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Inhibitors of type I MetAPs containing pyridine-2-carboxylic acid thiazol-2-ylamide. Part 1: SAR studies on the determination of the key scaffold

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Abstract—Systematic SAR studies on the HTS hit pyridine-2-carboxylic acid thiazol-2-ylamide (PACT) analogues revealed that the scaffold of PCAT is indispensable for the inhibition of type I MetAP. For effective inhibition of the enzyme, the most suitable position to modify is the 3-position of the pyridine ring of PCAT, and the best substituents are those containing O or N atoms connected directly with the pyridine ring. These findings provide useful information for the design and discovery of more potent inhibitors of type I MetAPs.

Belonging to the metalloprotease family, methionine aminopeptidases (MetAPs) remove the N-terminal Met from nascent proteins or polypeptides.[1] This N-terminus trimming is required for biological activity, subcellular location and eventual degradation of proteins.[2–5] Two types of MetAPs have been identified: type I and type II.[6] Eubacteria have only type I MetAPs, whereas eukaryotic cells contain both types. MetAP has important physiological functions, and disruption of the gene for MetAP in *Escherichia coli* (*Ec* MetAP1) or *Salmonella typhimurium* is a lethal event.[7,8] In *Saccharomyces cerevisiae*, disrupting either type I or type II MetAP (*Sc* MetAP1 or *Sc* MetAP2) renders a slow growth phenotype, and those with both removed are nonviable.[9] Therefore, MetAPs are potential targets for the development of antibacterial and antifungal drugs, and inhibitors of MetAPs offer promise as new treatments for bacterial and fungal infections.[10]

The natural product fumagillin and its derivatives[11,12] are the potent MetAP inhibitors; they are alkylating agents that covalently modify type II MetAP with highly specificity. Some reversible inhibitors of MetAPs show excellent potency against human MetAP2, but are generally of only weak to moderate activity (IC_{50} in the micromolar range) against MetAP1.[13–16]

We have previously reported that the derivatives of pyridine-2-carboxylic acid thiazol-2-ylamide (PCAT, **1**) are potent inhibitors of both *Ec* MetAP1 and *Sc* MetAP1.[17] Here, we report systematic SAR studies on the PCAT analogues to reaffirm the indispensability of this scaffold in the inhibition of type I MetAP, and to identify inhibitors that are more potent.

Around the key structure of PCAT (compound **1**), we initially prepared compounds **2**–**10** (Fig. 1, Scheme 1), in which the thiazole ring in compound **1** was replaced with its isosteric structure of pyridine, pyrimidine, isoxazole or [1,3,4]-thiadiazole, or with a substituted thiazole ring with different substituents (Scheme 1 and Figure 1). None of these compounds showed any activity against *Ec* MetAP1 or *Sc* MetAP1 up to 100 μM.

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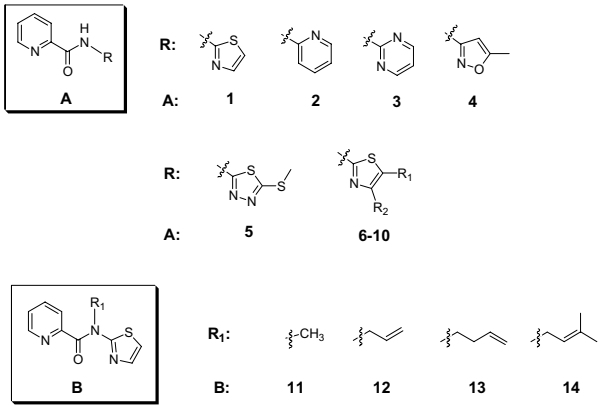
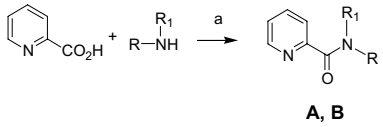


Figure 1. Compounds **1**–**14**.



Scheme 1. Reagents and conditions: (a) DCC, HOBt, DMF, 15–95%.

These results indicated that, no matter what isosteric displacement or what type of modification with various substitute groups on the thiazole ring, the compound loses its inhibitory activity against the enzymes. Therefore, the thiazole ring in PCAT is essential for effective inhibition of *Ec* MetAP1 and *Sc* MetAP1. This unit is, therefore, not for displacement or modification in these inhibitors. This can be interpreted as indicating that the thiazole ring matches a special pocket in the type 1 enzymes. Of note is that resolution of an X-ray structure of an inhibitor with a thiazole ring in the S1′ pocket in *Sa* MetAP1, an enzyme similar to *Ec* MetAP1,[18] partially supports this hypothesis.

Following the above attempts, we synthesized compounds **11**–**14**, in which the N atom of the amide bond was alkylated with different size groups. Alkylation of the amide bond transforms the H-bond donor into H-bond acceptor, and N-modification would confirm the importance of the NH as an H-bond donor. The four compounds did not show any inhibitory activities towards *Ec* MetAP1 or *Sc* MetAP1, even at high concentration (100 μM); therefore, any N-substitution will decrease the interaction between the enzyme and small molecules. Hence, we believe that the N atom of the amide bond of PCAT is not suitable for modification.

Finally, our attention focused on the pyridine ring of PCAT. A library was designed by replacement of the pyridine ring with saturated heterocycles or substituted phenyl rings, or changing the substitute position of the carbonyl group in the pyridine ring from the 2-position to the 3- or 4-position (Fig. 2). These compounds exemplify the replacement of the pyridine ring with a saturated heterocycle containing an N atom or with an aromatic group without an N atom within the aromatic ring but containing different substituents. However,

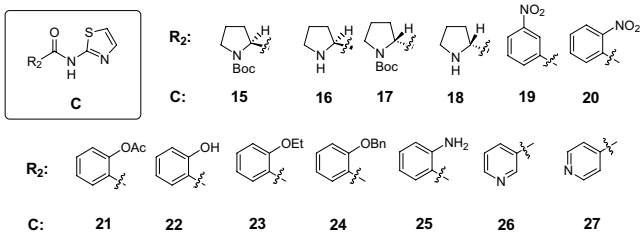


Figure 2. Compounds **15**–**27**.

none of the 13 compounds could inhibit *Ec* MetAP1 or *Sc* MetAP1, so we are convinced of the importance of both the N heteroatom and the aromaticity of the pyridine of PCAT for effective inhibition of the enzymes.

The above results indicated that the N atom in the pyridine ring, the direct connection of the carbonyl group to pyridine ring 2-position, and the N and/or S atoms in the thiazole ring are essential for effective inhibition of type I MetAPs. Therefore, our attention focused on modification of the pyridine ring, and a small library with the general structure as that for **D** (Table 1, compounds **28**–**48**) was designed and synthesized.

Scheme 2 shows the synthesis of compounds **28**–**31**. Prepared from dicarboxylic acid **28a**, acyl chloride **28b** coupling with 2 equiv of 2-aminothiazole yielded compound **28**, and with 1 equiv yielded **29**. Compound **29** reacted with isobutyl chloroformate to give in situ the mixed anhydride, which, when followed by $NaBH_4$ reduction, gave **30**. Acetylation of **30** with Ac_2O gave **31**.

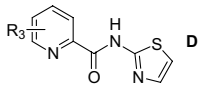
The synthesis of compounds **32**–**36** is summarized in Scheme 3. Compounds **32** and **33** were both produced through the coupling of 2-aminothiazole with the corresponding mixed anhydride yielded in situ from the corresponding acid, and **34** was from the DCC coupling with 2-aminothiazole. After removing the Boc protection group from **32**, we obtained **35**, and **36** was smoothly obtained from **35** by the coupling method used in the synthesis of **32** and **33**.

The preparation of compounds **37**–**43** is introduced in Scheme 4. Compound **37** was produced from the corresponding acyl chloride. Compounds **38**–**41** were directly formed by DCC coupling. Displacement of the nitro group of **41** with dry ammonia gas or benzyl amine in dry DMF yielded **42** and **43**, respectively.

The carboxyl group of **44a** was esterified while the hydroxy group was alkylated (Scheme 5). The two esters were hydrolyzed and then combined with 2-aminothiazole to yield **44** and **45**, respectively. The preparation of the other three compounds, **46**–**48** in Table 1, was as previously described.[17]

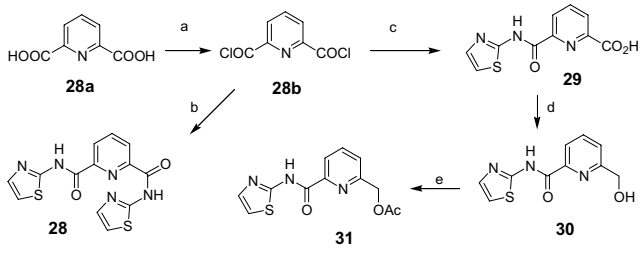
The inhibitory activities of these compounds towards *Ec* MetAP1 and *Sc* MetAP1 are summarized in Table 1. Unlike compounds **2**–**27**, which were derived from the replacement of the thiazole ring of PCAT, several compounds resulting from the N-modification of PCAT's

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



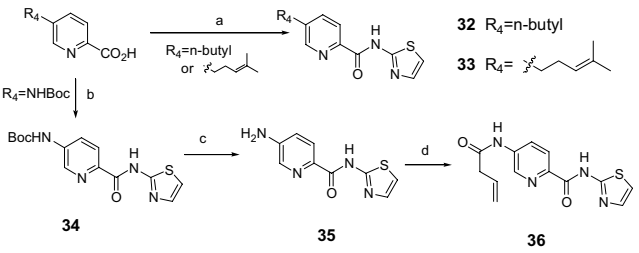
Compound	R_3	IC_{50} (μM)	
		EcMetAP1	ScMetAP1
1[a]	—	5.00 ± 0.80	7.00 ± 0.10
28	6-	>100	>100
29	6-COOH	>100	>100
30	6-CH_2OH	5.82 ± 0.58	ND
31	6-CH_2OAc	>100	ND
32	5-*n*-Butyl	>100	>100
33	5-	>100	>100
34	5-NHBoc	12.78 ± 0.97	62.86 ± 1.18
35	5-NH_2	2.09 ± 0.01	2.43 ± 0.01
36	5-	1.01 ± 0.02	>100
37	4-Cl	>100	>100
38	4-OMe	9.22 ± 0.04	>100
39	4-OBn	>100	>100
40	4-NHAc	1.98 ± 0.01	17.04 ± 1.94
41	4-NO_2	4.51 ± 0.36	>100
42	4-NH_2	6.71 ± 0.64	>100
43	4-NHBn	2.32 ± 0.03	>100
44	3-OBn	1.41 ± 0.3	0.77 ± 0.28
45	3-OMe	4.49 ± 0.28	ND
46[a]	3-	1.22 ± 0.12	1.03 ± 0.09
47[a]	3-	0.26 ± 0.04	0.35 ± 0.03
48[a]	3-	0.38 ± 0.03	0.62 ± 0.06

Assays were carried out as previously described.[17] ND: not determination.
[a] From Ref. 17.

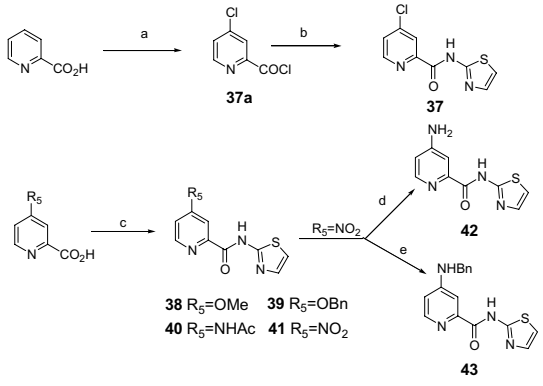


Scheme 2. Reagents and conditions: (a) $(COCl)_2$, CH_2Cl_2, rt; (b) 2-aminothiazole (2 equiv), TEA, CH_2Cl_2, 55%; (c) 2-aminothiazole (0.8 equiv), TEA, CH_2Cl_2, 61%; (d) (i) isobutyl chloroformate, *N*-methyl morpholine, THF, (ii) $NaBH_4$, MeOH; (e) Py, Ac_2O, rt.

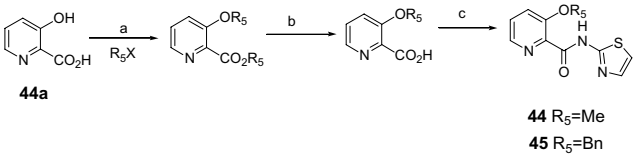
amide bond, or the replacement of the pyridine ring (**C**), most of this series of compounds showed high efficacy as inhibitors of MetAP1s. Of the 21 compounds tested (compounds **28**–**48**), nearly half showed inhibition of *Ec* MetAP1 with IC_{50} values of less than 5 μM, that is,



Scheme 3. Reagents and conditions: (a) (i) pivaloyl chloride, benzene, Py, (ii) 2-aminothiazole, DMF, rt; (b) DCC, HOBt, DMAP, DMF, 75%; (c) TFA, CH_2Cl_2; (d) 2-butenic acid, pivaloyl chloride, CH_2Cl_2, Py, 85%.



Scheme 4. Reagents and conditions: (a) $SOCl_2$, DMF (cat.), 40–45 °C; (b) CH_2Cl_2, TEA, 2-aminothiazole; (c) 2-aminothiazole, DCC, HOBt, DMF; (d) DMF, NH_3 (g), 110 °C, 15%; (e) DMF, $BnNH_2$, rt, 32%.



Scheme 5. Reagents and conditions: (a) Ag_2O, DMSO, R_5X = MeI or BnCl, rt; (b) KOH, H_2O, reflux; (c) $(COCl)_2$, CH_2Cl_2, 0 °C; (d) 2-aminothiazole, CH_2Cl_2, TEA, rt.

half of them are better than the lead molecule (**1**). In particular, when the 3-position of the pyridine ring of PCAT (**1**) was substituted with an amido of a short fat chain, such as a propionamido (**47**) or hexanamido group (**48**), the potencies against *Ec* MetAP1 increased almost 20- and 13-fold, respectively. Against *Sc* MetAP1, potencies increased 20- and 11-fold, respectively. This strongly indicates that the pyridine ring of PCAT is suitable for modification to improve the activity of inhibitors.

As far as the substitutional position on the pyridine ring of PCAT is concerned, the 3-position is much more favorable to inhibitory potency than the 4-, 5-, or 6-positions. All five compounds (**44**–**48**) revealed better inhibition than **1**, whereas other compounds derived from the modification of the 4-, 5-, or 6-position did

not show this tendency, no matter what kind of substituent was present. For example, the most active derivative at the 5-position (**36**) was not as potent as its 3-position analogues (**47**, **48**), and the most active derivative at the 4-position (**40**) could not match its 3-position analogue (**47**). Comparison of compound **38** with **45**, and compound **39** with **44** showed the same trend. These results may be attributed to the correct position of the enzyme's pocket being near the active site, thus accommodating the inhibitor's substituents.

In addition, the various substituents in the pyridine ring showed different priority in inhibitory activities. Compounds with substituents containing O or N atoms connected directly with the pyridine ring seemed to be more favorable than those with substituted groups such as halogen atoms (**37**), or acyl (**28**), carboxyl (**29**), nitro (**41**) or alkyl (**30**–**33**) groups. These results can be interpreted as indicating that N- or O-substituents increased the electron density of the pyridine ring, so that they modulated the cooperation of the N atom with the metal ions in the enzyme's active site, which can be regarded as increasing the binding affinity of the inhibitor to the protein.

In conclusion, systematic SAR studies on the PCAT analogues revealed that the scaffold of PCAT is indispensable to the inhibition of type I MetAP. For effective inhibition of the enzyme, the most suitable position to modify is the 3-position of pyridine ring of PCAT, and the most favorable substituents are those containing O or N atoms connected directly with the pyridine ring. These findings provide useful information for the design and discovery of more potent inhibitors of type I MetAPs.

Acknowledgements

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