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Synthesis and Biological Evaluation of 23-Oxa-, 23-Thia- and 23-Oxa-24-oxo-1α,25-dihydroxyvitamin D_3

K. Allewaert, H. Van Baelen and R. Bouillon*

Laboratorium voor Experimentele Geneeskunde en Endocrinologie, K.U. Leuven, Onderwijs en Navorsing Gasthuisberg, Herestraat, 49, B-3030 LEUVEN (Belgium)

Xu-yang Zhao, P. De Clercq, M. Vandewalle*

University of Gent, Department of Organic Chemistry, Laboratory for Organic Synthesis, Krijgslaan, 281 (S.4), B-9000 GENT (Belgium)

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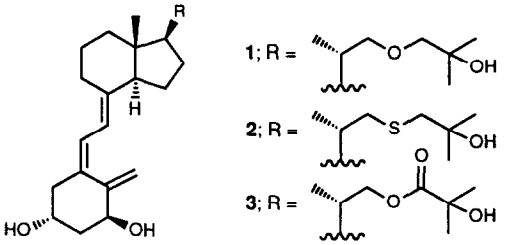
***Abstract** : The synthesis includes the side-chain construction starting from the Inhoffen-Lythgoe diol and coupling with the A ring. Both 23-oxa- and 23-thia-analogues showed a decreased cell differentiating effect but even a more decreased calcemic effect compared with 1α,25-$(OH)_2D_3$.*

Introduction

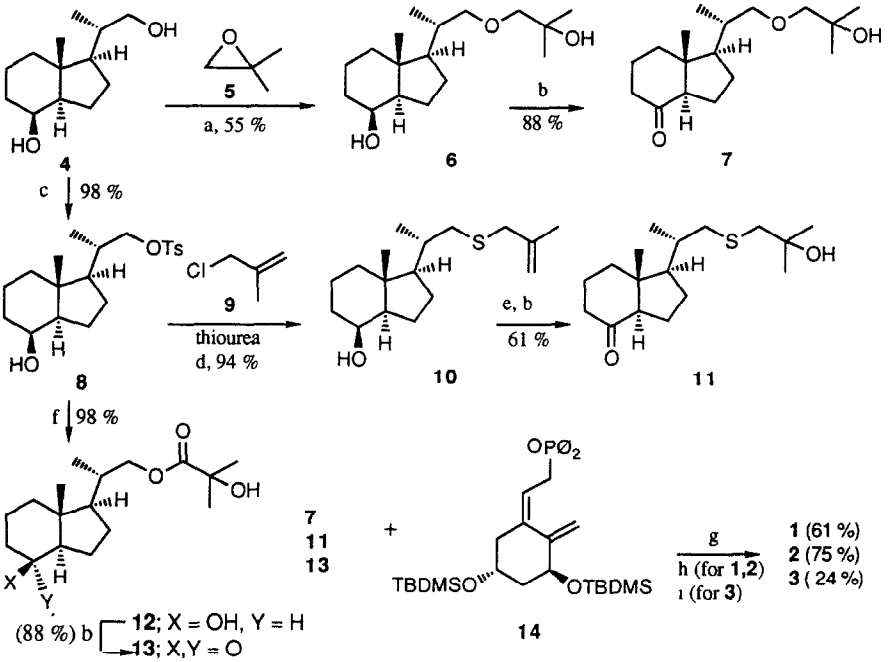
The active form of vitamin D, 1α,25-$(OH)_2D_3$, regulates serum calcium homeostasis by promoting intestinal calcium transport and bone mineral turnover.[1] The hormone 1α,25-$(OH)_2D_3$ has also an important effect on cell proliferation and cell differentiation.[2] In search of separating the hypercalcemic from the differentiating activity, new analogues have been synthesized with variations in the side chain, known to be a major discriminating part of the molecule.[3] Such interesting separation was found with 22-oxa-1α,25-$(OH)_2D_3$ which was more active in suppressing proliferation and inducing differentiation of HL-60 cells whereas the calcemic effects are less than 1 % of 1α,25-$(OH)_2D_3$.[4] These results stimulated the synthesis of three analogues with a heteroatom at the 23-position, namely 23-oxa-1α,25-$(OH)_2D_3$ (**1**) [5,6], 23-thia-1α,25-$(OH)_2D_3$ (**2**)[7] and 23-oxa-24-oxo-1α,25-$(OH)_2D_3$ (**3**). The differentiation inducing activity on HL-60 cells of **1** and **2** has already been tested in vitro by Kubodera et al..[7] No remarkable differences were observed between these analogues which had circa 20 % activity of 1α,25-$(OH)_2D_3$.



Synthesis

The analogue 23-oxa-1α,25-$(OH)_2D_3$ (**1**) has already been described by a few research groups.[5,6] The approaches involve alkylation of the 22-hydroxyl function by 3-chloro-2-methyl-1-propene **9** or bromo t.butylacetate and subsequent formation of the 25-hydroxyl group; the starting material being either the Inhoffen-Lythgoe diol **4**[5] or a 1α-hydroxy-isovitamin derivative.[6] In the case of the alkylation of **4** with **9**[5] a

low chemoselectivity was observed. This necessitated selective protection of the secondary hydroxyl group resulting in a 7-step sequence for the synthesis of ketone **7**. In search for a shorter sequence we decided to study the nucleophilic displacement on epoxide **5** which is less electrophilic than allylic chloride **9** and could thus show a higher selectivity. Several conditions were examined for this transformation; the only successful method involved powdered KOH in DMSO. This gave a clean reaction as only ether **6** was obtained next to starting material. Subsequent oxidation afforded keto-alcohol **7**. Ketone **7** was then coupled with the known A-ring phosphine oxide **14**,[8] after *in situ* protection of the 25-hydroxyl group as a TMS-ether. Deprotection using Amberlyst-15^R led to analogue **1**.



(a) powdered KOH, DMSO, 30°C, 17.5 h; (b) PDC, CH_2Cl_2, rt, 3 h; (c) p-TsCl, pyridine, 0°C, 15 h; (d) DMSO, 50-60°C, 2 h and then aq. NaOH, 45-50°C, 2 h; (e) $Hg(OAc)_2$, H_2O/THF, 40-50°C, 2 h and then 3 M NaOH, $NaBH_4$, rt, 96 % (based on recovered **10**); (f) $(CH_3)_2C(OH)COOH$, K_2CO_3, DMSO, 35°C, 42 h; (g) *(i)* N-TMS-imidazole, THF, rt, 2 h, *(ii)* n-BuLi, THF, -78°C, 2 h and then rt, 0.5 h; (h) Amberlyst-15^R, THF/MeOH, rt, 16 h; (i) $(n\text{-}Bu)_4NF$, THF, rt, 11 h.

SCHEME 1

The 23-thia analogue **2** has already been described; the synthesis involves the nucleophilic substitution of the 22-mesylate in a provitamin skeleton with 1-mercapto-2-methyl-2-hydroxypropane.[7] We independently developed a synthesis starting from the known monotosylate **8** while avoiding the use of a mercaptan. We therefore developped a one-pot process taking advantage of the thiourea-method[9] for producing thiols from halides. Indeed, upon mixing **8**, **9** and thiourea in DMSO in the presence of NaOH, the desired sulfide **10** was produced in high yield. The oxymercuration-reductive demercuration of **10** led to the tertiary alcohol in 61 % yield next to starting material (36 %); the reaction did not reach completion due to complexation of the sulfur

atom and the mercuric ion. Finally, oxidation of the secondary hydroxyl group afforded ketone **11**, which was coupled with **14** as described for **7**.

The synthesis of the analogue **3** is straightforward and involves formation of the α-hydroxy-butyrate **12** from tosylate **8**; subsequent oxidation to ketone **13** and coupling with **14**, as described for **7**, yielded the desired product **3**.

Biochemical evaluation

To evaluate the affinity of the analogues **1**, **2** and **3** to the vitamin D receptor, [^{3}H]1α,25-$(OH)_2D_3$ (specific activity 180 Ci/mmol Amersham, Buckinghamshire, United Kingdom) and increasing amounts of 1α,25-$(OH)_2D_3$ or of **1**, **2** and **3** were incubated with the rat intestinal mucosa cytosol. The relative affinity of the analogues was calculated from their concentration needed to displace 50 % of [^{3}H]1α,25-$(OH)_2D_3$ (assigned 100 %). All three had a decreased receptor binding (Table I).

Biological evaluation

The cell differentiating effect of **1**, **2** and **3** was evaluated in human promyeloid leukemia cells (HL-60 cells) by the induction of superoxide production measured by 4-nitro-blue-tetrazolium (NBT) reduction.[10] Their relative potency was compared to that of 1α,25-$(OH)_2D_3$ (Table 1).

The calcemic effects on vitamin D deficient chicks was evaluated by measuring serum calcium after 10 days of daily intramuscular injection of increasing amounts of analogues.[11,12] All three had reduced calcemic effects as their activity was less than 2 % of that of 1α,25-$(OH)_2D_3$. The cell differentiating effect of the 23-heteroatoms analogs was relatively higher than their calcemic effect. The dissociation of their effects was less than that of 22-oxa-1α,25-$(OH)_2D_3$

TABLE 1. Biological activity of the analogues as assessed by their relative affinity for the intestinal mucosal vitamin D receptor and their capability to induce differentiation of human promyeloid leukemia cells (HL-60). The activities are compared with those of 1α,25-$(OH)_2$-D_3 (assigned a value of 100 %).

Analogue	Receptor binding	HL-60 cell differentiation
1	2	9
2	13	29
3	26	6

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