Design and Synthesis of Antitumor Compounds Based on the Cytotoxic Diterpenoids from the Genus *Rabdosia*

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Two active sites responsible for antitumor activity, an oxirane ring and an α-methylene-cyclopentanone moiety, have been extracted from studies on the structure–activity relationship of the cytotoxic diterpenoids isolated from *Rabdosia shikokiana*. Series of the simplified cyclopentanone derivatives containing both of the two active sites in the molecule have been synthesized and evaluated for cytotoxicity against P 388 cells. The compounds possessing both of two active sites displayed cytotoxicity at a concentration of 1 μg/ml, while those possessing a single active site showed no activity.

Keywords diterpenoid; antitumor activity; drug design; *Rabdosia*; cytotoxity

Plants of the genus *Rabdosia* (Labiatae) are a treasury of biologically active diterpenoids. The potent cytotoxicity of *Rabdosia* diterpenoids against HeLa cells,[1,2] KB cells,[3] mammary cancer FM 3A/B cells,[4] and Ehrlich carcinoma cells,[5] has been reported. Some diterpenoids possess *in vivo* activity against Ehrlich ascites carcinoma,[2,6] Walker intramuscular carcinoma,[3c] and P 388 lymphocytic leukemia.[7] A clinical trial with oridonin, a major diterpenoid of *R. trichocarpa*, has been reported.[8] In a series of studies on the cytotoxic diterpenoids of the genus *Rabdosia*, we isolated antitumor diterpenoids of the 8,9-secokaurene-type from *R. shikokiana* var. *occidentalis*,[9] and report *in vivo* activity against Ehrlich ascites carcinoma in mice.[2a] In those studies, we found a remarkable increase in activity by converting shikoccin (**1**) into epoxyketone **2**.[10] This compound has both an α-methylene cyclopentanone moiety and an epoxide on the same 5-membered ring. An α,β-unsaturated carbonyl group has been claimed to be a Michael acceptor strong enough to react with bionucleophiles such as sulfhydryl groups to exert biological activities, including antitumor activity.[11] The oxirane ring has been known to open easily by attack of the nucleophiles. Thus, the marked increase in antitumor activity of epoxyketone **2** may be attributed to the intramolecular synergism[2b] of two active sites, an α,β-unsaturated ketone moiety a and the oxirane ring b.

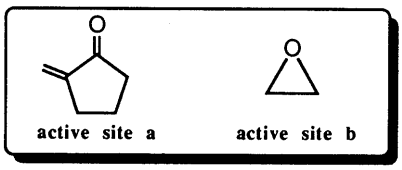


Chart 1. Two Active Sites Extracted from the Structure–Activity Relationship of Diterpenoids from *Rabdosia shikokiana*

The western parts of molecules **1** and **2**, shown by the thick line in Chart 2, are totally identical to each other, if C(6)–C(7) and C(12)–C(13) bonds are cleaved. In other words, all of the structural units necessary for antitumor activity are located on the 5-membered ring. Thus, we selected **7a** as a lead compound for a new type of antitumor agent, in which two key functional groups, a and b, are exquisitely deployed as in the eastern part of **2**. Here we describe syntheses of **7a** and its related compounds, and their *in vitro* activity against P 388 lymphocytic leukemia.

Synthesis Phenylselenylation of 2-methoxycarbonylcyclopentanone (**3a**) afforded **4a**, which was directly used for subsequent reactions. The oxidation, elimination of the resulting selenoxide, and the epoxidation were carried out in one pot to give the epoxide **5a**. The reaction of **5a** with formaldehyde provided the desired product **7a** in poor yield. An alternative two-step sequence *via* **6a** followed by the reduction with diisobutylaluminum hydride (DIBAH) increased the yield of **7a**. The related compounds **7b** and **7c** were prepared by the same route starting from **3b** and **3c**, respectively. Addition of ethanethiol and benzenethiol to **7a** gave **8** and **9**, respectively in high yield.

Aldol condensation of **3a** with benzaldehyde gave **10**,

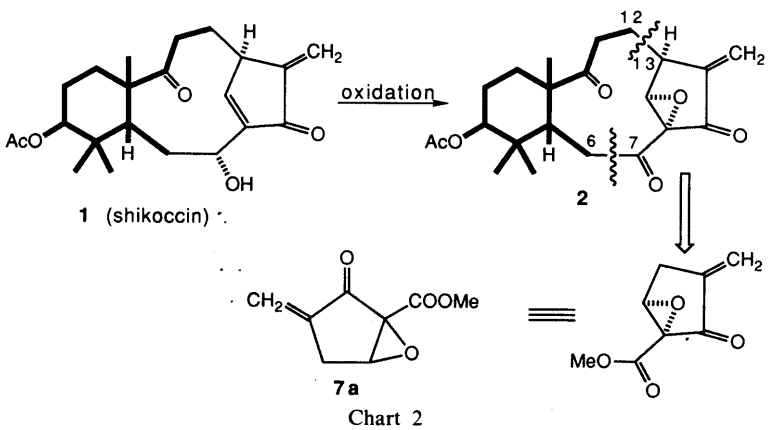


Chart 2

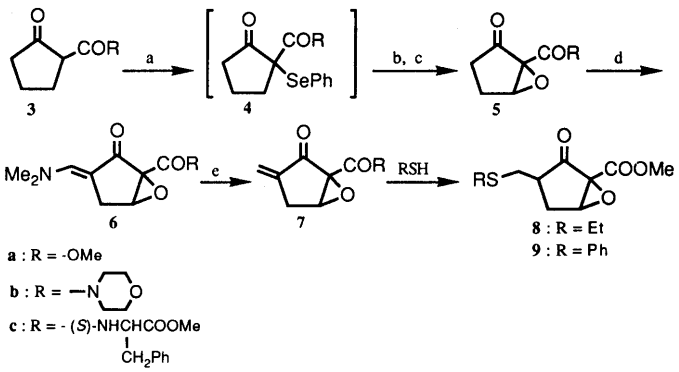
which was dehydrated with hydrochloric acid giving **12**. Phenylselenylation of **12** proceeded smoothly to afford **13**. A one-pot procedure involving hydrogen peroxide oxidation converted **13** into **14**. Compound **11** was prepared from **10** by phenylselenylation followed by oxidation. The reaction of **3a** with dimethylformamide dimethyl acetal afforded **15**. Compound **16** was prepared from **6a** by amidation with lithium benzylamide.

Transesterification of **3a** with ethylene glycol by Seebach's method[12] afforded **17**, which was condensed with **18** to give a symmetrical dimer **19**. Dimeric epoxide **20** and the corresponding α-methylenecyclopentanone **21** were obtained from **19** through a sequence of reactions similar to that for **3a**. Amidation of **18** with piperazine provided a dimeric amide **22** which was transformed to **23**, **24**, and **25** in a similar manner to that described for **3a**.

Phenylselenylation of β-estradiol derivative **26**, followed by oxidation with hydrogen peroxide in dichloromethane (CH_2Cl_2) provided **27**. Further oxidation of **27** with 30% hydrogen peroxide afforded an epoxide **28** which was converted to **29** through the two-step sequence described for **7a**. Syntheses of testosterone derivatives **31**, **32**, and **33** from **30** were similar to the preparation of β-estradiol derivatives.

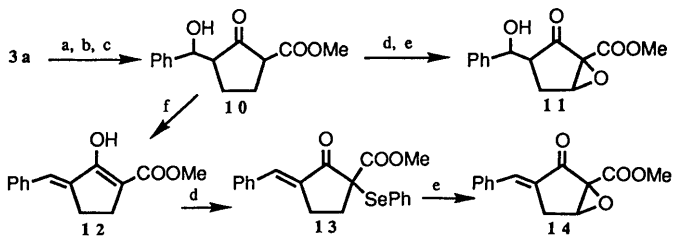
Biological Evaluation The cytotoxicity of the monomeric and dimeric compounds against P 388 leukemia cells was determined and the results are listed Tables I and II. Compound **7a** displayed rather strong cytotoxicity as expected, since it possesses both of two active sites a and b. Other derivatives with two active sites, **7b**, **7c**, and **14**, were active at a concentration of 1 μg/ml. The compounds having an enaminoketone moiety displayed no activity, though both the two active sites a and b were present on the same 5-membered ring (entries 10—12 and 17, Table I). Electrophilicity at the β-carbon to the carbonyl group in these compounds decreases due to the electron-donation from the lone pair on the nitrogen atom, as shown in Chart 7, to suppress the Michael addition of bionucleophiles. It is worthy to note that compounds **8** and **9** showed strong activity. This may be ascribed to the regeneration of active site a by the elimination of thiol under physiological conditions.

The most active compound in the dimeric series was not **21** but **20**. This unexpected result may be due to the chemical instability of **21**. Dimeric compounds connected with amide



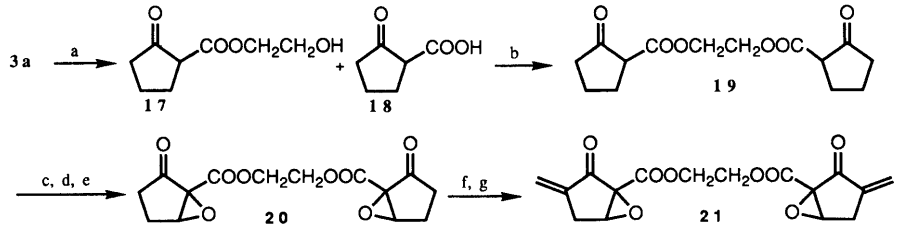
a) PhSeCl/pyridine/CH_2Cl_2, b) 15% H_2O_2/CH_2Cl_2, c) 15%H_2O_2/10% Na_2CO_3, d) $Me_2NCH(OMe)_2$, e) DIBAH/Et_3N

Chart 3



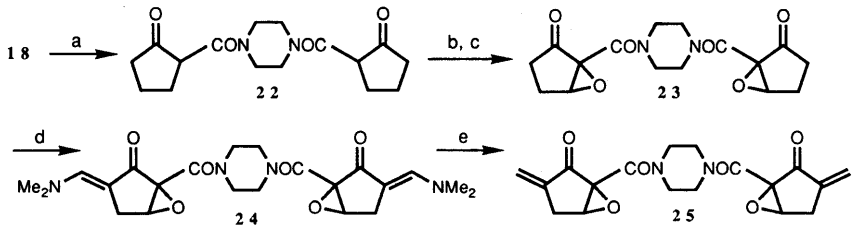
a) NaH, b) *n*-BuLi, c) PhCHO, d) PhSeCl/pyridine, e) 15%H_2O_2/10% Na_2CO_3, f) HCl

Chart 4



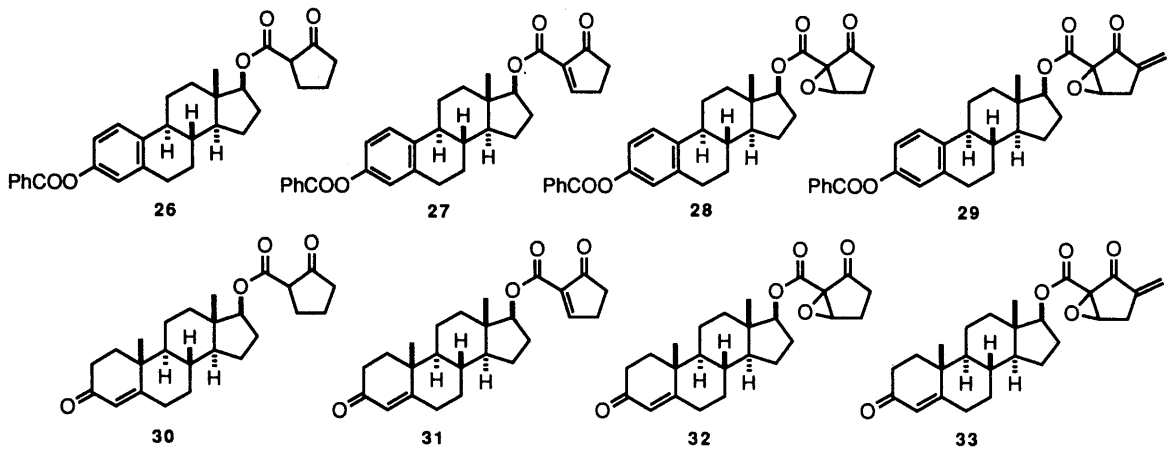
a) $HOCH_2CH_2OH$/$Ti(OEt)_4$, b) 1,1'-carbonyldiimidazole, c) PhSeCl/pyridine, d) 15%H_2O_2/CH_2Cl_2, e) 15%H_2O_2/10%Na_2CO_3, f) $Me_2NCH(OMe)_2$, g) DIBAH/Et_3N

Chart 5



a) piperazine/DCC, b) PhSeCl/pyridine, c) 15%H_2O_2/10% Na_2CO_3, d) $Me_2NCH(OMe)_2$, e) DIBAH/Et_3N

Chart 6



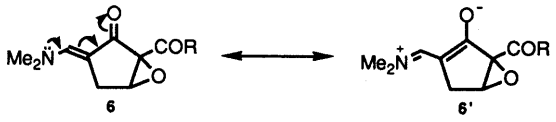


Chart 7

TABLE I. *In Vitro* Activity of the Monomeric Compounds against P388 Leukemia Cells

Entry	Compound	Growth rate (*T*/*C*%) Concentration μg/ml 10	1	0.1	Active site a	b
1	**5a**	26	105	110	No	Yes
2	**5b**	79	95	100	No	Yes
3	**5c**	0	63	91	No	Yes
4	**11**	0	23	72	No	Yes
5	**8**	0	4	66	No	Yes
6	**9**	0	0	62	No	Yes
7	**12**	36	93	102	Yes	No
8	**13**	0	73	98	Yes	No
9	**15**	100	101	104	Yes	No
10	**6a**	85	98	107	Yes	Yes
11	**6b**	85	100	108	Yes	Yes
12	**6c**	65	92	95	Yes	Yes
13	**7a**	0	0	69	Yes	Yes
14	**7b**	0	108	102	Yes	Yes
15	**7c**	0	52	44	Yes	Yes
16	**14**	0	57	101	Yes	Yes
17	**16**	54	94	93	Yes	Yes

TABLE II. *In Vitro* Activity of the Dimeric Compounds against P388 Leukemia Cells

Entry	Compound	Growth rate (*T*/*C*%) Concentration μg/ml 10	1	0.1
1	**20**	0	14	75
2	**21**	1	49	94
3	**22**	78	84	97
4	**23**	24	80	92
5	**24**	80	94	99
6	**25**	23	94	90

linkage displayed no significant activity (entries 3—6, Table II).

Estrogens and androgens have been clinically used for hormone dependent cancer. We synthesized **27**, **28**, and **29** from 17β-estradiol and **31**, **32**, and **33** from testosterone. Though *in vitro* antitumor activity was observed for all compounds at high concentrations, except for **27**, all compounds were inactive at the concentration of 1 μg/ml. Thus, further modification of the structures seems to be necessary for increasing the activity of those types of compound.

Though it is clear that more sophisticated chemical modifications are required, we have shown that our design of antitumor agents was successful in an *in vitro* system.

TABLE III. *In Vitro* Activity of Steroidal Derivatives

Compound	Dose (μg/ml)	Tumor cell GAC 3	GAC 4	MKN-28	Kato 3	P388
27	10	68.8	91.3	23.9	36.4	4.6
	1	132.3	115.7	110.2	105.3	102.7
	0.1	135.2	110.9	103.3	98.8	106.5
28	10	1.8	5.3	−0.5	0.0	−1.8
	1	64.1	92.2	24.7	28.5	4.8
	0.1	100.6	118.2	79.4	102.5	99.2
29	10	3.5	68.0	3.6	7.5	3.7
	1	99.5	101.2	68.7	90.5	84.4
	0.1	115.6	102.5	85.4	104.8	100.0
31	10	0.2	28.8	−1.3	−1.1	−2.1
	1	59.4	98.3	81.3	82.0	116.3
	0.1	89.5	103.5	73.7	100.3	93.1
32	10	3.4	22.3	5.2	2.7	4.6
	1	88.6	115.9	69.0	97.4	127.3
	0.1	92.5	104.0	83.2	106.6	106.1
33	10	3.3	33.6	−1.0	−0.3	−1.9
	1	85.8	103.5	34.6	93.8	4.9
	0.1	83.5	101.8	88.6	103.2	103.6

Experimental

General Method Melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded on a JOEL JMN-GX 400 or JMN-FX 100 spectrometer. Infrared (IR) spectra were measured with a Jasco IR-180 spectrophotometer. Mass spectra (MS) were measured with a JEOL JMS-DX 300 mass spectrometer.

2,3-Epoxy-2-methoxycarbonylcyclopentanone (5a) To a solution of PhSeCl (7.4 g, 39 mmol) and pyridine (3.3 ml, 41 mmol) in anhydrous CH_2Cl_2 (150 ml) was added dropwise a solution of 2-methoxycarbonylcyclopentanone (**3a**) (4.8 g, 34 mmol) at 0 °C, and the mixture was stirred under N_2 at room temperature. The reaction mixture was washed successively with 10% HCl, saturated $NaHCO_3$ solution, and brine, dried, and evaporated to give 10.1 g of 2-methoxycarbonyl-2-

phenylselenocyclopentanone (**4a**). This was immediately dissolved in CH_2Cl_2 (350 ml), 14 ml of 15% H_2O_2 was added to this solution, and the mixture was stirred for 1.5 h at 0 °C. Vigorous stirring was continued for 20 min after the addition of another 14 ml of 15% H_2O_2 and 30 ml of 10% aq. Na_2CO_3. The mixture was washed with 10% aq. Na_2CO_3 and brine, dried, and evaporated. The residue was crystallized from AcOEt–hexane to yield 4.0 g (75%) of **5a**. An analytical sample was recrystallized from AcOEt–hexane, mp 50—50.5 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.13—2.52 (m, 4H), 3.85 (s, 3H), 4.18 (s, 1H). *Anal.* Calcd for $C_7H_8O_4$: C, 53.84; H, 5.16. Found: C, 53.81; H, 5.16.

2,3-Epoxy-2-morpholinocarbonylcyclopentanone (5b) and *N*-(1,2-Epoxy-5-oxocyclopentylcarbonyl)-L-phenylalanine Methyl Ester (5c) Compounds **5b** and **5c** were prepared from **3b** and **3c** through a sequence similar to that for **5a** in 72 and 83% overall yields, respectively. **5b**: mp 75—77 °C (from AcOEt–hexane). *Anal.* Calcd for $C_{10}H_{13}NO_4$: C, 56.86; H, 6.20; N, 6.63. Found: C, 56.85; H, 6.17; N, 6.67. **5c**: Oil. *Anal.* Calcd for $C_{16}H_{17}NO_5$: C, 63.34; H, 5.65; N, 4.62. Found: C, 62.94; H, 5.65; N, 4.57.

5-Dimethylaminomethylene-2,3-epoxy-2-methoxycarbonylcyclopentanone (6a) A mixture of **5a** (4.7 g, 30 mmol) and dimethylformamide dimethylacetal (7.8 ml, 60 mmol) in dimethylformamide (DMF, 15 ml) was stirred at room temperature for 24 h. After the evaporation of the solvent, the residue was chromatographed over silica gel to yield 3.0 g (48%) of **6a**: mp 125.5—127 °C (from MeOH). ^{1}H-NMR ($CDCl_3$) δ: 2.84—3.31 (m, 2H), 3.07 (s, 6H), 3.85 (s, 3H), 4.05 (d, 1H, J=2.5 Hz), 7.31 (s, 1H). *Anal.* Calcd for $C_{10}H_{13}NO_4$: C, 56.86; H, 6.20; N, 6.63. Found: C, 56.86; H, 6.17; N, 6.63.

5-Dimethylaminomethylene-2,3-epoxy-2-morpholinocarbonylcyclopentanone (6b) and *N*-(4-Dimethylaminomethylene-1,2-epoxy-5-oxocyclopentylcarbonyl)-L-phenylalanine Methyl Ester (6c) Compounds **6b** and **6c** were prepared from **5b** and **5c** through a sequence similar to that for **6a** in 44 and 40% yields, respectively. **6b**: mp 199—200 °C (from EtOH). *Anal.* Calcd for $C_{13}H_{18}N_2O_4$: C, 58.63; H, 6.81; N, 10.52. Found: C, 58.58; H, 6.84; N, 10.62. **6c**: Oil. High resolution MS m/z: Calcd for $C_{19}H_{22}N_2O_5$: 358.1528. Found: 358.1529.

2,3-Epoxy-2-methoxycarbonyl-5-methylenecyclopentanone (7a) To a stirred solution of **6a** (870 mg, 4.1 mmol) in anhydrous tetrahydrofuran (THF, 60 ml) were added 3.3 ml of DIBAH (25 g/100 ml in hexane) and Et_3N (0.8 ml, 5.8 mmol) under N_2 at −78 °C, and the mixture was stirred for 2 h. After addition of 20 ml of saturated NH_4Cl solution, the mixture was extracted with CH_2Cl_2, and the combined CH_2Cl_2 phases were washed with saturated NH_4Cl solution, dried, and evaporated to afford an oily residue, which was chromatographed on silica gel to yield **7a** (525 mg, 76%), oil. ^{1}H-NMR ($CDCl_3$) δ: 2.94 (m, 2H), 3.88 (s, 3H), 4.18 (br s, 1H), 5.56 (t, 1H, J=2 Hz), 6.28 (t, 1H, J=3 Hz). *Anal.* Calcd for $C_8H_8O_4$: C, 57.14; H, 4.80. Found: C, 57.10; H, 4.80.

2,3-Epoxy-5-methylene-2-morpholinocarbonylcyclopentanone (7b) and *N*-(1,2-Epoxy-4-methylene-5-oxocyclopentylcarbonyl)-L-phenylalanine Methyl Ester (7c) Compounds **7b** and **7c** were prepared from **6b** and **6c** under conditions similar to those for **7a** in 39 and 36% yield, respectively. **7b**: Oil. ^{1}H-NMR ($CDCl_3$) δ: 2.95 (m, 2H), 3.46—3.92 (m, 8H), 4.18 (br s, 1H), 5.56 (t, 1H, J=2Hz), 6.25 (t, 1H, J=2 Hz). High resolution MS m/z: Calcd for $C_{11}H_{13}NO_4$: 223.0844. Found: 223.0845. **7c**: Oil. High resolution MS m/z: Calcd for $C_{17}H_{17}NO_5$: 315.1107. Found: 315.1106.

2,3-Epoxy-5-ethylthiomethyl-2-methoxycarbonylcyclopentanone (8) To a solution of **7a** (252 mg, 1.5 mmol) in 10 ml of anhydrous toluene were added EtSH (122 μl, 1.7 mmol) and Et_3N (230 μl, 1.7 mmol) and the mixture was stirred for 2.5 h. Evaporation of the solvent gave an oily residue, which was chromatographed on silica gel to yield 316 mg (92%) of oily **8**. ^{1}H-NMR ($CDCl_3$) δ: 1.25 (t, 3H, J=7 Hz), 2.32—3.08 (m, 5H), 2.53 (q, 2H, J=7 Hz), 3.85 (s, 3H), 4.16 (s, 1H). *Anal.* Calcd for $C_{10}H_{14}O_4S$: C, 52.16; H, 6.13. Found: C, 52.03; H, 6.09.

2,3-Epoxy-2-methoxycarbonyl-5-(phenylthiomethyl)cyclopentanone (9) Compound **9** was prepared in a similar manner to that described for **8** in 74% yield. **9**: mp 104—105 °C (from ether). *Anal.* Calcd for $C_{14}H_{14}O_4S$: C, 60.41; H, 5.07. Found: C, 60.26; H, 5.05.

5-α-Hydroxybenzyl-2-methoxycarbonylcyclopentanone (10) To a suspension of NaH (60% in mineral oil, 176 mg, 4.4 mmol) in anhydrous THF was added **3a** (570 mg, 4.0 mmol), and the mixture was stirred at room temperature for 15 min. After addition of *n*-BuLi (1.35 M in hexane, 3.3 ml, 4.4 mmol) and benzaldehyde (470 mg, 4.4 mmol), the mixture was stirred for 1 h at room temperature, poured into ice-water, acidified with 10% HCl, and extracted with ether. Combined ether phases were washed with brine, dried, and evaporated to give an oil, which was chromatographed on silica gel to yield **10** (452 mg, 46%) as a yellow oil. ^{1}H-NMR ($CDCl_3$) δ: 1.40—1.95 (m, 2H), 2.00—2.35 (m, 2H), 2.40—2.80 (m, 1H), 3.20 (t, J=10 Hz, 1H), 3.70 (s, 3H), 4.00 (s, 1H, OH), 4.70 (d, J=8.5 Hz, 1H), 7.22 (s, 5H). High resolution MS m/z: Calcd for $C_{14}H_{16}O_4$: 248.1048. Found: 248.1030.

2,3-Epoxy-5-α-hydroxybenzyl-2-methoxycarbonylcyclopentanone (11) Compound **11** was prepared from **10** through a sequence of the reactions similar to those for **5a** in 19% overall yield, mp 116—117 °C (from ether). *Anal.* Calcd for $C_{14}H_{14}O_5$: C, 64.11; H, 5.38. Found: C, 64.44; H, 5.43.

5-Benzylidene-2-methoxycarbonylcyclopentene-1-ol (12) A solution of 804 mg (3.2 mmol) of **10** in $CHCl_3$ (50 ml) saturated with HCl was stirred for 1 h, washed with aq.$NaHCO_3$ and brine, dried, and evaporated to give a residue, which was crystallized from AcOEt–hexane to yield **12** (497 mg, 67%), mp 114—115 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.56—2.96 (m, 4H), 3.81 (s, 3H), 6.92 (t, 1H, J=2 Hz), 7.19—7.50 (m, 5H), 10.19 (s, 1H). IR ($CHCl_3$) ν: 3300, 1650, 1600, 1445, 1250 cm^{-1}. *Anal.* Calcd for $C_{14}H_{14}O_3$: C, 73.02; H, 6.13. Found: C, 72.82; H, 6.21.

5-Benzylidene-2-methoxycarbonyl-2-phenylselenylcyclopentanone (13) A solution of PhSeCl (934 mg, 4.7 mmol) in 25 ml of anhydrous CH_2Cl_2 was stirred with pyridine (415 μl, 5.2 mmol) for 15 min. After addition of a solution of **12** (990 mg, 4.3 mmol) in anhydrous CH_2Cl_2 (5 ml), the mixture was stirred for 2 h, washed with 10% HCl, aq.$NaHCO_3$ and brine, dried, and evaporated to afford an oil. Chromatography on silica gel yielded 1.5 g (87%) of **13**, oil. ^{1}H-NMR ($CDCl_3$) δ: 2.18 (m, 1H), 2.46 (m, 1H); 2.74—3.01 (m, 2H), 3.78 (s, 3H), 7.21—7.69 (m, 11H). IR ($CHCl_3$) ν: 1740, 1705, 1610 cm^{-1}. *Anal.* Calcd for $C_{20}H_{18}O_3Se$: C, 62.34; H, 4.71. Found: C, 62.09; H, 4.67.

5-Benzylidene-2,3-epoxy-2-methoxycarbonylcyclopentanone (14) To a solution of 1.4 g (3.7 mmol) of **13** in CH_2Cl_2 (50 ml) was added 15% H_2O_2 (1.5 ml) under ice-cooling and the mixture was stirred for 1.5 h. After addition of another 1.5 ml of 15% H_2O_2 and 6 ml of 10% aq.Na_2CO_3, the mixture was stirred vigorously for 2 h, washed with aq.Na_2CO_3 and brine, dried, and evaporated to give a residue, which was crystallized from MeOH to yield **14** (547 mg, 61%), mp 115—116 °C. ^{1}H-NMR ($CDCl_3$) δ: 3.04 (dt, 1H, J=18, 2.5 Hz), 3.37 (dd, 1H, J=18, 2 Hz), 3.90 (s, 3H), 4.28 (d, 1H, J=2 Hz), 7.27—7.06 (m, 6H). *Anal.* Calcd for $C_{14}H_{12}O_4$: C, 68.84; H, 4.95. Found: C, 69.11; H, 4.98.

2-Dimethylaminomethylene-5-methoxycarbonylcyclopentanone (15) Compound **15** was prepared from **3a** by a method similar to that described for **6a** in 43% yield, mp 89.5—90 °C (from AcOEt–hexane). *Anal.* Calcd for $C_{10}H_{15}NO_3$: C, 60.89; H, 7.67; N, 7.10. Found: C, 60.83; H, 7.79; N, 7.07.

2-Benzylcarbamoyl-5-dimethylaminomethylene-2,3-epoxycyclopentanone (16) To a solution of 634 mg (3.0 mmol) of **6a** in 30 ml of anhydrous THF was added under ice-cooling 3.3 mmol of lithium benzylamide in anhydrous THF (10 ml), and the mixture was stirred for 2 h. After addition of brine, the reaction mixture was extracted with CH_2Cl_2 and the combined phases were washed with brine, dried, and evaporated to afford a residue, which was chromatographed on silica gel to yield 405 mg (47%) of **16**, mp 135—136 °C (from AcOEt). ^{1}H-NMR ($CDCl_3$) δ: 2.84 (br s, 1H, J=16 Hz), 3.19 (d, 1H, J=16 Hz), 3.07 (s, 6H), 4.27 (d, 1H, J=2 Hz), 4.49 (br d, 2H, J=16 Hz), 7.28 (s, 1H), 7.31 (s, 5H), 8.72 (br s, 1H). *Anal.* Calcd for $C_{16}H_{18}N_2O_3$: C, 67.11; H, 6.34; N, 9.78. Found: C, 67.03; H, 6.35; N, 9.73.

2-(β-Hydroxyethyloxycarbonyl)cyclopentanone (17) A mixture of 2.3 g (16 mmol) of **3a**, 0.8 ml of Ti $(OEt)_4$, and 5 ml of ethylene glycol was stirred at 105 °C for 9 h. After addition of water at room temperature, the mixture was extracted with CH_2Cl_2. The combined organic layer was washed with aq.$NaHCO_3$ and brine, dried, and evaporated to give a residue which was purified by column chromatography on silica gel to yield 2.0 g of **17** (73%), oil. ^{1}H-NMR ($CDCl_3$) δ: 1.75—2.50 (m, 6H), 2.70 (m, 1H, OH), 3.25 (t, 1H, J=10 Hz), 3.85 (m, 2H), 4.25 (m, 1H), 4.48 (m, 1H). High resolution MS m/z: Calcd for $C_8H_{12}O_4$: 172.0736. Found: 172.0737.

1,2-Bis(2-oxocyclopentylcarbonyloxy)ethane (19) After a mixture of 550 mg (4.3 mmol) of **18** and 600 mg (3.7 mmol) of 1,1′-carbonyldiimidazole in CH_2Cl_2 (10 ml) was stirred for 30 min, a solution of 605 mg (3.5 mmol) of **17**, 0.5 ml of pyridine, and 0.6 ml (6 mmol) of sodium imidazole (1 M solution in dimethyl sulfoxide (DMSO) in CH_2Cl_2 (10 ml) was added and the mixture was stirred for 1 h at room temperature, washed with 10% HCl and water, dried, and evaporated to give a crude oil. Purification by column chromatography on silica gel yielded oily **19** (623 mg, 63%). ^{1}H-NMR ($CDCl_3$) δ: 1.70—1.95 (m, 4H), 2.00—2.40 (m, 8H), 3.15 (t, 2H, J=9 Hz), 4.33 (s, 4H). High resolution MS m/z: Calcd for $C_{14}H_{18}O_6$: 282.1102. Found: 282.1092.

1,2-Bis(1,2-epoxy-5-oxocyclopentylcarbonyloxy)ethane (20) and 1,2-Bis(1,2-epoxy-4-methylene-5-oxocyclopentylcarbonyl)ethane (21) Com-

pound **20** and **21** were prepared from **19** in a manner similar to those for **5a** and **7a** in 60 and 3% yields, respectively. **20**: Oil. ^{1}H-NMR ($CDCl_3$) δ: 2.10—2.50 (m, 8H), 4.25 (s, 2H), 4.50 (m, 4H). *Anal.* Calcd for $C_{14}H_{14}O_8$: C, 54.19; H, 4.55. Found: C, 53.76; H, 4.69. **21**: Unstable oil. ^{1}H-NMR ($CDCl_3$) δ: 2.82—3.10 (m, 4H), 4.25 (br s, 2H), 4.55 (m, 4H), 5.55 (s, 2H), 6.25 (s, 2H).

1,4-Bis(2-oxocyclopentylcarbonyl)piperazine (22) A mixture of **18** (955 mg, 7.5 mmol), dicyclohexylcarbodiimide (DCC, 2.3 g, 11.2 mmol), and piperazine (321 mg, 3.7 mmol) in CH_2Cl_2 (60 ml) was stirred at room temperature for 15 h. After filtration, an organic phase was washed with 5% HCl and brine, dried, and evaporated to give a residue, which was chromatographed on silica gel to yield 537 mg (47%) of **22**, mp 140—142 °C (from AcOEt). *Anal.* Calcd for $C_{16}H_{22}N_2O_4$: C, 62.72; H, 7.24; N, 9.14. Found: C, 62.74; H, 7.19; N, 9.16.

1,4-Bis(1,2-epoxy-5-oxocyclopentylcarbonyl)piperazine (23), 1,4-Bis(4-dimethylaminomethylene-1,2-epoxy-5-oxocyclopentylcarbonyl)piperazine (24), and 1,4-Bis(1,2-epoxy-4-methylene-5-oxocyclopentylcarbonyl)piperazine (25) Compounds **23**, **24**, and **25** were prepared through a sequence of reactions similar to **3a** to **7a** in 66, 41, and 46% yields, respectively. **23**: mp 237—240 °C (dec.) (from acetone). *Anal.* Calcd for $C_{16}H_{18}N_2O_6$: C, 57.48; H, 5.43; N, 8.38. Found: C, 57.87; H, 5.58; N, 8.55. **24**: mp > 300 °C (from CH_2Cl_2–MeOH). High resolution MS *m/z*: Calcd for $C_{22}H_{28}N_4O_6$: 444.2008. Found: 444.2009. **25**: mp > 300 °C (from CH_2Cl_2–acetone). *Anal.* Calcd for $C_{18}H_{18}N_2O_6$: C, 60.33; H, 5.06; N, 7.82. Found: C, 60.04; H, 5.05; N, 7.72.

3-Benzoyloxy-17-(5-oxocyclopentylcarbonyloxy)-$\Delta^{1,3,5}$-estratriene (26) A mixture of estradiol 3-benzoate (2.0 g, 2.7 mmol), **18** (1.0 g, 7.8 mmol), 1-ethyl-3-(dimethylaminopropyl)carbodiimide hydrochloride (2.2 g, 11.5 mmol), and 4-dimethylaminopyridine (DMAP, 0.1 g, 0.8 mmol) in CH_2Cl_2 (60 ml) was stirred at 0 °C for 3.5 h. The reaction mixture was washed with 10% HCl and brine, dried, and evaporated to give a residue. Chromatography on silica gel yielded 1.3 g (99%) of **26**, mp 155—156 °C (from AcOEt–hexane). High resolution MS *m/z*: Calcd for $C_{31}H_{34}O_5$: 486.2406. Found: 486.2418.

3-Benzoyloxy-17-(5-oxocyclopentenylcarbonyloxy)-$\Delta^{1,3,5}$-estratriol (27) A solution of PhSeCl (620 mg, 3.1 mmol) in 40 ml of anhydrous CH_2Cl_2 was stirred with pyridine (0.8 ml, 10 mmol) for 20 min. After addition of a solution of 1.3 g (2.6 mmol) of **26** in anhydrous CH_2Cl_2 (45 ml), the mixture was stirred for 20 h, washed with 10% HCl and brine, dried, and evaporated to give an oil which was chromatographed on silica gel. Elution with CH_2Cl_2 afforded an oil (980 mg), which was dissolved with CH_2Cl_2 (80 ml) and stirred with 3 ml of 15% H_2O_2 for 1 h. The reaction mixture was washed with H_2O, dried, and evaporated to afford a crystalline residue (768 mg, 61%), mp 177—179 °C (from CH_2Cl_2–hexane). High resolution MS *m/z*: Calcd for $C_{31}H_{32}O_5$: 484.2308. Found: 484.2279.

3-Benzoyloxy-17-(1,2-epoxy-5-oxocyclopentylcarbonyloxy)-$\Delta^{1,3,5}$-estratriol (28) and 3-Benzoyloxy-17-(1,2-epoxy-4-methylene-5-oxocyclopentylcarbonyloxy)-$\Delta^{1,3,5}$-estratriol (29) Compounds **28** and **29** were prepared from **26** in a manner similar to those for **5a** and **7a** in 66 and 69% yields, respectively. **28**: mp 179—180 °C (from AcOEt–hexane). *Anal.* Calcd for $C_{31}H_{32}O_6$: C, 74.38; H, 6.44. Found: C, 74.09; H, 6.29. **29**: Amorphous. *Anal.* Calcd for $C_{32}H_{32}O_6$: C, 74.97; H, 6.29. Found: C, 74.87; H, 6.27.

17-(5-Oxocyclopentylcarbonyloxy)-Δ^4-androstene-3-one (30) and 17-(5-Oxocyclopentenylcarbonyloxy)-Δ^4-androstene-3-one (31) Compounds **30** and **31** were prepared from testosterone as described for **26** and **27** in 99 and 71% yields, respectively. **30**: mp 118—119 °C (from AcOEt–hexane). *Anal.* Calcd for $C_{25}H_{32}O_4$: C, 75.34; H, 8.60. Found: C, 74.99; H, 8.48. **31**: mp 179—181 °C (from CH_2Cl_2–hexane). *Anal.* Calcd for $C_{25}H_{32}O_4$: C, 75.72; H, 8.14. Found: C, 75.37; H, 8.17.

17-(1,2-Epoxy-5-oxocyclopentylcarbonyloxy)-Δ^4-androstene-3-one (32) and 17-(1,2-Epoxy-4-methylene-5-oxocyclopentylcarbonyloxy)-Δ^4-androstene-3-one (33) Compounds **32** and **33** were prepared from **30** through a sequence of reactions similar to those for **28** and **29**, in 87 and 62% yields, respectively. **32**: mp 153—154 °C (from AcOEt–hexane). *Anal.* Calcd for $C_{25}H_{32}O_5$: C, 72.79; H, 7.82. Found: C, 72.60; H, 7.85. **33**: mp 174—177 °C (from AcOEt–hexane). *Anal.* Calcd for $C_{26}H_{32}O_5$: C, 73.56; H, 7.60. Found: C, 73.36; H, 7.60.

Assessment of the Activity of the Drug against Tumor Cells Growth Assay: RPMI 1640 supplemented with 10% fetal bovine serum, 10 μM 2-hydroxyethyldisufide and 100 μg/ml kanamycin was used as a culture medium. P388 cells were suspended in the culture medium containing the drug (final concentration: 0.1, 1 or 10 μg/ml), plated at a final cell density of 5×10^4 cells/ml, and incubated in a CO_2 incubator at 37 °C for 48 h. The number of cells was counted in a model ZBI Coulter Counter after a 5-min incubation with 0.25% trypsin to dissociate the cells. T/C(%) was calculated according to the equation: (treated cells − starting cells)/(untreated cells − starting cells) × 100.

MTT Assay: The above culture medium was also used in this assay. Human stomach cancer lines, GAC-3 and GAC-4, were kindly supplied by Dr. Morikawa, Shimane Medical College, Izumo. P388 and human stomach cancer (GAC-3, GAC-4, Kato-III and MKN-28) cells were plated at a cell density of 500 and 2000 cells/100 μl, respectively, in a 96-well microtiter plate on day 0, and 100 μl of the drug solution (final incubation in CO_2 incubator. On day 5, 50 μl of 3-(4,5-dimethylthiazol-2-yl)-2,5-phenyltetrazolium bromide (MTT) solution (1 mg/ml) was added and incubated for an additional 4 h. The MTT formazan formed was dissolved in 150 μl of DMSO after removing the culture medium, and its optimal density at 540 nm was measured. T/C(%) was calculated according to the equation: (OD of treated cells − OD of medium only)/(OD of untreated cells − OD of medium only) × 100.

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