



Synthesis and biological evaluation of salicylate-based compounds as a novel class of methionine aminopeptidase inhibitors

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ABSTRACT

A series of salicylate-based compounds were designed and synthesized based on the simple function group replacement from our previously reported catechol-containing inhibitors of methionine aminopeptidase (MetAP). Some of these salicylate derivatives showed similar potency and metalloform selectivity, and some showed considerable antibacterial activity. These findings are consistent with our previous conclusion that Fe(II) is the likely metal used by MetAP in bacterial cells and provide new lead structures that can be further developed as novel antibacterial agents.

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Proteins are synthesized in cells with a methionine at the N-terminus, and the methionine is removed co-translationally in over 50% of the newly synthesized proteins.¹ Methionine aminopeptidase (MetAP) is responsible for hydrolysis of the initiator methionine, and this critical protein maturation process is ubiquitously found in all prokaryotes and eukaryotes.^{1,2} The essential role that MetAP plays in cell growth and survival is reflected in the lethal effect of MetAP gene deletion in *Escherichia coli*,³ *Salmonella typhimurium*,⁴ and *Saccharomyces cerevisiae*.⁵ Consequently, MetAP has become an appealing target for the development of broad-spectrum antibacterial and antifungal drugs with a novel mechanism of action.⁶

Hydrolytic cleavage of the peptide bond by MetAP is assisted by divalent metal ions that serve as co-factors for the catalytic reaction.⁷ When purified as an apoenzyme, MetAP can be reproducibly activated by a number of divalent metal ions, including Co(II), Mn(II), Ni(II), and Fe(II).^{8,9} Based on X-ray structures, two metal ions are found at the bottom of a shallow catalytic site in a dinuclear arrangement.¹⁰ Most of the current MetAP inhibitors were discovered and characterized by using MetAP enzyme reconstituted with Co(II), because several MetAPs were initially identified as Co(II) enzymes,¹⁰ and this metal is among the best MetAP activators. Due to its relative chemical stability and consistent MetAP activation, Mn(II) has also been used extensively in the screening

of MetAP inhibitors.^{11,12} Even though several small molecules showed potent inhibition of the purified MetAP enzyme, almost all of them failed to show any significant antibacterial activity.^{13–15} We have shown that potent MetAP inhibitors of one metalloform may not inhibit the same MetAP enzyme in another metalloform.^{8,11} Therefore, the failure may indicate that these MetAP inhibitors may not be able to effectively inhibit MetAP in a physiologically relevant metalloform in bacterial cells.

We have discovered potent inhibitors that selectively inhibit MetAP in either the Co(II), Mn(II), or Fe(II) form,^{8,11,16} and we demonstrated that only the Fe(II)-form selective MetAP inhibitors were able to halt bacterial growth.¹⁶ The compounds that are selective for the Fe(II)-substituted enzyme share a common catechol moiety (**1**, Fig. 1), and X-ray structure of *E. coli* MetAP in complex with one of the inhibitors showed that the catechol compounds coordinate with the catalytic metal ions through their hydroxyl groups.¹⁷ Salicylates are structurally similar to catechols, and salicylate derivatives, such as aspirin, are widely used as anti-inflammatory drugs. We envisioned that the salicylate scaffold may have better chemical stability and favorable pharmacokinetic properties and could potentially serve as a starting point for the development of novel MetAP inhibitors with antibacterial activity. In this study, we designed and synthesized a series of compounds based on the salicylate scaffold (**2** and **3**, Fig. 1), characterized them against the purified MetAP in different metalloforms, and evaluated their antibacterial activity against *E. coli*.

Syntheses of compounds **4–21** were outlined in Schemes 1 and 2. Suzuki coupling¹⁷ of appropriate phenylboronic acids with corresponding brominated thiophenes yielded phenyl thiophene

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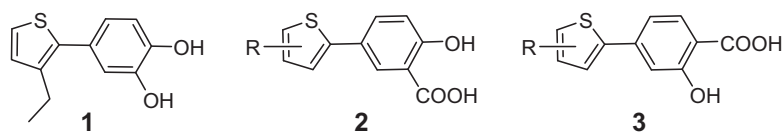
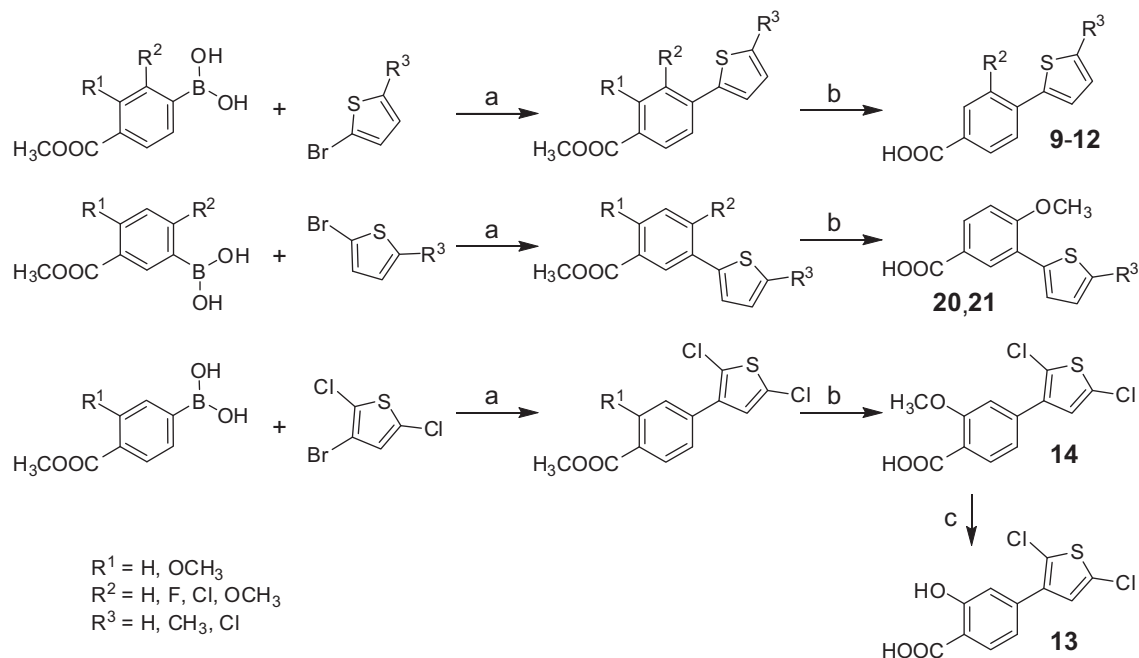
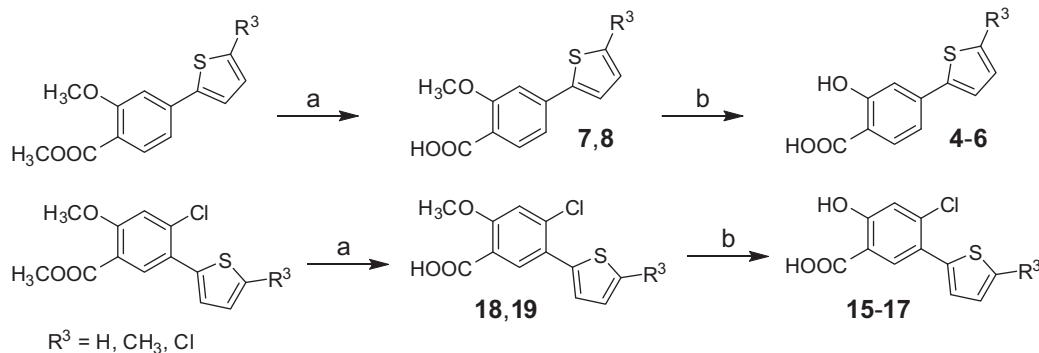


Figure 1. Salicylate derivatives **2** and **3** were designed as MetAP inhibitors, based on the antibacterial MetAP inhibitor **1** with a catechol moiety.



Scheme 1. Reagent and condition: (a) $\text{Pd(PPh}_3)_4$, 2 N Na_2CO_3 , DMF, 90 °C, yield 40–80%; (b) LiOH, MeOH/ H_2O , yield 90–100%; (c) BCl_3 , CH_2Cl_2 , –78 °C, yield 40–70%.



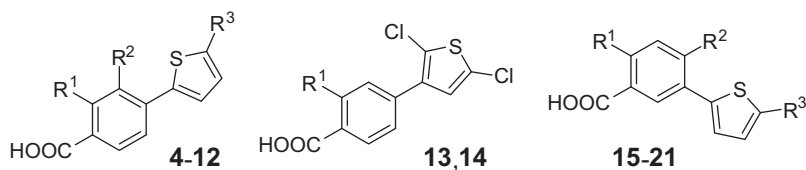
Scheme 2. Reagent and condition: (a) LiOH, MeOH/ H_2O , yield 90–100%; (b) BCl_3 , CH_2Cl_2 , –78 °C, yield 40–70%.

intermediates. Hydrolysis of methyl esters yielded carboxylic acids **7–12**, **14**, and **18–21**. Removal of methyl group from methoxyl compounds **7**, **8**, **14**, **18**, and **19** produced salicylate derivatives **4–6**, **13**, **15**, and **16**. Compounds **6** and **17** were similarly prepared by removing the methyl group in their precursors.

Assays for enzyme inhibition and bacterial cell growth inhibition were conducted as previously described.¹⁸ Inhibitory potencies of these salicylate derivatives on MetAP enzyme in the presence of either Co(II), Mn(II), or Fe(II) are presented in Table 1. For comparison, their inhibitory potencies on *E. coli* AS19 growth, as concentration at 50% inhibition (IC_{50}) and minimum inhibitory concentration (MIC), are also presented.

The relative inhibitory potencies on the three metalloforms of *E. coli* MetAP revealed the structural elements that are important

in inhibition and metalloform selectivity. Compounds **15–17** showed good inhibitory potency and good selectivity for the Fe(II)-form of MetAP. Importantly, these potent and selective inhibitors **15–17** also showed good antibacterial activity on the *E. coli* strain, consistent with the enzyme inhibition. Although compounds **4–6** and **13** were not selective among the metalloforms, they did show considerable antibacterial activity. This may be due to the fact that they do maintain some potency on the Fe(II) form. Because the Fe(II) form is the likely cellular metalloform for MetAP in *E. coli*,¹⁶ these results are consistent with the concept that effectively inhibition of the cellular MetAP is important and inhibitors of the Fe(II) form of MetAP are more likely to show antibacterial activity. Wang et al. reported that human type 2 MetAP uses Mn(II) as its native co-factor,¹⁹ and potentially, the Fe(II) form

Table 1Inhibition of *E. coli* MetAP enzyme activity and *E. coli* AS19 cell growth by salicylate-based derivatives

Compd	R ¹	R ²	R ³	Inhibition of enzymatic activity, IC ₅₀ , μM			Inhibition of bacterial growth, IC ₅₀ , μM ^a
				Co(II)	Mn(II)	Fe(II)	
4	OH	H	H	65.2	80.2	39.1	112 (100)
5	OH	H	CH ₃	81.0	90.7	109.1	73.1 (47)
6	OH	H	Cl	87.7	62.7	73.0	34.1 (28)
7	OCH ₃	H	H	254	89.8	500	>1000 (>234)
8	OCH ₃	H	CH ₃	256	160	270	550 (272)
9	H	H	H	249	159	>500	>1000 (>204)
10	H	F	H	251	143	267	>1000 (>222)
11	H	F	CH ₃	329	336	253	871 (306)
12	H	F	Cl	166	118	203	641 (297)
13	OH			118	97.5	29.9	51.4 (33)
14	OCH ₃			290	97.3	>500	829 (521)
15	OH	Cl	H	88.1	33.7	3.1	74.6 (38)
16	OH	Cl	CH ₃	77.4	37.8	6.8	161 (87)
17	OH	Cl	Cl	74.1	36.6	4.9	48.5 (30)
18	OCH ₃	Cl	H	203	19.1	208	>1000 (>268)
19	OCH ₃	Cl	CH ₃	70.9	11.4	106	>1000 (>282)
20	H	OCH ₃	H	>500	17.4	192	>1000 (>234)
21	H	OCH ₃	CH ₃	464	33.8	167	>1000 (>248)

^a MIC values in mg/L are in parentheses.

selective MetAP inhibitors are desired lead compounds to optimize their antibacterial activity, while minimizing their human toxicity. It is interesting to note that all of the compounds with good enzyme inhibition and antibacterial activity (**4–6**, **13**, and **15–17**) have a hydroxyl group, along with a carboxyl group at the adjacent position, indicating the importance of the salicylate moiety in interaction with MetAP, probably through metal chelation. Compounds missing this hydroxyl group (**9–12**, **20**, and **21**) or compounds with this hydroxyl group replaced by a methoxyl group (**7**, **8**, **14**, **18**, and **19**) all showed significantly lower enzyme inhibition and low or no antibacterial activity. While this hydroxyl group is indispensable, the hydroxyl group alone is not sufficient for inhibition, because we showed previously that mono-hydroxyl analogs of the catechol series displayed no enzyme inhibition.¹⁸ Therefore, both the hydroxyl group and the carboxyl group in the salicylate derivatives are required for effective inhibition of MetAP. Compounds **18–21** showed noticeable potency and selectivity on the Mn(II)-form of MetAP. Lacking the hydroxyl group, they all have a carboxyl group, and their selectivity towards the Mn(II) form is consistent with the results from furan carboxylates we reported earlier.¹¹ In that case, the carboxyl group directly coordinates with the catalytic metal ions, and the coordination contributes to their Mn(II)-form selectivity. However, their lack of antibacterial activity is possibly due to their poor potency on the Fe(II)-form of MetAP.

In summary, structure–function analysis of the newly synthesized salicylate derivatives as inhibitors of *E. coli* MetAP clearly indicates that replacement of one of the two hydroxyl groups to a carboxyl group on the catechol scaffold (change from **1** to **2** or **3**, Fig. 1) can be accommodated by MetAP, and the potency and selectivity on the Fe(II)-form can be maintained. Both the hydroxyl group and the carboxyl group in the salicylate derivatives are involved in enzyme inhibition and required for antibacterial activity.

The demonstrated activity of these salicylate derivatives on MetAP enzyme and on *E. coli* cells, coupled with their likely better chemical stability and favorable pharmacokinetic properties, provides a new starting point for the design and discovery of MetAP inhibitors as new antibacterial agents with a novel mechanism of action.

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