Design, Synthesis, and Antiangiogenic Effects of a Series of Potent Novel Fumagillin Analogues

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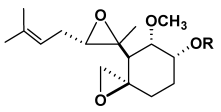
A series of fumagillin analogues containing the C6-substituted cinnamoyl moiety were designed, synthesized, and evaluated for antiangiogenic activity. Among them, 4-hydroxyethoxy-cinnamoyl fumagillol (4a) and 4-hydroxyethoxy-3,5-dimethoxycinnamoyl fumagillol (4d) exhibited more potent anti-proliferation activity in CPAE and HUVEC cells with low cytotoxicity *in vitro*. These compounds are presently under further pharmacological evaluation studies.

Key words *Aspergillus fumigatus*; semisynthesis; hydroxyethoxycinnamoyl fumagillol derivative; antiangiogenic activity; TNP-470

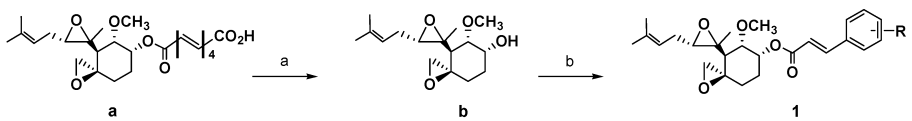
Angiogenesis, the process by which malignant tumors visualizes, is essential for growth and metastasis.[1,2] Endothelial cells respond to angiogenic signals produced by tumors by proliferating and migrating to neighboring tissues. After undergoing differentiation, the endothelial cells generate the inner lining of blood vessels that provide oxygen and nutrients to the growing tumor. Therefore inhibition of endothelial cell growth can block angiogenesis and prevent tumor growth. This approach to cancer treatment is orthogonal to conventional chemotherapy, in which the tumor is directly targeted, and therefore may entail the lower toxicity and drug resistance. Compounds with a similar action mechanism may also be used against other angiogenesis-dependent diseases such as rtheumatoid arthritis.[3,4] Fumagillin (**a**), a natural product from *Aspergillus fumigatus*, was discovered serendipitously and found to inhibit angiogenesis by blocking endothelial cell proliferation (Fig. 1).[5] Fumagillin is rapidly hydrolyzed to fumagillol (**b**) under basic conditions (Chart 1). Subsequently, a number of fumagillin analogues were prepared[5] including TNP-470 (**c**), which resulted from the subsequent search for improved fumagillin analogues and was found to have greater potency and lower toxicity than fumagillin,[6] and is one of the first inhibitors of angiogenesis to reach clinical trials. CKD-732 (**d**)[7] is currently undergoing clinical trial and exhibited better potency and less cytotoxicity compared with TNP-470.[8] Fumagillin derivatives (**a**—**d**) were proposed to inhibit angiogenesis by selective inhibition of methionine aminopeptidase type 2 (MetAP-2)[9] through covalently binding to the His-231 residue by opening the spiro-epoxide (Fig. 1).[10]



Fumagillin (**a**) : R = $CO(CH^t{=}CH)_4CO_2H$
Fumagillol (**b**) : R = H
TNP-470 (**c**) : R = $CONHCOCH_2Cl$
CKD-732 (**d**) : R = $COCH^t{=}CHC_6H_5OCH_2CH_2N(CH_3)_2$
CKD-731 (**e**) : R = $COCH^t{=}CHC_6H_5(OCH_3)_3$

Fig. 1

Significant correlation has been reported between inhibition of MetAP-2 and inhibition of human umbilical vein endothelial cell (HUVEC) proliferation.[11] From the crystal structure of fumagillin, it is obvious that the substitutent at C6 of fumagillin is exposed to solvent. TNP-470 has encountered some problems in human clinical trails including poor pharmacokinetic behavior (*i.e.* short serum half-life) and dose-limiting neurotoxicity, which make it difficult to use as an anticancer agent.[12,13] The lower chemical stability and solubility as well as high toxicity of TNP-470 and CKD-732 are likely due, at least in part, to their intact chemical structures with chloroacetyl groups at the C6 position of TNP-470 and dimethyamino moiety of CKD-732. The goal of our program was to explore the tolerability of MetAP-2 toward inhibitors with lower cytotoxicity and better solubility. We envisioned modification of the side chain at the C6 position, which was associated with the observed cytotoxicity of the compounds. Since the spiroepoxide of the fumagillin family was shown to be essential for MetAP-2 binding, we decided initially to keep this moiety intact. Previously, we reported that CKD-732[7] elicits good inhibitory activity on cell proliferation against EL-4, CPAE, and P388D1 cells *in vitro*[7,8] and that 5-demethoxyfumagillol is a potent antiangiogenic agent isolated from *Aspergillus fumigatus*.[14] Herein, we focused our efforts on the modification of dimethylaminoethoxycinnamoyl side chain (CKD-732) at the C6 to hydroxycinnamoyl, hydroxyethoxycinnamoyl, or aminocinnamoyl groups. As a result, in this paper we describe the synthesis



a **b** **1**

a) 0.1 N NaOH, 5 h, rt, 85%, b) a cinnamic acid (8-10 equiv.), DCC (8-10 equiv.), DMAP (8-10 equiv.), CH_2Cl_2, rt, 4-5 h,78-85%

Chart 1

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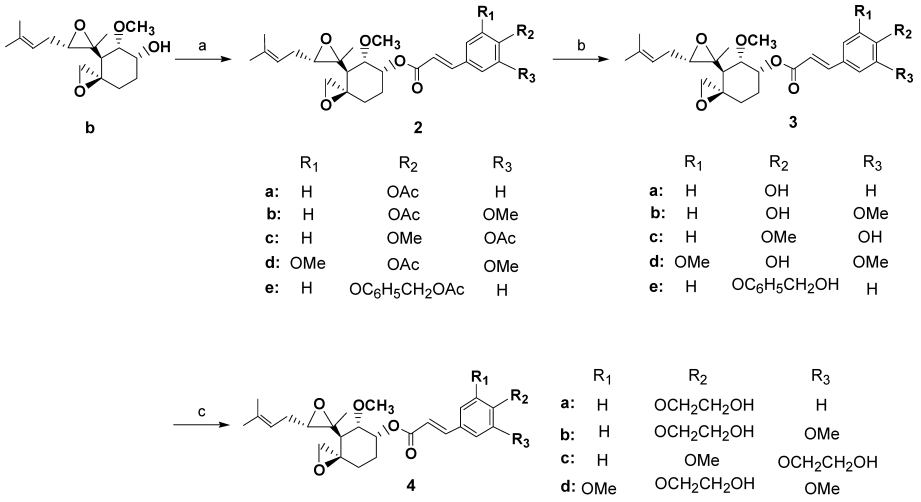
and anti-proliferation activity on calf pulmonary artery endothelial (CPAE) cells and HUVEC cells *in vitro*. Also we report the novel substituted cinnamoyl fumagillin derivatives (**3a**—**e**, **4a**—**d**, **6a**—**c**), which showed lower cytotoxicity compared with reference, TNP-470, reported by Takeda. Among them, 4-hydroxyethoxycinnamoyl fumagillin (**4a**) and 4-hydroxyethoxy-3,5-dimethoxyfumagillin (**4d**) showed not only more potent antiangiogenic activity but also lower cytotoxicity and better chemical stability than the reference compound, TNP-470.

Chemistry Several substituted fumagillin derivatives were prepared. A general synthetic scheme is shown in Chart 2. The starting material, fumagillol (**b**), was obtained in high yield (>85%) by hydrolysis with 0.1 N NaOH solution at room temperature for 5 h from fumagillin (**a**).[15] First, novel substituted cinnamoyl fumagillol derivatives (**3a**—**e**) were synthesized as shown in Chart 2. Condensation of the starting fumagillol (**b**) with several cinnamic acids (8—10 eq) using DCC (8—10 eq) and DMAP (8—10 eq) in CH_2Cl_2 gave the acetoxycinnamoyl fuamgillols (**2a**—**e**) in good yields (78—85%), which transformed to the desired novel cinnamoyl fumagillol derivatives (**3a**—**e**) by hydrolysis with K_2CO_3 in MeOH at room temperature for 1—2.5 h. To obtain novel fumagillol compounds containing longer aliphatic chain moieties, the obtained fumagillin derivatives (**3a**—**e**) were substituted by iodoethanol with K_2CO_3 in DMF at 80 °C for 2—5 h to give desired hydroxyethoxycinnamoyl fumagillols (**4a**—**d**).

Synthesis of aminocinnamoyl fumagillol (**6a—c**) (Chart 3) was carried out *via* regioselective reduction of the nitro group using Borane Exchange Resin (BER)–Ni(OAc)$_2$ with nitrocinnamoyl fumagillols (**6a**—**c**) in good yields (80—95%) without over-reduction in two epoxide sites (including one spiro-epoxide) at C6 as shown in Chart 3.

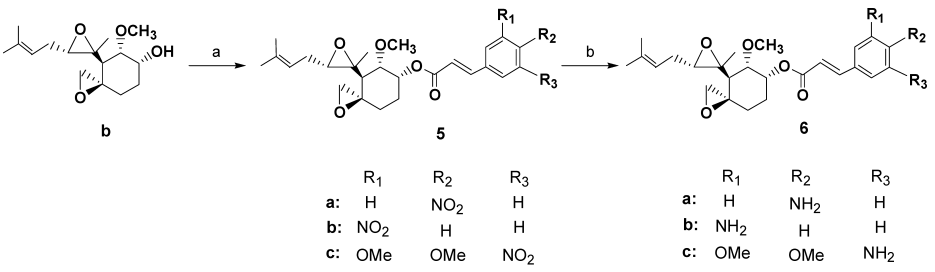
Results and Discussion

When this work started, most biologically active fumagillin analogues only differed from the parent molecule (fumagillin) in the nature of the ester at the C6 position (Chart 1). This is the case for TNP-470 (**a**)[5]—the most studied fumagillin derivative—and also for recently described novel fumagillin analogues (CKD-731 and CKD-732) reported from our group,[7,8] which showed strongly increased cell proliferation inhibitory activity compared with fumagillin and therefore may be considered as "state of the art" in this research area. Despite their potent antiangiogenic activity, fumagillin analogues are associated with considerable cytotoxicity.[16] For example, TNP-470 itself has been reported unstable and cytotoxic probably due, at least in part, to the presence of the chloroacetyl moiety at the C6 side chain. Basically, CKD-



a) a cinnamic acid (8-10 equiv.), DCC (8-10 equiv.), DMAP (8-10 equiv.), CH_2Cl_2, rt, 4-5 h, 78-85%, b) K_2CO_3, MeOH, rt, 1-2.5 h, quantitative c) Iodoethanol, K_2CO_3, DMF, 80 ^oC, 2-5 h, 72-85%

Chart 2

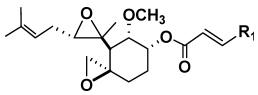


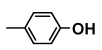
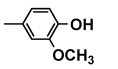
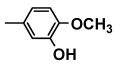
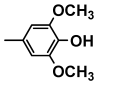
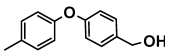
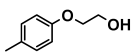
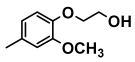
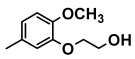
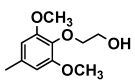
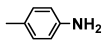
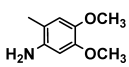
a) a cinnamic acid (8-10 equiv.), EDC (8-10 equiv.), DMAP (8-10 equiv.), CH_2Cl_2, rt, 4-5 h, 65-78%, b) BER-Ni(OAc)$_2$, 0 ^oC, 1-3 h, 80-95%

Chart 3

732 and CKD-731, reported from our group, showed good antiangiogenic activity and low cytotocixity but exhibited poor pharmacokinetic character due to low solubility. Therefore to prevent and overcome these problems we tried to modify the cinnamoyl moiety based on the C6 position of CKD-732 showing excellent anti-proliferation activity against EL-4, CPAE, and P388D1 cells *in vitro*.[7,8] First, to explore the anti-proliferation activity of the substituent, chloroacetyl group or dimethylaminoethoxycinnamoyl moiety was replaced by hydroxycinnamoyl, hydroxyethoxycinnamoyl, and aminocinnamoyl groups at the C6 position maintaining the two epoxides (spiro-epoxide upon the C3 position and the one on C4 side chain) of fumagillol structure (see Fig. 1). As a consequence, several substituted novel fumagillol derivatives (**3a**—**e**, **4a**—**d**, **6a**—**c**) were prepared from fumagillol *via* condensation and evaluated for their activity. Cell-based *in vitro* assay monitoring inhibition of proliferation for CPAE and HUVEC were performed using SRB dye (Table 1).[17] Also, their cytotoxicity was measured by murine thymus lymphoma (L5178Y) cell assay depicted as safety index value (S/I) ratio dividing CPAE IC_{50} into L5178Y IC_{50} and the results are also shown in Table 1. Almost all novel cinnamoyl fumagillol derivatives exhibited excellent anti-proliferation activity. However, among them, hydroxycinnamoyl group (**3a**) and hydroxymethylphenoxycinnamoyl group (**3e**) showed weak anti-proliferation activity, whereas the other novel compounds (**3b**—**d**, **4a**—**d**, **6a**—**c**) showed potent ani-proliferation activity for *in vitro* CPAE and HUVEC assay versus reference compounds TNP-470 and CKD-732 (Table 1). In the case of methoxycinnamoyl or aminocinnamoyl moiety at C6 of fumagillol, the increased lipophilic character (**3b**—**d**) showed more potency and less cytotoxicity comparable to TNP-470 and CKD-732; however, in compounds wherein the the methoxy group was exchanged from 4-position to 3-position (from **3b** to **3c**), there was little anti-proliferation activity, although the reason for this is obscure. Novel fumagillol compounds (**6a**—**c**) with aminocinnamoyl groups had much less anti-proliferation activity (CPAE and HUVEC) and cytotoxicity (S/I value) as compared with methoxycinnamoyl fumagillols (**3b**—**d**). The poor correlation between hydroxyethoxycinnamoyl fumagillol derivatives (**4a**—**d**) was observed in the anti-prolifera-

Table 1. Anti-proliferation Activities of Novel Cinnamoyl Fumagillol Derivatives



Compound	Structure	Activities and cytotoxicity (IC_{50}, nM)			S/I ($L5178Y\ IC_{50}/CPAE\ IC_{50}$)
		CPAE	HUVEC	L5178Y	
3a		2.22×10^{0}	7.60×10^{-3}	4060	1.83×10^{3}
3b		3.46×10^{-3}	1.69×10^{-5}	14786	4.29×10^{6}
3c		4.10×10^{-3}	7.24×10^{-5}	36332	8.77×10^{6}
3d		2.96×10^{-3}	1.65×10^{-5}	31315	1.09×10^{7}
3e		8.57×10^{-1}	4.56×10^{-3}	60190	7.03×10^{4}
4a		2.56×10^{-3}	9.11×10^{-6}	15532	6.22×10^{6}
4b		4.56×10^{-3}	1.74×10^{-5}	56386	1.26×10^{7}
4c		6.01×10^{-3}	3.76×10^{-3}	27298	4.53×10^{6}
4d		2.56×10^{-3}	1.36×10^{-5}	25027	1.00×10^{7}
6a		6.10×10^{-3}	1.04×10^{-3}	7110	1.16×10^{5}
6b		7.10×10^{-3}	2.22×10^{-3}	27670	3.88×10^{6}
6c		5.00×10^{-3}	1.20×10^{-5}	8449	1.70×10^{6}
	CKC-732[a]	6.48×10^{-1}	6.60×10^{-3}	8721	1.35×10^{4}
	CKD-731[a]	4.6×10^{-3}	5.60×10^{-4}	58157	1.35×10^{7}
	TNP-470[a]	1.12×10^{0}	2.20×10^{-2}	4304	3.85×10^{3}

a) The preparation of reference compound TNP-470[5] and CKD-732[7,8] were carried out following reported procedure and in house method. *b*) Anti-proliferation activities were colorimetrically measured CPAE cells (Calf pulmonary artery endothelial cells) and HUVEC (Human umbilical vein endothelial cells), and Cytotoxicity was measured by MTT assay (L5178Y) method, and IC_{50} values were estimated by Probits method (Pharm/PCS^{R}).

tion activity assay for CPAE and HUVEC cells, and cytotoxicity assay for L5178Y cells. Especially, hydroxyethoxycinnamoyl compound (**4a**) and dimethoxyhydroxyethoxycinnamoyl (**4d**) exhibited not only the most excellent anti-proliferation activity but also lower cytotoxicity than the other two hydroxyethoxy fumagillol compounds (**4b**, **4c**) and references TNP-470 and CKD-732. In conclusion, among the tested compounds, 4-hydroxyethoxycinnamoyl fumagillol (**4a**) and 3,5-dimethoxy-4-hydroxyethoxycinnamoyl fumagillol (**4d**) exhibited the best anti-proliferation activity in the CPAE (**4a**: 2.56 pM, **4d**: 2.56 pM) and HUVEC (**4a**: 9.11 fM, **4d**: 13.6 fM) cell-based assay, and also showed less cytotoxicity (**4a**: 6.22×10^6, **4d**: 1.00×10^7) comparable to reference compounds TNP-470 (CPAE: 1.12 nM, HUVEC: 22 pM, S/I value: 3.85×10^3) and CKD-732 (CPAE: 0.648 nM, HUVEC: 6.6 pM, S/I value: 1.35×10^4). On the basis of these results, two novel compounds (**4a**, **4d**) were selected for further evaluation and are presently under biological studies.

Experimental

General Methods All manipulations were carried out in dry solvents under dry nitrogen atmosphere and performed using oven-dried glassware. Commercially available reagents were used without further purification. Anion exchange resin (Amberlite IRA-400) was used supporting the polymer of borohydride exchange resin (BER). Melting points were determined on a Buchi 510 capillary apparatus and are uncorrected. IR spectra were recorded on a Bruker Vector 22 FT-IR spectrometer. NMR spectra were recorded on a Bruker DPX 400 MHz instrument operating at 400 MHz for proton and 100 MHz for carbon NMR and were performed in $CDCl_3$ solutions using tetramethylsilane as the internal reference except where indicated otherwise. The coupling constants (*J*) are reported in Hz. Mass spectra were recorded on an HP 5989B instrument. Flash chromatography was performed using Merck silica gel 60 (230—400 mesh) according to the published procedure.[18)]

Hydrolysis of Fumagillin To a solution of 0.1 N NaOH (60 ml) was added fumagillin (600 mg, 1.30 mmol) at ice-bath temperature then the mixture was stirred at room temperature for 5 h. The reaction mixture was diluted with diethyl ether (250 ml), and the organic layer was separated, washed with water (100 ml) and brine (100 ml), and dried over anhydrous $MgSO_4$. After filtering, the filtrate was condensed by evaporation under reduced pressure. The obtained residue was purified by SiO_2 chromatography with EtOAc/*n*-hexane (1 : 1, v/v) to obtain the fumagillol (**b**) compound as colorless oil (314 mg, 85% yield). mp 42—45 °C. IR $(KBr)_{max}$ cm^{-1}: 3480, 2964, 2932, 1105, 934, 835. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 5.14 (m, 1H), 4.31 (m, 1H), 3.55 (dd, 1H, J=8.4, 2.7 Hz), 3.43 (s, 3H), 2.88 (d, 1H, J=4.3 Hz), 2.52 (t, 1H, J=6.4 Hz), 2.47 (d, 1H, J=4.3 Hz), 2.39 (s, 1H), 2.28 (m, 1H), 2.12 (m, 2H), 1.91 (m, 1H), 1.87 (d, 1H, J=11 Hz), 1.70 (s, 3H), 1.59 (s, 3H), 1.15 (s, 3H), 0.91 (m, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 13.94, 17.99, 25.72, 26.52, 27.36, 28.49, 46.99, 50.69, 56.47, 58.51, 59.83, 61.16, 64.02, 80.96, 118.54, 134.00.

General Procedure for Preparation of 3-(4-Hydroxy-phenyl)-acrylic Acid, 5-Methoxy-4-[2-methyl-3-(3-methyl-but-2-enyl)-oxiranyl]-1-oxa-spiro[2,5]oct-6-yl Ester (3a) *via* 3-(4-Acetoxy-phenyl)-acrylic Acid, 5-Methoxy-4-[2-methyl-3-(3-methyl-but-2-enyl)-oxiranyl]-1-oxa-spiro[2,5]oct-6-yl Ester (2a) A solution of 4-acetoxycinnamic acid (6.40 g, 32 mmol) followed by DMAP (4.39 g, 32 mmol) and DCC (4.57 g, 32 mmol) were added to a solution of fumagillol (1.14 g, 4.0 mmol) in dry dichloromethane (100 ml). The reaction mixture was stirred for 5 h at room temperature then the mixture was passed over a short pad of silica gel using EtOAc/*n*-hexane (1 : 2, v/v) and the filtered solvent was removed *in vacuo* to leave an oil residue that was purified by column chromatography on SiO_2 with EtOAc/*n*-hexane (1 : 4, v/v) as elution solvent to give the 4-acetoxycinnamoyl fumagillol **2a** compound as a pale yellowish foam (1.49 g, 80% yield). The obtained compound **2a** (1.49 g, 3.18 mmol) was added to a solution of methanol (60 ml) and K_2CO_3 (439 mg, 3.18 mmol). The mixture was stirred at ambient temperature for 3 h then the reaction mixture was filtered through Celite pad and washed through with EtOAc (500 ml). The mixture was partitioned between EtOAc-aqueous layer and the combined organic layer was rinsed with brine and water, then the combined organic layer was dried by $MgSO_4$, and concentrated *in vacuo*. The residue was chromatographed on SiO_2 with EtOAc/*n*-hexane (1 : 4, v/v) as elution solvent to afford pure title compound **3a** (1.12 g, 82% yield), mp 60—63 °C. IR $(KBr)_{max}$ cm^{-1}: 3405, 2917, 1710, 1589, 1515, 1153. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.44 (d, 1H, J=16 Hz), 7.21 (d, 2H, J=8.6 Hz), 6.82 (d, 2H, J=8.6 Hz), 6.50 (br, 1H), 6.00 (d, 1H, J=16 Hz), 5.76 (m, 1H), 5.21 (m, 1H), 3.74 (dd, 1H, J=11, 2.7 Hz), 3.50 (s, 3H), 3.01 (d, 1H, J=4.4 Hz), 2.70 (t, 1H, J=6.4 Hz), 2.58 (d, 1H, J=4.4 Hz), 2.41 (m, 1H), 2.20—1.87 (m, 5H), 1.87 (s, 3H), 1.75 (s, 3H), 1.27 (s, 3H), 1.06 (m, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 13.81, 18.07, 25.76, 27.32, 29.40, 48.19, 50.83, 56.59, 59.66, 60.02, 61.35, 65.80, 79.57, 114.35, 115.70, 118.17, 126.33, 130.08, 135.35, 144.88, 158.69, 166.95. HR-MS Calcd for $C_{25}H_{32}O_6$: MH^+, 429.2277. Found: 429.2282 (MH^+).

3-(4-Hydroxy-3-methoxy-phenyl)-acrylic Acid, 5-Methoxy-4-[2-methyl-3-(3-methyl-but-2-enyl)-oxiranyl]-1-oxa-spiro[2,5]oct-6-yl Ester (3b) *via* 3-(4-Acetoxy-3-methoxy-phenyl)-acrylic Acid, 5-Methoxy-4-[2-methyl-3-(3-methyl-but-2-enyl)-oxiranyl]-1-oxa-spiro[2,5]oct-6-yl Ester (2b) The title compound was prepared by a similar procedure as described for **3a** (1.10 g, 85% yield), mp 60—62 °C. IR $(KBr)_{max}$ cm^{-1}: 3421, 2927, 1705, 1516, 1290, 1153, 1101. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.59 (d, 1H, J=16 Hz), 7.03 (m, 2H), 6.90 (d, 1H, J=7.9 Hz), 6.34 (d, 1H, J=16 Hz), 5.86 (s, 1H), 5.72 (m, 1H), 5.21 (m, 1H), 3.94 (s, 3H), 3.71 (dd, 1H, J=11.2, 2.8 Hz), 3.45 (s, 3H), 3.01 (d, 1H, J=4 Hz), 2.60 (t, 1H, J=6.3 Hz), 2.57 (d, 1H, J=4 Hz), 2.35 (m, 1H), 2.20—2.04 (m, 4H), 1.88 (m, 1H), 1.75 (s, 3H), 1.66 (s, 3H), 1.22 (s, 3H), 1.11 (m, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 13.58, 18.11, 25.65, 25.95, 27.33, 29.44, 48.23, 50.90, 56.14, 56.66, 56.88, 58.66, 59.51, 61.23, 66.44, 79.89, 105.33, 116.88, 118.55, 126.02, 134.93, 137.27, 145.11, 147.99, 166.12.

3-(3-Hydroxy-4-methoxy-phenyl)-acrylic Acid, 5-Methoxy-4-[2-methyl-3-(3-methyl-but-2-enyl)-oxiranyl]-1-oxa-spiro[2,5]oct-6-yl Ester (3c) *via* 3-(3-Acetoxy-4-methoxy-phenyl)-acrylic Acid, 5-Methoxy-4-[2-methyl-3-(3-methyl-but-2-enyl)-oxiranyl]-1-oxa-spiro[2,5]oct-6-yl Ester (2c) The title compound was prepared by a similar procedure as described for **3a** (1.08 g, 83% yield), mp 57—59 °C. IR $(KBr)_{max}$ cm^{-1}: 3431, 2920, 1731, 1511, 1282, 1155, 1115. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.58 (d, 1H, J=16 Hz), 7.12 (m, 1H), 7.04 (m, 1H), 6.84 (d, 1H, J=8.5 Hz), 6.34 (d, 1H, J=16 Hz), 5.74 (s, 1H), 5.61 (s, 1H), 5.22 (m, 1H), 3.92 (s, 3H), 3.70 (m, 1H), 3.45 (s, 3H), 3.00 (d, 1H, J=4 Hz), 2.62 (t, 1H, J=6.3 Hz), 2.57 (d, 1H, J=4 Hz), 2.34 (m, 1H), 2.19—2.01 (m, 4H), 1.90 (m, 1H), 1.84 (s, 3H), 1.74 (s, 3H), 1.23 (s, 3H), 1.12 (m, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 18.11, 25.22, 25.45, 27.27, 29.88, 48.31, 50.66, 56.11, 56.39, 57.00, 58.66, 59.33, 61.80, 66.49, 79.39, 105.11, 116.90, 118.14, 126.02, 134.47, 137.36, 145.83, 147.76, 166.01.

3-(4-Hydroxy-3,5-dimethoxy-phenyl)-acrylic Acid, 5-Methoxy-4-[2-methyl-3-(3-methyl-but-2-enyl)-oxiranyl]-1-oxa-spiro[2,5]oct-6-yl Ester (3d) *via* 3-(4-Acetoxy-3,5-dimethoxy-phenyl)-acrylic Acid, 5-Methoxy-4-[2-methyl-3-(3-methyl-but-2-enyl)-oxiranyl]-1-oxa-spiro[2,5]oct-6-yl Ester (2d) The title compound was prepared by a similar procedure as described for **3a** (1.11 g, 80% yield), mp 58—60 °C. IR $(KBr)_{max}$ cm^{-1}: 3424, 2933, 1705, 1514, 1282, 1153, 1111. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.58 (d, 1H, J=16 Hz), 6.78 (s, 2H), 6.37 (d, 1H, J=16 Hz), 5.72 (m, 2H), 5.21 (m, 1H), 3.93 (s, 6H), 3.71 (dd, 1H, J=11, 2.8 Hz), 3.45 (s, 3H), 3.00 (d, 1H, J=4 Hz), 2.62 (t, 1H, J=6.4 Hz), 2.57 (d, 1H, J=4 Hz), 2.36 (m, 1H), 2.20—2.04 (m, 4H), 1.88 (m, 1H), 1.74 (s, 3H), 1.66 (s, 3H), 1.23 (s, 3H), 1.11 (m, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 13.88, 18.03, 25.70, 25.75, 27.38, 29.39, 48.37, 50.90, 56.14, 56.39, 56.61, 58.66, 59.57, 61.11, 66.49, 79.18, 105.11, 116.38, 118.64, 126.02, 134.93, 137.08, 145.08, 147.21, 166.76. HR-MS Calcd for $C_{27}H_{36}O_8$: MH^+, 488.2410. Found: 488.2403 (MH^+).

3-(4-(4-Hydroxymethyl-4-phenoxy-phenyl)-acrylic Acid, 5-Methoxy-4-[2-methyl-3-(3-methyl-but-2-enyl)-oxiranyl]-1-oxa-spiro[2,5]oct-6-yl Ester (3e) *via* 3-(4-[4-Acetoxymethyl-4-phenoxy-phenyl]-acrylic Acid, 5-Methoxy-4-[2-methyl-3-(3-methyl-but-2-enyl)-oxiranyl]-1-oxa-spiro[2,5]oct-6-yl Ester (2e) The title compound was prepared by a similar procedure as described for **3a** (1.18 g, 78% yield), mp 55—58 °C. IR $(KBr)_{max}$ cm^{-1}: 3442, 2937, 1732, 1522, 1284, 1175, 1104. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.63 (d, 1H, J=16 Hz), 7.48 (d, 2H, J=8.6 Hz), 7.36 (d, 2H, J=8.4 Hz), 7.03 (d, 2H, J=8.4 Hz), 6.98 (d, 2H, J=8.6 Hz), 6.40 (d, 1H, J=16 Hz), 5.74 (m, 1H), 5.21 (m, 1H), 4.69 (s, 2H), 3.70 (dd, 1H, J=11, 2.7 Hz), 3.45 (s, 3H), 3.01 (d, 1H, J=4 Hz), 2.61 (t, 1H, J=6.3 Hz), 2.57 (d, 1H, J=4 Hz), 2.36 (m, 1H), 2.19—1.88 (m, 5H), 1.74 (s, 3H), 1.65 (s, 3H), 1.23 (s, 3H), 1.11 (m, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 13.88, 18.49, 25.46, 25.75, 27.87, 29.39, 48.67, 50.34, 56.68, 56.64, 56.80, 58.25, 59.44, 61.24, 66.44, 79.59, 105.28, 116.38, 118.26, 126.54, 134.40, 137.30, 145.29, 147.89, 166.36.

General Procedure of 3-(4-(2-Hydroxy-ethoxy)-phenyl)-acrylic Acid, 5-Methoxy-4-[2-methyl-3-(3-methyl-but-2-enyl)-oxiranyl]-1-oxa-spiro[2,5]oct-6-yl Ester (4a) To a solution of 4-hydroxycinnamoyl fumagillol (**3a**) (1.12 g, 2.61 mmol) in anhydrous DMF (20 ml) were added K_2CO_3 (2.16 g, 15.7 mmol) and iodoethanol (1.80 g, 10.4 mmol). The mixture was warmed to 80 °C and stirred at that temperature for 5 h. The reaction mixture was filtered through Celite pad and washed through with EtOAc (150 ml). The filtrate was rinsed in brine and water, and the combined organic layer was dried by $MgSO_4$. The filtrate was concentrated under reduced pressure to leave an oil residue that was purified by chromatography on SiO_2 with EtOAc/*n*-hexane (1 : 4, v/v) as elution solvent to give the title compound **4a** as a white foam product (938 mg, 72% yield), mp 47—49 °C. IR $(KBr)_{max}$ cm^{-1}: 3453, 2928, 1708, 1603, 1254, 1169. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.61 (d, 1H, J=16 Hz), 7.46 (m, 2H), 6.89 (m, 2H), 6.35 (d, 1H, J=16 Hz), 5.73 (m, 1H), 5.21 (m, 1H), 4.10 (m, 2H), 3.96 (m, 2H), 3.69 (dd, 1H, J=11, 2.7 Hz), 3.45 (s, 3H), 2.99 (d, 1H, J=4.4 Hz), 2.60 (t, 1H, J=6.4 Hz), 2.56 (d, 1H, J=4.4 Hz), 2.35 (m, 1H), 2.19—2.0 (m, 4H), 1.90 (m, 1H), 1.74 (s, 3H), 1.65 (s, 3H), 1.22 (s, 3H), 1.08 (m, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 13.92, 18.04, 25.76, 27.40, 29.43, 48.33, 50.91, 56.68, 58.62, 59.57, 61.03, 61.30, 66.37, 69.32, 79.27, 114.88, 116.20, 118.66, 127.58, 129.82, 134.88, 144.45, 160.42, 166.85.

3-(4-(2-Hydroxy-ethoxy)-3-methoxy-phenyl)-acrylic Acid, 5-Methoxy-4-[2-methyl-3-(3-methyl-but-2-enyl)-oxiranyl]-1-oxa-spiro[2,5]oct-6-yl Ester (4b) The title compound was prepared by a similar procedure as described for **4a** (1.03 g, 85% yield), mp 62—64 °C. IR $(KBr)_{max}$ cm^{-1}: 3455, 2930, 1706, 1632, 1164. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.61 (d, 1H, J=16 Hz), 7.06 (m, 2H), 6.89 (d, 1H, J=7.9 Hz), 6.38 (d, 1H, J=16 Hz), 5.72 (m, 1H), 5.21 (m, 1H), 4.16 (m, 2H), 3.98 (m, 2H), 3.91 (s, 3H), 3.71 (dd, 1H, J=11.2, 2.8 Hz), 3.45 (s, 3H), 3.01 (d, 1H, J=4 Hz), 2.62 (t, 1H, J=6.3 Hz), 2.57 (d, 1H, J=4 Hz), 2.38 (m, 1H), 2.25—2.04 (m, 4H), 1.88 (m, 1H), 1.75 (s, 3H), 1.66 (s, 3H), 1.66 (s, 3H), 1.23 (s, 3H), 1.11 (m, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 13.88, 18.03, 25.71, 25.74, 27.38, 29.40, 48.35, 50.90, 55.90, 56.63, 58.65, 59.56, 61.08, 61.14, 66.49, 70.87, 79.21, 110.00, 113.49, 116.59, 118.63, 122.76, 128.22, 134.91, 144.64, 149.69, 150.18, 166.76. HR-MS Calcd for $C_{28}H_{38}O_8$: MH^+, 502.2567. Found: 502.2567 (MH^+).

3-(4-(4-Hydroxy-3-(2-hydroxy-ethoxy)-phenyl)-acrylic Acid, 5-Methoxy-4-[2-methyl-3-(3-methyl-but-2-enyl)-oxiranyl]-1-oxa-spiro-[2,5]oct-6-yl Ester (4c) The title compound was prepared by a similar procedure as described for **4a** (945 mg, 80% yield), mp 55—58 °C. IR $(KBr)_{max}$ cm^{-1}: 3507, 2931, 1706, 1633, 1262, 1162. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.59 (d, 1H, J=16 Hz), 7.12 (m, 2H), 6.88 (d, 1H, J=8.5 Hz), 6.37 (d, 1H, J=16 Hz), 5.73 (m, 1H), 5.22 (m, 1H), 4.18 (m, 2H), 3.97 (m, 2H), 3.90 (s, 3H), 3.71 (m, 1H), 3.45 (s, 3H), 3.00 (d, 1H, J=4 Hz), 2.62 (t, 1H, J=6.3 Hz), 2.57 (d, 1H, J=4 Hz), 2.35 (m, 1H), 2.20—2.03 (m, 4H), 1.90 (m, 1H), 1.84 (s, 3H), 1.74 (s, 3H), 1.23 (s, 3H), 1.12 (m, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 13.89, 18.03, 25.74, 27.38, 29.40, 36.49, 48.34, 50.89, 55.92, 56.63, 58.63, 59.56, 61.07, 61.20, 66.44, 71.18, 79.22, 111.47, 112.70, 116.45, 118.64, 123.54, 127.63, 134.90, 144.50, 148.31, 151.71, 166.75.

3-(4-(2-Hydroxy-ethoxy)-3,5-dimethoxy-phenyl)-acrylic Acid, 5-Methoxy-4-[2-methyl-3-(3-methyl-but-2-enyl)-oxiranyl]-1-oxa-spiro-[2,5]oct-6-yl Ester (4d) The title compound was prepared by a similar procedure as described for **4a** (941 mg, 78% yield), mp 47—50 °C. IR $(KBr)_{max}$ cm^{-1}: 3451, 2934, 1710, 1583, 1275, 1155. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.58 (d, 1H, J=16 Hz), 6.76 (s, 3H), 6.42 (d, 1H, J=16 Hz), 5.72 (m, 1H), 5.19 (m, 1H), 4.16 (m, 2H), 3.90 (s, 3H), 3.72 (m, 2H), 3.45 (s, 3H), 2.99 (d, 1H, J=4 Hz), 2.61 (t, 1H, J=6.4 Hz), 2.57 (d, 1H, J=4 Hz), 2.41 (m, 1H), 2.20—2.03 (m, 4H), 1.92 (m, 1H), 1.74 (s, 3H), 1.65 (s, 3H), 1.23 (s, 3H), 1.11 (m, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 13.85, 18.02, 25.67, 25.74, 27.36, 29.36, 48.35, 50.87, 56.21, 56.61, 58.62, 59.51, 61.10, 61.42, 66.66, 75.47, 79.15, 155.12, 118.20, 118.60, 130.48, 134.92, 138.23, 144.57, 153.45, 166.44. HR-MS Calcd for $C_{29}H_{40}O_9$: MH^+, 532.2672. Found: 532.2661 (MH^+).

Preparation of Borohydride Exchange Resin (BER)[19,20] An aqueous solution of sodium borohydride (1.0 mol, 1.0 l) was stirred with wet chloride-form anion exchange resin (Amberlite IRA-400 [20—50 mesh], 200 g) for 1 h. The resulting resin was washed thoroughly with distilled water until free from excess $NaBH_4$. The borohydride from anion exchange resin was analyzed for borohydride content by hydride evolution on acidification with 2.0 N HCl, and the average hydride content of BER was found to be 3.3 mmol of BH_4^- per gram. The dried resin was stored under nitrogen in a refrigerator (*ca.* 4 °C). The hydride content was constant over 6 weeks.

Preparation of 3-(4-Nitro-phenyl)-acrylic Acid, 5-Methoxy-4-[2-methyl-3-(3-methyl-but-2-enyl)-oxiranyl]-1-oxa-spiro[2,5]oct-6-yl Ester (5a) A solution of 4-nitrocinnamic acid (700 mg, 3.96 mmol) followed by DMAP (480 mg, 3.96 mmol) and EDC (500 mg, 3.96 mmol) were added to a solution of fumagillol (140 mg, 0.49 mmol) in dry dichloromethane (10 ml). The reaction mixture was stirred at room temperature for 3 h, then the mixture was passed over a short pad of silica gel using EtOAc/*n*-hexane (1 : 2, v/v) and the filtered solvent was removed *in vacuo* to leave an oil residue that was purified by column chromatography on SiO_2 with EtOAc/*n*-hexane (1 : 4, v/v) as elution solvent to give the 4-nitrocinnamoyl fumagillol **5a** compound as a pale yellowish foam (146 mg, 65% yield). mp 62—64 °C. IR $(KBr)_{max}$ cm^{-1}: 3451, 3355, 2933, 1707, 1611, 1160. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.25 (m, 2H), 7.67 (m, 2H), 6.74 (m, 1H), 5.79 (d, 1H, J=16.0 Hz), 5.21 (m, 1H), 3.69 (m, 1H), 3.45 (s, 3H), 3.00 (d, 1H, J=4.2 Hz), 2.64 (t, 1H, J=6.3 Hz), 2.56 (d, 1H, J=4.2 Hz), 2.36 (m, 1H), 2.17—2.01 (m, 4H), 1.88 (m, 1H), 1.74 (s, 3H), 1.65 (s, 3H), 1.24 (s, 3H), 1.11 (m, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 19.44, 19.55, 22.14, 25.43, 26.26, 29.45, 42.87, 49.99, 51.23, 52.08, 53.66, 61.43, 65.80, 73.22, 115.18, 117.36, 124.09, 125.48, 127.30, 133.42, 142.86, 145.39, 165.80.

3-(3-Nitro-phenyl)-acrylic Acid, 5-Methoxy-4-[2-methyl-3-(3-methyl-but-2-enyl)-oxiranyl]-1-oxa-spiro[2,5]oct-6-yl Ester (5b) The title compound was prepared by a similar procedure as described for **5a** (175 mg, 78% yield), mp 63—66 °C. IR $(KBr)_{max}$ cm^{-1}: 3455, 3325, 2932, 1702, 1599, 1153. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.37 (t, 1H, J=6.3 Hz), 8.23 (m, 1H), 7.81 (d, 1H, J=7.8 Hz), 7.69 (d, 1H, J=16.0 Hz), 7.58 (m, 1H), 6.61 (d, 1H, J=15.9 Hz), 5.73 (m, 1H), 5.19 (m, 1H), 3.65 (m, 1H), 3.45 (s, 3H), 3.00 (d, 1H, J=4.2 Hz), 2.62 (t, 1H, J=6.3 Hz), 2.56 (d, 1H, J=4.2 Hz), 2.38 (m, 1H), 2.17—2.01 (m, 4H), 1.88 (m, 1H), 1.76 (s, 3H), 1.64 (s, 3H), 1.25 (s, 3H), 1.12 (m, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 19.33, 19.45, 22.77, 25.30, 26.83, 29.33, 42.81, 49.64, 51.76, 52.80, 53.17, 61.56, 65.44, 73.57, 115.92, 117.37, 124.48, 125.48, 127.65, 133.37, 142.39, 145.93, 166.47.

3-(2-Nitro-4,5-dimethoxy-phenyl)-acrylic Acid, 5-Methoxy-4-[2-methyl-3-(3-methyl-but-2-enyl)-oxiranyl]-1-oxa-spiro[2,5]oct-6-yl Ester (5c) The title compound was prepared by a similar procedure as described for **5a** (185 mg, 73% yield), mp 62—65 °C. IR $(KBr)_{max}$ cm^{-1}: 3336, 2938, 2812, 1711, 1609, 1517, 1144. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.22 (d, 1H, J=15.7 Hz), 7.64 (s, 1H), 7.03 (s, 1H), 6.34 (d, 1H, J=15.7 Hz), 5.78 (m, 1H), 5.20 (m, 1H), 4.04 (s, 3H), 3.96 (s, 3H), 3.73 (m, 1H), 3.46 (s, 3H), 2.99 (m, 1H), 2.61 (m, 1H), 2.57 (m, 1H), 2.34 (m, 1H), 2.17—2.01 (m, 4H), 1.91 (m, 1H), 1.74 (s, 3H), 1.63 (s, 3H), 1.22 (s, 3H), 1.12 (m, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 20.56, 20.44, 22.68, 26.43, 26.68, 29.55, 43.72, 50.16, 51.60, 52.23, 53.47, 56.88, 62.21, 65.47, 73.41, 101.26, 113.66, 114.22, 118.83, 125.18, 133.46, 138.88, 134.86, 143.72, 147.46, 168.18.

General Procedure for 3-(4-Amino-phenyl)-acrylic Acid, 5-Methoxy-4-[2-methyl-3-(3-methyl-but-2-enyl)-oxiranyl]-1-oxa-spiro[2,5]oct-6-yl Ester (6a) To a stirred suspension of BER (4.39 g, 13.1 mmol) in methanol (20 ml) was added $Ni(OAc)_2 4H_2O$ (82 mg, 0.328 mmol) at 0 °C. The mixture was stirred for at the same temperature 30 min. When black coating of nickel boride was observed, 4-nitrocinnamoyl fumagillol (1.0 g, 2.19 mmol) in methanol (10 ml) was added at the same temperature. After stirring at 0 °C, insoluble BER-Ni_2B was removed by filtration through Celite pad, and the methanol solution was concentration under reduced pressure. The residue was purified by column chromatography on SiO_2 with EtOAc/*n*-hexane (1 : 2, v/v) to give the title compound **6a** as a white foam product (860 mg, 95% yield), mp 65—68 °C. IR $(KBr)_{max}$ cm^{-1}: 3461, 3365, 2927, 1702, 1599, 1160. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.55 (d, 1H, J=16 Hz), 7.34 (m, 2H), 6.74 (m, 2H), 6.29 (d, 1H, J=16 Hz), 5.73 (m, 1H), 5.19 (m, 1H), 3.69 (m, 1H), 3.45 (s, 3H), 3.00 (d, 1H, J=4.2 Hz), 2.64 (t, 1H, J=6.3 Hz), 2.56 (d, 1H, J=4.2 Hz), 2.36 (m, 1H), 2.17—2.01 (m, 4H), 1.88 (m, 1H), 1.74 (s, 3H), 1.65 (s, 3H), 1.24 (s, 3H), 1.11 (m, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 19.32, 19.40, 22.71, 25.03, 26.38, 29.45, 42.38, 49.99, 51.67, 52.08, 53.71, 61.65, 65.78, 73.11, 115.09, 117.63, 124.90, 125.84, 127.03, 133.24, 142.86, 145.93, 165.08. HR-MS Calcd for $C_{25}H_{33}NO_5$: MH^+, 428.2428. Found: 428.2389 (MH^+). *Anal.* Calcd for: C, 70.72; H, 7.99; N, 3.17. Found: C, 70.77; H, 8.06; N, 3.22.

3-(3-Amino-phenyl)-acrylic Acid, 5-Methoxy-4-[2-methyl-3-(3-methyl-but-2-enyl)-oxiranyl]-1-oxa-spiro[2,5]oct-6-yl Ester (6b) The title compound was prepared by a similar procedure as described for **6a** (748 mg, 80% yield), mp 65—68 °C. IR $(KBr)_{max}$ cm^{-1}: 3461, 3365, 2927, 1702, 1599, 1160. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.55 (d, 1H, J=16 Hz), 7.34 (m, 2H), 6.74 (m, 2H), 6.29 (d, 1H, J=16 Hz), 5.73 (m, 1H), 5.19 (m, 1H), 3.69 (m, 1H), 3.45 (s, 3H), 3.00 (d, 1H, J=4.2 Hz), 2.64 (t, 1H, J=6.3 Hz), 2.56 (d, 1H, J=4.2 Hz), 2.36 (m, 1H), 2.17—2.01 (m, 4H), 1.88 (m, 1H), 1.74 (s, 3H), 1.65 (s, 3H), 1.24 (s, 3H), 1.11 (m, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$)

δ: 19.32, 19.40, 22.71, 25.03, 26.38, 29.45, 42.38, 49.99, 51.67, 52.08, 53.71, 61.65, 65.78, 73.11, 115.09, 117.63, 124.90, 125.84, 127.03, 133.24, 142.86, 145.93, 165.08. HR-MS Calcd for $C_{25}H_{33}NO_5$: MH^+, 428.2428. Found: 428.2389 (MH^+). *Anal.* Calcd for: C, 70.72; H, 7.99; N, 3.17. Found: C, 70.77; H, 8.06; N, 3.22.

3-(2-Amino-4,5-dimethoxy-phenyl)-acrylic Acid, 5-Methoxy-4-[2-methyl-3-(3-methyl-but-2-enyl)-oxiranyl]-1-oxa-spiro[2,5]oct-6-yl Ester (6c) The title compound was prepared by a similar procedure as described for **6a** (980 mg, 92% yield). IR $(KBr)_{max}$ cm^{-1}: 3367, 2929, 2831, 1702, 1609, 1515, 1160. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.81 (d, 1H, J=16.0 Hz), 6.91 (s, 1H), 6.30 (d, 2H, J=16 Hz), 5.72 (s, 1H), 5.19 (t, 1H, J=7.6 Hz), 3.86 (s, 6H), 3.72 (dd, 1H, J=2.8, 11.2 Hz), 3.46 (s, 3H), 2.99 (d, 1H, J=4.3 Hz), 2.64 (m, 1H), 2.56 (d, 2H, J=4.2 Hz), 2.35 (m, 1H), 2.01—2.18 (m, 4H), 1.91 (m, 2H), 1.74 (s, 3H), 1.65 (s, 3H), 1.23 (s, 3H), 1.07 (d, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 20.56, 20.67, 22.78, 26.58, 26.68, 29.43, 43.30, 50.16, 51.69, 52.23, 53.70, 56.99, 62.12, 65.74, 73.14, 101.62, 113.66, 114.82, 118.23, 125.12, 133.64, 138.94, 133.15, 142.83, 147.64, 167.09. HR-MS Calcd for $C_{27}H_{37}NO_7$: MH^+, 488.2638. Found: 488.2715 (MH^+). *Anal.* Calcd for: C, 67.04; H, 7.84; N, 2.79. Found: C, 66.91; H, 7.80; N, 2.75.

Biological. Anti-proliferation Activities The anti-proliferation activities of a series of novel fumagillol derivatives (**3a**—**e**, **4a**—**d**, **6a**—**c**) were evaluated as follows. Calf pulmonary artery endothelial cells (CPAE; ATCC CRL-209) were plated on 96-well microtiter plates (2×10^4 cells/ml) and incubated with MEM medium supplement (ECGS). Murine leukemia L5178Y cells were plated on 96-well microtiter plates (2×10^4 cells/ml) and incubated with RPMI 1640 medium supplement at 37 °C in a 5% carbon dioxide incubator for 3—4 d. Anti-proliferation activities were calorimetrically measured by SRB (CPAE cells) or MTT (L5178Y cells) method and the IC_{50} values were estimated by Probits method (Pharm/PCS^R). The biological data for novel cinnamoyl fumagillol derivatives are summarized in Table 1.

Inhibitory Effect on Proliferation of HUVEC Cells *in Vitro* HUVEC cells were isolated by perfusion of an umbilical vein with a trypsin-containing medium and were grown in GIT medium (Daigo Eiyl Kagaku) supplemented with 2.5% fetal bovine serum and 2.0 ng/ml of recombinant human basic fibroblast growth factor (rhbFGF; prepared by the Biotechnology Research Laboratories of Takeda Chemical Industries, Ltd.). Compounds to be tested were dissolved in dimethylsulfoxide (DMSO) then diluted with culture medium so that the final DMSO concentration did not exceed 0.25%. HUVEC cells were seeded onto 96-well microtiter plates at a concentration of 2×10^3 cells per well and were allowed to grow at 37 °C in a humidified atmosphere on 5% CO_2 and 95% air. On the following day, 100 μl/well of medium containing rhbFGF (final concentration of 2.0 ng/ml) and compounds at various concentrations were added. After 5 d of incubation, the inhibitory effect on proliferation was determined by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide) assay. Cell viability was measured as a function of the ability of the cells to form a blue formazon product, and the concentration required for 50% inhibition (IC_{50}) was estimated from the optical density (590 nm). The biological data for novel cinnamoyl fumagillol derivatives are summarized in Table 1.

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