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Synthesis and Biological Evaluation of Cytogenin Derivatives

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To enhance the stability *in vivo*, new derivatives of cytogenin were synthesized, and their biological activity and stability in mice were estimated. 2-(8-Hydroxy-6-methoxy-1-oxo-1*H*-2-benzopyran-3-yl)propionic acid (NM-3) was found to be the most stable among them. It modified collagen-induced arthritis in mice. It also showed potent anti-angiogenic activity in a mouse dorsal air sac assay.

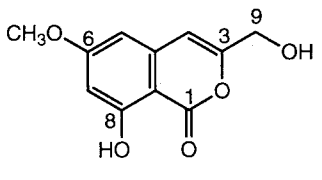
Cytogenin **1** (Fig. 1) was first found as an immunomodulative antitumor substance produced by *Streptoverticillum eurocidium*[1]. Later, it was found that cytogenin had an anti-inflammatory activity against collagen-induced arthritic model in mice[2,3]. However, cytogenin readily transformed to the less active sulfate or glucronide, or was oxidized to the 3-carboxylic acid (MC-1) in the biological assessment in mice. These metabolic conversions seemed to reduce efficacy of cytogenin. So we prepared *O*-acyl and *O*-alkyl derivatives of cytogenin to prevent glucuronide or sulfate conjugation, and the 3-side chain modified analogues to avoid oxidation. Among these derivatives, NM-3 (**2a** in Fig. 1) showed the best pharmacological properties. In this paper, we report the syntheses, stability *in vivo*, and the pharmacological activities of the cytogenin derivatives.

Materials and Methods

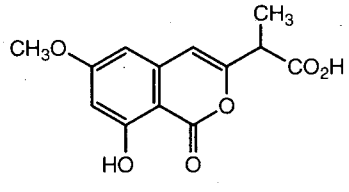
O-Acyl Derivatives of Cytogenin

As shown in Scheme 1, *O*-acyl derivatives **4**~**11** were prepared simply by acylation with acylating agent in the presence of base (method A), or by nucleophilic displacement of the chloride **3**, which was easily prepared by chlorination of cytogenin with PPh_3-CCl_4, with the corresponding nucleophilic reagent (method B).

Fig. 1. Structures of cytogenin and NM-3.



Cytogenin **1**



NM-3 **2a**

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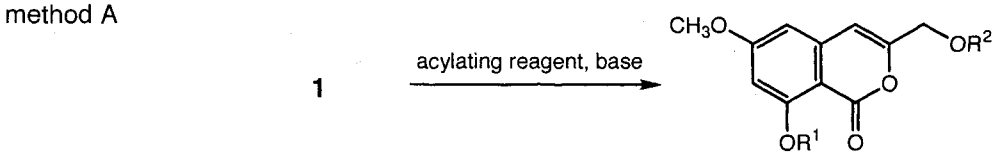
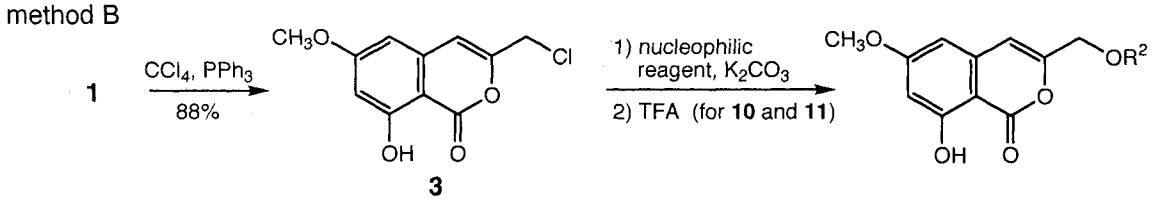
Scheme 1. *O*-Acyl derivatives of cytogenin.

method A



Compound	R^1	R^2	acylating reagent	base	yield (%)
4	H	Ac	Ac_2O (1.2 eq)	pyridine	51
5	Ac	Ac	Ac_2O (12 eq)	pyridine	53
6	H	$CO(CH_2)_2CO_2H$	succinic anhydride	none	74
7	H	$CO(CH_2)_3CO_2H$	glutamic anhydride	none	77
8	H	$COCH_2Ph$	phenacyl chloride	pyridine	74

method B



Compound	R^1	R^2	nucleophilic reagent	yield (%)
9	H	COCH(OH)Ph	DL-manderic acid	79
10	H	$COCH(NH_2)CH(CH_3)_2$	Boc-valine	55
11	H	$COCH(NH_2)CH_2(CH_2)_3NH_2$	*N,N'*-di-Boc-lysine	77

In the method A, acylation generally occurs at the 9-position first, and acylation at the 8-position was slow because of the hydrogen bond with the 1-carbonyl group. In the method B, after the nucleophilic displacement, the Boc group was deprotected by TFA, if necessary.

O-Alkyl Derivatives of Cytogenin

O-Alkyl derivatives **12**, **13**, **14** and **16** were prepared as shown in Scheme 2. Treatment of cytogenin with excess methyl iodide and sodium hydride gave the 6,8,9-*O*-trimethyl derivative **12**. Compound **13** was obtained after the selective demethylation of **12** at 8-position by boron trichrolide in dichloromethane. Treatment of cytogenin with *t*-butylbromoacetate and K_2CO_3 in dimethylformamide gave selective *O*-alkylation at the 8-position. Removal of the *t*-butyl group by TFA gave the 8-*O*-carboxymethyl ether **14**.

On the other hand, the 9-*O*-carboxymethyl derivative **16** was prepared as follows. The intermediate, 8-monomethoxymethyl cytogenin **15**, was prepared successively by methoxymethylation of **3**, nucleophilic displacement of a 9-chlorine atom with sodium formate, and hydrolysis of the formate. After alkylation of **15** with *t*-butylbromoacetate, the protective groups was removed, affording the 9-*O*-carboxymethyl ether **16**.

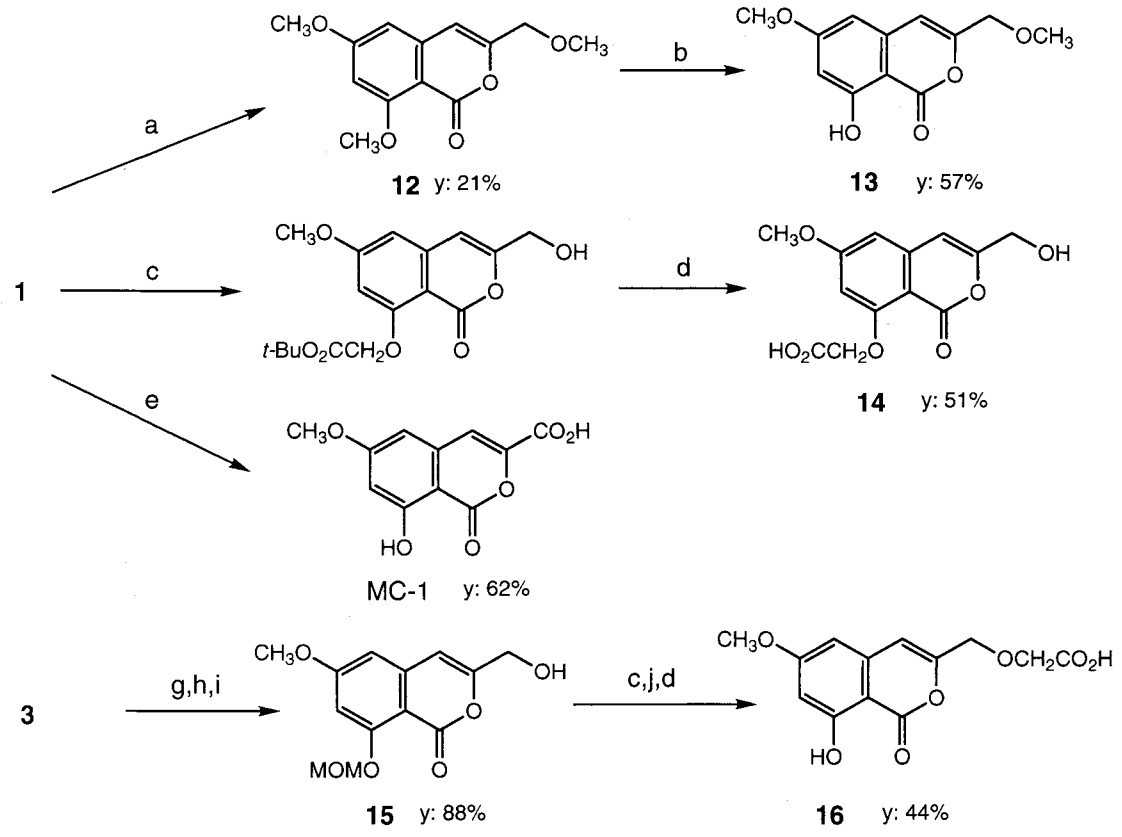
Oxidized Derivative of Cytogenin (MC-1)

Oxidation of cytogenin with Jones' reagent provided the 3-carboxyisocoumarine, MC-1, in 62% yield (Scheme 2), which was identical with one of the metabolites of cytogenin in mice.

Modification of the Side Chain

We designed the homologue of MC-1 not to be subjected to decarboxylation. The reaction of **3** with sodium cyanide in dimethylsulfoxide afforded the nitrile **17**. The desired 9-

Scheme 2. *O*-Alkyl derivatives of cytogenin.



a: CH_3I, NaH/DMF; b: BCl_3/CH_2Cl_2; c: $BrCH_2CO_2t$-Bu, K_2CO_3/DMF; d: CF_3CO_2H/CH_2Cl_2; e: CrO_3, H_2SO_4; f: Ph_3P/CCl_4; g: $ClCH_2OCH_3$, NaH/DMF; h: HCO_2Na/DMF; i: $NaHCO_3/CH_3CN$; j: 1 M HCl/CH_3CN

carboxy derivative **2** was obtained by heating of the nitrile **17** in a 1 : 1 mixture of concentrated hydrochloric acid and acetic acid (Scheme 3).

C-Alkyl Derivative of **2**

The preparation of *C*-alkyl derivatives are shown in Scheme 3. First, the 8-hydroxyl group in **17** was protected as the *tert*-butyldimethylsilyl ether **18**. Usual alkylation procedures such as sodium hydride and alkyl halide gave the dialkyl product as a main product. So we employed the two phase system of aqueous sodium hydroxide and dichloromethane in the presence of tetrabutyl ammonium bromide as a phase transfer catalyst to obtain the monoalkyl product. Even under these conditions, the reaction should be stopped generally within an hour before the dialkyl compound become a major product. Thus, mono-alkyl compounds **19a~c** were prepared along with the recovery of about a half the amount of the starting material **18**. Mono alkyl nitriles **19a~c** were hydrolyzed to **2a** (NM-3), **2b** and **2c**, respectively.

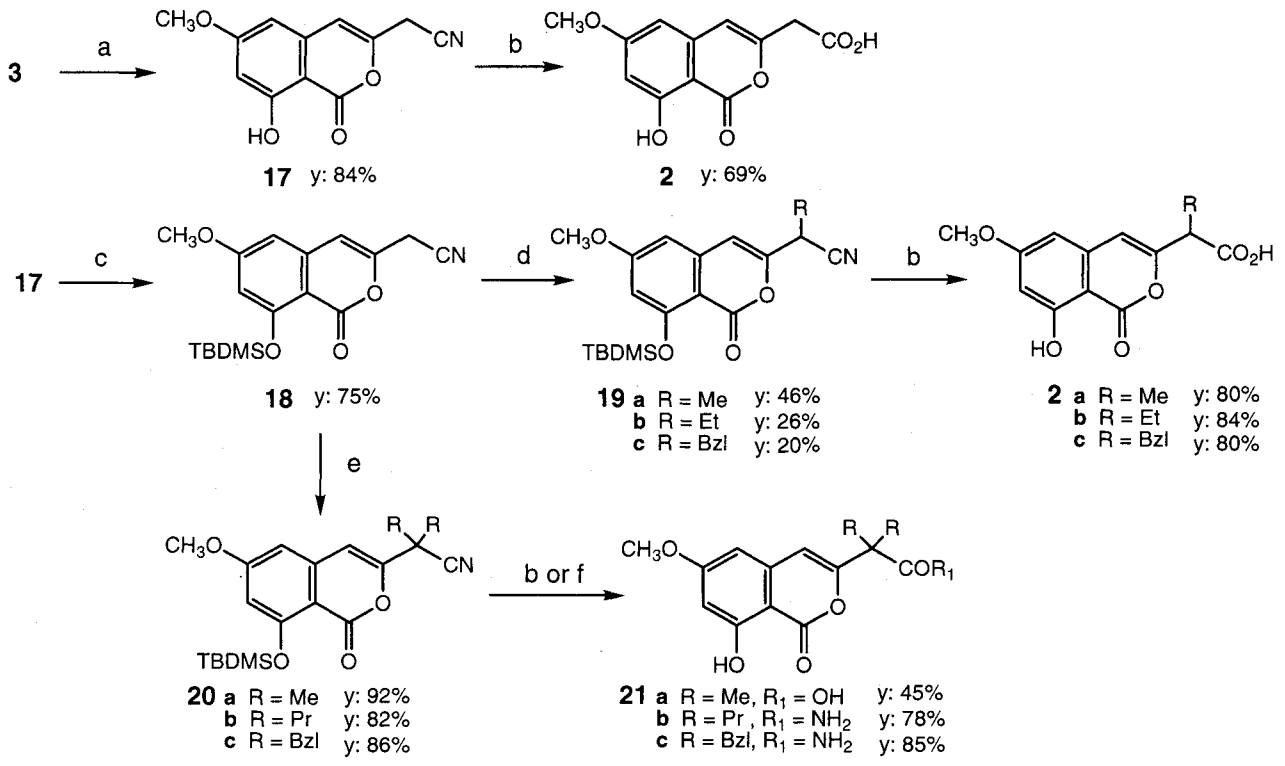
Dialkyl derivatives **20a~c** were obtained by using sodium hydride and alkyl halide, concomitant with partial desilylation as a side reaction. The 9,9-dimethylnitrile **20a** was hydrolyzed to give the carboxylic acid **21a** in 45% yield. However, the complete hydrolysis proved more resistant on the more hindered dialkyl compounds **20b** and **20c** under the same or even more drastic conditions, affording only the carbamoyl compounds **21b** and **21c**, respectively.

Results and Discussion

Metabolite Profile

Orally administered cytogenin was rapidly metabolized in mice and excreted in urine mainly as the 8 (or 9)-*O*-

Scheme 3. Modification of the side chain.



a: NaCN/DMSO; b: HCl, AcOH; c: *t*-butyldimethylchlorosilane, imidazole/DMF; d: RX, NaOH, Bu_4NBr/CH_2Cl_2; e: RX, NaH/THF; f: H_2SO_4, AcOH

sulfonate (MCS), the 8 (or 9)-*O*-glucuronate (MCG) and the 3-carboxylic acid (MC-1). That was confirmed by the HPLC analysis of the metabolites before and after hydrolysis with sulfatase and/or glucuronidase. The rate of these compounds, MCS, MCG and MC-1, found in urine was: 26% (retention time=4 minutes), 22% (4.5 minutes) and 24% (18 minutes), respectively, only 7% cytogenin remained at 5.5 minutes. Another experiment showed that about 70% of cytogenin was conjugated in a plasma.

In vitro and *in vivo* stability are shown in Table 1. *O*-acyl derivatives **4**, **5**, **8**, **9** and **10** were unstable in a plasma and deacylated to cytogenin. Although the *O*-acyl derivatives having a carboxyl group such as **6** and **7** were stable in a plasma, they were deacylated *in vivo* to cytogenin and gave the same metabolites as in the case of cytogenin. Based on these results and from the fact that MC-1 existed without conjugation, we assumed that other compounds having a carboxyl function in the molecule would be stable both *in vitro* and *in vivo*. Therefore, the stability of the carboxymethyloxy derivative **16** and carboxylic acids **2**, **2a**~**c** and **21a** were examined. They showed high stability and remained largely intact both *in vitro* and *in vivo*. On the other hand, 9,9-dialkyl carboxamides **21b** and **21c** were unstable. These findings showed that the carboxylic acid function is crucial for the *in vivo* stability.

Pharmacological Activities

Anti-arthritic activities of the derivatives with good *in vivo* stability were evaluated using type II collagen-induced arthritic mice at a dose of 20 mg/kg or 30 mg/kg, p.o. The inhibitory activities are shown in Table 2. The ED_{50} values of NM-3 and cytogenin were 4.4 mg/kg/day and 44 mg/kg/day, respectively.

As indicated by the data in Table 2, the anti-arthritic activity was exhibited by only a few limited compounds, by oral administration. The $-C^9-COOH$ linkage at C-3 is essential for exhibition of the activity. Substitution at the C-9 position was effective in strengthening the activity, and the methyl group was most efficacious. The bulkier

Table 1. Biological stability of cytogenin derivatives in plasma.

Compounds	*in vitro* (%)[1]	*in vivo*[2] Conjugation rate (%)
Cytogenin	98.0	70
MC-1	99.3	0
2	92.5	0
2a (NM-3)	97.6	0
2b	84.0	4
2c	90.6	8
4	0*	n.t.
5	0*	n.t.
6	95.4	*
7	92.9	*
8	0*	n.t.
9	0*	n.t.
10	41.1*	n.t.
12	99.7	6
13	95.3	90
14	99.2	25
16	101	25
21a	98.4	0
21b	95.2	20
21c	80.3	70

1): Stability in mouse plasma
2): Metabolism in mice (at 30 min after 25 mg/kg, po)
* : Hydrolized to cytogenin
n.t.: not tested

Table 2. Pharmacological activities of cytogenin derivatives.

Compounds	Inhibiting activity (%)	
	Anti-arthritic[a]	Anti-Angiogenic[b]
Cytogenin	38.1	60**
MC-1	32.6	50**
2	3.7*	n.t.
2a (NM-3)	73.6	77.8
2b	6.7	n.t.
2c	46.4	47.3
16	-1.9	n.t.
21a	37.6	66.4
21b	18.1	n.t.

a) Inhibitory effect on collagen-induced arthritis at 20 mg/kg (*: at 30 mg/kg).
b) Inhibitory effect on S-180-induced angiogenesis at 10 mg/kg (**: at 100mg /kg).

substituents such as ethyl for compound **2b** or dimethyl for compound **21a** showed less potent activity.

Anti-angiogenic activity has been found for cytogenin[4]. So a mouse dorsal air sac assay, as described in the previous report[5], was also examined for cytogenin derivatives. As shown in Table 2, compounds **2a** (NM-3) and **21a** were more active than cytogenin. NM-3 showed 78% inhibitory activity against the angiogenesis induced by S-180 tumor cells in mice at a single dose of 10 mg/kg, p.o.

When 25 mg/kg of NM-3 was orally administered, the maximal blood concentration (C_{max}) reached 129.3 μg/ml ($T_{1/2}$: 3.43 hour, T_{max}: 0.141 hour), which was about 100 times higher than that of cytogenin. The area under the curve (AUC) value was 777 mg·hr/ml.

Furthermore, the acute toxicity test in mice showed that NM-3 had extremely low toxicity (the LD_{50} value was more than 1 g/kg by p.o.). Consequently, NM-3 is the most promising candidate as an orally available therapeutic agent for the treatment of angiogenesis-associated diseases and rheumatoid arthritis.

Experimental

General

NMR spectra were recorded on a JEOL JNM-GSX400 spectrometer. Chemical shifts are expressed in δ-value down field from TMS as an internal standard. FAB-MS spectra were measured on a JEOL JMS-SX102A spectrometer using *m*-nitrobenzyl alcohol as a matrix. Silica gel column chromatography was carried out on silica gel 60 (Merck). IR and UV spectra were recorded on a JASCO FT-IR 5300 spectrophotometer and Hitachi model U-3210 spectrophotometer, respectively.

Metabolites of Cytogenin

Male Sprague-Dawley rats weighing 150~160 g (Japan SLC Inc, Shizuoka, Japan) were fasted overnight before and 8 hours after dosing. After oral administration of cytogenin (200 mg/kg), rats were kept separately in a glass metabolism cage. Urine was collected for up to 8 hours and analyzed before and after hydrolysis with sulfatase and/or α-glucronidase. Briefly, 0.5 ml of urine sample was incubated with either 5 mg of sulfatase (Sigma, S-8629) in 2.0 ml of 0.2 M acetate buffer (pH 5.0), 2 mg of α-glucronidase (Sigma, G-7646) in 2.0 ml of 0.1 M phosphate buffer (pH 6.8) or 5 mg of sulfatase containing α-

glucronidase (Sigma, S-9626) in 2.0 ml of 0.2 M acetate buffer (pH 5.0) at 37°C for 2 hours. After adding 2.0 ml of saturated ammonium sulfate, the reaction mixture was mixed with 5 ml of ethyl acetate and centrifuged at 1700 *g* at 4°C for 10 minutes. The resultant supernatant was evaporated *in vacuo* and the residue was re-dissolved with 1.0 ml of 50% methanol. Cytogenin and its metabolites in the solution were analyzed by HPLC.

In Vitro and *In Vivo* Stability Estimation of Cytogenin Analogue

In vitro stability of cytogenin analogs was examined using the plasma taken from ICR mice (Japan SLC Inc, Shizuoka, Japan). The compound was spiked to the plasma (2.0 ml) at 10 μg/ml. After adding saturated ammonium sulfate (1.5 ml), the plasma was mixed with 3 ml of ethyl acetate and centrifuged at 1700 *g* at 4°C for 10 minutes. The resultant supernatant was collected and evaporated *in vacuo*. The residue was re-dissolved with 0.2 ml of 50% methanol for the HPLC analysis.

In vivo stability of cytogenin analogue was examined in mice. Male ICR mice weighing 15~17 *g* (Japan SLC Inc., Shizuoka, Japan) were fasted overnight before dosing. After oral administration of the compounds (25 mg/kg), mice were anesthetized with diethyl ether at 5, 15 and 30 minutes, and blood was drawn from the abdominal aorta. The plasma samples (0.2 ml) obtained by centrifugation of the blood were analyzed before and after hydrolysis with 5 mg sulfatase contains α-glucronidase (Sigma, S-9626) in 1.0 ml of 0.2 M acetate buffer (pH 5.0) at 37°C for 2 hours. After adding 1.5 ml of saturated ammonium sulfate, the reaction mixture was mixed with 3 ml of ethyl acetate and centrifuged at 1700 *g* at 4°C for 10 minutes. The resultant supernatant was evaporated *in vacuo* and the residue was re-dissolved with 0.2 ml of 50% methanol for the HPLC analysis. The conjugation rate (%) was calculated as [(Area of the compound after deconjugation by the mixed enzymes−Area of the compound before deconjugation)/ Area of the compound after deconjugation]×100.

HPLC Analysis

The HPLC apparatus included two 6-AD HPLC pumps (Shimadzu), a 484 tunable absorbance detector (Waters) and a CHROMATOPAC C-R6A (Shimadzu). YMC-Pack ODS-A A-312 column (6.0×150 mm, YMC) was used for analysis. The mobile phase was consisted of a solution of 40% MeOH as solution A and MeOH as solution B, and the columns were eluted with a linear gradient from 0% to 100% solution B (15~20 minutes) in a mixture of solution A. The flow rate of the mobile phase was 1.0 ml/minute. The column effluent was monitored by the ultraviolet absorption at 244 nm.

Anti-arthritic Activity

Male DBA/1J mice (Charles River Japan, Atsugi, Japan) were used at 8~12 weeks of age for the collagen-induced arthritis experiment. The animals were housed under standard laboratory conditions and were fed food and water *ad libitum*. All compounds used were dissolved or suspended in physiological saline or 0.5% carboxymethyl-cellulose (CMC) (Wako Pure Chemical Industries, Osaka, Japan). Bovine type II collagen (Cosmo-Bio, Tokyo, Japan) was dissolved in 0.01 M acetic acid at a concentration of 2 mg/ml before use. Mice were immunized by intradermal injection at a base of the tail with 100 μg of native collagen emulsified in an equal volume of Freund's complete adjuvant (Difco Labs., Detroit, MI, USA). Three weeks later, mice were boosted by i.p. injection with 100 μg of the same emulsified native collagen. Groups of 6 to 10 mice were daily treated p.o. with the compounds at a dose of 20 mg/kg/day for 3 weeks after the injection of booster. Groups of 3 and 6 to 10 mice were used for normal control (normal) and arthritis control (control), respectively. Normal and control mice received vehicle alone in the same manner. The mice were observed for clinical arthritis and were scored by grading each paw from 0 to 4 based on erythema and swelling of the joint (0=no erythema or swelling; 1=erythema or swelling of one toe; 2=erythema or swelling of two or more of the toe; 3=erythema and swelling of the entire paw; 4=complete erythema and swelling of the entire paw and incapacity to bend the ankle). All four legs were scored; the highest score reached 16. Inhibitory percent on arthritis was calculated as [(C−T) /C]×100, where C was the maximal score of control group and T was that of treatment group.

Anti-angiogenic Activity

Anti-angiogenic activity was evaluated using a mouse dorsal air sac assay. The assay was performed as described previously[5]. The compound was administered orally once daily (10 or 100 mg/kg) for 5 consecutive days to ICR mice (Charles River Japan). And the angiogenic response was assessed by determining the number of newly formed blood vessels, induced by inplanted chamber containing S-180 tumor cells.

3-Chloromethyl-8-hydroxy-6-methoxy-1-oxo-1*H*-2-benzopyran (**3**)

To a solution of **1** (20.0 g, 90.09 mmol) in THF (100 ml), carbon tetrachloride (150 ml) and triphenylphosphine (40.3

g, 153.2 mmol) were added. The solution was refluxed for 2 hours and then concentrated. The residue was crystallized from ethanol to give **3** (18.9 g, 88% yield).

IR (KBr) cm^{-1} 1685, 1165, 690; UV (MeOH) λ_{max} nm (ε) 246 (47,900), 333 (7,700); ^1H NMR ($CDCl_3$) δ 10.98 (1H, s), 6.54 (1H, d, J=2.2 Hz), 6.52 (1H, s), 6.41 (1H, d, J=2.2 Hz), 4.34 (2H, s), 3.88 (3H, s); HR-FABMS *m/z* calcd for $C_{11}H_{10}O_4Cl$ $(M+H)^+$ 241.0268, found 241.0275.

8-Acetoxy-3-hydroxymethyl-6-methoxy-1-oxo-1*H*-2-benzopyran (**4**)

A mixture of **1** (500 mg, 2.25 mmol), pyridine (0.24 ml, 2.92 mmol) and acetic anhydride (0.26 ml, 2.70 mmol) in THF (2 ml) were stirred at room temperature for 15 hours. The reaction mixture was diluted with water and extracted with EtOAc (50 ml). The organic layer was washed with water and brine, dried over Na_2SO_4, and then concentrated. The residue was crystallized from methanol to give **4** (302 mg, 51% yield).

IR (KBr) cm^{-1} 1735, 1680, 1245; UV (MeOH) λ_{max} nm (ε) 244 (46,400), 330 (6,500); ^1H NMR ($CDCl_3$) δ 11.01 (1H, s), 6.53 (1H, d, J=2.2 Hz), 6.48 (1H, s), 6.40 (1H, d, J=2.2 Hz), 4.87 (2H, s), 3.88 (3H, s), 2.15 (3H, s); HR-FABMS *m/z* calcd for $C_{13}H_{13}O_6$ $(M+H)^+$ 265.0712, found 265.0704.

8-Acetoxy-3-acetoxymethyl-6-methoxy-1-oxo-1*H*-2-benzopyran (**5**)

Compound **5** was prepared by use of a similar procedure analogous to that described for the preparation of **4** except for the amount of reagent (Ac_2O, 12 eq; pyridine, 12 eq).

IR (KBr) cm^{-1} 1765, 1740, 1720, 1225; UV (MeOH) λ_{max} nm (ε) 246 (57,200), 316 (3,600); ^1H NMR ($CDCl_3$) δ 6.72 (1H, d, J=2.2 Hz), 6.71 (1H, d, J=2.2 Hz), 6.46 (1H, s), 4.85 (2H, s), 3.90 (3H, s), 2.41 (3H, s), 2.14 (3H, s); HR-FABMS *m/z* calcd for $C_{15}H_{15}O_7$ $(M+H)^+$ 307.0818, found 307.0812.

8-Hydroxy-6-methoxy-1-oxo-3-succinoyloxymethyl-1*H*-2-benzopyran (**6**)

To a solution of **1** (300 mg, 1.35 mmol) in toluene (5 ml) was added succinic anhydride (270 mg, 5.40 mmol). After refluxing for 8 hours, the solution was concentrated. The residue was crystallized from methanol to give **6** (320 mg, 74% yield).

IR (KBr) cm^{-1} 1720, 1680, 1650, 1165; UV (MeOH) λ_{max} nm (ε) 244 (53,200), 330 (6,600); ^1H NMR (acetone-d_6) δ 11.04 (1H, s), 6.78 (1H, s), 6.64 (1H, d, J=2.2 Hz), 6.55 (1H, d, J=2.2 Hz), 4.96 (2H, s), 3.93 (3H, s), 2.70~2.73 (2H, m), 2.64~2.68 (2H, m); HR-FABMS *m/z* calcd for $C_{15}H_{15}O_8$ $(M+H)^+$ 323.0767, found 323.0759.

3-Glutamoyloxymethyl-8-hydroxy-6-methoxy-1-oxo-1*H*-2-benzopyran (**7**)

By use of a procedure analogous to that described for the preparation of **6**, compound **7** was prepared from **1** and glutamic anhydride (77% yield).

IR (KBr) cm^{-1} 1735, 1680, 1650, 1170; UV (MeOH) λ_{max} nm (ε) 244 (45,600), 330 (6,200); ^1H NMR (acetone-d_6) δ 11.04 (1H, s), 6.78 (1H, s), 6.65 (1H, d, J=2.2 Hz), 6.55 (1H, d, J=2.2 Hz), 4.96 (2H, s), 3.93 (3H, s), 2.51~2.54 (2H, m), 2.38~2.42 (2H, m), 1.89~1.96 (2H, m); HR-FABMS *m/z* calcd for $C_{16}H_{17}O_8$ $(M+H)^+$ 337.0923, found for 337.0927.

8-Hydroxy-6-methoxy-3-phenylacetoxymethyl-1-oxo-1*H*-2-benzopyran (**8**)

By use of a procedure analogous to that described for the preparation of **4**, compound **8** was prepared from **1** and phenacyl chloride (74% yield).

IR (KBr) cm^{-1} 1745, 1680, 1165; UV (MeOH) λ_{max} nm (ε) 244 (52,200), 330 (7,100); ^1H NMR ($CDCl_3$) δ 10.99 (1H, s), 7.28~7.37 (5H, m), 6.51 (1H, d, J=2.2 Hz), 6.31 (1H, s), 6.30 (1H, d, J=2.2 Hz), 4.90 (2H, s), 3.87 (3H, s), 3.72 (2H, s); HR-FABMS *m/z* calcd for $C_{18}H_{17}O_6$ $(M+H)^+$ 341.1025, found 341.1017.

8-Hydroxy-3-manderoyloxymethyl-6-methoxy-1-oxo-1*H*-2-benzopyran (**9**)

To a solution of **3** (200 mg, 0.83 mmol) in DMF (1 ml), potassium carbonate (285 mg, 2.08 mmol) and DL-manderic acid (630 mg, 4.15 mmol) were added. After stirring at 60°C for 2 hours, the reaction mixture was diluted with water and extracted with EtOAc (30 ml). The organic layer was washed with water and brine, dried over Na_2SO_4, and then concentrated. The residue was purified by silica gel column chromatography to give **9** (234 mg, 79% yield).

IR (KBr) cm^{-1} 1690, 1165; UV (MeOH) λ_{max} nm (ε) 245 (52,300), 330 (7,300); ^1H NMR ($CDCl_3$) δ 10.93 (1H, s), 7.35~7.47 (5H, m), 6.51 (2H, d, J=2.2 Hz), 6.24 (1H, d, J=2.2 Hz), 6.09 (1H, s), 5.29 (1H, d, J=5.8 Hz), 4.96 (2H, s), 3.87 (3H, s), 3.31 (1H, d, J=5.8 Hz); HR-FABMS *m/z* calcd for $C_{19}H_{17}O_7$ $(M+H)^+$ 357.0974, found 357.0949.

3-(2-Aminoisovaleroyloxy)methyl-8-hydroxy-6-methoxy-1-oxo-1*H*-2-benzopyran (**10**)

A solution of **3** (300 mg, 1.35 mmol), potassium carbonate (273 mg, 2.03 mmol) and Boc-valine (580 mg, 2.70 mmol) in DMF (5 ml) was stirred at 80°C for 2 hours. The reaction mixture was diluted with water and extracted with

EtOAc (50 ml). The organic layer was washed with water and brine, dried over Na_2SO_4, and then concentrated. The residue was purified by silica gel column chromatography to give *N*-Boc derivative of **10** (510 mg). To a solution of this compound in CH_2Cl_2 (10 ml) was added TFA (1 ml). After stirring at room temperature for 10 hours, the reaction mixture was concentrated. The residue was diluted with EtOAc (50 ml), washed successively with saturated sodium bicarbonate, water and brine. The organic layer was dried over Na_2SO_4 and concentrated to give **10** (237 mg, 55% yield).

IR (KBr) cm^{-1} 1690, 1620, 1165; UV (MeOH) λ_{max} nm (ε) 244 (50,500), 330 (7,100); ^1H NMR (CD_3OD) δ 6.86 (1H, s), 6.70 (1H, d, J=2.2 Hz), 6.60 (1H, d, J=2.2 Hz), 4.99 (1H, d, J=13.2 Hz), 4.94 (1H, d, J=13.2 Hz), 3.87 (3H, s), 3.21 (1H, d, J=5.9 Hz), 1.84~1.92 (1H, m), 0.89 (3H, d, J=6.6 Hz), 0.84 (3H, d, J=6.6 Hz); HR-FABMS *m/z* calcd for $C_{16}H_{20}NO_6$ $(M+H)^+$ 322.1291, found 322.1310.

3-(2,6-Diaminohexanoyloxy)methyl-8-hydroxy-6-methoxy-1-oxo-1*H*-2-benzopyran (**11**)

By use of a procedure analogous to that described for the preparation of **10**, compound **11** was prepared from **1** and *N*,*N*′-di-Boc-lysine as trifluoroacetic acid salt (77% yield).

IR (KBr) cm^{-1} 1690, 1620, 1200; UV (MeOH) λ_{max} nm (ε) 245 (40,800), 331 (5,600); ^1H NMR (DMSO-d_6) δ 10.89 (1H, s), 8.48 (2H, br), 7.73 (2H, br), 6.93 (1H, s), 6.71 (1H, d, J=2.2 Hz), 6.64 (1H, d, J=2.2 Hz), 5.14 (1H, d, J=13.2 Hz), 5.09 (1H, d, J=13.2 Hz), 4.15 (1H, br), 3.88 (3H, s), 2.76 (2H, br), 1.79~1.84 (2H, m), 1.33~1.58 (4H, m); HR-FABMS *m/z* calcd for $C_{17}H_{23}N_2O_6$ $(M+H)^+$ 351.1556, found 351.1560.

6,8-Dimethoxy-3-methoxymethyl-1-oxo-1*H*-2-benzopyran (**12**)

To a solution of **1** (1.00 g, 4.50 mmol) in DMF (15 ml), sodium hydride (60%, in oil, 540 mg, 13.5 mmol) and methyl iodide (3.6 ml, 27.0 mmol) were added. After stirring at room temperature for 15 hours, the reaction mixture was diluted with water and extracted with EtOAc (100 ml). The organic layer was washed with water and brine, dried over Na_2SO_4, and then concentrated. Chromatographic separation followed by recrystallization from EtOH gave **12** (235 mg, 21% yield).

IR (KBr) cm^{-1} 1730, 1680, 1210, 1120; UV (MeOH) λ_{max} nm (ε) 244 (50,400), 326 (7,300); ^1H NMR ($CDCl_3$) δ 6.47 (1H, d, J=2.2 Hz), 6.39 (1H, d, J=2.2 Hz), 6.37 (1H, s), 4.23 (2H, s), 3.98 (3H, s), 3.90 (3H, s), 3.48 (3H, s); HR-FABMS *m/z* calcd for $C_{13}H_{15}O_5$ $(M+H)^+$ 251.0919, found 251.0917.

8-Hydroxy-6-methoxy-3-methoxymethyl-1-oxo-1*H*-2-benzopyran (**13**)

To a solution of **12** (431 mg, 1.72 mmol) in CH_2Cl_2 (10 ml) was added boron trichloride (1 M CH_2Cl_2 sol., 3.5 ml) at −35°C. After stirring at the same temperature for 30 minutes and further at room temperature for 1 hour, the reaction mixture was diluted with water and extracted with CH_2Cl_2 (10 ml). The organic layer was washed with water and brine, dried over Na_2SO_4, and then concentrated. The residue was recrystallized from EtOH to give **13** (231 mg, 57% yield).

IR (KBr) cm^{-1} 1700, 1240, 1120; UV (MeOH) λ_{max} nm (ε) 243 (47,000), 330 (6,700); ^1H NMR ($CDCl_3$) δ 11.05 (1H, s), 6.50 (1H, d, J=2.2 Hz), 6.46 (1H, s), 6.38 (1H, d, J=2.2 Hz), 4.24 (2H, s), 3.87 (3H, s), 3.48 (3H, s); HR-FABMS *m/z* calcd for $C_{12}H_{13}O_5$ $(M+H)^+$ 237.0763, found 237.0765.

3-Hydroxymethyl-8-carboxymethyloxy-6-methoxy-1-oxo-1*H*-2-benzopyran (**14**)

A solution of **1** (330 mg, 1.50 mmol), potassium carbonate (330 mg, 2.25 mmol) and *t*-butyl bromoacetate (1.1 ml, 7.50 mmol) in DMF (5 ml) was stirred at room temperature for 19 hours. The reaction mixture was diluted with water and extracted with EtOAc (100 ml). The organic layer was washed with water and brine, dried over Na_2SO_4, and then concentrated. The residue was purified by silica gel column chromatography to give *t*-butyl ester of **14** (501 mg). To a solution of the *t*-butyl ester (501 mg) in CH_2Cl_2 (5 ml) was added TFA (1 ml). After stirring at room temperature for 2 hours, the reaction mixture was concentrated. Crystallization from MeOH (5 ml) gave **14** (211 mg, 51% yield).

IR (KBr) cm^{-1} 1755, 1695, 1210, 1090; UV (MeOH) λ_{max} nm (ε) 244 (53,500), 333 (7,100); ^1H NMR (DMSO-d_6) δ 6.74 (1H, d, J=2.2 Hz), 6.54 (1H, s), 6.46 (1H, d, J=2.2 Hz), 5.59 (1H, s), 4.86 (2H, s), 4.22 (1H, s), 3.85 (3H, s). HR-FABMS *m/z* calcd for $C_{13}H_{13}O_7$ $(M+H)^+$ 281.0661, found 281.0662.

3-Hydroxymethyl-6-methoxy-8-methoxymethyloxy-1-oxo-1*H*-2-benzopyran (**15**)

To a solution of **3** (500 mg, 2.25 mmol) in DMF (10 ml), sodium hydride (60%, in oil, 166 mg, 4.15 mmol) and chloromethylmethylether (0.80 ml, 10.24 mmol) were added at 0°C. After stirring at room temperature for 2 hours, the reaction mixture was diluted with water and extracted with EtOAc (100 ml). The organic layer was washed with water

and brine, dried over Na_2SO_4, and then concentrated. To a solution of the residue in DMF (10 ml) was added sodium formate (1.40 g, 10.3 mmol) and the solution was stirred at 80°C for 2 hours. The reaction mixture was diluted with water and extracted with EtOAc (100 ml). The organic layer was washed with water and brine, dried over Na_2SO_4, and then concentrated. The residue in acetonitrile was treated with sodium bicarbonate (10 ml) at 40°C for 17 hours. The reaction mixture was diluted with EtOAc (100 ml), which was washed with water and brine, dried over Na_2SO_4 and concentrated to give **15** (526 mg, 88% yield).

IR (KBr) cm^{-1} 1700, 1160, 1050; UV (MeOH) λ_{max} nm (ε) 244 (40,700), 321 (4,100); ^1H NMR ($CDCl_3$) δ 6.77 (1H, d, J=2.2 Hz), 6.46 (1H, d, J=2.2 Hz), 6.37 (1H, s), 5.35 (2H, s), 4.44 (2H, d, J=6.6 Hz), 3.88 (3H, s), 3.55 (3H, s), 2.22 (1H, t, J=6.6 Hz). HR-FABMS *m/z* calcd for $C_{13}H_{15}O_6$ $(M+H)^+$ 267.0869, found 267.0869.

3-Carboxymethyloxymethyl-8-hydroxy-6-methoxy-1-oxo-1*H*-2-benzopyran (**16**)

To a solution of **15** (300 mg, 1.13 mmol) in DMF (10 ml), sodium hydride (136 mg, 3.40 mmol) and bromoacetic acid *t*-butyl ester (0.83 ml, 5.66 mmol) were added at 0°C. After stirring at room temperature for 2 hours, the reaction mixture was diluted with water and extracted with EtOAc (100 ml). The organic layer was washed with water and brine, dried over Na_2SO_4, and then concentrated. After chromatographic purification, the ester was dissolved in acetonitrile (10 ml) and was treated with 1 M HCl (10 ml) at 40°C for 1 hour. The reaction mixture was diluted with EtOAc (50 ml), which was washed with water and brine, dried over Na_2SO_4, and then concentrated. To a solution of the residue in CH_2Cl_2 (5 ml) was added TFA (1 ml), and the solution was stirred at room temperature for 2 hours. The reaction mixture was added water and extracted with EtOAc (50 ml). The organic layer was washed with water and brine, dried over Na_2SO_4 and concentrated. Crystallization from EtOAc (10 ml) gave **16** (140 mg, 44% yield).

IR (KBr) cm^{-1} 1730, 1680, 1165, 1130; UV (MeOH) λ_{max} nm (ε) 244 (48,500), 330 (6,700); ^1H NMR (DMSO-d_6) δ 10.94 (1H, s), 6.78 (1H, s), 6.69 (1H, d, J=2.2 Hz), 6.58 (1H, d, J=2.2 Hz), 4.39 (2H, s), 4.15 (2H, s), 3.87 (3H, s). HR-FABMS *m/z* calcd for $C_{13}H_{13}O_7$ $(M+H)^+$ 281.0661, found 281.0637.

3-Carboxy-8-hydroxy-6-methoxy-1-oxo-1*H*-2-benzopyran (MC-1)

To a solution of **1** (3.6 g, 16.2 mmol) in acetone (100 ml) was added Jones' reagent (14 ml) at 0°C. After stirring at the same temperature for 10 minutes, the mixture was diluted with water (500 ml) and extracted with EtOAc. The organic layer was washed with 20% aq. NaCl (200 ml×3). The extract was back-extracted with 5% $NaHCO_3$ (300 ml×4). The volume of the aqueous solution was adjusted to 1400 ml by addition of water, and acidified to pH 3 with conc. HCl. The white precipitate (2.36 g) formed was obtained by filtration, while 150 mg of MC-1 was recovered from EtOAc extract of the mother liquid. The combined solid was washed with hot 90% aq. MeOH (40 ml) to give pure MC-1 (2.36 g, 62% yield).

IR (KBr) cm^{-1} 3436, 1723, 1692, 1616, 1377, 1196, 1173; ^1H NMR (DMSO-d_6) δ 10.89 (1H, s), 7.59 (1H, s), 6.96 (1H, d, J=2.4 Hz), 6.71 (1H, d, J=2.4 Hz), 3.88 (3H, s); ^{13}C NMR (DMSO-d_6) δ 56.0, 100.9, 102.7, 104.5, 112.6, 137.1, 143.2, 160.7, 162.5, 163.8, 166.3; FAB-MS *m/z* 237 $(M+H)^+$.

3-Cyanomethyl-8-hydroxy-6-methoxy-1-oxo-1*H*-2-benzopyran (**17**)

A solution of **3** (5.00 g, 20.8 mmol) and sodium cyanide (4.09 g, 83.3 mmol) in DMSO (250 ml) was stirred at room temperature for 1 hour under a nitrogen atmosphere. The reaction mixture was diluted with water and extracted with EtOAc (500 ml×3). The organic layer was washed with 20% brine (400 ml×3), dried over Na_2SO_4, and then concentrated. The residue was dissolved in hot EtOH (50 ml) and the solution was stirred at room temperature for 1 hour. The precipitate was collected by filtration, dried *in vacuo* to give **17** (4.02 g, 84% yield).

IR (KBr) cm^{-1} 2260, 1680, 1195; UV (MeOH) λ_{max} nm (ε) 244 (44,700), 329 (6,600); ^1H NMR ($CDCl_3$) δ 10.82 (1H, s), 6.58 (1H, s), 6.54 (1H, d, J=2.2 Hz), 6.42 (1H, d, J=2.2 Hz), 3.89 (3H, s), 3.65 (2H, s); HR-FABMS *m/z* calcd for $C_{13}H_{10}NO_4$ $(M+H)^+$ 232.0610, found 232.0598.

(8-Hydroxy-6-methoxy-1-oxo-1*H*-2-benzopyran-3-yl)-acetic acid (**2**)

To a solution of **17** (1.50 g, 6.49 mmol) in AcOH (12 ml) was added conc. HCl (12 ml), and stirred at 80°C for 8 hours. Water (9 ml) was added to the solution and stirred at room temperature for 15 hours. The precipitate was collected by filtration, washed with water, and then dried *in vacuo* to give crude **2**. The crude **2** was dissolved in EtOH (30 ml) and added charcoal (1.00 g). The solution was stirred at 60°C for 1 hour and concentrated after filtration. Recrystallization from acetone-hexane (1 : 5) gave **2** (1.12 g, 69% yield).

IR (KBr) cm^{-1} 1720, 1690, 1240; UV (MeOH) λ_{max} nm (ε) 245 (47,200), 329 (6,300); ^1H NMR (DMSO-d_6) δ 10.93 (1H, s), 6.66 (1H, s), 6.63 (1H, d, J=2.2 Hz), 6.56

(1H, d, J=2.2 Hz), 3.86 (3H, s), 3.62 (2H, s). HR-FABMS m/z calcd for $C_{12}H_{11}O_6$ $(M+H)^+$ 251.0556, found 251.0557.

8-*t*-Butyldimethylsilyloxy-3-cyanomethyl-6-methoxy-1-oxo-1*H*-2-benzopyran (**18**)

To a solution of crude **17**, which was prepared from 21.6 g of **3**, in DMF (200 ml), imidazole (12.2 g, 180.2 mmol) and *t*-butyldimethylchlorosilane (21.8 g, 144.1 mmol) were added at 0°C and the solution was stirred at the same temperature for 2 hours. The reaction mixture was diluted with water and extracted with toluene (400 ml). The organic layer was washed with water and brine, dried over Na_2SO_4, and then concentrated. The residue was crystallized from ethanol to give **18** (24.3 g, 75% yield).

IR (KBr) cm^{-1} 2260, 1740, 1170, 835; UV (MeOH) λ_{max} nm (ε) 244 (56,600), 323 (6,100); ^1H NMR ($CDCl_3$) δ 6.48 (1H, s), 6.46 (1H, d, J=2.2 Hz), 6.45 (1H, d, J=2.2 Hz), 3.87 (3H, s), 3.59 (2H, s), 1.05 (9H, s), 0.27 (6H, s); HR-FABMS m/z calcd for $C_{18}H_{24}NO_4Si$ $(M+H)^+$ 346.1475, found 346.1454.

8-*t*-Butyldimethylsilyloxy-3-(1-cyanoethyl)-6-methoxy-1-oxo-1*H*-2-benzopyran (**19a**)

To a vigorously stirred solution of **18** (10.0 g, 29.0 mmol) in two phase system of CH_2Cl_2 (200 ml) - 1 M NaOH (200 ml), tetra-*n*-butyl ammonium bromide (2.34 g, 7.25 mmol) and methyl iodide (7.2 ml, 115.9 mmol) were added and the solution was stirred at 0°C for 1 hour. The organic layer was separated, washed with water and brine, dried over Na_2SO_4 and concentrated. The residue was purified by silica gel column chromatography to give **19a** (4.79 g, 46% yield).

IR (KBr) cm^{-1} 2240, 1740, 1170, 840; UV (MeOH) λ_{max} nm (ε) 244 (51,500), 323 (5,500); ^1H NMR ($CDCl_3$) δ 6.48 (1H, s), 6.46 (1H, d, J=2.2 Hz), 6.45 (1H, d, J=2.2 Hz), 3.87 (3H, s), 3.73 (1H, q, J=7.0 Hz), 1.69 (3H, d, J=7.0 Hz), 1.06 (9H, s), 0.28 (6H, s); HR-FABMS m/z calcd for $C_{19}H_{26}NO_4Si$ $(M+H)^+$ 360.1631, found 360.1617.

8-*t*-Butyldimethylsilyloxy-3-(1-cyanopropyl)-6-methoxy-1-oxo-1*H*-2-benzopyran (**19b**)

By use of a procedure analogous to that described for the preparation of **19a**, **19b** was prepared from **18** and ethyl bromide (26% yield).

IR (KBr) cm^{-1} 2250, 1740, 1170, 840; UV (MeOH) λ_{max} nm (ε) 245 (60,000), 324 (6,600); ^1H NMR ($CDCl_3$) δ 6.49 (1H, s), 6.46 (1H, d, J=2.2 Hz), 6.45 (1H, d, J=2.2 Hz), 3.87 (3H, s), 3.66 (1H, dd, J=5.1 and 8.8 Hz), 1.98~2.14 (2H, m), 1.12 (3H, t, J=7.3 Hz), 1.06 (9H, s), 0.28 (6H, s); HR-FABMS m/z calcd for $C_{20}H_{28}NO_4Si$ $(M+H)^+$ 374.1788, found 374.1784.

8-*t*-Butyldimethylsilyloxy-3-(1-cyano-2-phenyl)ethyl-6-methoxy-1-oxo-1*H*-2-benzopyran (**19c**)

By use of a procedure analogous to that described for the preparation of **19a**, **19c** was prepared from **18** and benzyl bromide (20% yield).

IR (KBr) cm^{-1} 2250, 1740, 1600, 1170, 840; UV (MeOH) λ_{max} nm (ε) 246 (55,000), 325 (6,200); ^1H NMR ($CDCl_3$) δ 7.24~7.33 (5H, m), 6.45 (1H, d, J=2.2 Hz), 6.39 (1H, d, J=2.2 Hz), 6.36 (1H, s), 3.90 (1H, dd, J=5.1 and 8.8 Hz), 3.85 (3H, s), 3.39 (1H, dd, J=5.1 and 13.6 Hz), 3.21 (1H, dd, J=8.8 and 13.6 Hz), 1.07 (9H, s), 0.29 (6H, s); HR-FABMS m/z calcd for $C_{25}H_{30}NO_4Si$ $(M+H)^+$ 436.1944, found 436.1917.

8-*t*-Butyldimethylsilyloxy-3-(1-cyano-1-methyl)ethyl-6-methoxy-1-oxo-1*H*-2-benzopyran (**20a**)

To a solution of **18** (400 mg, 1.16 mmol) in THF (4 ml), sodium hydride (60%, in oil, 116 mg, 2.90 mmol) and methyl iodide (0.44 ml, 6.96 mmol) were added at −30°C. After stirring at the same temperature for 1 hour, the reaction mixture was diluted with water and extracted with EtOAc (100 ml). The organic layer was washed with water and brine, dried over Na_2SO_4, and then concentrated. The residue was purified by silica gel column chromatography to give **20a** (398 mg, 92% yield).

IR (KBr) cm^{-1} 2240, 1745, 1600, 1170, 840; UV (MeOH) λ_{max} nm (ε) 245 (54,600), 323 (6,000); ^1H NMR ($CDCl_3$) δ 6.56 (1H, s), 6.47 (1H, d, J=2.2 Hz), 6.44 (1H, d, J=2.2 Hz), 3.87 (3H, s), 1.72 (6H, s), 1.07 (9H, s), 0.29 (6H, s); HR-FABMS m/z calcd for $C_{20}H_{28}NO_4Si$ $(M+H)^+$ 374.1788, found 374.1776.

8-*t*-Butyldimethylsilyloxy-3-(1-cyano-1-propyl)butyl-6-methoxy-1-oxo-1*H*-2-benzopyran (**20b**)

By use of a procedure analogous to that described for the preparation of **20a**, **20b** was prepared from **18** and propyl iodide (82% yield).

IR (KBr) cm^{-1} 1735, 1600, 840; UV (MeOH) λ_{max} nm (ε) 245 (54,500), 324 (6,100); ^1H NMR ($CDCl_3$) δ 6.60 (1H, s), 6.45 (1H, d, J=2.2 Hz), 6.43 (1H, d, J=2.2 Hz), 3.87 (3H, s), 2.04 (2H, dt, J=5.2 and 14.0 Hz), 1.82 (2H, dt, J=5.2 and 14.0 Hz), 1.49~1.59 (2H, m), 1.25~1.34 (2H, m), 1.07 (9H, s), 0.95 (3H, t, J=7.4 Hz), 0.91 (3H, t, J=7.4 Hz), 0.29 (6H, s); FAB-MS m/z 430 $(M+H)^+$

8-*t*-Butyldimethylsilyloxy-3-(1-benzyl-1-cyano-2-phenyl)-ethyl-6-methoxy-1-oxo-1*H*-2-benzopyran (**20c**)

By use of a procedure analogous to that described for the

preparation of **20a**, **20c** was prepared from **18** and benzyl bromide (86% yield).

IR (KBr) cm^{-1} 2240, 1745, 1600, 1170, 840; UV (MeOH) λ_{max} nm (ε) 247 (55,700), 325 (6,500); ^1H NMR ($CDCl_3$) δ 7.23 (10H, s), 6.43 (1H, d, J=2.2 Hz), 6.19 (1H, d, J=2.2 Hz), 6.06 (1H, s), 3.77 (3H, s), 3.49 (2H, d, J=13.6 Hz), 3.20 (2H, d, J=13.6 Hz), 1.10 (9H, s), 0.32 (6H, s); HR-FABMS *m/z* calcd for $C_{32}H_{36}NO_4Si$ $(M+H)^+$ 526.2414, found 526.2391.

2-(8-Hydroxy-6-methoxy-1-oxo-1*H*-2-benzopyran-3-yl)-2-methyl-propionic Acid (**21a**)

To a solution of **20a** (369 mg, 0.99 mmol) in AcOH (4 ml) was diluted with conc. HCl (4 ml). After stirring at 70°C for 15 hours, the solution was added water (15 ml) and stirred at room temperature for 3 hours. The precipitate was collected by filtration, washed with water and dried *in vacuo* to give the crude **21a**. The crude **21a** was decolorized with charcoal (100 mg) in hot EtOH (10 ml). Recrystallization from acetone - hexane (4 : 9) gave **21a** (126 mg, 45% yield).

IR (KBr) cm^{-1} 1725, 1685, 1170; UV (MeOH) λ_{max} nm (ε) 244 (54,100), 329 (7,500); ^1H NMR (DMSO-d_6) δ 10.87 (1H, s), 6.74 (1H, s), 6.71 (1H, d, J=2.2 Hz), 6.55 (1H, d, J=2.2 Hz), 3.86 (3H, s), 1.45 (6H, s); HR-FABMS *m/z* calcd for $C_{14}H_{15}O_6$ $(M+H)^+$ 279.0869, found 279.0872.

2-(8-Hydroxy-6-methoxy-1-oxo-1*H*-2-benzopyran-3-yl)-2-propyl-valeramide (**21b**)

To a solution of **20b** (346 mg, 0.81 mmol) in AcOH (2 ml) was added sulfuric acid (2 ml). After stirring at 70°C for 16 hours, the reaction mixture was diluted with water and extracted with EtOAc (50 ml). The organic layer was washed with water and brine, dried over Na_2SO_4, and then concentarated. The residue was purified by silica gel chromatography to give **21b** (210 mg, 78% yield).

IR (KBr) cm^{-1} 1680; UV (MeOH) λ_{max} nm (ε) 245 (60,000), 330 (8,200); ^1H NMR ($CDCl_3$) δ 10.97 (1H, s), 6.50 (1H, d, J=2.2 Hz), 6.43 (1H, s), 6.39 (1H, d, J=2.2 Hz), 5.73 (1H, br), 5.48 (1H, br), 3.87 (3H, s), 1.87~1.99 (4H, m), 1.18~1.33 (4H, m), 0.97 (6H, t, J=7.3 Hz); FAB-MS *m/z* 334 $(M+H)^+$

2-(8-Hydroxy-6-methoxy-1-oxo-1*H*-2-benzopyran-3-yl)-2-phenylmethyl-3-phenyl-propionamide (**21c**)

By use of a procedure analogous to that described for the preparation of **20b**, **21c** was prepared from **20c** (85% yield).

IR (KBr) cm^{-1} 1680; UV (MeOH) λ_{max} nm (ε) 247 (53,000), 331 (7,500); ^1H NMR ($CDCl_3$) δ 10.96 (1H, s), 7.14~7.26 (10H, m), 6.51 (1H, d, J=2.2 Hz), 6.25 (1H, s), 6.23 (1H, d, J=2.2 Hz), 5.60 (2H, s), 3.84 (3H, s), 3.34 (2H, d, J=14.0 Hz), 3.29 (2H, d, J=14.0 Hz); HR-FABMS *m/z* calcd for $C_{26}H_{24}NO_5$ $(M+H)^+$ 430.1654, found 430.1634.

2-(8-Hydroxy-6-methoxy-1-oxo-1*H*-2-benzopyran-3-yl)-propionic acid (**2a**, NM-3)

To a solution of **19a** (14.27 g, 39.75 mmol) in AcOH (120 ml) was diluted with conc. HCl (120 ml). After stirring at 80°C for 8 hours, the mixture was added water (90 ml), and stirred at room temperature for 15 hours. The precipitate was collected by filtration, washed with water and dried *in vacuo* to give crude **2a**. The crude **2a** was decolorized with charcoal (1 g) in hot EtOH (10 ml). Recrystallization from acetone - hexane (1 : 5) gave **2a** (8.40 g, 80% yield).

IR (KBr) cm^{-1} 1720, 1690, 1240, 1170; UV (MeOH) λ_{max} nm (ε) 244 (24,400), 330 (7,900); ^1H NMR (DMSO-d_6) δ 12.87 (1H, s), 10.90 (1H, s), 6.68 (1H, s), 6.67 (1H, d, J=2.2 Hz), 6.56 (1H, d, J=2.2 Hz), 3.86 (3H, s), 3.70 (1H, q, J=7.4 Hz), 1.39 (3H, d, J=7.4 Hz). HR-FABMS *m/z* calcd for $C_{13}H_{13}O_6$ $(M+H)^+$ 265.0712, found 265.0711.

2-(8-Hydroxy-6-methoxy-1-oxo-1*H*-2-benzopyran-3-yl)-butyric acid (**2b**)

By use of a procedure analogous to that described for the preparation of **2a**, **2b** was prepared from **19b** (84% yield).

IR (KBr) cm^{-1} 1715, 1690, 1165; UV (MeOH) λ_{max} nm (ε) 245 (47,200), 329 (6,300); ^1H NMR (DMSO-d_6) δ 10.90 (1H, s), 6.69 (1H, s), 6.67 (1H, d, J=2.2 Hz), 6.56 (1H, d, J=2.2 Hz), 3.86 (3H, s), 3.48 (1H, dd, J=7.3 and 8.1 Hz), 1.88~1.96 (1H, m), 1.77~1.86 (1H,m), 0.91 (3H, t, J=7.3 Hz). HR-FABMS *m/z* calcd for $C_{14}H_{15}O_6$ $(M+H)^+$ 279.0869, found 279.0841.

2-(8-Hydroxy-6-methoxy-1-oxo-1*H*-2-benzopyran-3-yl)-3-phenyl-propionic acid (**2c**)

By use of a procedure analogous to that described for the preparation **2a**, **2c** was prepared from **19c** (80% yield).

IR (KBr) cm^{-1} 1685, 1165; UV (MeOH) λ_{max} nm (ε) 246 (51,900), 331 (7,000); ^1H NMR (DMSO-d_6) δ 10.86 (1H, s), 7.12~7.27 (5H, m), 6.59 (1H, d, J=2.2 Hz), 6.58 (1H, s), 6.54 (1H, d, J=2.2 Hz), 3.93 (1H, dd, J=6.6 and 8.8 Hz), 3.83 (3H, s), 3.27 (1H, dd, J=6.6 and 14.0 Hz), 3.10 (1H, dd, J=8.8 and 14.0 Hz). HR-FABMS *m/z* calcd for $C_{19}H_{17}O_6$ $(M+H)^+$ 341.1025, found 341.1028.

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