Structure of Sch 528647: A New Antitumor Antibiotic Related to Fumagillin

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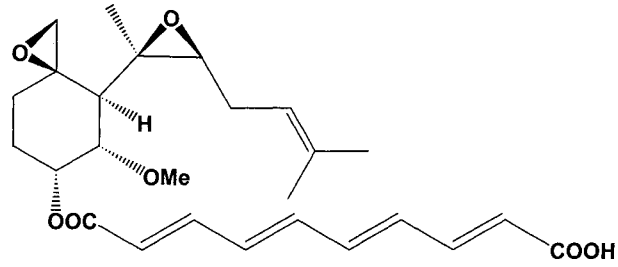
Fumagillin (**1**) was originally isolated from *Aspergillus fumigatus*[1] as an antiparasitic,[2] and belongs to the sesquiterpene family of compounds. In later biological studies, fumagillin and its analogs were reported as highly potent angiogenesis inhibitors, which inhibit endothelial cell proliferation *in vitro* and tumor-induced angiogenesis *in vivo*.[3~5] Methionine aminopeptidase-2 (MetAP-2), the specific enzyme was recently discovered to be the molecular target of fumagillin.[6,7] The binding mode of fumagillin to the enzyme was further revealed by X-ray crystallographic analysis.[8] Furthermore, a semisynthetic analog of fumagillin, TNP-470 (AGM-1470), is currently in phase II clinical trials for the treatment of patients with a variety of cancers, including Kaposi's sarcoma, breast, cervical, lung and renal cancer.[9~13] In order to provide pure material for biological studies in our anti-cancer program, as well as to search for some new analogs, an isolation project of fumagillin and related compounds was launched. In the process of large-scale preparation of fumagillin, Sch 528657 (**2**), a novel metabolite closely related to fumagillin was discovered from *Aspergillus fumigatus* as shown in Fig. 1. Herewith, we wish to report on the isolation, structure elucidation and biological activity of **2**.

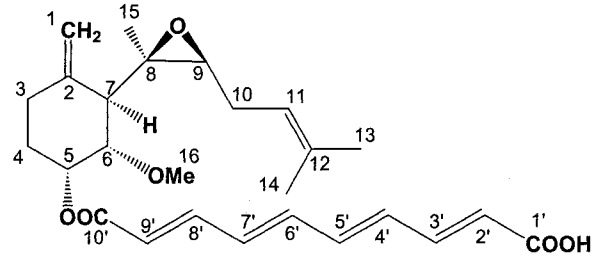
The crude ethyl acetate extract from 100 liters fermentation broth was chromatographed on silica gel column eluting with hexane and ethyl acetate (1 : 1) to obtain the enriched mixture of **1** containing a small amount of **2** (~2%). The mixture was further purified by reversed phase HPLC under following conditions: YMC-ODS preparative column, S-10P, 120 Å, 250×50 mm with a guard column 50×50 mm; 5~50% acetonitrile in NH_4OAc (10 mM, as is pH) with a linear gradient for 20 minutes, then 50~100% acetonitrile/NH_4OAc for 15 minutes, and followed by a 100% acetonitrile holding for 10 minutes; 50 ml/minute flow rate; UV detection at 390 nm. Pure **2** was obtained as a yellow solid (16 mg), m.p.=144~146°C, $[\alpha]_D^{25}$ −12.18° (*c* 0.1, MeOH).

The molecular weight of **2** was determined to be 442 Da based on LC-MS data that showed the protonated molecular ion at *m/z* 443 $(M+H)^+$ and a small water adduct ion at *m/z* 460 $(M+H_2O)^+$ in the positive atmospheric pressure chemical ionization (+APCI) mode. This was also confirmed by observation of *m/z* 441 $(M-H)^-$ in the negative APCI mode. The molecular formula was deduced by elemental analysis as $C_{26}H_{34}O_6$ (*Anal*: C, 70.52; H, 7.58; calcd for $C_{26}H_{34}O_6$: C, 70.59; H, 7.69). The UV spectral data of **2** showed a typical polyene pattern with maximum absorptions at 240 (72), 320 (sh. 1012), 335 (1478), 358 (1338) nm. As listed in Table 1 and 2, the ^1H and ^{13}C NMR spectra of **2** were strikingly similar to fumagillin. In the ^{13}C NMR spectrum of **2**, the olefinic methylene and quaternary carbon signals at 110.83 and 146.78 ppm indicated a terminal double bond formation in comparison with the spiro-epoxide carbon resonances at 51.66 and 60.28 ppm in **1**. The ^1H NMR data of **2** were consistent with the ^{13}C

Fig. 1. Structures of fumagillin and Sch 528647.



Fumagillin (**1**)



Sch 528647 (**2**)

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Table 1. ^{1}H NMR spectral data of **1** and **2**[a].

#	1	2	#	1	2
1a	2.56 d, J=5[b]	4.90 s	14	1.65 s	1.64 s
1b	2.97 d, J=5	4.71 s	15	1.17 s	1.27 s
3	1.11, 2.10 m,m	2.18, 2.29 m,m	16	3.37 s	3.33 s
4	1.90 m	1.57, 1.93 m,m	2'	6.02 d, J=15	6.01 d, J=15
5	5.67 m	5.54 m	3'	7.31 dd, J=12,15	7.28 dd, J=12,15
6	3.67 dd, J=3,10	3.40, dd, J=3,10	4'	6.65 t, J=12	6.64 t, J=12
7	1.98 d, J=10	2.26 d, J=10	5'	6.79, t, J=12	6.76 t, J=12
9	2.58 t, J=6	2.61 t, J=6	6'	6.82 t, J=12	6.81 t, J=12
10	2.22 m	2.28 m	7'	6.65 t, J=12	6.64 t, J=12
11	5.24 t, J=8	5.23 t, J=8	8'	7.39 dd, J=12,15	7.36 dd, J=12,15
13	1.72 s	1.70 s	9'	6.06 d, J=15	6.04 d, J=15

a. Recorded at 400 MHz in acetone -d_6

b. Coupling constants in Hz

Table 2. ^{13}C NMR spectral data of **1** and **2**[a].

#	1	2	#	1	2
1	51.66 t	110.83 t	14	18.09 q	18.07 q
2	60.28 s	146.78 s	15	14.59 q	14.09 q
3	30.23 t	31.32 t	16	56.77 q	56.73 q
4	26.50 t	29.03 t	1'	168.25 s	168.95 br.s
5	67.75 d	68.25 d	2'	124.87 br.d	125.43 br.d
6	80.40 d	81.46 d	3'	144.23 d	144.06 d
7	49.16 d	53.16 d	4'	134.85 d	134.34 d
8	59.08 s	60.33 s	5'	139.83 d	139.69 d
9	60.94 d	61.45 d	6'	140.53 d	140.66 d
10	28.20 t	28.47 t	7'	134.99 d	135.12 d
11	120.40 d	120.45 d	8'	144.74 d	144.87 d
12	134.85 s	134.53 s	9'	123.79 d	123.62 d
13	25.92 q	25.90 q	10'	166.44 s	166.65 s

a. Recorded at 100 MHz in acetone -d_6

b. Multiplicity was determined by APT and DEPT data.

Fig. 2. HMQC-TOCSY data of **2**.

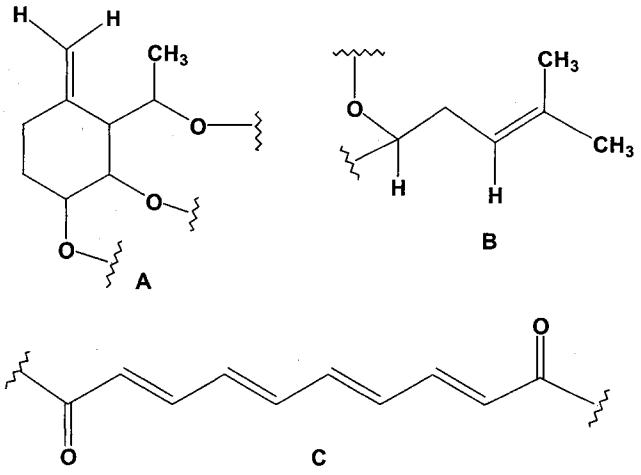
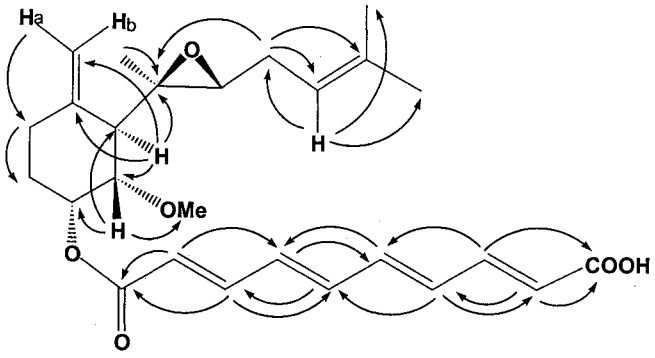


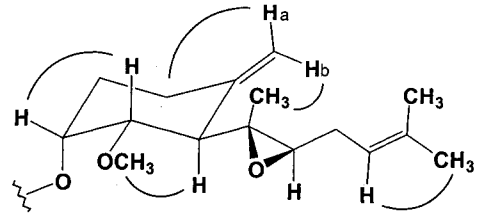
Fig. 3. Some important HMBC data of **2**.



NMR data showing two vinyl singlets at 4.71 and 4.90 ppm instead of the two epoxide doublets at 2.56 and 2.97 ppm in **1**. Extensive 2D-NMR experiments were carried out in order to elucidate the structure of **2**. Three fragments A, B and C were further established based on HMQC-TOCSY data as shown in Fig. 2. The assignments of protons and carbons were completed by the analysis of HMBC data as depicted in Fig. 3. The relative stereochemistry of **2** was determined by NOESY experiments, which revealed its similarity to fumagillin as shown in Fig. 4.

Compound **2** exhibited fairly potent antitumor activity against melanoma cells (SKMEL-5 cells) in the soft agar assay with an IC_{50}=250 nM. However, compound **1** elicited better potency with an IC_{50}=8 nM. This result of a >31-fold potency decrease strongly suggested that the

Fig. 4. NOSEY data of **2**.



spiro-epoxide functionality plays an important role for antitumor activity.

Soft agar assay: Dilutions of compounds are made in DMSO and applied to a mixture of agarose and complete media to form the bottom layer. The bottom layer is then placed into specific wells and left to solidify. SKMEL-5 cells are prepared at a concentration of 5000 cells/well. Additional dilutions of compounds are made and cells are added to this agarose/media mixture. This top mixture is placed into specific wells and left to solidify before placing to a 37°C incubator for 3 weeks. Cells are then strained with MTT (1 mg/ml) and scanned for colony counts. Total area is measured and calculated vs. compound concentration.

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