Chemical Modification of Fumagillin. II.[1] 6-Amino-6-deoxyfumagillol and Its Derivatives

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6-Amino-6-deoxyfumagillol (5) was synthesized by reductive amination of 6-oxo-6-deoxyfumagillol (4), which was obtained by oxidation of fumagillol (2). The reduction proceeded stereoselectively by the equatorial attack of hydride and 5 was found to have the same stereochemistry as that of 2. Several derivatives of 5 were prepared and most of them showed anti-angiogenic activity comparable to that of fumagillol derivatives.

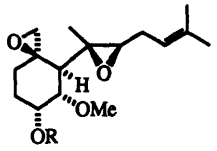
Keywords fumagillin; fumagillol; 6-amino-6-deoxyfumagillol; angiogenesis; anti-angiogenic activity; AGM-1470

Growth of solid tumors is thought to be dependent on angiogenesis and it has been suggested that regulation of tumor-angiogenesis may provide a new approach for cancer therapy.[2] Recently Ingber *et al.*[3] reported that fumagillin (**1**) showed potent anti-angiogenic activity.

In a previous paper,[1] we reported on acylation, sulfonylation, alkylation, and carbamoylation of fumagillol (**2**), a degradation product of **1**, and on the anti-angiogenic activity of the compounds obtained by means of these reactions. These compounds showed more potent anti-angiogenic activity than **1** and among them 6-*O*-chloroacetylcarbamoylfumagillol (**3**, AGM-1470) was found to have the most potent activity.

It was anticipated that replacement of the hydroxyl group of **2** by an amino group might cause some interesting changes in biological activities, owing to changes in pharmacokinetic behavior, and in physicochemical properties. Therefore the synthesis of 6-amino analogues of **2** was investigated. The results are reported herein, along with the anti-angiogenic activities of the compounds obtained.

Chemistry 6-Amino-6-deoxyfumagillol (**5**) was synthesized in 49% yield from 6-oxo-6-deoxyfumagillol (**4**) by reductive amination using $AcONH_4$ and $NaBH_3CN$ in MeOH (Chart 2). Compound **4** was obtained by chromate oxidation of fumagillol (**2**).[4]



fumagillin (1) : R = $CO(CH_2)_4CO_2H$

fumagillol (2) : R = H

AGM-1470 (3) : R = $CONHCOCH_2Cl$

Chart 1

No stereoisomer of **5** at the C-6 position was found and the stereochemistry of the amino group was determined by means of nuclear magnetic resonance (NMR) spectroscopy. The signal of 5-H is a double doublet at 3.60 ppm, which is coupled with 4-H (1.97 ppm) at 6-H (3.66 ppm) with coupling constants of 10 and 3 Hz, respectively. This indicates that 4-H and 5-H are both axial and 6-H is equatorial. Consequently, it was determined that the cyclohexane ring of **5** adopts a chair conformation with the substituent at C-4 in the equatorial position, as in fumagillin (**1**) and fumagillol (**2**),[5] while the amino group at the C-6 position points in an axial direction (Fig. 1).

It is known that axial attack of hydrides is usually predominant when cyclohexanones are reduced using $NaBH_4$, but if there is steric hindrance to axial attack, the proportion of the product obtained by equatorial attack increases.[6] In the case of compound **4**, considerable steric hindrance to axial attack at the C-6 position was suggested by the difficulty of acylation of fumagillol.[7] Furthermore, Wigfield and Phelps[8] reported that the presence of an equatorial substituent α to the carbonyl group has the effect of slowing the axial attack of hydride in the reduction of cyclohexanones. These effects may explain the stereo-

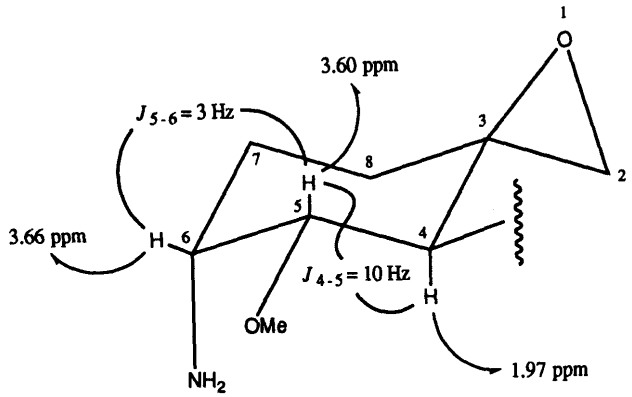
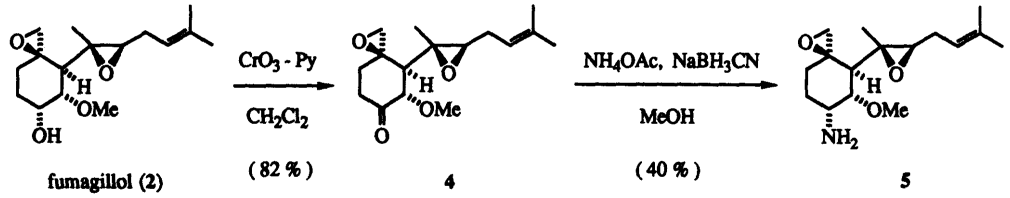


Fig. 1. Conformation of Compound **5**



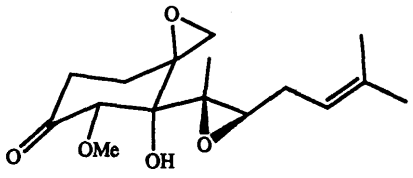
fumagillol (2) 4 5

Chart 2

selectivity in reductive amination of **4**. It is also known that ovalicin, which has a structure closely related to **4**, affords the axial alcohol on reduction with $NaBH_4$.[9]

Reductive amination of **4** using $MeNH_2$, AcOH, and $NaBH_3CN$ was carried out in a similar way to that described above for the preparation of **5**. Although the reaction proceeded in a similar fashion, isolation of 6-methylamino-6-deoxyfumagillol (**6**) was difficult because it was unstable and readily cyclized to the bicyclic compound (**7**) by intramolecular nucleophilic attack of the methylamino group on the spiro-epoxide. Dialkylamines are known to be more nucleophilic than monoalkylamines.[10] This may explain the instability of **6** compared to **5**. The *N*-acetyl derivative 6-(*N*-acetyl-*N*-methylamino)-6-deoxyfumagillol (**8**) could, however, be isolated as a stable compound when crude **6** was treated with acetic anhydride.

6-Phenylamino-6-deoxyfumagillol (**9**) was obtained in a



ovalicin

Chart 3

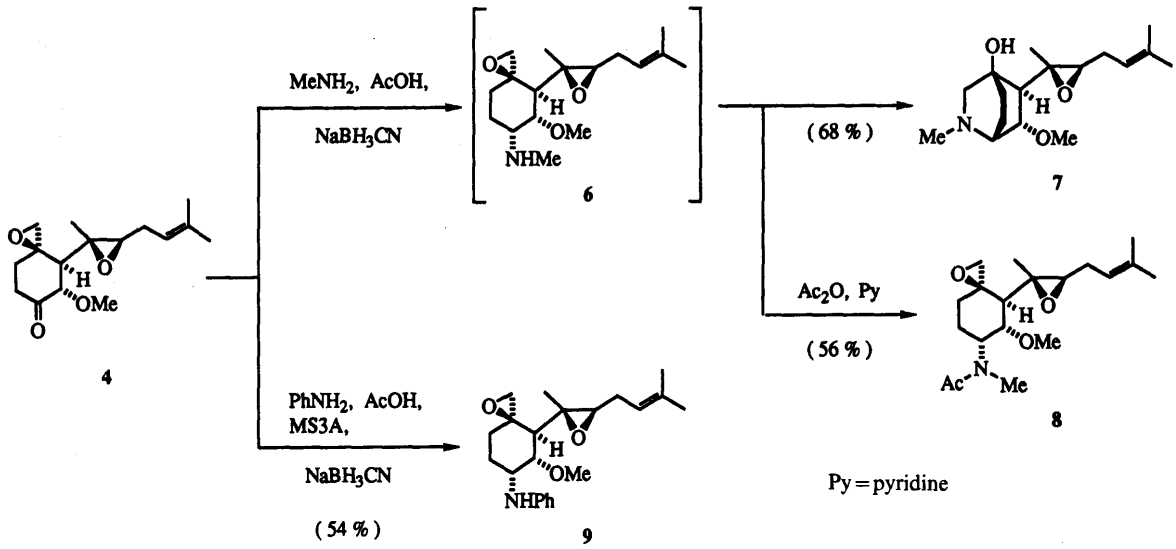


Chart 4

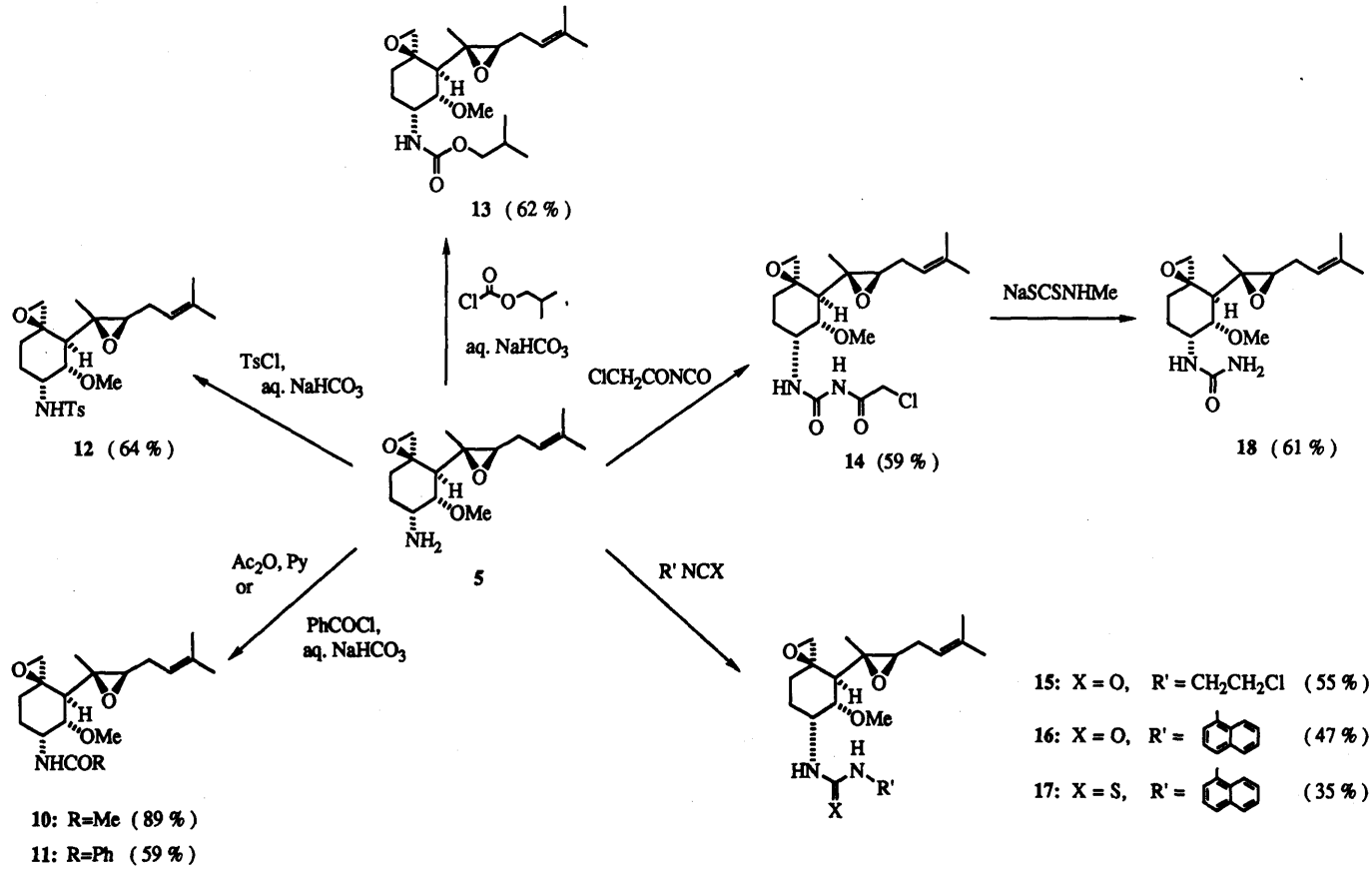
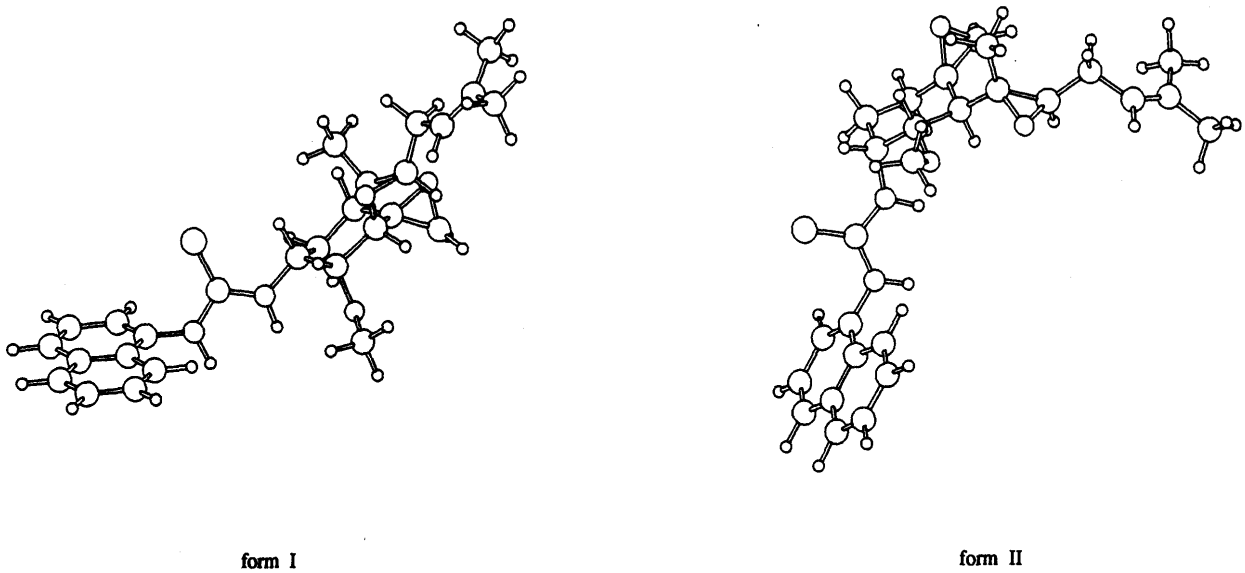


Chart 5



form I form II

Fig. 2. Conformations of Compound **17**

TABLE I. Anti-angiogenic Activity of Fumagillin Analogues in Rat Corneal Assay

Compound No.	Ratio of corneas inhibited/total (%)
1	100
11	86
12	75
13	100
14	100
16	100
17	40
18	100

similar manner, though the addition of molecular sieves 3A to the reaction mixture was required to take up water generated during the reaction. The product **9** was found to be a stable compound. Arylamines are known to be less reactive nucleophiles in the reaction with alkyl halides or epoxides than alkylamines,[10] and this may explain why the intramolecular nucleophilic attack on the spiro-epoxide did not occur in the case of **9** (Chart 4).

The amino group of 6-amino-6-deoxyfumagillol (**5**) was easily modified and compounds **10**—**17** were prepared by the usual methods (Chart 5). However, as compound **5** was not so stable and was therefore not easy to store, crude **5** obtained by reductive amination of **4** was immediately submitted to these reactions without purification, and the yields shown in Chart 5 were calculated from **4**. For example, 6-(*N'*-chloroacetylureido)-6-deoxyfumagillol (**14**) was prepared by treatment of crude **5** with chloroacetylisocyanate. The chloroacetyl group of **14** could be removed using sodium *N*-methyldithiocarbamate to give 6-ureido-6-deoxyfumagillol (**18**) in 61% yield. Though it is known that **1** is readily destroyed by photolysis,[11] these 6-amino analogues are not unstable photolytically.

The cyclohexane ring of 6-[*N'*-(1-naphthyl)thioureido]-6-deoxyfumagillol (**17**), which was obtained by the reaction of **5** with 1-naphthylisothiocyanate, was found to have a different conformation from the other derivatives. The signal for 6-H appears at 4.85 ppm, which is shifted upfield compared with that of 6-[*N'*-(1-naphthyl)ureido]-6-deoxyfumagillol (**16**) (5.39 ppm), and the coupling constants J_{4-5} and J_{5-6}, are both 4 Hz. Furthermore nuclear Overhauser effect (NOE) was observed between 4-H and 2-H (2.47 ppm). These data indicate that 4-H and 5-H are both equatorial whereas 6-H is axial. Consequently, it was concluded that the cyclohexane ring of **17** adopts a flipped chair conformation with the substituent at C-4 pointing axially. MNDO calculations also indicated that form I, which is consistent with the NMR data, is more stable than form II, which has the same conformation as the other compounds, by 2.68 kcal/mol (Fig. 2).

Biological Activity The rat corneal micropocket assay was carried out to examine the anti-angiogenic activity of some representative compounds (Table I). The effect of compounds on angiogenesis induced by a potent angiogenic factor, basic fibroblast growth factor, was evaluated by local administration.

All the compounds were found to possess anti-angiogenic activity. Among them, the thioureido compound **17** only showed a weak activity, whereas the other compounds showed potent anti-angiogenic activity comparable to that of fumagillin (**1**). The most distinguishing features of the fumagillin molecule are the two epoxides, and thus it might be thought that they play an important role for the expression of anti-angiogenic activity. As mentioned above, the cyclohexane ring of **17** adopts a different conformation from that of the other compounds and therefore the relative location of the epoxides of **17** is quite different (Fig. 2). This change in location of the epoxides may explain the relatively low activity of **17**.

Antitumor activity of these 6-amino analogues against solid tumors is under investigation.

Experimental

Melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. Optical rotations were measured with a JASCO DPI-181. Infrared (IR) spectra were taken on a JASCO IR-810 spectrometer. NMR spectra were recorded on a Varian Gemini-200, and chemical shifts are given in ppm with tetramethylsilane as the internal standard. Mass spectra were obtained on a Hitachi RMU-6D.

6-Oxo-6-deoxyfumagillol (4) Compound **4** was obtained by a method similar to that reported by Tarbell *et al.*[4] with some modification.

Chromium(III) oxide (25.4 g) was added to a stirred solution of pyridine (42 ml) in CH_2Cl_2 (500 ml) in several portions and the mixture was stirred for 15 min. Fumagillol (**2**, 10 g) was then added to the mixture. The reaction mixture was stirred for 1 h at room temperature and the supernatant solution was decanted from the tarry residue, which was washed with Et_2O. The organic solutions were combined and washed with 1 N NaOH solution, saturated aqueous $NaHCO_3$ solution, and brine. The solution was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The residue was chromatographed on silica gel (AcOEt–hexane, 1 : 2) to give **4** (8.2 g, 82%) as a colorless oil. $[\alpha]_D^{24}$ −64.9° (c=0.21, $CHCl_3$). IR (neat) cm^{-1}: 2920, 1725, 1440, 1380, 1110, 1085. NMR ($CDCl_3$) δ: 1.29 (3H, s), 1.66 (3H, s), 1.70 (1H, m), 1.75 (3H, s), 1.88 (1H, d, J=11 Hz), 2.00—2.80 (6H, m), 2.74 (1H, d, J=4 Hz), 3.07 (1H, d, J=4 Hz), 3.52 (3H, s), 4.10 (1H, dd, J=1, 11 Hz), 5.19 (1H, m). MS m/z: 280 (M^+), 249, 234, 219, 211, 205.

6-Amino-6-deoxyfumagillol (5) $NaBH_3CN$ (0.11 g) was added to a stirred solution of **4** (0.50 g) and $AcONH_4$ (1.40 g) in MeOH (15 ml). The reaction mixture was stirred for 1 h, then concentrated *in vacuo*, and the residue was dissolved in AcOEt (100 ml). The AcOEt solution was successively washed with saturated aqueous $NaHCO_3$ solution and brine, dried over anhydrous $MgSO_4$, and concentrated *in vacuo*. The residue was chromatographed on silica gel ($CHCl_3$–MeOH–concentrated NH_4OH, 20 : 1 : 0.1) to give **5** (0.20 g, 40%) as a colorless oil. $[\alpha]_D^{26}$ −23.5° (c=0.20, $CHCl_3$). IR (neat) cm^{-1}: 3430, 2920, 1450, 1380, 1250, 1080, 975. NMR ($CDCl_3$) δ: 1.05 (1H, m), 1.24 (3H, s), 1.66 (3H, s), 1.75 (3H, s), 1.80 (2H, m), 1.97 (1H, d, J=10 Hz), 2.10—2.50 (3H, m), 2.51 (1H, d, J=4 Hz), 2.59 (1H, t, J=6 Hz), 2.90 (1H, d, J=4 Hz), 3.44 (3H, s), 3.60 (1H, dd, J=3, 10 Hz), 3.66 (1H, m), 5.21 (1H, m). MS m/z: 281 (M^+), 264, 250, 212, 194.

5-(1,2-Epoxy-1,5-dimethyl-4-hexenyl)-4-hydroxy-6-methoxy-2-methyl-2-azabicyclo[2.2.2]octane (7) $NaBH_3CN$ (0.11 g) was added to a stirred solution of **4** (0.50 mg), methylamine (40% solution in methanol) (1.4 ml), and AcOH (1.1 ml) in MeOH (15 ml). The reaction mixture was stirred for 2 h, then concentrated *in vacuo*, and the residue was dissolved in AcOEt (100 ml). The AcOEt solution was successively washed with saturated aqueous $NaHCO_3$ solution and brine, dried over anhydrous $MgSO_4$, and concentrated *in vacuo*. The residue was chromatographed on silica gel ($CHCl_3$–MeOH–concentrated NH_4OH, 30 : 1 : 0.03) to give **7** (0.36 g, 68%) as a colorless oil. $[\alpha]_D^{24}$ −20.0° (c=0.10, $CHCl_3$). IR (neat) cm^{-1}: 3430, 2940, 1445, 1380, 1250, 1160, 1095, 1030, 970. NMR ($CDCl_3$) δ: 1.20—1.50 (2H, m), 1.47 (3H, s), 1.67 (3H, s), 1.75 (3H, m), 1.70—1.90 (1H, m), 2.00—2.50 (3H, m), 2.10 (1H, dd, J=2, 6 Hz), 2.26 (1H, d, J=9 Hz), 2.44 (3H, s), 2.75 (1H, m), 2.94 (1H, dd, J=4, 9 Hz), 3.15 (1H, t, J=7 Hz), 3.18 (1H, dd, J=2, 6 Hz), 3.36 (3H, s), 5.18 (1H, m). MS m/z: 295 (M^+), 280, 264, 226, 208.

6-(*N*-Acetyl-*N*-methylamino)-6-deoxyfumagillol (8) $NaBH_3CN$ (0.11 g) was added to a stirred solution of **4** (0.50 mg), methylamine (40% solution in methanol) (1.4 ml) and AcOH (1.1 ml) in MeOH (15 ml). The reaction mixture was stirred for 2 h, then concentrated *in vacuo*, and the residue was dissolved in $CHCl_3$ (50 ml). Pyridine (0.26 ml) and acetic anhydride (0.30 ml) were added to the solution and the mixture was stirred for 20 min. The reaction mixture was diluted with AcOEt (30 ml), washed with brine, dried over anhydrous $MgSO_4$, and concentrated *in vacuo*. The residue was chromatographed on silica gel (AcOEt) to give **8** (0.34 g, 56%) as a colorless oil. $[\alpha]_D^{26}$ +139.5° (c=0.20, $CHCl_3$). IR (neat) cm^{-1}: 2930, 1645, 1455, 1405, 1375, 1310, 1085. NMR ($CDCl_3$) δ: 1.28 (1H, m), 1.52 (3H, s), 1.65 (3H, s), 1.73 (3H, s), 1.50—1.90 (3H, m), 2.00—2.80 (7H, m), 2.95 (0.9H, s), 3.01 (2.1H, s), 3.20—3.30 (4H, m), 4.20 (0.3H, m), 4.68 (0.7H, m), 5.10—5.30 (1H, m). MS m/z: 337 (M^+), 322, 305, 294, 268, 236, 226, 206.

6-Phenylamino-6-deoxyfumagillol (9) Molecular sieves 3A (0.20 g) and $NaBH_3CN$ (0.11 g) were added to a stirred solution of **4** (0.30 mg), aniline (0.11 ml) and AcOH (1.1 ml) in MeOH (15 ml). The reaction mixture was stirred for 1 h, then concentrated *in vacuo*, and the residue was dissolved in AcOEt (100 ml). The AcOEt solution was successively washed with saturated aqueous $NaHCO_3$ solution and brine, dried over anhydrous $MgSO_4$, and concentrated *in vacuo*. The residue was chromatographed on silica gel (AcOEt–hexane, 1 : 9) to give **9** (0.20 g, 54%) as a colorless oil. $[\alpha]_D^{26}$ −25.5° (c=0.20, $CHCl_3$). IR (neat) cm^{-1}: 3380, 2920, 1660, 1500, 1445, 1380, 1315, 1255, 1105. NMR ($CDCl_3$) δ: 1.25 (1H, m), 1.31 (3H, s), 1.66 (3H, s), 1.75 (3H, s), 1.80—2.50 (6H, m), 2.55 (1H, d, J=4 Hz), 2.66 (1H, t, J=6 Hz), 2.88 (1H, d, J=4 Hz), 3.34 (3H, s), 3.78 (1H, dd, J=3, 10 Hz), 4.02 (1H, m), 5.21 (1H, m), 6.73 (3H, m), 7.19 (2H, m). MS m/z: 357 (M^+), 342, 326, 288, 270, 258, 242, 226, 200.

6-Acetylamino-6-deoxyfumagillol (10) $NaBH_3CN$ (67 mg) was added to a stirred solution of **4** (0.30 g) and $AcONH_4$ (0.80 g) in MeOH (10 ml). The reaction mixture was stirred for 1 h, then concentrated *in vacuo*, and the residue was dissolved in AcOEt (50 ml). The AcOEt solution was successively washed with saturated aqueous $NaHCO_3$ solution and brine, dried over anhydrous $MgSO_4$, and concentrated *in vacuo*. The residue was dissolved in CH_2Cl_2, and pyridine (0.26 ml) and acetic anhydride (0.30 ml) were added. The reaction mixture was stirred for 1 h, then diluted with AcOEt (50 ml). The AcOEt solution was successively washed with saturated aqueous $NaHCO_3$ solution and brine, dried over anhydrous $MgSO_4$, and concentrated *in vacuo*. The residue was chromatographed on silica gel ($CHCl_3$–MeOH–concentrated NH_4OH, 20 : 1 : 0.1) to give **10** (0.31 g, 89%) as a colorless oil. $[\alpha]_D^{26}$ −22.5° (c=0.20, $CHCl_3$). IR (neat) cm^{-1}: 3360, 2920, 1655, 1540, 1445, 1375, 1275, 1105. NMR ($CDCl_3$) δ: 1.28 (3H, s), 1.32 (1H, m), 1.66 (3H, s), 1.74 (3H, s), 1.60—1.90 (3H, m), 2.00 (3H, s), 2.00—2.50 (3H, m), 2.54 (1H, d, J=4 Hz), 2.66 (1H, t, J=6 Hz), 2.85 (1H, d, J=4 Hz), 3.38 (3H, s), 3.70 (1H, dd, J=4, 9 Hz), 4.46 (1H, m), 5.20 (1H, m), 5.97 (1H, m). MS m/z: 323 (M^+), 308, 292, 280, 254, 222.

6-Benzoylamino-6-deoxyfumagillol (11) $NaBH_3CN$ (45 mg) was added to a stirred solution of **4** (0.20 g) and $AcONH_4$ (0.55 g) in MeOH (10 ml). The reaction mixture was stirred for 1 h, then concentrated *in vacuo*, and the residue was dissolved in $CHCl_3$ (4 ml). Saturated aqueous $NaHCO_3$ solution (3 ml) and benzoyl chloride (0.17 ml) were added to the $CHCl_3$ solution and the mixture was stirred for 30 min. Water was then added to the reaction mixture and the products were extracted with $CHCl_3$. The extract was washed with brine, dried over anhydrous $MgSO_4$, and concentrated *in vacuo*. The residue was chromatographed on silica gel (AcOEt–hexane, 1 : 2) to give **11** (163 mg, 59%) as a colorless oil. $[\alpha]_D^{24}$ −24.3° (c=0.21, $CHCl_3$). IR (neat) cm^{-1}: 3350, 2920, 1715, 1655, 1525, 1490, 1380, 1350, 1105. NMR ($CDCl_3$) δ: 1.35 (3H, s), 1.51 (1H, m), 1.66 (3H, s), 1.75 (3H, s), 1.60—1.84 (3H, m), 2.05—2.50 (3H, m), 2.57 (1H, d, J=4 Hz), 2.74 (1H, t, J=6 Hz), 2.85 (1H, d, J=4 Hz), 3.41 (3H, s), 3.79 (1H, dd, J=4, 9 Hz), 4.63 (1H, m), 5.22 (1H, m), 6.44 (1H, m), 7.45 (2H, d, J=8 Hz), 7.78 (2H, d, 8 Hz). MS m/z: 385 (M^+), 367, 354, 342, 317, 300, 284, 254, 228, 212.

Compounds **12** and **13** were synthesized by the same method as that described for **11**.

6-Toluenesulfonylamino-6-deoxyfumagillol (12) Yield 64%. Colorless oil. $[\alpha]_D^{24}$ −40.0° (c=0.20, $CHCl_3$). IR (neat) cm^{-1}: 3290, 2925, 1600, 1455, 1435, 1380, 1335, 1180, 1090. NMR ($CDCl_3$) δ: 1.17 (3H, s), 1.18 (1H, m), 1.65 (3H, s), 1.75 (3H, s), 1.60—1.84 (3H, m), 2.05—2.50 (3H, m), 2.44 (3H, s), 2.53 (1H, d, J=4 Hz), 2.55 (1H, t, J=6 Hz), 2.86 (1H, d, J=4 Hz), 3.42 (3H, s), 3.50 (1H, dd, J=4, 9 Hz), 3.62 (1H, m), 4.83 (1H, m), 5.27 (1H, m), 7.33 (2H, d, J=8 Hz), 7.79 (2H, d, J=8 Hz). MS m/z: 435 (M^+), 417, 404, 366, 334, 304, 280, 262, 232.

6-Isobutoxycarbonylamino-6-deoxyfumagillol (13) Yield 62%. Colorless oil. $[\alpha]_D^{24}$ −16.6° (c=0.20, $CHCl_3$). IR (neat) cm^{-1}: 3350, 2950, 1710, 1530, 1460, 1380, 1240, 1105. NMR ($CDCl_3$) δ: 0.91 (3H, s), 0.95 (3H, s), 1.29 (3H, s), 1.32 (1H, m), 1.66 (3H, s), 1.74 (3H, s), 1.60—2.00 (4H, m), 2.05—2.50 (3H, m), 2.54 (1H, d, J=4 Hz), 2.65 (1H, t, J=6 Hz), 2.84 (1H, d, J=4 Hz), 3.40 (3H, s), 3.68 (1H, dd, J=4, 9 Hz), 3.84 (2H, d, J=7 Hz), 4.22 (1H, m), 5.04 (1H, m), 5.20 (1H, m). MS m/z: 381 (M^+), 350, 338, 312, 294, 280, 263, 250, 238, 224, 208.

6-(*N'*-Chloroacetylureido)-6-deoxyfumagillol (14) $NaBH_3CN$ (110 mg) was added to a stirred solution of **4** (0.50 g) and $AcONH_4$ (1.40 g) in MeOH (15 ml). The reaction mixture was stirred for 1 h, then concentrated *in vacuo*; and the residue was dissolved in AcOEt (50 ml). The AcOEt solution was successively washed with saturated aqueous $NaHCO_3$ solution and brine, dried over anhydrous $MgSO_4$, and concentrated *in vacuo*. The residue was dissolved in CH_2Cl_2 (5 ml), then chloroacetylisocyanate (0.30 ml) was added at 0 °C. The reaction mixture was stirred for 30 min and diluted with AcOEt (50 ml). The AcOEt solution was successively washed with saturated aqueous $NaHCO_3$ solution and then with brine, dried over anhydrous $MgSO_4$, and concentrated *in vacuo*. The residue was chromatographed on silica gel (AcOEt–hexane, 1 : 1) to give **14** (0.42 g 59%) as a white amorphous powder. $[\alpha]_D^{26}$ −5.9° (c=0.22, $CHCl_3$). IR (neat) cm^{-1}: 3300, 2980, 1710, 1545, 1500, 1450, 1380, 1200, 1105. NMR ($CDCl_3$) δ: 1.31 (3H, s), 1.32 (1H, m), 1.66 (3H, s), 1.75 (3H, s), 1.50—1.95 (4H, m), 2.00—2.50 (2H, m), 2.55 (1H, d, J=4 Hz), 2.68 (1H, t, J=6 Hz), 2.84 (1H, d, J=4 Hz), 3.43 (3H, s), 3.63 (1H, dd, J=4, 9 Hz), 4.12 (2H, s), 4.57 (1H, m), 5.20 (1H, m). *Anal.* Calcd for $C_{19}H_{29}ClN_2O_5 \cdot 0.3H_2O$: C, 56.17%; H, 7.34%; N, 6.89%. Found: C, 56.17%; H, 7.32%, N, 6.95%.

Compounds **15**—**17** were synthesized by the same method as that described for **14**.

6-[*N'*-(2-Chloroethyl)ureido]-6-deoxyfumagillol (15) Yield 55%. White amorphous powder. $[\alpha]_D^{26}$ −49.0° (c=0.20, $CHCl_3$). IR (neat) cm^{-1}:

3380, 2930, 1690, 1655, 1555, 1450, 1385, 1260, 1105. NMR ($CDCl_3$) δ: 1.20 (1H, m), 1.28 (3H, s), 1.65 (3H, s), 1.74 (3H, s), 1.60—1.95 (3H, m), 2.05—2.50 (3H, m), 2.53 (1H, d, J=4 Hz), 2.54 (1H, t, J=6 Hz), 2.84 (1H, d, J=4 Hz), 3.37 (3H, s), 3.40—3.70 (4H, m), 3.72 (1H, dd, J=4, 9 Hz), 4.31 (1H, m), 5.10—5.42 (3H, m). *Anal.* Calcd for $C_{19}H_{31}ClN_2O_4 \cdot 0.7H_2O$: C, 57.12%; H, 8.17%; N, 7.01%. Found: C, 57.10%; H, 8.05%; N, 7.26%.

6-[*N'*-(1-Naphthyl)ureido]-6-deoxyfumagillol (16) Yield 47%. White amorphous powder. $[\alpha]_D^{26}$ −23.5° (c=0.20, $CHCl_3$). IR (neat) cm^{-1}: 3370, 2930, 1700, 1650, 1550, 1345, 1225, 1105, 795, 775. NMR ($CDCl_3$) δ: 1.26 (3H, s), 1.25—1.80 (4H, m), 1.63 (3H, s), 1.73 (3H, s), 2.00—2.50 (3H, m), 2.48 (1H, d, J=4 Hz), 2.61 (1H, t, J=6 Hz), 2.73 (1H, d, J=4 Hz), 3.27 (3H, s), 3.65 (1H, dd, J=4, 8 Hz), 4.43 (1H, m), 5.17 (1H, m), 5.39 (1H, m), 7.02 (1H, m), 7.50 (3H, m), 7.71 (2H, m), 7.87 (1H, m), 8.04 (1H, m). *Anal.* Calcd for $C_{27}H_{34}N_2O_4 \cdot 0.5H_2O$: C, 70.56%; H, 7.68%; N, 6.10%. Found: C, 70.56%; H, 7.44%; N, 6.08%.

6-[*N'*-(1-Naphthyl)thioureido]-6-deoxyfumagillol (17) Yield 35%. Colorless needles from diisopropyl ether, mp: 151—152 °C. $[\alpha]_D^{26}$ +37.0° (c=0.20, $CHCl_3$). IR (neat) cm^{-1}: 3375, 3275, 2930, 1505, 1265, 1245, 1195, 1085, 795, 775. NMR ($CDCl_3$) δ: 1.20 (1H, m), 1.36 (1H, d, J=4 Hz), 1.41 (3H, s), 1.55—1.75 (1H, m), 1.65 (3H, s), 1.74 (3H, s), 1.90—2.50 (4H, m), 2.42 (1H, d, J=5 Hz), 2.47 (1H, d, J=5 Hz), 2.83 (1H, t, J=6 Hz), 2.93 (3H, s), 3.52 (1H, t, J=4 Hz), 4.85 (1H, m), 5.20 (1H, m), 6.10 (1H, m), 7.40—7.60 (4H, m), 7.80—8.10 (4H, m). *Anal.* Calcd for $C_{27}H_{34}N_2O_3S$: C, 69.50%; H, 7.34%; N, 6.00%. Found: C, 69.29%; H, 7.33%; N, 5.98%.

6-Ureido-6-deoxyfumagillol (18) Sodium *N*-methyldithiocarbamate (110 mg) was added to a stirred solution of **14** (170 mg) in tetrahydrofuran (THF) (2 ml). The reaction mixture was stirred for 15 min and diluted with AcOEt (30 ml). The AcOEt solution was successively washed with saturated aqueous $NaHCO_3$ solution and brine, dried over anhydrous $MgSO_4$, and concentrated *in vacuo*. The residue was chromatographed on silica gel ($CHCl_3$–MeOH–concentrated NH_4OH, 20 : 1 : 0.1) and recrystallized from benzene to give **18** (86 mg, 61%) as colorless needles, mp: 124—125 °C. $[\alpha]_D^{24}$ −36.2° (c=0.21, $CHCl_3$). IR (neat) cm^{-1}: 3440, 2930, 1655, 1545, 1450, 1380, 1105. NMR ($CDCl_3$) δ: 1.23 (1H, m), 1.26 (3H, s), 1.65 (3H, s), 1.78 (3H, s), 1.60—1.95 (3H, m), 2.00—2.50 (3H, m), 2.54 (1H, d, J=4 Hz), 2.59 (1H, t, J=6 Hz), 2.85 (1H, d, J=4 Hz), 3.93 (3H, s), 3.71 (1H, dd, J=4, 10 Hz), 4.32 (1H, m), 4.58 (2H, m), 5.19 (1H, m). *Anal.* Calcd for $C_{17}H_{28}N_2O_4$: C, 62.91%; H, 8.70%; N, 8.63%. Found: C, 63.41%; H, 8.67%; N, 8.33%.

Rat Corneal Micropocket Assay The rat corneal micropocket assay was carried out as described previously.[12] Both an ethylene vinylacetate copolymer (EVA) pellet containing bovine basic fibroblast growth factor (bFGF, R & D Systems) (250 ng) and an empty or a compound-containing (20 μg) EVA pellet were implanted into rat corneas. Ten days later, the anti-angiogenic activity of the compound was examined using a stereomicroscope.

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