Novel and Selective Calcitonin-Inducing Agents

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A series of xanthine sulfonamides is presented as a class of calcitonin (CT) inducers − a potentially new method for treating diseases associated with postmenopausal bone loss such as osteoporosis. We have found that certain di-*n*-butylxanthine sulfonamides **4** upregulate CT transcription in a CT-luciferase reporter gene assay (CT-luci) and increase the production and release of CT in a CT secretion/RIA assay (CTS). In addition, these compounds do not have potent PDE4 inhibitory activity as do the related xanthine methylene ketones such as denbufyllene **(2)**. One compound in particular **(9)** shows a transcription activation ratio (TAR) of 2.1 in CT-luci, a CTS increase of 3.6-fold, and a PDE4 (phosphodiesterase type IV) IC_{50} = 4.1 μM. In addition, this compound showed a statistically significant 47% trabecular bone protection in ovariectomized-induced osteopenia (OVX) rats as determined by assay when administered for 4 weeks at 30 mg/kg/day, i.p. by quantitative computed tomography (QCT). When administered p.o., compound **9** shows 50% trabecular bone protection when administered for 3 weeks at 50 mg/kg/day, i.p. This compared with salmon CT which shows 62% trabecular bone protection when administered at 50 IU/kg/day for 4 weeks.

Introduction

Calcitonin (CT) is a 32-amino acid polypeptide hormone secreted by the parafollicular or C cells of the thyroid gland in response to elevated blood levels of calcium. This hormone decreases blood calcium (hypocalcemic activity) primarily by inhibiting bone resorption through plasma membrane-associated receptors on the osteoclast. High-turnover bone loss, as seen with hypercalcemia of malignancy, estrogen withdrawal following the onset of menopause, and certain antiinflammatory or arthritis therapies, has recently been shown to be preventable by the administration of salmon calcitonin (s-CT).[1] As recently demonstrated for postmenopausal osteoporosis, s-CT treatment leads not only to a maintenance of bone mass and total body calcium but also to a decrease in the incidence of hip and vertebral fractures.[2] Thus, it is apparent that calcitonin is an appropriate therapeutic for the prevention and treatment of osteoporosis by virtue of its hypocalcemic activity.

Although s-CT has demonstrated efficacy in the prevention of high-turnover bone loss, a limitation for its widespread use is the lack of oral bioavailability necessitating administration by parental (intramuscular), intrapulmonary,[3] or, most commonly, intranasal routes.[4] References to oral delivery systems for s-CT have appeared in the patent literature: stable formulations,[5] polymer conjugation,[6] and delivery agent-mediated oral absorption,[7] but currently none have been approved for clinical use. However, the stimulation of endogenous calcitonin synthesis and release by inducer compounds would be expected to result in a similar therapeutic effect as the administration of nasal s-CT. To our knowledge, there have been no citings in the literature to a CT inducer. Related to this idea, one series of small organic molecules **(1)** has recently been claimed to act as CT mimetics (Chart 1).[8]

High-throughput screening of our compound library identified a potential CT-inducer compound, denbufyllene **(2)**, which was found to stimulate CT transcription as assessed by the measurement of luciferase activity from a CT promotor/luciferase receptor construct (CT-luci) as well as CT secretion and release in a CT secretion/RIA assay (CTS). However, we were aware that **2** displays polypharmacy, most notably phosphodiesterase type IV (PDE4) inhibition.[9] Moreover, xanthine PDE4 inhibitors such as **2** possess emetic side effects which prevent their clinical use due to their affinity for a hypothesized high-affinity rolipram **(3)** binding site in addition to their affinity for the catalytic site of PDE4.[10] There has been a suggested correlation between PDE4 inhibitory activity and bone-sparing activity in the literature: **2** has been reported to reduce bone loss in ovariectomy-induced osteopenic (OVX) rats;[11] however it is not clear whether the bone-sparing activity of **2** is due to its ability to increase CT transcription/CT secretion or whether it was due to its PDE4 inhibitory activity.

In this paper, we disclose a series of xanthine sulfonamides **4** that induce the expression and release of endogenous calcitonin without the potent PDE4 inhibitory activity of denbufylline **(2)**. In addition, some of these compounds were found to have bone-sparing

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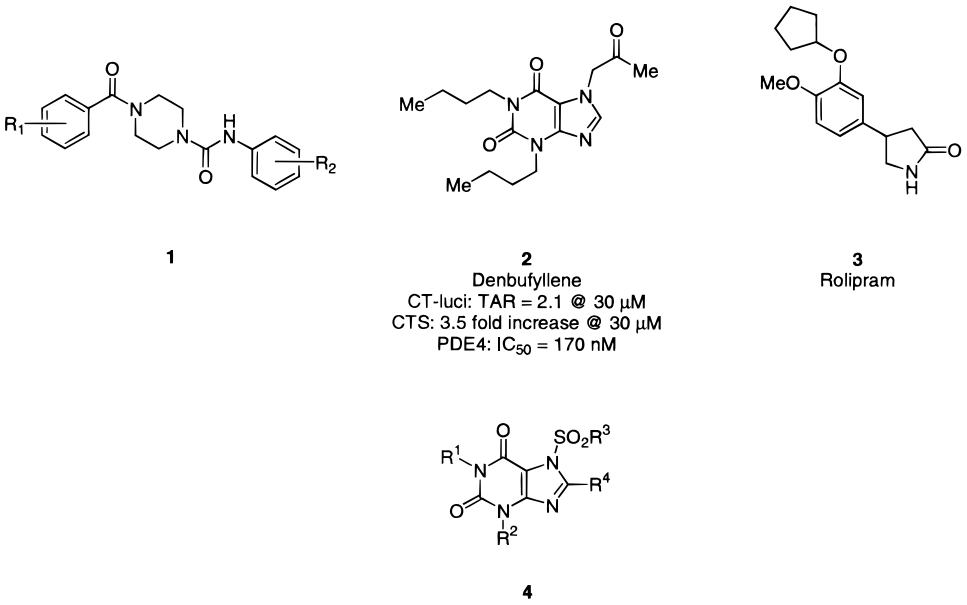
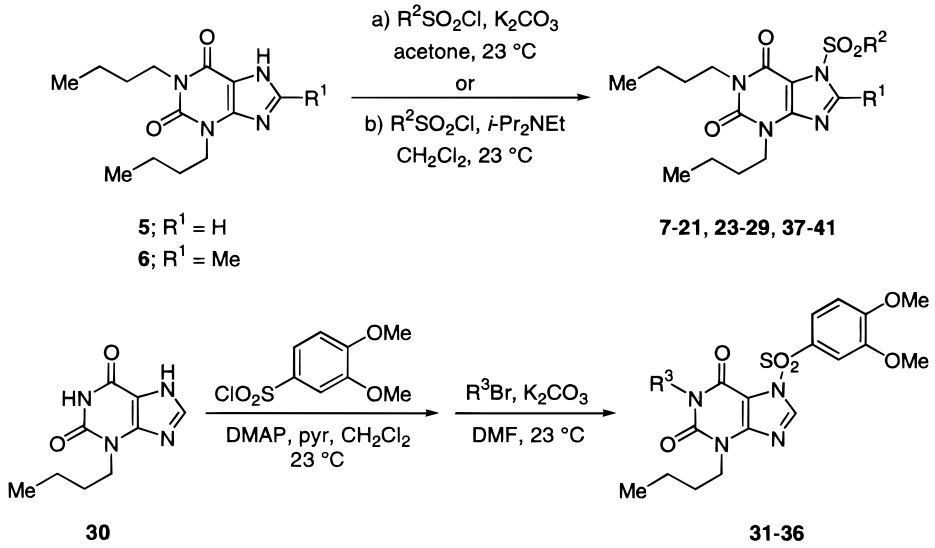
Chart 1



Scheme 1



activity in OVX rats which could lead to their use as a new class of antiosteoporotic agents.

Chemistry

Xanthine sulfonamides **7**−**21**, **23**−**29**, and **37**−**41** are prepared by sulfonating *N*(1),*N*(3)-di-*n*-butylxanthines **5**[12] and **6** with various sulfonyl chlorides using either (a) K_2CO_3 in acetone at 23 °C or (b) *i*-Pr_2NEt in CH_2Cl_2 at 23 °C. Sulfonamides **31**−**36** were prepared by selectively sulfonating *N*(3)-*n*-butylxanthine **30**[13] at N(7) with 3,4-dimethoxybenzenesulfonyl chloride in CH_2Cl_2 at 23 °C, followed by alkylation at N(3) with alkyl bromides using K_2CO_3 in DMF at 23 °C (Scheme 1).

Pharmacology

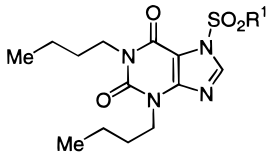
Newly synthesized compounds were evaluated in vitro using (1) a CT-luci reporter gene assay (CT-luci) to measure the ability of a compound to increase transcription of a luciferase receptor gene linked to the human CT gene, (2) a CT secretion/RIA assay (CTS) to measure the ability of a compound to increase cellular levels and secretion of human CT, and (3) a U937 cell PDE4 enzyme assay. In this assay, compounds were first tested for PDE4 activity at 30 μM; a percent rolipram PDE4 activity was determined with no rolipram inhibition set to 0% and full rolipram inhibition set at 100% (PDE4 30). Compounds of further interest were tested in this assay at multiple concentrations so that a proper IC_{50} could be determined.

Compounds which displayed a transcription activation ratio (TAR) equal to or greater than 2.0 at 30 μM, a CTS increase of equal to or greater than 2.0-fold at 30 μM, and a PDE4 $IC_{50} \geq 1.0$ μM were advanced to OVX studies to measure bone-sparing activity which was determined after 4 weeks of dosing using quantitative computed tomography (QCT) assessment of femoral bone mineral density.[14,15]

Results and Discussion

The *N*(1),*N*(3)-di-*n*-butylxanthine sulfonamides in Table 1 are representative of those compounds which

Table 1. In Vitro Profile of *N*(1),*N*(3)-Dibutylxanthine Sulfonamides



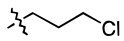
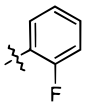
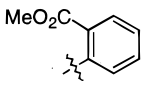
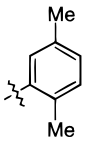
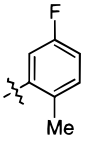
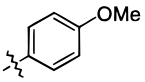
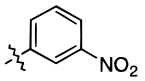
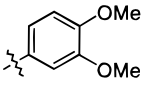
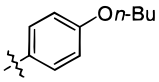
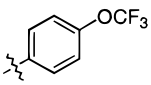
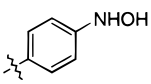
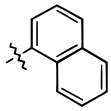
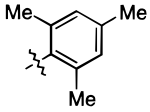
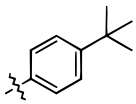
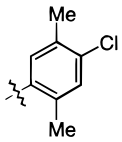
Compound	R^1	% Yield	Formula[a]	CT Luciferase TAR[b] (n)	CT Secretion Increase[c] (n)	PDE4 30 % rolipram activity[d](n)	PDE4 IC_{50}; μM[e] (n)
2	Denbufyllene	-	-	2.1 ± 0.3 (3)	3.5 ± 0.5 (3)	100 (3)	0.17 (2)
7	Me	77	$C_{14}H_{22}N_4O_4S$	1.9 (3)	4.7 (2)	84 (1)	25.2 (1)
8	*n*-Pr	27	$C_{16}H_{26}N_4O_4S$	2.3 (4)	2.2 (1)	54 (2)	-
9		90	$C_{16}H_{25}ClN_4O_4S$	2.1 ± 0.3 (3)	3.6 ± 0.4 (2)	71 (3)	4.1 (1)
10	*n*-Bu	82	$C_{17}H_{28}N_4O_4S$	2.0 (3)	4.7 (2)	15 (4)	-
11	*n*-octyl	98	$C_{21}H_{36}N_4O_4S$	2.1 (3)	4.7 (1)	12 (3)	-
12	Ph	61	$C_{19}H_{24}N_4O_4S$	1.9 (3)	3.9 (2)	5 (3)	-
13		86	$C_{19}H_{23}FN_4O_4S$	2.0 (4)	1.7 (1)	53 (1)	-
14		60	$C_{21}H_{26}N_4O_6S$	3.5 (2)	3.1 (1)	61 (1)	1.3 (1)
15		73	$C_{21}H_{28}N_4O_4S$	2.6 (3)	6.6 (1)	92 (1)	-
16		84	$C_{20}H_{25}FN_4O_4S$	2.8 (2)	6.6 (1)	86 (1)	-
17		80	$C_{20}H_{26}N_4O_5S$	1.9 (3)	4.6 (2)	49 (2)	-
18		79	$C_{19}H_{23}N_5O_6S$	2.0 (6)	2.9 (2)	37 (3)	-
19		81	$C_{21}H_{28}N_4O_6S$	2.5 (3)	4.7 (1)	59 (3)	1.2 (1)
20		88	$C_{23}H_{32}N_4O_5S$	2.2 (3)	5.0 (1)	54 (2)	-
21		79	$C_{20}H_{23}F_3N_4O_5S$	2.0 (3)	4.9 (1)	0 (3)	-
23		88	$C_{19}H_{25}N_5O_6S$	2.0 (3)	5.6 (1)	18 (3)	-
24		80	$C_{23}H_{26}N_4O_4S$	2.5 (3)	4.5 (1)	17 (3)	-

Table 1 (Continued)

Compound	R^1	% Yield	Formula[a]	CT Luciferase TAR[b] (n)	CT Secretion Increase[c] (n)	PDE4 30 % rolipram activity[d](n)	PDE4 IC_{50}; μM[e] (n)
25		63	$C_{17}H_{22}N_4O_4S_2$	2.2 (4)	2.9 (1)	48 (1)	-
26	Bn	56	$C_{20}H_{26}N_4O_4S$	2.3 (3)	5.1 (1)	7 (1)	3.2 (1)
27		90	$C_{22}H_{30}N_4O_4S$	1.9 (2)	4.0 (2)	58 (3)	6.0 (1)
28		57	$C_{23}H_{32}N_4O_4S$	1.8 (2)	5.1 (1)	33 (3)	-
29		77	$C_{21}H_{27}N_4O_4S$	2.5 (3)	5.3 (2)	72 (1)	23.3 (1)

[a] Structures of compounds confirmed by ^{1}H NMR, IR, and MS. Analytical results are within ±0.4% of the theoretical value. [b] Calcitonin luciferase assay (CT-luci) transcription activation ratio (TAR): transcription of a luciferase receptor gene linked to the human CT promotor gene expressed as a ratio of luciferase activity in the presence of test compound (at 30 μM) compared to the luciferase activity in the untreated control. [c] Calcitonin secretion assay (CTS): stimulation of CT secretion as expressed as a ratio of secretion activity in the presence of test compound compared to the secretion activity in the untreated control. [d] PDE4 30 assay: percent rolipram activity of the PDE4 assay at 30 μM concentration of test compound. [e] PDE4 assay: concentration at which 50% of the activity of the PDE4 assay is inhibited by test compound. *n*: number of experiments.

showed increased CT-luci/CTS values at 30 μM in conjunction with having reduced PDE4 inhibitory activity compared to that of denbufylline **(2)**. Practically all of these compounds have CT-luci results similar to the known PDE4 inhibitors denbufylline **(2)** and rolipram **(3)** as well as elevated CTS values. Other replacements for the sulfonamide moiety in this series − alkyl, ester, amide − produced molecules which fail to increase CT-luci/CTS and possessed moderate to potent PDE4 inhibitory activity.

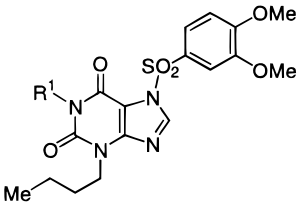
The sulfonamide substituent was varied in order to optimize the in vitro profile of the compounds in Table 1. Although the methyl sulfonamide **7** possesses good CT-luci activity (TAR: 1.9) as well as good activity in the CTS assay (CTS: 4.7), it also shows relatively potent PDE4 inhibitory activity when tested at 30 μM (PDE4 30: 84%). This was surprising considering that this compound had a relatively weak PDE4 IC_{50} (IC_{50}: 25.2 μM). Longer alkyl chains (**8**: *n*-Pr, **9**: (3-Cl)-*n*-Pr, **10**: *n*-Bu, and **11**: *n*-octyl) maintained CT-luci and CTS activity while showing reduced PDE4 inhibition. Sulfonamide **9** showed slightly more PDE4 inhibitory activity than the other alkyl analogues when tested in the PDE4 30 assay but only had an IC_{50} of 4.1 μM.

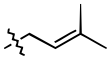
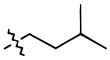
Aryl substituents on the sulfonamide give active compounds as well. The simple phenyl compound **12** possesses good CT-luci and CTS activity, with very little PDE4 inhibitory activity. Ortho substituents on the phenyl ring can lead to increased CT-luci and CTS activity but often at the expense of adding more PDE4 inhibition. For instance the *o*-F compound **13** maintains the good CT-luci activity of **12** (CT-luci: 2.0) but lacks much of its CTS activity (CTS: 1.7). However, the *o*-CO_2-Me compound **14** has excellent CT-luci and good CTS data while still having moderate PDE4 acivity (IC_{50} = 1.3 μM). Dramatic increases in CTS activity are seen with the 2,5-disubstituted phenyl compounds **15** and **16** (**15** CTS: 6.6, **16** CTS: 6.6) although additional PDE4 inhibition is seen as well (**15** PDE4 30: 92%, **16** PDE4 30: 86%).

4-Substituted phenyl sulfonamides give compounds with the desired in vitro profile − good CT-luci and CTS activity accompanied by weak to moderate PDE4 inhibition. All of the following 4-substituted aryl sulfonamides: the 4-OMe analogue **17**, the 3,4-dimethoxy analogue **19**, the 4-O*n*-Bu analogue **20**, the 4-OCF_3 analogue **21**, the 4-NHOH compound **21**, and the 4-*t*-Bu analogue **28** possess CT-luci TARs from 1.8 to 2.5, accompanied by CTS values from 4.6 to 5.6. All compounds were moderate to weak PDE4 inhibitors, particularly compound **21** which shows no PDE4 inhibitory activity. Combining ortho and para substitution leads to potent compounds as well. The 2,4,6-trimethyl analogue **26** possesses good CT-luci and CTS activity (CT-luci: 1.9, CTS: 4.0) and moderate PDE4 inhibition (IC_{50}: 6.0 μM). The 2,4,5-trisubstituted compound **29** also possesses an excellent activity profile (CT-luci: 2.5, CTS: 5.3, PDE4 IC_{50} = 23.3 μM) consistent with properties of an ortho- and a para-substituted sulfonamide.

In addition, other kinds of aromatic sulfonamides possess our desired in vitro activity profile. The 1-naphthyl compound **24** has an in vitro profile very similar to that of the Ph compound **12**. The isosteric thiophene sulfonamide **25** also shows a good in vitro profile although its CTS activity is lower than that of **12**.

Table 2. In Vitro Profile of 3-Substituted 3,4-Dimethoxybenzene Sulfonamides



Compound	R^1	% Yield	Formula[a]	CT Luciferase TAR[b] (n)	CT Secretion Increase[c] (n)	PDE4 30 % rolipram activity[d] (n)	PDE4 IC_{50}; μM[e] (n)
31	H	38	$C_{17}H_{20}N_4O_6S$	1.3 (2)	0.8 (1)	25 (1)	-
19	*n*-Bu	81	$C_{21}H_{28}N_4O_6S$	2.5 (3)	4.7 (1)	59 (3)	1.2 (1)
32	*n*-octyl	54	$C_{25}H_{36}N_4O_6S$	0.7 (2)	1.1 (1)	0 (1)	-
33		82	$C_{21}H_{26}N_4O_6S$	2.8 (3)	0.7 (1)	97 (1)	-
34		51	$C_{22}H_{28}N_4O_6S$	2.9 (3)	0.9 (1)	87 (1)	-
35		71	$C_{22}H_{28}N_4O_6S$	2.8 (3)	3.7 (1)	77 (2)	-
36		57	$C_{22}H_{30}N_4O_6S$	2.6 (3)	3.7 (1)	86 (1)	-

[a] Structures of compounds confirmed by ^{1}H NMR, IR, and MS. Analytical results are within ±0.4% of the theoretical value unless otherwise noted. [b] Calcitonin luciferase assay (CT-luci) transcription activation ratio (TAR): transcription of a luciferase receptor gene linked to the human CT promotor gene expressed as a ratio of luciferase activity in the presence of test compound (at 30 μM) compared to the luciferase activity in the untreated control. [c] Calcitonin secretion assay (CTS): stimulation of CT secretion as expressed as a ratio of secretion activity in the presence of test compound compared to the secretion activity in the untreated control. [e] PDE4 30 assay: percent rolipram activity of the PDE4 assay at 30 μM concentration of test compound. [e] PDE4 assay: concentration at which 50% of the activity of the PDE4 assay is inhibited by test compound. *n*: number of experiments.

Finally benzyl sulfonamide **26** shows excellent CT-luci and CTS activity with a moderate PDE4 IC_{50} of 3.2 μM. The CT-inducer activity of all of the aryl sulfonamides without substantial PDE4 inhibition is noteworthy since aryl sulfonamides (particularly 3,4-dimethoxyphenyl sulfonamides) have previously been published as having potent PDE4 inhibitory activity.[16]

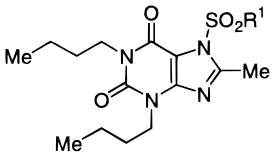
The observed CT-luci/CTS activity coupled with minimal to moderate PDE4 inhibition is due to a combination of the sulfonamide moiety in addition to the substituent at the N(3) position of the xanthine as shown in Table 2. While compound **19** (N(3): *n*-Bu) displays the desired in vitro profile, **31** (N(3): H) and **32** (N(3): *n*-octyl) have reduced PDE4 activity but at the expense of CT-luci and CTS. Compounds **33** and **34** display excellent CT-luci activity (**33** CT-luci: 2.8, **34** CT-luci: 2.9) but lack any CTS activity. In addition, both of these compounds containing branched alkyl chains at N(3) possess PDE4 inhibitory activity that approaches that of rolipram. Compounds **35** (N(3): allyl) and **36** (N(3): 3-methylbutyl) show both excellent CT-luci and CTS activity but have substantial PDE4 activity. The branching of the alkyl chains at N(3) leading to more PDE4 inhibition is consistent with known xanthine PDE4 SAR.[17]

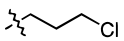
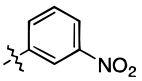
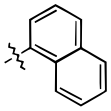
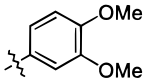
The hydrogen at C(6) in several xanthine sulfonamides was replaced with a Me group in an attempt to increase the CT-luci/CTS activity of these compounds. The results of this replacement are presented in Table 3. Compounds **37**−**41** all possess moderate CT-luci values (1.5−1.8) and moderate CTS values (2.2−4.1). However, all of these compounds have substantially more PDE4 inhibitory activity than the corresponding C(6) hydrogen compounds (see Table 1).

Several compounds from Table 1 with good CT-luci/CTS values and weak to moderate PDE4 activity were moved forward to OVX testing where bone-sparing activity was determined by QCT measurements after 4 weeks of compound administration at 30 mg/kg/day (Table 4). The 3-Cl-*n*-propyl sulfonamide **9** gave a statistically significant 47% bone mineral density (BMD) protection of trabecular bone when administered i.p., where 0% represents the BMD of the OVX rats and 100% is the BMD of sham-operated rats. This result compares favorably to both s-CT, the marketed form of CT, which gives a 62% bone-sparing effect when administered i.p. at 50 IU/kg and to denbufyllene **(2)** which gives a 49% bone-sparing effect at 30 mg/kg, i.p. Compound **9** was also tested on p.o. administration at 50 mg/kg and gave a statistically significant 50% BMD protection after 3 weeks of dosing. It is important to note that **9** has a PDE4 IC_{50} of 4.1 μM; thus it is doubtful that the bone-sparing activity could be due to PDE4 inhibition. In addition, further in vivo pharmacological characterization showed increases in both thyroidal calcitonin mRNA level and plasma calcitonin levels, consistent with induction of endogenous calcitonin (data not shown).

Other xanthine sulfonamides selected for OVX studies were phenyl sulfonamides. Compounds **10**, **18**, **19**, **24**,

Table 3. In Vitro Profile of *N*(1),*N*(3)-Di-*n*-butyl-6-methylxanthine Sulfonamides



Compound	R^1	% Yield	Formula[a]	CT Luciferase TAR[b] (n)	CT Secretion Increase[c] (n)	PDE4 30 % rolipram activity[d] (n)	PDE IV IC_{50}; μM[e] (n)
37		71	$C_{17}H_{27}ClN_4O_4S$	1.7 (2)	4.1 (1)	95 (1)	-
38		65	$C_{20}H_{25}N_5O_6S$	1.8 (2)	3.7 (1)	85 (1)	-
39		60	$C_{24}H_{25}N_4O_4S$	1.7 (1)	2.2 (1)	94 (1)	-
40		78	$C_{22}H_{30}N_4O_6S$	1.5 (1)	2.6 (1)	100 (1)	-
41	Ph	50	$C_{20}H_{26}N_4O_4S$	1.7 (1)	4.0 (1)	100 (1)	-

[a] Structures of compounds confirmed by ^{1}H NMR, IR, and MS. Analytical results are within ±0.4% of the theoretical value. [b] Calcitonin luciferase assay (CT-luci) transcription activation ratio (TAR): transcription of a luciferase receptor gene linked to the human CT promotor gene expressed as a ratio of luciferase activity in the presence of test compound (at 30 μM) compared to the luciferase activity in the untreated control. [c] Calcitonin secretion assay (CTS): stimulation of CT secretion as expressed as a ratio of secretion activity in the presence of test compound compared to the secretion activity in the untreated control. [d] PDE4 30 assay: percent rolipram activity of the PDE4 assay at 30 μM concentration of test compound. [e] PDE4 assay: concentration at which 50% of the activity of the PDE4 assay is inhibited by test compound. *n*: number of experiments.

Table 4. Ovariectomized-Induced Osteopenia Studies of Selected Compounds in Rats

Compound	Method of Administration	n^a	Duration of Assay (weeks)	Percent Protection (trabecular BMD, 30 mg/kg/day[b]
Salmon CT	i.p.	8	4	62 ± 5[c]*
2	i.p.	7	4	49 ± 9*
9	i.p.	7	4	47 ±8*
9	p.o.	8	3	50 ± 10[d]*
10	i.p.	7	4	23 ± 7
18	i.p.	8	4	30 ± 7
19	i.p.	8	4	35 ± 8
24	i.p.	8	4	37 ± 9
26	i.p.	8	4	25 ± 8
27	i.p.	8	4	50 ± 7*
29	i.p.	8	4	52 ± 9*

[a] Number of animals tested. [b] Trabecular bone mineral density measured by QCT. [c] 50 IU/kg. [d] 50 mg/kg/day. *$p < 0.05$: results are statistically significant.

and **26** all showed some bone protection activity at 30 mg/kg/day, i.p., but none of the results were statistically significant nor were these compounds as protective as **9**. Compounds **27** and **29** however gave substantial BMD protection (**27**: 50%, **29**: 52%) at 30 mg/kg/day, i.p. These compounds also have PDE4 IC_{50}s of 6.0 and 23.3 μM, respectively. It should be noted that all rats during the OVX studies did not suffer any species-specific PDE4 inhibitory effects (rats: apnea) when treated with any of the xanthine sulfonamides, an observation that correlates with the weak to moderate PDE4 inhibitory activity of these compounds.

Conclusions

We have demonstrated that a series of xanthine sulfonamides **4** can increase CT transcription secretion in vitro with only moderate to weak PDE4 inhibitory activity. Many of these compounds have CT-luci TARs of over 2.0 at 30 μM and CTS values of over 3.0 at 30 μM as well as moderate to weak PDE4 IC_{50}s (≥2.0 μM). Several of these molecules were put into OVX rats and were shown to be bone sparing. Compounds **9**, **27**, and **29** were shown to have statistically significant bone-sparing activity with **9** having oral bone-sparing activity at 50 mg/kg. All of these observations are consistent with compound **9** and possibly several of the xanthine sulfonamides presented above acting as CT inducers, which represents a potentially novel method of treating diseases of bone loss.

Experimental Section

Melting points were determined on a Thomas-Hoover Meltemp apparatus and are uncorrected. The proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded at 300

MHz on a Bruker DPX-300 spectrometer using tetramethylsilane (δ 0.0) as an internal standard. Infrared (IR) spectra were obtained as KBr pellets. Combustion analyses were obtained using a Perkin-Elmer series II 2400 CHNS/O analyzer. Mass spectra were obtained using a Micromass platform electrospray ionization quadrapole mass spectrometer. Flash chromatography was performed using EM Science 230−400 mesh silica gel. Thin-layer chromatography (TLC) was performed in Analtech silica gel GHLF 250-μm prescored plates.

1,3-Dibutyl-7-methanesulfonyl-3,7-dihydropurine-2,6-dione (7). Method A. To 500 mg (1.89 mmol) of 1,3-dibutyl-3,7-dihydropurine-2,6-dione[12] in 30 mL of acetone at 23 °C was added 1.04 g (7.56 mmol) of K_2CO_3 followed by 0.15 mL (216 mg, 1.89 mmol) of $MeSO_2Cl$. After stirring at 23 °C for 30 min, the reaction mixture was filtered, and the filtrate collected and evaporated to give a white solid. This solid was then triturated with 30 mL of petroleum ether to give 500 mg (1.46 mmol, a 77% yield) of the title compound as a white crystalline solid: mp 98−100 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.93−0.99 (m, 6H), 1.42−1.49 (m, 4H), 1.55−1.58 (m, 2H), 1.60−1.70 (m, 2H), 3.85 (s, 3H), 3.99−4.04 (m, 2H), 4.05−4.16 (t, 2H), 8.17 (s, 1H), 8.55; IR (KBr, cm^{-1}) 1715, 1663, 1537, 1397, 1182; MS 343 $(MH)^+$. Anal. $C_{14}H_{22}N_4O_4S$: C, H, N.

1,3-Dibutyl-7-propanesulfonyl-3,7-dihydropurine-2,6-dione (8). Method B. To 412 mg (1.56 mmol) of 1,3-dibutyl-3,7-dihydropurine-2,6-dione in 15 mL of CH_2Cl_2 at 23 °C were added 0.41 mL (302 mg, 2.34 mmol) of *i*-Pr_2NEt and 0.19 mL (245 mg, 1.72 mmol) of *n*-$PrSO_2Cl$. After stirring at 23 °C at 24 h, the reaction mixture as poured into 50 mL of brine and extracted with 2 × 30 mL of EtOAc. The combined organics were washed with 1 × 50 mL of H_2O and 1 × 50 mL of brine, dried over $MgSO_4$, filtered and evaporated to a light yellow oil. Flash chromatography on silica gel, eluting with CH_2Cl_2/EtOAc (40/1), gave the title compound as an off-white solid. Recrystallization from hot hexanes/EtOAc gave 156 mg (0.42 mmol, a 27% yield) of **8** as a white solid: mp 73−74 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.92−1.01 (m, 6H), 1.11 (t, J = 7.4 Hz, 3H), 1.32−1.48 (m, 4H), 1.60−1.79 (m, 4H), 1.92 (hex, J = 7.6 Hz, 2H), 3.96−4.06 (m, 4H), 4.12 (t, J = 7.6 Hz, 2H), 8.15 (s, 1H); IR (KBr, cm^{-1}) 1699, 1667, 1376; MS 371 $(MH)^+$. Anal. $C_{16}H_{26}N_4O_4S$: C, H, N.

1,3-Dibutyl-7-(3-chloropropane-1-sulfonyl)-3,7-dihydropurine-2,6-dione (9). Compound **9** was prepared according to method B using 1,3-dibutyl-3,7-dihydropurine-2,6-dione and $Cl(CH_2)_3SO_2Cl$: yield 90%; mp 71−73 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.90−1.01 (m, 6H), 1.32−1.49 (m, 4H), 1.56−1.80 (m, 4H), 2.30−2.42 (m, 4H), 3.69 (t, J = 6.1 Hz, 2H), 4.01 (t, J = 7.6 Hz, 2H), 4.12 (t, J = 7.4 Hz, 2H), 4.23 (t, J = 7.6 Hz, 2H), 8.15 (s, 1H); IR (KBr, cm^{-1}) 1704, 1670, 1385; MS 406 $(MH)^+$. Anal. $C_{16}H_{25}ClN_4O_4S$: C, H, N.

1,3-Dibutyl-7-butanesulfonyl-3,7-dihydropurine-2,6-dione (10). Compound **10** was prepared according to method A using 1,3-dibutyl-3,7-dihydropurine-2,6-dione and *n*-$BuSO_2Cl$: yield 82%; mp 185−187 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.93−0.99 (m, 9H), 1.39−1.88 (m, 11H), 3.98−4.09 (m, 6H), 4.10−4.19 (m, 4H), 8.14 (s, 1H); IR (KBr, cm^{-1}) 1717, 1672, 1466, 1194; MS 385 $(MH)^+$. Anal. $C_{17}H_{28}N_4O_4S$: C, H, N.

1,3-Dibutyl-7-octanesulfonyl-3,7-dihydropurine-2,6-dione (11). Compound **11** was prepared according to method B using 1,3-dibutyl-3,7-dihydropurine-2,6-dione and *n*-octylSO_2Cl: yield 98%; mp 59−60 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.89 (t, J = 7.5 Hz, 3H), 0.92−1.00 (m, 6H), 1.20−1.32 (m, 8 H), 1.34−1.49 (m, 6H), 1.57−1.68 (m, 2H), 1.70−1.79 (m, 2H), 1.79−1.89 (m, 2H), 3.97−4.05 (m, 4H), 4.12 (t, J = 7.5 Hz, 2H), 8.14 (s, 1H); IR (KBr, cm^{-1}) 1708, 1667, 1373, 1178; MS 441 $(MH)^+$. Anal. $C_{21}H_{36}N_4O_4S$: C, H, N.

7-Benzenesulfonyl-1,3-dibutyl-3,7-dihydropurine-2,6-dione (12). Compound **12** was prepared according to method A using 1,3-dibutyl-3,7-dihydropurine-2,6-dione and $PhSO_2Cl$: yield 61%; mp 144−146 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.92 (t, J = 7.5 Hz, 6H), 1.31−1.43 (m, 4H), 1.51−1.75 (m, 4H), 3.96 (t, J = 7.5 Hz, 2H), 4.03 (t, J = 7.5 Hz, 2H), 7.60 (t, J = 8.2 Hz, 2H), 7.68−7.76 (m, 1H), 8.29 (d, J = 8.1 Hz, 2H), 8.29 (s, 1H); IR (KBr, cm^{-1}) 1710, 1668; MS 405 $(MH)^+$. Anal. $C_{19}H_{24}N_4O_4S$: C, H, N.

1,3-Dibutyl-7-(2-fluorobenzenesulfonyl)-3,7-dihydropurine-2,6-dione (13). Compound **13** was prepared according to method B using 1,3-dibutyl-3,7-dihydropurine-2,6-dione and (2-F)$PhSO_2Cl$: yield 86%; mp 122−123 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.89 (t, J = 7.5 Hz, 3H), 0.96 (t, J = 7.5, 3H); 1.22−1.43 (m, 4H), 1.45−1.54 (m, 2H), 1.72 (pent, J = 7.5 Hz, 2H), 3.87 (t, J = 7.5 Hz, 2H), 4.08 (t, J = 7.4 Hz, 2H), 7.19 (t, J = 9.1 Hz, 1H), 7.44 (t, J = 7.9 Hz, 1H), 7.68−7.78 (m, 1H), 8.37 (s, 1H), 8.44 (td, J = 7.9, 1.7 Hz, 1H); IR (KBr, cm^{-1}) 1713, 1665; MS 423 $(MH)^+$. Anal. $C_{19}H_{23}FN_4O_4S$: C, H, N.

2-(1,3-Dibutyl-2,6-dioxo-1,2,3,6-tetrahydropurine-7-sulfonyl)benzoic Acid Methyl Ester (14). Compound **14** was prepared according to method A using 1,3-dibutyl-3,7-dihydropurine-2,6-dione and (2-CO_2Me)$PhSO_2Cl$: yield 60%; mp 106−109 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.88−1.0 (m, 6H), 1.30−1.43 (m, 4H), 1.50−1.60 (m, 2H), 1.66−1.77 (m, 2H), 3.92 (t, J = 7.6 Hz, 2H), 3.96 (s, 3H), 4.09 (t, J = 7.6 Hz, 2H), 7.65−7.81 (m, 3H), 8.32 (s, 1H), 8.86−8.94 (m, 1H); IR (KBr, cm^{-1}) 1711, 1668; MS 463 $(MH)^+$. Anal. $C_{21}H_{26}N_4O_6S$: C, H, N.

1,3-Dibutyl-7-(2,5-dimethylbenzenesulfonyl)-3,7-dihydropurine-2,6-dione (15). Compound **15** was prepared according to method A using 1,3-dibutyl-3,7-dihydropurine-2,6-dione and (2,5-Me_2)$PhSO_2Cl$: yield 73%; mp 120−122 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.89 (t, J = 7.3 Hz, 3H), 0.96 (t, J = 7.4 Hz, 3H), 1.23−1.43 (m, 4H), 1.46−1.56 (m, 2H), 1.63−1.78 (m, 2H), 2.46 (s, 6H), 3.88 (t, J = 7.5 Hz, 2H), 4.08 (t, J = 7.5 Hz, 2H), 7.17 (d, J = 7.8 Hz, 1H), 7.37 (dd, J = 1.9, 7.8 Hz, 1H), 8.32 (d, J = 2.0 Hz, 1H), 8.35 (s, 1H); IR (KBr, cm^{-1}) 1712, 1666; MS 433 $(MH)^+$. Anal. $C_{21}H_{28}N_4O_4S$: C, H, N.

1,3-Dibutyl-7-(5-fluoro-2-methylbenzenesulfonyl)-3,7-dihydropurine-2,6-dione (16). Compound **16** was prepared according to method A using 1,3-dibutyl-3,7-dihydropurine-2,6-dione and (5-F,2-Me)$PhSO_2Cl$: yield 84%; mp 88−90 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.90 (t, J = 7.3 Hz, 3H), 0.96 (t, J = 7.4 Hz, 3H), 1.26−1.46 (m, 4H), 1.48−1.56 (m, 2H), 1.68−1.74 (m, 2H), 2.48 (s, 3H), 3.88 (t, J = 7.6 Hz, 2H), 4.08 (t, J = 7.6 Hz, 2H), 7.26−7,33 (m, 2H), 8.24 (dd, J = 2.2, 8.0 Hz, 1H), 8.35 (s, 1H); IR (KBr, cm^{-1}) 1711, 1667; MS 437 $(MH)^+$. Anal. $C_{20}H_{25}FN_4O_4S$: C, H, N.

1,3-Dibutyl-7-(4-methoxybenzenesulfonyl)-3,7-dihydropurine-2,6-dione (17). Compound **17** was prepared according to method A using 1,3-dibutyl-3,7-dihydropurine-2,6-dione and (4-OMe)$PhSO_2Cl$: yield 80%; mp 68−70 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.91−0.99 (m, 6H), 1.38 (m, 4H), 1.53−1.75 (m, 4H), 3.89 (s, 3H), 3.94 (t, J = 7.5 Hz, 2H), 4.06 (t, J = 7.5 Hz, 2H), 7.03 (d, J = 8.1 Hz, 2H), 8.24 (d, J = 8.1 Hz, 2H), 8.27 (s, 1H); IR (KBr, cm^{-1}) 1714, 1667, 1167; MS 435 $(MH)^+$. Anal. $C_{20}H_{26}N_4O_5S$: C, H, N.

1,3-Dibutyl-7-(3-nitrobenzenesulfonyl)-3,7-dihydropurine-2,6-dione (18). Compound **18** was prepared according to method B using 1,3-dibutyl-3,7-dihydropurine-2,6-dione and (3-NO_2)$PhSO_2Cl$: yield 79%; mp 178−179 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.88−0.99 (m, 6H), 1.28−1.43 (m, 4H), 1.50−1.62 (m, 2H), 1.62−1.75 (m, 2H), 3.93 (t, J = 7.5 Hz, 2H), 4.10 (t, J = 7.5 Hz, 2H), 7.86 (t, J = 8.1 Hz, 1H), 8.34 (s, 1H), 8.56 (dd, J = 2.0, 8.1 Hz, 1H), 8.77 (d, J = 8.0 Hz, 1H), 9.01 (t, J = 2.0 Hz, 1H); IR (KBr, cm^{-1}) 1717, 1661, 1538; MS 450 $(MH)^+$. Anal. $C_{19}H_{23}N_5O_6S$: C, H, N.

1,3-Dibutyl-7-(3,4-dimethoxybenzenesulfonyl)-3,7-dihydropurine-2,6-dione (19). Compound **19** was prepared according to method B using 1,3-dibutyl-3,7-dihydropurine-2,6-dione and (3,4-OMe)$PhSO_2Cl$: yield 81%; mp 134−135 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.88−0.99 (m, 6H), 1.30−1.43 (m, 4H), 1.50−1.62 (m, 2H), 1.63−1.76 (m, 2H), 3.91−4.00 (m, 2H), 3.95 (s, 3H), 3.99 (s, 3H), 4.09 (t, J = 7.5 Hz, 2H), 6.98 (d, J = 8.7 Hz, 1H), 7.86 (dd, J = 2.3, 8.7 Hz, 1H), 7.97 (d, J = 2.3 Hz, 1H), 8.25 (s, 1H); IR (KBr, cm^{-1}) 1713, 1668, 1513, 1273; MS 465 $(MH)^+$. Anal. $C_{21}H_{28}N_4O_6S$: C, H, N.

7-(4-Butoxybenzenesulfonyl)-1,3-dibutyl-3,7-dihydropurine-2,6-dione (20). Compound **20** was prepared according to method B using 1,3-dibutyl-3,7-dihydropurine-2,6-dione and (4-*n*-OBu)$PhSO_2Cl$: yield 88%; mp 84−86 °C; ^{1}H NMR (300

MHz, $CDCl_3$) δ 0.85−1.00 (m, 9H), 1.10−1.41 (m, 4H), 1.42−1.53 (m, 2H), 1.53−1.61 (m, 2H), 1.61−1.75 (m, 2H), 1.75−1.81 (m, 2H), 3.94 (t, J = 7.6 Hz, 2H), 4.01−4.09 (m, 4H), 7.01 (d, J = 8.1 Hz, 2H), 8.18 (d, J = 8.1 Hz, 2H), 8.26 (s, 1H); IR (KBr, cm^{-1}) 1718, 1668, 1169; MS 477 $(MH)^+$. Anal. $C_{23}H_{32}N_4O_5S$: C, H, N.

1,3-Dibutyl-7-(4-trifluoromethoxybenzenesulfonyl)-3,7-dihydropurine-2,6-dione (21). Compound **21** was synthesized according to method B using 1,3-dibutyl-3,7-dihydropurine-2,6-dione and (4-OCF_3)$PhSO_2Cl$: yield 79%; mp 128−129 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 0.89−0.99 (m, 4H), 1.28−1.43 (m, 4H), 1.52−1.63 (m, 2H), 1.63−1.78 (m, 2H), 3.94 (t, J = 7.5 Hz, 2H), 4.07 (t, J = 7.5 Hz, 2H), 7.40 (d, J = 8.1 Hz, 2H), 8.28 (s, 1H), 8.37 (d, J = 8.1 Hz, 2H); IR (KBr, cm^{-1}) 1720, 1673, 1272, 1180, 1169; MS 489 $(MH)^+$. Anal. $C_{20}H_{23}F_3N_4O_5S$: C, H, N.

1,3-Dibutyl-7-(4-nitrobenzenesulfonyl)-3,7-dihydropurine-2,4-dione (22). Compound **22** was synthesized according to method B using 1,3-dibutyl-3,7-dihydropurine-2,6-dione and (4-NO_2)$PhSO_2Cl$: yield 69%; mp 138−139 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 0.89−0.98 (m, 6H), 1.29−1.42 (m, 3H), 1.52−1.62 (m, 3H), 1.65−1.74 (m, 2H), 3.92 (t, J = 7.5 Hz, 2H), 4.08 (t, J = 7.5 Hz, 2H), 8.31 (s, 1H), 8.42 (d, J = 8.1 Hz, 2H), 8.51 (d, J = 8.1 Hz, 2H); IR (KBr, cm^{-1}) 1717, 1671, 1178; MS 450 $(MH)^+$. Anal. $C_{19}H_{23}N_5O_6S$: C, H, N.

1,3-Dibutyl-7-(4-hydroxyaminobenzenesulfonyl)-3,7-dihydropurine-2,6-dione (23). To 4.75 g (10.57 mmol) of **22** in 100 mL EtOAc was added 1.0 g of 10% Pd/C. This heterogeneous mixture was hydrogenated under 1 atm of H_2 at 23 °C for 8 h (until H_2 uptake ceased). The reaction mixture was then filtered through Celite and evaporated to an off-white foam. Recrystallization from hot hexanes/EtOAc gave 3.91 g (8.98 mmol, an 88% yield) of **23** as a white solid: mp 148−150 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 0.88−0.98 (m, 6H), 1.35 (sept, J = 8.0 Hz, 4H), 1.51−1.61 (m, 2H), 1.61−1.73 (m, 2H), 3.93 (t, J = 7.5 Hz, 2H), 4.08 (t, J = 7.5 Hz, 2H), 5.85 (s, 1H), 7.03 (d, J = 8.1 Hz, 2H), 7.19 (brs, 1H), 8.11 (d, J = 8.1 Hz, 2H), 8.26 (s, 1H); IR (KBr, cm^{-1}) 1707, 1659, 1595, 1167; MS 420 $(MH)^+$. Anal. $C_{19}H_{25}N_5O_6S$: C, H, N.

1,3-Dibutyl-7-(naphthalene-1-sulfonyl)-3,7-dihydropurine-2,6-dione (24). Compound **24** was synthesized according to method B using 1,3-dibutyl-3,7-dihydropurine-2,6-dione and 1-naphthalenesulfonyl chloride: yield 80%; mp 135−136 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 0.82−0.97 (m, 6H), 1.20−1.39 (m, 4H), 1.41−1.53 (m, 2H), 1.60−1.72 (m, 2H), 3.83 (t, J = 7.5 Hz, 2H), 4.02 (t, J = 7.5 Hz, 2H), 7.57−7.78 (m, 3H), 7.98 (d, J = 8.2 Hz, 1H), 8.22 (d, J = 8.1 Hz, 1H), 8.47 (d, J = 8.2 Hz, 1H), 8.55 (s, 1H), 8.97 (d, J = 8.2 Hz, 1H); IR (KBr, cm^{-1}) 1712, 1664; MS 455 $(MH)^+$. Anal. $C_{23}H_{26}N_4O_4S$: C, H, N.

1,3-Dibutyl-7-(thiophene-2-sulfonyl)-3,7-dihydropurine-2,6-dione (25). Compound **25** was synthesized according to method B using 1,3-dibutyl-3,7-dihydropurine-2,6-dione and 2-thiophenesulfonyl chloride: yield 63%; mp 145−146 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 0.90−0.99 (m, 6H), 1.31−1.46 (m, 4H), 1.55−1.76 (m, 4H), 3.98 (t, J = 7.4 Hz, 2H), 4.08 (t, J = 7.4 Hz, 2H), 7.19 (t, J = 4.0 Hz, 1H), 7.83 (dd, J = 1.4, 5.0 Hz, 1H), 8.26 (s, 1H), 8.33 (dd, J = 1.4, 4.0 Hz, 1H); IR (KBr, cm^{-1}) 1713, 1670, 1392, 1176; MS 411 $(MH)^+$. Anal. $C_{17}H_{22}N_4O_4S_2$: C, H, N.

1,3-Dibutyl-7-phenylmethanesulfonyl-3,7-dihydropurine-2,6-dione (26). Compound **26** was synthesized according to method B using 1,3-dibutyl-3,7-dihydropurine-2,6-dione and benzylsulfonyl chloride: yield 56%; mp 117−118 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 0.90−1.01 (m, 6H), 1.31−1.52 (m, 4H), 1.62−1.79 (m, 4H), 4.00−4.13 (m, 4H), 5.23 (s, 2H), 7.21−7.43 (m, 5H), 7.77 (s, 1H); IR (KBr, cm^{-1}) 1705, 1672, 1438; MS 419 $(MH)^+$. Anal. $C_{20}H_{26}N_4O_4S$: C, H, N.

1,3-Dibutyl-7-(2,4,6-trimethylbenzenesulfonyl)-3,7-dihydropurine-2,6-dione (27). Compound **27** was synthesized according to method B using 1,3-dibutyl-3,7-dihydropurine-2,6-dione and 2,4,6-trimethylbenzenesulfonyl chloride: yield 90%; mp 125−127 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 0.87 (t, J = 7.2 Hz, 3H), 0.96 (t, J = 7.2 Hz, 3H), 1.15−1.30 (m, 2H), 1.30−1.48 (m, 2H), 1.49−1.56 (m, 2H), 1.65−1.78 (m, 2H), 2.32 (s, 3H), 2.61 (s, 6H), 3.83 (t, J = 7.4 Hz, 2H), 4.14 (t, J = 7.4 Hz, 2H), 7.00 (s, 2H), 8.35 (d, J = 2.1 Hz, 1H); IR (KBr, cm^{-1}) 1712, 1673, 1363; MS 447 $(MH)^+$. Anal. $C_{22}H_{30}N_4O_4S$: C, H, N.

1,3-Dibutyl-7-(4-*tert*-butylbenzenesulfonyl)-3,7-dihydropurine-2,6-dione (28). Compound **28** was synthesized according to method B using 1,3-dibutyl-3,7-dihydropurine-2,6-dione and 4-*tert*-butylbenzenesulfonyl chloride: yield 57%; mp 105−107 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 0.90−0.97 (m, 6H), 1.36 (s, 9H), 1.35−1.42 (m, 4H), 1.64−1.74 (m, 4H), 3.73 (t, J = 7.2 Hz, 2H), 4.06 (t, J = 7.5 Hz, 2H), 7.60 (d, J = 8.7 Hz, 2H), 8.18 (d, J = 8.8 Hz, 2H), 8.28 (s, 1H); IR (KBr, cm^{-1}) 1714, 1671, 1183, 1121; MS 461 $(MH)^+$. Anal. $C_{23}H_{32}N_4O_4S$: C, H, N.

1,3-Dibutyl-7-(4-chloro-2,5-dimethylbenzenesulfonyl)-3,7-dihydropurine-2,6-dione (29). Compound **29** was synthesized according to method A using 1,3-dibutyl-3,7-dihydropurine-2,6-dione and 4-chloro-2,5-dimethylbenzenesulfonyl chloride: yield 77%; mp 138−140 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 0.90 (t, J = 7.5 Hz, 3H), 0.95 (t, J = 7.5 Hz, 3H), 1.23−1.46 (m, 4H), 1.50−1.59 (m, 2H), 1.63−1.72 (m, 2H), 2.44 (s, 3H), 2.46 (s, 3H), 3.88 (t, J = 7.5 Hz, 2H), 4.07 (t, J = 7.5 Hz, 2H), 7.27 (s, 1H), 8.33 (s, 1H), 8.40 (s, 1H); IR (KBr, cm^{-1}) 1711, 1665, 1172; MS 467 $(MH)^+$. Anal. $C_{21}H_{27}N_4O_4S$: C, H, N.

1,3-Dibutyl-8-methyl-3,7-dihydropurine-2,6-dione (30). 6-Amino-1,3-di-*n*-butyl-5-nitrosouracil[12] (15.0 g, 52.8 mmol), 1.5 g 10% Pd/C and 150 mL DMF were hydrogenated at 40 psi in a Parr shaker for 4 h. After completion, the reaction mixture was filtered through Celite, and evaporated to give crude 5,6-diamino-1,3-di-*n*-butyluracil as a dark oil. To this oil were added 20 mL of acetic acid, 40 mL of DMF, 16.1 g (84 mmol) of 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride, and 177 mg (1.45 mmol) of DMAP, and the resulting mixture was stirred overnight. The reaction mixture was concentrated on a rotary evaporator, 100 mL of 2 N NaOH was added and this mixture was refluxed for 4 h. After cooling to 23 °C, the reaction mixture was adjusted to pH ∼ 7 with 6 N HCl which caused a precipitate to form. The solid was collected to give 14.1 g (50.7 mmol, a 96% yield) of the title compound as a yellow solid: mp 144−146 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 0.91−0.98 (m, 6H), 1.36−1.46 (m, 4H), 1.64−1.70 (m, 2H), 1.71−1.80 (m, 2H), 2.58 (s, 3H), 4.06−4.14 (m, 4H), 13.10 (brs, 1H); IR (KBr, cm^{-1}) 1701, 1650; MS 279 $(MH)^+$.

3-Butyl-7-(3,4-dimethoxybenzenesulfonyl)-3,7-dihydropurine-2,6-dione (31). To a suspension of 5.0 g (26.0 mmol) of 3-*N*-butylxanthene[13] in 50 mL CH_2Cl_2 were added 3.0 mL (2.93 g, 38.0 mmol) of pyridine, 7.4 g (31.0 mmol) of 3,4-dimethoxybenzenesulfonyl chloride and 3.5 g (28.65 mmol) of DMAP. The suspension was stirred at 23 °C for 5 days. The remaining precipitate was filtered and discarded. The resulting solution was washed with 1 × 100 mL of brine, dried over $MgSO_4$, filtered and evaporated to give a white solid. This solid was crystallized from hot ethyl acetate to give 4.0 g (9.8 mmol, a 38% yield) of the title compound as a white solid: mp 195−198 °C; ^1H NMR (300 MHz, DMSO-d_6) δ 0.90 (t, J = 7.5 Hz, 3H), 1.23−1.33 (m, 2H), 1.53−1.63(m, 4H), 3.85 (s, 3H), 3.87 (s, 3H), 7.24 (d, J = 8.6 Hz, 1H), 7.81 (d, J = 2.2 Hz, 1H), 7.84 (dd, J = 2.2, 8.6 Hz, 1H), 8.70 (s, 1H) 11.38 (s, 1H); IR (KBr, cm^{-1}) 1710, 1597, 1031; MS 409 $(MH)^+$. Anal. $C_{17}H_{20}N_4O_6S$: C, H, N.

3-Butyl-7-(3,4-dimethoxybenzenesulfonyl)-1-octyl-3,7-dihydropurine-2,6-dione (32). To a suspension of 408 mg (1.0 mmol) of 3-*N*-butyl-7-(3,4-dimethoxybenzenesulfonyl)-1*H*-3,7-dihydropurine-2,6-dione in 4 mL of DMF was added 165 mg (1.2 mmol) K_2CO_3, followed by 212 mg (1.02 mmol) of 1-bromooctane. The reaction was stirred at 50 °C for 5 h and then at 23 °C overnight. After pouring into 100 mL water, the product was extracted with 2 × 50 mL of EtOAc. The combined organics were dried over $MgSO_4$, filtered and evaporated to give a white solid. The solid was triturated with Et_2O and filtered to give 280 mg (0.54 mmol, a 54% yield) of the title compound as a while solid: mp 110−112 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 0.87 (t, J = 7.4 Hz, 3H), 0.94 (t, J = 7.5 Hz,

3H), 1.22−1.43 (m, 12H), 1.61−1.72 (m, 4H), 3.95 (s, 3H), 3.98 (s, 3H), 4.07 (t, J = 7.5 Hz, 4H), 8.97 (d, J = 8.7 Hz, 1H), 7.86 (dd, J = 2.3, 8.6 Hz, 1H), 7.97 (d, J = 2.2 Hz, 1H), 8.25 (s, 1H); IR (KBr, cm^{-1}) 1716, 1667, 1272; MS 521 $(MH)^+$. Anal. $C_{25}H_{36}N_4O_6S$: C, H, N.

3-Butyl-1-cyclopropylmethyl-7-(3,4-dimethoxybenzenesulfonyl)-3,7-dihydropurine-2,6-dione (33). Compound **33** was prepared according to the procedure for **32** using bromomethylcyclopropane in place of 1-bromooctane: yield 82%; mp 139−141 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.41−0.52 (m, 4H), 0.95 (t, J = 7.5 Hz, 3H), 1.15−1.26 (m, 1H), 1.38 (sex, J = 7.4 Hz, 2H), 1.61−1.74 (m, 2H), 3.87 (d, J = 7.1 Hz, 2H), 3.95 (d, 3H), 3.99 (s, 3H), 4.07 (t, J = 7.5 Hz, 2H), 7.86 (d, J = 2.3, 8,6 Hz, 1H), 7.96 (d, J = 2.3 Hz, 1H), 8.26 (s, 1H); IR (KBr, cm^{-1}) 1713, 1666, 1270; MS 463 $(MH)^+$. Anal. $C_{21}H_{26}N_4O_6S$: C, H, N.

3-Butyl-1-cyclobutylmethyl-7-(3,4-dimethoxybenzenesulfonyl)-3,7-dihydropurine-2,6-dione (34). Compound **34** was prepared according to the procedure for **32** using bromomethylcyclobutane in place of 1-bromooctane: yield 51%; mp 150−151 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.93 (t, J = 7.5 Hz, 3H), 1.38 (sex, J = 7.4 Hz, 2H), 1.57−1.72 (m, 2H), 1.76−1.85 (m, 4H), 1.91−2.00 (m, 2H), 2.60−2.72 (m, 1H), 3.95 (s, 3H), 3.99 (s, 3H), 4.06 (t, J = 7.5 Hz, 2H), 6.96 (d, J = 8.5 Hz, 1H), 7.86 (dd, J = 2.2, 8.6 Hz, 1H), 7.97 (d, J = 2.2 Hz, 1H), 8.25 (s, 1H); IR (KBr, cm^{-1}) 1712, 1667, 1272; MS 477 $(MH)^+$. Anal. $C_{22}H_{28}N_4O_6S$: C, H, N.

3-Butyl-7-(3,4-dimethoxybenzenesulfonyl)-1-(3-methylbut-2-enyl)-3,7-dihydropurine-2,6-dione (35). Compound **35** was prepared according to the procedure for **32** using 1-bromo-3-methylbutene in place of 1-bromooctane: yield 71%; mp 92−95 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.94 (t, J = 7.5 Hz, 3H), 1.25−1.41 (m, 2H), 1.54−1.59 (m, 4H), 1.68 (s, 3H), 1.71 (s, 3H), 3.95 (s, 3H), 3.98 (s, 3H), 4.06 (t, J = 9 Hz, 2H), 4.57 (d, J = 6 Hz, 1H), 5.20 (t, J = 12 Hz, 1H), 6.95 (d, J = 9 Hz, 1H), 7.82 (dd, J = 2.1, 10.8 Hz, 1H), 8.05 (d, J = 2.1 Hz, 1H) 8.24 (s, 1H); IR (KBr, cm^{-1}) 1711, 1677; MS 477$(MH)^+$. Anal. $C_{22}H_{28}N_4O_6S$: C, H, N.

3-Butyl-7-(3,4-dimethoxybenzenesulfonyl)-1-(3-methylbutyl)-3,7-dihydropurine-2,6-dione (36). Compound **36** was prepared according to the procedure for **32** using 1-bromo-3-methylbutane in place of 1-bromooctane: yield 57%; mp 134−135 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.87−0.93 (m, 3H), 0.89 (d, J = 7.3 Hz, 6H), 1.21−1.40 (m, 6H), 1.43−1.65 (m, 3H), 3.85 (s, 3H), 3.87 (s, 3H), 3.92 (t, J = 7.5 Hz, 2H), 7.24 (d, J = 8.3 Hz, 1H), 7.81−7.87 (m, 2H), 8.73 (s, 1H); IR (KBr, cm^{-1}) 1712, 1668, 1273; MS 479 $(MH)^+$. Anal. $C_{22}H_{30}N_4O_6S$: C, H, N.

1,3-Dibutyl-7-(3-chloropropane-1-sulfonyl)-8-methyl-3,7-dihydropurine-2,6-dione (37). Compound **37** was prepared according to method A using 1,3-di-*n*-butyl-8-methyl-3,7-dihydropurine-2,6-dione and $Cl(CH_2)_3SO_2Cl$: yield 71%; mp 76−78 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.92−0.99 (m, 6H), 1.33−1.42 (m, 4H), 1.57−1.65 (m, 2H), 1.67−1.74 (m, 2H), 2.32−2.41 (m, 2H) 2.74 (s, 3H), 3.69 (t, J = 6.2 Hz, 2H), 3.99 (t, J = 7.6 Hz, 2H), 4.08 (t, J = 7.5 Hz, 2H), 4.23 (t, J = 7.5 Hz, 2H); IR (KBr, cm^{-1}) 1705, 1522; MS 419 $(MH)^+$. Anal. $C_{17}H_{27}ClN_4O_4S$: C, H, N.

1,3-Dibutyl-8-methyl-7-(3-nitrobenzenesulfonyl)-3,7-dihydropurine-2,6-dione (38). Compound **38** was prepared according to method A using 1,3-di-*n*-butyl-8-methyl-3,7-dihydropurine-2,6-dione and (3-NO_2)$PhSO_2Cl$: yield 65%; mp 166−168 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.88−0.97 (m, 6H), 1.21−1.43 (m, 4H), 1.48−1.77 (m, 4H), 2.92 (s, 3H), 3.90 (t, J = 7.5 Hz, 2H), 4.04 (t, J = 7.4 Hz, 2H), 7.84 (t, J = 8.1, 1H), 8.55 (dd, J = 2.0, 8.2 Hz, 1H), 8.71 (dd, J = 2.0, 8.2 Hz, 1H), 8.95 (t, J = 2.0 Hz, 1H); IR (KBr, cm^{-1}) 1705, 1669; MS 464 $(MH)^+$. Anal. $C_{20}H_{25}N_5O_6S$: C, H, N.

1,3-Dibutyl-8-methyl-7-(naphthalene-1-sulfonyl)-3,7-dihydropurine-2,6-dione (39). Compound **39** was prepared according to method A using 1,3-di-*n*-butyl-8-methyl-3,7-dihydropurine-2,6-dione and 1-naphthalenesulfonyl chloride: yield 60%; mp 96−98 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.84 (t, J = 7.4 Hz, 3H), 0.93 (t, J = 7.4 Hz, 3H), 1.15−1.22 (m, 2H), 1.32−1.49 (m, 4H), 1.60−1.69 (m, 2H), 3.06 (s, 3H), 3.77 (t, J = 7.4 Hz, 2H), 4.01 (t, J = 7.5 Hz, 2H), 7.57−7.71 (m, 3H), 7.97 (dd, J = 2.1, 8.2 Hz, 1H), 8.19 (d, J = 8.1 Hz, 1H), 8.23 (d, J = 8.2 Hz, 1H), 8.76 (d, J = 8.2 Hz, 1H); IR (KBr, cm^{-1}) 1711, 1672; MS 469 $(MH)^+$. Anal. $C_{24}H_{25}N_4O_4S$: C, H, N.

1,3-Dibutyl-7-(3,4-dimethoxybenzenesulfonyl)-8-methyl-3,7-dihydropurine-2,6-dione (40). Compound **40** was prepared according to method A using 1,3-di-*n*-butyl-8-methyl-3,7-dihydropurine-2,6-dione and (3,4-OMe)$PhSO_2Cl$: yield 78%; mp 145−147 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.89−0.97 (m, 6H), 1.29−1.43 (m, 4H), 1.51−1.72 (m, 4H), 2.83 (s, 3H), 3.95 (s, 3H), 3.98 (s, 3H), 3.97 (t, 2H, obscured by the δ 3.95 and 3.98 singlets), 4.03 (t, J = 7.4 Hz, 3H); IR (KBr, cm^{-1}) 1707, 1659; MS 479 $(MH)^+$. Anal. $C_{22}H_{30}N_4O_6S$: C, H, N.

7-Benzenesulfonyl-1,3-dibutyl-8-methyl-3,7-dihydropurine-2,6-dione (41). Compound **41** was prepared according to method A using 1,3-di-*n*-butyl-8-methyl-3,7-dihydropurine-2,6-dione and $PhSO_2Cl$: yield 50%; mp 87−89 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.88−0.98 (m, 6H), 1.26−1.43 (m, 4H), 1.50−1.61 (m, 2H), 1.62−1.73 (m, 4H), 2.88 (s, 3H), 3.93 (t, J = 7.5 Hz, 2H), 4.03 (t, J = 7.3 Hz, 2H), 7.61 (t, J = 8.4 Hz, 2H), 7.66−7.73 (m, 1H), 8.25 (d, J = 8.2 Hz, 2H); IR (KBr, cm^{-1}) 1711, 1667; MS 419 $(MH)^+$. Anal. $C_{20}H_{26}N_4O_4S$: C, H, N.

Pharmacological Methods. 1. Calcitonin/Luciferase Reporter Gene Expression Assay/Calcitonin Secretion Assay. Assessment of the ability of compounds to stimulate human calcitonin expression was performed using a reporter cell line, designated C3 which has been previously described in the patent literature.[18] This human medullary thyrocarcinoma cell line (TT; ATCC) was stably transfected with 3 kb (from the translation start site) of the upstream elements of the human calcitonin gene fused to a luciferase reporter construct. For assay of transcriptional activation ability, C3 cells were plated at a density of 6500−7500 cells/well in a 96-plate microtiter plate. Twenty-four to forty-eight hours later, compounds were added to the wells in triplicate. Compounds were tested at a concentration of 10 μg/mL in 0.5% DMSO. Compounds that exhibited a transcription activation ratio (TAR) equal to or greater than 1.5 (equivalent to a 50% increase in transcription or greater) advanced to primary followup (CA-FUP), in which compounds were rescreened at four concentrations: 10, 2, 0.4, and 0.08 μg/mL. Controls were distributed throughout the plate and included (1) unstimulated cells for basal luciferase expression and (2) cells stimulated with 1 mM 8-CPT-*c*-AMP (results in 2.1 ± 0.3-fold induction). The plates were incubated for 12 h in a humidified CO_2 incubator, washed and then lysed in luciferase assay buffer as previously described.[18] The luciferase activity was determined with a commercially available assay (Promega) by measurement of the light signal in a luminometer.

TAR ratio: Stimulation of calcitonin promoter-dependent transcription is expressed as a ratio of luciferase activity (CT-luci) in the presence of test compound compared to the CT-luci activity in the untreated control.

Following stimulation of the C3 cells for the determination of transcription activation, culture media was aspirated and assayed for calcitonin levels in duplicate using a commercially available radioimmunoassay (RIA; Nichols Institute Diagnostics, kit #40-2125) according to the manufacturers instructions.

Secretion ratio: Stimulation of calcitonin secretion is expressed as a ratio of secretion activity in the presence of test compound compared to the secretion activity in the untreated control.

2. Ovariectomy-Induced Osteopenia in Rats. The ability of compounds to prevent ovariectomy-induced bone loss in female rats was determined in mature Sprague−Dawley CD rats, weighing 225−250, and the time of ovariectomy. Rats were either bilaterally ovariectomized (OXV) or sham-operated (SHAM) and allowed 4−5 days recovery before initiation of dosing. Rats were housed in metal cages (3−5 rats/cage) with access to food and water ad libitum on a 14/10 h day/night cycle. The concentration of the drug stock was calculated to

be delivered in a volume of 0.1 mL/100 g of body weight and was administered either intraperitoneally (i.p.) or orally (p.o.). The drug solution or a uniform suspension was prepared in 1% Tween 80 in normal saline and was administered daily (five times a week) for 6 weeks.

A sequential double labeling of mineralized tissue with a fluorochrome was employed to determine the osseous changes (especially the bone formation) and the mineralization rates. Each animal was administered either 90 or 15 mg/kg calcein (Sigma Chemical Co.), subcutaneously approximately 2 days and then 9 days prior to the termination of the study, respectively.

Four weeks after the initiation of treatment, each rat was evaluated for total and trabecular density (TotD and TrabD) of the proximal tibia. TotD and TrabD were measured in anesthetized rats using a XCT-960M (Stratec Medizintechnik, Pforzheim, Germany). Measurements were performed as follows: 10 min prior to scanning, animals were anesthetized with an i.p. injection of 50 mg/kg ketamine (Bristol Laboratories, Syracuse, NY), xylazine (5 mg/kg), and 1 mg/kg acepromazine (Aveco, Ft. Dodge, IA). The right hind limb was passed through a polycarbonate tube with a diameter of 25 mm and taped to an acrylic frame with the ankle joint at a 90° angle and the knee joint at 180°. The polycarbonate tube was affixed to a sliding platform that maintained it parallel to the aperture of the XCT. The platform was adjusted so that the distal end of the femur and the proximal end of the tibia would be in the scanning field. A two-dimensional scout view was run for a length of 10 mm and a line resolution of 0.2 mm. After the scout view was displayed on the monitor, the proximal end of the tibia was located. The CT scan was initiated 3.4 mm distal from this point. The CT scan was 1 mm thick, had a voxel (three-dimensional pixel) size of 0.140 mm, and consisted of 145 projections through the slice.

The difference in bone mineral density percent of the proximal tibia between a treatment group and the vehicle group was analyzed using a one-way analysis of variance with Dunnett's test or other multiple comparison methods. Compounds were designated active if $p < 0.05$ vs vehicle value.

3. Inhibition of Phosphodiesterase IV (PDE4) Isolated from Human U937 Cells. The human leukemic cell line (U937) was maintained in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum and penicillin/streptomycin (100 units/100 μg/mL) in a humidified 5% CO_2 atmosphere at 37 °C. Starter cells were maintained in continuous logarithmic growth by seeding them in 75 cm^2 vented tissue culture flasks at a concentration of 3×10^4 cells/mL and passing them every 3−4 days when the cells reached approximately 8×10^5 cells/mL. For experiments, 3−4 day starter cells were seeded in 225 cm^2 flasks at 1×10^5 cells/mL and harvested 3−4 days later at approximately 6×10^5 cells/mL.

For isolation of PDE4 enzyme activity, U937 cells were activated with 10 μM dibutyryl cAMP for a period of 4 h (to upregulate PDE4) and then harvested by centrifugation at 1200*g* for 10 min in 250-mL conical centrifuge tubes. The pellet from each 200-mL flask was resuspended in 5 mL of buffer A [10 mM Tris-HCl, 5 mM $MgCl_2$, 4 mM EGTA, 5 mM 2-mercaptoethanol, 1 μM leupeptin, 1 μM pepstatin A, and 5 μM phenylmethanesulfonyl fluoride (PMSF) (pH 7.8)], and the cells were lysed using three cycles of freezing (3 min in dry ice/acetone) followed by thawing (warm water). The extract was centrifuged for 20 min at 1200*g* to remove cell debris and the supernatant was immediately loaded onto a 1.6×70 cm DEAE-Sepharose CL-6B anion-exchange column equilibrated with buffer A. The column was next washed with 2.5 column volumes of buffer B [10 mM Tris-HCl, 5 mM 2-mercaptoethanol, 0.1 μM leupeptin, 0.1 μM pepstatin A, and 0.1 μM PMSF (pH 7.8)], and PDEs were eluted with a step gradient consisting of 80 mL each of buffer B containing 0.4 or 0.7 M sodium acetate (80 mL/h, 8 mL/fraction). To determine which families of PDEs were present, fractions were assayed for hydrolytic activity with 1 μM [^{3}H]cAMP or 1 μM [^{3}H]cGMP using a commercially available assay (Amersham) according to the manufacturer's instructions. Additionally, 1 μM [^{3}H]cAMP assays were conducted in the absence and presence of 10 μM of the selective PDE4 inhibitor rolipram. Fractions which showed cAMP hydrolytic activity which was inhibitable by rolipram were pooled.

When prepared in this manner, approximately 80% of total cAMP PDE activity is eluted by buffer B containing 0.7 M sodium acetate. This PDE activity, which consists of >90% PDE4 (as evidenced by its susceptibility to inhibition by rolipram), was stored in 30% ethylene glycol at 20 °C; 15 μL of the PDE was typically required to obtain sufficient activity for inhibition assays (approximately 25000 dpm). Enzyme activity was determined at 37 °C. The amount of enzyme and duration of assay were adjusted to ensure that less than 25% of the substrate was consumed under these conditions. PDE activities of U937 cell PDE4 preparations have been found to be linear for at least 30 min. To test inhibition of PDE4, a test compound was added to the reaction mixture, usually at concentrations ranging from 0.001 to 10 μM. Inhibition by a test compound was measured as a percent reduction of total PDE activity and calculated as follows:

$$\frac{A}{B} \times 100 = \%\ \text{inhibition of PDE4}$$

where A is the PDE activity (mean dpm − background dpm) in the presence of test compound and B is the total PDE4 activity (mean dpm − background dpm) in the absence of test compound. Percent inhibition of PDE4 values were then normalized to rolipram values where the rolipram percent inhibition of PDE IV is set to 100%.

IC_{50}s were estimated by linear regression analysis using the percent inhibition data bracketing 50% inhibition.

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