γ-Pyrones from *Gonystylus keithii*, as New Inhibitors of Parathyroid Hormone (PTH)-Induced Ca Release from Neonatal Mouse Calvaria

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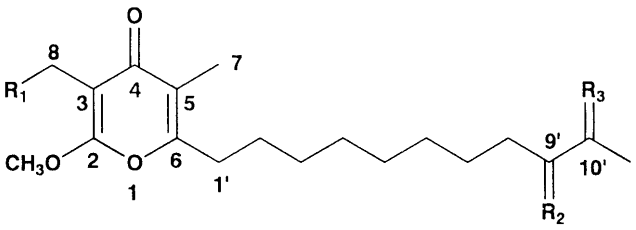
New γ-pyrones, 9′-oxopodopyrone (3) and 8-methyl-9′-oxopodopyrone (4) were isolated from the leaves of *Gonystylus keithii*, along with known γ-pyrones, 10′-oxopodopyrone (1) and 8-methyl-10′-oxopodopyrone (2). These γ-pyrones markedly inhibited the bovine parathyroid hormone (PTH)-induced Ca release from neonatal mouse calvaria *in vitro*. It is the first time that γ-pyrones showed inhibitory effects on bone resorption, and these compounds may be seed compounds of new drugs for osteoporosis.

Key words γ-pyrone; *Gonystylus keithii*; bone resorption; Thymeliaceae; podopyrone

The occurrence of osteoporosis has increased with the growing population of aged people. Osteoporosis is said to be caused by a disorder of the balance between bone formation and resorption.[4] There are two ways to research new drugs for osteoporosis: one is to stimulate bone formation and the other is to suppress bone resorption. No drug is known to stimulate bone formation, although vitamin K_2[5] and parathyroid hormones (PTH)[6] are assumed to stimulate bone formation. On the other hand, there are many drugs which suppress bone resorption, and the following drugs have already been used or are under investigation: calcitonins,[7] osteostachines,[8] estradiols,[9] bisphosphonates[10] and ipriflavone.[11] Osteoporosis will become an even more serious problem for aged people in developed countries, especially for women, so it is important to investigate new drugs for treating osteoporosis. We have planned to screen for bone resorption inhibitor using an assay system of bovine PTH (bPTH)-induced Ca release from neonatal mouse calvaria. In the course of our screening of one hundred plant extracts, the methanol extract of the leaves of *Gonystylus keithii* (Thymeliaceae), collected at Sabah (Malaysia), exhibited a remarkable inhibitory effect. Its active compounds (**1**—**4**) were isolated using solvent fractionation and preparative HPLC.

Compound **1**, colorless oil, showed the molecular formula $C_{19}H_{30}O_4$ by HR-EI-MS. In the ^{1}H-NMR spectrum, four methyl signals were observed at δ 3.94, 2.13, 1.94 and 1.85 (Table 1). The signal at δ 3.94 in the ^{1}H-NMR spectrum, and that of δ 55.3 in the ^{13}C-NMR spectrum (Table 2) indicate the presence of a methoxy group on sp^2 carbon. The signal at δ 2.13 in the ^{1}H-NMR



	R_1	R_2	R_3
1	H	= H,H	= O
2	CH_3	= H,H	= O
3	H	= O	= H,H
4	CH_3	= O	= H,H

Fig. 1. Structure of Isolated Compounds

Table 1. ^{1}H-NMR Spectral Data of Compounds **1**—**4** in $CDCl_3$

	1	**2**	**3**	**4**
7	1.94 (3H, s)	1.93 (3H, s)	1.94 (3H, s)	1.94 (3H, s)
8	1.85 (3H, s)	2.38 (2H, q, J=7.5 Hz)	1.85 (3H, s)	2.39 (2H, q, J=7.5 Hz)
9	—	1.03 (3H, t, J=7.5 Hz)	—	1.03 (3H, t, J=7.5 Hz)
1′	2.57 (2H, t, J=7.5 Hz)	2.56 (2H, t, J=7.5 Hz)	2.57 (2H, t, J=7.5 Hz)	2.57 (2H, t, J=7.5 Hz)
2′	1.64 (2H, m)	1.64 (2H, m)	1.63 (2H, m)	1.64 (2H, m)
3′				
4′				
5′	1.28, 1.32 (10H, m)	1.28, 1.32 (10H, m)	1.28, 1.32 (8H, m)	1.28, 1.32 (8H, m)
6′				
7′			1.58 (2H, m)	1.58 (2H, m)
8′	1.56 (2H, m)	1.57 (2H, m)	2.38 (2H, t, J=7.5 Hz)	2.39 (2H, t, J=7.5 Hz)
9′	2.41 (2H, t, J=7.5 Hz)	2.41 (2H, t, J=7.5 Hz)	—	—
10′	—	—	2.41 (2H, q, J=7.5 Hz)9	2.41 (2H, q, J=7.5 Hz)
11′	2.13 (3H, s)	2.13 (3H, s)	1.05 (3H, t, J=7.5 Hz)	1.05 (3H, t, J=7.5 Hz)
OMe	3.94 (3H, s)	3.94 (3H, s)	3.94 (3H, s)	3.94 (3H, s)

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spectrum, signals at δ 209.2 and 29.8 in the ^{13}C-NMR spectrum, and the absorption at 1720 cm^{-1} in the IR spectrum indicate the presence of a methyl ketone moiety. The absorptions at 1650 and 1550 cm^{-1} in the IR spectrum, the absorption (λ_{max}) at 252 nm in the UV spectrum, and the signals at δ 99.3, 118.2, 158.4, 162.1 and 181.0 in the ^{13}C-NMR spectrum indicate the presence of γ-pyrone moiety. This assumption was supported by the results of two dimensional (2D)-NMR experiments; correlation spectroscopy (COSY), heteronuclear single quantum coherence (HSQC) and heteronuclear multiple bond connectivity (HMBC). The precise analysis of 2D-NMR spectra revealed that the structure of **1** is 6-substituted 2-methoxy-3,5-dimethyl-4*H*-pyrane-4-one, and the substitution at the 6 position was shown to be a 10-oxoundecanyl group. Therefore, **1** was identified as 10′-oxopodopyrone, 2-methoxy-3,5-dimethyl-6-(10′-oxoundecanyl)-4*H*-pyrane-4-one, which has been isolated from *Podolepis longipedata*.[12] The structure of **1** was further confirmed by synthesis, as shown in Chart 1.

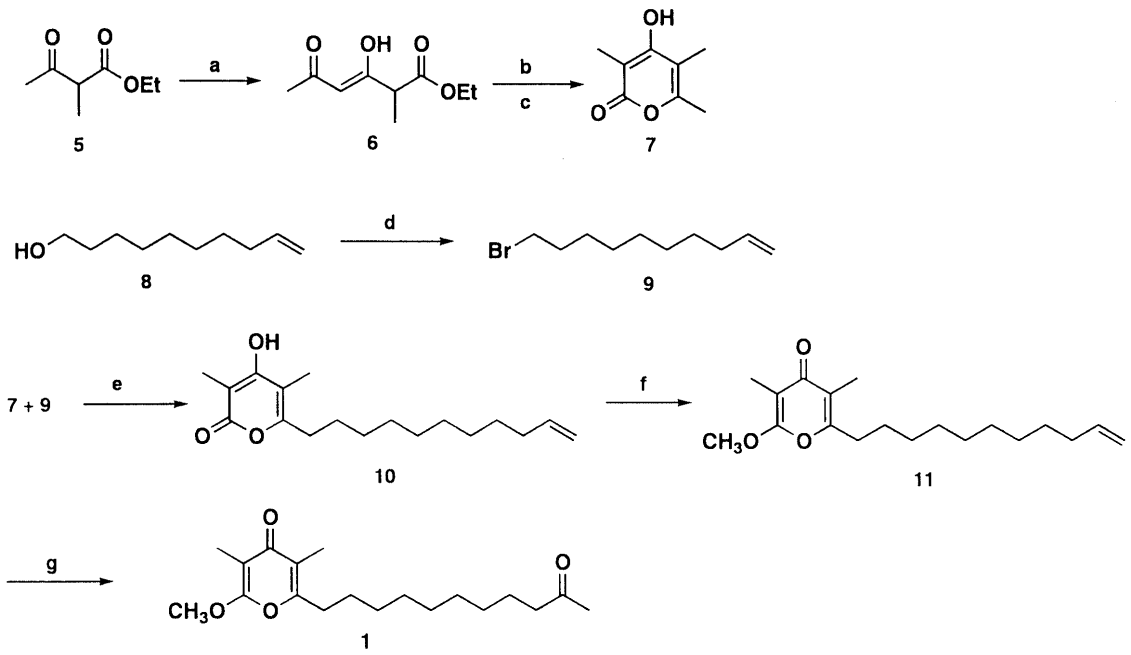
Table 2. ^{13}C-NMR Spectral Data of Compounds **1**—**4** in $CDCl_3$

Carbon No.	1	2	3	4
2	162.1	162.2	162.1	162.1
3	99.3	105.4	99.4	105.4
4	181.0	180.5	181.0	180.5
5	118.2	118.6	118.3	118.6
6	158.4	158.3	158.3	158.3
7	9.9	9.9	9.9	9.9
8	6.8	15.2	6.8	15.2
9	—	12.9	—	12.9
1′	30.7	30.7	30.7	30.7
2′	27.0	27.0	27.0	27.0
3′	29.32[a)]	29.32[b)]	29.26[c)]	29.24[d)]
4′	29.28[a)]	29.28[b)]	29.17[c)]	29.15[d)]
5′	29.26[a)]	29.26[b)]	29.14[c)]	29.11[d)]
6′	29.1[a)]	29.11[b)]	29.0[c)]	29.0[d)]
7′	29.0[a)]	29.08[b)]	23.8	23.8
8′	23.8	23.8	42.3	42.3
9′	43.7	43.7	211.8	211.8
10′	209.2	209.2	35.9	35.9
11′	29.8	29.8	7.8	7.8
OMe	55.3	55.3	55.3	55.3

a—*d*) Interchangeable.

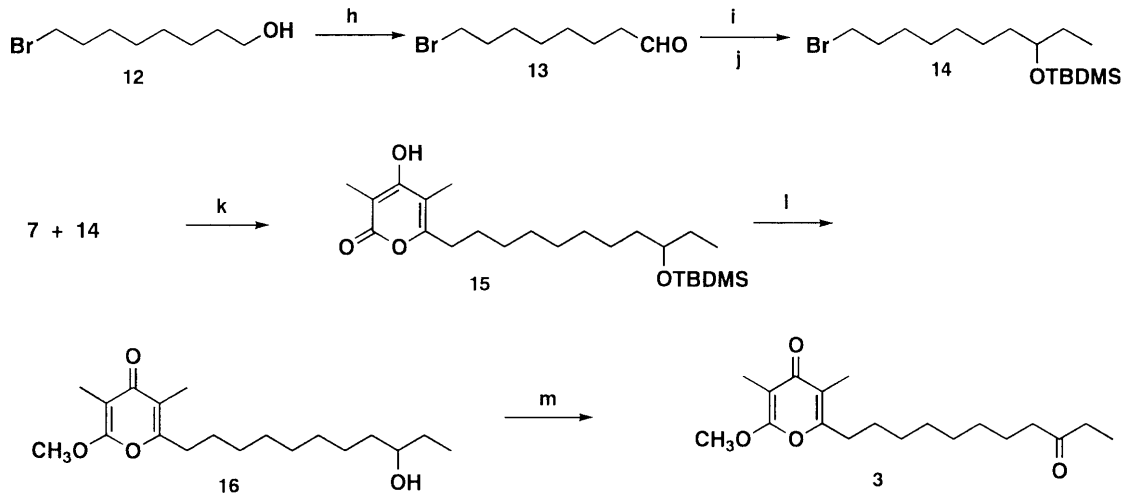
Compound **2**, colorless oil, showed the molecular formula $C_{20}H_{32}O_4$ by HR-EI-MS, which was 14 mass units higher than that of **1**. The ^{1}H- and ^{13}C-NMR spectra of **2** were similar to those of **1** (Tables 1, 2). However, the signal of a singlet methyl at δ 1.85 of **1** was replaced with a triplet methyl signal at δ 1.03 and a quartet methylene signal at δ 2.38. The signals due to the side chain were identical with those of **1**. Therefore, the structure of **2** was assumed to be 8-methyl-10′-oxopodopyrone, 2-methoxy-3-ethyl-5-methyl-6-(10′-oxoundecanyl)-4*H*-pyrane-4-one, which was confirmed by the comparison of spectral data with those of *Podolepis hieracioides*.[13]

Compound **3**, colorless oil, showed the molecular formula $C_{19}H_{30}O_4$ by HR-EI-MS, the same as **1**. In the ^{1}H- and ^{13}C-NMR spectra, the signals due to a γ-pyrone moiety corresponded to those of **1** (Tables 1, 2); however, signals due to a side chain differed. The singlet at δ 2.13 disappeared, whereas a triplet methyl signal at δ 1.05 and a quartet at δ 2.41 were observed. These results indicated that the carbonyl group of the side chain of **3** is located at the 9′ position. The 2D-NMR spectra of **3** supports this assumption. Therefore, **3** is assumed to be 9′-oxopodopyrone, 2-methoxy-3,5-dimethyl-6-(9′-oxoundecanyl)-4*H*-pyrane-4-one, and its structure was confirmed by the synthesis shown in Chart 2.



a: NaH, n-BuLi, CH_3COOCH_3, 0°C (60%). b: CH_3I, K_2CO_3, acetone, reflux. c: polyphosphoric acid, 100°C (31% from **6**). d: CBr_4, Ph_3P, CH_2Cl_2, r.t. (80%). e: 2 equiv. n-BuLi, HMPA, THF, -70°C~0°C~-5°C (27%). f: CH_3OSO_2F, CH_2Cl_2, r.t. (95%). g: $PdCl_2$, CuCl, O_2, DMF/H_2O=7:1, r.t. (54%).

Chart 1



h: $(C_6H_5N)_2Cr_2O_7$, CH_2Cl_2, r.t. (48%). i: EtMgBr, THF, followed by sat. NH_4Cl. j: TBDMSCl, imidazole, DMF(30% from **12**).
k: 2 equiv. n-BuLi, HMPA, THF, -70°C~0°C~-20°C (26%). l: $(CH_3)_2SO_4$, K_2CO_3, acetone, reflux (28%).
m: $(C_6H_5N)_2Cr_2O_7$, CH_2Cl_2, r.t. (48%).

Chart 2

Table 3. Effects of Compounds **1**—**4** and Reference Drugs on bPTH-Induced Calcium Release from Neonatal Mouse Calvaria

Compounds	Concentration (μg/ml)	Inhibition of Ca release (%)
1	0.01	31
	0.1	37
	1.0	109
	10.0	105
2	0.01	19
	0.1	11
	1.0	87
	10.0	104
3	0.01	21
	0.1	33
	1.0	107
	10.0	105
4	0.01	21
	0.1	18
	1.0	94
	10.0	104
s-Calcitonin	0.03	14
	0.34	31
	3.43	50
h-Calcitonin	0.03	18
	0.34	48
	3.42	59
Etidronate	2.68	20
	10.0	88
Ipriflavone	1.0	21
	2.8	38
	10.0	50

Inhibition (%) of Ca release $=(C_P-C_D)/(C_P-C_O)\times 100$. C_P: Ca concentration treated with bPTH. C_D: Ca concentration treated with test compounds and bPTH. C_O: Ca concentration treated with vehicle (control).

Compound **4**, colorless oil, showed the molecular formula $C_{20}H_{32}O_4$ by HR-EI-MS, which was the same as that of **2**. In the ^{1}H- and ^{13}C-NMR spectra, signals due to a side chain corresponded to those of **3** (Tables 1, 2). However, the signals due to a γ-pyrone moiety were similar to those of **2**. These results indicate that the structure of **4** is 8-methyl-9′-oxopodopyrone, 2-methoxy-3-ethyl-5-methyl-6-(9′-oxoundecanyl)-4*H*-pyrane-4-one.

Jaensch *et al.*[12] have reported many derivatives of podopyrone; however, this is the first report on the isolation of podopyrones with an oxo group at the 9′ position.

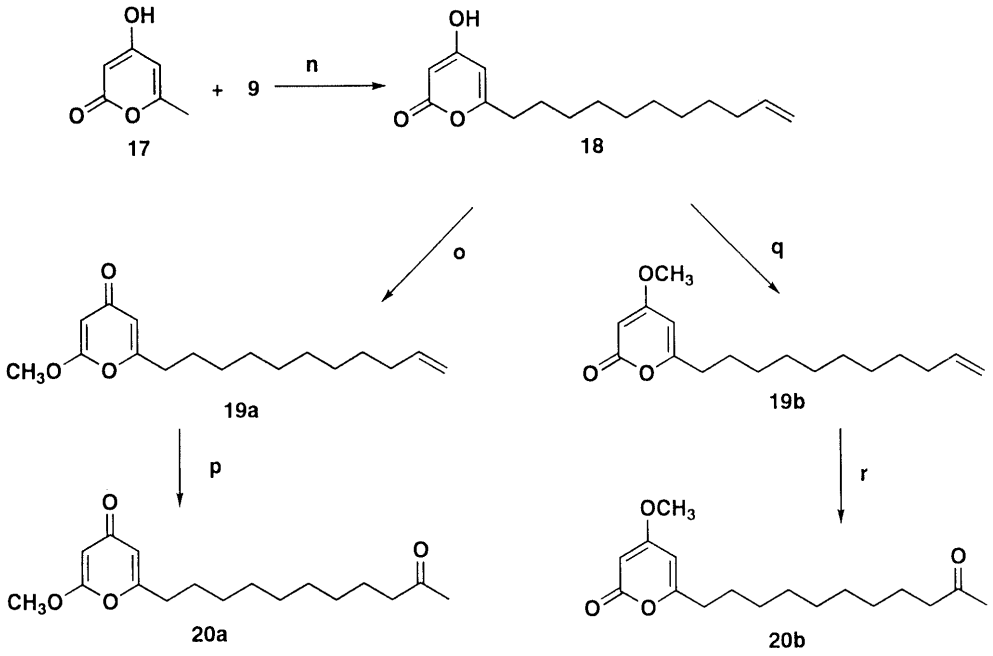
The effects of these compounds and reference drugs on *in vitro* bPTH-induced bone resorption[14] are summarized in Table 3. Compounds **1**—**4** markedly inhibited bPTH-induced Ca release from neonatal mouse calvaria. The inhibitory activities of **1**—**4** were assumed to be the same, and were shown to be more potent than those of calcitonins, etidronate and ipriflavone.

To clarify the structural requirement for this inhibitory activity, the effects of **11**, **20a** and **20b** on bPTH-induced Ca release were tested. Compound **11**, which has an end methylene group instead of an oxo group at the 10′ position of **1**, was obtained in the course of the synthesis of **1**. Compound **20a** has no methyl groups on the γ-pyrone ring, and **20b** is the α-pyrone derivative of **20a**. As shown in Table 4, the carbonyl group at the 9′ or 10′ position of the side chain, and methyl groups at the 3 and/or 5 position are necessary for the inhibitory effect on bPTH-induced bone resorption.

As reported above, podopyrones with a carbonyl group at the 9′ or 10′ position of the side chain strongly inhibited the bPTH-induced Ca release *in vitro*. The effects of compounds **1** and **3** on human PTH-induced hypercalcemia in rats are under investigation, and they may be potent prototypes of new drugs for osteoporosis.

Experimental

IR and UV spectra were recorded on a Shimadzu FT-IR 4300 spectrometer and a Shimadzu UV-160A spectrometer, respectively. Mass spectra were taken with a JEOL JMS-AX505H spectrometer and a Shimadzu/Kratos Profile spectrometer. The NMR spectra were measured with a JEOL JNM-A500 spectrometer at 500 MHz (^{1}H) and at 125.65 MHz (^{13}C) in $CDCl_3$, with tetramethylsilane as an internal standard. HPLC were carried out using a Hitachi L-6200 pump, L-4000 UV detector and D-2500 recorder using a TSK gel ODS-120T (Tosoh;



n: 2 equiv. n-BuLi, HMPA, THF, -70°C~0°C~-5°C (23%). o: CH_3OSO_2F, CH_2Cl_2, r.t. (96%).
p: $PdCl_2$, CuCl, O_2, DMF/H_2O=7:1, r.t. (38%). q: $(CH_3)_2SO_4$, K_2CO_3, 2-butanone, reflux (66%).
r: $PdCl_2$, CuCl, O_2, DMF/H_2O=7:1, r.t. (52%).

Chart 3

Table 4. Effects of Compounds **11**, **20a**, **20b** and Reference Drug on bPTH-Induced Calcium Release from Mouse Neonatal Calvaria

Compounds	Concentration (μg/ml)	Inhibition of Ca release (%)
11	0.3	27
	1.0	80
20a	1.0	9
20b	1.0	21
Etidronate	25.0	136

Inhibition (%) of Ca release: see Table 3.

5 μm, 4.6 mmϕ × 250 mm), and preparative HPLC by a Shimadzu LC-8A system using a TSK gel ODS-120T (Tosoh; 5 μm, 7.8 mmϕ × 300 mm). Column chromatography was performed on Kiesel gel 60 (Merck). TLC were carried out on Kiesel gel $60F_{254}$ (Merck).

Material The methanol extract (125 mg) of the leaves of *Gonystylus keithii* (Thymeliaceae) collected at Sabah prefecture in Malaysia was obtained from the Strathclyde Institute of Drug Research (Glasgow, U.K.). bPTH (1—34) was purchased from Bachem California (Torrance, CA, U.S.A.). Salmon and human calcitonins were obtained from Peptide Institute, Inc. (Osaka). Both ipriflavone (Takeda Chemical, Osaka) and etidronate (Sumitomo Pharmaceuticals, Osaka) were extracted from commercially available tablets. Other reagents were of analytical grade and were obtained from Wako Pure Chemical (Osaka), Tokyo Kasei (Tokyo) and Aldrich (Milwaukee, WI, U.S.A.).

Isolation The methanol extract (64.2 mg) was partitioned by $CHCl_3$/MeOH/H_2O (30 ml/15 ml/15 ml). The lower layer (47.4 mg) was dissolved in MeOH (15 ml) and extracted with *n*-hexane (30 ml × 2). The MeOH layer was subjected to preparative HPLC (detection at 254 nm) using 55% acetonitril containing 0.1% acetic acid as an eluent, and compounds **1** (1.9 mg), **2** (1.3 mg), **3** (1.0 mg) and **4** (0.7 mg) were obtained.

Compound **1** (10′-Oxopodopyrone): Colorless oil, HR-EI-MS: *m/z* 322.2127 (Calcd for $C_{19}H_{30}O_4$: 322.2144). EI-MS *m/z*: 322 (M^+), 304, 279, 265, 195, 181, 168. IR (neat) cm^{-1}: 1715 (carbonyl), 1670, 1600 (pyrone). UV λ_{max} (MeOH) nm (log ε): 252 (3.96). The ^1H- and ^{13}C-NMR data: see Tables 1 and 2, respectively.

Compound **2** (8-methyl-10′-Oxopodopyrone): Colorless oil, HR-EI-MS: *m/z* 336.2281 (Calcd for $C_{20}H_{32}O_4$: 336.2300). EI-MS *m/z*: 336 (M^+), 321, 279, 209, 197, 195, 182. IR (neat) cm^{-1}: 1715 (carbonyl), 1670, 1600 (pyrone). UV λ_{max} (MeOH) nm (log ε): 254 (3.94). The ^1H- and ^{13}C-NMR data: see Tables 1 and 2, respectively.

Compound **3** (9′-Oxopodopyrone): Colorless oil, HR-EI-MS: *m/z* 322.2121 (Calcd for $C_{19}H_{30}O_4$: 322.2144). EI-MS *m/z*: 322 (M^+), 307, 293, 265, 251, 195, 181, 168. IR (neat) cm^{-1}: 1715 (carbonyl), 1670, 1600 (pyrone). UV λ_{max} (MeOH) nm (log ε): 252 (3.96). The ^1H- and ^{13}C-NMR data: see Tables 1 and 2, respectively.

Compound **4** (8-Methyl-9′-oxopodopyrone): Colorless oil, HR-EI-MS: *m/z* 336.2288 (Calcd for $C_{20}H_{32}O_4$: 336.2300). EI-MS *m/z*: 336 (M^+), 321, 307, 279, 265, 209, 195, 182, 168. IR (neat) cm^{-1}: 1715 (carbonyl), 1670, 1600 (pyrone). UV λ_{max} (MeOH) nm (log ε): 253 (4.00). The ^1H- and ^{13}C-NMR data: see Tables 1 and 2, respectively.

Synthesis of 1. Ethyl 2-Methyl-3-hydroxy-5-oxo-3-hexenoate (6) Ethyl 2-methylacetoacetate (5.88 g, 40 mmol) was added dropwise to a solution of NaH (2.01 g, 44 mmol, 60% oil dispersion) in tetrahydrofuran (THF) (160 ml), then cooled in ice under a nitrogen atmosphere. The solution was stirred for 20 min after the addition was complete, then *n*-butyl lithium (40 mmol, 28 ml of 1.69 M *n*-hexane solution) was added dropwise. After stirring for 20 min, methyl acetate (1.55 g, 20 mmol) was added in one portion.[15] The latter reaction was repeated using the same amounts of the reagents, and the reaction was quenched by conc. HCl (16 ml). The products were worked up by the addition of H_2O (50 ml) and Et_2O (50 ml). The water layer was further extracted with Et_2O (50 ml). The combined extract was washed with sat. $NaHCO_3$ and sat. brine, then dried (Na_2SO_4). Removal of the solvent gave an oil. The oil was distilled under reduced pressure to give **6** (4.48 g, 60%). Colorless oil, EI-MS *m/z*: 186 (M^+), 102, 85. IR (neat) cm^{-1}: 1740, 1615, 760. ^1H-NMR ($CDCl_3$) δ: 5.57 (1H, s), 4.18 (2H, q, J=7 Hz), 3.35 (1H, q, J=7 Hz), 2.05 (3H, s), 1.37 (3H, d, J=7 Hz), 1.25 (3H, t, J=7 Hz).

4-Hydroxy-3,5,6-trimethyl-2*H*-pyrane-2-on (7) A suspension of K_2CO_3 (3.15 g, 22.8 mmol), **6** (3.50 g, 20 mmol) and methyl iodide (4.03 g, 28.6 mmol) in acetone was refluxed for 1 h.[16] After removal of the solid by filtration, the filtrate was concentrated under reduced pressure to give an oil (3.05 g). The oil was mixed with polyphosphate (10.3 g) and heated at 100 °C for 1 h.[17] After the addition of ice water (30 ml) to the reaction mixture, the aqueous layer was extracted with EtOAc (30 ml × 2), and the combined extract was washed with H_2O, sat. brine, dried ($MgSO_4$), and concentrated to give an oil. The addition of *n*-hexane to the oil gave a precipitate (**7**), which was separated by repeated decantation (891 mg, 31% over all from **6**). An amorphous powder, EI-MS *m/z*: 154 (M^+, base peak), 126, 111, 99. IR (KBr) cm^{-1}: 1670,

1640, 1570, 1410, 1240, 1220, 1175, 1140, 750. ^{1}H-NMR ($CDCl_3$) δ: 2.22 (3H, s), 1.98 (3H, s), 1.95 (3H, s).

1-Bromo-9-decene (9) Triphenylphosphine (19.7 g, 75 mmol) and CCl_4 (36.3 g, 110 mmol) were added to a solution of 9-decen-1-ol (**8**, 10.2 g, 70 mmol) in dry CH_2Cl_2 (100 ml) at 0 °C, and the mixture was stirred for 1 h at room temperature. The reaction mixture was washed with sat. $NaHCO_3$ and sat. brine, dried ($MgSO_4$), and concentrated to give an oil. EtOAc was added to the oil, and the resulting white precipitate was removed by filtration. The filtrate was chromatographed on silica gel (100 g), and eluted with *n*-hexane (200 ml) and 1% EtOAc–*n*-hexane (500 ml) to give **9** (11.51 g, 79%). Colorless oil, EI-MS *m/z*: 220, 218, 164, 162, 150, 148, 97, 83, 69, 55. IR (neat) cm^{-1}: 2930, 1640, 1460, 1430, 910. ^{1}H-NMR ($CDCl_3$) δ: 5.81 (1H, ddt, J=17, 10, 7 Hz), 4.99 (1H, br d, J=17 Hz), 4.93 (1H, br d, J=10 Hz), 3.40 (2H, t, J=7 Hz), 2.06 (2H, q, J=7 Hz), 1.85 (2H, tt, J=7, 7 Hz), 1.30 (6H, br s).

3,5-Dimethyl-4-hydroxy-6-(10-undecenyl)-2*H*-pyrane-2-one (10) A 1.69 M *n*-butyl lithium solution in *n*-hexane (7.5 ml, 12.8 mmol) was added to a solution of **7** (883.5 mg, 5.7 mmol) and hexamethylphosphoramide (HMPA) (3 ml) in THF (20 ml) at −70 °C under a nitrogen atmosphere. The mixture was allowed to warm to 0 °C, and was then stirred for 2 h. After the addition of **9** (1248.3 mg, 5.7 mmol), the solution was stirred for 15 h at −5 °C.[17] After the addition of ice–water (20 ml), the upper layer was separated. The aqueous layer was further extracted with *n*-hexane (15 ml × 3). The aqueous layer was then acidified with 5% HCl to pH 6, and extracted with Et_2O (15 ml × 3). The Et_2O layer was washed with sat. $NaHCO_3$ and sat. brine, dried ($MgSO_4$), and concentrated to give an oil. This oil was chromatographed on silica gel (40 g), and eluted with $CHCl_3$ to give **11** (452.6 mg, 27% from **7**). Colorless oil, EI-MS: *m/z* 292 (M^+), 251, 237, 167, 154, 125, 83. IR (neat) cm^{-1}: 3080 (br), 2930, 1685, 1640, 1570, 1230. ^{1}H-NMR ($CDCl_3$) δ: 5.81 (1H, ddt, J=17, 10, 7 Hz), 4.99 (1H, br d, J=17 Hz), 4.93 (1H, br d, J=10 Hz), 2.49 (2H, t, J=7 Hz), 2.03 (2H, q, J=7 Hz), 1.99 (3H, s), 1.96 (3H, s), 1.62 (2H, m), 1.37 (2H, m), 1.27 (10H, m).

2-Methoxy-3,5-dimethyl-6-(10-undecenyl)-4*H*-pyrane-4-one (11) Methyl fluorosulfonate (1 ml) was added to a solution of **10** (450.2 mg, 1.5 mmol) in dry CH_2Cl_2 (3 ml).[18] After stirring for 1 h at room temperature, methyl fluorosulfonate was removed under reduced pressure. The residue was dissolved in 1 N NaOH, then extracted with EtOAc (20 ml × 3). The combined extract was washed with sat. brine, dried ($MgSO_4$), and concentrated to give **12** (460.7 mg, 98%). Colorless oil, EI-MS *m/z*: 306 (M^+), 291, 265, 251, 195, 181, 168. IR ($CHCl_3$) cm^{-1}: 2930, 1670, 1600, 1460, 1410, 1380, 1320, 1250, 1170. ^{1}H-NMR ($CDCl_3$) δ: 5.80 (1H, ddt, J=17, 10, 7.5 Hz), 4.99 (1H, br d, J=17 Hz), 4.93 (1H, br d, J=10 Hz), 3.94 (3H, s), 2.57 (2H, t, J=7.5 Hz), 2.03 (2H, q, J=7.5 Hz), 1.93 (3H, s), 1.85 (3H, s), 1.64 (2H, m), 1.36 (2H, m), 1.32—1.27 (10H, m).

2-Methoxy-3,5-dimethyl-6-(10-oxoundecanyl)-4*H*-pyrane-4-one (1) A mixture of palladium chloride (30.0 mg, 0.17 mmol) and cuprous chloride (148.8 mg, 1.5 mmol) in aqueous dimethylformamide (DMF) (10 ml, DMF/water = 7/1) was stirred under an oxygen atmosphere at room temperature for 1 h, then an aqueous DMF (5 ml, DMF/water = 7/1) containing **11** (460.7 mg, 1.5 mmol) was added. After standing for 19 h, the reaction mixture was poured into cold 3 N HCl (20 ml) and extracted with Et_2O (40 ml × 4). The combined extract was washed with sat. $NaHCO_3$, sat. brine, dried ($MgSO_4$) and concentrated. The residue was chromotographed on silica gel (70 g), and eluted with *n*-hexane : acetone = 9 : 1 (300 ml), 5 : 1 (480 ml) and 4 : 1 (500 ml) to give **1** (Colorless oil, 262.1 mg, 54%). The spectral data were identical with those of the isolated compound.

Synthesis of 3. 8-Bromo-1-octanal (13) A mixture of 8-bromo-1-octanol (**12**, 5.01 g, 24 mmol) and pyridinium dichromate (9.13 g, 24 mmol) in 30 ml of CH_2Cl_2 was stirred for 1 d at room temperature. To the reaction mixture, Et_2O (150 ml) was added, and the resulting precipitate was filtered off. The filtrate was concentrated, and the residue was chromatographed on silica gel (100 g), and eluted with 10% EtOAc–*n*-hexane (200 ml), 15% EtOAc–*n*-hexane (200 ml) and 20% EtOAc–*n*-hexane (200 ml) to give **13** (2.37 g, 48%). Colorless oil, EI-MS *m/z*: 207, 205, 192, 190, 164, 162, 150, 148, 125, 111, 97, 83, 69, 55. IR (neat) cm^{-1}: 2930, 1725, 1470, 1250. ^{1}H-NMR ($CDCl_3$) δ: 9.77 (1H, d, J=2 Hz), 3.40 (2H, t, J=7 Hz), 2.45 (1H, dt, J=2.7 Hz). 1.85 (2H, m), 1.64 (2H, m), 1.44 (2H, m), 1.34 (4H, m).

10-Bromo-3-decanyl *tert*-Butyl-dimethylsilyl Ether (14) A solution of **13** (2.30 g, 11 mmol) in 20 ml of THF was dropped into a 0.92 M solution of ethyl magnesium bromide in THF (12 ml, 11.4 mmol), and the reaction mixture was stirred for 1 h. To this mixture, sat. NH_4Cl (20 ml) was added dropwise. After standing 40 min, the organic layer was separated, then the water layer was extracted with Et_2O (20 ml × 2). The combined Et_2O layer was washed with sat. $NaHCO_3$ (20 ml), sat. brine, and dried (Na_2SO_4). Removal of the solvent gave an oil. The oil was chromatographed on silica gel (70 g), and eluted with *n*-hexane (100 ml), 10% EtOAc–*n*-hexane (800 ml) and 17% EtOAc–*n*-hexane (420 ml) to give 10-bromo-3-decanol (1.0578 g, 40.5%). Colorless oil, EI-MS *m/z*: 209, 207, 109, 59. IR (neat) cm^{-1}: 3390, 2920, 2855, 1460, 1250, 1120, 960. ^{1}H-NMR ($CDCl_3$) δ: 3.51 (1H, m), 3.40 (2H, t, J=7 Hz), 1.85 (2H, q, J=7 Hz). 1.38—1.56 (6H, m), 1.34 (6H, m), 0.94 (3H, t, J=7.5 Hz).

To a solution of 10-bromo-3-decanol (1.05 g, 5 mmol) in dry DMF (10 ml), *tert*-butyl dimethylsilyl chloride (3.92 g, 26 mmol) and imidazole (3.54 g, 52 mmol) were added, and the mixture was stirred for 2 h at room temperature. The reaction mixture was extracted with Et_2O (20 ml × 3), and the extract was washed with sat. $NaHCO_3$, sat. brine, and was dried ($MgSO_4$). Removal of the solvent gave an oil. The oil was chromatographed on silica gel (31 g), and eluted with *n*-hexane (200 ml) to give **14** (1.1528 g, 30% over all from **12**). Colorless oil, EI-MS *m/z*: 351, 349, 337, 335, 323, 321, 197, 195, 173, 151, 139, 115, 97, 83. IR (neat) cm^{-1}: 2930, 1465, 1380, 1255, 1060. ^{1}H-NMR ($CDCl_3$) δ: 3.58 (1H, quintet, J=7 Hz), 3.40 (2H, t, J=7 Hz), 1.86 (2H, q, J=7 Hz), 1.43 (6H, m), 1.30 (6H, m), 0.89 (3H, t, J=7 Hz), 0.85 (9H, s), 0.04 (6H, s).

3,5-Dimethyl-4-hydroxy-6-(9-*tert*-butyldimethylsilyloxyundecanyl)-2*H*-pyrane-2-one (15) A 1.69 M solution of *n*-butyl lithium in *n*-hexane (4 ml, 6.8 mmol) was added to a solution of **7** (498.5 mg, 3.2 mmol) in THF (25 ml) and HMPA (2 ml) at −70 °C under a nitrogen atmosphere, and the mixture was allowed to warm to 0 °C. After stirring for 2 h, **14** (1.05 g, 3.0 mmol) was added, and the reaction mixture was stirred for 12 h at −20 °C.[17] The reaction mixture was then treated with ice–water (25 ml), and the upper layer was separated. The aqueous layer was further extracted with *n*-hexane (25 ml), then acidified with 5% HCl to pH 6, and was extracted with Et_2O (20 ml × 3). The Et_2O extract was washed with water (20 ml), sat. brine (20 ml), and dried (Na_2SO_4). Removal of the solvent gave an oil, and the oil was chromatographed on silica gel (40 g), and eluted with $CHCl_3$ (200 ml) and 1% MeOH–$CHCl_3$ (500 ml) to give **15** (359.2 mg, 26%). Colorless oil, EI-MS *m/z*: 367, 353, 339, 173. IR (neat) cm^{-1}: 3190, 2930, 1665, 1565, 1465, 1410, 1250, 1125, 1075, 835. ^{1}H-NMR ($CDCl_3$) δ: 3.56 (1H, quintet, J=6 Hz), 2.50 (2H, br t, J=7.5 Hz), 1.98 (3H, s), 1.96 (3H, s), 1.63 (2H, m), 1.37—1.50 (6H, m), 1.28—1.32 (8H, m), 0.89 (9H, s), 0.86 (3H, t, J=7.5 Hz), 0.04 (6H, s).

2-Methoxy-3,5-dimethyl-6-(9′-hydroxyundecanyl)-4*H*-pyrane-4-one (16) The mixture of K_2CO_3 (70.3 mg, 0.5 mmol), **15** (212.7 mg, 0.5 mmol) and $(CH_3)_2SO_4$ (94 mg, 0.67 mmol) in acetone (25 ml) was refluxed for 1 h.[17] After filtration, the solvent was removed under reduced pressure to give an oil. The oil was chromatographed on silica gel (40 g), and eluted with *n*-hexane–EtOAc = 3 : 1 (200 ml), 2 : 1 (180 ml), 1 : 1 (200 ml), and 1 : 2 (180 ml) to give **16** (42.3 mg, 28%). Colorless oil, EI-MS *m/z*: 324, 309, 295, 195, 181, 168. IR (neat) cm^{-1}: 3390, 2960, 2860, 1665, 1585, 1465, 1415, 1380, 1320, 1250, 1215, 1170. ^{1}H-NMR ($CDCl_3$) δ: 3.94 (3H, s), 3.51 (1H, m), 2.57 (2H, t, J=7.5 Hz), 1.93 (3H, s), 1.84 (3H, s), 1.64 (2H, m), 1.37—1.50 (6H, m), 1.30—1.32 (8H, m), 0.93 (3H, t, J=7.5 Hz).

2-Methoxy-3,5-dimethyl-6-(9′-oxoundecanyl)-4*H*-pyrane-4-one (3) A mixture of **16** (24.3 mg, 0.08 mmol) and pyridinium dichromate (36.2 mg, 0.09 mmol) in CH_2Cl_2 (5 ml) was stirred for 2 h at room temperature. To the mixture, Et_2O (50 ml) was added. After filtration, the solvent was removed and the residue was chromatographed on silica gel (10 g), and eluted with 17% aceton–*n*-hexane (400 ml) to give **3** (colorless oil, 12.9 mg, 48%). The spectral data were identical with those of the isolated compound.

Synthesis of 20a. 4-Hydroxy-6-(10′-undecenyl)-2*H*-pyrane-2-one (18) A 1.69 M solution of *n*-butyl lithium in hexane (19.5 ml, 33 mmol) was added to a solution of **17** (1.96 g, 16 mmol) in THF (40 ml) and HMPA (4 ml) at −70 °C under a nitrogen atmosphere. The mixture was allowed to warm to 0 °C. After stirring for 2 h, **9** (6.04 g, 28 mmol) was added. The solution was stirred for 13 h at −5 °C,[17] then treated with ice–water (20 ml), and the organic layer was separated. The aqueous layer was further extracted with *n*-hexane (30 ml × 3), then was acidified with 5% HCl to pH 6, and was extracted with Et_2O (30 ml × 3). The combined extract was washed with water (30 ml), sat. brine (30 ml × 2), and then dried (Na_2SO_4). Removal of the solvent gave an oil, and the oil was chromatographed on silica gel (70 g), and eluted with $CHCl_3$ (300 ml), 1% MeOH–$CHCl_3$ (300 ml), 3% MeOH–$CHCl_3$ (300 ml), and 5%

MeOH–$CHCl_3$ (300 ml) to give **18** (947.8 mg, 23%). Colorless needles, EI-MS *m/z*: 264 (M^+), 139, 126, 111, 98, 84, 69. IR (KBr) cm^{-1}: 2980, 2850, 1690, 1650, 1615, 1570, 1540, 1494, 1270, 1145, 840. ^{1}H-NMR ($CDCl_3$) δ: 5.96 (1H, d, J=2 Hz), 5.81 (1H, ddt, J=17, 10, 7 Hz), 5.56 (1H, d, J=2 Hz), 5.00 (1H, br d, J=17 Hz), 4.93 (1H, br d, J=10 Hz), 2.48 (2H, t, J=7.5 Hz), 2.04 (2H, q, J=7.5 Hz), 1.64 (2H, m), 1.37 (2H, m), 1.28 (10H, m).

2-Methoxy-6-(10′-undecenyl)-4*H*-pyrane-4-one (19a) Methyl fluorosulfonate (2 ml) was added to a solution of **18** (498.2 mg, 1.9 mmol) in dry CH_2Cl_2 (3 ml), and the mixture was stirred for 3 h at room temperature.[18] Then, methyl fluorosulfonate was removed under reduced pressure and 1 N NaOH was added to the residue. The aqueous solution was extracted with EtOAc (20 ml × 3). The combined extract was washed with sat. brine (20 ml × 2), dried ($MgSO_4$), and concentrated to give an oil. The oil was chromatographed on silica gel (41.5 g), and eluted with $CHCl_3$ (300 ml) and 1% MeOH–$CHCl_3$ (300 ml) to give **19a** (505.1 mg, 96%). Colorless needles, EI-MS *m/z*: 264 (M^+), 139, 126, 111, 98, 84, 69. IR (KBr) cm^{-1}: 2980, 2850, 1690, 1650, 1615, 1570, 1540, 1494, 1270, 1145, 840. ^{1}H-NMR ($CDCl_3$) δ: 5.97 (1H, d, J=2 Hz), 5.81 (1H, ddt, J=17, 10, 7 Hz), 5.44 (1H, d, J=2 Hz), 4.98 (1H, br d, J=17 Hz), 4.93 (1H, br d, J=10 Hz), 3.85 (3H, s), 2.46 (2H, t, J=7.5 Hz), 2.03 (2H, br q, J=7.5 Hz), 1.63 (2H, m), 1.36 (2H, m), 1.28 (10H, m).

2-Methoxy-6-(10′-oxoundecanyl)-4*H*-pyrane-4-one (20a) A mixture of palladium chloride (35.6 mg, 0.2 mmol) and cuprous chloride (181.8 mg, 1.8 mmol) in aqueous DMF (10 ml, DMF/water = 7/1) was stirred under an oxygen atmosphere at room temperature. After stirring for 1 h, an aqueous DMF (5 ml, DMF/water = 7/1) solution containing **19a** (500.2 mg, 1.8 mmol) was added, and the mixture was allowed to stand for 15 h. The reaction mixture was poured into cold 3 N HCl (20 ml) and extracted with Et_2O (25 ml × 4). The combined extract was washed with sat. $NaHCO_3$, sat. brine, dried ($MgSO_4$) and concentrated under reduced pressure. The residue was chromatographed on silica gel (40 g), and eluted with *n*-hexane : acetone = 9 : 1 (300 ml), 5 : 1 (300 ml), 4 : 1 (300 ml), 2 : 1 (300 ml) and 1 : 1 (300 ml) to give **20a** (199.3 mg, 38%). Colorless needles, EI-MS *m/z*: 294 (M^+), 251, 237, 153, 140. IR (KBr) cm^{-1}: 3070, 2930, 1710, 1660, 1620, 1585, 1400, 1260, 1160, 930. ^{1}H-NMR ($CDCl_3$) δ: 6.02 (1H, br s), 5.49 (1H, br d, J=2 Hz), 3.90 (3H, s), 2.50 (2H, t, J=7.5 Hz), 2.45 (2H, t, J=7.5 Hz), 2.17 (3H, s), 1.67 (2H, m), 1.37 (2H, m), 1.32 (10H, m).

Synthesis of 20b. 4-Methoxy-6-(10′-undecenyl)-2*H*-pyrane-2-one (19b) A mixture of K_2CO_3 (940.0 mg, 6.81 mmol), **18** (274.0 mg, 1.0 mmol), and $(CH_3)_2SO_4$ (158.0 mg, 1.24 mmol) in 2-butanone (50 ml) was refluxed for 3 h. After filtration, the solvent was removed under reduced pressure to give an oil. The oil was chromatographed on silica gel (27 g), and eluted with $CHCl_3$ (200 ml), and 1% MeOH–$CHCl_3$ (200 ml) to give **19b** (191.0 mg, 66%). Colorless needles, EI-MS *m/z*: 264 (M^+), 139, 126, 111, 98, 84, 69. IR (KBr) cm^{-1}: 2980, 2850, 1690, 1650, 1615, 1570, 1540, 1494, 1270, 1145, 840. ^{1}H-NMR ($CDCl_3$) δ: 5.81 (1H, ddt, J=17, 10, 7 Hz), 5.76 (1H, d, J=2 Hz), 5.40 (1H, d, J=2 Hz), 4.99 (1H, br d, J=17 Hz), 4.93 (1H, br d, J=10 Hz), 3.79 (3H, s), 2.43 (2H, t, J=7.5 Hz), 2.04 (2H, q, J=7.5 Hz), 1.64 (2H, m), 1.37 (2H, m), 1.27 (10H, m).

4-Methoxy-6-(10′-oxoundecanyl)-2*H*-pyrane-2-one (20b) A mixture of palladium chloride (20.5 mg, 0.11 mmol) and cuprous chloride (105.2 mg, 1.1 mmol) in aqueous DMF (5 ml, DMF/water = 7/1) was stirred under an oxygen atmosphere at room temperature. After stirring for 1 h, an aqueous DMF (5 ml, DMF/water = 7/1) solution containing **19b** (185.2 mg, 0.5 mmol) was added, and the mixture was allowed to stand for 15 h. The reaction mixture was poured into cold 3 N HCl (20 ml) and extracted with Et_2O (25 ml × 4). The combined extract was washed with sat. $NaHCO_3$, sat. brine, dried ($MgSO_4$) and concentrated. The residue was chromatographed on silica gel (40 g), and eluted with *n*-hexane : acetone = 9 : 1 (200 ml), 5 : 1 (200 ml), 4 : 1 (200 ml) and 2 : 1 (200 ml) to give **20b** (100.4 mg, 52%). Colorless needles, EI-MS *m/z*: 294 (M^+), 251, 237, 153, 140, 125. IR (KBr) cm^{-1}: 2995, 2850, 1710, 1650, 1580, 1460, 1250, 1140, 1045, 865. ^{1}H-NMR ($CDCl_3$) δ: 5.80 (1H, d, J=2 Hz), 5.44 (1H, d, J=2 Hz), 3.83 (3H, s), 2.47 (2H, t, J=7.5 Hz), 2.45 (2H, t, J=7.5 Hz), 2.17 (3H, s), 1.68 (2H, m), 1.60 (2H, m), 1.32 (10H, m).

***In Vitro* bPTH-Induced Bone Resorption Assay** This experiment was conducted according to the modified method described by Gary *et al.*[14] A calvaria was obtained sterilely from 5- or 6-d-old ddY mouse (Japan SLC, Shizuoka), and was washed with saline phosphate buffer (Gibco BRL, U.S.A.). The calvaria was cut into halves, and was then incubated in 1.5 ml of Dulbecco's Modified Eagle medium (Gibco BRL, U.S.A.) containing 15% heat- (56 °C, 60 min) inactivated horse serum, 2.5% bovine serum and an antibiotic–antimycotic solution. The test compounds were dissolved in EtOH, and added to the medium (the final concentration of EtOH : 1%). bPTH, which was dissolved in 0.15 M NaCl containing 0.01% acetic acid, was applied to the medium (the final concentration: 1×10^{-8} M). The medium was maintained at 37 °C under 5% CO_2. After 48 h incubation, the medium was changed and incubated for another 48 h. The concentration of Ca in the medium was determined using the Calcium C Test Wako (Wako Pure Chemicals). Inhibitory activities of test compounds on Ca release from calvaria were calculated using the following formula.

$$\text{inhibition (\%) of Ca release} = (C_P - C_D)/(C_P - C_O) \times 100$$

Where C_P, C_D and C_O were defined as follows: C_D, total Ca concentration in medium treated with test compounds plus bPTH; C_P, total Ca concentration in medium treated with bPTH; C_O, total Ca concentration in medium treated with vehicle (control).

References and Notes

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