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Lead optimization of methionine aminopeptidase-2 (MetAP2) inhibitors containing sulfonamides of 5,6-disubstituted anthranilic acids

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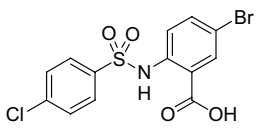
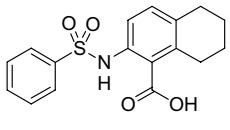
Abstract—A series of aryl sulfonamides of 5,6-disubstituted anthranilic acids were identified as potent inhibitors of methionine aminopeptidase-2 (MetAP2). Small alkyl groups and 3-furyl were tolerated at the 5-position of anthranilic acid, while $-OCH_3$, CH_3, and Cl were found optimal for the 6-position. Placement of 2-aminoethoxy group at the 6-position enabled interaction with the second Mn^{2+} but did not result in enhancement in potency. Introduction of a tertiary amino moiety at the *ortho*-position of the sulfonyl phenyl ring gave reduced protein binding and improved cellular activity, but led to lower oral bioavailability.

The intracellular enzyme methionine aminopeptidase-2 (MetAP2) came into the focus as a potential anti-tumor therapeutic target when it was identified as the molecular target of the anti-cancer natural product fumagilin and its synthetic analog TNP-470.[1–3] TNP-470 has exhibited potent anti-tumor efficacy in a variety of animal xenograft models as well as in transgenic tumor models and has shown varying degrees of efficacy against several human cancers in clinical trials.[4] Both fumagilin and TNP-470 exert their anti-tumor effect primarily through inhibition of endothelial cell proliferation, even though these molecules also inhibit the proliferation of a subset of tumor cells.[5,6] This anti-angiogenic activity of fumagilin and TNP-470 has been attributed to the irreversible inhibition of MetAP2.[1–3] More recently, a rationally designed reversible inhibitor of MetAP2 based on bestatin has also been reported to have potent anti-angiogenic activity both in vitro and in vivo, and potent anti-tumor efficacy in several animal models, further supporting the validity of MetAP2 as an anti-cancer target.[7–9] Several other series of reversible inhibitors of MetAP2 have also been reported.[10,11] Biochemically, MetAP2 is a metalloproteinase responsible for the removal of the *N*-terminal initiator methionine residue of nascent proteins. It is required for protein co- and/or post-translational modifications, such as NH_2-terminal myristoylation, and for protein stability.[12–14] The active site of MetAP2 is believed to contain two metal cations, either Co^{2+} or Mn^{2+}, with Mn^{2+} being characterized as the physiologically relevant cofactor.[14]

Recently, we have described a series of sulfonamides of anthranilic acid as MetAP2 inhibitors.[15–17] Starting from a screening lead **1** (Fig. 1), initial lead optimization led to the tetrahydronaphthyl sulfonamide **2** (Fig. 1), which is a potent MetAP2 inhibitor but lacks cellular activity and is highly protein bound. The X-ray crystallographic structure of compound **2** complexed to



1 (IC_{50}=11,000 nM)



2 (IC_{50}=9 nM)

Figure 1.

Keywords: Methionine aminopeptidase-2 (MetAP2) inhibitors; Sulfonamides of 5,6-disubstituted anthranilic acids.
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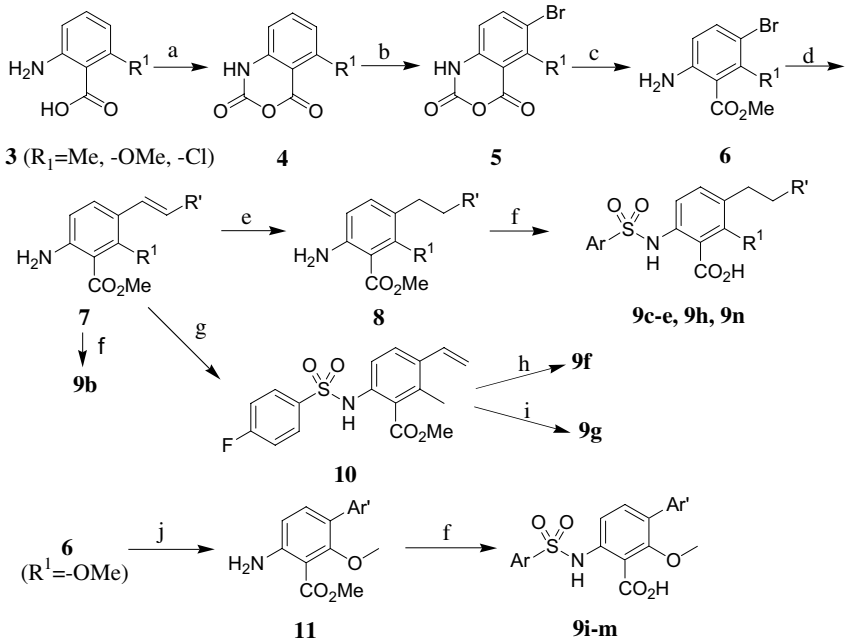
MetAP2 revealed that the carboxylate chelates one of the two manganese ions and the rigid tetrahydronaphthyl moiety fits rather tightly into a hydrophobic pocket of the active site.[16] Consistent with this observation, in a few limited examples with substitution on the cyclohexyl ring of the tetrahydronaphthyl moiety, reduction in enzyme inhibition IC_{50} was observed.[15] We reasoned that breaking open the cyclohexyl ring to form 5,6-disubstituted anthranilic acid derivatives would offer more flexibility and allow for fully optimized interaction with the key hydrophobic binding pocket of the enzyme. This added flexibility might also offer the potential to form an interaction with the second manganese in the active site. In this paper, we describe the results of this effort.

Synthesis of the required 5,6-disubstituted anthranilic acid sulfonamides followed one of the general routes shown in Scheme 1.[17] Isatoic anhydrides **4** were prepared from 6-substituted anthranilic acids **3** in high yield. Bromination of **4** with NBS gave modest yields of compounds **5**, which were converted to the anthranilic acid methyl esters **6** by refluxing in MeOH. Suzuki coupling of **6** with vinyl boronic acids or boronates, followed by Pd-catalyzed hydrogenation, sulfonylation, and saponification, gave the 5-alkyl compounds **9c**–**e**, **9h**, and **9n**. Suzuki coupling of **6** with heteroaryl boronic acids gave **11**, which led to compounds **9i**–**m** upon sulfonylation and saponification. The 5-hydroxymethyl compound **9f** and 5-(1-hydroxyethyl) compound **9g** were obtained from the 5-vinyl compound **10** using standard chemistry (Fig. 2).

The compounds were tested in MetAP2 inhibition assays and cellular proliferation assays described previously.[14] We have reported previously that, for sulfonamides of 5-alkyl mono-substituted anthranilic acids, an ethyl group at the 5-position such as in the reference compound **9o** is optimal.[15] As shown in Table 1, this trend holds for the current 5,6-disubstituted anthranilic acid series: 5-alkyl lower (**9a**) or higher (**9d**, **9e**) than 5-ethyl (**9c**) gave less potent MetAP2 inhibitors. In comparison with the reference compound **9o**, adding a C_6 methyl (**9c**), methoxy (**9h**) or chloro- (**9n**) results in 3- to 4-fold increase in MetAP2 inhibitory potency. Several five-membered ring heteroaryls were also examined as the C_5-substituents (**9i**–**m**). Interestingly, 3-furyl was found to give compounds equi-potent to the 5-ethyl counterpart (**9i** vs **9h**), whereas 2-furyl, 4-imidazolyl, and 4-pyrazolyl all gave much less active compounds (**9k**–**m**).

X-ray crystallographic structures of 4-fluorophenyl sulfonamide of 5-ethyl-6-methyl-anthranilic acid **9c** and tetrahydronaphthyl sulfonamide **2** complexed with MetAP2 (Fig. 1) indicated essentially identical binding interactions between these two molecules. The X-ray structure of **9c** also revealed the possibility of reaching to the second Mn^{2+} atom by extending the 6-methyl group of **9c**. We choose to explore this possibility by extending the 6-methoxy group of **9h** with the chemistry shown in Scheme 2, with the results summarized in Table 2.

Extending the 6-methoxy group of **9h** to 6-ethoxy group of **16a** resulted in a modest loss of potency (from 10 to 40 nM), whereas further extending to 6-propoxy (as 2-fluorophenyl-sulfonamide) abolished the activity



Scheme 1. Reagents and conditions: (a) phosgene, NaOH, H_2O, 90%; (b) NBS, DMF, DCM, 45%; (c) MeOH, reflux, 80%; (d) R′CH=CHB(OH)$_2$, Pd(PPh$_3$)$_4$, CsF, DME, MeOH, 150 °C (μwave), 5 min, ~80%; (e) H_2, Pd/C, MeOH, 90%; (f) i—4-fluorophenyl sulfonyl chloride, pyridine, DCM, 90–95%; ii—LiOH (10×), dioxane, H_2O, 160 °C (μwave), 15 min, 40–70%; (g) 4-fluorophenyl sulfonyl chloride, pyridine, DCM, 90%; (h) i—OsO_4, $NaIO_4$, dioxane, H_2O; ii—$NaBH_4$, EtOH; iii—LiOH (10×), dioxane, H_2O, 160 °C (μwave), 15 min; (i) i—OsO_4, $NaIO_4$, dioxane, H_2O; ii—CH_3MgBr, THF; iii—LiOH (10×), dioxane, H_2O, 160 °C (μwave), 15 min; (j) Ar′B(OH)$_2$, Pd(PPh$_3$)$_4$, CsF, DME, MeOH, 150 °C (μwave), 5 min, ~80%.

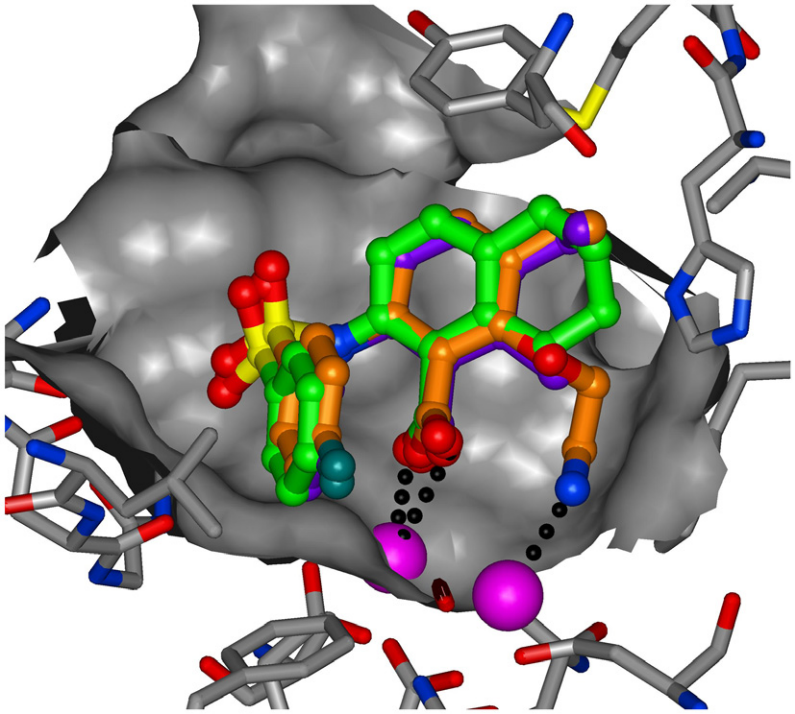
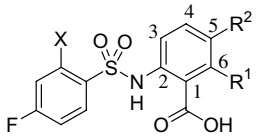
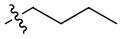


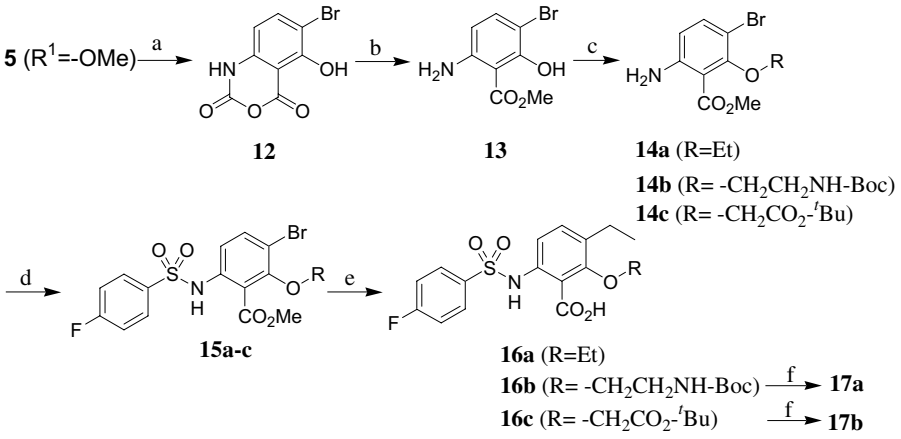
Figure 2. X-ray crystallographic structure of MetAP2 complexed with compound **2** (green), **9c** (purple), and **17a** (brown). The atomic coordinates have been deposited with the Protein Data Bank (1YW8, 2EA2, 2EA4).

Table 1. Initial optimization of 5,6-disubstituted anthranilic acid sulfonamides



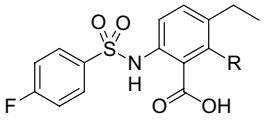
Compound ID	X	R^1	R^2	Enzyme IC_{50} (μM)	Enzyme IC_{50} w/HSA[a](μM)	HT-1080 prol. EC_{50} (μM)
9a	H	CH_3–	CH_3–	0.078	4.2	
9b	H	CH_3–	CH_2=CH_2–	0.060	4.0	
9c	H	CH_3–	CH_3CH_2–	0.016	0.76	4.9
9d	H	CH_3–		0.15	12	
9e	H	CH_3–		1.3		
9f	H	CH_3–		8.0	>100	
9g	H	CH_3–		2.5	>100	
9h	H	CH_3O–	CH_3CH_2–	0.011	1.7	1.4
9i	H	CH_3O–		0.008	1.1	3.8
9j	Br	CH_3O–		0.02	20	11
9k	Br	CH_3O–		1.0	>100	
9l	Br	CH_3O–		36	>100	
9m	H	CH_3O–		0.28	14	
9n	H	Cl–	CH_3CH_2–	0.015	1.8	
9o	H	H	CH_3CH_2–	0.055	4.2	

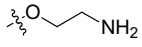
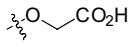
[a] MetAP2 inhibition activity in the presence of 40 mg/mL of human serum albumin (HSA).



Scheme 2. Reagents and conditions: (a) $AlCl_3$, DCM, rt; (b) MeOH, reflux, 3 h, 80% for two steps; (c) EtI or Boc–NH–CH_2CH_2Br or $BrCH_2CO_2{}^tBu$, Cs_2CO_3, DMF, 0 °C, ~90%; (d) 4-fluorophenylsulfonyl chloride, pyridine, DCM, 90–95%; (e) i—$CH_2{=}CHB(OBu)_2$, $Pd(PPh_3)_4$, CsF, DME, MeOH, 150 °C (μwave), 5 min, ~80%; ii—H_2, Pd/C, MeOH, ~90%; iii—LiOH (10×), dioxane, H_2O, 160 °C (μwave), 15 min, 40–70%; (f) 80% TFA in DCM, rt, 3 h, ~90%.

Table 2. Efforts to reach the second manganese ion via C_6 substitution

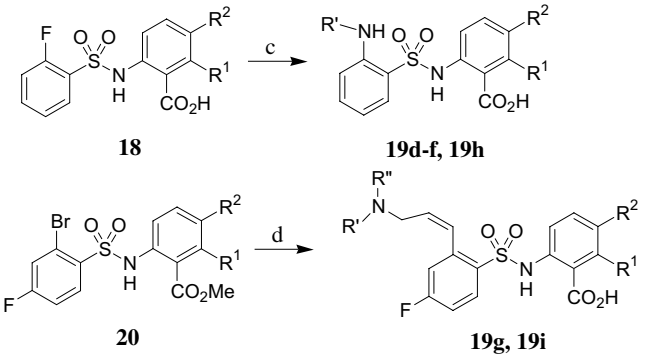


Compound ID	R	Enzyme IC_{50} (μM)	Enzyme IC_{50} w/HSA[a] (μM)
9c	$-CH_3$	0.016	0.76
9h	$-OCH_3$	0.011	1.7
16a		0.038	40
17a		0.046	0.12
17b		5.8	>100

[a] MetAP2 inhibition activity in the presence of 40 mg/mL of HSA.

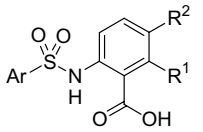
completely (IC_{50} > 10 μM, data not shown in Table 2). However, the 6-(2-aminoethoxy) compound **17a** turned out to be a MetAP2 inhibitor about equi-potent to the 6-ethoxy compound **16a**. The X-ray structure of MetAP2 complexed to **17a** revealed that the side-chain amino group of **17a** displaced a water molecule and indeed was oriented to interact with the second Mn^{2+} (Fig. 1), with the distance from the aminoethoxy nitrogen of **17a** to the second Mn^{2+} being 2.5 Å. In comparison, the distances from the two oxygen atoms of the carboxylate to the first Mn^{2+} were 2.2 and 3.5 Å, respectively, for all three compounds in Figure 1. Thus, we were disappointed to find that there was no gain in potency from **16a** to **17a**. One possible explanation for this result is that the NH_2–Mn^{2+} interaction is weak relative to the interaction between this Mn^{2+} and the displaced water. Another plausible explanation resides in the very recent report of catalytically active monometalated bacterial MetAP1,[18] which suggests that the dimetalated MetAP2 observed in X-ray crystallographic structures might be a crystallization artifact, while the relevant form of intracellular MetAP2, as well as the active MetAP2 present in our assays, may lack a second active site metal.[18]

The MetAP2 inhibitory potency of most compounds in Tables 1 and 2 showed a large reduction when 40 mg/mL of human serum albumin (HSA) was included in the enzyme assay (e.g., 48-fold, 154-fold, and 177-fold for compounds **9c**, **9h**, and **9i**, respectively). Additionally, most of these compounds had poor cellular activity in the HT-1080 proliferation assay. We have reported previously that introduction of tertiary amino functionalities at the *ortho*-position of the sulfonyl phenyl ring significantly reduced protein binding and improved cellular activity in related compounds.[16] Thus, similar structural modifications were made to the current series. The requisite compounds were prepared following the general procedure depicted in Scheme 3,[16,17] and the biological results are summarized in Table 3. These compounds showed only 4- to 10-fold reduction in the IC_{50} in the enzymatic assay when 40 mg/mL of HSA was present. In terms of cellular anti-proliferation activity, compounds with the aminoalkyl moieties attached to

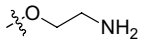
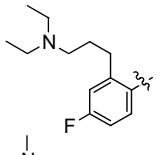
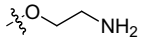
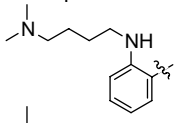
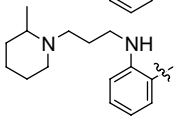
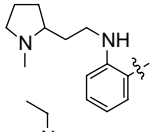
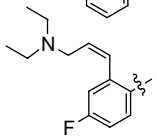
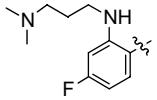
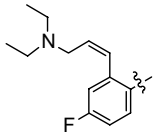


Scheme 3. Reagents and conditions: (c) $R'NH_2$ (8×), Et_3N (5×), acetonitrile, 90 °C, 3 days, ~50%; (d) i—*Z*-(2-diethylaminomethyl)-vinyl *tri*-butyltin, $(t\text{-}Bu_3P)_2Pd$, $Pd_2(dba)_3$, toluene, 90 °C, 50–80%; ii—LiOH (10×), dioxane, H_2O, 160 °C (μwave), 15 min, 40–70%.

Table 3. Sulfonamides containing amino groups



19

Compound ID	Ar	R^1	R^2	Enzyme IC_{50} (μM)		HT-1080 prol. EC_{50} (μM)	
				No HSA	w/HSA[a]	No HSA	w/HSA[b]
19a			CH_3CH_2–	0.016	0.23	83	
19b		CH_3–	CH_3CH_2–	0.067	0.555	12	
19c			CH_3CH_2–	0.024	0.085	30	
19d		CH_3O–	CH_3CH_2–	0.010	0.097	0.25	0.37
19e		CH_3O–	CH_3CH_2–	0.018	0.175	0.37	1.2
19f		CH_3O–	CH_3CH_2–	0.015	0.085	0.48	1.9
19g		CH_3O–	CH_3CH_2–	0.026	0.080	0.07	0.15
19h		CH_3O–		0.017	0.096	0.32	2.9
19i		CH_3O–		0.015	0.087	0.06	0.20

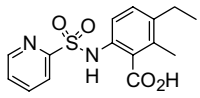
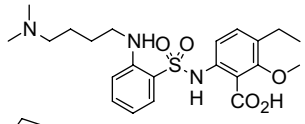
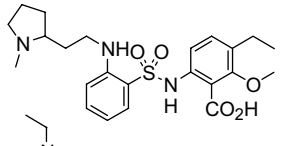
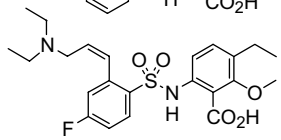
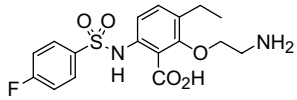
[a] Enzyme inhibition activity in the presence of 40 mg/mL of HSA.
[b] Anti-proliferative activity in the presence of 40 mg/mL of HSA.

the *ortho*-position of the sulfonyl phenyl ring via a nitrogen atom (**19d–f**, **19h**) all showed $EC_{50} < 0.5$ μM. In particular, compounds with an amino-*Z*-alkenyl moiety attached to the *ortho*-position of the sulfonyl phenyl ring (**19g** and **19i**) showed potent anti-proliferation activity (0.07 and 0.06 μM, respectively), consistent with the earlier report.[16]

The mouse pharmacokinetic data for selected compounds are summarized in Table 4. The pyridyl sulfonamide **19b** has a relatively large volume of distribution and low clearance and consequently good oral bioavailability (86%). Installation of an amino group as in **19d**, **19f**, and **17a** resulted in lower volume of distribution, increased clearance, and much lower oral bioavailability. The *Z*-alkenyl substituted **19g** had a larger volume of distribution relative to **19b** but very high clearance. As a result, its oral bioavailability was also very low (16%).

In summary, we have identified a series of sulfonamides of 5,6-disubstituted anthranilic acids as potent inhibitors of MetAP2. The most potent compounds, **19g** and **19i**, display potent MetAP2 inhibition ($IC_{50} \sim 20$ nM) and anti-proliferation activity against HT-1080 cells ($EC_{50} \sim 60$ nM).

Table 4. Mouse pharmacokinetics of selected compounds

Compound		IV (10 mg/kg)				PO (30 mg/kg)		
ID	Structure	$t_{1/2}$ (h)	V_d (L/kg)	CL (L/h kg)	AUC (mg h/mL)	C_{max} (μM)	AUC (mg h/mL)	F (%)
19b		2.4	5.5	1.6	19.8	34	60.9	86
19d		0.3	0.8	2.1	10.8	5.9	9.9	31
19f		0.3	2.0	5.0	4.3	0.9	1.7	13
19g		0.2	13.8	40.5	0.5	0.1	0.26	16
17a		0.3	1.1	3.0	8.6	2.4	9.8	38

Abbreviations: V_d, volume of distribution; CL, clearance; AUC, area under the curve; C_{max}, maximum concentration; F, oral bioavailability.

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