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Letters

Fumarranol, a Rearranged Fumagillin Analogue That Inhibits Angiogenesis in Vivo

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Abstract: The fumagillin family of natural products inhibits angiogenesis through the irreversible inhibition of the type 2 methionine aminopeptidase (MetAP2). Herein is reported a novel fumagillin analogue named fumarranol. It is shown that, like fumagillin, fumarranol selectively inhibits MetAP2 and endothelial cell proliferation. It is also active in a mouse model of angiogenesis in vivo. Unlike TNP-470, fumarranol does not covalently bind to MetAP2. Fumarranol may serve as a lead for a new class of angiogenesis inhibitors.

Angiogenesis, the formation of new blood vessels, has been implicated in the pathogenesis of several important human diseases, including cancer, diabetic retinopathy, and age-related macular degeneration. Inhibition of angiogenesis is emerging as an effective new strategy for the treatment of angiogenesis-dependent diseases.[1,2] One of the most potent classes of small molecule inhibitors is from the fumagillin family.[3] Fumagillin, its synthetic analogue TNP-470, and ovalicin have been shown to specifically bind to type 2 methionine aminopeptidase (MetAP2).[4,5] In a mechanism that remains to be completely elucidated, inhibition of MetAP2 by these small molecule inhibitors led to the transcriptional activation of p53, which in turn activates the expression of p21 that inhibits cyclinE·Cdk2, accounting for the cell cycle blockade by these inhibitors.[6,7] Since the identification of MetAP2 as the target for fumagillin and ovalicin, a number of attempts have been made to find new and reversible inhibitors of this enzyme through either the structural modification of fumagillin[8−12] or high-throughput screening.[13−15] Herein, we report a new fumagillin analogue with a completely rearranged skeleton, named fumarranol, that inhibits MetAP2 and blocks angiogenesis (Figure 1).

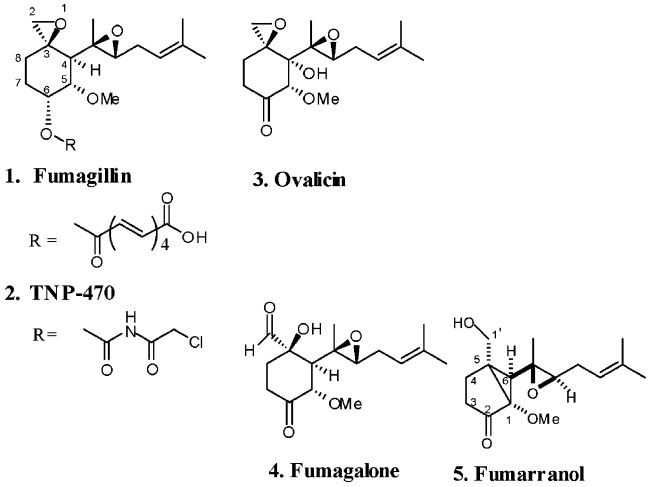

Figure 1. Structures of fumagillin and synthetic analogues.

Fumarranol is an unexpected product of a reaction intended to produce another fumagillin analogue when fumaginone (**6**) was reacted with potassium hydroxide in an attempt to open the spiroepoxide group to produce a diol. A single product distinct from the starting material (**6**), as judged by thin-layer chromatography, was isolated. The molecular mass as determined by high-resolution mass spectrometry was 280 Da, 18 Da smaller than that for the expected diol, suggesting that the hydroxide group might have failed to be incorporated into the final product. Careful analysis of ^{1}H and ^{13}C NMR suggested that the product is more consistent with the bicyclic structure **5** (Scheme 1). To verify the assigned structure of **5**, a number of derivatives were made. One derivative, **7**, formed crystals. The crystal structure of **7** (Figure 2) confirmed the existence of the bicyclic ring system in **5**, which can be explained by the formation of a carbanion at the α-position of the 6-ketone group in the starting material (**6**), which undergoes an intramolecular SN2 reaction to open the spiroepoxide group (Scheme 1). As is apparent from the crystal structural of **7**, the presence of the cyclopropyl ring and the concurrent loss of the spiroepoxide group in **5** rendered it structurally distinct from fumagillin and TNP-470. As a result, it was not expected that fumarranol would

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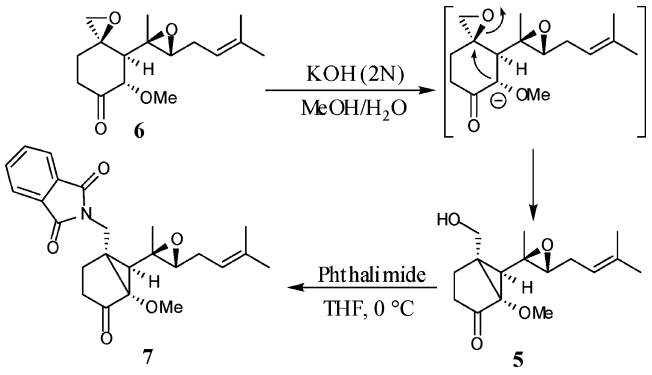
Scheme 1. Formation of Fumarranol and Its Conversion to Analogue **7**



retain the ability to bind human MetAP2 (hMetAP2) or to inhibit endothelial cell proliferation.

Surprisingly, however, fumarranol remained active against hMetAP2 in vitro, albeit with lower potency than fumagillin. Its IC_{50} value is 3.2 μM (Table 1). Importantly, it is also active at the cellular level, inhibiting the proliferation of bovine aortic endothelial cells (BAEC) with an IC_{50} of 34 nM (Table 1). The apparently higher cellular activity of fumarranol is similar to those seen with TNP-470 and fumagalone (**4**) and can be attributed to both the likely lower cellular concentration of MetAP2 in comparison to that required for in vitro enzymatic assays and the accumulation of fumarranol inside cells. Moreover, fumarranol remained specific for hMetAP2, as it did not inhibit the enzymatic activity of recombinant hMetAP1 at 100 μM, suggesting that the inhibition of BAEC was likely to be caused by the inhibition of hMetAP2.

A structure/activity (SAR) study of fumarranol analogues was then pursued to determine the key structural elements in fumarranol that are essential for its activity. Different substitutions on the bicyclic ring system were designed and synthesized, and the resultant analogues were tested against hMetAP2 and in the BAEC proliferation assay (Table 1). The primary alcohol group at C1′ is essential, as its conversion into an aldehyde (**10**) caused a 10-fold increase in IC_{50} value against recombinant hMetAP2. Not surprisingly, the replacement of the alcohol group with the bulky phthalyl group (**7**) or methoxy group (**12**) led to complete inactivation. In contrast to the C1′ position, which requires an alcohol group, reduction of the C2 carbonyl group to an alcohol (**11**) caused a complete inactivation. Similar decreases in activity upon reduction of the carbonyl group to an alcohol in the fumagillin and ovalicin core structures have also been observed.[4] Like fumagillin, the C6 side chain in fumarranol is also sensitive to chemical modifications. Thus, removal of the side-chain epoxide (**8**, **9**) caused a significant decrease in activity. Further extension of the side chain ending with a carboxylic ester (analogue **14**) resulted in complete inactivation, which is likely due to the inability of the enzyme active site to accommodate the excessively long side chain. Together, these results indicate that fumarranol remains an optimal structure among the analogues tested with this unique bicyclic core for the inhibition of hMetAP2 or endothelial cell proliferation.

We next determined whether fumarranol is capable of inhibiting angiogenesis in vivo in a mouse matrigel plug assay.

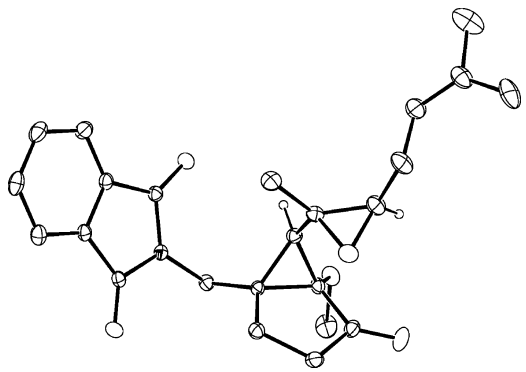
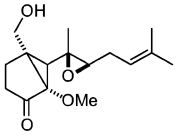
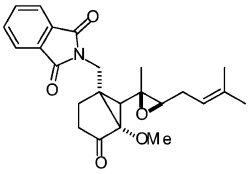
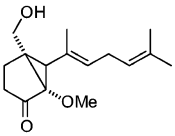
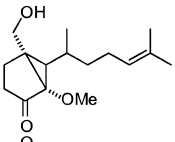
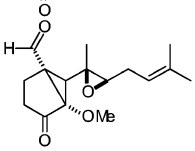
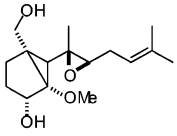
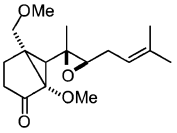
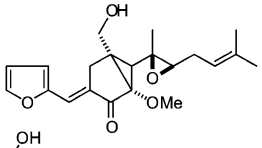
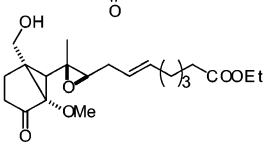


Figure 2. X-ray crystal structure of fumarranol analogue **7**.

Table 1. Inhibition of BAEC Proliferation and Human MetAP2 Enzymatic Activity by Fumarranol and Analogues[a]

	Compound	IC_{50} (μM)	
		BAEC	MetAP2
2	TNP-470	3.7×10^{-5}	0.0010
4	Fumagalone	0.052	8.0
5		0.034	3.23
7		Inactive	Inactive
8		1.78	33.8
9		4.0	57.5
10		0.37	13.2
11		Inactive	Inactive
12		Inactive	Inactive
13		Inactive	Inactive
14		Inactive	Inactive

[a] Inactive, no activity at 100 μM.

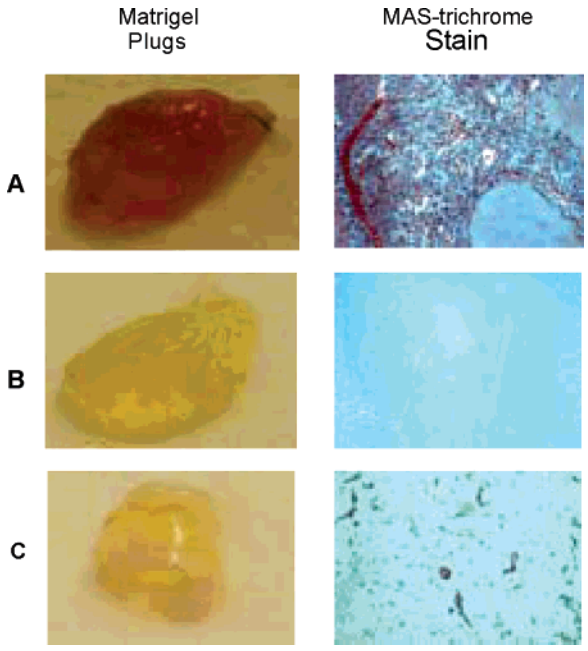


Figure 3. Effects of fumarranol on angiogenesis in mouse matrigel plug assays in vivo: (A) control; (B) TNP-470 at 30 mg/kg/day; (C) fumarranol at 90 mg/kg/day.

In this assay, matrigel containing VEGF and basic FGF were injected subcutaneously into mice and allowed to incubate for 10 days, during which new blood vessels grew into the matrigel in response to stimulation by VEGF and bFGF. As shown in Figure 3A, matrigel from control animals contained new blood vessels as judged both by the red color of the matrigel and by staining the matrigel slices in MAS-trichrome to visualize blood vessels (Figure 3A). As expected, treatment of the animals with TNP-470 at 30 mg/kg almost completely inhibited the growth of new blood vessels into the matrigel implant (Figure 3B). Importantly, administration of fumarranol at 90 mg/kg also blocked the growth of new blood vessels (Figure 3C). The higher amount of fumarranol that is required to achieve a comparable degree of inhibition of angiogenesis in vivo is consistent with the lower potency of fumarranol in comparison to TNP-470.

TNP-470, despite its reduced toxicity compared to fumagillin,[3] still has side effects. In animal experiments, this is exhibited in part by the ulceration in animals given TNP-470 (Figure S1, Supporting Information). Unlike TNP-470, however, administration of fumarranol at 90 mg/kg did not result in the same ulceration of skins as seen with TNP-470. These observations suggest that fumarranol is likely to be less toxic than TNP-470. It remains to be determined whether fumarranol also possesses a better pharmacokinetic profile than TNP-470, i.e., with a longer half-life in humans, because the most reactive spiroepoxide group in TNP-470 has been eliminated in fumarranol.

Fumagillin irreversibly inhibits MetAP2 through covalent modification of His231 in the active site of the enzyme using its spiroepoxide group.[16,17] In fumarranol, the spiroepoxide group is replaced by the cyclopropyl group and a primary alcohol. It is therefore unlikely for fumarranol to form a covalent bond with the His231 side chain in the same manner as fumagillin and TNP-470. In addition, the chemically reactive chloroacetyl side chain at C6 in TNP-470 (Figure 1) is absent from fumarranol. These structural differences may account for the lower toxicity of fumarranol in mice compared with that of TNP-470.

Given the significant structural difference between fumarranol with a bicyclic core and fumagillin containing a cyclohexyl core, it is surprising that fumarranol retains binding affinity as well as isoform specificity of fumagillin. From the SAR data (Table 1), it is clear that several positions on the bicyclic core of fumarranol are quite sensitive to further chemical modifications, including the hydroxyl group at C1′ and the carbonyl group at C6. It can be envisaged that a key structural element involved in the binding of fumarranol to hMetAP2 is the epoxide-containing side chain that interacts with the same hydrophobic pocket in the enzyme as its counterpart in fumagillin.[17] However, the bicyclic core of fumarranol may have to reorient itself relative to the cyclohexyl core of fumagillin to fit the remainder of the active site of the enzyme. This may allow the primary alcohol group to form a new interaction with either the active site metal ion or a hydrogen-bond acceptor in the active site. The reorientation will also necessarily lead to the repositioning of the C6 carbonyl group of fumarranol in the active site of MetAP2, making any modification at this position, including reduction to an alcohol, intolerable. It will be interesting to see whether the fumarranol core can be further modified to enhance its affinity for MetAP2 and its potency as an angiogenesis inhibitor.

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Supporting Information Available: Figure S1, synthetic procedures and the procedure for the determination of X-ray crystal structure of analogue **7**. This material is available free of charge via the Internet at http://pubs.acs.org.

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