Studies on Disease-Modifying Antirheumatic Drugs. III.[1] Bone Resorption Inhibitory Effects of Ethyl 4-(3,4-Dimethoxyphenyl)-6,7-dimethoxy-2-(1,2,4-triazol-1-ylmethyl)quinoline-3-carboxylate (TAK-603) and Related Compounds

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In the course of our studies aimed at obtaining new drugs for treatment of bone and joint diseases, chemical modification of the potent bone resorption inhibitors justicidins, was performed and various naphthalene lactones, quinoline lactones and quinoline derivatives bearing an azole moiety at the side chain were prepared. Their inhibitory effects on bone resorption were evaluated by Raisz's method, and several compounds, including ethyl 4-(3,4-dimethoxyphenyl)-6,7-dimethoxy-2-(1,2,4-triazol-1-ylmethyl)quinoline-3-carboxylate (6c, TAK-603), were found to have activities comparable with or superior to the justicidins. The 4-(3-isopropoxy-4-methoxy)-phenyl derivative (6d), in particular, displayed a marked increase in potency. TAK-603 and compound 6d were very effective in preventing osteoclast formation and bone resorption by mature osteoclasts. Further, TAK-603 was shown to be effective in preventing bone loss in ovariectomized mice.

Key word TAK-603; justicidin; bone and joint disease; antiresorptive activity; bone resorption

Rheumatoid arthritis (RA) is a serious, chronic, and systemic disease characterized by inflammation and progressive joint destruction. Since RA is an autoimmune disease, disease-modifying antirheumatic drugs (DMARDs), which have selective and direct effects on the abnormal immune system, have attracted a great deal of attention as potentially effective treatments for RA.[2] Development of osteopenia in the region of inflamed joints is a common clinical feature of RA. Previous histological studies of bone specimens obtained from patients with chronic RA have demonstrated an increase in bone resorption, suggesting increased bone turnover.[3] Therefore, DMARDs possessing antiresorptive activity as well as immune system modulating effects are expected to be useful in the treatment of RA.

Our search for a new type of DMARD with antiresorptive activity began with chemical modification of the potent bone resorption inhibitors justicidins (**1**, Fig. 1),[4] which were isolated from *Justicia Procumbes*, since factors such as interleukin (IL)-1β and prostaglandin (PG) E_2 play important roles in both RA and bone metabolism.[5] Although justicidins possess only weak anti-inflammatory activity,[6] these compounds with antiresorptive activity are very attractive lead compounds for the development a new type of DMARD. Thus, the justicidin structure was modified to obtain compounds with better pharmacological properties. Naphthalene lactones (**2**), quinoline lactones (**3**), and quinoline derivatives (**4**—**6**), bearing a heteroaryl moiety at the side chain of the 2-position, were prepared to evaluate their antiresorptive and anti-inflammatory activities. We previously reported structure–activity relationships (SAR) with respect to the anti-inflammatory effect of these derivatives, leading to the finding of a new type of DMARD, TAK-603 (**6c**).[7] This report details some SAR information concerning the bone resorption inhibitory effects of this series of compounds.[8]

Chemistry

The naphthalene lactone derivatives (**2**, Table 1) were prepared using the method of Stevenson and co-workers,[9,10] as shown in Chart 1. Condensation of carboxylic acids (**7**) with alcohols (**8**) gave the esters (**9**), which were converted to the dihydronaphthalene lactones (**10**) by heating in acetic anhydride. Aromatization of **10** yielded **2**.

Friedländer reaction[11] of 2-aminobenzophenones (**11**) with tetronic acid afforded quinoline lactones (**3**) (Chart 2).[12]

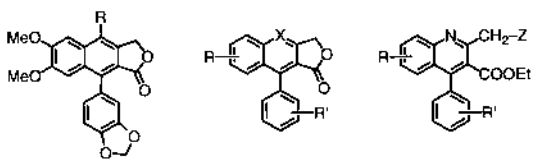
The syntheses of 2-(heteroarylthiomethyl)-(**4**), 2-(1-methylimidazol-2-ylethyl)-(**5**) and 2-(azolylmethyl)quinolines (**6**), the structures of which are shown in Table 2, were detailed in our previous reports.[1,7] Compounds **4c**, **g**, **i** and **m** were newly prepared for this study by the same methods, and their physical and spectral data are shown in Table 3.

Results and Discussion

The structures and physical data for the naphthalene and quinoline derivatives prepared are shown in Tables 1—3. Since we selected the potent bone resorption inhibitors, the justicidins, as lead compounds aiming at a new type of DMARD, these compounds were first evaluated for their inhibition of ^{45}Ca release from a fetal long-bone culture system (Raisz's assay).[13] The results are summarized in Table 4.

Modification was begun by changing the alkoxy substituents on the naphthalene skeleton and the pendent phenyl moiety. In general, these justicidin analogues (**2a**—**c**) were fully active.

Replacement of the naphthalene ring by a quinoline ring



Justicidin A (1a) : R= OMe **2** : X= CH **4** : Z= S–Heteroaryl
Justicidin B (1b) : R= H **3** : X= N **5** : Z= CH_2–(1-methylimidazol-2-yl)
6 : Z= 1-imidazolyl, 1-triazolyl *etc.*

Fig. 1

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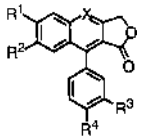
Table 1. Physical Data for Naphthalene Lactones **2** and Quinoline Lactones **3**



Compd.	R^1	R^2	R^3	R^4	X	Yield (%)[a]	Fomula	mp (°C)	Recrystn. solvent
2a	OCH_2O		OCH_2O		CH	31	$C_{20}H_{12}O_6$	203—205	AcOEt–acetone
2b	OCH_2O		OMe	OMe	CH	36	$C_{21}H_{16}O_6$	230—231	AcOEt
2c	OCH_2O		H	OMe	CH	37	$C_{20}H_{14}O_5$	209—210	AcOEt
3a	OCH_2O		OMe	OMe	N	84	$C_{20}H_{15}NO_6$	252—253	$CHCl_3$–MeOH
3b	OMe	OMe	OMe	OMe	N	90	$C_{21}H_{19}NO_6$	241—242	$CHCl_3$–MeOH
3c	OMe	OMe	H	H	N	65	$C_{19}H_{15}NO_4$	210—211	EtOH

a) Yields of **2a**—**c** were based on **10**. Yields of **3a**—**c** were based on **11**.

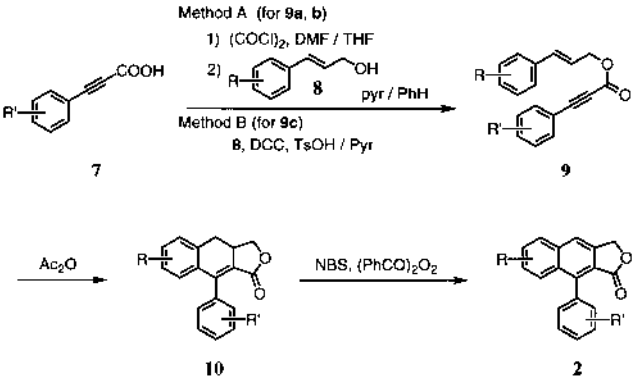


Chart 1

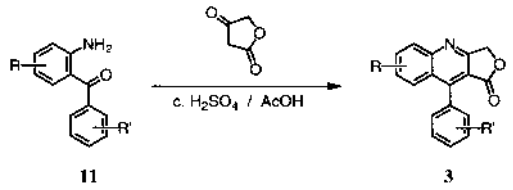
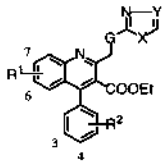


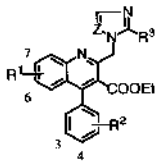
Chart 2

was tried next in an attempt to improve the pharmacological properties, and it was found that activity was retained in the quinoline lactone derivatives (**3a**—**c** *vs*. **2a**—**c**). This finding suggested that the quinoline ring was an effective core structure, encouraging us to introduce various heteroaryl moieties to the 2-methyl position, generated by ring opening of the lactone. The 2-(1-methylimidazol-2-ylthiomethyl)quinoline derivative (**4a**) was the first compound found with the desired biological activity in this series of compounds. Thus, further modification of **4a** was performed.

Firstly, the effect of changing a heteroaryl moiety on the side chain at the 2-position of the quinoline skeleton was evaluated for compounds with a 1-methylimidazolyl (**4a**), a 4-methyltriazolyl (**4b**) or a thiazolyl (**4c**) moiety. These 2-azolylthio derivatives exhibited potent activity comparable with that of **4a**. Compounds **4d**—**g** represent variations of the 6,7-dimethoxy moiety on the quinoline ring possessing the 2-(1-methylimidazol-2-ylthio)methyl substituent. Among these derivatives, compounds bearing a 6,7-dialkoxy moiety (**4a**, **4d**, **4e**) had potent activity. This result is similar to that for the lactone derivatives. Concerning the substituents on the pendent phenyl ring at the 4-position, potent activity was

Table 2. Structures of 2-(Heteroarylthiomethyl)quinoline Derivatives **4**, 2-(1-Methylimidazol-2-ylethyl)quinoline Derivative **5** and 2-(Azolylmethyl)-quinoline Derivatives **6**





Compd.	R^1	R^2	G	X	Y	Z	R^3
4a	6,7-$(MeO)_2$	3,4-$(MeO)_2$	S	N-Me	CH		
4b	6,7-$(MeO)_2$	3,4-$(MeO)_2$	S	N-Me	N		
4c	6,7-$(MeO)_2$	3,4-$(MeO)_2$	S	S	CH		
4d	6,7-$(EtO)_2$	3,4-$(MeO)_2$	S	N-Me	CH		
4e	6,7-$O(CH_2)_2O$–	3,4-$(MeO)_2$	S	N-Me	CH		
4f	H	3,4-$(MeO)_2$	S	N-Me	CH		
4g	6-Cl	3,4-$(MeO)_2$	S	N-Me	CH		
4h	6,7-$(MeO)_2$	4-MeO	S	N-Me	CH		
4i	6,7-$(MeO)_2$	4-EtO	S	N-Me	CH		
4j	6,7-$(MeO)_2$	4-Me	S	N-Me	CH		
4k	6,7-$(MeO)_2$	4-Cl	S	N-Me	CH		
4l	6,7-$(MeO)_2$	H	S	N-Me	CH		
4m	6-Cl	2-Cl	S	N-Me	CH		
5	6,7-$(MeO)_2$	3,4-$(MeO)_2$	CH_2	N-Me	CH		
6a	6,7-$(MeO)_2$	3,4-$(MeO)_2$				CH	H
6b	6,7-$(MeO)_2$	3,4-$(MeO)_2$				CH	Et
6c	6,7-$(MeO)_2$	3,4-$(MeO)_2$				N	H
6d	6,7-$(MeO)_2$	3-iso-PrO, 4-MeO				N	H

observed for compounds with 4-methoxy (**4h**) and 4-methyl (**4j**) substituents, despite the reduction in activity with the unsubstituted compound (**4l**).

Although more data need to be accumulated for further discussion, we proceeded to explore the SAR around **4a**. The activities of compounds **5** and **6a**—**c** illustrate the influence of the linker between the quinoline and the heteroaryl rings. The sulfide moiety of **4a** can be substituted with a methylene (**5**) or can be removed (**6b**, **c**). An abrupt increase in potency was achieved through replacement of the 3,4-dimethoxyphenyl group of **6c** by a 3-isopropoxy-4-methoxyphenyl group (**6d**).

Compounds **2**—**6**, which were derived from justicidins as lead compounds and found to possess antiresorptive activity, were evaluated for anti-inflammatory activity using an adjuvant arthritis model in the rat. Compounds **4**, **5** and **6** had

Table 3. Physical and Spectral Data for Compounds **4c**, **g**, **i** and **m**

Compd.	mp (°C)	Solvent[a]	^{1}H-NMR (ppm, in $CDCl_3$, J in Hz)	IR (KBr, cm^{-1})	Fomula	*Anal*. Calcd (Found) C	H	N	Yield[b] (%)
4c	145—146	EA–H	0.98 (3H, t, J=7.2), 3.80 (3H, s), 3.88 (3H, s), 3.97 (3H, s), 4.05 (2H, q, J=7.2), 4.06 (3H, s), 4.89 (2H, s), 6.91—7.02 (4H, m), 7.22 (1H, d, J=3.4), 7.45 (1H, s), 7.69 (1H, d, J=3.4)	1710	$C_{26}H_{27}N_3O_6S$	59.30 (59.10)	4.98 (5.03)	5.32 (5.45)	81
4g	119—120	EA–H	0.99 (3H, t, J=7.2), 3.51 (3H, s), 3.88 (3H, s), 3.98 (3H, s), 4.08 (2H, q, J=7.2), 4.65 (2H, s), 6.85—7.03 (4H, m), 7.10 (1H, s), 7.64—7.69 (2H, m), 7.96 (1H, d, J=9.6)	1716	$C_{25}H_{24}ClN_3O_4S$	60.30 (60.39)	4.86 (5.00)	8.44 (8.34)	66
4i	117—118	EA–H	0.96 (3H, t, J=7.2), 1.47 (3H, t, J=7.0), 3.43 (3H, s), 3.78 (3H, s), 4.02 (2H, q, J=7.2), 4.04 (3H, s), 4.11 (2H, q, J=7.2), 4.61 (2H, s), 6.86 (1H, s), 6.88 (1H, d, J=1.2), 7.00 (2H, d, J=8.8), 7.09 (1H, d, J=1.2), 7.26 (2H, d, J=8.8), 7.37 (1H, s)	1714	$C_{27}H_{29}N_3O_5S$	63.89 (64.01)	5.76 (5.88)	8.28 (8.36)	83
4m	137—138	A–E	0.93 (3H, t, J=7.2), 3.32 (3H, s), 4.04 (2H, q, J=7.2), 4.59 (1H, d, J=13.6), 4.70 (1H, d, J=13.6), 6.85 (1H, d, J=1.2), 7.10 (1H, d, J=1.2), 7.20—7.30 (2H, m), 7.36—7.60 (3H, m), 7.66 (1H, dd, J=9.0, 2.0), 7.97 (1H, d, J=9.0)	1739	$C_{23}H_{19}C_{l2}N_3O_2S$	58.48 (58.20)	4.05 (4.12)	8.90 (8.73)	48

a) Recrystallization solvent, EA=ethyl acetate, H=hexane, A=acetone, E=Et_2O. *b*) Yield from the corresponding 2-chloromethyl quinoline derivative (see ref.7).

Table 4. Bone Resorption Inhibitory Effect of the Compounds Prepared[a)]

Compd.	Conct. (μM)	^{45}Ca release (% *vs.* control)	Compd.	Conct. (μM)	^{45}Ca release (% *vs.* control)
2a	10	50***	**4h**	10	63**
2b	10	57**	**4i**	10	>80
2c	10	48***	**4j**	10	67*
3a	27[b)]	74*	**4k**	10	>80
3b	26[b)]	>80	**4l**	10	>80
3c	31[b)]	59**	**4m**	10	>80
4a	30	57***	**5**	10	79**
4b	10	76*	**6a**	10	>80
4c	10	59***	**6b**	10	76**
4d	10	72*	**6c**	30	59**
4e	10	66***	**6d**	3	44**
4f	10	>80	**1a** (Justicidin A)	25	48**
4g	10	70***	**1b** (Justicidin B)	25	47**

a) Bone resorption inhibitory effects were evaluated by Raisz's method (see Biological Procedures). *b*) Actual assay was performed at a concentration of 10 μg/ml, which is equivalent to this μM value. Statistically significant at * $p<0.05$, ** $p<0.01$ and *** $p<0.001$ by Student's t-test.

Table 5. Inhibitory Effect of **6c** and **6d** on FBS, hPTH and IL-1β Stimulated Release of ^{45}Ca from Fetal Rat Long Bones in Organ Culture

Stimulator	Compd.	Conct. (μM)	^{45}Ca release (%)[a)]
FBS	**6d**	0	28.9±2.0 (100.0)
		0.3	23.1±2.3 (79.9)***
		1	14.8±1.3 (51.2)***
		3	10.8±0.3 (37.4)***
	6c (TAK-603)	30	17.4±1.6 (60.2)**
hPTH	**6d**	Basal	20.0±2.7 (58.3)**
		0	34.3±2.1 (100.0)
		0.5	23.6±1.3 (68.8)**
		1	17.8±1.3 (51.9)***
	6c (TAK-603)	30	21.8±3.2 (63.6)**
IL-1β	**6d**	Basal	16.9±1.7 (48.6)**
		0	34.8±3.3 (100.0)
		0.3	26.9±1.4 (77.3)
		1	20.0±1.3 (57.5)**
	6c (TAK-603)	30	19.1±0.4 (54.9)**

a) These effects were evaluated by Raisz's method (see Biological Procedures). Data are the mean±S.E. (n=5—6). Statistically significant at * $p<0.05$, ** $p<0.01$ and *** $p<0.001$ by Student's t-test.

potent anti-inflammatory activity.[7)] Among compounds possessing both antiresorptive and anti-inflammatory activities, **6c** (TAK-603) was selected for clinical studies based on its pharmacological profile.[14)] Compound **6d**, which was synthesized during study of the metabolites of **6c**,[1)] exhibited reduced anti-inflammatory activity,[15)] suggesting that some factors other than bone-resorbing function participate in adjuvant-induced inflammation.

Considering the biological results described above, two compounds, **6c** (TAK-603) and **6d**, were selected for detailed evaluation of antiresorptive activity. Ca release from bone induced by bone-resorbing factors,[16)] human IL-1β, human parathyroid hormone (hPTH) and fetal bovine serum (FBS), was reduced by **6c** and **6d** (Table 5). Both compounds inhibited pit formation caused by pre-existing tartrate-resistant acid phosphatase (TRAP)-positive multinucleated cells (MNCs) on dentin slices.[17)] Furthermore, new TRAP–positive MNC formation in culture dishes, without dentin slices, was also inhibited (Table 6). These results indicate that **6c** and **6d** inhibited both the activation of mature osteoclasts and the formation of new ones.

As shown in Table 7, **6c** was effective in an *in vivo* model, ovariectomized mice. In this model, **6c** (TAK-603) had a preventive effect at an oral dose of 10 mg/kg/d.[18)] Since the final stage of RA is bone destruction, the antiresorptive activity of **6c** (TAK-603) may be useful in controlling cartilage destruction. Concerning **6d**, *in vivo* studies using this model are presently under way.

In conclusion, we have found that quinoline derivatives bearing an azole moiety at the side chain of the 2-position have effects on bone metabolism as well as anti-inflammatory activity. Ethyl 4-(3,4-dimethoxyphenyl)-6,7-dimethoxy-2-(1,2,4-triazol-1-ylmethyl)quinoline-3-carboxylate (TAK-603), which had the most promising profile[19)] in this series of compounds, is expected to be useful as a new type of DMARD. Although several approaches to elucidate the detailed mechanism of TAK-603 are now in progress, we are convinced that the antiresorptive activity of this compound will be one of its most characteristic features as an antirheumatic drug.

Table 6. Effect of **6c** (TAK-603) and **6d** on Pit Formation and New Osteoclast Formation by Mature Osteoclasts

	Conct. (M)	Pit formation		New osteoclast formation
		No. of pits/dentin slice[a)]	No. of TRAP(+)-MNCs/dentin slice[b)]	No. of TRAP(+)-MNCs/culture dish[c)]
Control		70.5±4.7	57.5±2.4	66.3±3.0
6c (TAK-603)	10^{-7}	71.3±6.7	55.3±7.3	62.3±7.0
	10^{-6}	47.0±7.0*	38.0±3.9**	46.8±3.2**
	10^{-5}	31.8±5.0**	26.0±2.9**	23.0±3.4**
6d	10^{-9}	69.0±6.0	50.8±5.8	67.8±3.2
	10^{-7}	34.0±6.7**	41.3±3.8**	63.8±3.4
	10^{-5}	2.5±1.0**	21.0±2.4**	13.0±2.9**

a) Effect on the number of resorption pits in mouse unfractionated bone cell cultures. *b*) Effect on the number of pre-existing TRAP-positive MNCs in mouse unfractionated bone cell cultures. *c*) Effect on the formation of new TRAP-positive MNCs in culture dishes without dentin slices. Data are the mean±S.E. (n=4). Statistically significant at * $p<0.05$, ** $p<0.01$ by Student's t-test.

Table 7. Effect of **6c** (TAK-603) on Changes in Femoral Bone Dry Weight in Ovariectomized Mice

	Ovx	Dose (mg/kg)	Femur dis. 1/3 (mg)	(%)
Sham-operated	−		13.0±0.2**	100.0**
Ovx Control	+		11.7±0.2	0.0
6c (TAK-603)	+	10	12.8±0.3*	86.7*

Data are the mean±S.E. (n=6—7). Statistically significant at * $p<0.05$, ** $p<0.01$ by Student's t-test.

Experimental

Chemistry Melting points were determined on a Yanagimoto micromelting point apparatus and are uncorrected. Elemental analyses (C, H, N) were carried out by the Analytical Department of Takeda Chemical Industries, Ltd. ^{1}H-NMR spectra of deuterochloroform ($CDCl_3$) or dimethyl sulfoxide (DMSO-d_6) solutions (internal standard tetramethylsilane (TMS), δ 0) were recorded on a Varian EM-390 (CW–60 MHz) or a Gemini-200 (FT-200 MHz) spectrometer. Infrared (IR) spectra were recorded on a Hitachi IR-215 spectrometer. All compounds exhibited ^{1}H-NMR, IR, and analytical data consistent with the proposed structures. Column chromatography was performed with E. Merck Silica gel 60 (0.063—0.200 mm).

General Procedure for Quinoline Lactones 3. 4-(3,4-Dimethoxyphenyl)-2-hydroxymethyl-6,7-methylenedioxyquinoline-3-carboxylic Acid Lactone (3a) A mixture of 2-amino-3′,4′-dimethoxy-4,5-methylenedioxybenzophenone (200 mg, 0.66 mol), tetronic acid (80 mg, 0.79 mmol), concentrated H_2SO_4 (1 drop) and AcOH (3 ml) was stirred at 100 °C for 1 h, and then concentrated *in vacuo*. The residue was neutralized with saturated aqueous $NaHCO_3$, and extracted with CH_2Cl_2. The extract was washed successively with H_2O and brine, dried over $MgSO_4$, and concentrated *in vacuo* to give crystals. Recrystallization from $CHCl_3$–MeOH afforded **3a** as colorless needles (175 mg, 84%). ^{1}H-NMR ($CDCl_3$) δ: 3.90 (3H, s), 3.99 (3H, s), 5.18 (2H, s), 5.37 (2H, s), 6.95 (1H, d, J=2.0 Hz), 6.98 (1H, dd, J=8.0, 2.0 Hz), 7.06 (1H, d, J=8.0 Hz), 7.22 (1H, s), 7.46 (1H, s). IR (KBr) ν: 1768 cm^{-1}. *Anal*. Calcd for $C_{20}H_{15}NO_6$: C, 65.75; H, 4.14; N, 3.83. Found: C, 65.45; H, 4.06; N, 3.64.

4-(3,4-Dimethoxyphenyl)-2-hydroxymethyl-6,7-dimethoxyquinoline-3-carboxylic Acid Lactone (**3b**): Colorless prisms. ^{1}H-NMR ($CDCl_3$) δ: 3.85 (3H, s), 3.91 (3H, s), 4.01 (3H, s), 4.11 (3H, s), 5.39 (2H, s), 6.98—7.14 (3H, m), 7.23 (1H, s), 7.50 (1H, s). IR (KBr) ν: 1769 cm^{-1}. *Anal*. Calcd for $C_{21}H_{19}NO_6$: C, 66.14; H, 5.02; N, 3.67. Found: C, 66.13; H, 5.06; N, 3.71.

2-Hydroxymethyl-6,7-dimethoxy-4-phenylquinoline-3-carboxylic Acid Lactone (**3c**): Colorless prisms. ^{1}H-NMR ($CDCl_3$) δ: 3.82 (3H, s), 4.10 (3H, s), 5.39 (2H, s), 7.10 (1H, s), 7.40—7.65 (6H, m). IR (KBr) ν: 1758 cm^{-1}. *Anal*. Calcd for $C_{19}H_{15}NO_4$: C, 71.02; H, 4.71; N, 4.36. Found: C, 71.17; H, 4.68; N, 4.34.

3,4-Methylenedioxycinnamyl 3′,4′-Methylenedioxyphenylpropiolate (9a) *N*,*N*-dimethylformamide (DMF) (1 drop) was added to a stirred mixture of 3,4-methylenedioxyphenylpropiolic acid (2.0 g, 11.0 mmol), $(COCl)_2$ (1.7 g, 13.2 mmol) in tetrahydrofuran (THF) (10 ml) at room temperature. The resultant mixture was stirred at room temperature for 1.5 h, and then concentrated *in vacuo*. A mixture of the residue obtained, pyridine (1.3 g, 16.0 mmol), 3,4-methylenedioxycinnnamyl alcohol (1.9 g, 11.0 mmol) and benzene (20 ml) was refluxed for 40 min. After cooling, the mixture was washed successively with 2 N aqueous HCl, H_2O, saturated aqueous $NaHCO_3$ and brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was chromatographed on SiO_2 with AcOEt–hexane (2 : 3) to give **9a** as pale yellow powder (1.4 g, 36%), mp 100—101 °C. ^{1}H-NMR ($CDCl_3$) δ: 4.84 (2H, d, J=7 Hz), 5.97 (2H, s), 6.02 (2H, s), 6.16 (1H, dt, J=16, 7 Hz), 6.63 (1H, d, J=16 Hz), 6.76 (1H, d, J=8 Hz), 6.80 (1H, d, J=8 Hz), 6.85 (1H, dd, J=8, 2 Hz), 6.95 (1H, d, J=2 Hz), 7.01 (1H, d, J=2 Hz), 7.17 (1H, dd, J=8, 2 Hz). IR (KBr) ν: 1710 cm^{-1}.

3,4-Methylenedioxycinnamyl 3′,4′-Dimethoxyphenylpropiolate (**9b**): The title compound was prepared by the same method for **9a**. Yellow powder (yield: 44%), mp 113—115 °C. ^{1}H-NMR ($CDCl_3$) δ: 3.86 (3H, s), 3.90 (3H, s), 4.84 (2H, d, J=6 Hz), 5.93 (2H, s), 6.15 (1H, dt, J=16, 6 Hz), 6.64 (1H, d, J=16 Hz), 6.77—6.93 (4H, m), 7.07 (1H, d, J=2 Hz), 7.23 (1H, dd, J=9, 2 Hz). IR (KBr) ν: 1700 cm^{-1}.

3,4-Methylenedioxycinnamyl 4′-Methoxyphenylpropiolate (9c) A solution of *N*,*N*′-dicyclohexylcarbodiimide (DCC) (2.6 g, 12.7 mmol) in pyridine (9 ml) was added dropwise to a stirred mixture of 3,4-methylenedioxycinnamyl alcohol (2.0 g, 11.5 mmol), 4-methoxyphenylpropiolic acid (2.0 g, 11.5 mmol), *p*-TsOH·H_2O (120 mg, 0.63 mmol) and pyridine (29 ml) at room temperature. After stirring at room temperature for 5 h, AcOH (12 ml) was added, the mixture cooled to 0 °C, and stirred for a further 2 h. The insoluble product was filtered off, the filtrate was diluted with H_2O (100 ml), acidified with concentrated HCl, and then extracted with AcOEt. The combined extracts were washed successively with H_2O, saturated aqueous $NaHCO_3$ and brine, dried over $MgSO_4$ and concentrated *in vacuo* to afford **9c** as pale brown powder (72%), mp 100—102 °C. ^{1}H-NMR ($CDCl_3$) δ: 3.82 (3H, s), 4.84 (2H, d, J=6 Hz), 5.94 (2H, s), 6.15 (1H, td, J=16, 6 Hz), 6.65 (1H, d, J=16 Hz), 6.79 (1H, d, J=2 Hz), 6.87 (4H, d, J=9 Hz), 7.54 (2H, d, J=9 Hz). IR (KBr) ν: 1700 cm^{-1}.

General Procedure for Dihydronaphthalene Lactones 10. 3,4-Dihydro-3-hydroxymethyl-6,7-methylenedioxy-1-(3,4-methylenedioxyphenyl)-naphthalene-2-carboxylic Acid Latone (10a) A solution of **9a** (1.4 g, 4.0 mmol) in Ac_2O (25 ml) was refluxed for 13 h. The mixture was concentrated *in vacuo*, and the residue was chromatographed on SiO_2 with AcOEt–hexane (1 : 1) to give crystals. Recrystallization from AcOEt–EtOH afforded **10a** as pale yellow needles (1.1 g, 78%), mp 140—141 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.77—2.95 (2H, m), 3.33—3.41 (1H, m), 4.00 (1H, t, J=9 Hz), 4.69 (1H, t, J=9 Hz), 5.97 (2H, s), 6.02 (2H, s), 6.52 (1H, s), 6.74—6.90 (4H, m). IR (KBr) ν: 1745 cm^{-1}. *Anal*. Calcd for $C_{20}H_{14}O_6$: C, 68.57; H, 4.03. Found: C, 68.35; H, 4.28.

1-(3,4-Dimethoxyphenyl)-3,4-dihydro-3-hydroxymethyl-6,7-methylenedioxynaphthalene-2-carboxylic Acid Latone (**10b**): Yellow prisms (yield: 34%), mp 225—226 °C (EtOH). ^{1}H-NMR ($CDCl_3$) δ: 2.78—2.92 (2H, m), 3.34—3.42 (1H, m), 3.87 (3H, s), 3.94 (3H, s), 4.01 (1H, t, J=9 Hz), 4.70 (1H, t, J=9 Hz), 5.97 (2H, s), 6.75—6.92 (5H, m). IR (KBr) ν: 1745 cm^{-1}. *Anal*. Calcd for $C_{21}H_{18}O_6$: C, 68.85; H, 4.95. Found: C, 68.88; H, 4.88.

3,4-Dihydro-3-hydroxymethyl-1-(4-methoxyphenyl)-6,7-methylenedioxynaphthalene-2-carboxylic Acid Lactone (**10c**): Pale yellow prisms (yield: 53%), mp 233—234 °C (AcOEt). ^{1}H-NMR ($CDCl_3$) δ: 2.78—2.91 (2H, m),

3.37—3.42 (1H, m), 3.86 (3H, s), 3.99 (1H, t, J=9 Hz), 4.69 (1H, t, J=9 Hz), 5.96 (2H, s), 6.49 (1H, s), 6.77 (1H, s), 6.96 (2H, d, J=10 Hz), 7.24 (2H, d, J=10 Hz). IR (KBr) ν: 1740 cm^{-1}. *Anal*. Calcd for $C_{20}H_{16}O_5$: C, 71.42; H, 4.79. Found: C, 71.60; H, 4.67.

General Procedure for Naphthalene Lactones 2. 3-Hydroxymethyl-6,7-methylenedioxy-1-(3,4-methylenedioxyphenyl)naphthalene-2-carboxylic Acid Lactone (2a) A mixture of **10a** (840 mg, 2.4 mmol), *N*-bromosuccinimide (NBS) (510 mg, 2.9 mmol), benzoyl peroxide (70 mg, 0.29 mmol) and CCl_4 (120 ml) was refluxed for 3 h, and the solvent was evaporated. The residue was chromatographed on SiO_2 with AcOEt–hexane (1 : 1) to give crystals. Recrystallization from AcOEt–acetone afforded **2a** as pale yellow prisms. ^{1}H-NMR ($CDCl_3$) δ: 5.38 (2H, s), 6.07 (2H, s), 6.09 (2H, s), 6.79 (1H, dd, J=8, 2 Hz), 6.81 (1H, d, J=2 Hz), 6.97 (1H, d, J=8 Hz), 7.12 (1H, s), 7.20 (1H, s), 7.70 (1H, s). IR (KBr) ν: 1760 cm^{-1}. *Anal*. Calcd for $C_{20}H_{12}O_6$: C, 68.97; H, 3.47. Found: C, 68.34; H, 3.51.

1-(3,4-Dimethoxyphenyl)-3-hydroxymethyl-6,7-methylenedioxynaphthalene-2-carboxylic Acid Lactone (**2b**): Pale yellow needles. ^{1}H-NMR ($CDCl_3$) δ: 3.87 (3H, s), 3.98 (3H, s), 5.38 (2H, s), 6.08 (2H, s), 6.87 (1H, d, J=2 Hz), 6.91 (1H, dd, J=8, 2 Hz), 7.03 (1H, d, J=8 Hz), 7.13 (1H, s), 7.21 (1H, s), 7.70 (1H, s). IR (KBr) ν: 1750 cm^{-1}. *Anal*. Calcd for $C_{21}H_{16}O_6$: C, 69.23; H, 4.43. Found: C, 69.10; H, 4.40.

3-Hydroxymethyl-1-(4-methoxyphenyl)-6,7-methylenedioxynaphthalene-2-carboxylic Acid Lactone (**2c**): Yellow prisms. ^{1}H-NMR ($CDCl_3$) δ: 3.91 (3H, s), 5.37 (2H, s), 6.08 (2H, s), 7.06 (2H, dd, J=7, 2 Hz), 7.11 (1H, s), 7.20 (1H, s), 7.28 (2H, dd, J=7, 2 Hz), 7.69 (1H, s). IR (KBr) ν: 1765 cm^{-1}. *Anal*. Calcd for $C_{20}H_{14}O_5$: C, 71.85; H, 4.22. Found: C, 71.52; H, 4.43.

Biological Procedures. Bone Resorption Inhibitory Effect The bone resorption inhibitory effect was determined by Raisz's method[13]: ^{45}Ca (radioisotope of calcium in $^{45}CaCl_2$ solution) (50 μCi) was subcutaneously injected into a Sprague–Dawley rat on the 18th day of pregnancy. On the next day, the abdomen was opened, and a fetus was aseptically removed. The left and right humeri (radii and ulnae) were removed under a dissecting microscope, and as much connective tissue and cartilage as possible were also removed. Bone culture samples were then prepared. The bone was incubated in 0.6 ml BCJ_b medium (Fitton-Jackson modification; GIBCO Laboratories) containing 2 mg/ml bovine serum albumin at 37 °C for 24 h in an atmosphere of 5% CO_2 air. The bones were transferred to further fresh medium containing the appropriate final dose of test compound, with or without stimulator, 3% FBS or hPTH (1—34, 100 ng/ml) or IL-1β (300 U/ml)). Bone was cultivated for 2 d in the resulting medium. The ^{45}Ca radioactivity in the medium and the ^{45}Ca radioactivity in the bone were determined. The ratio (%) of ^{45}Ca released from the bone into the medium was calculated from the following equation:

$$\text{ratio of } {}^{45}\text{Ca released from bone into medium (\%)} = \frac{{}^{45}\text{Ca released into medium}}{({}^{45}\text{Ca released into medium}) + ({}^{45}\text{Ca incorporated in bone})} \times 100$$

Unfractionated Bone Cell Preparation Unfractionated bone cells were prepared according to the method of Takeda *et al*.[17] In brief, the femur and tibia of 11- to 13-day-old ICR mice (Japan Charles River, Tokyo, Japan) were aseptically isolated and minced with scalpel blades into 20 ml culture medium consisting of α-minimum essential medium (α-MEM) containing 5% FBS, 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) (pH 7.0), 100 μg/ml kanamycin, and 80 μg/ml gentamicin. The mixture of cell suspension and bone fragments was gently pipetted for 5 min and allowed to stand for 5 min. The resulting supernatant was used for the experiments. To determine the number of osteoclasts in the supernatant, an aliquot was smeared on a slide glass and stained for TRAP, a marker enzyme for osteoclasts, according to the method of Burstone.[20] TRAP-positive MNCs with three or more nuclei (roughly corresponding to 0.05% of the total cells) were counted as osteoclast-like cells.

Pit-Formation Assay Dentin slices of 0.1 mm-thickness and 6 mm-diameter were prepared by our previous method[21] and placed in a 96-well culture dish (Nunclon; Nunc, Roskilide, Denmark). The unfractionated bone cell suspension (4×10^5 cells/dentin slice) with a TRAP-positive MNC density of 200 cells/dentin slice was seeded on to dentin slices and allowed to stand for 2 h at 37 °C in a CO_2 incubator. After removal of nonadherent cells, the cells were further cultured for 4 d in medium containing several concentrations of test compound in the presence of 10^{-8} M 1α,25-dihydroxyvitamine D_3 (1α,25$(OH)_2D_3$). Experiments were completed by removing the culture medium and adding 0.1 M cacodylate buffer solution (pH 7.4) containing 2% paraformaldehyde. The total number of TRAP-positive MNC on each dentin slice was counted after TRAP staining of the cells. Cells were then removed from the dentin slices by ultrasonication for 30 s in distilled water, and air-dried. The slices were stained with hematoxylin and the number of densely stained pits was counted under light microscopy.

Osteoclast-Formation Assay[22] The unfractionated bone cell suspension (2×10^5 cells/well) with a TRAP-positive MNC density of 100 cells/well was cultured in a 96-well plate without dentin slices in the absence of 1α,25$(OH)_2D_3$ for 4 d. After depletion of TRAP-positive MNC was confirmed, the cells were further incubated for 6 d in culture medium containing test compound in the presence of 10^{-8} M 1α,25$(OH)_2D_3$. The number of newly formed TRAP-positive MNC in the culture medium was then counted after TRAP staining of the cells. Our previous study demonstrated that preexisting TRAP-positive MNC were depleted by culturing in the absence of 1α,25$(OH)_2D_3$, but new TRAP-positive MNC formation is induced by the addition of 1α, 25$(OH)_2D_3$ in culture dishes even after complete depletion of TRAP-positive MNCs.

Effect on Changes in Femoral Bone Weight in Ovariectomized Mice Thirteen-week-old female C3H mice were ovariectomized by a dorsal approach under ether anesthesia. Three weeks later, the right femur of each mouse was removed and cleaned of soft tissue. The dry weight of the femur, which was cut one-third from the distal end, was determined after heating at 110 °C for 3 h in an oven. **6c** was administered orally once a day for three weeks after the ovariectomy.

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