Discovery and Structural Modification of Inhibitors of Methionine Aminopeptidases from *Escherichia coli* and *Saccharomyces cerevisiae*

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A series of pyridine-2-carboxylic acid derivatives were synthesized according to the leads from the screening, and potent inhibitors have been obtained by structural modification. They have shown submicromolar inhibition of the enzymes (for example, for **9n**, IC_{50} = 130 nM for EcMetAP1 and IC_{50} = 380 nM for ScMetAP1). They represent small-molecule MetAP inhibitors with novel structures different from alkylating fumagillin derivatives and peptidic bestatin-based MetAP inhibitor.

Introduction

Emerging bacterial resistance to current antibiotic drugs has driven the search for novel prokaryotic targets and for novel small molecules to modulate the activity of these targets. The methionine aminopeptidases (MetAPs) are a unique class of cobalt-containing metalloproteinases that facilitate the removal of the N-terminal initiator methionine from nascent polypeptides in a nonprocessive manner.[1] MetAPs exist in both prokaryotic and eukaryotic cells, and their gene sequences have been determined in several clinically important pathogens, such as *Mycobacterium tuberculosis* in tuberculosis, *Enterococcus faecalis* in urinary tract infection and endocarditis, *Streptococcus pneumoniae* in pneumonia, *Haemophilus influenzae* in respiratory tract infection, and *Helicobacter pylori* in ulcers. The pathogenic *Escherichia coli* strain O157:H7 has the exact MetAP sequence as the *E. coli* laboratory strain K12.

There are two types (types I and II) of MetAP that have been identified. Eubacteria (for example, *E. coli*, *Bacillus subtilis*, and *Salmonella typhimurium*) have only type I MetAPs, while archaea (for example, *Methanobacterium thermoautotrophicum*, *Sulfolobus solfataricus*, and *Pyrococcus furiosis*) have only type II MetAPs. Eukaryotic cells contain both type I and type II MetAPs.[2] The presence of MetAPs is essential for cell viability, and disruption of the gene for MetAP in *E. coli* (EcMetAP1) or *Salmonella typhimurium* is a lethal event.[3] Since the yeast *Saccharomyces cerevisiae* has both type I and type II MetAPs (ScMetAP1 and ScMetAP2), both genes need to be blocked to affect cell viability, and disruption of either gene alone resulted in only a slow-growth phenotype.[2] Therefore, the MetAP enzymes present good targets for new antibiotic drug discovery, and inhibitors against MetAPs offer hope for a new treatment of bacterial and fungal infections.[3a]

Although there are intensive efforts in searching for MetAP inhibitors in both academia and industry, natural product fumagillin and its derivatives[4] with specificity for type II MetAPs are the only potent MetAP inhibitors described in the literature so far, and they are alkylating agents and covalently modify MetAPs. A peptidic inhibitor AHHpA-Ala-Leu-Val-Phe-OMe designed on the basis of leucine peptidase inhibitors with a bestatin moiety showed only weak activity against EcMetAP1 (IC_{50} = 5.0 μM),[5] and there are few inhibitors reported for type I MetAPs,[6] although it is the only and essential enzyme in bacteria. Clearly, there are needs for potent inhibitors of bacterial and fungal MetAPs for antibiotic applications.

In the present work, we report our discovery of inhibitors of EcMetAP1 through high-throughput screening (HTS) and the structural modifications based on the screening leads. Inhibitors with submicromolar inhibitory potencies in vitro against the bacterial EcMetAP1 and the yeast ScMetAP1 have been obtained.

Results and Discussion

The starting point of this work was a random screening of a diverse small molecule library of 4500 compounds using a recombinant EcMetAP1 expressed and purified in our laboratory. Compound **1** (Figure 1), with an IC_{50} of 5.0 μM, was identified along with several other leads. For understanding the structure−activity relationship, we initially prepared compounds **2**−**4**, in which we replaced the N atom in the pyridine ring of compound **1** with CH, the S atom in the thiazole ring by its isosteric ethylenic group, or inserted an NH between the pyridine ring and carbonyl group (Figure 1). None of these compounds showed any activities to the EcMetAP1 up to 100 μM. These results indicated that the N atom in the pyridine ring, the direct connection of the carbonyl group to the pyridine ring, and the

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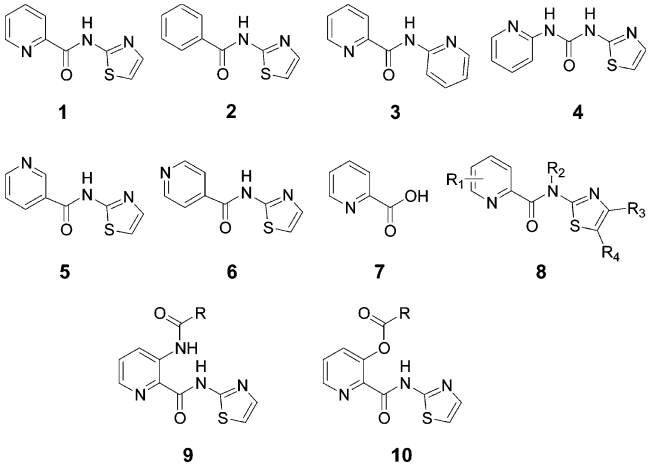


Figure 1. Compound **1** and prepared compounds **2**−**10**.

N and/or S atoms in the thiazole ring are essential for effective inhibition of EcMetAP1.

To further understand the key structural scaffold, compounds **5** and **6** were synthesized, which changed the substitute position of the carbonyl group in the pyridine ring from the 2- to the 3- and 4-positions. Surprisingly, neither **5** nor **6** showed any activity. Pyridine-2-carboxylic acid **7** also showed no activity. These results strongly suggested that pyridine-2-carboxylic acid thiazol-2-ylamide (PCAT) is a key structural scaffold for effective inhibition of the enzyme activity.

With this assumption, we reasoned that to improve its inhibitory potency, an increase of the van der Waals contacts was needed between the ligand and the hydrophobic surface of the MetAP, such as the S1 pocket, which serves as the specificity pocket for the NH_2 terminal methionine side chain of natural substrates. We introduced a number of hydrophobic substitutions at various positions on the pyridine ring of **1**, and a small library with a general structure as that for **8** was designed. In this series of compounds, we focused our attention on 3-substituted pyridine-2-carboxylic acid thiazol-2-ylamides **9** and **10**, because we observed inhibitory activity from the initial derivatives on EcMetAP1 and ScMetAP1.

The syntheses of 3-*N*-acyl-substituted compounds **9** could be accomplished by the well-precedented condensation of 3-*tert*-butoxycarbonylaminopyridine-2-carboxylic acid with 2-aminothiazole in the presence of DCC in DMF, followed by the amine deprotection and acylation with various carboxylic acids or chlorides under neutral or basic conditions (Scheme 1). By employment of a similar procedure, the 3-*O*-acyl-substituted compounds **10** were obtained from 3-hydroxypyridine-2-carboxylic acid in good yields.

Forty-one 3-substituted compounds **9** and **10** were synthesized and evaluated for their inhibitory activity against both EcMetAP1 and ScMetAP1. Of the 41 compounds tested, all of compounds showed inhibition of EcMetAP1 with IC_{50} values of less than 10 μM, and most of them showed better activity than compound **1** (Table 1). In particular, compounds **9n**, **9m**, and **9c** are the most active against EcMetAP1, with IC_{50} values of 130, 150, and 180 nM, respectively, and compounds **9o** and **10g** were the most active against ScMetAP1, with IC_{50} values of 140 and 200 nM, respectively. They were

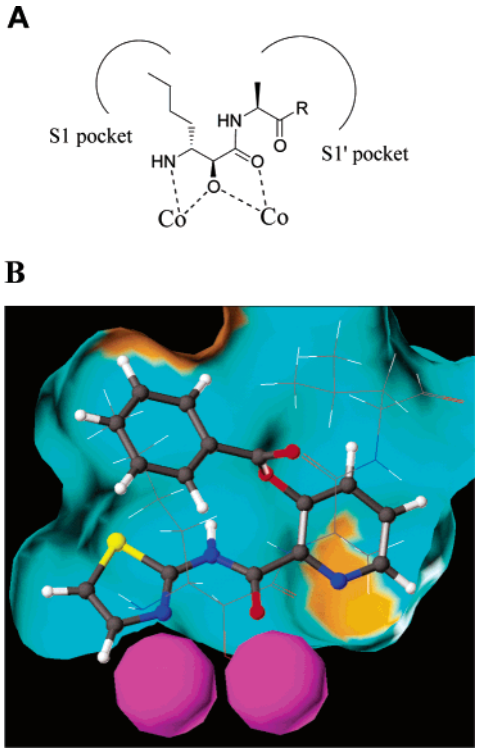


Figure 2. (A) Active site of interactions of EcMetAP1 with a bestatin-based inhibitor. (B) Docked structure of molecule **10k** inside the EcMetAP1 model (cut-away Connolly surface with polar regions shown in blue; hydrophobic in gold) determined from X-ray crystallography.[7] Rendered in ball-and-stick form, the predicted conformation of **10k** is contrasted with the bestatin analogue (wire model, oriented according to the crystal structure[7]).

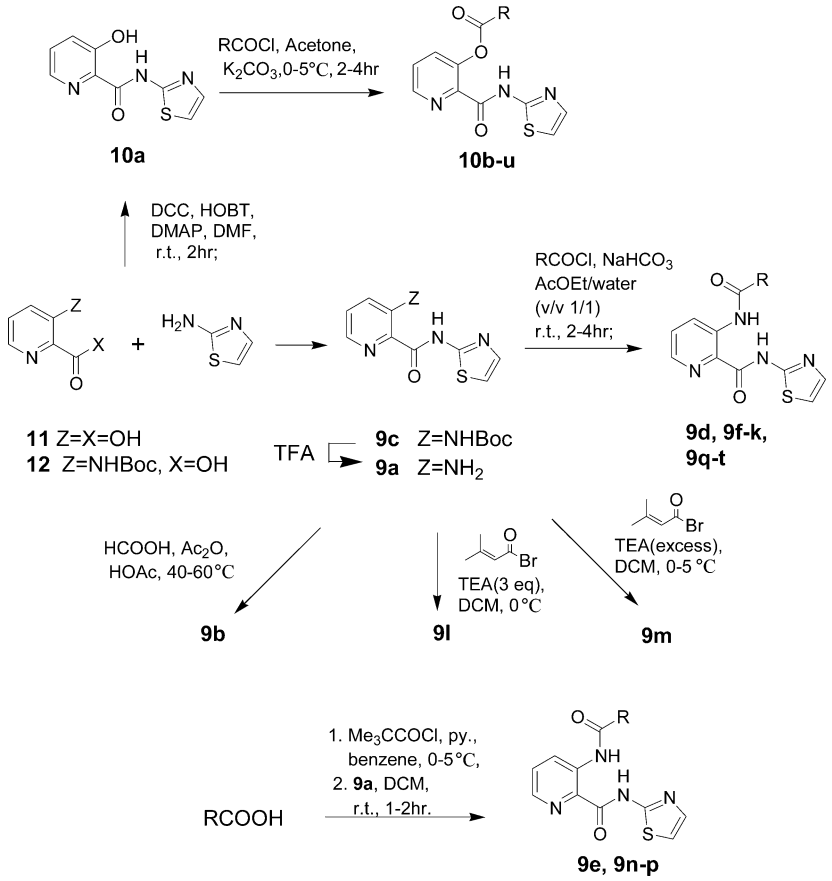
the most potent EcMetAP1 and ScMetAP1 inhibitors described in the literature to date (Table 1).

When compounds **9** and **10** with the same R group (**9g**−**l**, **9q**−**t** vs **10d**−**i**, **10k**−**n**) were compared, the 3-N-substituted derivatives **9** were often more active than the 3-O-substituted derivatives against EcMetAP1, indicating that an N is preferred at that position. However, it is just opposite against ScMetAP1, and the 3-O-substituted derivatives **10** were often more active than the 3-N-substituted derivatives.

It seems that EcMetAP1 is less sensitive to the substitution at the 3-position of the pyridine ring, and compounds with either an aliphatic or an aromatic group often showed submicromolar potency. This could not hold true for ScMetAP1 though, and we observed a much bigger variation of IC_{50} values on ScMetAP1. None of the four compounds **9** with aromatic groups directly connected to the carbonyl carbon (**9q**, **9r**, **9s**, and **9t**) showed measurable activity against ScMetAP1. However, the aromatic substitutions at this position for compounds **10** gave mixed results, and compounds **10q** and **10s** even showed submicromolar potencies. Among compounds **10** with the aromatic substitutions, ScMetAP1 prefers a group at the ortho position on the aromatic ring to meta or para positions (**10l**, **10q**, **10s**, for example).

The X-ray structure of EcMetAP1 complexed with the bestatin-based inhibitor AHHpA-Ala-Leu-Val-Phe-OMe has been reported, and the peptidic inhibitor interacts with the two cobalt ions (Figure 2A).[7] From molecular docking studies carried out via the AutoDock program[8]

Scheme 1. Syntheses of Pyridine-2-carboxylic Acid Thiazol-2-ylamides **9** and **10**



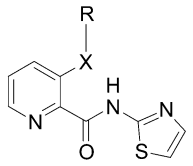
for a sample set of the PCAT scaffold (compounds **1**, **9a**, **9d**, **9n**, **10a**, **10b**, and **10k** given in Table 1) bound to the EcMetAP1, one finds that the inhibitors consistently occupy a region spatially similar to that chosen by the bestatin-based ligand. For example, Figure 2B showed the docked structure of molecule **10k** inside the EcMetAP1 model (cut-away Connolly surface with polar regions shown in blue; hydrophobic in gold) determined from X-ray crystallography.[7] Rendered in ball-and-stick form, the predicted conformation of **10k** is contrasted with the bestatin analogue (wire model, oriented according to the crystal structure[7]), suggesting affinity for the same spatial region. In both cases, the presence of the two Co^{2+} ions (rendered as magenta space filling spheres at the bottom of the cavity) appears to provide a strong electropositive environment capable of fixing the ligand's most electronegative features. Not all molecules in the PCAT scaffold have the shape and flexibility necessary to direct three electronegative groups toward the ions; many instead orient two in that direction. Compounds **1**, **9n**, and **10g** all appear to bind optimally by orienting their pyridine N and central (i.e., non side chain) carbonyl O toward the cobalts, **10a** employs the central O and thiazol S for this purpose, and compounds **9a** and **10b** couple via their side chain electronegative (amino and ester, respectively) atoms. Compound **9d** is unusual in that it appears to only bind to the Co's via a single atom, the pyridine N. Although this information partially supported our assumption, the exact binding mode of the PCAT derivative with MetAPs should be obtained from X-ray crystal experiments.

The differences in sensitivity to the substitution at the 3-position of the pyridine ring may reflect the differences at the active sites of EcMetAP1 and ScMetAP1 and the differences in the interaction between the inhibitors and the MetAPs. From docking studies, it appears that the substituted group at this position can influence the molecule's docked conformation, but the relative similarity among docking free energies among the PCAT analogues (all between −8.7 to −11.9 kcal/mol) agrees with the relative similarity among IC_{50} values and indicated that side chain substitutions may not greatly influence binding efficacy within the EcMetAP1 receptor. Without a corresponding crystal structure for the ScMetAP1 receptor, we are unable at this point to offer sound speculation on the reason for the much greater variation in IC_{50} data for this latter system.

The ratio of the IC_{50} of EcMetAP1 to the IC_{50} of ScMetAP1 (Table 1) is another indication of the differences in the inhibitor binding to the enzymes. Compound **9r** (IC_{50} = 280 nM for EcMetAP1; IC_{50} > 100 μM for ScMetAP1; ratio, <0.0028) is the most selective for EcMetAP1, and compound **10c** (IC_{50} = 5.4 μM for EcMetAP1; IC_{50} = 380 nM for ScMetAP1; ratio, 14) is the most selective for ScMetAP1. The selectivity for either EcMetAP1 or ScMetAP1 gives us confidence in obtaining the antibiotic drugs specific for the targeting microorganisms, and the selectivity will become more important if we want to develop antibiotics without affecting human MetAPs for low toxicity.

We only tested a few compounds for antibacterial activity (Table 2). Like most antibiotics that target protein synthesis, these are bacteriostatic agents. Compound **1** was active against Gram-positive bacteria, including *Staphylococcus aureus* and *Enteropathogenic*

Table 1. Inhibition of EcMetAP1 and ScMetAP1[a]



9 X=NH
10 X=O

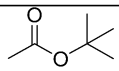
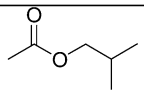
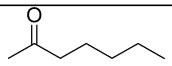
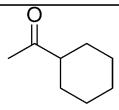
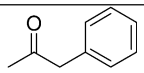
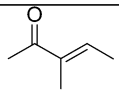
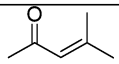
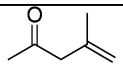
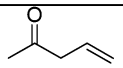
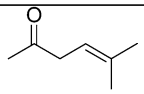
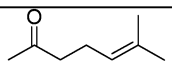
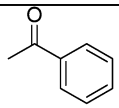
Cmpd	R	IC_{50} (μM)		Ratio (Ec/Sc)
		EcMetAP1	ScMetAP1	
1		5.0±0.8	7.0±0.1	0.71
AHHpA-Ala-Leu-OMe*		22.9±2.40	95.14±1.95	
Fumagillin		>100	>100	
9a	—H	1.7±0.1	32.8±0.1	0.052
9b		0.33±0.08	0.32±0.01	1
9c		0.18±0.02	3.35±1.51	0.054
9d		1.92±0.30	53.7±0.65	0.036
9e		0.34±0.04	4.92±1.45	0.069
9f		0.26±0.40	0.35±0.03	0.74
9g		0.54±0.10	0.34±0.01	1.59
9h		0.38±0.30	0.62±0.06	0.61
9i		0.87±0.10	>100	<0.0087
9j		0.40±0.10	0.82±0.01	0.49
9k		0.28±0.04	10.9±0.12	0.026
9l		0.22±0.07	0.77±0.07	0.29
9m		0.15±0.03	4.67±0.36	0.032
9n		0.13±0.01	0.38±0.02	0.34
9o		0.24±0.02	0.14±0.01	1.71
9p		0.61±0.08	0.31±0.02	2
9q		0.89±0.06	>100	<0.0089

Table 1. (Continued)

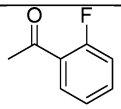
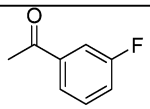
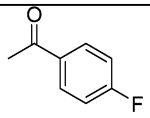
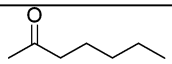
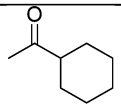
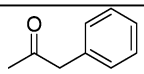
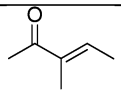
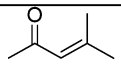
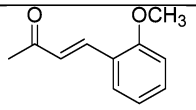
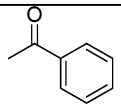
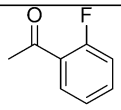
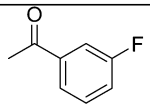
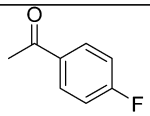
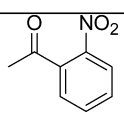
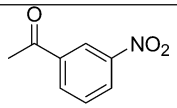
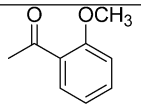
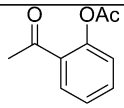
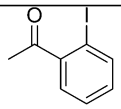
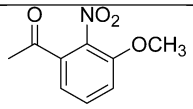
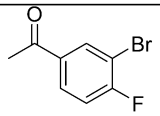
Cmpd	R	IC_{50} (μM)		Ratio (Ec/Sc)
		EcMetAP1	ScMetAP1	
9r		0.28±0.04	>100	<0.0028
9s		0.71±0.01	>100	<0.0071
9t		1.36±0.14	>100	<0.0136
10a	—H	1.87±0.30	4.86±0.19	0.38
10b		8.2±0.1	-	
10c		5.4±2.8	0.38±0.03	14
10d		0.68±0.02	0.78±0.15	0.87
10e		1.02±0.20	1.74±0.05	0.59
10f		2.39±0.40	0.32±0.08	7.5
10g		0.54±0.05	0.20±0.04	2.7
10h		4.4±0.1	0.39±0.01	11
10i		2.6±0.4	0.49±0.08	5.3
10j		2.1±0.5	9.9±1.5	0.21
10k		1.22±0.12	1.03±0.09	1.2
10l		0.83±0.06	16±2	0.052
10m		0.95±0.03	>100	<0.0095
10n		1.1±0.3	62±1	0.018
10o		2.1±0.5	>100	<0.021

Table 1. (Continued)

Cmpd	R	IC_{50} (μM)		Ratio (Ec/Sc)
		EcMetAP1	ScMetAP1	
10p		0.6±0.02	>100	<0.006
10q		1.7±0.4	0.55±0.06	3.1
10r		1.6±0.5	15±3	0.11
10s		1.3±0.4	0.89±0.22	1.5
10t		1.7±0.5	3.02±0.35	0.56
10u		2.5±0.7	3.40±0.35	0.74

[a] The asterisk (∗) indicates the positive control. Similar potency was reported as AHHpA-Ala-Leu-Val-Phe-OMe.[5]

Table 2. Antibacterial Activities of Pyridine-2-carboxylic Acid Derivatives

strain	MIC [a] (μg/mL)					
	1	**9n**	**10c**	**9b**	**9r**	ciprofloxacin
Staphylococcus aureus ATCC25923	2.0	62.5	125	62.5	15.6	0.125
Enteropathogenic E. coli ATCC25922	12.5	125	250	125	250	0.06
Pseudomonas aeruginosa ATCC27853	250	500	500	500	250	0.125

[a] MIC = minimum inhibitory concentration, determined after 24 h.

E. coli. Compounds **9n, 9b**, and **9r** also showed moderate activity against these strains. We were puzzled that although we have improved their inhibitory potencies for the MetAP inhibitors by structural modification, we did not see improvement in in vivo activity against these bacteria. One possibility is the differences of these inhibitors in their ability in penetrating the bacterial cell wall, which we have not tested. However, the failure in correlating the in vitro and in vivo activities may point to the big question in MetAP research: Which is the physiologically relevant metal in the enzyme? MetAPs have been traditionally studied in vitro as Co(II) enzymes, and the EcMetAP1 we used for screening and characterization was a Co(II) enzyme. Recent data suggest that the MetAPs may have a metal other than Co(II) at their active sites, such as Zn(II),[9] Fe(II),[10] or Mn(II),[11] and our preliminary data showed that the metal substitution could cause significant changes in specificity toward substrates and inhibitors. We are in the process of assessing these and other inhibitors on the metal-substituted EcMetAP1s with the hope of improving their in vivo potencies.

Bacteria have only one MetAP and its inhibition could be sufficient in controlling bacterial infections. Fungi as eukaryotic cells have type II MetAPs in addition to the type I MetAPs, such as ScMetAP1. Fumagillin and its analogues are MetAP inhibitors specific for type II MetAPs. However, they are alkylating agents, and other type II MetAP inhibitors need to be developed. Combined use of the inhibitor for type I MetAPs and the inhibitor for type II MetAPs is required for antifungal applications. Alternatively, a dual inhibitor for MetAPs of both types is needed.

Conclusion

In summary, pyridine-2-carboxylic acid thiazol-2-ylamide **1** was discovered as an EcMetAP1 inhibitor through HTS, and various pyridine-2-carboxylic acid thiazol-2-ylamide analogues **9** and **10** were synthesized and identified as EcMetAP1 and ScMetAP1 inhibitors. The introduction of the 3-*N*-acyl group (compounds **9**) or 3-*O*-acyl group (compounds **10**) greatly enhanced the activity against EcMetAP1 and ScMetAP1. This is the first identification of small-molecule MetAP inhibitors with structural units totally different from known fumagillin or bestatin-based MetAP inhibitors. A few compounds were tested and showed moderate antibacterial activity. These results open a new avenue to finding new MetAP inhibitors with potential antibiotic activity. Further efforts in modifying these and other lead structures with the aim of improving potency as well as specificity in vitro and efficacy in vivo are in progress.

Experimental Section

General Methods. ^{1}H (300 MHz) and ^{13}C (75 MHz) NMR spectra were recorded on a Varian Mercury-VX300 Fourier transform spectrometer. The chemical shifts were reported in δ (ppm) using the δ 7.26 signal of $CDCl_3$ (^{1}H NMR) and the δ 77.23 signal of $CDCl_3$ (^{13}C NMR) as internal standards. Low-resolution mass spectra were obtained on a SHIMADZU GCMS-QP5050A spectrometer, and high-resolution MS data were obtained on a MAT-711 mass spectrometer, an APEXIII 7.0 TESLA FTMS mass spectrometer, or a Concept 1H series mass spectrometer. HPLC analyses were performed on an HP1100 series LC system (HP ChemStation A.06.03; column, Zorbax SB-C18, 4.6 × 150 mm, 5 μm; mobile phase, MeOH/H_2O, 80:20; flow rate, 1.0 mL/min; UV wavelength, 254 nm; temperature, ambient temperature; injection volume, 10 μL). The reaction mixture was generally poured into water, and the separated aqueous phase was then thoroughly extracted with the specified solvent. After the mixture was washed with 10% aqueous HCl and/or $NaHCO_3$ (if required), water, and saturated aqueous NaCl, the combined organic phases were dried over anhydrous Na_2SO_4 or $MgSO_4$ and then filtered and concentrated under reduced pressure to yield the crude reaction product.

Pyridine-2-carboxylic Acid Thiazol-2-ylamide (1). To a mixture of pyridine-2-carboxylic acid (1.23 g, 10 mmol), thiazol-2-ylamine (1.01 g, 10 mmol), and HOBT (1.45 g, 10.75 mmol) in dry DMF (10 mL) was added DCC (2.16 g, 10.05 mmol). The reaction mixture was stirred at room temperature for 24 h. The reaction mixture was diluted with 50 mL of EtOAc and 20 mL of H_2O. The aqueous phase was extracted with EtOAc. The combined organic phases were then processed in the usual way and chromatographed (3:1 petroleum ether/EtOAc) to yield **1** (1.50 g, 73%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 11.20 (br, 1H), 8.66 (dd, J = 0.9, 4.2 Hz, 1H), 8.29 (dd, J = 0.6, 7.5 Hz, 1H), 7.94 (ddd, J = 0.9, 7.5, 7.8 Hz, 1H), 7.53 (m, 2H), 7.05 (d, J = 3.3 Hz, 1H). EI-MS m/z: 205 (M^+). HREI-MS: exact mass calcd for $C_9H_7N_3OS$ (M^+), 205.0304; found, 205.0306. HPLC purity, 99.88%.

3-*tert*-Butoxycarbonylaminopyridine-2-carboxylic Acid (12). 3-Aminopyridine-2-carboxylic acid (5.02 g, 36 mmol) was suspended in 60 mL of dry DMF, and Et_3N (15.2 mL, 108 mmol) was added dropwise at room temperature. To the resulting brown solution was added Boc_2O (11.80 g, 54 mmol). After being stirred for 10 min, the mixture was heated at 40−50 °C overnight. The reaction mixture was poured into water and was then extracted with EtOAc (2 × 50 mL). The aqueous phase was acidified to pH 4−5 with 2 M aqueous HCl and then extracted with CH_2Cl_2 (3 × 50 mL). The combined organic phases were then processed in the usual way and chromatographed (13:1 $CHCl_3$/MeOH) to yield **12** (4.2 g, 49%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 10.27 (br, 1H), 8.96 (d, J = 8.7 Hz, 1H), 8.23 (d, J = 4.2 Hz, 1H), 7.55 (dd, J = 4.4, 8.9 Hz, 1H), 1.53 (s, 9H).

3-*tert*-Butoxycarbonylaminopyridine-2-carboxylic Acid Thiazol-2-ylamide (9c). To a mixture of **12** (2.51 g, 10.5 mmol), thiazol-2-ylamine (1.05 g, 10.5 mmol), and HOBT (1.61 g, 11.9 mmol) in dry DMF (20 mL) was added DCC (3.36 g, 16.3 mmol). The reaction mixture was stirred at room temperature for 24 h. The reaction mixture was diluted with 50 mL of EtOAc and 20 mL of H_2O. The aqueous phase was extracted with EtOAc. The combined organic phases were then processed in the usual way and chromatographed (4:1 petroleum ether/EtOAc) to yield **9c** (1.56 g, 44%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 11.50 (br, 1H), 10.66 (br, 1H), 8.92 (dd, J = 1.2, 8.7 Hz, 1H), 8.23 (dd, J = 1.2, 4.2 Hz, 1H), 7.55 (d, J = 3.6 Hz, 1H), 7.47 (dd, J = 4.2, 8.7 Hz, 1H), 7.06 (d, J = 3.6 Hz, 1H), 1.55 (s, 9H). EI-MS m/z: 320 (M^+). HREI-MS: exact mass calcd for $C_{14}H_{16}N_4O_3S$ (M^+), 320.0943; found, 320.0972. HPLC purity, 99.76%.

3-Aminopyridine-2-carboxylic Acid Thiazol-2-ylamide (9a). To a stirred solution of **9c** (1.56 g, 4.6 mmol) in CH_2Cl_2 (10 mL) at −10 °C was added TFA (4.0 mL) dropwise. The reaction mixture was stirred for 4 h with gradual warming to room temperature. TLC monitoring shows complete consumption of starting material. The solvents were removed, and then the residue was dissolved in saturated aqueous $NaHCO_3$ and extracted with CH_2Cl_2. The combined organic phases were then processed in the usual way to yield **9a** (940 mg, 91%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 11.32 (br, 1H), 7.94 (d, J = 4.2 Hz, 1H), 7.51 (d, J = 3.6 Hz, 1H), 7.24 (dd, J = 4.2, 8.1 Hz, 1H), 7.05 (d, J = 8.1 Hz, 1H), 7.00 (d, J = 3.6 Hz, 1H), 5.97 (br, 2H). EI-MS m/z: 220 (M^+). HREI-MS: exact mass calcd for $C_9H_8N_4OS$ (M^+), 220.0419; found, 220.0421. HPLC purity, 99.29%.

3-Formylaminopyridine-2-carboxylic Acid Thiazol-2-ylamide (9b). A mixture of 1 mL of Ac_2O and 0.5 mL of HCOOH was heated at 60 °C under N_2 for 2 h, and then 1.2 mL of HOAc was added. The solution was cooled to 40 °C, and **9a** (40 mg, 0.18 mmol) was added. The mixture was stirred at 60 °C for an additional 2 h before 2 g of ice was added. The aqueous phase was neutralized to pH 7 with saturated aqueous $NaHCO_3$ and then extracted with EtOAc (3 × 10 mL). The combined organic phases were then processed in the usual way to yield the crude product, which was recrystallized from EtOAc give pure **9b** (9 mg, 20%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 11.50 (s, 1H), 11.00 (br. 1H), 9.17 (d, J = 8.1 Hz, 1H), 8.60 (s, 1H), 8.36 (d, J = 3.3 Hz, 1H), 7.55 (m, 2H), 7.09 (d, J = 3.3 Hz, 1H). EI-MS m/z: 248(M^+). HREI-MS: exact mass calcd for $C_{10}H_8N_4O_2S$ (M^+), 248.0368; found, 248.0366. HPLC purity, 98.63%.

General Procedure for Preparation of Compounds 9d, 9f−k, and 9q−t. To a solution of **9a** (0.1 mmol) in 3 mL of a 1:1 EtOAc/H_2O mixture was added excess $NaHCO_3$ (160 mg, 1.9 mmol). The appropriate acid chloride (0.15 mmol) was added at 0 °C. The mixture was stirred for 2−24 h with gradual warming to room temperature. The reaction mixture was diluted with 5 mL of EtOAc and 2 mL of H_2O. The aqueous phase was extracted with EtOAc. The combined organic phases were then processed in the usual way and chromatographed to yield the desired products.

3-*iso*-Butoxycarbonylaminopyridine-2-carboxylic Acid Thiazol-2-ylamide (9d). Yield 38%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 11.49 (br, 1H), 10.90 (s, 1H), 8.93 (dd, J = 1.5, 8.7 Hz, 1H), 8.26 (dd, J = 1.5, 4.5 Hz, 1H), 7.55 (d, J = 3.6 Hz, 1H), 7.51 (dd, J = 4.5, 8.7 Hz, 1H), 7.07 (d, J = 3.6 Hz, 1H), 4.01 (d, J = 6.9 Hz, 2H), 2.03 (m, 1H), 1.01 (d, J = 6.9 Hz, 6H). EI-MS m/z: 320 (M^+). HREI-MS: exact mass calcd for $C_{14}H_{16}N_4O_3S$ (M^+), 320.0943; found, 320.0971. HPLC purity, 99.93%.

3-Propionylaminopyridine-2-carboxylic Acid Thiazol-2-ylamide (9f). Yield 18%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 11.52 (s, 1H), 9.20 (dd, J = 1.5, 8.7 Hz, 1H), 8.30 (dd, J = 1.5, 4.5 Hz, 1H), 7.56 (d, J = 3.6 Hz, 1H), 7.51 (dd, J = 4.5, 8.7 Hz, 1H), 7.07 (d, J = 3.6 Hz, 1H), 2.57 (q, J = 7.5 Hz, 2H), 1.28 (t, J = 7.5 Hz, 3H). EI-MS m/z: 276 (M^+). HREI-MS: exact mass calcd for $C_{12}H_{12}N_4O_2S$ (M^+), 276.0681; found, 276.0681. HPLC purity, 98.81%.

3-(2,2-Dimethylpropionylamino)pyridine-2-carboxylic Acid Thiazol-2-ylamide (9g). Yield 57%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 11.80 (br, 2H), 9.23 (dd, J = 1.5, 8.7 Hz, 1H), 8.30 (dd, J = 1.5, 4.5 Hz, 1H), 7.56 (d, J = 3.6 Hz, 1H), 7.50 (dd, J = 4.5, 8.7 Hz, 1H), 7.07 (d, J = 3.6 Hz, 1H), 1.38 (s, 9H). EI-MS m/z: 304 (M^+). HREI-MS: exact mass calcd for $C_{14}H_{16}N_4O_2S$ (M^+), 304.0994; found, 304.0988. HPLC purity, 99.76%.

3-Hexanoylaminopyridine-2-carboxylic Acid Thiazol-2-ylamide (9h). Yield 40%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 11.49 (s, 1H), 9.20 (dd, J = 1.2, 8.7 Hz, 1H), 8.29 (dd, J = 1.2, 4.2 Hz, 1H), 7.55 (d, J = 3.6 Hz, 1H), 7.50 (dd, J = 4.2, 8.7 Hz, 1H), 7.07 (d, J = 3.6 Hz, 1H), 2.51 (t, J = 7.5 Hz, 2H), 1.79 (m, 2H) 1.39 (m, 4H), 0.90 (t, J = 6.3 Hz, 3H). EI-MS m/z: 318 (M^+). HREI-MS: exact mass calcd for $C_{15}H_{18}N_4O_2S$ (M^+), 318.1150; found, 318.1136. HPLC purity, 97.52%.

3-(Cyclohexanecarbonylamino)pyridine-2-carboxylic Acid Thiazol-2-ylamide (9i). Yield 61%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 11.49 (br, 1H), 9.23 (dd, J = 1.2, 8.4 Hz, 1H), 8.30 (dd, J = 1.2, 4.5 Hz, 1H), 7.60 (d, J = 3.6 Hz, 1H), 7.50 (dd, J = 4.5, 8.4 Hz, 1H), 7.08 (d, J = 3.6 Hz, 1H), 2.41 (m, 1H), 2.04 (m, 2H), 1.86 (m, 2H), 1.76 (m, 1H), 1.58 (m, 2H),

1.33 (m, 3H). EI-MS *m/z*: 330 (M^+). HREI-MS: exact mass calcd for $C_{16}H_{18}N_4O_2S$ (M^+), 330.1150; found, 330.1144. HPLC purity, 99.76%.

3-Phenylacetylaminopyridine-2-carboxylic Acid Thiazol-2-ylamide (9j). Yield 52%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 11.57 (br, 2H), 9.18 (dd, J = 1.2, 0.7 Hz, 1H), 8.28 (dd, J = 1.2, 7.2 Hz, 1H), 7.54 (d, J = 3.6 Hz, 1H), 7.49 (dd, J = 4.2, 8.7 Hz, 1H), 7.41 (m, 5H), 7.07 (d, J = 3.6 Hz, 1H), 3.84 (s, 1H). EI-MS *m/z*: 338 (M^+). HREI-MS: exact mass calcd for $C_{17}H_{14}N_4O_2S$ (M^+), 338.0837; found, 338.0833. HPLC purity, 99.24%.

3-(2-Methylbut-2-enoylamino)pyridine-2-carboxylic Acid Thiazol-2-ylamide (9k). Yield 29%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 11.86 (br, 1H), 9.25 (dd, J = 1.2, 8.7 Hz, 1H), 8.29 (dd, J = 1.2, 4.5 Hz, 1H), 7.55 (d, J = 3.6 Hz, 1H), 7.51 (dd, J = 4.5, 8.7 Hz, 1H), 7.06 (d, J = 3.6 Hz, 1H), 6.90 (m, 1H), 2.04 (s, 3H), 1.90 (d, J = 3.0 Hz, 3H). EI-MS *m/z*: 302 (M^+). HREI-MS: exact mass calcd for $C_{14}H_{14}N_4O_2S$ (M^+), 302.0837; found, 302.0827. HPLC purity, 96.66%.

3-Benzoylaminopyridine-2-carboxylic Acid Thiazol-2-ylamide (9q). Yield 60%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 12.49 (s, 1H), 11.55 (br, 1H), 9.40 (dd, J = 1.5, 8.4 Hz, 1H), 8.36 (dd, J = 1.5, 4.5 Hz, 1H), 8.12−8.09 (m, 2H), 7.62−7.55 (m, 5H), 7.09 (d, J = 3.6 Hz, 1H). EI-MS *m/z*: 324 (M^+). HREI-MS: exact mass calcd for $C_{16}H_{12}N_4O_2S$ (M^+), 324.0681; found, 324.0688. HPLC purity, 99.72%.

3-(2-Fluorobenzoylamino)pyridine-2-carboxylic Acid Thiazol-2-ylamide (9r). Yield 19%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 12.37 (s, 1H), 9.37 (dd, J = 0.9, 8.7 Hz, 1H), 8.38 (dd, J = 0.9, 4.2 Hz, 1H), 8.07 (dd, J = 1.8, 7.8 Hz, 1H), 7.60−7.57 (m, 2H), 7.55 (d, J = 3.6 Hz, 1H), 7.29 (m, 2H), 7.06 (d, J = 3.6 Hz, 1H). EI-MS *m/z*: 342 (M^+). HREI-MS: exact mass calcd for $C_{16}H_{11}FN_4O_2S$ (M^+), 342.0587; found, 342.0561. HPLC purity, 99.47%.

3-(3-Fluorobenzoylamino)pyridine-2-carboxylic Acid Thiazol-2-ylamide (9s). Yield 18%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 12.52 (s, 1H), 11.54 (s, 1H), 9.36 (d, J = 8.4 Hz, 1H), 8.39 (d, J = 4.5 Hz, 1H), 7.88 (d, J = 7.8 Hz, 1H), 7.80 (d, J = 9.3 Hz, 1H), 7.63−7.53 (m, 3H), 7.32 (dt, J = 8.4, 6.0 Hz, 1H), 7.11 (d, J = 3.6 Hz, 1H). EI-MS *m/z*: 342 (M^+). HREI-MS: exact mass calcd for $C_{16}H_{11}FN_4O_2S$ (M^+), 342.0587; found, 342.0583. HPLC purity, 99.57%.

3-(4-Fluorobenzoylamino)pyridine-2-carboxylic Acid Thiazol-2-ylamide (9t). Yield 22%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 12.49 (s, 1H), 11.38 (br, 1H), 9.36 (dd, J = 0.9, 8.4 Hz, 1H), 8.36 (dd, J = 0.9, 4.5 Hz, 1H), 8.14−8.10 (m, 2H), 7.61−7.57 (m, 2H), 7.28−7.22 (m, 2H), 7.10 (d, J = 3.6 Hz, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 167.27−163.91 (J = 252.8 Hz), 165.53, 165.12, 157.06, 142.79, 139.75, 139.47, 138.62, 131.52, 130.33, 130.21, 129.17−129.04 (J = 9.8 Hz), 116.45−116.16 (J = 21.8 Hz), 114.46. EI-MS *m/z*: 342 (M^+). HREI-MS: exact mass calcd for $C_{16}H_{11}FN_4O_2S$ (M^+), 342.0587; found, 342.0583. HPLC purity, 99.81%.

3-(3-Methylbut-2-enoylamino)pyridine-2-carboxylic Acid Thiazol-2-ylamide(9l). To a solution of **9a** (78 mg, 0.35 mmol) in 4 mL of CH_2Cl_2 was added Et_3N (0.16 mL, 1.15 mmol). A solution of 3-methyl-but-2-enoyl bromide (110 μL) in 1 mL of CH_2Cl_2 was added at 0 °C. The mixture was stirred for 2 h with gradual warming to room temperature. The reaction mixture was diluted with 5 mL of EtOAc and 2 mL of H_2O. The aqueous phase was extracted with EtOAc. The combined organic phases were then processed in the usual way to yield the crude product, which was recrystallized from EtOAc/petroleum ether to give compound **9l** (12.5 mg, 12%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 11.38 (s, 1H), 9.26 (dd, J = 1.5, 9.0 Hz, 1H), 8.28 (dd, J = 1.5, 4.5 Hz, 1H), 7.56 (d, J = 3.6 Hz, 1H), 7.49 (dd, J = 4.5, 9.0 Hz, 1H), 7.07 (d, J = 3.6 Hz, 1H), 5.90 (s, 1H), 2.25 (s, 3H), 1.97 (s, 3H). EI-MS *m/z*: 302 (M^+). HREI-MS: exact mass calcd for $C_{14}H_{14}N_4O_2S$ (M^+), 302.0837; found, 302.0825. HPLC purity, 97.80%.

3-(3-Methylbut-3-enoylamino)pyridine-2-carboxylic Acid Thiazol-2-ylamide (9m). To a solution of **9a** (22 mg, 0.1 mmol) in 1 mL of CH_2Cl_2 was added excess of Et_3N (0.5 mL, 3.5 mmol). A solution of newly prepared 3-methyl-but-2-enoyl bromide (50 μL) in 0.5 mL of CH_2Cl_2 was added at 0 °C. The mixture was stirred for 8 h with gradual warming to room temperature. The reaction mixture was diluted with 5 mL of EtOAc and 2 mL of H_2O. The aqueous phase was extracted with EtOAc. The combined organic phases were then processed in the usual way and chromatographed (50:10:1:1 petroleum ether/EtOAc/acetone/HOAc) to yield compound **9m** (3.2 mg, 11%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 11.62 (s, 1H), 9.18 (d, J = 9.0 Hz, 1H), 8.31 (d, J = 4.5 Hz, 1H), 7.55 (d, J = 3.6 Hz, 1H), 7.52 (dd, J = 4.5, 9.0 Hz, 1H), 7.07 (d, J = 3.6 Hz, 1H), 5.11 (d, J = 8.7 Hz, 1H), 3.24 (s, 2H), 1.89 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 171.01 (C), 164.88 (C), 158.92 (C), 142.73 (CH), 139.18 (C), 138.99 (C), 137.01 (CH), 130.98 (C), 129.12 (CH), 129.05 (CH), 116.73 (CH_2), 114.28 (CH), 48.29 (CH_2), 22.74 (CH_3). EI-MS *m/z*: 302 (M^+), HREI-MS: exact mass calcd for $C_{14}H_{14}N_4O_2S$ (M^+), 302.0837; found, 302.0833. HPLC purity, 95.21%.

General Procedure for Preparation of Compounds 9e and 9n−p. Pyridine (0.2 mL) was added to a solution of 2,2-dimethylpropionyl chloride in 1.5 mL of dry benzene at 0 °C. The mixture was stirred for 5 min, and the appropriate acid (1 mmol) was added. After 15 min, a solution of **9a** (0.1 mmol) in 1 mL of dry DMF was added. The reaction mixture was stirred at room temperature for 8 h. The mixture was partitioned between H_2O (20 mL) and EtOAc (20 mL). The phases were separated, and the aqueous phase was extracted with EtOAc. The combined organic phases were then processed in the usual way and chromatographed to yield the desired products.

3-(2,2,2-Trifluoroacetylamino)pyridine-2-carboxylic Acid Thiazol-2-ylamide (9e). Yield 22%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 12.70 (s, 1H), 9.13 (d, J = 8.7 Hz, 1H), 8.51 (d, J = 4.5 Hz, 1H), 7.64 (dd, J = 4.5, 8.7 Hz, 1H), 7.59 (d, J = 3.6 Hz, 1H), 7.14 (d, J = 3.6 Hz, 1H). EI-MS *m/z*: 316 (M^+). HREI-MS: exact mass calcd for $C_{11}H_7F_3N_4O_2S$ (M^+), 316.0242; found, 316.0263. HPLC purity, 95.05%.

3-(But-3-enoylamino)pyridine-2-carboxylic Acid Thiazol-2-ylamide (9n). Yield 76%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 11.59 (s, 1H), 11.50 (br, 1H), 9.18 (d, J = 8.7 Hz, 1H), 8.30 (d, J = 4.2 Hz, 1H), 7.55 (d, J = 3.6 Hz, 1H), 7.51 (dd, J = 4.2, 8.7 Hz, 1H), 7.07 (d, J = 3.6 Hz, 1H), 6.08 (m, 1H), 5.39 (d, J = 4.8 Hz, 1H), 5.35 (s, 1H), 3.30 (d, J = 6.6 Hz, 2H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 170.97, 164.88, 142.84, 142.57, 139.07, 138.37, 131.28, 130.25, 129.12, 128.81, 120.53, 114.23, 43.55. EI-MS *m/z*: 288 (M^+), HREI-MS: exact mass calcd for $C_{13}H_{12}N_4O_2S$ (M^+), 288.0681; found, 288.0683. Anal. ($C_{15}H_{16}N_4O_2S$) C, H, N, S.

3-(4-Methylpent-3-enoylamino)pyridine-2-carboxylic Acid Thiazol-2-ylamide (9o). Yield 63%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 11.65 (br, 2H), 9.21 (dd, J = 1.5, 8.7 Hz, 1H), 8.28 (dd, J = 1.5, 4.5 Hz, 1H), 7.55 (d, J = 3.3 Hz, 1H), 7.49 (dd, J = 4.5, 8.7 Hz, 1H), 7.06 (d, J = 3.3 Hz, 1H), 5.45 (t, J = 7.2 Hz, 1H), 3.23 (d, J = 7.2 Hz, 2H), 1.97 (s, 3H), 1.78 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 172.16, 164.65, 157.35, 142.48, 139.75, 139.06, 138.43, 131.39, 128.97, 128.77, 115.64, 114.15, 38.13, 26.14, 18.28. EI-MS *m/z*: 316 (M^+). HREI-MS: exact mass calcd for $C_{15}H_{16}N_4O_2S$ (M^+), 316.0994; found, 316.0999. Anal. ($C_{15}H_{16}N_4O_2S$) C, H, N, S.

3-(5-Methylhex-4-enoylamino)pyridine-2-carboxylic Acid Thiazol-2-ylamide (9p). Yield 47%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 11.50 (br, 2H), 9.19 (dd, J = 1.2, 8.7 Hz, 1H), 8.28 (dd, J = 1.2, 4.5 Hz, 1H), 7.55 (d, J = 3.3 Hz, 1H), 7.50 (dd, J = 4.5, 8.7 Hz, 1H), 7.07 (d, J = 3.3 Hz, 1H), 5.18 (t, J = 7.2 Hz, 1H), 2.56 (m, 3H), 2.48 (m, 3H), 1.69 (s, 3H), 1.65 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 173.02, 164.87, 157.20, 142.43, 139.24, 138.48, 133.76, 130.95, 129.09, 128.92, 122.35, 114.27, 38.85, 25.94, 24.14, 17.97. EI-MS *m/z*: 330 (M^+). HREI-MS: exact mass calcd for $C_{16}H_{18}N_4O_2S$ (M^+), 330.1150; found, 330.1170. HPLC purity, 98.71%.

3-Hydroxypyridine-2-carboxylic Acid Thiazol-2-ylamide (10a). A mixture of **11** (1.39 g, 10 mmol), HOBT (1.35 g, 10 mmol), DCC (2.27 g, 11 mmol), and Et_3N (0.4 mL, 30 mmol) in dry DMF (20 mL) was stirred at room temperature for 20 min, and then thiazol-2-ylamine (1.05 g, 10.5 mmol) was

added. The reaction mixture was stirred at room temperature for 2 h and was then diluted with 100 mL of EtOAc and 40 mL of H_2O. The aqueous phase was extracted with EtOAc. The combined organic phases were then processed in the usual way and chromatographed (3:1 petroleum ether/EtOAc) to yield **10a** (1.41 g, 64%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.17 (dd, J = 1.5, 4.2 Hz, 1H), 7.56 (d, J = 3.6 Hz, 1H), 7.44 (dd, J = 4.2, 8.4 Hz, 1H), 7.39 (dd, J = 1.5, 8.4 Hz, 1H), 7.05 (d, J = 3.6 Hz, 1H). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 166.43, 158.45, 156.84, 140.59, 138.56, 130.11, 130.00, 126.83, 114.58. EI-MS *m/z*: 221 (M^+). HREI-MS: exact mass calcd for $C_9H_7N_3O_2S$ (M^+), 221.0259; found, 221.0235. HPLC purity, 98.46%.

General Procedure for Preparation of Compounds 10b−u. To solution of **10a** (0.1 mmol) in 2 mL of acetone was added excess of anhydrous K_2CO_3 (0.5 g). The appropriate acid chloride (0.15 mmol) was added at 0 °C. The mixture was stirred for 2−24 h with gradual warming to room temperature. The mixture was concentrated under reduced pressure to remove acetone, and the residue was diluted with 5 mL of EtOAc and 2 mL of H_2O. The aqueous phase was extracted with EtOAc. The combined organic phases were then processed in the usual way and chromatographed to yield the desired products.

3-Acetyloxypyridine-2-carboxylic Acid Thiazol-2-ylamide (10b). Yield 23%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 11.24 (br, 1H), 8.54 (dd, J = 1.8, 4.2 Hz, 1H), 7.59 (d, J = 4.2 Hz, 1H), 7.58 (d, J = 1.8 Hz, 1H), 7.52 (d, J = 3.6 Hz, 1H), 7.02 (d, J = 3.6 Hz, 1H), 2.47 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 169.57, 160.40, 157.67, 148.38, 145.98, 139.71, 138.20, 133.71, 128.78, 113.39, 21.28. EI-MS *m/z*: 263 (M^+). HREI-MS: exact mass calcd for $C_{11}H_9N_3O_3S$ (M^+), 263.0365; found, 263.0357. HPLC purity, 95.79%.

3-Propionyloxypyridine-2-carboxylic Acid Thiazol-2-ylamide (10c). Yield 80%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 11.30 (br, 1H), 8.55 (dd, J = 1.8, 4.2 Hz, 1H), 7.58 (dd, J = 2.7, 4.2 Hz, 2H), 7.52 (d, J = 3.6 Hz, 1H), 7.02 (d, J = 3.6 Hz, 1H), 2.80 (q, J = 7.5 Hz, 2H), 1.33 (t, J = 7.5 Hz, 3H). EI-MS *m/z*: 277 (M^+). HREI-MS: exact mass calcd for $C_{12}H_{11}N_3O_3S$ (M^+), 277.0521; found, 277.0514. HPLC purity, 98.23%.

3-(2,2-Dimethylpropionyloxy)pyridine-2-carboxylic Acid Thiazol-2-ylamide (10d). Yield 97%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 11.50 (br, 1H), 8.55 (dd, J = 1.5, 4.2 Hz, 1H), 7.57 (m, 2H), 7.51 (d, J = 3.6 Hz, 1H), 7.01 (d, J = 3.6 Hz, 1H), 1.46 (s, 9H). EI-MS *m/z*: 305 (M^+). HREI-MS: exact mass calcd for $C_{14}H_{15}N_3O_3S$ (M^+), 305.0829; found, 305.0829. HPLC purity, 99.42%.

3-Hexanoyloxypyridine-2-carboxylic Acid Thiazol-2-ylamide (10e). Yield 62%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.53 (dd, J = 1.2, 4.2 Hz, 1H), 7.61−7.51 (m, 3H), 7.01 (dd, J = 0.9, 3.6 Hz, 1H), 2.76 (t, J = 7.5 Hz, 2H), 1.82 (m, 2H), 1.45−1.40 (m, 4H), 0.95 (t, J = 6.9 Hz, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 172.36, 160.44, 157.79, 148.45, 145.83, 139.85, 138.13, 133.71, 128.66, 113.84, 34.27, 31.42, 24.34, 22.57, 14.16. EI-MS *m/z*: 319 (M^+). HREI-MS: exact mass calcd for $C_{15}H_{17}N_3O_3S$ (M^+), 319.0985; found, 319.0984. HPLC purity, 98.03%.

3-(Cyclohexanecarbonyloxy)pyridine-2-carboxylic Acid Thiazol-2-ylamide (10f). Yield 82%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.55 (dd, J = 1.8, 4.2 Hz, 1H), 7.58 (d, J = 4.2 Hz, 1H), 7.55 (d, J = 3.9 Hz, 1H), 7.52 (d, J = 3.6 Hz, 1H), 7.02 (d, J = 3.6 Hz, 1H), 2.70 (m, 1H), 2.19 (m, 2H), 1.85 (m, 2H), 1.65 (m, 3H), 1.35 (m, 3H). EI-MS *m/z*: 331 (M^+). HREI-MS: exact mass calcd for $C_{16}H_{17}N_3O_3S$ (M^+), 331.0985; found, 331.0985. HPLC purity, 99.11%.

3-Phenylacetyloxypyridine-2-carboxylic Acid Thiazol-2-ylamide (10g). Yield 77%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 11.40 (br, 1H), 8.55 (dd, J = 1.5, 4.2 Hz, 1H), 7.57 (d, J = 4.2 Hz, 1H), 7.54 (d, J = 3.6 Hz, 1H), 7.52 (d, J = 3.6 Hz, 1H), 7.45 (m, 2H), 7.36 (m, 3H), 7.05 (d, J = 3.6 Hz, 1H), 4.10 (s, 2H). EI-MS *m/z*: 339 (M^+). HREI-MS: exact mass calcd for $C_{17}H_{13}N_3O_3S$ (M^+), 339.0678; found, 339.0668. HPLC purity, 96.57%.

3-(2-Methylbut-2-enoyloxy)pyridine-2-carboxylic Acid Thiazol-2-ylamide (10h). Yield 76%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.56 (dd, J = 2.7, 4.2 Hz, 1H), 7.61 (d, J = 3.3 Hz, 2H), 7.51 (d, J = 3.6 Hz, 1H), 7.24 (m, 1H), 7.01 (d, J = 3.6 Hz, 1H), 2.02 (s, 3H), 1.94 (d, J = 7.2 Hz, 3H). EI-MS *m/z*: 303(M^+). HREI-MS: exact mass calcd for $C_{14}H_{13}N_3O_3S$ (M^+), 303.0678; found, 303.0661. HPLC purity, 97.49%.

3-(3-Methylbut-2-enoyloxy)pyridine-2-carboxylic Acid Thiazol-2-ylamide (10i). Yield 56%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 11.40 (br, 1H), 8.52 (dd, J = 3.0, 3.3 Hz, 1H), 7.58 (d, J = 3.3 Hz, 2H), 7.50 (d, J = 3.6 Hz, 1H), 6.99 (d, J = 3.6 Hz, 1H), 6.10 (s, 1H), 2.22 (s, 3H), 2.03 (s, 3H). EI-MS *m/z*: 303-(M^+). HREI-MS: exact mass calcd for $C_{14}H_{13}N_3O_3S$ (M^+), 303.0678; found, 303.0686. HPLC purity, 97.94%.

3-[3-(2-Methoxyphenyl)acryloyloxy]pyridine-2-carboxylic Acid Thiazol-2-ylamide (10j). Yield 28%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 11.24 (br, 1H), 8.55 (dd, J = 1.5, 4.2 Hz, 1H), 8.25 (d, J = 15.9 Hz, 1H), 7.69−7.59 (m, 3H), 7.51 (d, J = 3.6 Hz, 1H), 7.40 (dt, J = 1.5, 8.1 Hz, 1H), 7.03−6.94 (m, 2H), 6.99 (d, J = 3.6 Hz, 1H), 6.89 (d, J = 16.2 Hz, 1H), 3.92 (s, 3H). EI-MS *m/z*: 381 (M^+). HREI-MS: exact mass calcd for $C_{19}H_{15}N_3O_4S$ (M^+), 381.0778; found, 381.0777. HPLC purity, 99.63%.

3-Benzoyloxypyridine-2-carboxylic Acid Thiazol-2-ylamide (10k). Yield 47%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 11.33 (br, 1H), 8.60 (d, J = 4.5 Hz, 1H), 8.29 (d, J = 7.5 Hz, 2H), 7.75−7.63 (m, 3H), 7.56 (t, J = 4.5 Hz, 2H), 7.50 (d, J = 3.0 Hz, 1H), 7.05 (d, J = 3.0 Hz, 1H). EI-MS *m/z*: 325 (M^+). HREI-MS: exact mass calcd for $C_{16}H_{11}N_3O_3S$ (M^+), 325.0521; found, 325.0541. HPLC purity, 97.40%.

3-(2-Fluorobenzoyloxy)pyridine-2-carboxylic Acid Thiazol-2-ylamide (10l). Yield 52%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 11.38 (br, 1H), 8.60 (dd, J = 1.6, 4.5 Hz, 1H), 8.27 (ddd, J = 1.6, 7.2, 7.8 Hz, 1H), 7.74−7.60 (m, 3H), 7.50 (d, J = 3.6, 1H), 7.36−7.20 (m, 2H), 6.98 (d, J = 3.6 Hz, 1H). EI-MS *m/z*: 343 (M^+). HREI-MS: exact mass calcd for $C_{16}H_{10}FN_3O_3S$ (M^+), 343.0421; found, 343.0429. HPLC purity, 99.55%.

3-(3-Fluorobenzoyloxy)pyridine-2-carboxylic Acid Thiazol-2-ylamide (10m). Yield 34%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 11.38 (br, 1H), 8.62 (dd, J = 1.4, 4.3 Hz, 1H), 8.07 (d, J = 7.8 Hz, 1H), 7.96 (ddd, J = 1.5, 2.5, 8.7 Hz, 1H), 7.73 (dd, J = 1.7, 8.0 Hz, 1H), 7.60 (dd, J = 4.5, 8.4 Hz, 1H), 7. 57−7.52 (m, 1H), 7.50 (d, J = 3.6 Hz, 1H), 7.38 (ddd, J = 1.9, 7.8 Hz, 8.2 Hz, 1H), 6.98 (d, J = 3.6 Hz, 1H). EI-MS *m/z*: 343(M^+). HREI-MS: exact mass calcd for $C_{16}H_{10}FN_3O_3S$ (M^+), 343.0421; found, 343.0423. HPLC purity, 99.22%.

3-(4-Fluorobenzoyloxy)pyridine-2-carboxylic Acid Thiazol-2-ylamide (10n). Yield 33%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 11.29 (br, 1H), 8.59 (dd, J = 1.5, 4.5 Hz, 1H), 8.33−8.28 (m, 2H), 7.71 (dd, J = 1.5, 8.4 Hz, 1H), 7.65 (dd, J = 4.5, 8.4 Hz, 1H), 7.50 (d, J = 3.6 Hz, 1H), 7.22 (dt, J = 4.2, 8.4 Hz, 2H), 6.97 (d, J = 3.6 Hz, 1H). EI-MS *m/z*: 343(M^+). EI-MS *m/z*: 343 (M^+). HREI-MS: exact mass calcd for $C_{16}H_{10}FN_3O_3S$ (M^+), 343.0421; found, 343.0417. HPLC purity, 99.78%.

3-(2-Nitrobenzoyloxy)pyridine-2-carboxylic Acid Thiazol-2-ylamide (10o). Yield 42%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.64 (dd, J = 1.2, 4.2 Hz, 1H), 8.31 (dd, J = 0.9, 7.5 Hz, 1H), 8.16 (d, J = 7.8 Hz, 1H), 7.88 (t, J = 7.5 Hz, 2H), 7.77−7.68 (m, 2H), 7.54 (d, J = 3.6 Hz, 1H), 7.04 (d, J = 3.6 Hz, 1H). EI-MS *m/z*: 370 (M^+). HREI-MS: exact mass calcd for $C_{16}H_{10}N_4O_5S$ (M^+), 370.0372; found, 370.0367. HPLC purity, 96.19%.

3-(3-Nitrobenzoyloxy)pyridine-2-carboxylic Acid Thiazol-2-ylamide (10p). Yield 15%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 11.28 (br, 1H), 9.12 (dd, J = 1.5, 1.8 Hz, 1H), 8.64 (dd, J = 1.5, 4.5 Hz, 1H), 8.60 (dt, J = 1.5, 7.5 Hz, 1H), 8.54 (ddd, J = 1.5, 2.4, 8.1 Hz, 1H), 7.76 (m, 2H), 7.69 (dd, J = 4.5, 8.1 Hz, 1H), 7.50 (d, J = 3.6HZ, 1H), 6.97 (d, J = 3.6 Hz, 1H). EI-MS *m/z*: 370 (M^+). HREI-MS: exact mass calcd for $C_{16}H_{10}N_4O_5S$ (M^+), 370.0366; found, 370.0364. HPLC purity, 96.58%.

3-(2-Methoxybenzoyloxy)pyridine-2-carboxylic Acid Thiazol-2-ylamide (10q). Yield 56%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 11.23 (br, 1H), 8.56 (dd, J = 1.5, 4.2 Hz, 1H), 8.32 (dd, J = 1.5, 7.8 Hz, 1H), 7.72 (dd, J = 1.5, 8.4 Hz, 1H), 7.64−7.56 (m, 2H), 7.50 (d, J = 3.6 Hz, 1H), 7.12 (dt, J = 1.2, 7.8 Hz, 1H), 7.05 (d, J = 8.4 Hz, 1H), 6.97 (d, J = 3.6 Hz, 1H),

3.95 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 163.55, 160.41, 160.38, 157.85, 148.54, 145.81, 140.12, 138.10, 134.88, 134.14, 133.27, 128.55, 120.62, 118.45, 113.75, 112.33, 56.29. EI-MS *m/z*: 355 (M^+). HREI-MS: exact mass calcd for $C_{17}H_{13}N_3O_4S$ (M^+), 355.0621; found, 355.0621. HPLC purity, 99.81%.

3-(2-Acetyloxybenzoyloxy)pyridine-2-carboxylic Acid Thiazol-2-ylamide (10r). Yield 35%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 11.22 (br, 1H), 8.60 (dd, J = 1.5, 4.2 Hz, 1H), 8.44 (dd, J = 1.5, 7.8 Hz, 1H), 7.72−7.62 (m, 3H), 7.51 (d, J = 3.6 Hz, 1H), 7.45 (t, J = 7.8 Hz, 1H), 7.20 (d, J = 7.8 Hz, 1H), 6.99 (d, J = 3.6 Hz, 1H), 2.28 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 169.81, 162.65, 160.16, 157.77, 151.42, 148.16, 146.15, 139.84, 138.09, 134.94, 134.01, 132.95, 128.76, 126.51, 124.01, 122.34, 113.91, 21.24. EI-MS *m/z*: 383 (M^+). HREI-MS: exact mass calcd for $C_{18}H_{13}N_3O_5S$ (M^+), 383.0570; found, 383.0573. HPLC purity, 99.51%.

3-(2-Iodobenzoyloxy)pyridine-2-carboxylic Acid Thiazol-2-ylamide (10s). Yield 41%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.55 (dd, J = 1.5, 4.2 Hz, 1H), 8.35 (dd, J = 1.8, 7.5 Hz, 1H), 8.04 (d, J = 7.8 Hz, 1H), 7.79−7.59 (m, 3H), 7.51 (d, J = 3.6 Hz, 1H), 7.24 (dt, J = 1.5, 8.1 Hz, 1H), 6.99 (d, J = 3.6 Hz, 1H). EI-MS *m/z*: 451 (M^+). HREI-MS: exact mass calcd for $C_{16}H_{10}IN_3O_3S$ (M^+), 450.9482; found, 450.9483. HPLC purity, 98.99%.

3-(3-Methoxy-2-nitrobenzoyloxy)pyridine-2-carboxylic Acid Thiazol-2-ylamide (10t). Yield 50%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.73 (d, J = 4.2 Hz, 1H), 8.03 (d, J = 8.4 Hz, 1H), 7.91−7.81 (m, 4H), 7.56 (d, J = 3.6 Hz, 1H), 7.31 (d, J = 3.6 Hz, 1H), 3.98 (s, 3H). EI-MS *m/z*: 400 (M^+). HREI-MS: exact mass calcd for $C_{17}H_{12}N_4O_6S$ (M^+), 400.0472; found, 400.0474. HPLC purity, 95.20%.

3-(3-Bromo-4-fluorobenzoyloxy)pyridine-2-carboxylic Acid Thiazol-2-ylamide (10u). Yield 30%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 11.30 (br, 1H), 8.62 (dd, J = 1.5, 4.5 Hz, 1H), 8.51 (dd, J = 2.1, 6.6 Hz, 1H), 8.27−8.21 (m, 1H), 7.75−7.64 (m, 2H), 7.51 (d, J = 3.6 Hz, 1H), 7.29 (dd, J = 8.4, 8.7 Hz, 1H), 6.98 (d, J = 3.6 Hz, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 172.5, 164.6−161.2 (J = 254.6 Hz), 163.9, 160.3, 148.7, 146.2, 139.8, 138.2, 136.6−136.5 (J = 1.7 Hz), 133.7, 132.2−132.0 (J = 8.6 Hz), 128.8, 126.8−126.7 (J = 3.6 Hz), 117.2−116.9 (J = 23.1 Hz), 113.9, 110.0−109.7 (J = 21.6 Hz). EI-MS *m/z*: 421, 423 (M^+). HREI-MS: exact mass calcd for $C_{16}H_9BrFN_3O_3S$ (M^+), 420.9527; found, 420.9536. HPLC purity, 96.45%.

MetAP Activity Assays. The thiopeptolide Met-S-Gly-Phe-OH was synthesized in this lab according to the literature procedure,[12] and the chromogenic assays using the thiopeptolide were carried out as described with minor modification. The enzymatic assay was carried out in a 100 μL system containing 50 mM MOPS (pH 7.0), 1 mM DTNB, 100 μM thiopeptolide, and active enzyme (final protein concentrations of 150 nM for EcMetAP1 and 330 nM for ScMetAP1). The activation of EcMetAP1 or ScMetAP1 was carried out by incubating the MetAP enzyme with 25 mM HEPES (pH 7.5), 150 mM KCl, 15 mM methionine, and 500 μM $CoCl_2$ for 30 min prior to the assay. The hydrolysis of thiopeptolide was monitored by a microplate reader SpectraMax 340 (Molecular Devices, Sunnyvale, CA) at 412 nm for 60 s. In the IC_{50} determinations, the inhibitors were diluted from 10 μM to 1 nM.

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