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Design and evaluation of new antipsoriatic antedrug candidates having 16-en-22-oxa-vitamin D_3 structures

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Abstract—Design, synthesis, and in vitro and in vivo evaluation of a series of antipsoriatic antedrugs having 16-en-22-oxa-vitamin D_3 are described. Among the seven compounds examined, two are promising: ester **5c** and amide **5f**, both of which exhibit greater potent antiproliferation activity with lessened calcemic activity than the presently prescribed maxacalcitol (**2**).

As therapeutic agents for intractable psoriasis with an unknown cause, various steroids, retinoids, and immunosuppressants such as cyclosporine and methotrexate have been developed. However, these medicines have severe side effects including rebound phenomenon,[1] atrophy of skin,[2,3] teratogenesis,[4] nephrotoxicity,[5] liver fibrosis,[6,7] and interstitial pneumonia.[8] Since the recognition of the therapeutic effect of the naturally occurring hormone calcitriol (1α,25-dihydroxyvitamin D_3; **1**, Chart 1) was reported in the early 1980s,[9] a number of studies[10] have focused on the separation of the antipsoriatic effect from the undesirable calcemic activity by modification of the natural structure in order to develop a more favorable therapeutic index. Side-chain analogs of calcitriol (**1**) such as maxacalcitol (**2**),[11] tacalcitol (**3**),[12] and calcipotriol (**4**)[13] have been developed for clinical treatment of psoriasis. Although they require careful administration due to their calcemic effects, such vitamin D_3 analogs have been used extensively because they are safer than other treatments.

However, these vitamin D agents show less efficacy than steroids which belong to the 'very strong'[14] or 'strongest' class. High doses of vitamin D_3 analogs induce an undesirable calcemic action not found in steroids but exhibit an efficacy closer to that of the potent steroids.[15] These findings prompted us to improve the safety of vitamin D agents which would allow higher dosages.

Recently, we reported a series of 16-en-22-oxa-vitamin D_3 analogs[16] which showed reduction of calcemic activity. This reduction is assumed to be due to the rapid oxidative metabolism of enzymes in liver that rapidly converted the absorbed 16-en-22-oxa-vitamin D_3 analogs into inactive metabolites after exhibiting cell antiproliferative activity. The 16-en-22-oxa-vitamin D_3 analogs were absorbed topically and assumed to be oxidized at the allylic C20 or C23 center adjacent to the oxygen atom rapidly after entering into liver through systemic circulation, and gave rise, presumably via the hemiacetal intermediates **6** or **8** from metabolic enzymes in the liver, such as CYPs, to the inactive metabolite **7** or **9**.[16,17] This mode of action is in accordance with the definition of an 'antedrug,'[18–20] an active synthetic derivative designed to undergo biotransformation, proposed in the early 1980s (Chart 2).

With the antedrug concept in mind, we decided to design a new 16-en-22-oxa-vitamin D_3 antedrug having 24-ester

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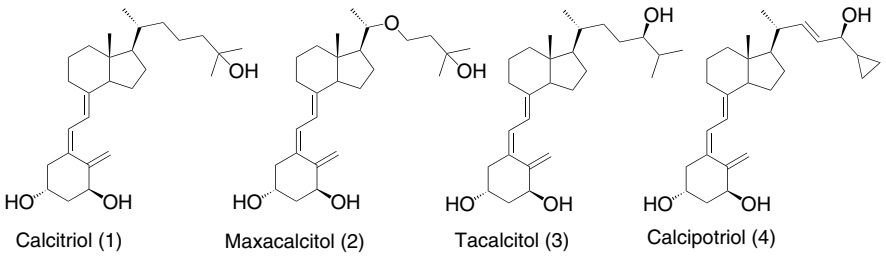


Chart 1. Structure of **1** and its analogs.

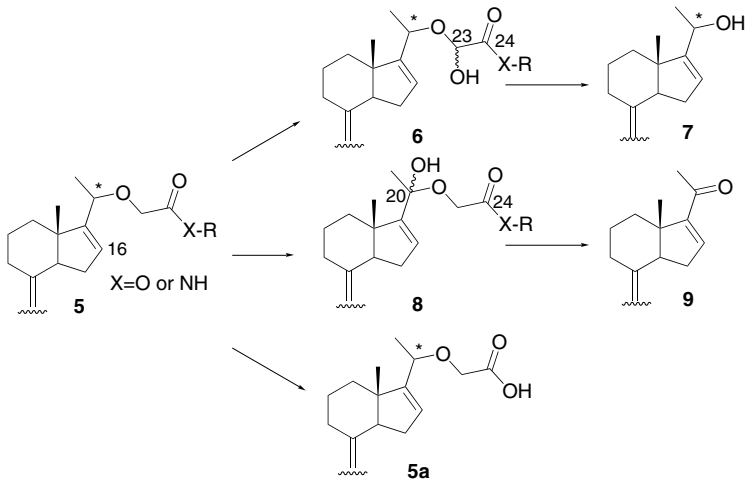


Chart 2. Presumed multipath metabolism of 16-en-22-oxa-24-carboalkoxy or carbamide analogs.

and 24-amide functionality. As it was known that calcitriol (**1**) metabolized into biologically inactive calcitronic acid (**10**),[21] installing additional metabolic functionality leading to inactive carboxy functionality would improve the 'antedrug' profile (Chart 3). We were most interested in a combination of the changes in the activities of the newly combined analogs of 16-en, 22-oxa, and 24-carboalkoxide or 24-carbamide moieties and reduction of the metabolic stability by multipath metabolism. With modification at the terminal part of the side-chain, the undesirable calcemic activity would be reduced through faster metabolism in systemic circulation.

In this paper, we describe the synthesis and biological profiles of 16-en-22-oxa-24-carboalkoxy, carbamide analogs **5b–f**, and the presumed metabolites **7** and **5a**. For comparison, **5g–i** were also prepared and their activities evaluated. The results from compounds **5c** and **5f** provide promise of potent and selective topical vitamin D antipsoriatic agents (Fig. 1).

The synthesis of 16-en-22-oxa-24-carboalkoxy and 16-en-22-oxa-24-carbamide analogs **5b–f** is shown in Scheme 1. Treatment of the allyl alcohol **11**[22] was first transformed into the secosteroidal allyl alcohol **12** by photocycloreversion and thermal hydrogen migration, in 64% yield. The etherification of the allyl alcohol **12** with *tert*-butylacetate under standard Williamson conditions gave the desired *tert*-butyl ester **13b**, followed by hydrolysis to obtain the common intermediate secosteroidal carboxylic acid **14**. The condensation with appropriate alcohols or amines under standard conditions,

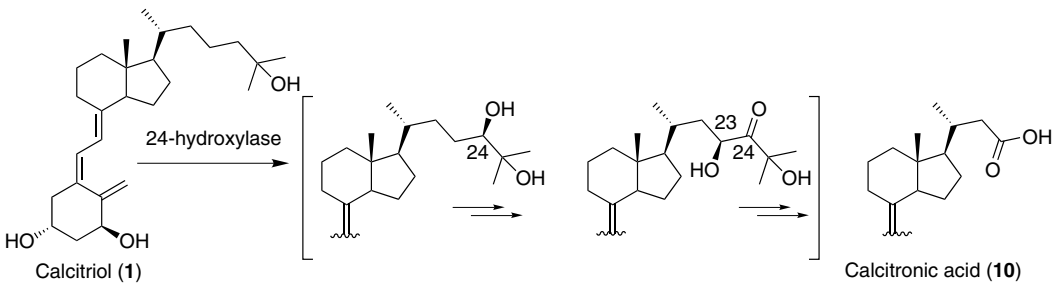


Chart 3. Metabolic pathway of calcitriol (**1**).

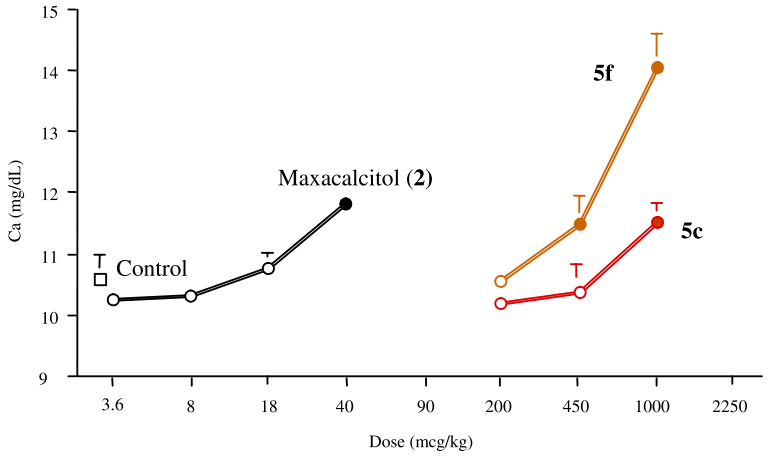


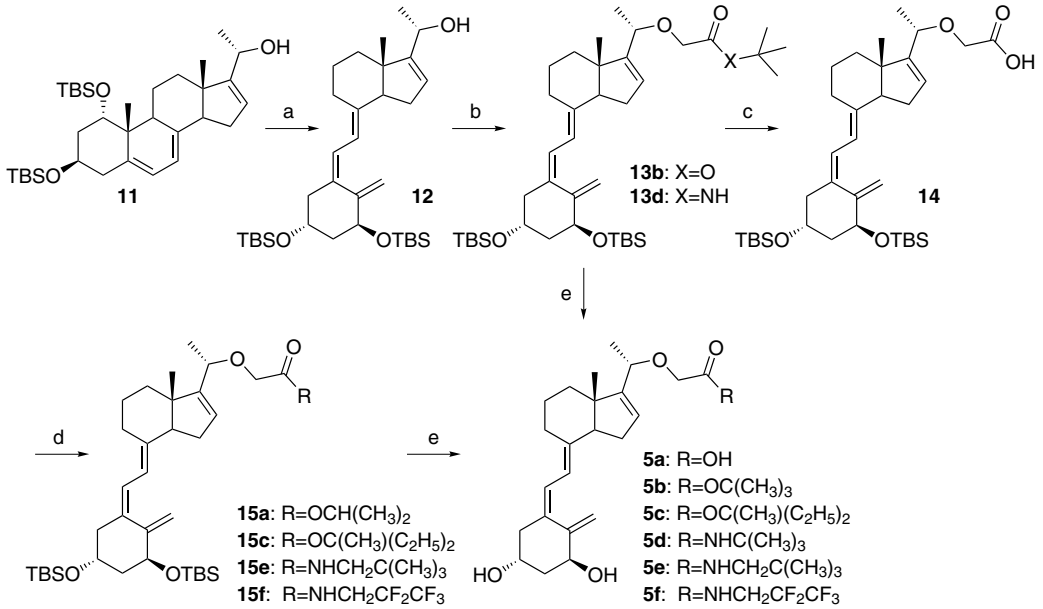
Figure 1. In vivo serum total calcium concentration after 8-day topical administration of maxacalcitol (**2**), **5c**, and **5f** in rats.

followed by desilyation, furnished 16-en-22-oxa-24-carboalkoxy and 16-en-22-oxa-24-carbamide analogs **5b–f** in good yield without difficulty.

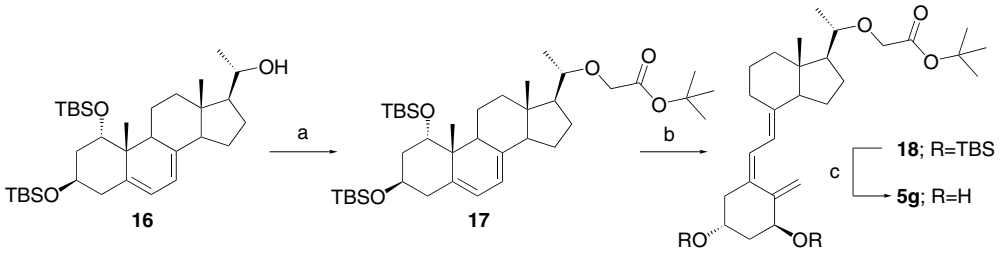
We also synthesized the 16-en-22-oxa-24-carboxylic acid **5a**, which is presumed to be the common metabolite of esters **5b** and **5c**, and amides **5d–f** for a comparison in the biological evaluation. The synthesis utilized the intermediate **13b** in the syntheses of **5b**, **5c**, **5e**, and **5f**. Thus, the carboxylic acid **14** afforded the desired seco-steriod **5a** through **15a** after removal of its bis TBS ether.

Synthesis of 22-oxa-24-carboalkoxy analog **5g** utilized the secondary alcohol **16** formed in the synthesis[23] of maxacalcitol (**2**) shown in Scheme 2. With the same Williamson condition as step b in Scheme 1, the precursor of photolysis, 5,7-dien-ester **17**, was obtained. After photolysis and deprotection, steroidal ester **17** furnished 22-oxa-24-carboalkoxy analog **5g**.

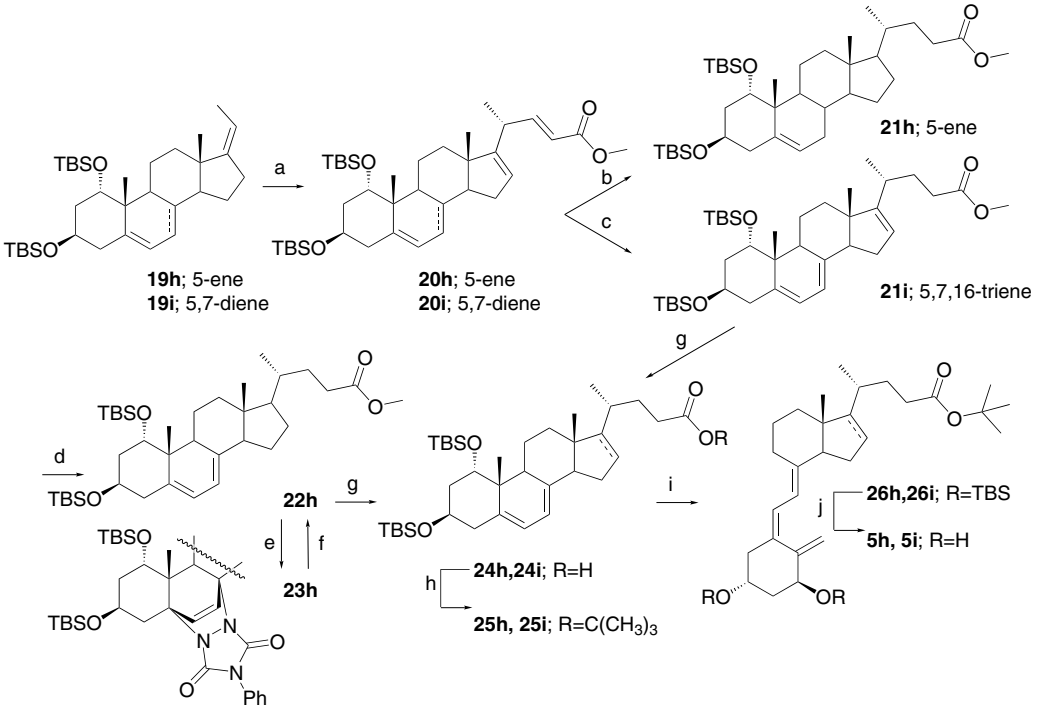
The 22-carbon analogs **5h** and **5i** were prepared starting from the known diene **19h**[24] or triene **19i**[23] (Scheme 3). Ene reaction of **19** with methyl propiolate using Et_2AlCl provided 22-en-steroidal ester **20**. Reduction of triene **20h** gave C16–C17 saturated analog **21h** by H_2 and Pd–C, and reduction of tetraene **20i** gave 5,7,16-trien analog **21i** by $NiCl_2$ and $NaBH_4$, respectively. 5-En analog **21h** converted into 5,7-diene by standard



Scheme 1. Reagents and conditions: (a) hν, THF; heating; (b) method for **13b**: NaH, 15-crown-5, *tert*-butyl bromoacetate, THF, method for **13d**: NaH, 15-crown-5, *N*-*tert*-butyl bromoacetamide, DMF, THF; (c) 1 M NaOMe, aq 1 M NaOH, MeOH, THF (69% from **12**); (d) method for **15a**: propan-2-ol,2-chloro-1,3-dimethylimidazolinium chloride, pyridine, CH_2Cl_2, method for **15c**: 3-methyl-3-pentanol, DCC, DMAP, CH_2Cl_2, method for **15e**: 2,2-dimethyl-propylamine,1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride salt (WSCI·HCl), HOBt·H_2O, CH_2Cl_2, method for **15f**: 2,2,3,3,3-pentafluoro-propylamine, WSCI·HCl, HOBt·H_2O, CH_2Cl_2, (e) 1 M TBAF, THF (**5a**: 33% from **14**, **5b**: 30%, **5c**: 50% from **14**, **5d**: 20% from **12**, **5e**: 40% from **14**, and **5f**: 77% from **14**).



Scheme 2. Reagents and conditions: (a) NaH, 15-crown-5, *tert*-butyl bromoacetate, THF (17%); (b) hν, THF; heating; (c) HF–pyridine, THF (9% from **17**).



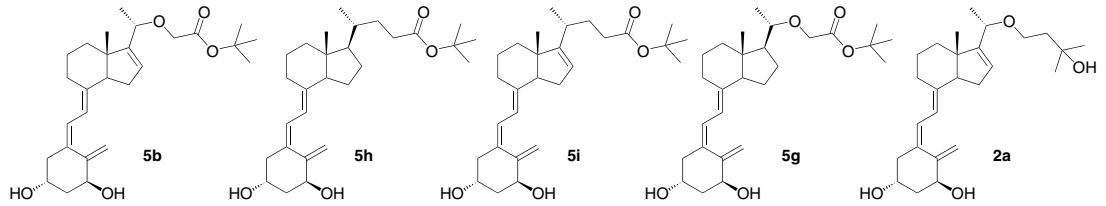
Scheme 3. Reagents and conditions: (a) Et_2AlCl in *n*-hexane, methyl propiolate, CH_2Cl_2 (**20h**: 84%, **20i**: 23%); (b) 5% Pd–C, H_2, AcOEt (98%); (c) $NaBH_4$, $NiCl_2{\cdot}6H_2O$, THF, MeOH, (quant.); (d) NBS, AIBN, *n*-hexane; then γ-collidine, toluene; (e) PTAD, CH_2Cl_2; (f) DMI, heating (17% from **21h**); (g) aq 2 N NaOH, THF, MeOH (**24h**: 99%, **24i**: 99%) (h) *tert*-butanol, DCC, DMAP, CH_2Cl_2; (i) hν, THF; heating; (j) method for **5h**: HF–pyridine, THF; (8% from **24h**), method for **5i**: 1 M TBAF, THF (1% from **24i**).

conditions established in the synthesis of vitamin D_3 derivatives,[25,26] via allyl bromides. The crude dienes **22h** were first treated with 4-phenyl-1,2,4-triazoline-3,5-dione (PTAD)[27] to give the corresponding PTAD adduct **23h** for purification. After retro-Diels–Alder reaction, the pure 5,7-diene **22h** was obtained. The 5,7-dienes **22h** and **21i** furnished 22-carbon esters **5h** and **5i** after hydrolysis, ester condensation, photolysis, thermal isomerization, and deprotection.

Biological activities of the compounds prepared in the present study were evaluated as follows using **2** as the reference: (i) in vitro metabolic stability, (ii) in vivo calcemic activity, and (iii) in vitro pharmacological activity. For in vitro metabolic stability, compounds were incubated with rat liver microsomes or human liver S9 in the presence of NADPH and the metabolic elimination rate, k_e, was determined by time course for concentrations in the reaction mixture. The calcemic activity was estimated based on the calcemic increment levels from in vivo mouse or rat percutaneous administration. The pharmacological activity was estimated by measurement of human keratinocyte growth inhibitory activity.[28] A chicken VDR affinity evaluation confirmed that the compounds function as vitamin D analogs.

Table 1 shows the profiles of compound **5b** and its analogs **5i**, **5g**, and **5h**. According to our previous studies,[16] 16-en-22-oxa-vitamin D_3 analogs metabolized faster than **2**, shown by the profiles of the representative analog **2a** in Table 1. Introduction of a 24-ester moiety into 16-en-22-oxa analogs like **5b** increased the metabolic rate. This result might be due to the multipath metabolism, oxidative metabolism, and hydrolysis. A comparison of **5b** with **5i** and of **5g** with **5h** clearly showed that 22-oxa functionality largely contributed to the poor metabolic stability. Interestingly, the

Table 1. Biological activity of 16-en-22-oxa-vitamin D_3 analogs



Compound	VDR affinity (%)[a]	Metabolic stability in rat liver microsome k_e ratio versus **2**[b]	In vivo blood ionized Ca (mmol/L)[c]	Ratio of Ca change to vehicle C (%)[j]	Antiproliferatior activity IC_{50} (nM)[k]	Antiproliferatior activity A (%) versus **1**[l]
Calcitriol (**1**)	100	n.d.	n.d.	n.d.	27.3	100
Maxacalcitol (**2**)	20.0	1	2.60 ± 0.22[d]	98.5	22.6	120.5
2a[16]	n.d.	1.3	2.16 ± 0.07[e]	66.2	50.4	54.1
5b	40.1	3.9	1.65 ± 0.05[f]	22.2	8.9	305.6
5h	n.d.	0.4	1.77 ± 0.11[g]	28.3	17.0	160.4
5i	n.d.	2.9	2.31 ± 0.18[h]	67.4	6.3	432.1
5g	n.d.	11.6	1.49 ± 0.09[i]	9.6	88.4	30.8

n.d., not determined.

[a] Each point represents the average of duplicate measurements.

[b] Metabolic stability was calculated as the ratio of the k_e value of the analog to **2**. Values are means of two experiments.

[c] The in vivo calcemic activity of test compounds determined by measuring serum calcium level in mice after single percutaneous administration ($n = 3$).

[d] Vehicle levels (mmol/L): 1.31 ± 0.04.

[e] Vehicle levels (mmol/L): 1.30 ± 0.05.

[f] Vehicle levels (mmol/L): 1.35 ± 0.02.

[g] Vehicle levels (mmol/L):1.38 ± 0.02.

[h] Vehicle levels (mmol/L): 1.38 ± 0.03.

[i] Vehicle levels (mmol/L): 1.36 ± 0.01.

[j] Calcemic activity (%) = {(blood ionized Ca [mmol/L] of the analogs) − (blood ionized Ca [mmol/L] of the vehicle)}/(blood ionized Ca [mmol/L] of the vehicle)} × 100.

[k] The in vitro effect is expressed as percentage activity at IC_{50} ($n = 2$, mean value) in comparison with **1**. IC_{50} is the molar concentration of the test compound that causes 50% of the maximal inhibition of proliferation by suppression of [^{3}H]TdR uptake.

[l] Antiproliferation activity (%) = {(IC_{50} [nM] of **1**)/(IC_{50} [nM] of the analogs)} × 100.

antiproliferative activity appeared to be subjected to 16-en substructure in comparing **5g** with **5b** and **5h** with **5i**. Finally, the combination of 16-en, 22-oxa, and 24-ester moieties is effective for enhancing the metabolic instability as well as the pharmacological activity.

Table 2 shows the profiles of substrates **5c**–**f** and their presumed metabolites **5a** and **7**. The 3-(3-methyl)-pentyl ester **5c** was designed based on the structure of the 'super agonist' lexacalcitol[10] having three substructures—epimerization at C20, insertion of methylene group at C24, and insertion of methylene group at C26 and C27—which seem to contribute to its potent activity. The increase of terminal hydrophobicity in **5c** with a 26,27-dihomo substitution obviously enhanced the metabolic instability, reduced the calcemic activity, and increased the antiproliferative activity greatly. It occurred to us that the higher hydrophobicity enhanced the affinity to CYP[29] and the terminal bulky substituent had some impact on VDR signaling.[30]

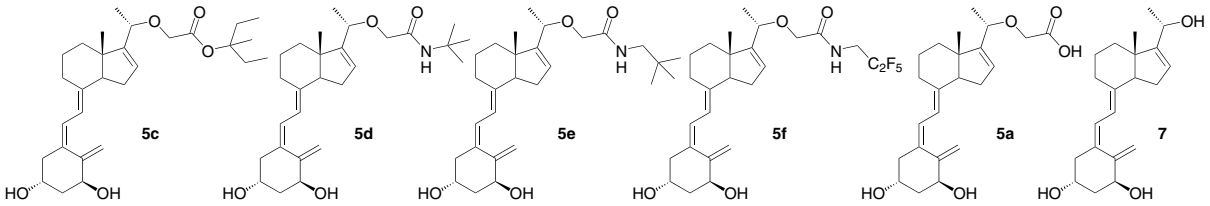
Although amide **5d** was metabolically unstable, calcemic activity remained relatively potent. Undesirable calcemic activity seems to be explained by a balance of metabolic stability with biological activity. In the case of **5d**, this balance seems to be responsible for improved activity. However, the metabolic stability of **5b** and **5d** was inconsistent with the general tendency of metabolic rates in esters and amides.

To lower the calcemic activity of amide **5d**, we promoted metabolism by steric and electronic effects on the carbamide moiety. In **5e**, steric hindrance around the nitrogen atom was removed, and in **5f**, the elimination ability of the amino group was accelerated by attachment of an electronegative fluorine atom, and retained terminal hydrophobicity in both. The enhancement of metabolism and the reduction of calcemic activity in **5e** and **5f** suggested that both steric and electronic effects may have contributed to the promotion of hydrolysis. Additionally, the modifications of **5f** may have markedly increased antiproliferative activity.

Carboxylic acid **5a** and alcohol **7** exhibited few activities, desirable or undesirable, indicating that these two presumed metabolites were inactive, in accordance with the antedrug concept.

Of the antipsoriatic vitamin D_3 analogs form this series, the above results show that ester **5c** and amide **5f** were found to have beneficial pharmacological activity and only slight calcemic activity. Therefore, compounds **5c**

Table 2. Biological activity of 16-en-22-oxa-vitamin D_3 analogs



Compound	VDR affinity (%)[a]	Metabolic stability in rat liver microsome k_e ratio versus **2**[b]	In vivo blood ionized Ca (mmol/L)[c]	Ratio of Ca change to vehicle C[j] (%)	Antiproliferatio activity IC_{50}[k] (nM)	Antiproliferatior activity A (%) versus **1**[l]
Maxacalcitol (**2**)	20.0	1	2.60 ± 0.22[d]	98.5	22.6	120.5
5c	45.1	>10	1.57 ± 0.03[e]	9.8	0.8	3519.8
5d	22.2	8.2	2.16 ± 0.28[f]	63.6	4.4	619.2
5e	18.0	>10	1.82 ± 0.08[g]	31.9	17.4	157.2
5f	34.4	14.4	1.58 ± 0.11[h]	16.2	1.9	1470.7
5a	0.04	n.d.	1.38 ± 0.04[i]	No elevation	>1000	<2.7
7[16]	0.32	n.d.	1.40 ± 0.04[i]	No elevation	>1000	<2.7

n.d., not determined.
[a,b,c,j,k,l] See Table 1.
[d] Vehicle levels (mmol/L): 1.31 ± 0.04.
[e] Vehicle levels (mmol/L): 1.43 ± 0.07.
[f] Vehicle levels (mmol/L): 1.32 ± 0.02.
[g] Vehicle levels (mmol/L): 1.38 ± 0.02.
[h] Vehicle levels (mmol/L): 1.36 ± 0.01.
[i] Vehicle levels (mmol/L): 1.44 ± 0.03.

Table 3. In vitro and in vivo pharmacokinetic parameters of maxacalcitol **2**, **5c**, and **5f**

Compound	In vivo pharmacokinetic in rats[a]		In vivo skin concentration after percutaneous administration to rats (3 mg/0.3 g ointment/kg)[b] concentration ratio versus **2** (4 h)	In vitro metabolic clearance in human liver S9 (mL/min/mg S9 protein)[c]
	Dose (μg/kg)	CL_{tot} (mL/h/kg)		
Maxacalcitol (**2**)	10	1257	1	0.007
5c	1000	5691	0.8	1.421
5f	1000	7853	0.6	0.278

[a] Values represent means of several rats: **2**: $n = 4$, **5c**: $n = 3$, **5f**: $n = 3$.
[b] Single percutaneous administration (after 4 h).Values are means of two rats.
[c] Values are means of two experiments.

and **5f** were further evaluated in pharmacokinetic studies and their calcemic activities examined in detail.

Table 3 shows the pharmacokinetic profiles for the in vitro and in vivo studies of maxacalcitol (**2**), **5c**, and **5f**. After intravenous dosing to rats, **5c** and **5f** had greater clearance than **2**, similar to the results of the stability evaluation in rat microsomes. The in vivo rat skin concentrations after percutaneous administration were almost equal to **2**, indicating that highly potent analogs **5c** and **5f** would exhibit higher efficacy than **2** at the same dose.

In human liver S9, **5c** and **5f** proved quite unstable metabolically compared with **2**, indicating that these two compounds also metabolized rapidly in human liver.

The calcemic activity of **5c** and **5f** were further assessed in an 8-day percutaneous administration study. The results from rats treated with **5c** and **5f** at several dosages exhibited clear reduced calcemic activity over maxacalcitol (**2**).

In summary, we prepared and profiled a series of 16-en-22-oxa vitamin D_3 analogs possessing a 24-carboalkoxide or 24-carbamide moiety in the side-chain, with the expected enhancement of the metabolic instability followed by conversion to inactive metabolites. This series has shown to be extremely potent agonists of VDR with low calcemic action. Further biological evaluation of these analogs identified compounds **5c** and **5f** to be extremely potent, low-calcemic vitamin D_3 analogs, with concentration in rat skin comparable to maxacalcitol (**2**). Compounds **5c** and **5f** are currently being evaluated in further detail for their clinical application as antipsoriatic agents.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2006.03.014.

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