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Potent, low-calcemic, selective inhibitors of CYP24 hydroxylase: 24-sulfone analogs of the hormone 1α,25-dihydroxyvitamin D_3 ☆

Gary H. Posner [a,*], Kenneth R. Crawford [a], Hong Woon Yang [a], Mehmet Kahraman [a], Heung Bae Jeon [a], Hongbin Li [a], Jae Kyoo Lee [a], Byung Chul Suh [a], Mark A. Hatcher [a], Tanzina Labonte [a], Aimee Usera [a], Patrick M. Dolan [b], Thomas W. Kensler [b], Sara Peleg [c], Glenville Jones [d], Anqi Zhang [e], Bozena Korczak [e], Uttam Saha [e], Samuel S. Chuang [e]

[a] *Department of Chemistry, School of Arts and Sciences, The Johns Hopkins University, 3400 N. Charles Street, Baltimore, MD 21218-2685, USA*
[b] *Department of Environmental Health Sciences, Division of Toxicological Sciences, Bloomberg School of Hygiene, The Johns Hopkins University, Baltimore, MD 21205, USA*
[c] *Department of Medical Specialities, The University of Texas, M.D. Anderson Cancer Center, Houston, TX 77030, USA*
[d] *Department of Biochemistry, Queens University, Kingston, Ont., Canada K7L 3N6*
[e] *Cytochroma Inc., Markham, Ont., Canada L3R 8E4*

Abstract

The new 24-phenylsulfone **4a**, a low-calcemic analog of the natural hormone 1α,25-dihydroxyvitamin D_3, is a potent (IC_{50} = 28 nM) and highly selective inhibitor of the human 24-hydroxylase enzyme CYP24.

Keywords: Vitamin D analogs; Low-calcemic; Catabolism inhibitor

1. Introduction

The natural hormone 1α,25-dihydroxyvitamin D_3 [1,25$(OH)_2D_3$, calcitriol, **1**] binds to the Vitamin D receptor (VDR) and thus acts as a ligand to activate transcription. This process controls a wide range of biological functions including immunomodulation, cell proliferation, and cell differentiation [1,2]. Supraphysiological levels of calcitriol required for effective chemotherapy of human diseases, however, usually cause severe hypercalcemia and soft tissue calcifications, thereby limiting medical use of calcitriol [3]. To avoid these undesirable effects, medicinal chemists have designed and prepared thousands of analogs of calcitriol with the goal of separating the beneficial properties of calcitriol from its harmful calcemic effects [3]. Some conceptually new side-chain sulfone analogs **2** and **3** of calcitriol, even though lacking the traditional side-chain terminal OH group, were shown recently to be transcriptionally active and to have desirably low-calcemic activity [4,5]. Now we report a new series of side-chain sulfone analogs **4**–**9**, also lacking the classical side-chain terminal OH group, some of which are low-calcemic, potent, and selective inhibitors of the human cytochrome P450C24 (CYP24) hydroxylase enzyme. The CYP24 enzyme catabolizes calcitriol into its less active major initial metabolite 1,24,25-trihydroxyvitamin D_3 and ultimately into calcitroic acid that is excreted [6]. Blocking or slowing such 24-hydroxylation of calcitriol prolongs its biological lifetime and thus should allow smaller amounts of calcitriol to be used for effective chemotherapy of some human diseases while minimizing or even avoiding hypercalcemia.

1.1. Chemistry

Synthesis of sulfone analogs **4** is outlined in Scheme 1. The 22-iodide **10** [7] undergoes a substitution reaction with the required sulfonylmethyllithium reagents to form C,D-ring side-chain sulfones **11** with a new linkage between carbons 22 and 23. Desilylation and oxidation at C-8 produces C-8 ketones **12**. Horner–Wadsworth–Emmons coupling of ketone **12** with A-ring α-lithio phosphine oxide **13** followed by desilylation [8] generates the target sulfones **4**. New sulfone analogs **5**–**7** were prepared in a similar fashion.

The 16-ene-24-sulfone analog **8** was prepared as shown in Scheme 2. A highlight of this synthesis is a high-yielding

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* Corresponding author. Tel.: +1-410-516-4670; fax: +1-410-516-8420.
E-mail address: ghp@jhu.edu (G.H. Posner).

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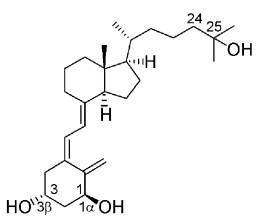
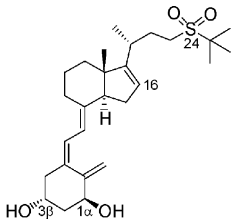
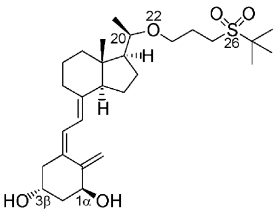


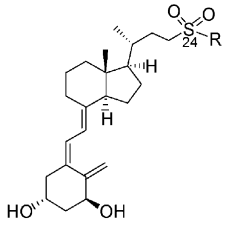
1 (Calcitriol)



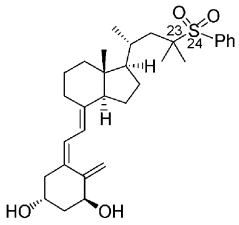
(+)-**2** (JK-1624SO_2)



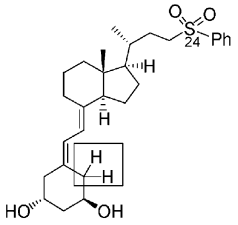
(-)-**3** (KRC-20-epi-22-oxa-26-SO2-1)



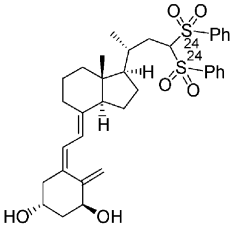
(+)-**4a**, R = Ph (KRC-24SO_2Ph-1)
(+)-**4b**, R = 4-ClPh (HWY-24SO2-pCP-1)
(+)-**4c**, R = 2,4-Cl_2Ph (HWY-24SO_2-m, p-DCP)
(+)-**4d**, R = 4-FPh (HWY-24SO_2-pFP-1)
(+)-**4e**, R = 4-MePh (HWY-24SO_2-p-Tolyl-1)
(+)-**4f**, R = Naphthyl (MK-24SO_2-Naph)
(+)-**4g**, R = 4'-Pyridyl (HL-24SO_2-4'-Pyr-1)
(+)-**4h**, R = *i*-Pr (JK-24SO_2-IP-1)



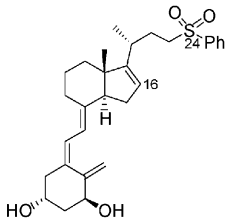
(+)-**5**, (BCS-23-dimethyl-SO_2-Ph-1)



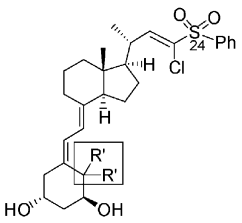
(+)-**6**, (HBJ-19-nor-24SO_2Ph)



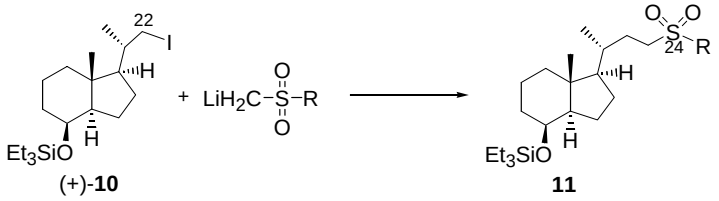
(+)-**7**, (AU-24,24(SO_2Ph)$_2$)



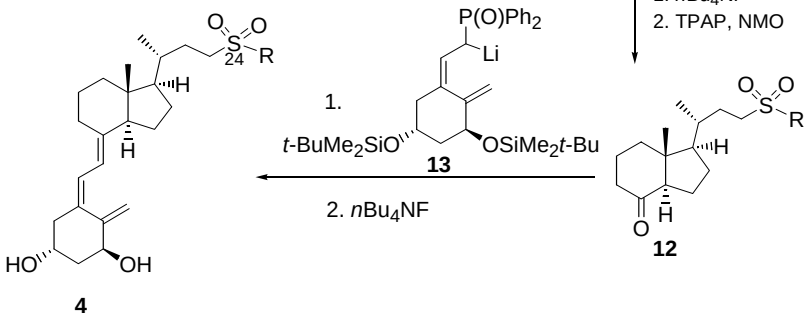
(+)-**8**, (MH-1624-SO_2Ph-1)



(-)-**9a**, R' = R' = CH_2 (MK-22Z-ene-23-Cl-24SO2Ph-1)
(+)-**9b**, R' = R' = H (TM-19-nor-22Z-ene-23-Cl-SO2Ph-1)

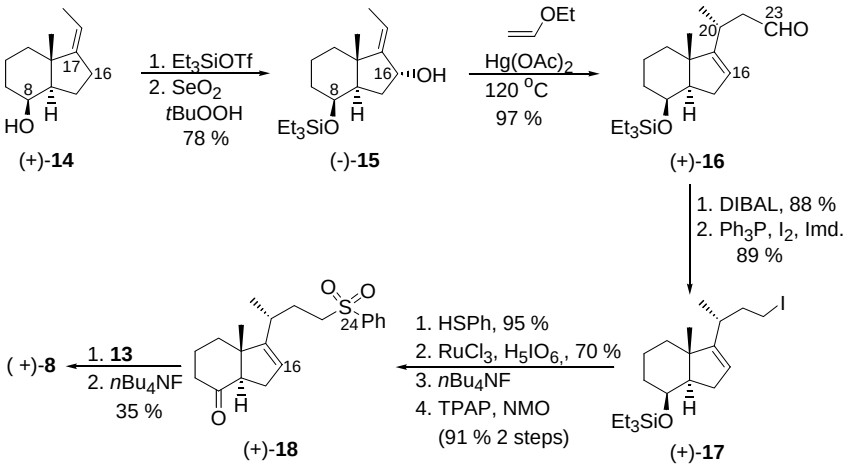


(+)-**10** **11**

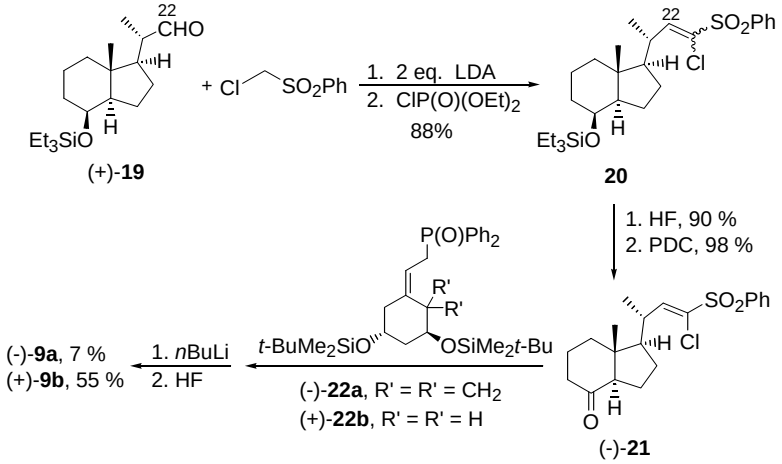


4 **12**

Scheme 1.



Scheme 2.



Scheme 3.

sequence of three reactions converting D-ring ethylidene **14** into allylic alcohol **15** and then into Claisen rearrangement product 23-aldehyde **16** with natural absolute stereochemistry at C-20 [9]. The 22-ene-23-chloro analogs **9a** and **9b** were prepared from 22-aldehyde **19** [7] as shown in Scheme 3.

1.2. Biology

Biochemical analysis showed that, among the analogs **4**–**9**, 24-sulfone **4a** is the strongest inhibitor of human CYP24 (Table 1) [10]. Sulfone **4a** is at least 10 times more potent than the commonly used CYP24 inhibitor ketoconazole. We analyzed sulfone **4a**'s ability to inhibit the enzyme activity of CYP27A and CYP24B, human cytochromes that are critical in the Vitamin D biosynthesis pathway. Sulfone **4a** failed to suppress 1α-hydroxylation by CYP27B and showed a >35/1 selectivity for inhibition of CYP24/CYP27B. Sulfone **4a** does not inhibit CYP27A enzyme activity; the extraordinary selectivity of sulfone **4a** for inhibition of CYP24/CYP27A is >350/1. For comparison, the recently reported leading azole inhibitor of CYP24 has a selectivity of only ∼2/1 CYP24/CYP27A [11].

Sulfones **4**–**9** represent a diverse series of new analogs from which some important chemical *structure*—CYP24

Table 1
CYP24 inhibitory activities

Analog	IC_{50} (nM)
(−)-**3**	>3000
(+)-**4a**	28
(+)-**4b**	94
(+)-**4c**	92
(+)-**4d**	>1000
(+)-**4e**	212
(+)-**4f**	>1000
(+)-**4g**	188
(+)-**5**	>300
(+)-**6**	160
(+)-**7**	>1000
(+)-**8**	>300
(−)-**9a**	>300
(+)-**9b**	>300
Ketoconazole	300

inhibitory *activity relationships* (SAR) can be gleaned, as follows: (1) the size of the side-chain terminal R group is important, with CYP24 inhibition potency decreasing as R gets larger than phenyl (CYP24 inhibitory activity R = Ph > naphthyl); (2) the electronic nature of the R group is important, with CYP24 inhibitory activity decreasing as the terminal aromatic ring acquires lower electron density (CYP24 inhibitory activity R = Ph > 4-ClPh ≫ 4-FPh) or higher electron density (R = Ph > 4-MePh); (3) a terminal phenyl group is much better than a terminal isopropyl group (**4a** > **4g**); (4) sulfone **5**, the 23-dimethylated version of sulfone **4a**, is inactive; (5) sulfone **6**, the 19-nor version of **4a**, is considerably less potent than **4a**; (6) bis-sulfone **7** is inactive; (7) sulfone **8**, the 16-ene version of **4a**, is much less potent than **4a**; and (8) 22-ene-23-chloro sulfone analogs **9a** and **9b**, designed to be inert toward C-22 or C-23 hydroxylation, are much less potent than **4a** as CYP24 inhibitors.

Using our previously reported protocol in which rats are treated orally with calcitriol and with the new analogs daily for 1 week [12], sulfones **4a** and **4b** produced no statistically significant urinary calcium elevation above control even at a 20-fold higher dose than calcitriol (Fig. 1).

To examine transcriptional activity of the analogs, the monkey kidney cells CV1 were transfected by the DEAE-dextran method with 1 mg/dish expression plasmid of the human Vitamin D receptor and 2 μg/dish of a reporter construct containing the human osteocalcin Vitamin D responsive element (ocVDRE) linked to the thymidine kinase promoter and the growth hormone reporter gene [13]. One day after transfection, the cells were treated with the analogs at concentrations ranging from 1 to 1000 nM, for 24 h, and then medium was collected and growth hormone was measured by using a radioimmunoassay as described by the manufacturer (Nichols Institute, San Clemente, CA). The ED_{50} for the transcriptional activity of sulfone **4a** was

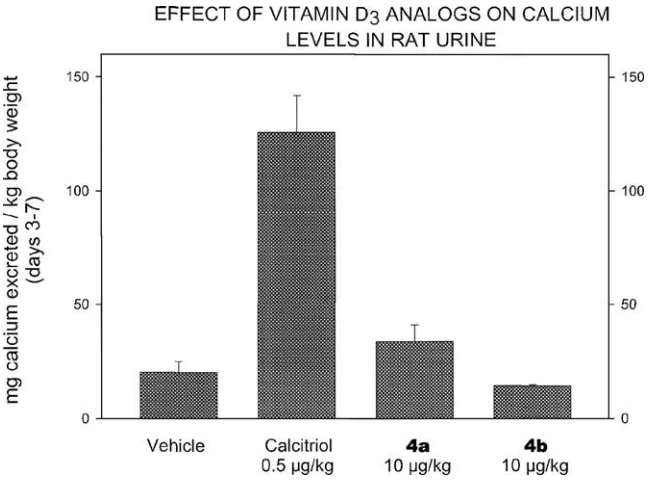


Fig. 1. Effects of Vitamin D_3 analogues on urinary calcium excretion in rats. Animals were treated with 0.5–10 μg/kg body weight of test compound for 7 consecutive days and urinary excretion of calcium was measured during days 3–7. Values are mean ± S.E. from three animals in each group.

1000 nM, whereas sulfone **4b** had non-detectable transcriptional activity at any concentration, up to 1000 nM. To determine if **4b** lacked transcriptional activity because it was an antagonist of the natural hormone, the transcription assay was repeated using $1,25(OH)_2D_3$ without or with 1000 nM of **4b**. Sulfone **4b** did not have any effect on transcriptional activity of $1,25(OH)_2D_3$, thus suggesting it is not an antagonist. To determine the VDR affinity of the transcriptionally active sulfone **4a**, we performed competition assays using recombinant human VDR from COS-1 cells transfected with the VDR expression plasmid. Homogenates from transfected cells were incubated with ^3H-$1,25(OH)_2D_3$ and graded concentration of non-radioactive competitors. Ligand-bound VDR was separated from the free ligand by HAP. The results of this assay showed that the IC_{50} for **4a** was 24 nM, whereas the IC_{50} for $1,25(OH)_2D_3$ was 1 nM. These results indicated that binding affinity of **4a** for VDR is approximately 4% that of $1,25(OH)_2D_3$.

None of the sulfone analogs **4** was strongly antiproliferative in our standard murine keratinocyte assay (data not shown).

In summary, 24-sulfone **4a** is a new chemical entity that is a potent (IC_{50} = 28 nM) and selective inhibitor of CYP24. The high inhibitory selectivity of sulfone **4a** for CYP24/CYP27B is >35/1. Sulfone **4a** also has a very high inhibitory selectivity for CYP24 over CYP27A (>350/1). Thus, new sulfone **4a** has desirable physiological characteristics, importantly including also low-calcemic activity, that make it a good candidate for further preclinical drug development to treat human diseases involving defective cell growth and differentiation.

2. Experimental [5]

2.1. Preparation of Sulfone *4a* (Scheme 1)

A flame-dried 15 mL recovery flask, equipped with a magnetic stir bar, was charged with methyl phenyl sulfone (125 mg, 0.802 mmol) and dissolved in 2.25 mL freshly distilled THF. The flask was cooled down to −78 °C in an isopropyl alcohol/dry ice bath. To this solution was added *n*-BuLi (556 μL, 0.802 mmol, 1.44 M in hexanes), dropwise over several minutes. After 15 min, HMPA (0.1–0.2 mL) was added and allowed to stir for an additional 15 min at −78 °C. Then, a pre-cooled solution of iodide (+)-**10** (100 mg, 0.229 mmol, prepared by sodium iodide displacement of the corresponding tosylate [7]) in 0.75 mL THF was added via cannula. The reaction mixture was then gradually warmed up to room temperature. TLC showed the completion of the reaction. This reaction mixture was quenched with water and then rinsed into a separatory funnel with ethyl acetate and was extracted with ethyl acetate (3× 25 mL). The combined organic extracts were washed with water (1× 25 mL), brine solution (1× 25 mL), dried over $MgSO_4$ and filtered. The filtrate was concentrated in vacuo

to give the crude product that was purified by flash column chromatography eluted with first 5% ethyl acetate, then 20% ethyl acetate in hexanes or preparative TLC plates to afford 91 mg of C,D-ring sulfone **11** in 85% as a colorless oil. Data for **11** (R = Ph): $[\alpha]_D^{25} + 36.7$ (*c* 4.3, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 7.92–7.85 (m, 2H), 7.67–7.59 (tt, $J = 7.4$, 1.5 Hz, 1H), 7.59–7.53 (m, 2H), 4.04–3.96 (m, 1H), 3.16–3.04 (m, 1H), 3.02–2.91 (m, 1H), 1.88–1.75 (m, 2H), 1.66–1.61 (m, 2H), 1.50–0.95 (m, 11H), 0.92 (t, $J = 8.0$ Hz, 9H), 0.84 (s, 3H), 0.83 (d, $J = 6.8$ Hz, 3H), 0.52 (q, $J = 8.0$ Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 139.2, 133.5, 129.1, 128.0, 69.2, 55.9, 53.6, 52.9, 42.1, 40.6, 34.4, 34.2, 28.2, 26.9, 22.8, 18.2, 17.5, 13.4, 6.9, 4.9; IR (neat cm^{-1}) 2949, 2912, 2873, 1446, 1317, 1306, 1234, 1148, 1087, 1021, 803, 740, 724, 689; HRMS: calcd for $C_{26}H_{44}O_3SSiNa^+[M + Na]$: 487.2678, found 487.2672.

A flame-dried 10 mL recovery flask, equipped with a magnetic stir bar, was charged with (triethylsilyl)-oxy aryl sulfone **11** (R = Ph) (0.109 mmol) in THF (~0.7 M) and to this solution was added tetrabutylammonium fluoride (TBAF, 0.436 mmol, 1.0 M in THF) at room temperature. The reaction mixture was stirred for 18 h and concentrated under reduced pressure to a brown syrup. This brown syrup was then dissolved in CH_2Cl_2 and treated with pyridinium dichromate (PDC, 0.327 mmol) and celite® for 12 h. Crude product was purified by flash column chromatography eluted with first 5% ethyl acetate, then 20% ethyl acetate in hexanes or preparative TLC plates to afford C,D-ring ketone. Data for **12** (R = Ph): $[\alpha]_D^{25} + 17.7$ (*c* 4.3, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 7.92–7.84 (m, 2H), 7.67–7.60 (tt, $J = 7.6$, 1.7 Hz, 1H), 7.59–7.51 (m, 2H), 3.16–3.04 (m, 1H), 3.03–2.91 (m, 1H), 2.45–2.33 (dd, $J = 11.4$, 7.4 Hz, 1H), 2.29–2.11 (m, 2H), 2.07–1.91 (m, 2H), 1.89–1.61 (m, 4H), 1.54–1.31 (m, 5H), 1.25–1.15 (m, 1H), 0.89 (d, $J = 6.4$ Hz, 3H), 0.52 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 211.4, 139.0, 133.6, 129.2, 127.9, 61.6, 55.7, 53.4, 49.6, 40.7, 38.7, 34.3, 28.1, 27.1, 23.8, 18.9, 18.3, 12.4; IR (neat, cm^{-1}) 2956, 2875, 1709, 1446, 1306, 1145, 1086, 747, 690; HRMS: calcd for $C_{20}H_{28}O_3SNa^+[M + Na]$: 371.1657, found 371.1664.

Prior to reaction, phosphine oxide and C,D-ring ketones **12** were azeotrophically dried with benzene and left under vacuum for 48 h. A solution of *n*-BuLi in hexanes (0.129 mmol) was added dropwise to a cold (−78 °C) solution of phosphine oxide (0.129 mmol) in THF (1.30 mL) under dry argon. The resulting deep red solution was stirred for 1 h, at which time a cold (−78 °C) solution of C,D-ring ketone **12** (0.064 mmol) in THF (1.2 mL) was added dropwise via cannula. The resulting solution was stirred at −78 °C in the dark for approximately 3 h. Upon observation of a light yellow color, the reaction was quenched at −78 °C by addition of 5 mL of pH 7 buffer, and then allowed to warm to room temperature. The mixture was then rinsed into a separatory funnel with ethyl acetate and extracted with ethyl acetate (3× 25 mL). The combined organic extracts were washed with water (1× 25 mL) and brine solution (1× 25 mL), dried over Na_2SO_4 and filtered. The filtrate was concentrated in vacuo to give the crude product which was purified by column chromatography eluted with 20–50% ethyl acetate in hexanes in the presence of 1% triethylamine to afford the coupled products as a clear oil. This oil was immediately dissolved in THF (5.0 mL) and treated with TBAF (0.322 mmol, 1.0 M in THF) in the dark for 16 h. Concentration of the reaction mixture and column chromatography (EtOAc) in the presence of 1% triethylamine yielded a mixture of diastereomers. This diastereomeric mixture was separated by HPLC (CHIRALCEL® OJ semipreparative column, 15% EtOH/hexanes, 3 mL/min) affording analog **4a** (9 mg, 43%, 1α,3β, R_f 37.2 min). Data for (+)-**4a** (1α,3β): $[\alpha]_D^{25} + 31.8$ (*c* 8.3, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 7.95–7.88 (m, 2H), 7.70–7.62 (tt, $J = 7.6$, 1.7 Hz, 1H), 7.62–7.53 (m, 2H), 6.35 (d, $J = 11.2$ Hz, 1H), 5.99 (d, $J = 11.2$ Hz, 1H), 5.32 (m, 1H), 4.98 (m, 1H), 4.47–4.38 (m, 1H), 4.27–4.17 (m, 1H), 3.18–3.06 (m, 1H), 3.06–2.92 (m, 1H), 2.86–2.75 (dd, $J = 12.6$, 4.2 Hz, 1H), 2.64–2.53 (dd, $J = 13.6$, 3.2 Hz, 1H), 2.36–2.25 (dd, $J = 13.4$, 6.6 Hz, 1H), 1.95–1.44 (m, 13H), 1.30–1.19 (m, 5H), 0.88 (d, $J = 6.0$ Hz, 3H), 0.49 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 147.6, 142.5, 139.2, 133.6, 133.2, 129.2, 128.0, 124.8, 117.2, 111.8, 70.8, 66.8, 56.1, 55.7, 53.6, 45.8, 45.2, 42.8, 40.3, 35.0, 28.9, 28.2, 27.3, 23.4, 22.1, 18.5, 12.0; IR (neat, cm^{-1}) 3647–3119, 3020, 2943, 2871, 1446, 1304, 1216, 1143, 1086, 1055, 753, 688, 534; UV (MeOH) λ_{max}: 263 nm (ε 6,723); HRMS: calcd for $C_{29}H_{40}O_4Na^+[M + Na]$: 507.2545, found 507.2533.

2.2. Preparation of Sulfone *4b*

Data for (+)-**4b**: $[\alpha]_D^{25} + 33.5$ (*c* 0.88, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 7.83–7.86 (m, 2H), 7.54–7.57 (m, 2H), 6.36 (d, $J = 11.2$, 1H), 6.00 (d, $J = 11.2$ Hz, 1H), 5.33 (s, 1H), 4.99 (s, 1H), 4.43 (m, 1H), 4.23 (m, 1H), 3.13 (ddd, $J = 4.4$, 12.0, 14.0 Hz, 1H), 2.99 (ddd, $J = 4.8$, 11.6, 14.0 Hz, 1H), 2.82 (dd, $J = 12.4$, 4.0 Hz, 1H), 2.59 (dd, $J = 13.6$, 3.6 Hz, 1H), 2.31 (dd, $J = 13.6$, 6.8 Hz, 1H), 1.44–2.05 (m, 15H), 1.16–1.30 (m, 3H), 0.89 (d, $J = 6.4$ Hz, 3H), 0.51 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 147.6, 142.5, 140.4, 137.7, 133.2, 129.6, 129.5, 124.8, 117.3, 111.8, 70.8, 66.8, 56.2, 55.7, 53.7, 45.8, 45.2, 42.8, 40.3, 35.0, 28.9, 28.3, 27.4, 23.4, 22.2, 18.5, 12.0; IR (neat, cm^{-1}) 3382, 2926, 1583, 1313, 1148, 1088, 756; UV (MeOH) λ_{max}: 264 nm (ε 14,000); HRMS calcd for $C_{29}H_{39}ClO_4SNa^+[M + Na]$: 541.2150, found 541.2139.

2.3. Preparation of Sulfone *5*

Data for (+)-**5**: $[\alpha]_D^{24} + 25.1$ (*c* 0.12, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 7.88–7.85 (m, 2H), 7.67–7.63 (m, 1H), 7.57–7.54(m, 2H), 6.36 (d, $J = 11.2$ Hz, 1H), 6.00 (d, $J = 11.2$ Hz, 1H), 5.32 (s, 1H), 4.98 (s, 1H), 4.43 (m, 1H), 4.23 (m, 1H), 2.82 (m, 1H), 2.60 (m, 1H), 2.31 (m, 1H), 2.03–1.82 (m, 8H), 1.70–1.44 (m, 10H), 1.34 (s, 3H),

1.30 (s, 3H), 1.00 (d, $J = 5.6$ Hz, 3H), 0.54 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 147.6, 142.7, 135.4, 133.5 133.1, 130.7, 128.6, 124.8, 117.2, 111.8, 70.8, 66.8, 64.0, 57.5, 56.3, 45.8, 45.1, 42.8, 40.4, 39.5, 32.9, 29.0, 28.1, 23.5, 22.4, 22.1, 21.3, 21.1, 11.9; IR (neat, cm^{-1}) 3436, 2931, 2861, 1719, 1649, 1443, 1296, 1155, 1126, 1073, 756, 568; UV (MeOH) λ_{max}: 264 nm (ε 5,774); HRMS: calcd for $C_{31}H_{44}O_4SNa^+$[M + Na]: 535.2853, found 535.2898.

2.4. Preparation of Sulfone **6**

Data for (+)-**6**: $[\alpha]_D^{24}$ + 91.2 (c 0.19, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.93–7.90 (m, 2H), 7.67 (m, 1H), 7.60–7.56 (m, 2H), 6.29 (d, $J = 11.2$ Hz, 1H), 5.83 (d, $J = 11.2$ Hz, 1H), 4.11 (m, 1H), 4.05 (m, 1H), 3.14 (ddd, $J = 13.6$, 12.0, 4.0 Hz, 1H), 3.00 (ddd, $J = 13.6$, 11.2, 4.8 Hz, 1H), 2.78 (dd, $J = 12.4$, 4.0 Hz, 1H), 2.72 (dd, $J = 13.2$, 4.0 Hz, 1H), 2.47 (dd, $J = 13.2$, 3.6 Hz, 1H), 2.43–2.17 (m, 2H), 1.99–1.74 (m, 6H), 1.68–1.44 (m, 9H), 1.30–1.17 (m, 3H), 0.89 (d, $J = 6.0$ Hz, 3H), 0.50 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 142.4, 139.2, 133.6, 131.4, 129.2, 128.0, 123.7, 115.5, 67.4, 67.2, 56.1, 55.7, 53.6, 45.7, 44.6, 42.1, 40.3, 37.1, 35.0, 28.8, 28.3, 27.3, 23.3, 22.1, 18.5, 12.0; IR (neat, cm^{-1}) 3362, 2943, 1447, 1306, 1145, 1086, 1048, 753, 689, 537; UV (MeOH) λ_{max}: 251 nm (ε 37,888); HRMS: calcd for $C_{28}H_{40}O_4SNa^+$[M + Na]: 495.2539, found 495.2526.

2.5. Preparation of Sulfone **7**

Data for (+)-**7**: $[\alpha]_D^{24}$ + 16.3 (c 0.08, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.00–7.93 (m, 4H), 7.74–7.68 (m, 2H), 7.62–7.56 (m, 4H), 6.36 (d, $J = 11.0$ Hz, 1H), 6.01 (d, $J = 11.7$ Hz, 1H), 5.33 (s, 1H), 4.99 (s, 1H), 4.46–4.43 (m, 2H), 4.28–4.19 (m, 1H), 2.84–2.80 (d, $J = 11.9$ Hz, 1H), 2.67–2.58 (m, 1H), 2.31 (dd, $J = 13.7$, 6.6 Hz, 1H), 2.25–2.22 (m, 1H) 2.06–1.43 (m, 13H), 1.31–1.16 (m, 4H), 0.80 (d, $J = 6.2$ Hz, 3H), 0.50 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 147.6, 142.5, 138.0, 137.7, 133.2, 129.7, 129.1, 124.8, 117.3, 111.9, 81.8, 70.8, 66.8, 57.0, 56.1, 46.0, 45.2, 42.8, 40.3, 35.2, 31.6, 28.9, 27.1, 23.4, 22.2, 17.5, 12.1; IR (neat, cm^{-1}) 3528, 3390, 2929, 2871, 1445, 1330, 1312, 1151, 1076, 1053, 1019, 748, 736, 685; UV (MeOH) λ_{max}: 267 nm (ε 11,195); HRMS: calcd for $C_{35}H_{44}O_6S_2Na^+$[M + Na]: 647.2471, found 647.2505.

2.6. Preparation of Sulfone **8**

Data for (+)-**8**: $[\alpha]_D^{25}$ + 14.7 (c 0.23, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 (m, 2H), 7.66 (m, 1H), 7.58 (m, 2H), 6.35 (d, $J = 11.2$ Hz, 1H), 6.08 (d, $J = 11.2$ Hz, 1H), 5.34 (m, 1H), 5.18 (m, 1H) 5.00 (m, 1H), 4.44 (m, 1H), 4.24 (m, 1H), 3.08 (ddd, $J = 14$, 10.8, 4.8 Hz, 1H), 2.97 (ddd, $J = 14.0$, 10.8, 4.8 Hz, 1H), 2.79 (m, 1H), 2.59 (dd, $J = 13.6$, 3.2 Hz, 1H), 2.32 (m, 2H), 2.17 (m, 2H), 2.07–2.01 (m, 1H), 1.97 (m, 1H), 1.92–1.86 (m, 1H), 1.74 (m, 2H), 1.67–1.51 (m, 2H), 1.40 (m, 1H), 1.02 (d, $J = 6.8$ Hz, 3H), 0.60 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.3, 147.6, 141.9, 139.1, 133.6, 133.3, 129.2, 128.1 124.7, 122.0, 117.1, 111.7, 70.7, 66.9, 58.3, 54.5, 49.8, 45.2, 42.9, 35.1, 32.0, 29.4, 28.6, 28.5, 23.5, 21.5, 16.8; IR (neat, cm^{-1}) 3283, 2948, 2874, 1486, 1326, 1163, 1093; UV (MeOH) λ_{max}: 264 nm (ε 12,507); HRMS: calcd for $C_{29}H_{38}O_4SNa^+$[M + Na]: 505.2383, found 505.2365.

2.7. Preparation of Sulfone **9**

Data for (−)-**9a**: $[\alpha]_D^{25}$ − 27.6 (c 0.12, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92–7.90 (m, 2H), 7.68–7.64 (m, 1H), 7.58–7.54 (m, 2H), 6.36 (d, $J = 11.2$ Hz, 1H), 6.00 (d, $J = 11.2$ Hz, 1H), 5.32–5.31 (m, 1H) 4.98–4.97 (br, m, 1H), 4.45–4.41 (m, 1H), 4.24–4.20 (m, 1H), 2.84 (dd, $J = 12.0$, 3.6 Hz, 1H), 2.76–2.66 (m, 1H), 2.59 (dd, $J = 13.6$, 3.6 Hz, 1H), 2.32 (dd, $J = 13.6$, 6.8 Hz, 1H), 2.06–1.88 (m, 4H), 1.73–1.66 (m, 3H), 1.60 (q, $J = 9.2$ Hz, 1H), 1.56–1.45 (m, 5H), 1.40–1.24 (m, 2H), 1.10 (d, $J = 6.4$ Hz, 3H), 0.54 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 147.6, 145.0, 144.9, 142.1, 133.9, 133.4, 130.2, 129.2, 128.3, 124.7, 117.4, 111.7, 70.7, 66.8, 55.8, 55.7, 46.1, 45.2, 42.8, 40.1, 36.6, 28.9, 25.9, 23.3, 22.3,18.5, 12.5; IR (neat, cm^{-1}) 3360, 2927, 2870, 1448, 1323, 1157, 1089, 1055, 923, 752; UV (MeOH) λ_{max}: 266 nm (ε 6,022); HRMS: calcd for $C_{29}H_{37}ClO_4SNa^+$[M + *Na*]: 539.1993, found 539.1969. Data for (+)-**9b**: $[\alpha]_D^{25}$ + 4.92 (c 0.36, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92–7.89 (m, 2H), 7.69–7.64 (m, 1H), 7.58–7.54 (m, 2H), 7.08 (d, $J = 10.4$ Hz, 1H), 6.29 (d, $J = 11.2$ Hz, 1H), 5.84 (d, $J = 11.2$ Hz, 1H), 4.12 (m, 1H), 4.06–4.03 (m, 1H), 2.83–2.79 (m, 1H), 2.76–2.67 (m, 2H), 2.50–2.45 (dd, $J = 13.6$, 3.20 Hz, 1H), 2.23–2.18 (m, 2H), 2.05–1.92 (m, 3H), 1.83–1.64 (m, 5H), 1.62–1.33 (m, 6H), 1.25–1.14 (m, 2H), 1.10 (d, $J = 6.8$ Hz, 3H), 0.53 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 144.9, 142.0, 137.5, 131.7, 130.2, 129.2, 128.7, 128.3, 123.5, 115.7, 67.4, 67.2, 55.8, 55.7, 46.0, 44.6, 42.1, 40.0, 37.1, 36.7, 28.8, 26.0, 23.3, 22.3, 18.5, 12.5. IR (neat, cm^{-1}) 3366, 2931, 2872, 1443, 1325, 1220, 1155, 1085, 1049, 926, 867, 808, 756, 720, 685; UV (MeOH) λ_{max}: 251 nm (ε 14,376); HRMS: calcd for $C_{28}H_{37}ClO_4SNa^+$[M + Na]: 527.1993, found 527.1971.

3. Biochemical studies

3.1. Generation of stable cell line expressing recombinant CYP24 and CYP27A

Human CYP24 cDNAs were obtained from HPK-1A ras cells treated with 10 nM 1α,25(OH)$_2$D$_3$ for 18 h. Cell culture reagents were purchased from Invitrogen (Carlsbad, CA), 1α,25(OH)$_2$D$_3$ and other chemicals (unless specified otherwise) were purchased from Sigma (St. Louis,

MO). Total RNA was extracted from the cells using Trizol reagent (Invitrogen) as per manufacturer's instructions. The full length cDNA was amplified using one-step RT-PCR kit (Clontech, Palo Alto, CA) according to the protocol provided by the supplier. The oligonucleotides 5′-TACGCTAGCACCATGAGCTCCCCCATCAGCAA-3′ and 5′-AGGCTCGAGTTATCGCTGGCAAAACGCGATGG-3′ were used as PCR primers on the basis of the human CYP24 cDNA sequence described by Chen [14]. An *Nhe*I site and *Xho*I site (underlined) were engineered in the forward and reverse primer before and after the start codon, respectively. The amplified PCR fragment was *Nhe*I and *Xho*I digested and the resulting 1560 bp product was cloned into the mammalian expression vector pcDNA3.1-Hygro (+) (Invitrogen) that had been digested with the same enzymes to yield the plasmid pcDNA3.1-CYP24.

V79 hamster cells were transfected with pcDNA3.1-CYP24 using FuGENE reagent (Roche, Indianapolis, IN) as per manufacturer's instructions. The hygromycin-resistant clones were selected after 2 weeks propagation in the presence of 100 μg/ml hygromycin. Single clones (V79-CYP24) were isolated and the presence of recombinant protein verified by RT-PCR (data not shown) and enzymatic reaction (see below).

Human CYP27A cDNAs were obtained from *Eco*RI digestion of a pBluescript SK (−) plasmid containing the 2.1 kb human cDNA of CYP27A1 [15]. The CYP27A1 cDNA clone was then ligated into the mamalian expression vector pcDNA3.1-Hygro (+) (Invitrogen) that had been digested with the same enzyme to yield the plasmid pcDNA3.1-CYP27A.

V79 hamster cells were transfected with pcDNA3.1-CYP27A using FuGENE reagent (Roche, Indianapolis, IN) as per manufacturer's instructions. The hygromycin-resistant clones were selected after 2 weeks in the presence of 100 μg/mL hygromycin. Single clones (V79-CYP27A) were isolated and the presence of recombinant protein verified by RT-PCR (data not shown) and enzymatic reaction (see below).

3.2. Measurement of enzyme activity of CYP24

V79-CYP24 cells were cultured in DMEM media supplemented with 100 μg/ml hygromycin and 10% fetal bovine serum at 37 °C humidified atmosphere plus 5% CO_2. On the day of the assay, V79-CYP24 cells were collected by washing with 1× PBS buffer, trypsinization and centrifugation. The cell pellet was resuspended in DMEM +1% BSA media and 250,000 cells in 150 μL were added to wells of a 48-well plate. After the cells were left for a preincubation period of 30 min at 37 °C in a humidified atmosphere containing 5% CO_2, 25 μL of sulfone analog (10^{-6} to 10^{-9} M) was added to each well. After 10 min, 25 μL of substrate [^{3}H-1β]-1α,25(OH)$_2$D$_3$ (20 nM) was added and the plate incubated for 2 h at 37 °C in a humidified atmosphere containing 5% CO_2. [^{3}H-1β]-1α,25(OH)$_2$D$_3$ (40 Ci/mmol) was synthesized by sodium boro[^{3}H]hydride reduction of 1-keto, 25-hydroxyprevitamin D$_3$ according to the method of Makin et al. [16]. Both sulfone analog and radiolabeled substrate were prepared in DMEM with 1% BSA media in the absence and presence of 100 μM 1,2 dianilinoethane, respectively. The reaction was terminated by the addition of 500 μL of methanol and transferred to glass tube. The aqueous phase was extracted by standard Bligh–Dyer extraction in which we substituted dichloromethane for chloroform [17]. Samples were then spun at 4000 rpm for 5 min. Triplicate 100 μL aliquots of aqueous fraction containing water-soluble CYP24 products were mixed with 600 μL of scintillation fluid and the radioactivity was measured using a scintillation counter. All values were normalized for background.

3.3. Measurement of enzyme activity of CYP27A1

CYP27A1 activity was measured as described by Dilworth et al. with exception of the use of V79-CYP27A cells [18].

3.4. Measurement of enzyme activity of CYP27B

Keratinocytes were purchased from Cambrex (East Rutherford, NJ) and grown as per supplier's instructions. CYP27B enzyme activity was measured as described by Schuster using human epidermal keratinocytes [19] with a few modifictions. Normal phase HPLC of $CHCl_3$ extracts was done on a fully automated Waters Alliance 2695 HPLC system (Milford, MA) and Radioflow detector LB509 (EG&G Berthold, Bundoora, Australia).

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