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Development of sulfonamide compounds as potent methionine aminopeptidase type II inhibitors with antiproliferative properties

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Abstract—We have screened molecules for inhibition of MetAP2 as a novel approach toward antiangiogenesis and anticancer therapy using affinity selection/mass spectrometry (ASMS) employing MetAP2 loaded with Mn^{2+} as the active site metal. After a series of anthranilic acid sulfonamides with micromolar affinities was identified, chemistry efforts were initiated. The micromolar hits were quickly improved to potent nanomolar inhibitors by chemical modifications guided by insights from X-ray crystallography.
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Methionine aminopeptidases (MetAPs) play an important role in removing the initiator methionine residue from nascent polypeptide chains and are believed to be one of the essential enzymes involved in protein maturation. There are three known mammalian methionine aminopeptidases, MetAP1, MetAP2, and the recently reported MetAP1D.[1,2] The latter is closely related to MetAP1 and is located in the mitochondria. The well-studied types 1 and 2 remove methionine from the N-terminus of proteins during protein synthesis, but appear to play different roles in cell function.[3] Eukaryotic cells contain two types, (MetAP1 and MetAP2), while prokaryotes have only MetAP1. MetAP2 appears to play a critical role in cell proliferation and tumor growth, and is expressed at higher concentrations in tumors as compared to normal cells.[4] Many efforts have been focused on the inhibition of MetAP2 since the enzyme was identified as an endogenous target for the anti-angiogensis/antitumor agent TNP-470, a well-studied semi-synthetic product under clinical development.[5] Recently, it has been reported that an irreversible MetAP2 inhibitor potently inhibits the proliferation of human fibroblast-like synoviocytes (HFLS-RA), suggesting the possibility of usage for the treatment of rheumatoid arthritis.[6]

Since the finding of clinical implication of an inhibition of MetAP2 as an anti-tumor agent, several classes of compounds have been reported to possess inhibitory activities against MetAP2.

Various synthetic analogues of the natural products fumagillin, ovacillin, and bengamide have been evaluated as MetAP2 inhibitors.[7] We have also previously examined bestatin analogues as MetAP2 inhibitors, and revealed that a reversible inhibitor effectively suppresses tumor growth and inhibits angiogenesis in animal models.[8] After establishing that MetAP2 inhibitors are promising anti-cancer agents, we shifted our focus to search for orally active reversible inhibitors. In this study, we have used an affinity selection/mass spectrometry (ASMS) screening method[9] to identify molecules that bind to human MetAP2 containing Mn^{2+} in the enzyme active site.[10] Activity was confirmed by evaluation of enzyme inhibitory activity and inhibition of cell proliferation using assay described previously.[8b]

From several classes of small molecule inhibitors possessing less than 10 μM inhibitory activities against

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MetAP2, anthranilic acid sulfonamide **1** (IC_{50} = 9 μM for MetAP2) was chosen as a starting point to develop a structure–activity relationship study. We envisioned that a sulfonamide is an attractive lead, showing promising pharmacokinetics (data not shown) as well as a high synthetic feasibility.

Based on the X-ray structures shown in Figure 1, we felt the active site would be able to accommodate substituted anthranilic acids with substituents at 5 and 6 positions. We also observed that the sulfonamide backbone is angled and fits tightly to allow both aromatic rings to reside in a hydrophobic environment. Chloro substitution at the *para*-position on the sulfonyl phenyl shows a relatively tight binding due to a narrow hydrophobic region of the enzyme.

Compounds **1**–**5** were prepared from commercially available anthranilic acids and well-established chemistry. Compounds **6**–**8** were synthesized by the route illustrated in Scheme 1 (I). Commercially available 2-amino-5-bromo benzoic acid was methylated, followed by benzenesulfonylation to yield the 5-bromo methyl ester. Organometallic couplings, followed by the saponification of the ester under microwave conditions, provided the final products.

Compounds **9** and **10** were synthesized by the procedure illustrated in Scheme 1 (II). 2-Aminonaphthalene was reacted with diethyl ketomalonate in acetic acid to form an isatin derivative.[12] Ring opening was carried out by treatment with 30% hydrogen peroxide in 1 N sodium hydroxide to yield 2-aminonaphthalene-1-carboxylic acid.[13] Sulfonamide formation was carried out with in situ protection of the carboxyl group as its TMS ester, followed by reaction with appropriate arylsulfonyl chloride.

Sulfonamides **12** and **14** were prepared by the route shown in Scheme 2.[14] Commercially available 7-nitro-1-tetralone was reduced, followed by bromination to yield its hydrobromic acid salt. The resulting amine was reacted with the aryl sulfonyl chloride to generate sulfonamides, and the ketone was reacted with MeMgBr to insert a methyl group. Carbonylation was carried out in methanol to yield methoxycarbonyl group insertion, followed by hydrogenation, and the ester was then removed. The enantiomeric mixture was separated by a chiral column to obtain compound **14** (*S*-configuration). Compound **12** was synthesized from the same route except omitting the hydrogenation step.

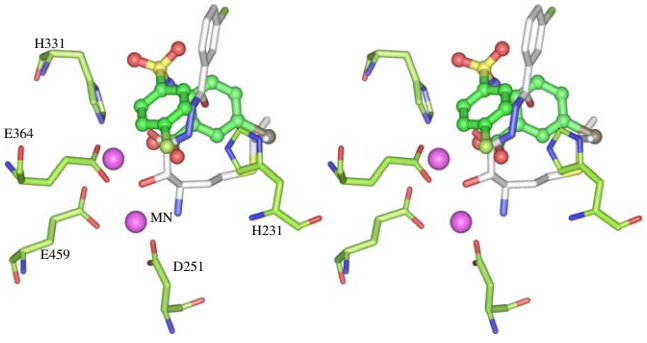
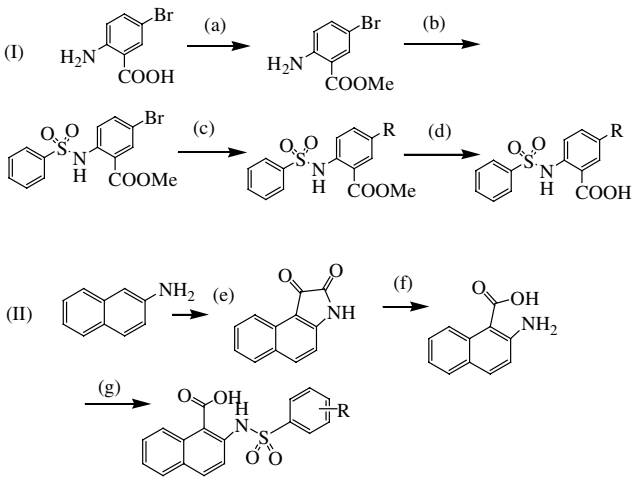
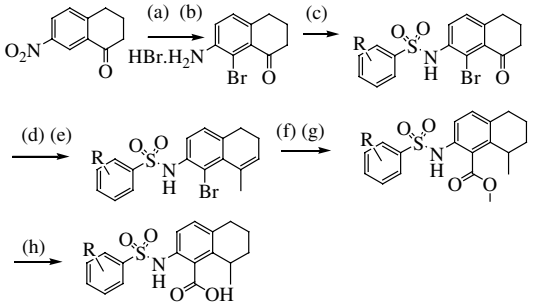


Figure 1. Stereo view of MetAP2 complex of compound **1** from an X-ray crystal structure of an inhibitor–enzyme complex.[11] Mn^{2+} ions are shown as pink spheres.



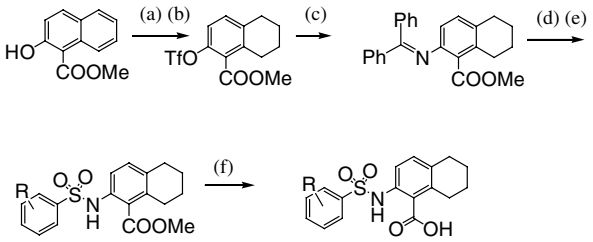
Scheme 1. Reagents and conditions: (a) HCl in MeOH; (b) $ArSO_2Cl$, Py–CH_2Cl_2; (c) R ZnBr, CuI, $PdCl_2$(dppf), THF, μwave, 150 °C, 10 min; (d) LiOH, dioxane–water, μwave, 160 °C, 10 min; (e) diethyl ketomalonate, AcOH, 120 °C, 3 h; (f) 30% H_2O_2, 1 N NaOH; (g) TMS–Cl, $ArSO_2Cl$, Py–CH_2Cl_2.



Scheme 2. Reagents and conditions: (a) Fe, NH_4Cl, EtOH–H_2O (4:1); (b) Br_2, $CHCl_3$–DMF; (c) $ArSO_2Cl$, Py–CH_2Cl_2; (d) 3 M MeMgBr, THF–Et_2O; (e) *p*-TosOH toluene; (f) $PdCl_2$(dppf)-CH_2Cl_2, CO, Et_3N, MeOH (g) Pd/C, H_2, EtOAc; (h) LiOH, dioxane–water, μwave, 160 °C, 10 min.

Tetrahydro naphthalene derivatives **11** and **13** were prepared from the route shown in Scheme 3. A substituted naphthalene derivative was hydrogenated to obtain the tetrahydronaphthalene derivative, followed by treatment with trifluoromethane sulfonic acid anhydride to activate the hydroxyl group. After formation of a Schiff base, it was reacted with hydrochloric acid to obtain the amine. Final analogues were then prepared according to the method described in Scheme 1. Compounds **11** and **13** can also be made by hydrogenation of **9** and **10** (PtO_2, H_2, and HOAc).

Enzyme inhibition assay results ([*E*] IC_{50}) as well as proliferation inhibition data on human microvascular endothelial cells (HMVEC, IC_{50}) are shown in Table 1. Some analogues were examined in the enzymatic assay to



Scheme 3. Reagents and conditions: (a) Pd/C, H_2, MeOH–H_2O; (b) Tf_2O, Py–CH_2Cl_2; (c) $Pd(OAc)_2$, xantphos, Cs_2CO_3, HN=C(Ph)$_2$, dioxane; (d) HCl, THF–H_2O; (e) $ArSO_2Cl$, Py–CH_2Cl_2; (f) μwave, LiOH, dioxane–water, 160 °C, 10 min.

Table 1. Inhibition of human methionine aminopeptidase type-2

Compound	[*E*] IC_{50} (μM)	[*E*] IC_{50}[a] (μM)	IC_{50}[b] (μM)
1	9.1	>100	3
2	3.9	>100	2
3	11		0.3
4	1		0.1
5	0.35	>100	0.4
6	0.09	>100	10
7	1.1		60
8	10		100
9	0.019	3.8	0.5
10	0.015		0.5
11	0.009	3.3	0.4
12	0.02	1.9	0.15
13	0.01	2.6	0.18
14	0.027	9.2	0.13

[a] Enzyme inhibition in the presence of 40 mg/mL of human serum albumin.[8b]

[b] HMVEC cell line growth inhibition.[8b]

determine the effect of 40 mg/mL of human serum albumin. As was expected from the X-ray data, relatively larger hydrophobic groups were accepted at the active site of the enzyme. Interestingly, even weak inhibitors, such as **3**–**5**, showed relatively potent inhibition of HMVEC proliferation, which might be due to off-target effects. On the other hand, compounds **9**–**11** were potent inhibitors of MetAP2 with good selectivity against MetAP1 (e.g., **9**: MetAP1 IC_{50} = 47μM) and other aminopeptidases (e.g., **9**, Pfu-MetAP, AP, AP-1, and AP-M; IC_{50} > 100 μM), and displayed moderate activity in the cellular proliferation (HMVEC) assay.

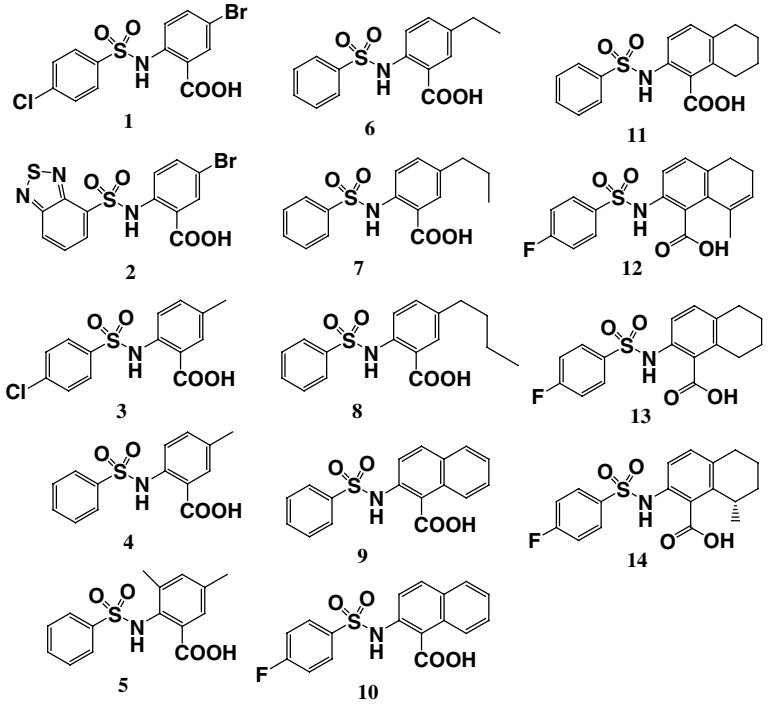
Table 2. % protein binding of some MetAP2 inhibitors

Compound	Rat plasma binding
3	98.1% ± 0.2
4	99.7% ± 0.1
6	99.8% ± 0.1
9	99.8% ± 0.1
10	99.2% ± 0.2

Compounds **11**–**14** possess excellent inhibitory activity. Among them, an *S*-configuration on the compound **14** was preferred over the *R*-configuration in every assay tested (data not shown).

In light of the propensity of serum albumin to bind hydrophobic anionic compounds, we examined the extent of protein binding of selected MetAP2 inhibitors, as shown in Table 2. Rat plasma (pH 7.4) was spiked with compounds to a concentration of 10 μM. The plasma sample was centrifuged and the sample was fractionated, extracted and unbound compound was measured in the supernatant by LC methods. These compounds are found to be highly protein bound.

In conclusion, we have shown that the modification of anthranilic acid sulfonamide **1** has led to inhibitors of human MetAP2 with good to potent activities against



the enzyme and against HMVEC proliferation. The compounds also display high affinity to serum albumin. Therefore, our efforts shifted to identifying molecules having less protein binding, while maintaining potent biological activity.

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