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Inhibitors of type I MetAPs containing pyridine-2-carboxylic acid thiazol-2-ylamide. Part 2: SAR studies on the pyridine ring 3-substituent

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Abstract—Systematic SAR studies on the pyridine ring 3-substituent of PCAT, an inhibitor of *Ec*MetAP1 and *Sc*MetAP1, revealed that 3-substituents have different selectivity for *Ec*MetAP1 and *Sc*MetAP1. The selective inhibitors of type I MetAP are useful tools for investigating the detailed interactions between the enzymes and their inhibitors. In addition, these findings provide useful information for the design and discovery of more potent inhibitors of type I MetAPs.

Methionine aminopeptidases (MetAPs) are intracellular metalloproteases responsible for removing the N-terminal initiator methionine residue of nascent proteins.[1] They are broadly distributed through all living organisms, and play a critical role in the maturation of proteins for proper function, targeting, and degradation.[2–5] This class of enzyme exists in two forms: type I (MetAP1) and type II (MetAP2).[6] MetAPs present good targets for new antibiotic and antitumor drug discovery because of their important physiological functions.[7–10] Moreover, inhibitors of MetAPs offer hope as new treatments for bacterial and fungal infections and cancers.[11–16]

In our preceding paper, we introduced a novel class of small-molecule inhibitors of type I MetAP with the indispensable scaffold, pyridine-2-carboxylic acid thiazol-2-ylamide (PCAT, **1**). Systematic SAR studies on PCAT analogues demonstrated that the 3-position of the pyridine ring of PCAT is suitable for modification and the more effective substituents are those containing O or N atoms connected directly with the pyridine ring (Fig. 1). These discoveries provided a starting point for us to prepare a set of PCAT derivatives with various substituents at the 3-position of the pyridine ring, with the aim of discovering inhibitors with improved activity as well as more selectivity toward different type I MetAPs, based on SAR studies.

In our previous work,[17] we prepared a series of compounds that share substructural diversity at the 3-position of the pyridine ring of PCAT, including aromatic acyloxy or acylamino groups and fatty acyloxy or acylamino groups. From these results, we can anticipate the effect of aromatic substituents on the inhibitory activity. These results also validated the earlier finding that introducing a substituent containing an O or N atom

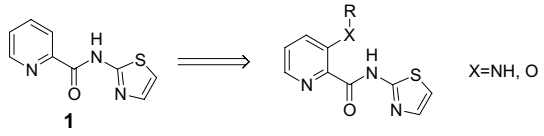

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Figure 1.

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connected directly with the pyridine ring increases the inhibitory activity against *Ec*MetAP1. Aromatic acylamino derivatives exhibited special selectivity toward *Ec*MetAP1, and acyloxy derivatives showed greater inhibition of *Sc*MetAP1 compared with their acylamino analogues.

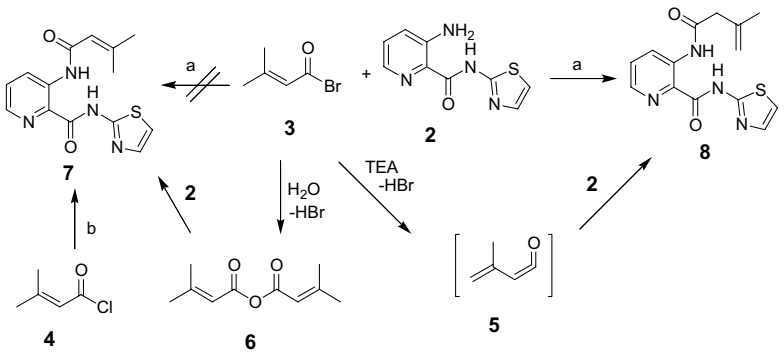
Compounds with fatty acyl substituents at the 3-position of the pyridine ring of PCAT, in general, showed strong inhibitory activity toward both *Ec*MetAP1 and *Sc*MetAP1. At the same time, the fatty acylamino derivatives were more active against *Ec*MetAP1 than their fatty acyloxy analogues; however, this trend was reversed for *Sc*MetAP1. In other words, *Sc*MetAP1 was remarkably inclined to select the fatty acyloxy derivatives substituted at the 3-position of the pyridine ring of PCAT, and *Ec*MetAP1 was inclined to the fatty acylamino derivatives. When PCAT (compound **1**) was transformed into its fatty acyloxy derivative substituted at the 3-position of the pyridine ring, the potency of the inhibition of *Sc*MetAP1 increased 9- to 35-fold, and when transformed into the fatty acylamino derivatives, the potency of the inhibition of *Ec*MetAP1 increased 9- to 38-fold. These results revealed the different selectivities of MetAP1s from different biological sources, and may reflect subtle differences in the active sites of *Ec*MetAP1 and *Sc*MetAP1 and differences in the interaction between the inhibitors and the enzymes. These specific and potent inhibitors give us confidence in obtaining antibiotic drugs with lower toxicity while being specific for the targeted microorganisms. Moreover, selective *Sc*MetAP1 inhibitors are competent tools for investigating the detailed interaction of *Sc*MetAP1 with its inhibitors, as its X-ray structure is currently not available.

Compounds with fatty acyl substituents were much more potent than compounds with aromatic acyl substituents in inhibiting both enzymes. That is, the fatty side chain of the 3-position acyl of the pyridine ring is more favorable than the aromatic side chain. Therefore, further modification of PCAT should focus on those with fatty acyl substituents.

We intended to prepare compound **7** by amidation of **2** with newly made acyl bromide **3** (Scheme 1); however, compound **8** was obtained as an unexpected product. Combination of acyl chloride **4** with **2** in basic conditions with pyridine gave **7**, and with acyl bromide **3** that had been refrigerated for weeks in basic conditions with triethylamine gave the mixed products, **7** and **8**. Therefore, we can reason that the active properties of **3** and the stronger basicity of triethylamine produced the ketene **5**[7] and led to the unexpected product, **8**. Once the kinetic product **3** was stored for sufficient time, it became stable because of the formation of the anhydride **6**. Compound **7** interested us because of its prominent potency against *Ec*MetAP1, as well as the structural feature of an isolated C=C double bond from a carbonyl group, which stimulated us to investigate the effect of the double bond on the inhibition of MetAP1s. Therefore, compounds **9**–**19** were prepared. In addition, compounds **20** and **21**, respectively, were obtained from the corresponding unsaturated analogues **13** and **14**. At the same time, to evaluate the effect of a cycloalkyl side chain on MetAP1 inhibition, compounds **22**–**25** were synthesized (Table 1).

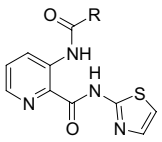
The synthesis of compounds **9**–**25** is summarized in Schemes 2–4. Simple amidation of **2** directly afforded compounds **9** and **22**–**24** (Scheme 2). Compounds **10**, **12**–**14**, **18**–**19**, and **25** were produced through the coupling of **2** with the corresponding mixed anhydrides yielded from the corresponding acids and pivaloyl chloride (Scheme 3). Compound **10** was treated with LiOH in a MeOH–H_2O solution to afford a clean product, **11**. Compounds **20** and **21**, respectively, formed from the epoxidation of **13** and **14** with *m*-CPBA. The other compounds, **15**–**17**, were synthesized through a coupling method using mixed anhydrides from isobutyl chloroformate, as shown in Scheme 4.

Most of the 17 compounds tested showed excellent inhibition of *Ec*MetAP1 (IC_{50} values in the range of tens to hundreds of nanomolar) (Table 1). Five compounds (**10**, **13**, **18**, **19** and **22**) had IC_{50} values less than 0.25 μM, which means their potency was increased more than 20-fold compared with the original HTS hit **1**. These compounds shared the segment 3-butenoyl, except **22**, which contained a cyclopropyl group, a classical isostere of vinyl. The relative position of the isolated C=C double bond and the carbonyl of the side chain of the pyridine ring is quite important. Any changes in this



Scheme 1. Reagents and conditions: (a) triethylamine, CH_2Cl_2; (b) Py, CH_2Cl_2, **2**.

Table 1. Inhibitory activity of PCAT derivatives on *Ec*MetAP1 and *Sc*MetAP1[a]



Compound	R	IC_{50} (μM)	
		*Ec*MetAP1	*Sc*MetAP1
1	—	5.00 ± 0.80	7.00 ± 0.10
7[b]		0.22 ± 0.04	0.77 ± 0.07
8[b]		0.15 ± 0.07	4.67 ± 0.36
9		>100	0.62 ± 0.13
10[b]		0.13 ± 0.01	0.38 ± 0.02
11		>100	0.82 ± 0.09
12		0.51 ± 0.08	12.25 ± 2.38
13[b]		0.24 ± 0.02	0.14 ± 0.01
14[b]		0.61 ± 0.08	0.31 ± 0.02
15		0.36 ± 0.06	5.17 ± 1.85
16		0.97 ± 0.09	21.87 ± 6.91
17		0.81 ± 0.21	15.08 ± 4.86
18		0.19 ± 0.03	5.07 ± 1.95
19		0.12 ± 0.02	8.91 ± 1.96
20		0.70 ± 0.01	3.37 ± 0.65
21		0.56 ± 0.01	3.30 ± 0.50
22		0.13 ± 0.04	2.56 ± 1.24
23		>100	>100
24		0.35 ± 0.08	2.58 ± 0.75
25		0.79 ± 0.25	6.72 ± 2.61

[a] Assays were performed as previously described.[17]
[b] See Ref. 17.

position resulted in the inhibition of *Ec*MetAP1 decreasing sharply (**11**, **16** vs **10**, **14** vs **13**, **17** and **19** vs **16**). When the C=C bond was oxidized into an epoxide, the potency also decreased 3-fold (**13** vs **20**). Therefore, *Ec*MetAP1 has a strong tendency to select the 3-butenoyl motif on the 3-position of the pyridine ring of PCAT.

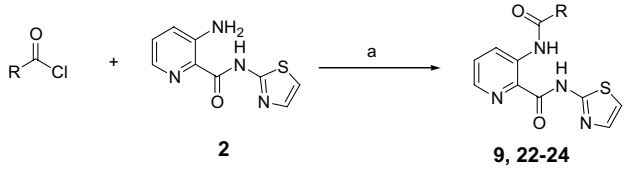
The 17 compounds showed much greater variation of IC_{50} values against *Sc*MetAP1. Most of them were weaker than the fatty acyloxy derivatives, which further proved that fatty acyloxy derivatives substituted at the 3-position of the pyridine ring were more active against *Sc*MetAP1 than their fatty acylamino analogues. Compounds with chain unsaturated fatty acylamino substituents were generally more active than those with ring unsaturated fatty acylamino substituents (**9**–**11** and **13**–**14** vs **15**–**19**, and **22**–**25**). Molecules with unsaturated chain fatty acylamino substituents showed quite good activity, with IC_{50} values against *Sc*MetAP1 being equivalent to those of the fatty acyloxy derivatives (**9**–**11** and **13**–**14**) (Table 1).

From the above results, the introduction of small unsaturated fatty acylamino substituents at the 3-position of PCAT significantly improved the inhibitory potency toward to *Ec*MetAP1 and *Sc*MetAP1, as expected, because these substituents increased the van der Waals contacts between ligands and the hydrophobic surface of the protein. Hydrogen bonds are important noncovalent interactions in biological systems, and this kind of interaction has directional preferences. To further improve inhibitory potency, we considered strengthening the hydrogen bond between MetAP1 and inhibitors. Therefore, a focused library, as shown in Figure 2, was designed with the purpose of forming extra hydrogen bonds between MetAP1 and inhibitors by introducing function groups, including carboxyl, ester alkyl, amino, pyridyl, hydroxy, alkoxy, and carbamyl groups.

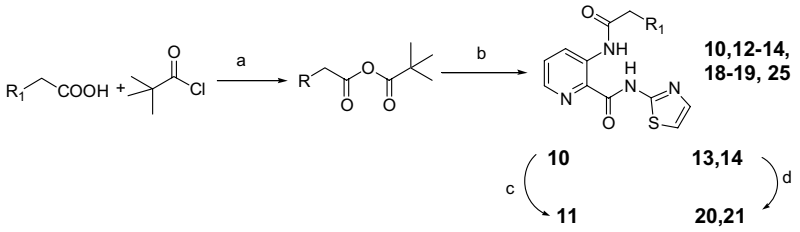
The preparation of these compounds is summarized in Scheme 5. Compound **2** reacted with corresponding dicarboxylic esters to give compounds **26** and **27**, followed by basic hydrolysis to afford compounds **28** and **29**. Compounds **30** and **31** were synthesized by aminolysis of corresponding anhydrides by **2**. Compounds **32** and **33** were produced via the coupling of **2** with the mixed anhydrides yielded from the corresponding acids and pivaloyl chloride. Deprotection of **32** and **33** resulted in compounds **34** and **35**, respectively.

Direct amidation of **2** produced compounds **36** and **43**. Compounds **37**–**42** were prepared via aminolysis of **43** by the corresponding amines (Scheme 5).

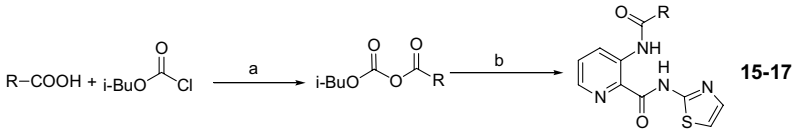
The inhibitory activities of these compounds to *Ec*MetAP1 and *Sc*MetAP1 are summarized in Table 2. Of the 17 compounds (**26**–**42**), all showed good inhibition of *Ec*MetAP1 with IC_{50} values less than 0.625 μM (8-fold better than the HTS hit, compound **1**). More than half of them are far more active than **1**, with IC_{50} values less than 100 nM (50-fold better than **1**). In particular, a



Scheme 2. Reagents and conditions: (a) Py, DMAP, EtOAc, 0 °C to rt.



Scheme 3. Reagents and conditions: (a) Py (excess), PhH; (b) **21a**, DMF, rt; (c) LiOH, MeOH/H_2O; (d) *m*-CPBA, $CHCl_3$.



Scheme 4. Reagents and conditions: (a) triethylamine (excess), THF, 0 °C; (b) **2**, THF, rt.

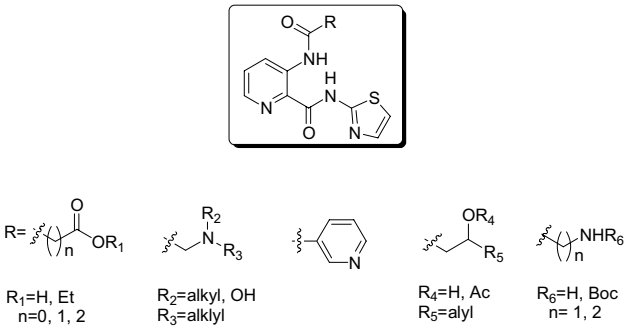
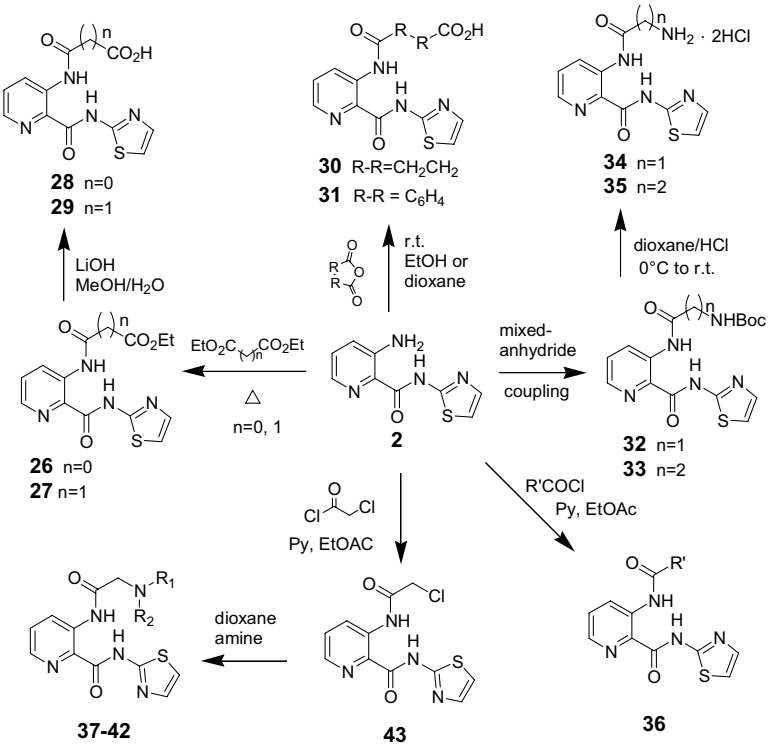


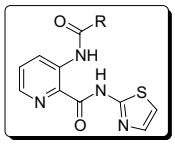
Figure 2. Focused library of MetAP1 inhibitors.

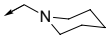
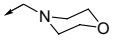
quarter of them (**26**, **32**, **33**, **39**) are most active against *Ec*MetAP1, with IC_{50} values less than 50 nM (100-fold better than **1**). Inhibition activities against *Sc*MetAP1 are not as outstanding as those for *Ec*MetAP1, but the results are satisfying, because two-thirds of them showed activities against *Sc*MetAP1 with IC_{50} values less than 0.7 μM (10-fold better than **1**) and the efficacy of a third of these compounds increased 30-fold (IC_{50} values less than 0.23 μM) compared with **1**. These results strongly demonstrate that strengthening the hydrogen bond between MetAP1 and the inhibitors could efficiently increase the binding affinity. Moreover, these compounds are the most potent *Ec*MetAP1 and *Sc*MetAP1 inhibitors described to date.



Scheme 5. Synthesis of compounds **26**–**42**.

Table 2. Inhibition of *Ec*MetAP1 and *Sc*MetAP1[a]



Compound	R	IC_{50} (μM)	
		*Ec*MetAP1	*Sc*MetAP1
1	—	5.00 ± 0.80	7.00 ± 0.10
26	$-CO_2Et$	0.050 ± 0.004	0.77 ± 0.12
27	$-CH_2CO_2Et$	0.075 ± 0.018	0.72 ± 0.08
28	$-CO_2H$	0.62 ± 0.15	>100
29	$-CH_2CO_2H$	0.14 ± 0.03	1.52 ± 0.01
30	$-CH_2CH_2CO_2H$	0.14 ± 0.02	2.27 ± 0.20
31		0.33 ± 0.06	7.78 ± 0.26
32	$-CH_2NHBoc$	0.049 ± 0.003	0.38 ± 0.05
33	$-CH_2CH_2NHBoc$	0.049 ± 0.003	0.15 ± 0.02
34	$-CH_2NH_2{\cdot}2HCl$	0.092 ± 0.008	0.21 ± 0.01
35	$-CH_2CH_2NH_2{\cdot}2HCl$	0.074 ± 0.003	0.13 ± 0.01
36		0.060 ± 0.006	0.33 ± 0.04
37		0.12 ± 0.01	0.42 ± 0.03
38		0.090 ± 0.015	0.15 ± 0.02
39		0.049 ± 0.004	0.23 ± 0.01
40		0.053 ± 0.001	0.19 ± 0.01
41		0.059 ± 0.001	0.51 ± 0.01
42		0.057 ± 0.002	0.23 ± 0.01

[a] Assays were performed as previously described.[17]

When compounds **26**–**42** with different R groups were compared, the carboxyl-substituted derivatives **28**–**31** were apparently less active against both MetAP1s than the other analogues, indicating that a free carboxyl on the side chain of PCAT is unfavorable. The compounds with free carboxyl groups were less active than the other analogues, further supported by the counterexamples, compounds **34** and **35**. These two salts will be dissociated into neutral molecules in solution in water if the pH value exceeds 5, and they showed excellent inhibition toward both enzymes.

When compounds with different substituent chain lengths were compared (**29** and **30** vs **28** and **31**, **26** vs **27**, **34** vs **35**, **32** vs **33**), we found that the length of the substituent chain demonstrated distinct effects on the inhibition activity against *Sc*MetAP1, but less so against *Ec*MetAP1. The compounds whose hydrogen bond-forming substituent was separated from the carbonyl of the acylamino by one or two methylenes showed excellent efficacy, with improved inhibition. This result may be attributed to the space in and the shape of the pockets in which *Sc*MetAP1 accommodates inhibitors. Considering the substrate specificity of MetAPs, a substituent chain that is too long or too large may decrease the binding affinity. Therefore, we did not attempt to prepare analogues containing substituents of increased chain length.

In summary, in systematic SAR studies of the pyridine ring 3-substituents of PCAT toward *Ec*MetAP1 and *Sc*MetAP1, the 3-substituents showed different selectivity against *Ec*MetAP1 and *Sc*MetAP1: (1) fatty acylamino substituents preferentially inhibited *Ec*MetAP1; (2) fatty acyloxy substituents preferentially inhibited *Sc*MetAP1; (3) substituents containing a 3-butenoyl motif had remarkably increased inhibition potency against *Ec*MetAP1; (4) small acylamino substituents with unsaturated fatty chains were generally more effective against *Sc*MetAP1 than other acylaminos were; and (5) the hydrogen bond formed between the enzyme and 3-position substituents of PCAT importantly contributed to the binding affinity. These findings provide useful information for the design and discovery of more potent inhibitors of type I MetAPs.

Acknowledgements

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