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Design, Synthesis and Evaluation of a Series of Novel Fumagillin Analogues

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Abstract—A series of fumagillin analogues targeted at understanding tolerability of MetAP2 toward substitution at C4 and C6 were synthesized. Initially, the C6 side chain was maintained as cinnamoyl ester and C4 was modified. It was concluded that replacing the natural C4 of fumagillin with a benzyl oxime at C4 resulted in moderate loss of activity toward binding to MetAP2. Placement of a primary or secondary carbamate at C6 did not improve the potency of compounds toward inhibition of MetAP2. However, the inhibitory activity against MetAP2 was gained back by placing polar groups such as piperazinyl carbamate at C6. Small alkyl substituents on the amine of piperazinyl carbamate were well tolerated.

Introduction

Angiogenesis, the process by which the tumor vascularizes, is essential for tumor 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 the conventional chemotherapy, in which the tumor is directly targeted, and therefore may benefit from lower toxicity and drug resistance. Compounds with a similar mechanism of action may also be used for other angiogenesis-dependent diseases such as rheumatoid arthritis.

Fumagillin, a natural product from *Aspergillus fumigatus* was discovered serendipitously and found to inhibit angiogenesis by blocking endothelial cell growth (Fig. 1).[3] Fumagillin is rapidly hydrolyzed to fumagillol under basic conditions. Subsequently, a number of analogues of fumagillol were prepared[4] including CKD-731[5] and TNP 470 which was advanced to phase III clinical trials. Ovalicin, the oxidation product of fumagillol, also shows potent antiangiogenic activity.[6] It was proposed that fumagillin analogues inhibit angiogenesis by covalently binding to His-231 of the enzyme, methionine aminopeptidase type 2 (MetAP2).[7,8] During protein translation, a methionine (or N-formyl methionine) is installed at the N-terminus of most proteins. Removal of the terminal methionine as a part of the translocation process, is performed by methionine amino peptidases. MetAP2 cleaves the initiator methionine from specific proteins that are essential for cell cycle progression in endothelial cells.[9] Therefore, inhibition of MetAP2 may result in cell cycle arrest and subsequent apoptosis.[10]

A high resolution crystal structure of fumagillin bound MetAP2 confirmed ring opening of the spirocyclic epoxide by His-231 and subsequent covalent binding to MetAP2.[11] From the crystal structure it is evident that the substituent at C6 of fumagillin is exposed to the solvent.

Angiogenic activity of fumagillin analogues are associated with mild cytotoxicity.[12] The goal of our program

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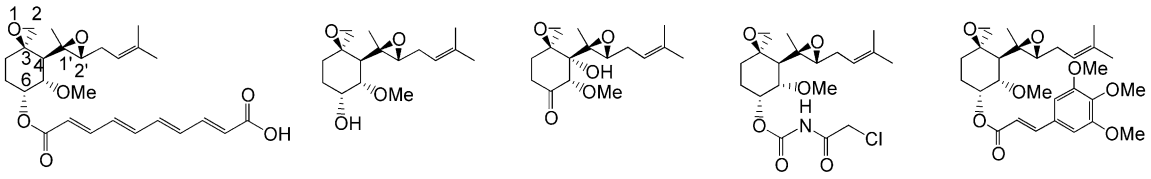


Figure 1.

was to explore the tolerability of MetAP2 toward inhibitors with lower cytotoxicity and better solubility. We envisioned modifying the two epoxides which we hypothesized to be associated with the observed cytotoxicity of the compounds. Since the spiroepoxide of the fumagillin family was shown to be essential for MetAP2 binding,[13] we decided to initially keep this moiety intact. It was reported that the chlorohydrin analogue of the fumagillin spiroepoxide is as active as the parent compound.[14] Hence, a few chlorohydrins were also synthesized and evaluated. Previously reported SAR studies of fumagillin analogues showed that the exocyclic epoxide was not essential.[13] Other studies suggested the need for the planar geometry at the 1′ position of the molecule.[15] Taken together, we decided to replace the exocyclic epoxide with an oxime functionality which would provide the desired geometry, substitution pattern, and easy access to further analogues. A number of C4 oxime analogues were synthesized and evaluated.[16] Oximes such as compounds **5** and **6** carrying a benzyl substituent, proved to be an effective isostere for the natural C4 side chain (Fig. 2). In this paper, we describe the relationship between modification at C4 and C6 positions of fumagillin analogues. We found that C4 modifications lead to changes in SAR of the compound. A series of analogues with C6 carbamates were prepared that demonstrated equipotent enzymatic activity to TNP-470.

Results and Discussion

We initially prepared a series of analogues carrying the cinnamoyl group at C6, since CKD-731 (**1**) was reported to be one of the most potent inhibitors of MetAP2.[5] Maintaining the cinnamoyl group at C6 allowed us to better compare the analogues with various C4 groups.

In order to examine the effect of replacing the exocyclic epoxide with the oxime moiety, compounds **3** and **4** were prepared in which the epoxide was changed to the olefin (Fig. 2). The purpose of this series was to learn the effect of modification of C1′ from sp^3 hybridization, as in compound 1, to sp^2, as in compound **3**. We then explored the effect of replacing the C4 olefin in compound **3** with the C4 oxime of **5**. Simultaneously, modification of the spirocyclic epoxide to the chlorohydrin was evaluated.

Compound **1** was prepared by a peptide coupling strategy using EDC and DMAP (Scheme 1). A small amount of the chlorohydrin side product was isolated due to the chloride ion present in EDC. Longer reaction times resulted in lower overall yields. Therefore, the reactions were generally halted after 15 h. In some cases, when the amount of the chlorohydrin was insignificant, the cinnamoyl compound was treated with LiCl and HOAc to provide the desired chlorohydrin quantitatively.

Activity of the C6 cinnamoyl analogues were evaluated against MetAP2[17] in vitro. Cell based assay monitoring inhibition of proliferation of human umbilical vein endothelial cells (HUVEC) was performed using the SRB dye (Table 1).[18] Removal of the exocyclic olefin seems to have a large impact in activity in the cell based assay, but minor changes were observed in the in vitro enzyme assay. It was also apparent that substitution of the oxime for the olefin is an acceptable modification as observed by the similar activity numbers for compounds **3** and **5**.

The C4 diene fumagillol **10**, precursor to compound **3**, was prepared by a four-step synthesis from fumagillol (Scheme 2).[16] It was found that conversion of fumagillol to the diene **10** is best achieved by opening the spiroepoxide before the reduction of the exocyclic epoxide to avoid generating a mixture of olefins in the reaction. Closure of the chlorohydrin to the epoxide proceeds well to generate compound **10**. Removal of the acetate group was accomplished using the standard K_2CO_3 and MeOH conditions. Formation of **3** and **4** was achieved using the EDC, DMAP conditions as before.

Generation of the benzyl oxime precursor to **5** was performed starting from compound **10**.[16] Formation of the cinnamoyl **5** and the subsequent chlorohydrin **6** was achieved utilizing the peptide coupling method.

A second series of compounds were prepared with the aim of improving solubility of the benzyloxime analogues

Table 1. Activity of a series of cinnamoyl derivatives of fumagillin in enzymatic and cell-based assays[a]

Compound	MetAP2IC$_{50}$ (nM)	HUVEC assay EC$_{50}$ (nM)
Fumagillin	0.63	0.82
Fumagillol	230	49
TNP470	0.43	0.52
1 (CKD 731)	0.96	2.6
2	15.7	4.1
3	1.67	170
4	54.4	310
5	1.6	110
6	161	160

[a]Values are average of duplicate of triplicate data.

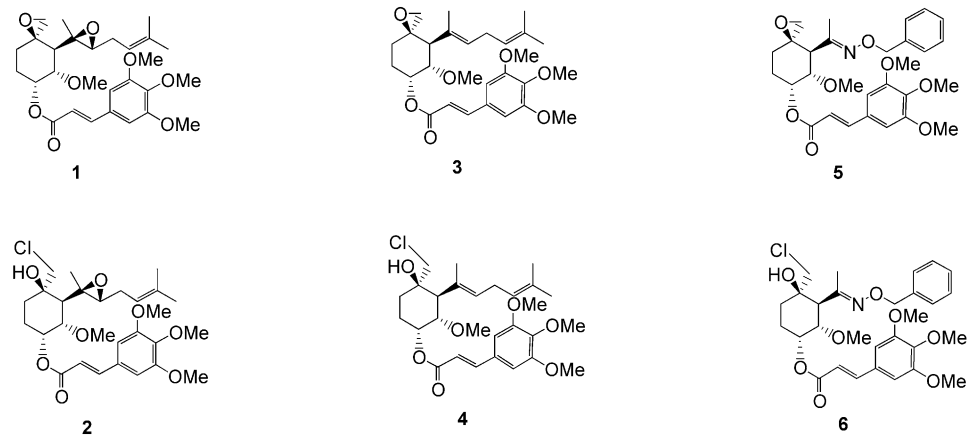
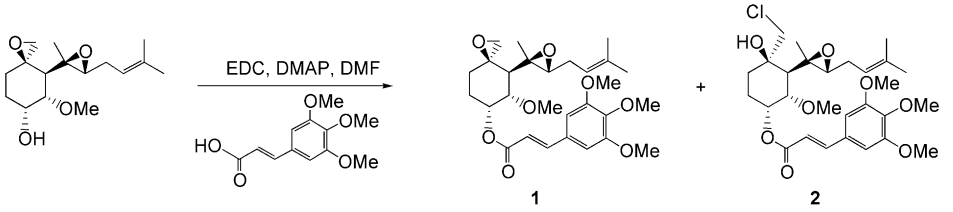
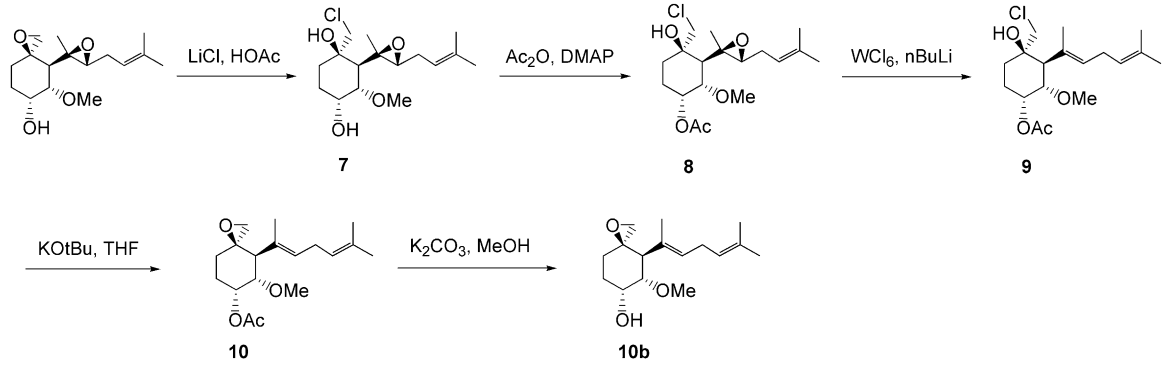


Figure 2.



Scheme 1.



Scheme 2.

(Fig. 3). Considering the encouraging results obtained from the benzyloxime modifications, we kept the C4 substitution as the oxime moiety while we changed the C6 group. We initially explored the carbamate group as the linkage at C6. It was found that *N*-methyl and *N*,*N*-dimethyl carbamates were moderately active ($EC_{50} = 570$ nM and 550 nM in that order). To improve the activity, we decided to attach a polar group carrying a nitrogen, such as a piperazinyl onto our benzyloxime compounds. *N*-methyl, ethyl, and benzyl piperazinyl analogues were synthesized to develop an SAR around the C6 position of the molecule. In order to examine the effect of the 3′ amine, the carbon analogue **12** was also prepared. Importance of the cyclic structure was evaluated by preparing the *N*,*N*-dimethyl ethylene compound **14**.

Formation of the carbamate bond at C6 was attempted under different conditions. Synthesis of intermediate *p*-nitrophenyl carbonate gave the highest yield of the desired product (Scheme 3). The carbonate **16** was purified and could be stored for a few months.

Activity of the C6 piperazinyl benzyloximes were very promising and resembled the parent fumagillin and TNP-470 (Table 2). Comparing compounds **11** and **12** demonstrated the importance of the basic amine present in the molecule. Some tolerance around the 3′ amine was observed as is apparent from compounds **14** and **15**. A small improvement of activity was observed for the cyclic amine **13** over the acyclic one, **14**, as evident from the enzymatic and cell-based assays. Solubility of compound **13** was measured at 144 μM which is an improvement over CKD-732,[19] a highly soluble analogue of fumagillin. In-house measurement of solubility of CKD-732 was about 120 μM.

Ovalicin, a natural product structurally related to fumagillin, has also shown inhibition of MetAP2 activity.[20] Therefore, analogues of fumogillol with an sp^2

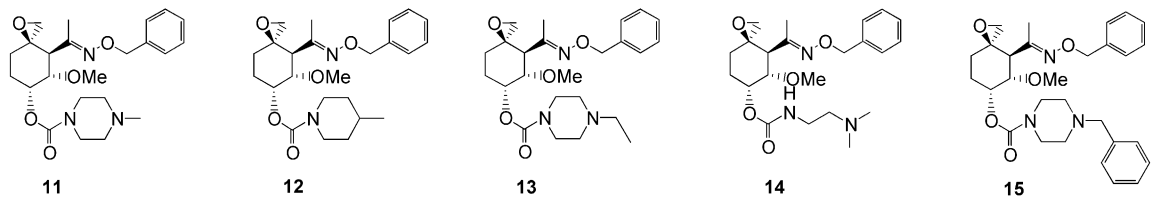
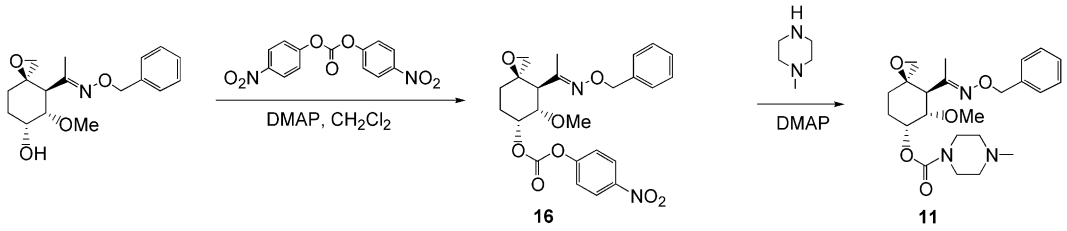


Figure 3.



Scheme 3.

Table 2. Activity of a series of the piperazinyl derivatives of fumagillin in enzymatic and cell-based assays

Compound	MetAP2IC$_{50}$ (nM)	HUVEC Assay EC$_{50}$ (nM)
Fumagillin	0.63	0.82
Fumagillol	230	49
TNP470	0.43	0.52
11	0.85	0.80
12	0.55	4.8
13	0.77	0.90
14	0.89	3.2
15	0.95	12

Table 3. Activity of a series of the ovalicin analogues in enzymatic and cell-based assays

Compound	MetAP2IC$_{50}$ (nM)	HUVEC Assay EC$_{50}$ (nM)
Fumagillin	0.63	0.82
Fumagillol	230	49
17	0.35	0.46
18	5.4	0.64
19	0.51	36
20	50	15
21	43	21

center at C6 were of interest. Also, since the C6 position of fumagillol seems to be exposed to the solvent, as shown by crystallography,[11] we decided to exploit this position further. Fumaginone **17** was synthesized by oxidation of fumagillol using PCC (Fig. 4). Presence of the C6 ketone provides a handle for further modification. Furthermore, analogues carrying C4 benzyloxime and the corresponding chlorohydrins were prepared by the same oxidation method from the benzyloxime alcohol starting material and evaluated. Formation of oxime from ketone **19** using 50% aqueous NH_2OH and AcOH went smoothly to provide compound **21**. Attempts to define the regioisomer of oxime **21** by NMR were unsuccessful. No NOE's were detected between oxime OH and any of the ring protons. In other similar ring systems though, E oxime was reported as the dominant product.[21] Activity of this class of compounds is shown in Table 3. The following modifications did not improve the activity of the parent compound.

Conclusions

A series of compounds, **1–6**, aimed at understanding the effect of the exocyclic epoxide of fumagillin were prepared. These compounds carry the cinnamoyl moiety at C6 and were prepared to compare the effect of modifications at C4. It was demonstrated that C4 alteration from the exocyclic epoxide to the E-oxime was tolerated, although activity of these compounds was slightly decreased. Therefore, further analogues with the benzyloxime at the C4 position were of interest.

Overall, it appears that the exocyclic olefin of fumagillin can be replaced with other functional groups. The activity of the compounds may drop slightly, but further SARs at the C6 position can result in restoration of the activity. Furthermore, solubility of this class of compounds can be improved by substituting more polar functional groups at the solvent exposed C6 position.

Experimental

All reactions involving moisture sensitive reagents were carried out under dry nitrogen in oven dried apparati. THF was distilled over sodium and benzophenone. Other solvents were reagent grade quality and from Aldrich Sureseal bottles and were used as received. The separations using column chromatography were performed using Merck Silica Gel 230–400 mesh. TLC was performed with Merck silica gel 60 F_{254} precoated plates and the products were visualized with UV and phosphomolibdic acid as purchased from Aldrich and diluted with EtOH.

NMR spectra were acquired using $CDCl_3$ filtered through basic alumina, unless otherwise noted, using

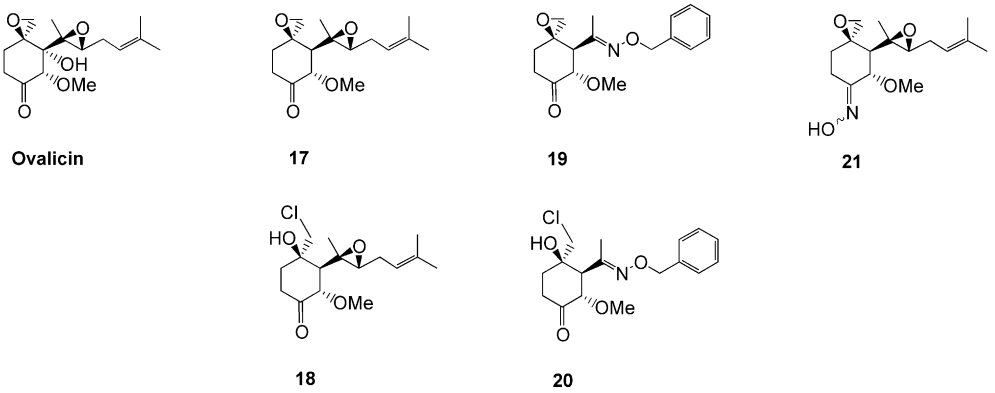


Figure 4.

300 or 500 MHz Varian instruments. The ^{13}C NMR spectra were recorded at 75 MHz. Chemical shifts are reported in ppm with residual $CHCl_3$ (7.26 ppm) and $CDCl_3$ (77 ppm) as references. Mass spectra were obtained with a Finnigan MAT LCQ machine under electrospray ionization conditions.

3-(3,4,5-Trimethoxy-phenyl)-acrylic acid 5-methoxy-4-[2-methyl-3-(3-methyl-but-2-enyl)-oxiranyl]-1-oxa-spiro [2.5]oct-6-yl ester (2) and 3-(3,4,5-trimethoxy-phenyl)-acrylic acid 4-chloromethyl-4-hydroxy-2-methoxy-3-[2-methyl-3-(3-methyl-but-2-enyl)-oxiranyl]-cyclohexyl ester (2). Under a N_2 atmosphere, a mixture of 3,4,5-trimethoxycinnamic acid (84.3 mg, 0.354 mmol), EDC (68 mg, 0.354 mmol), and DMAP (43.2 mg, 0.345 mmol) was stirred in 3.5 mL of DMF for 10 min. Fumagillol (100 mg, 0.345 mmol) was added to the yellow solution and the mixture was stirred over night. The reaction mixture was diluted with EtOAc and the organic layer was washed with H_2O, NH_4Cl, and $NaHCO_3$, dried ($MgSO_4$) and concentrated. Flash chromatography (30–40% EtOAc–hexane) yielded 60 mg (34%) of product **1** as well as 8 mg (5%) of the chlorohydrin **2**. Data for compound **1**: ^1H NMR (300 MHz, $CDCl_3$) δ 1.05–1.17 (m, 1H), 1.24 (s, 3H), 1.66 (s, 3H), 1.75 (s, 3H), 1.87–1.98 (m, 1H), 2.07 (d, 1H, $J=11$ Hz), 2.08–2.25 (m, 3H), 2.32–2.44 (m, 1H), 2.57 (d, 1H, $J=4$ Hz), 2.63 (t, 1H, $J=6$ Hz), 3.02 (d, 1H, $J=4$ Hz), 3.46 (s, 3H), 3.72 (dd, 1H, $J=11$, 2 Hz), 3.90 (s, 3H), 3.91 (s, 6H), 5.22 (br t, 1H, $J=8$ Hz), 5.73 (d, 1H, $J=2$ Hz), 6.42 (d, 1H, $J=16$ Hz), 6.76 (s, 2H), 7.59 (d, 1H, $J=16$ Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.6, 17.8, 25.4, 25.5, 27.1, 29.1, 48.1, 50.7, 55.9, 56.4, 58.4, 59.3, 60.7, 60.9, 60.3, 78.9, 105.0, 117.6, 118.4, 129.7, 134.7, 139.8, 144.5, 153.1, 166.3. Data for compounds **2**: ^1H NMR (300 MHz, $CDCl_3$) δ 1.07–1.17 (m, 1H), 1.26 (s, 3H), 1.38–1.48 (m, 1H), 1.67 (s, 3H), 1. 74 (s, 3H), 1.85–1.92 (m, 1H), 2.08–2.13 (m, 2H), 2.41–2.53 (m, 1H), 2.56 (d, 1H, $J=11$ Hz), 2.00 (t, 1H, $J=7$ Hz), 3.34 (s, 3H), 3.34–3.42 (m, 1H), 3.52 (d, 1H, $J=11$ Hz), 3.90 (s, 3H), 3.91 (s, 6H), 3.88–3.93 (m, 1H), 4.24 (br s, 1H), 5.20 (br t, 1H, $J=7$ Hz), 5.60 (d, 1H, $J=3$ Hz), 6.38 (d, 1H, $J=16$ Hz), 6.77 (s, 2H), 7.64 (d, 1H, $J=16$ Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.9, 17.7, 23.3, 25.6, 27.2, 29.5, 43.0, 50.3, 56.0, 56.4, 60.8, 62.1, 65.7, 75.9, 78.4, 105.1, 117.2, 117.9, 129.7, 134.6, 140.0, 144.9, 153.2, 166.1; EI MS (m/z) 539.2 [MH^+], 561.2 [M + Na].

3-(3,4,5-Trimethoxy-phenyl)-acrylic acid 4-(1,5-dimethyl-hexa-1,4-dienyl)-5-methoxy-1-oxa-spiro[2.5] oct-6-yl ester (3). Under a N_2 atmosphere, a mixture of 3,4,5-trimethoxycinnamic acid (43 mg, 0.18 mmol), EDC (34.5 mg, 0.18 mmol), and DMAP (22 mg, 0.18 mmol) was stirred in 1.2 mL of DMF for 10 min. Fumagillol diene **10b** (33 mg, 0.12 mmol) was added to the yellow solution and the mixture was stirred over night. The reaction mixture was diluted with EtOAc and the organic layer was washed with H_2O, NH_4Cl, and $NaHCO_3$, dried ($MgSO_4$) and concentrated. Flash chromatography (30% EtOAc–hexane) yielded 29 mg (99% based on the recovered starting materials) of product as a white solid as well as 18 mg of the starting materials back. ^1H NMR (300 MHz, $CDCl_3$) δ 1.05–1.17 (m, 1H), 1.61 (s, 3H), 1.62 (s, 3H), 1.68 (s, 3H), 1.84–1.98 (m, 1H), 2.03–2.14 (m, 1H), 2.15–2.28 (m, 1H), 2.49 (d, 1H, $J=5$ Hz), 2.62–2.82 (m, 3H), 2.96 (d, 1H, $J=11$ Hz), 3.38 (s, 3H), 3.65 (dd, 1H, $J=11$, 3 Hz), 3.89 (s, 3H), 3.90 (s, 6H), 5.09 (t, 1H, $J=7$ Hz), 5.26 (t, 1H, $J=7$ Hz), 5.72 (br s, 1H), 6.42 (d, 1H, $J=16$ Hz), 6.76 (s, 2H), 7.61 (d, 1H, $J=16$ Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.7, 17.5, 25.4, 26.7, 28.5, 29.5, 49.1, 51.2, 56.0, 56.8, 60.4, 60.7, 66.8, 78.8, 105.0, 117.5, 122.8, 129.4, 129.8, 130.9, 131.4, 140.0, 144.6, 153.2, 166.3.

3-(3,4,5-Trimethoxy-phenyl)-acrylic acid 4-chloromethyl-3-(1,5-dimethyl-hexa-1,4-dienyl)-4-hydroxy-2-methoxy-cyclohexyl ester (4). A solution of compound **3** (6.0 mg, 0.012 mmol) in 0.3 mL of THF was stirred with 2.1 mg (0.051 mmol) of LiCl and Acetic acid (2.9 μL, 0.051 mmol) for 1 day when the starting materials had been completely consumed as judged by TLC. The reaction mixture was worked up by removal of THF, followed by addition of EtOAc. The organic layer was washed with H_2O, NH_4Cl, and $NaHCO_3$, dried ($MgSO_4$) and concentrated. Flash chromatography (30% EtOAc–hexane) yielded 6.5 mg (100%) of the product as a film. ^1H NMR (300 MHz, $CDCl_3$) δ 1.07–1.17 (m, 1H), 1.34–1.46 (m, 1H), 1.64 (s, 3H), 1.70 (s, 3H), 1.77 (s, 3H), 1.92-2.05 (m, 2H), 2.63 (d, 1H, $J=11$ Hz), 2.79 (ddd, 2H, $J=7$, 7, 8 Hz), 3.37 (s, 3H), 3.45 (d, 1H, $J=11$ Hz), 3.60–3.79 (m, 1H), 3.86–3.93 (m, 1H), 3.90 (s, 3H), 3.91

(s, 6H), 4.70 (br s, 1H), 5.12 (t, 1H, $J=7$ Hz), 5.30 (t, 1H, $J=7$ Hz), 5.65 (br s, 1H), 6.40 (d, 1H, $J=16$ Hz), 6.76 (s, 2H), 7.61 (d, 1H, $J=16$ Hz); EI MS (m/z) 523.2 [MH^+], 545.2 [M + Na].

3-(3,4,5-Trimethoxy-phenyl)-acrylic acid 4-(1-benzyloxy-imino-ethyl)-5-methoxy-1-oxa-spiro[2.5]oct-6-yl ester (5). A mixture of 3,4,5-trimethoxycinnamic acid (29.3 mg, 0.123 mmol), EDC (24.0 mg, 0.123 mmol), DMAP (15.0 mg, 0.123 mmol), and 4-benzyloxime fumagillol[16] (25.0 mg, 0.082 mmol) in 0.82 mL of DMF was stirred for 1 day. The reaction mixture was diluted with EtOAc and washed with H_2O, NH_4Cl, and $NaHCO_3$, dried ($MgSO_4$) and concentrated. Flash chromatography (30% EtOAc–hexane) yielded 24.7 mg (85% based on the recovered starting materials) of the product as a film as well as 8 mg of the starting material. ^1H NMR (300 MHz, $CDCl_3$) δ 1.10–1.28 (m, 1H), 1.86 (s, 3H), 1.94 (br d, 1H, $J=13$ Hz), 2.05–2.16 (m, 1H), 2.23 (dt, 1H, $J=5$, 14 Hz), 2.52 (d, 1H, $J=4$ Hz), 2.56 (d, 1H, $J=4$ Hz), 3.29 (d, 1H, $J=11$ Hz), 3.39 (s, 3H), 3.75 (dd, 1H, $J=3$, 11 Hz), 3.90 (s, 3H), 3.91 (s, 6H), 5.11 (d, 1H, $J=13$ Hz), 5.16 (d, 1H, $J=13$ Hz), 5.78 (br s, 1H), 6.42 (d, 1H, $J=16$ Hz), 6.77 (s, 2H), 7.27–7.40 (m, 5H), 7.62 (d, 1H, $J=16$ Hz).

3-(3,4,5-Trimethoxy-phenyl)-acrylic acid 3-(1-benzyloxyimino-ethyl)-4-chloromethyl-4-hydroxy-2-methoxy-cyclohexyl ester (6). A solution of compound **5** (5.3 mg, 0.010 mmol) in 0.10 mL of THF was stirred with 5.0 mg (0.12 mmol) of LiCl and acetic acid (7.0 μL, 0.12 mmol) for 1 day when the starting materials had been completely consumed as judged by TLC. The reaction mixture was worked up by removal of THF, followed by addition of EtOAc. The organic layer was washed with H_2O, NH_4Cl, and $NaHCO_3$, dried ($MgSO_4$) and concentrated to give 5.7 mg (100%) of the product as a film. ^1H NMR (300 MHz, $CDCl_3$) δ 1.17–1.29 (m, 1H), 1.33–1.45 (m, 1H), 1.90–2.07 (m, 2H), 2.07 (s, 3H), 3.05 (d, 1H, $J=11$ Hz), 3.16–3.27 (m, 2H), 3.23 (s, 3H), 3.56 (dd, 1H, $J=3$, 11 Hz), 3.89 (s, 3H), 3.91 (s, 6H), 4.62 (br s, 1H), 5.08 (d, 1H, $J=12$ Hz), 5.15 (d, 1H, $J=12$ Hz), 5.53 (br s, 1H), 6.39 (d, 1H, $J=16$ Hz), 6.77 (s, 2H), 7.27–1.43 (m, 5H), 7.63 (d, 1H, $J=16$ Hz); EI MS (m/z) 562.2 [MH^+], 584.2 [M + Na].

1-Chloromethyl-3-methoxy-2-[2-methyl-3-(3-methyl-but-2-enyl)-oxiranyl]-cyclohexane-1,4-diol (7). A mixture of 7.13 g (25.2 mmol) of fumagillol and 4.40 g (103.7 mmol) of LiCl in 70 mL of THF was stirred at 0 °C, as 7 mL (122.3 mmol) of acetic acid was added. After 10 min, the cold bath was removed and the mixture was stirred at ambient temperature for 15 h. The reaction mixture was diluted with water and saturated aq $NaHCO_3$ and the product was extracted with ethyl acetate. The organic extracts were washed with water, dried ($MgSO_4$), and concentrated to obtain 7.75 g (96%) of the crude title compound as an oil. The crude product was used in the next reaction without purification. ^1H NMR (300 MHz, $CDCl_3$) δ 1.33 (br d, 1H, $J=13.8$ Hz), 1.47 (s, 3H), 1.66 (s, 3H), 1.74 (s, 3H), 1.68–1.87 (m, 2H), 2.05–2.23 (m, 3H), 2.32–2.49 (m, 2H), 2.98 (t, 1H, $J=6.0$ Hz), 3.26 (br d, 1H, $J=10.2$ Hz), 3.33 (s, 3H), 3.49 (d, 1H, $J=11.1$ Hz), 3.80 (d, 1H, $J=11.1$ Hz), 3.90 (br, 1H), 4.22 (m, 1H), 5.19 (t, 1H, $J=6.6$ Hz).

Acetic acid 4-chloromethyl-4-hydroxy-2-methoxy-3-[2-methyl-3-(3-methyl-but-2-enyl)-oxiranyl]-cyclohexyl ester (8). A solution of 7.75 g (24.3 mmol) of compound **7** and 5.94 g (48.6 mmol) of DMAP in 150 mL of CH_2Cl_2 was stirred at 0 °C as 3.45 mL (36.5 mmol) of acetic anhydride was added. After 1 h at 0 °C, the solution was diluted with water and the product was extracted with ethyl acetate. The extracts were washed with water and brine, dried ($MgSO_4$), and concentrated. The product was purified by column chromatography to obtain 7.85 g (86% from fumagillol) of the title compound. ^1H NMR (300 MHz, $CDCl_3$) δ 1.40 (br d, 1H, $J=13.2$ Hz), 1.50 (s, 3H), 1.67 (s, 3H), 1.75 (s, 3H), 1.75–1.83 (m, 2H), 1.95–2.03 (m, 1H), 2.13 (s, 3H), 2.13–2.20 (m, 1H), 2.41–2.51 (m, 2H), 2.97 (t, 1H, $J=6.6$ Hz), 3.27 (br, 1H), 3.30 (s, 3H), 3.51 (d, 1H, $J=10.8$ Hz), 3.86 (d, 1H, $J=10.8$ Hz), 4.14 (br, 1H), 5.19 (br t, 1H, $J=7.5$ Hz), 5.47 (m, 1H).

Acetic acid 4-chloromethyl-3-(1,5-dimethyl-hexa-1,4-dienyl)-4-hydroxy-2-methoxy-cyclohexyl ester (9). Under a N_2 atmosphere, to a solution of 7.70 g (19.4 mmol) of WCl_6 in 60 mL of THF at −78 °C was added 27 mL (38.8 mmol) of 1.43 M *n*-BuLi in hexane over 15 min. After 15 min at −78 °C, the solution was stirred at rt for 35 min and a solution of 2.26 g (6.26 mmol) of compound **8** in 20 mL of THF was added over 10 min. After 1 h, 1.5 M sodium tartrate (80 mL) and 2.0 M NaOH (80 mL) were added to the reaction mixture and the product was extracted with ether. The extracts were washed with a mixture of 100 mL of 1.5 M sodium tartrate and 100 mL of 2 M NaOH, saturated aq $NaHCO_3$, and water. The residue was purified by column chromatography (25% EtOAc–hexane) to yield 1.84 g (86%) of the product as a clear oil. ^1H NMR (300 MHz, $CDCl_3$) δ 1.62 (br s, 1H), 1.63 (s, 3H), 1.70 (s, 3H), 1.73 (s, 3H), 1.68–1.79 (m, 2H), 1.75–1.83 (m, 2H), 2.10 (s, 3H), 2.51 (d, 1H, $J=11.1$ Hz), 2.68–2.87 (m, 2H), 3.31 (s, 3H), 3.41 (d, 1H, $J=11.1$ Hz), 3.65 (m, 2H), 5.11 (br t, 1H, $J=7.1$ Hz), 5.25 (br t, 1H, $J=6.2$ Hz), 5.54 (m, 1H).

Acetic acid 4-(1,5-dimethyl-hexa-1,4-dienyl)-5-methoxy-1-oxa-spiro[2.5]oct-6-yl ester (10). To a stirring solution of 5.11 g (14.8 mmol) of **9** in THF was added 15.6 mL (15.6 mmol) of 1 M potassium *tert*-butoxide solution in THF at 0 °C. After 1 h, an additional 0.75 mL (0.75 mmol) of 1 M potassium *tert*-butoxide solution was added and the resulting solution was stirred for 5 min at 0 °C. The solution was diluted with saturated aq $NaHCO_3$ and the product was extracted with ethyl acetate. The combined extracts were washed with water and saturated aq NaCl, dried ($MgSO_4$), and concentrated. The product was purified by chromatography to afford the title compound as a white solid (3.51 g, 77%). ^1H NMR (300 MHz, $CDCl_3$) δ 1.15 (ddd, 1H, $J=11.1$, 3.9, 3.0 Hz), 1.59 (s, 3H), 1.62 (s, 3H), 1.69 (s, 3H), 1.56–1.75 (m, 1H), 1.79–2.00 (m, 1H), 2.12 (s, 3H), 2.17 (td, 1H, $J=13.5$, 4.8 Hz), 2.47 (d, 1H, $J=5.1$ Hz), 2.70 (d, 1H, $J=5.1$ Hz), 2.63–2.82 (m, 2H), 2.87 (d, 1H,

$J = 11.1$ Hz), 3.36 (s, 3H), 3.58 (dd, 1H, $J = 11.1$, 2.7 Hz), 5.09 (br t, 1H, $J = 7.1$ Hz), 5.23 (br t, 1H, $J = 7.1$ Hz), 5.63 (m, 1H).

4-Methyl-piperazine-1-carboxylic acid 4-(1-benzyloxyimino-ethyl)-5-methoxy-1-oxa-spiro[2.5]oct-6-yl ester (11). Under a N_2 atmosphere, a solution of compound **16** (16.5 mg, 0.035 mmol), 1-methylpiperazine (7.03 mg, 0.070 mmol) and DMAP (1.3 mg, 0.011 mmol) in 0.350 mL of dry CH_2Cl_2 was stirred for 1.5 h. The reaction mixture was worked up by diluting with CH_2Cl_2 and washing the organic layer with H_2O. Removal of the solvent followed by flash chromatography (0–15% MeOH–EtOAc) gave 10.4 mg (69%) of the product as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.13–1.25 (m, 1H), 1.83–1.92 (m, 1H), 1.84 (s, 3H), 1.98–2.19 (m, 2H), 2.13 (s, 3H), 2.40 (br s, 4H), 2.49 (d, 1H, $J = 4$ Hz), 2.55 (d, 1H, $J = 4$ Hz), 3.10 (d, 1H, $J = 11$ Hz), 3.38 (s, 3H), 3.53 (br s, 4H), 3.68 (dd, 1H, $J = 11$, 3 Hz), 5.09 (d, 1H, $J = 12$ Hz), 5.15 (d, 1H, $J = 12$ Hz), 5.58 (br s, 1H), 7.25–7.38 (m, 5H); EI MS (m/z) 432.2 [MH^+], 454.2 [M + Na].

4-Methyl-piperidine-1-carboxylic acid 4-(1-benzyloxyimino-ethyl)-5-methoxy-1-oxa-spiro[2.5]oct-6-yl ester (12). Preparation was same as for compound **11**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.95 (d, 3H, $J = 6$ Hz), 1.16 (br d, 1H, $J = 14$ Hz), 1.02–1.12 (m, 2H), 1.46–1.70 (m, 3H), 1.79–1.92 (m, 1H), 1.84 (s, 3H), 1.98–2.08 (m, 1H), 2.15 (dt, 1H, $J = 5$, 14 Hz), 2.49 (d, 1H, $J = 4$ Hz), 2.56 (d, 1H, $J = 4$ Hz), 2.67–2.91 (m, 2H), 3.12 (d, 1H, $J = 11$ Hz), 3.38 (s, 3H), 3.67 (dd, 1H, $J = 3$, 11 Hz), 4.04–4.20 (m, 2H), 5.09 (d, 1H, $J = 12$ Hz), 5.15 (d, 1H, $J = 12$ Hz), 5.58 (br s, 1H), 7.26–7.37 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 12.2, 21.6, 25.6, 28.1, 30.7, 33.6, 44.1, 46.4, 51.1, 56.7, 60.0, 66.6, 75.1, 78.4, 127.4, 127.4, 128.1, 138.0, 156.5; EI MS (m/z) 431.3 [MH^+], 453.3 [M + Na].

4-Ethyl-piperazine-1-carboxylic acid 4-(1-benzyloxyimino-ethyl)-5-methoxy-1-oxa-spiro[2.5]oct-6-yl ester (13). Preparation was same as for compound **11**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.01 (t, 3H, $J = 7.4$ Hz), 1.17 (br d, 1H, $J = 15.3$ Hz), 1.84 (s, 3H), 1.80–1.92 (m, 1H), 2.00–2.09 (m, 1H), 2.13 (td, 1H, $J = 13.8$, 1.8 Hz), 2.40–2.48 (m, 6H), 2.49 (d, 1H, $J = 4.2$ Hz), 2.56 (d, 1H, $J = 4.2$ Hz), 3.11 (d, 1H, $J = 11.1$ Hz), 3.38 (s, 3H), 3.53 (m, 4H), 3.68 (dd, 1H, $J = 11.1$, 2.7 Hz), 5.12 (ABqt, 2H, $J = 12.0$ Hz), 5.58 (br, 1H), 7.29–7.35 (m, 5H); MS (ESI) 446.2 (M + H).

(2-Dimethylamino-ethyl)-carbamic acid 4-(1-benzyloxyimino-ethyl)-5-methoxy-1-oxa-spiro[2.5]oct-6-yl ester (14). Under a N_2 atmosphere, a solution of compound **16** (19.3 mg, 0.041 mmol), *N*,*N*-dimethylethylenediamine (6.8 μL, 0.062 mmol) and DMAP (1.0 mg, 0.008 mmol) in 0.40 mL of dry CH_2Cl_2 was stirred for 1.5 h. The reaction mixture was worked up by diluting with CH_2Cl_2 and washing the organic layer with H_2O. Removal of the solvent followed by flash chromatography (0–1% TEA–EtOAc followed by a second column using 0–10% MeOH–CH_2Cl_2) gave 11.2 mg (66%) of the product as a white solid. ^{1}H NMR (300 MHz, 80 °C, DMSO-d_6) δ 1.09–1.20 (m, 1H), 1.70–1.78 (m, 1H), 1.72 (s, 3H), 1.82–1.95 (m, 1H), 1.97–2.05 (m, 1H), 2.15 (s, 6H), 2.27–2.37 (m, 2H), 3.08 (br s, 3H), 3.25 (s, 3H), 3.35–3.48 (m, 2H), 3.66 (br d, 1H, $J = 12$ Hz), 5.03 (s, 2H), 5.09 (br s, 1H), 5.30–5.39 (m, 1H), 7.25–7.38 (m, 5H); EI MS (m/z) 420.3 [MH^+], 442.3 [M + Na].

4-Benzyl-piperazine-1-carboxylic acid 4-(1-benzyloxyimino-ethyl)-5-methoxy-1-oxa-spiro[2.5]oct-6-yl ester (15). Preparation was same as for compound **11**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.16 (br d, 1H, $J = 14$ Hz), 1.79–1.93 (m, 1H), 1.83 (s, 3H), 1.97–2.10 (m, 1H), 2.14 (dd, 1H, $J = 14$, 4 Hz), 2.44 (br s, 4H), 2.49 (d, 1H, $J = 4$ Hz), 2.54 (d, 1H, $J = 4$ Hz), 3. 09 (d, 1H, $J = 11$ Hz), 3.38 (s, 3H), 3.53 (br s, 6H), 3.67 (dd, 1H, $J = 11$, 3 Hz), 5.09 (d, 1H, $J = 12$ Hz), 5.15 (d, 1H, $J = 12$ Hz), 5.57 (br s, 1H), 7.25–7.36 (m, 10H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 12.2, 25.5, 28.1, 43.6, 46.4, 51.1, 52.5, 56.7, 60.0, 62.8, 66.9, 75.1, 78.3, 127.0, 127.4, 127.5, 128.1, 128.9, 137.5, 138.0, 154.6, 156.3; EI MS (m/z) 508.2 [MH^+], 530.2 [M + Na].

Carbonic acid 4-(1-benzyloxyimino-ethyl)-5-methoxy-1-oxa-spiro[2.5]oct-6-yl ester 4-nitro-phenyl ester (16). Under a N_2 atmosphere, a mixture of 4-benzyloxime fumagillol (56.6 mg, 0.185 mmol), bis(4-nitrophenyl)carbonate (169 mg, 0.555 mmol) and DMAP (68 mg, 0.555 mmol) in 0.10 mL of dry CH_2Cl_2 was stirred for 6 h. The reaction mixture was diluted with CH_2Cl_2 and washed with H_2O. The organic layer was dried ($MgSO_4$) and concentrated. Flash chromatography (10–25% EtOAc–hexane) yielded 75.3 mg (87%) of the product as a white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.20–1.30 (m, 1H), 1.86 (s, 3H), 1.91–2.05 (m, 1H), 2.11–2.26 (m, 2H), 2.53 (s, 2H), 3.20 (d, 1H, $J = 11$ Hz), 3.42 (s, 3H), 3. 76 (dd, 1H, $J = 3$, 11 Hz), 5.11 (d, 1H, $J = 12$ Hz), 5.16 (d, 1H, $J = 12$ Hz), 5.61 (br s, 1H), 7.26–7.42 (m, 7H), 8.28 (d, 1H, $J = 7$ Hz), 8.29 (d, 1H, $J = 7$ Hz).

5-Methoxy-4-[2-methyl-3-(3-methyl-but-2-enyl)-oxiranyl]-1-oxa-spiro[2.5]octan-6-one (17). A mixture of fumagillol (37 mg, 0.13 mmol), pyridinium chlorochromate (198 mg, 0.917 mmol) and pyridine (0.36 mL, 4.5 mmol) in 2.6 mL dry CH_2Cl_2 was stirred for 8 h at room temperature when all of the starting materials disappeared on TLC. The reaction mixture was loaded onto a silica gel column and the product was eluted with 0–30% EtOAc–CH_2Cl_2 to give 25.8 mg (70%) of the product as a clear oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.29 (s, 3H), 1.66 (s, 3H), 1.66–1.75 (m, 1H), 1.75 (s, 3H), 1.88 (d, 1H, $J = 11$ Hz), 2.00–2.22 (m, 2H), 2.40 (ddd, 1H, $J = 6.5$, 7.5, 13.0 Hz), 2.52 (ddd, 1H, $J = 14.1$, 5.4, 3.9), 2.61 (t, 1H, $J = 6.5$ Hz), 2.63–2.72 (m, 1H), 2.73 (d, 1H, $J = 4.5$ Hz), 3.06 (d, 1H, $J = 4.5$ Hz), 3.51 (s, 3H), 4.09 (d, 1H, $J = 11$ Hz), 5.19 (br t, 1H, $J = 7.5$ Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.7, 17.8, 25.5, 27.2, 33.0, 36.6, 51.6, 53.4, 58.4, 58.4, 60.3, 83.0, 118.0, 138.0, 206.9; EI MS (m/z) 281.2 [MH^+], 303.2 [M + Na].

4-Chloromethyl-4-hydroxy-2-methoxy-3-[2-methyl-3-(3-methyl-but-2-enyl)-oxiranyl]-cyclohexanone (18). During the large-scale synthesis of compound **17** starting from fumagillol (104.7 mg, 0.371 mmol) using the procedure

described above, a small amount (7.8 mg, 7%) of the above compound was also obtained in addition to 68% of **17**. Data for **18**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.50 (s, 3H), 1.68 (s, 3H), 1.76 (s, 3H), 1.89 (ddd, 1H, J = 14, 6, 2 Hz), 2.05–2.14 (m, 1H), 2.14–2.23 (m, 1H), 2.28 (ddd, 1H, J = 2, 13, 5 Hz), 2.47 (d, 1H, J = 12 Hz), 2.42–2.55 (m, 1H), 2.78 (dt, 1H, J = 6, 13 Hz), 3.06 (t, 1H, J = 6 Hz), 3.42 (s, 3H), 3.55 (d, 1H, J = 11 Hz), 3.84 (d, 1H, J = 12 Hz), 3.91 (d, 1H, J = 11 Hz), 4.31 (br s, 1H), 5.19 (br t, 1H, J = 7 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 17.9, 21.5, 25.8, 27.3, 29.7, 35.1, 35.3, 49.2, 50.3, 59.1, 62.5, 75.7, 82.3, 117.8, 135.1, 208.4; EI MS (m/z) 317.4 [MH^+], 339.4 [M + Na].

4 - (1 - Benzyloxyimino - ethyl) - 5 - methoxy - 1 - oxa - spiro[2.5]octan-6-one (19). Preparation was same as for compound **17** from C-4 benzyloxime fumagillol. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.76 (ddd, 1H, J = 6, 6, 12 Hz), 1.95–2.02 (m, 1H), 1.90 (s, 3H), 2.51–2.63 (m, 2H), 2.70 (s, 2H), 2.92 (d, 1H, J = 9 Hz), 3.39 (s, 3H), 4.06 (d, 1H, J = 9 Hz), 5.08 (s, 2H), 2.26–7.39 (m, 5H); EI MS (m/z) 304.3 [MH^+], 326.2 [M + Na].

3-(1-Benzyloxyimino-ethyl)-4-chloromethyl-4-hydroxy-2-methoxy-cyclohexanone (20). A solution of 4-benzyloxime fumaginone **19** (10.2 mg, 0.034 mmol) in 0.33 mL of THF was stirred with 17 mg (0.41 mmol) of LiCl and HOAc (23 μL, 0.41 mmol) for 1 day at room temperature when the starting materials was completely consumed. The reaction mixture was worked up by diluting with CH_2Cl_2 and washing the organic layer with H_2O. Removal of the solvent followed by flash chromatography (25% EtOAc–hexane) gave 11.5 mg (100%) of the product as a film. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.87 (ddd, 1H, J = 2, 6, 14 Hz), 2.04 (s, 3H), 2.05–2.11 (m, 1H), 2.31 (ddd, 1H, J = 2, 4, 14 Hz), 2.84 (ddd, 1H, J = 6, 14, 14), 2.93 (d, 1H, J = 12 Hz), 3.16 (d, 1H, J = 11 Hz), 3.20 (d, 1H, J = 11 Hz), 3.32 (s, 3H), 4.02 (d, 1H, J = 12 Hz), 4.84 (d, 1H, J = 2 Hz), 5.10 (d, 1H, J = 12 Hz), 5.17 (d, 1H, J = 12 Hz), 7.26–7.42 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 18.6, 34.0, 35.4, 48.6, 53.3, 59.8, 74.4, 76.0, 85.3, 128.0, 128.1, 128.6, 137.7, 160.6, 207.7; EI MS (m/z) 346.3 [M + Li].

5-Methoxy-4-[2-methyl-3-(3-methyl-but-2-enyl)-oxiranyl]-1-oxa-spiro[2.5]octan-6-one oxime (21). A mixture of compound **17** (15.3 mg, 0.055 mmol) in 0.180 mL of *i*-PrOH was stirred with 27 μL (0.44 mmol) of 50% aqueous NH_2OH and 13 μL of AcOH for 2 h. The reaction mixture was worked up by diluting with EtOAc and washing the organic layer with H_2O. Removal of the solvent left 14.1 mg (88%) of the product as a clear oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.32 (s, 3H), 1.64 (s, 3H), 1.73 (s, 3H), 1.72–1.76 (m, 1H), 1.80–1.88 (m, 2H), 2.17 (ddd, 1H, J = 7, 7, 14 Hz), 2.32 (ddd, 1H, J = 7, 7, 14 Hz), 2.43 (dt, 1H, J = 17, 6 Hz), 2.66 (d, 1H, J = 5 Hz), 2.73-2.79 (m, 2H), 3.02 (dt, 1H, J = 17, 6 Hz), 3.28 (s, 3H), 4.04 (d, 1H, J = 3 Hz), 5.18 (br t, 1H, J = 7 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 15.6, 17.8, 19.3, 25.5, 27.1, 28.8, 53.7, 53.9, 56.0, 56.6, 59.6, 62.6, 78.9, 118.2, 134.4, 156.0; EI MS (m/z) 296.2 [MH^+], 318.3 [M + Na].

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