Pergamon

Bioorganic & Medicinal Chemistry 9 (2001) 2365–2371

BIOORGANIC & MEDICINAL CHEMISTRY

A Non-Calcemic Sulfone Version of the Vitamin D_3 Analogue Seocalcitol (EB 1089): Chemical Synthesis, Biological Evaluation and Potency Enhancement of the Anticancer Drug Adriamycin

Gary H. Posner,[a,*] Kenneth R. Crawford,[a] Sara Peleg,[b] Jo-Ellen Welsh,[c] Saara Romu,[c] David A. Gewirtz,[d] Mona S. Gupta,[d] Patrick Dolan[e] and Thomas W. Kensler[e]

[a]*Department of Chemistry, School of Arts and Sciences, The Johns Hopkins University, Baltimore, MD 21218, USA*
[b]*Department of Medical Specialties, The University of Texas, M.D. Anderson Cancer Center, Houston, TX 77030, USA*
[c]*Department of Biological Sciences, University of Notre Dame, Notre Dame, IN 46556, USA*
[d]*Department of Pharmacology and Toxicology, Virginia Commonwealth University, Medical College of Virginia, Richmond, VA 23298, USA*
[e]*Department of Environmental Health Sciences, School of Hygiene and Public Health, The Johns Hopkins University, Baltimore, MD 21205, USA*

Received 1 February 2001; accepted 30 April 2001

In honor of Peter Dervan, winner of the 2000 Tetrahedron Prize.

Abstract—Novel side-chain diene sulfones **5**, analogues of the natural hormone 1α,25-dihydroxyvitamin D_3 (calcitriol, **1**), were designed to incorporate some of the therapeutically most favorable structural features of the Leo Pharmaceutical Company's drug candidate diene EB 1089 (seocalcitol, **4**) and of the Hopkins' non-calcemic side-chain sulfone analogues **2** and **3**. Synthesis of diene sulfones **5** features selective Swern oxidation of a primary silyl ether in the presence of a secondary silyl ether (**9**→**10**) and Horner–Wadsworth–Emmons aldehyde addition by a 1-phosphonyl-3-sulfonyl stabilized carbanion regiospecifically at the 1-position to form *E,E*-diene sulfone **11**. Sulfone diene analogue **5a** with natural 1α,3β-diol functionality, but not its diastereomer **5b** with unnatural A-ring stereochemistry, is antiproliferative in vitro toward murine keratinocytes and malignant melanoma cells, as well as toward MCF-7 human breast cancer cells. Combining diene sulfone **5a** with the currently used anticancer drug adriamycin (ADR) caused a noteworthy 3-fold enhancement of ADR antiproliferative potency in MCF-7 cells. Sulfone diene analogue **5a** is weakly active transcriptionally in MCF-7 and ROS 17/2.8 cells, binds poorly but measurably to the vitamin D receptor (VDR), and desirably is non-calcemic in vivo at a daily dose (7 days) of 10 μg/kg of rat body weight.

Introduction

Using vitamin D_3 analogues for chemotherapy against various human diseases (e.g., cancer, osteoporosis, psoriasis) requires separating desirable antiproliferative and prodifferentiating activities from undesirable calcemic activity.[1–3] The natural hormone 1α,25-dihydroxyvitamin D_3 (calcitriol, **1**), unfortunately, is too strongly calcemic for such general medicinal applications. As molecular architects, synthetic organic chemists worldwide have structurally altered small portions of the natural hormone's skeleton to produce therapeutically valuable analogues.[4] Most of these analogues retain the natural hormone's tertiary alcohol group located toward the end of the side chain for effective binding to the vitamin D receptor (VDR).[4] In 1999, we showed for the first time that replacing the side-chain tertiary hydroxyl (OH) group by an unorthodox terminal sulfone group produced analogues (e.g., **2**) that maintained the natural hormone's antiproliferative potency in vitro but without significant calcemic activity in vivo.[5] In 2000, we showed for the first time that sulfone analogue **3** with a different type of side-chain modification had similarly desirable separation of antiproliferative from calcemic activities while being relatively stable metabolically.[6] One of the current leading calcitriol (**1**) analogue drug candidates is Leo Pharmaceutical Company's side-chain conjugated diene allylic alcohol EB 1089 (seocalcitol, **4**);

*Corresponding author. Fax: +1-410-516-8420; e-mail: ghp@jhu.edu

0968-0896/01/$ - see front matter
PII: S0968-0896(01)00159-6

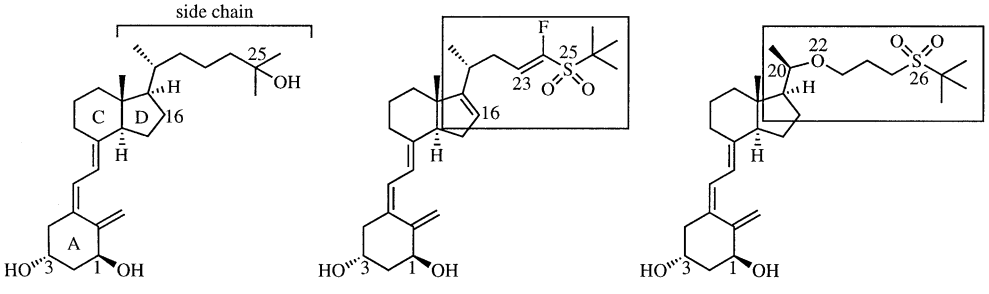
such side-chain unsaturation was incorporated, at least in part, to retard metabolic deactivation (i.e., P-450 oxidation) near the 26-hydroxyl group.[7,8] Incorporating some of the therapeutically best structural features of this successful Leo analogue EB 1089 (**4**) and also of our promising sulfone analogues **2** and **3**, we have now designed, prepared, and evaluated side-chain diene sulfones **5**.

Chemistry

Arbusov substitution[9] on allylic bromide **6** cleanly produced phosphonate sulfone **7** (Scheme 1). Selective Swern oxidation[10] of the primary triethylsilyl ether group of bis-silyl ether **9** produced aldehyde **10** without disturbing the secondary silyl ether functionality. Horner–Wadsworth–Emmons (HWE) condensation of aldehyde **10** with the conjugate base of phosphonate sulfone **7** proceeded via attack of this allylic carbanion regiospecifically by the carbanionic center adjacent to phosphorus to form *E,E*-diene sulfone **11**.[11] Final HWE condensation of the conjugate base of racemic A-ring allylic phosphine oxide **13**, prepared as described previously,[12] with enantiomerically pure C,D-ring ketone **12** and HF-promoted desilylation gave the desired side-chain analogues **5** as a mixture of diastereomers. This desilylation step was successful with HF, without disrupting the conjugated triene system, whereas more commonly used nucleophilic tetrabutylammonium fluoride was avoided because of its tendency to add fluoride anion in a Michael fashion to α,β-unsaturated sulfones.[5] Diene sulfones **5** were separated by semipreparative HPLC to afford pure target compounds **5a** and **5b**, each diastereomer enantiomerically pure, characterized fully and distinguished from each other as in similar cases[5,6] by their characteristic ^{1}H NMR resonances and optical rotations (Table 1).

Biology

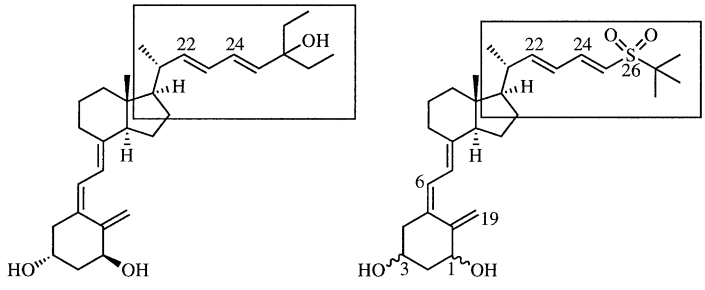
The in vitro antiproliferative potencies of sulfones **5** and of calcitriol (**1**), determined using our previously described murine keratinocyte protocol,[12] are shown in Figure 1. Diastereomer **5a** having the natural A-ring hydroxyl group stereochemistry (i.e., 1α,3β) is much more potent than the corresponding unnatural A-ring diastereomer **5b**, a trend found previously in related diastereomeric pairs of sulfone analogues.[5,6] At 100-nM concentration, diastereomer **5a** and calcitriol (**1**) have comparable antiproliferative potencies in murine keratinocytes. Similar results were observed in vitro in murine malignant melanoma cells (data not shown). In MCF-7 human breast cancer cells, analogue **5a** at 100 nM reduced cell proliferation by 40% after 96 h, compared directly with EB 1089 (**4**) at 100 nM causing a 50% reduction in cell proliferation. At a concentration of only 10 nM, however, diene **5a** inhibited MCF-7 cell growth only weakly, by approximately 10%. Combining this analogue **5a** (10 nM) with adriamycin (ADR), a currently used anticancer drug,[13] shifted the growth inhibition dose–response curve (Fig. 2) for ADR favorably by approximately 3-fold (from IC_{50} of 230 to 85 nM). The magnitude of this desirable shift is similar to that reported previously for the combination of ADR with the 16-ene-23-yne-25-OH drug candidate ILX-23-7553.[14] Whether this synergistic effect of diene **5a** plus ADR is due to the capacity of diene **5a** to confer susceptibility to apoptosis, as shown previously for other calcitriol (**1**) analogues,[14,15] remains to be determined. When administered to rats orally for seven consecutive days at a 20-times higher dose than calcitriol (**1**), diene **5a** was not statistically different from vehicle control in terms of levels of urinary calcium excretion (Fig. 3). Furthermore, unlike calcitriol and unlike EB 1089 (**4**),[7] *non-calcemic* diene sulfone **5a** did not compromise animal weight gain when administered orally



1α,25-dihydroxyvitamin D_3 (calcitriol, **1**)

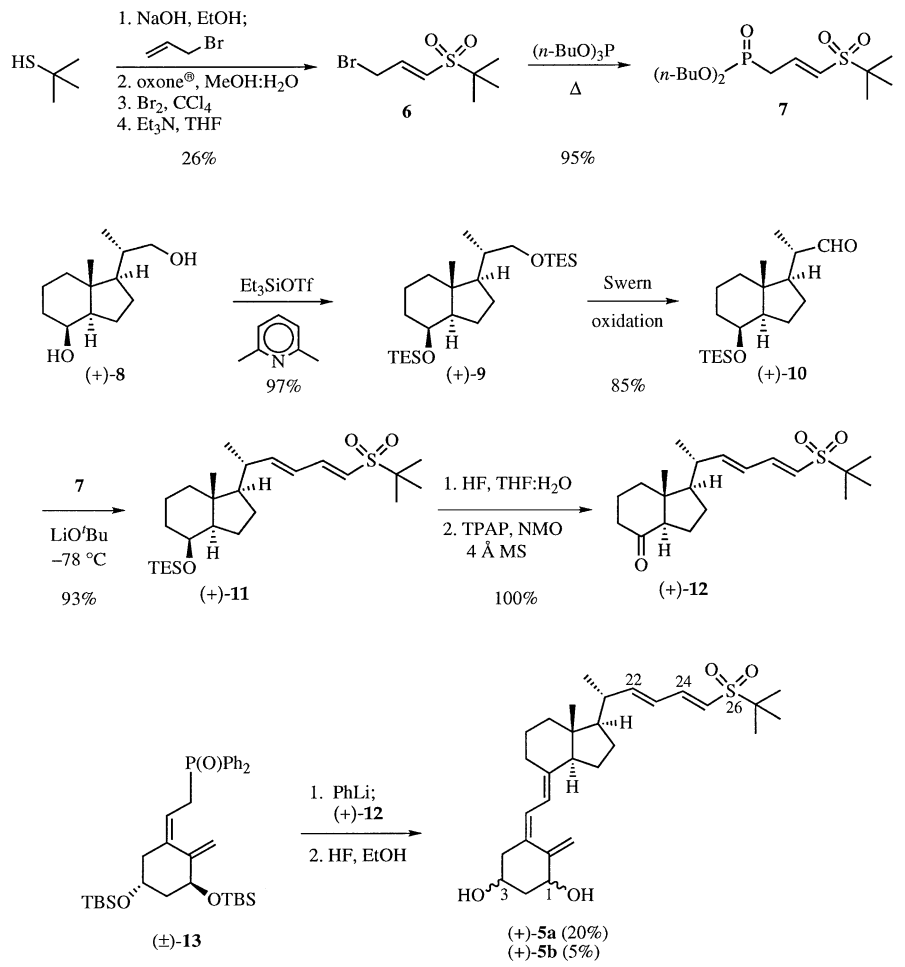
2 (QW-1623*E*-diene-24F-25-SO_2-1)

3 (KRC-20-epi-22-oxa-26-SO_2-1)



4 (seocalcitol, EB 1089)

(+)-**5a** (1α,3β), KRC-22*E*,24*E*-diene-26-SO_2-1
(+)-**5b** (1β,3α), KRC-22*E*,24*E*-diene-26-SO_2-2



Scheme 1.

Table 1. ^{1}H NMR (δ) and optical rotation (°) characteristics of sulfones **5**

Analogue	C6-H	C19a	$[\alpha]_D^{25}$
5a	6.37	5.32	+96.6
5b	6.38	5.31	+31.6

for seven consecutive days at a dose of 10 μg/kg of body weight.

The in vitro vitamin D receptor-mediated transcriptional potencies of diene analogues **5** were determined, as described previously,[16] in rat osteosarcoma ROS 17/2.8 cells using a reporter gene containing the osteocalcin vitamin D responsive element. The ED_{50} values for transcriptional activities are as follows: calcitriol (**1**), 0.4 nM; **5a**, 20 nM; **5b**, no activity. The ED_{50} for sulfone **5a** was calculated from a dose–response plot ranging from 0.01 to 1000 nM. This compound **5a** induces 80% of calcitriol (**1**) maximal response at concentrations of 100 nM and 100% of calcitriol (**1**) maximal response at 1000 nM. These data mean that sulfone **5a** has the same efficacy as that of calcitriol (**1**) but the potency of sulfone **5a** is lower than that of calcitriol (**1**). These results imply that sulfone **5a** is not a partial agonist in ROS 17/2.8 cells. To determine the transcriptional potency of the various vitamin D_3 analogues also in MCF-7 human breast cancer cells, we transiently transfected MCF-7 cells with a vitamin D_3 reporter construct derived from the rat 24-hydroxylase (24-OHASE) promoter. This construct, which consists of approximately 300 bp of the native rat 24-OHASE promoter linked to a luciferase reporter gene, is one of the most potent vitamin D_3 responsive reporter systems characterized to date.[17] Transactivation of the 24-OHASE reporter gene in response to increasing doses of calcitriol (**1**) and two vitamin D_3 analogues is shown in Figure 4. As expected, the natural hormone **1** dose-dependently enhanced reporter gene activity, with an 18-fold induction relative to vehicle-treated cells at the 100-nM dose. Analogue **4** (EB 1089) exhibited a higher potency than the natural hormone **1**, with significant induction of reporter gene activity (10-fold over vehicle-treated cells) at a dose as low as 1 nM. However, higher doses of analogue **4** (10–100 nM) did not further enhance reporter gene activity, suggesting that analogue **4** (EB 1089) is a partial agonist. Thus, despite the higher potency of analogue **4** at low doses, the maximal reporter gene activity for analogue **4** was not higher than that of the natural hormone **1** (compare 100-nM doses for each compound). Although low (1–10 nM) doses of analogue **5a** were inactive, this analogue did up-regulate vitamin D_3 responsive reporter gene activity 5-fold compared to

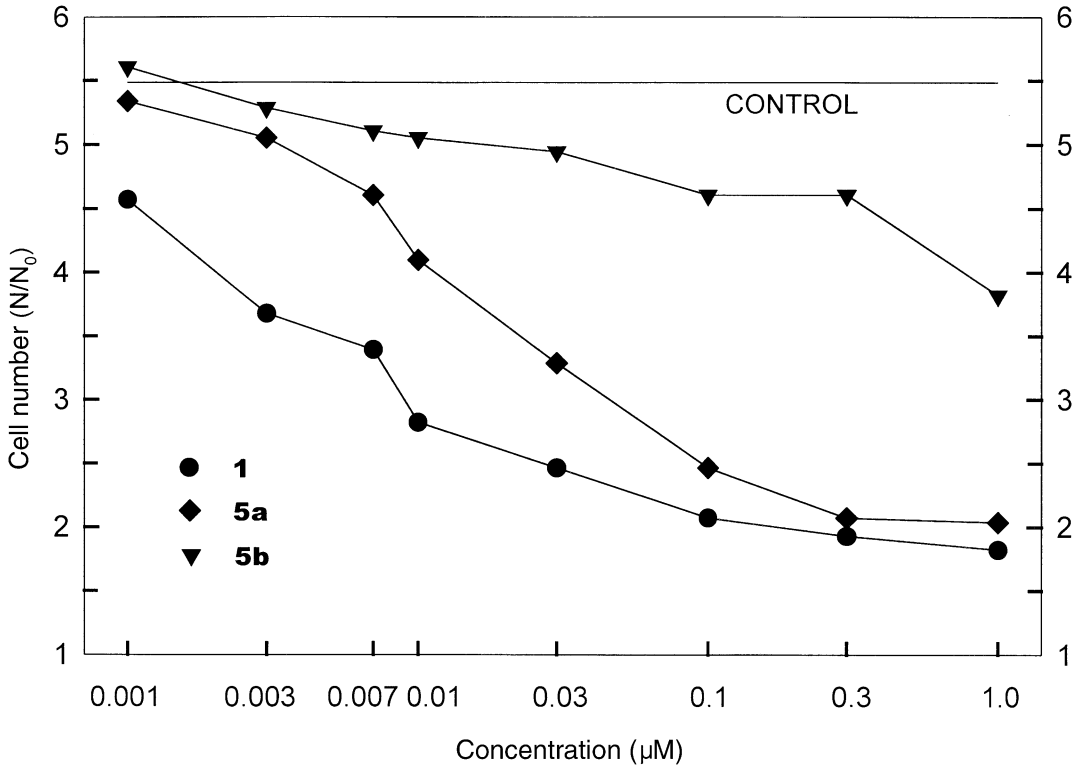


Figure 1. Dose–response effects of analogues on keratinocyte proliferation (96 H).

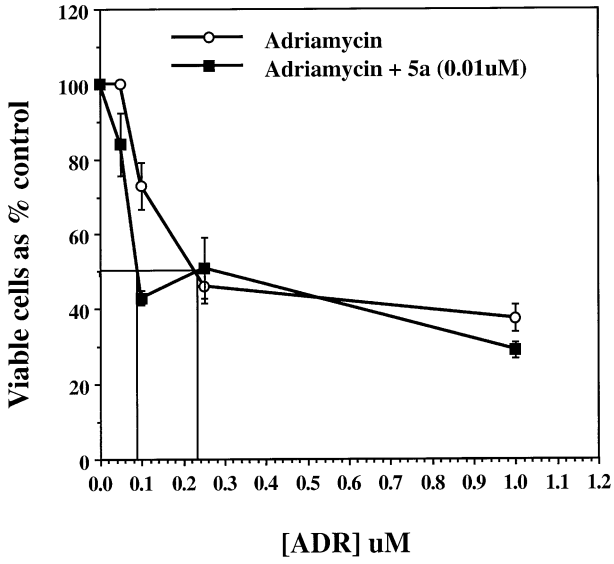


Figure 2. Growth inhibition in MCF-7 cells.

ethanol-treated cells when used at the 100-nM dose level. By extrapolation, we estimate that the transcriptional potency of analogue **5a** was approximately equal to that which would be observed with 5 nM of the natural hormone **1** in this system. In comparing sulfone analogue **5a** to Leo analogue **4**, we conclude that the structural modifications in **5a** clearly reduced, but did not eliminate, its ability to transactivate the vitamin D_3 responsive reporter gene. Consistent with these transcriptional potencies, relative binding affinities[5] to VDR are as follows: calcitriol (**1**) 100%; **5a**, 2%.

In conclusion, diene **5a** represents a new type of side-chain sulfone analogue that, despite lacking the classical 25-OH group,[5,6] has selective biological activities, including especially considerable in vitro anti-proliferative potency; unlike calcitriol (**1**) and unlike EB 1089 (**4**), diene **5a** is non-calcemic in vivo. This new sulfone **5a** has potential as a sensitive molecular probe of ligand–receptor interactions. Further biological evaluation of this new chemical entity will reveal its full biological profile and its possible medicinal value, alone or combined with established anticancer drugs, especially in comparison with Leo's promising side-chain diene drug candidate seocalcitol (EB 1089, **4**).

Experimental

Unless otherwise noted, all reactions were performed in oven-dried glassware stirred under an atmosphere of ultra-high-purity Ar. Et_2O and THF were distilled from Na/benzophenone ketyl immediately prior to use. DMSO, CH_2Cl_2, and Et_3N were distilled from CaH_2. Organolithiums were titrated prior to use following known methods. All other reagents were used as received from commercial suppliers. Analytical TLC analysis was conducted on precoated glass-backed silica gel plates (Merck Kieselgel 60 F_{254}, 250 μm thickness) and visualized with *p*-anisaldehyde or $KMnO_4$ stains.

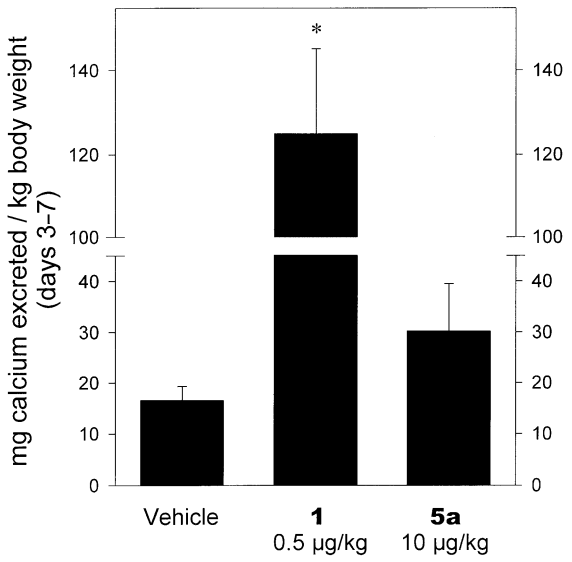


Figure 3. Effects of vitamin D_3 analogues on urinary calcium excretion in rats. Animals were treated with 0.5–1.0 µg/kg body weight of test compound po for seven consecutive days, and urinary excretion of calcium was measured during days 3–7. Values are mean ± SE from three animals in each group. *Signifies statistically different ($p > 0.05$) from vehicle.

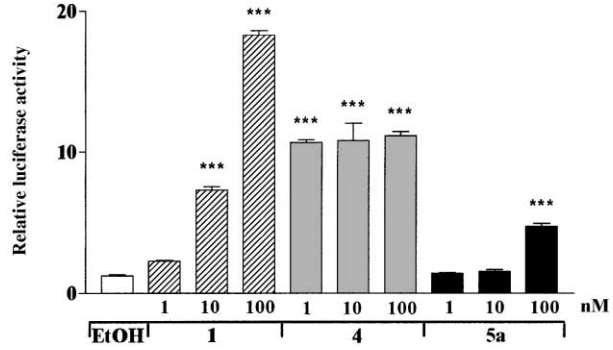


Figure 4. Data are expressed as relative luciferase activity (i.e., values of treated cells relative to that of ethanol-treated control cells, which were normalized to 1). Each bar represents the mean ± SEM of values obtained from three independent cell extracts. *$p < 0.001$, treated versus ethanol control values. Similar data were obtained with these three compounds in independent experiments utilizing a distinct clone of MCF-7 cells.

Column chromatography was performed using flash silica gel (particle size 230–400 mesh). HPLC was carried out using a Rainin HPLX system equipped with two 25-mL/min preparative pump heads using Rainin Dynamax 10×250-mm (semipreparative) columns packed with 60 Å silica gel (8 µm pore size) as C-18-bonded silica and a Rainin Dynamax UV-C dual-beam variable-wavelength detector set at 264 nm. Yields are reported for pure products (>95% based on their chromatographic and spectroscopic homogeneity) and are unoptimized. Melting points were determined in open capillaries using a Mel-Temp metal-block apparatus and are uncorrected. Optical rotations were measured at the Na line using a Perkin-Elmer 141 Polarimeter. NMR spectra were obtained on a Varian XL-400 spectrometer, operating at 400 MHz for ^1H and 100 MHz for ^{13}C. Chemical shifts are reported in ppm (δ) and are referenced to $CDCl_3$ (7.26 and 77.0 ppm). UV spectra were obtained using a Beckman DU®-70 spectrophotometer at ambient temperature. IR spectra were obtained using a Perkin-Elmer 1600 Series FT-IR instrument. HRMS were obtained with electronic or chemical ionization (EI or CI) at the University of Illinois at Urbana—Champaign on a Finnigan-MAT CH5, a Finnigan-MAT 731, or a VG Instruments 70-VSE spectrometer run at an ionizing voltage of 70 eV for EI and run with methane (CH_4) as a carrier gas for CI. Elemental analyses were performed by Atlantic Microlab Inc., Norcross, GA.

***E*-3-Bromo-1-propenyl *t*-butyl sulfone 6.** To a solution of sodium hydroxide (2.40 g, 60.0 mmol) in ethanol (50 mL) at 50 °C was added dropwise via syringe 2-methyl-2-propanethiol (6.76 mL, 60 mmol) over 2 min. The resulting solution was stirred for 30 min while cooling to rt. Upon dropwise addition of allylbromide (5.19 mL, 60 mmol), the reaction mixture was heated to reflux and stirred overnight. The resulting suspension was filtered to remove NaBr, which was rinsed thoroughly with Et_2O. The filtrate was neutralized with 1 M HCl (50 mL), diluted with H_2O (100 mL), extracted with Et_2O (3×25 mL), washed with H_2O, dried over Na_2SO_4, filtered, and concentrated to a pale-yellow oil by removing EtOH and Et_2O via gentle distillation.

This pale-yellow oil (7.81 g theor) was dissolved in 215 mL of methanol and cooled to 0 °C. To this solution was added a 26% oxone® solution (55.3 g, 90.0 mmol in 150 mL H_2O) dropwise via addition funnel. The resulting suspension was allowed to stir with gradual warming to room temperature overnight. The reaction mixture was then diluted with 150 mL H_2O, extracted with $CHCl_3$ (3×75 mL), washed with H_2O and brine (150 mL each), dried over Na_2SO_4, filtered, and concentrated to give essentially pure allyl *t*-butyl sulfone as a clear oil.

A portion of this sulfone (1.00 g, 6.16 mmol) was dissolved in CCl_4 (40 mL), treated with bromine (0.317 mL, 6.16 mmol) for 3 h, and concentrated to give the desired dibromide (1.46 g, 74%) as a pale-orange solid (recryst from cold Et_2O/hexanes; mp 75–80 °C) clean enough for the following transformation.

The dibromide was dissolved in THF (50 mL), cooled to 0 °C, and treated with Et_3N (0.945 mL, 6.78 mmol) overnight, while gradually warming to room temperature. The reaction mixture was neutralized with dilute HCl, diluted with H_2O, extracted with Et_2O (3×25 mL), dried over Na_2SO_4, filtered, concentrated, and purified by column chromatography (10–20% Et_2O/pentane) to give **6** (385 mg, 26% over two steps from crude allyl *t*-butyl sulfone) as white crystals: mp 60–62 °C; ^1H NMR ($CDCl_3$) δ 7.00 (dt, $J = 15$, 6.8 Hz, 1H), 6.55 (dt, $J = 15$, 1.2 Hz, 1H), 4.07 (dd, $J = 6.8$, 1.2 Hz, 2H), 1.38 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 143.8, 127.6, 58.9, 27.4, 23.2; IR (neat) 3068, 3053, 2976, 1636, 1305, 1275, 1109, 982.

Anal. calcd for $C_7H_{13}BrO_2S$: C, 34.86; H, 5.43. Found: C, 34.98; H, 5.32.

Di-*n*-butylphosphonate 7. A stirred solution of tri-*n*-butyl phosphite (5 mL) and sulfone **6** (370 mg, 1.53 mmol) was heated to 130 °C for 12 h, cooled to room temperature, concentrated and purified on silica gel (75% EtOAc/hexanes) to give 519 mg (95%) of **7** as a clear oil: ^{1}H NMR ($CDCl_3$) δ 6.74 (dt, J = 15, 8.0 Hz, 1H), 6.38 (ddt, J = 15, 4.6, 1.2 Hz, 1H), 4.03–3.92 (m, 4H), 2.76 (ddd, J = 25, 8.0, 1.2 Hz, 2H), 1.62–1.53 (m, 4H), 1.38–1.30 (m, 4H) 1.29 (s, 9H), 0.85 (t, J = 7.2 Hz, 6H); ^{13}C NMR ($CDCl_3$) δ 140.2 (d, J = 11 Hz), 128.2 (d, J = 14 Hz), 66.2 (d, J = 6.8 Hz), 58.7, 32.5 (d, J = 6.1 Hz), 30.0 (d, J = 140 Hz), 23.2, 18.7, 13.5; IR (neat) 2961, 2935, 2874, 1633, 1477, 1464, 1289, 1246, 1114, 1066, 1024, 983. Anal. calcd for $C_{15}H_{31}O_5PS$: C, 50.83; H, 8.82; S, 9.05. Found: C, 50.64; H, 8.65; S, 8.91.

Acetaldehyde (+)-10. To a cold (−78 °C) solution of diol (+)-**8**[6] (0.751 g, 3.54 mmol) in THF (50 mL) was added 2,6-lutidine (2.10 mL, 18.0 mmol) followed by TESOTf (1.76 mL, 7.78 mmol). After 10 min, the reaction mixture was quenched with H_2O (10 mL), warmed to room temperature, extracted with CH_2Cl_2 (3×10 mL), dried ($MgSO_4$), filtered, and concentrated in vacuo. The residue was filtered through a short plug of silica (20% EtOAc/hexanes) to give the desired bis-triethylsilyl ether (+)-**9**[6] (1.51 g, 97%) as a colorless oil. To a cold (−60 °C) solution of DMSO (0.664 mL, 9.35 mmol) in CH_2Cl_2 (3.00 mL) was added oxalyl chloride (0.424 mL, 4.86 mmol) in CH_2Cl_2 (22.1 mL). After 2 min, a cold (−60 °C) solution of the aforementioned bis-triethylsilyl ether (+)-**9** (1.65 g, 3.74 mmol) in CH_2Cl_2 (3.00 mL) was added via cannula. The resulting mixture was stirred at −60 °C for 1 h, quenched with Et_3N (2.81 mL, 20.2 mmol), and warmed to room temperature. Upon dilution with H_2O (10 mL), the reaction mixture was extracted with CH_2Cl_2 (3×10 mL), dried ($MgSO_4$), filtered, concentrated, and purified by flash column chromatography (2–5% EtOAc/hexanes) to give the desired aldehyde (+)-**10** (1.03 g, 85%) which was carried forward directly.

26-*tert*-Butyl sulfone (+)-11. A solution of lithium *tert*-butoxide (1.0 M in THF, 0.491 mL) was added via syringe to a cold (−78 °C) solution of phosphonate **7** (175 mg, 0.493 mmol) in THF (1.0 mL). The mixture was warmed slightly to effect solution and returned to −78 °C. The resulting yellow solution was delivered via cannula to a stirred solution of aldehyde (+)-**10** (64.0 mg, 0.197 mmol) in THF (1.5 mL) at rt. After 10 min, the solvent was removed and the residual brown oil was flash chromatographed (8% EtOAc/hexanes) to give 86 mg (93%) of (+)-**11** as a moist solid: $[\alpha]_D^{25}$ +78° (*c* 4.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.16–7.06 (m, 1H), 6.14 (t, J = 15 Hz, 1H), 6.11–6.06 (m, 2H), 4.05–4.00 (m, 1H), 2.27–2.17 (m, 1H), 1.96–1.89 (m, 1H), 1.89–1.74 (qt, J = 13, 3.8 Hz, 1H), 1.35 (s, 9H), 1.04 (d, J = 6.8 Hz, 3H), 0.93 (s, 3H), 0.93 (t, J = 8.0 Hz, 9H), 0.54 (q, J = 8.0 Hz, 6H); ^{13}C NMR ($CDCl_3$) δ 153.7, 147.2, 123.7, 120.4, 69.2, 58.6, 55.8, 52.9, 42.4, 40.6, 40.1, 34.5, 27.4, 23.3, 22.9, 19.4, 17.6, 13.8, 6.9, 4.9; IR (neat) 2950, 2935, 2873, 1638, 1458, 1300, 1113, 1018, 1004; HRMS: calcd for $C_{26}H_{48}O_3SSi$: 468.3093, found 468.3094.

C,D-Ring ketone (+)-12. An aqueous solution of HF (0.500 mL, 10% wt) was added dropwise to a stirred solution of (+)-**11** in H_2O:THF (1.00 mL H_2O:few drops of THF to effect solution) and the resulting solution stirred at room temperature for 2 days [two additional 0.500 mL portions of 10% $HF_{(aq)}$ were added over this period to consume starting material]. The reaction mixture was then carefully neutralized (satd. $NaHCO_3$), diluted with H_2O, and extracted with CH_2Cl_2 (3×10 mL). The combined organics were dried (Na_2SO_4), filtered, and concentrated to give the desired hydroxy sulfone as a white solid.

To this crude hydroxy sulfone in CH_2Cl_2 (3 mL) was added 4-methymorpholine *N*-oxide (NMO, 58.0 mg, 0.492 mmol) and powdered (4 Å) molecular sieves, followed by tetrapropylammonium perruthenate (TPAP, 4.30 mg, 0.0123 mmol). After stirring for 15 min, the reaction mixture was filtered, concentrated and passed through a short pad of flash silica gel (20% EtOAc/hexanes) to afford the desired keto sulfone (+)-**12** as a white foam [79 mg, quantitative from (+)-**11**]: $[\alpha]_D^{25}$ +15 (*c* 1.9, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.08 (dd, J = 15, 10 Hz, 1H), 6.15 (t, J = 15 Hz, 1H), 6.17–6.00 (m, 2H), 2.43 (dd, J = 11, 8 Hz, 1H), 2.29–2.15 (m, 3H), 1.32 (s, 9H), 1.08 (d, J = 6.4 Hz, 3H), 0.63 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 211.4, 152.0, 146.6, 124.4, 121.2, 61.6, 58.6, 55.6, 49.9, 40.9, 40.1, 38.7, 27.5, 23.9, 23.3, 19.6, 19.1, 12.7; IR (neat) 2955, 2934, 2872, 1708, 1638, 1587, 1461, 1296, 1278, 1111, 1001, 824; HRMS: calcd for $C_{20}H_{32}O_3S$: 352.2072, found 352.2066.

Diene-26-*tert*-butyl sulfone analogues 5a and 5b. Racemic phosphine oxide (±)-**13** (78.0 mg, 0.134 mmol) was dissolved in 1.34 mL of THF and cooled to −78 °C under argon. To this solution was added 97.0 μL of PhLi (0.134 mmol, 1.38 M in cyclohexane/Et_2O) dropwise via syringe. The deep orange solution was stirred for 30 min, at which time a cold solution of C,D-ring ketone (+)-**11** (31.0 mg, 0.0880 mmol) in 1.00 mL of THF was added dropwise via cannula. The resulting solution was stirred in the dark at −78 °C for approximately 4 h, then slowly warmed to −40 °C over 2 h. The reaction mixture was quenched with 3 mL of a 2:1 (v/v) mixture of 2 N sodium potassium tartrate and 2 N potassium carbonate. Upon warming to room temperature, the reaction mixture was diluted with H_2O, extracted with EtOAc (4×20 mL), dried over $MgSO_4$, filtered, concentrated and purified by silica gel column chromatography (10–50% EtOAc/hexanes/<0.1% Et_3N) to afford the coupled product [33 mg, 90% based on recovered (+)-**11**] as a yellow oil.

This oil was immediately dissolved in ethanol (1.50 mL), cooled to 0 °C, and treated with HF (0.100 mL, 49% aqueous). The solution was slowly warmed to room temperature and treated with additional HF (0.100 mL) to complete the deprotection. After aqueous workup the resulting white film was flash chromatographed (1% Et_3N/EtOAc) to afford 17.3 mg (77% for deprotection)

of diastereomers **5a** and **5b**. This diastereomeric mixture was purified by reversed-phase HPLC (C-18 semi-preparative column, 46% MeCN/H_2O, 3 mL/min) giving 8.8 mg of **5a** (20%, t_R 115 min) and 2.1 mg of **5b** (5%, t_R 111 min). **5a** (1α, 3β): $[\alpha]_D^{25}$ +97 (*c* 7.5, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.18–7.06 (m, 1H), 6.37 (d, *J* = 11 Hz, 1H), 6.16 (t, *J* = 15 Hz, 1H), 6.13–6.05 (m, 2H), 6.01 (d, *J* = 11 Hz, 1H), 5.35–5.29 (m, 1H), 5.01–4.95 (m, 1H), 4.46–4.40 (m, 1H), 4.26–4.19 (m, 1H), 2.83 (dd, *J* = 12, 3.7 Hz, 1H), 2.59 (dd, *J* = 14, 3.2 Hz, 1H), 1.36 (s, 9H), 1.09 (d, *J* = 6.8 Hz, 3H), 0.57 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 153.1, 147.6, 147.0, 142.4, 133.2, 124.8, 124.0, 120.7, 117.3, 111.8, 70.8, 66.8, 58.6, 56.1, 55.6, 46.1, 45.2, 42.8, 40.7, 40.3, 29.0, 27.5, 23.5, 23.4, 22.2, 19.7, 12.3; IR (neat) 3598–3148 (br), 3013, 2935, 2870, 1637, 1588, 1457, 1295, 1108, 756; UV (MeOH) λ_{max} 287 nm (ε 7640); HRMS: calcd for $C_{29}H_{44}O_4S$ 488.2960, found 488.2950. **5b** (1ß, 3α): $[\alpha]_D^{25}$ 32 (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.18–7.07 (m, 1H), 6.38 (d, *J* = 11 Hz, 1H), 6.16 (t, *J* = 15 Hz, 1H), 6.15–6.06 (m, 2H), 6.01 (d, *J* = 11 Hz, 1H), 5.33–5.29 (m, 1H), 5.01–4.96 (m, 1H), 4.47–4.40 (m, 1H), 4.26–4.18 (m, 1H), 2.84 (dd, *J* = 12, 3.8 Hz, 1H), 2.61 (dd, *J* = 14, 3.2 Hz, 1H), 1.36 (s, 9H), 1.09 (d, *J* = 6.8 Hz, 3H), 0.57 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 153.1, 147.3, 147.0, 142.5, 133.1, 124.8, 124.0, 120.8, 117.3, 112.6, 71.3, 66.8, 58.6, 56.1, 55.6, 46.1, 45.4, 42.8, 40.7, 40.3, 29.0, 27.4, 23.45, 23.38, 22.3, 19.7, 12.3; IR (neat) 3568–3087 (br), 3016, 2954, 2928, 2872, 1637, 1588, 1457, 1295, 1109, 755; UV (MeOH) λ_{max} 286 nm (ε 4442); HRMS: calcd for $C_{29}H_{44}O_4S$ 488.2960, found 488.2944.

MTT assay

The influence of the vitamin D_3 analogues on the antiproliferative activity of adriamycin in MCF-7 cells was determined using the MTT tetrazolium dye assay as described previously.[13] Cells were seeded in 96-well plates at 37 °C for 24 h, treated with the vitamin D_3 analogues for 48 h and washed twice with 200 μL of PBS before exposure to various concentrations of adriamycin for 2 h. Adriamycin was removed and cells were washed prior to an additional incubation period of 72 h. MTT (2 mg/mL in PBS) was added, cells were incubated at 37 °C for 3 h, MTT was removed, 100 μL of DMSO added to dissolve the crystallized dye and absorbance was determined at 490 nm. Each condition involved 6–8 replicate samples.

MCF-7 human breast cancer cells

These were plated in phenol red free Hams F12 media containing 5% charcoal stripped serum at a density of 2.5×10^5 cells per well in six well plates. After plating (24 h), cells were co-transfected using Fugene (Gibco) with 0.75 μg of pGL3/24-OHASE, a vitamin D_3 responsive 24-hydroxylase luciferase reporter construct (generously provided by Dr. J. Omdahl, University of New Mexico, Albuquerque, USA) and 0.25 μg of pRL SV40, a control luciferase vector (Promega). Triplicate wells were treated with ethanol vehicle (EtOH), calcitriol (**1**), or vitamin D_3 analogues **4** or **5a** at 1-, 10-, or 100-nM concentration for 22 h. Luciferase activity was analyzed with the Dual Luciferase Assay Kit (Promega) on a Victor 2 microplate reader. Luciferase activity of the pGL3/24-OHASE was corrected for transfection effeciency using the luciferase values obtained with the co-transfected control vector (pRL SV40). Data were statistically evaluated by ANOVA followed by Dunnetts post-hoc test using GraphPad Instat software.

Acknowledgements

The authors thank the NIH (CA 44530 to G.H.P. and T.W.K., CA 69700 to J.W., and DK 50583 to S.P.) and the American Institute for Cancer Research (99A091 to D.A.G.) for support of this research and H. Brem and S. Zand for the malignant melanoma assay.

References and Notes

1. Holick, M. F. *Vitamin D: Molecular Biology, Physiology, and Clinical Applications*; Humana: Totowa, 1999.
2. Feldman, D.; Glorieux, F.; Pike, J. W.; *Vitamin D*; Academic: San Diego, 1997.
3. Kutner, A., Ed., *Curr. Pharm. Des*. **2000**, *6*, 701.
4. Bouillon, R.; Okamura, W. H.; Norman, A. W. *Endocrine Rev*. **1995**, *16*, 200.
5. Posner, G. H.; Wang, Q.; Han, G.; Lee, J. K.; Crawford, K.; Zand, S.; Brem, H.; Peleg, S.; Dolan, P.; Kensler, T. W. *J. Med. Chem*. **1999**, *42*, 3425.
6. Posner, G. H.; Crawford, K.; Siu-Caldera, M.-L.; Reddy, G. S.; Sarabia, S. F.; Feldman, D.; van Etten, E.; Mathieu, C.; Gennaro, L.; Vouros, P.; Peleg, S.; Dolan, P.; Kensler, T. W. *J. Med. Chem*. **2000**, *43*, 3581.
7. Hansen, C. M.; Hamberg, K. J.; Binderup, E.; Binderup, L. *Curr. Pharm. Des* **2000**, *6*, 803.
8. Diaz, G. D.; Paraskeva, C.; Thomas, M. G.; Binderup, L.; Hague, A. *Cancer Res*. **2000**, *60*, 2304.
9. Bhattacharya, A.; Thyagarajan, G. *Chem. Rev*. **1981**, *81*, 415.
10. Tolstikov, G. A.; Miftakhov, M. S.; Adler, M. E.; Komissarova, N. G.; Kuznetsov, O. M.; Vostrikov, N. S. *Synthesis* **1989**, 940.
11. Corey, E. J.; Roberts, B. E.; Dixon, B. R. *J. Am. Chem. Soc*. **1995**, *117*, 193.
12. Posner, G. H.; Nelson, T. D.; Guyton, K. Z.; Kensler, T. W. *J. Med. Chem*. **1992**, *35*, 3280.
13. Munger, C.; Ellis, A.; Woods, K.; Randolph, J.; Yanovich, S.; Gewirtz, L. *Cancer Res*. **1988**, *48*, 2404.
14. Chaudry, M.; Sundaram, S.; Gewirtz, D. *Cancer Chemother. Pharmacol*. **2001**, *47*, 429.
15. Sundaram, S.; Chaudry, M.; Reardon, D.; Gupta, M.; Gewirtz, D. *Breast Cancer Res. Treat* **2001**, *63*, 1.
16. Posner, G. H.; Lee, J. K.; Wang, Q.; Peleg, S.; Burke, M.; Brem, H.; Dolan, D.; Kensler, T. W. *J. Med. Chem*. **1998**, *41*, 3008.
17. Kerry, D. M.; Dwivedi, P. P.; Hah, C. N.; Morris, H. A.; Omdahl, J. L.; May, B. K. *J. Biol. Chem*. **1996**, *271*, 29715.