Fumagalone, a Reversible Inhibitor of Type 2 Methionine Aminopeptidase and Angiogenesis

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Abstract: Fumagillin and ovalicin constitute a family of structurally related natural products that possess antiangiogenic activity. We report the synthesis of a new fumagillin analogue, fumagalone, in which the spiroepoxide group is replaced with an aldehyde. Fumagalone inhibits type 2 methionine aminopeptidase (MetAP2) with $IC_{50} = 8\ \mu M$ and endothelial cell proliferation with $IC_{50} = 52$ nM. With dialysis and competition assays, it was unambiguously demonstrated that binding of fumagalone to MetAP2 is reversible.

Fumagillin (**1**) and ovalicin (**3**) are structurally related natural products of fungal origin[1,2] that have been shown to possess potent antiangiogenic activity.[3,4] An analogue of fumagillin, TNP-470 (**2,** also known as AGM-1470), has undergone clinical trials for the treatment of a variety of cancers. At the cellular level, these compounds inhibit angiogenesis by blocking the cell cycle progression of endothelial cells primarily in the G1-to-S phase transition.[5−8] At the molecular level, the direct target for both fumagillin[9,10] and ovalicin[9] has been identified as the type 2 methionine aminopeptidase (MetAP2). Recent chemical, biochemical, and structural studies have revealed that these natural products are bound to MetAP2 irreversibly by covalently modifying a histidine side chain at the active site of MetAP2 with the spiroepoxide group.[11−13] The binding of these natural products to MetAP2 leads, in a yet unknown mechanism, to the activation of the transcription factor p53, causing the transcriptional up-regulation of p21 that inhibits cyclin E/Cdk2 kinase activity required for cell cycle progression.[14,15]

TNP-470 has encountered some problems in human clinical trails. Among them are short serum half-life and dose-limiting neurotoxicity.[16,17] The spiroepoxide in TNP-470 is prone to hydrolysis in a reaction catalyzed by epoxide hydrolases in vivo. The irreversible binding of TNP-470 to MetAP2 could also be responsible, at least in part, for its dose-limiting side effects. A reversible inhibitor of MetAP2 thus became not only an interesting intellectual challenge but also a good candidate for developing new angiogenesis inhibitors that are less toxic than TNP-470. As a first step toward a reversible MetAP2 inhibitor, we converted the spiroepoxide in fumagillin responsible for covalent modification of MetAP2 into an aldehyde. The new fumagillin analogue, named fumagalone (**4**, Figure 1), was shown to be active, albeit with decreased potency, as an inhibitor of MetAP2 in vitro and endothelial cell proliferation in vivo.

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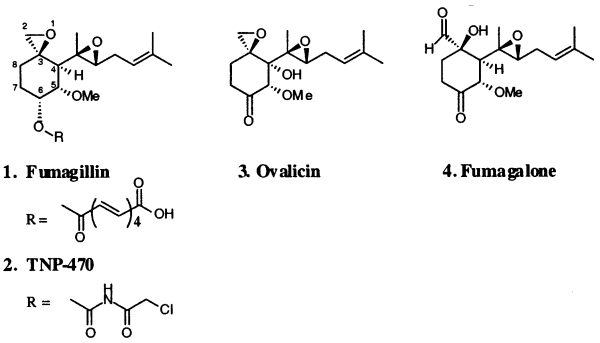
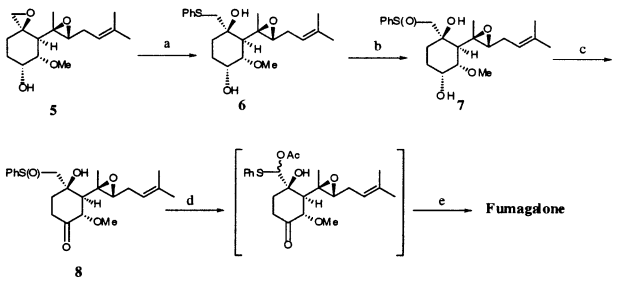


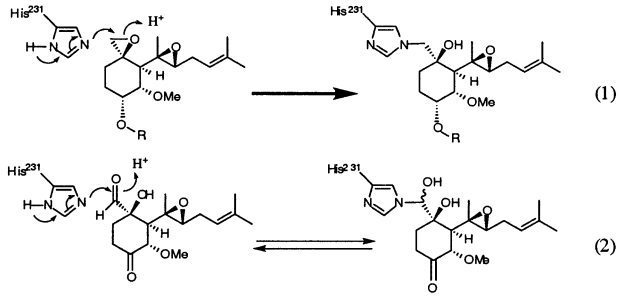
Figure 1. Structures of fumagillin, TNP-470, ovalicin, and fumagalone.

Scheme 1[a]



[a] (a) NaSPh/DMF, room temp, 2.5 h; (b) mCPBA/$CHCl_3$, −78 °C, 1 h; (c) TPAP/NMO, 1 h; (d) NaOAc/Ac_2O, 155 °C (oil bath), 1 h; (e) K_2CO_3/MeOH/H_2O, 15 min.

Because the spiroepoxide group in fumagillin and ovalicin reacts with His231 imidazole side chain (eq 1),[11,12] we reasoned that conversion of the spiroepoxide into an aldehyde would allow for the retention of the bonding distance between the C2 carbon in fumagillin and the ϵN of His231 in MetAP2. Since the carbonyl group of an aldehyde remains electrophilic, it was anticipated that the nucleophilic nitrogen of His231 would react with the aldehyde by forming an aminal intermediate (eq 2). It was not certain, however, whether the additional hydroxyl group formed as part of the aminal could be accommodated by the active site of MetAP2.



The synthesis of fumagalone commences with fumagillol, a fumagillin analogue in which the lower polyene side chain has been removed by hydrolysis (Scheme 1).[18] Opening of the spiroepoxide with sodium benzenethiol in DMF afforded adduct **6**, which was then oxidized by mCPBA to yield the sulfoxide **7**. The secondary alcohol in **7** was converted into a ketone (**8**) by treatment with TPAP/NMO. Intermediate **8** underwent a Pummerer

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rearrangement upon treatment with NaOAc and acetic anhydride to give rise to an intermediate that, upon hydrolysis in the presence of potassium carbonate, yielded fumagalone.[19,20] The overall yield of this five-step process is 16%.

Fumagalone is active in both the MetAP2 enzymatic assay in vitro and in endothelial cell proliferation assay. By use of 100 nM of MetAP2 enzyme and the tetrapeptide Met-Gly-Met-Met as a substrate, the IC_{50} for fumagalone was determined to be 8 μM. For the bovine aortic endothelial cell proliferation assay, fumagalone had an IC_{50} of about 52 nM. In comparison with its natural precursor fumagillin (IC_{50} of 200 nM for MetAP2 and 1 nM for bovine aortic endothelial cell proliferation), the potency of fumagalone is decreased by 40- to 50-fold. We have also determined the effect of fumagalone on type 1 human methionine aminopeptidase. Like fumagillin and ovalicin, fumagalone has little effect on human MetAP1 at the highest concentration tested (50 μM), indicating that fumagalone remains highly specific for human MetAP2.

It is noteworthy that the IC_{50} for inhibition of MetAP2 in vitro by fumagalone is over 100-fold higher than that for inhibition of endothelial cell proliferation in cell culture. The same is true for fumagillin and most other fumagallin analogues tested to date.[9] This difference in IC_{50} for MetAP2 and endothelial cell inhibition may be attributable to two factors. First, the sensitivity of the MetAP2 assay in vitro is such that it requires 20−100 nM of recombinant MetAP2 to detect the product signal. And the minimum IC_{50} for an inhibitor as determined by the in vitro MetAP2 enzyme assay is at least half of the enzyme concentration used. In contrast, the total MetAP2 concentration in a cellular assay is determined by the level of expression of MetAP2 in endothelial cells, which could be much lower. Second, because both fumagillin and fumagalone (presumably) form a covalent adduct with MetAP2, intracellular MetAP2 could serve as a trap for both inhibitors in a cellular assay, significantly raising intracellular concentration of each inhibitor in comparison to the concentrations of inhibitors in the culture media.

Two distinct biochemical assays were performed to determine whether fumagalone is bound to MetAP2 reversibly. In the first assay, fumagalone was preincubated with purified recombinant human MetAP2.[11] The reaction mixture containing fumagalone and MetAP2 was then subjected to dialysis in 1000 volumes of buffer at 4 °C, with the dialysis buffer changed twice during a 24 h period. The dialyzed mixture was then checked for the recovery of the enzymatic activity of MetAP2, in comparison with MetAP2 samples that are preincubated with DMSO as a solvent carrier control (normalized to 100%). As shown in Figure 2A, the presence of 50 μM fumagalone inhibited MetAP2 activity by over 96% (Figure 2A, bar 2). Upon dialysis, close to 80% of the activity of MetAP2 was recovered (Figure 2A, bar 3) in comparison to the solvent control (Figure 2A, bar 1), strongly suggesting that fumagalone is bound to MetAP2 reversibly. In an alternative assay, we determined whether fumagalone is capable of competing with the biotin−fumagillin conjugate for binding to MetAP2 in a reversible manner. Thus, recombinant MetAP2 was preincubated with fumagalone or TNP-470 for 1 h. This

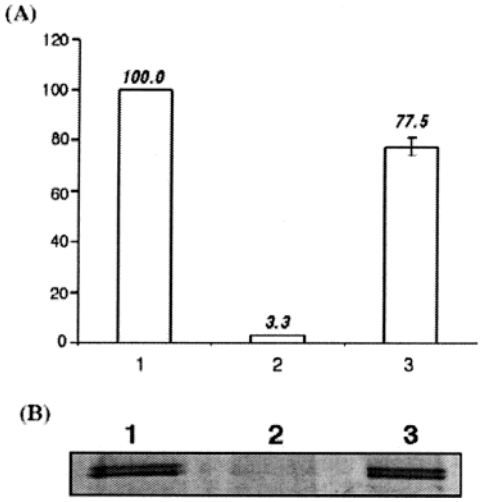


Figure 2. Reversible binding of fumagalone to MetAP2. (A) Relative MetAP2 enzymatic activity upon preincubation with DMSO (bar 1) or 50 μM fumagalone (bar3), followed by dialysis. As a control, MetAP2 activity was determined in the presence of 50 μM fumagalone without dialysis (bar 2). (B) Commassie blue stained polyacrylamide gel for MetAP2 bound by biotin−fumagillin after preincubation with solvent (lane 1), TNP-470 (lane 2), or fumagalone (lane 3).

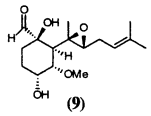
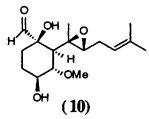
is followed by the addition of the biotin−fumagillin conjugate, which binds to MetAP2 irreversibly. The MetAP2 protein bound by the biotin−fumagillin conjugate is captured using streptavidin−sepharose, which binds to the biotin moiety of the conjugate,[9] followed by SDS−polyacrylamide gel electrophoresis and subsequent staining with commassie blue to visualize the bound MetAP2 protein. Biotin−fumagillin was able to compete effectively for MetAP2 after its preincubation with fumagalone (Figure 2B, lane 3) but not with TNP-470 (Figure 2B, lane 2). These results unambiguously demonstrate that fumagalone, unlike TNP-470, is bound to MetAP2 reversibly.

Although we have shown that fumagalone (**4**) inhibits MetAP2 reversibly, in contrast to fumagillin or its synthetic derivative TNP-470, it remains to be proven that fumagalone forms the tetrahedral adducts as envisioned. The final proof will have to await the X-ray structure of the complex between fumagalone and human MetAP2.

In addition to fumagalone, we have also synthesized several other aldehyde-containing analogues of fumagillin. There appears to be a stringent structural requirement for the activity of the reversible inhibitor because reduction of the ketone in fumagalone to an alcohol (Table 1, **9**) significantly decreased its activity against MetAP2 and almost completely abrogated its activity in endothelial cells. Inversion of stereochemistry at C6 (Table 1, **10**) did not recover any activity. It is possible that an intramolecular hydrogen bond is formed between the newly formed hydroxyl group of the aminal and a yet-to-be-determined residue in MetAP2, which may place a severe conformational constraint on the juxtaposition of the cyclohexyl ring within the active site of MetAP2.

The synthesis of fumagalone as a reversible inhibitor of MetAP2 paves the way to designing, synthesizing, and identifying more potent reversible inhibitors of this enzyme using the fumagillin core structure as a leading

Table 1. Activity of Fumgalone and Its Structurally Related Analogues

	IC_{50} (μM)	
	BAEC Proliferation[a]	MetAP2
4	0.052	8.0
8	Inactive[b]	Inactive
 **(9)**	Inactive	53.6
 **(10)**	Inactive	Inactive

[a] BAEC = bovine aortic endothelial cell. [b] No inhibition at 50 μM or above.

pharmacophore. While the decrease in potency upon conversion of an irreversible inhibitor into a reversible one may be an inevitable price to pay, there is relatively large room to accommodate such a loss in activity, given the extremely high potency of the epoxide-containing precursors. The ready availability of fumagillin as a fermentation product and the relatively short synthetic route from fumagillin to fumagalone should facilitate large-scale production of related analogues. On the basis of the results reported in this manuscript, we have begun to synthesize the second generation of derivatives to further probe the structure−activity relationship of this new class of inhibitors of MetAP2. It will be interesting to see whether fumagalone-based reversible inhibitors of MetAP2 will have improved pharmacological properties and decreased side effects in comparison with TNP-470. Progress along these lines will be reported in due course.

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Supporting Information Available: Synthetic procedures for and characterization of fumagalone and intermediates **6**−**8** as well as the protocols for the biochemical assays. This material is available free of charge via the Internet at http://pubs.acs.org.

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