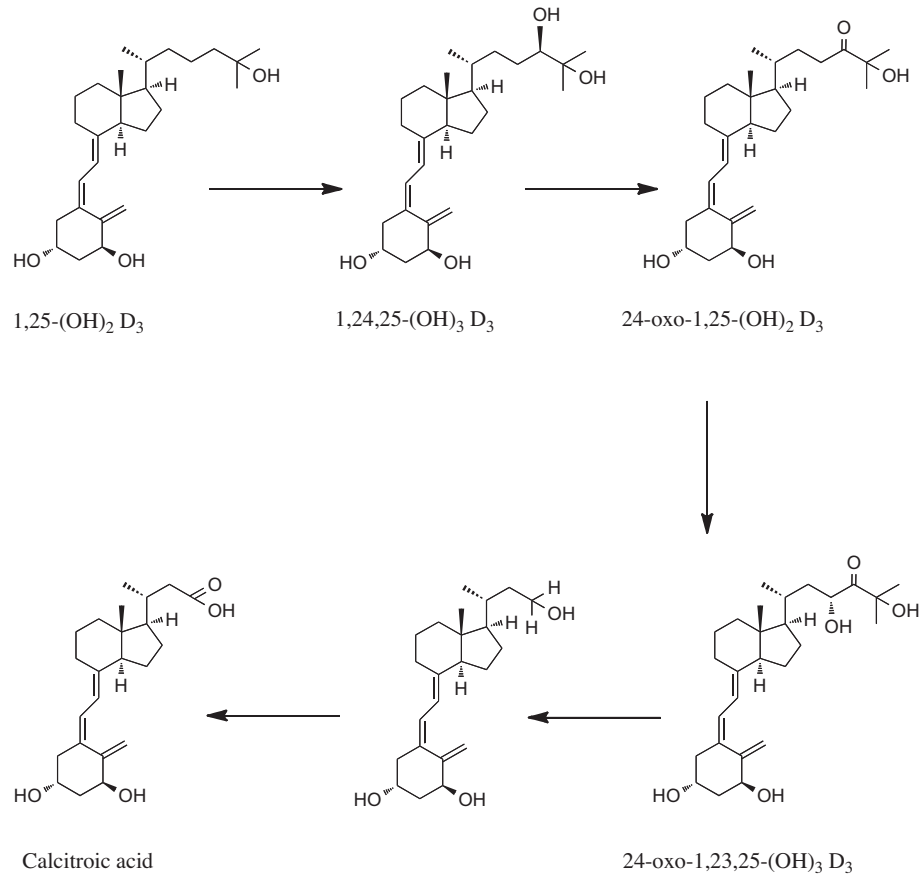

Fig. 2. CYP24A1 oxidation pathway.

other parts of the substrate site. Currently, the non-selective CYP inhibitor ketoconazole (Fig. 3) has been shown to inhibit CYP24 and act synergistically with vitamin D analogs in cell cultures [8] and is being tried in combination with Calcitriol in a phase I clinical trial [9]. Liarazole (Fig. 3) has also been shown to inhibit 1,25$(OH)_2D_3$ hydroxylation and act synergistically with 1,25$(OH)_2D_3$ in androgen independent DU-145 prostate cancer cells [10]. However, a major problem of many azole inhibitors is their low selectivity.

Due to these limitations, selective CYP24 inhibitors offer a therapeutic advantage, and several groups have developed compounds to this end [11–16]. Aiming at increased/sustained hormone levels, a project to develop azole-type inhibitors of CYP24A1 was started in Novartis in the early 1990s. More than 400 structurally different azole-type compounds were tested, using primary human keratinocytes as model system. A compound termed VID 400 (Fig. 3) showed the desired qualities as a strong selective CYP24A1 inhibitor, and was chosen for development in the indication of psoriasis [11,12]. Simons' group reported CYP24A1 inhibitory activity of tetralone derivatives and *N*-[2-(1*H*-imidazol-1-yl)-2-phenylethyl] arylamides, but their selectivity has not been assessed [13,14]. C-24 analogs of 1,25$(OH)_2D_3$ licensed to Cytochroma Inc. and termed "vitamin D signal amplifiers" are both, potent inhibitors of CYP24A1 and agonists of the vitamin D signaling pathway. The lead compound CTA018, where C24 is replaced with a sulfoximine functional group [15,16] (Fig. 3), is at the beginning of clinical Phase II in the indication: topical treatment of mild to moderate psoriasis.

Cyclopropylamine derivatives are also known to have interesting and sometimes useful properties as enzyme inhibitors [17]. Since the initial reports that *N*-benzyl-*N*-cyclopropylamine