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Methionine Analogues as Inhibitors of Methionyl-tRNA Synthetase

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Abstract: A series of methionine analogues have been synthesized as inhibitors of methionyl-tRNA synthetase and evaluated for their inhibitory activities of *E. coli* methionyl-tRNA synthetase and bacterial growth . Among them, L-methionine hydroxamate **20** has proved to be the best inhibitor of the enzyme with K_i = 19 μM and showed a growth inhibition against *E.coli* JM 109, *P. vulganis* 6059 and *C. freundii* 8090.

Introduction

Aminoacyl-tRNA synthetases (aaRS) catalyze the transfer of a particular amino acid to its corresponding specific tRNA to form each aminoacyl tRNAs which are used for protein synthesis. Selective inhibitors of bacterial aaRS can be potential antibacterial candidates especially to overcome the problem of antibacterial resistance (AR).[1,2] The enzyme reaction proceeds in two steps. In the first step, the amino acid is activated by reaction with ATP to form an enzyme-bound aminoacyl adenylate (aminoacyl AMP, aa-AMP). In the second step, the activated amino acid is transferred to the 3'-adenosine of the cognate tRNA that directs its placement within a growing polypeptide. Two different mechanisms of the latter step classify aaRSs into two classes in which, for class I enzymes, the aminoacyl group of aa-AMP is transferred initially to the 2'-OH of the terminal adenylate in tRNA, then moved to the 3'-OH by a transesterification while, for class II, the aminoacyl group is transferred directly to the 3'-OH.[3,4] Among aaRS inhibitors, only pseudomonic acid (mupirocin) is currently used as a topical antibiotic[5] and its mechanism is known to inhibit bacterial isoleucyl-tRNA synthetase selectively as a bifunctional-analogue.[6] Recently, a number of synthetic inhibitors of aaRS which were designed as mimetics of aminoacyl AMP or transition states have been reported in arginyl-tRNA[7], glutamyl-tRNA[8], isoleucyl-tRNA[9], prolyl-tRNA[10], seryl-tRNA[11], tryptophanyl-tRNA synthetase[12].

As our programs directed toward developing novel antibacterial agents as inhibitors of aminoacyl tRNA synthetases, we are interested in inhibitors of bacterial methionyl-tRNA synthetase (MetRS) acting on its active site. The native*E. coli* MetRS is a homodimer of 676 amino acid protomer and the removal of about 100 amino acids in the C-terminal appendix results in the active monomer.[13] The X-ray structure of the active monomeric enzyme was determined at the resolution of 2.5 Å.[14] In this paper we describe the synthesis, the inhibition of *E. coli* methionyl-tRNA synthetase and antibacterial activity of methionine analogues as potential antibacterial agents.

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Synthesis of Methionine Analogues

Synthesis of methylthioalkyl derivatives (**1-9**) as methionine analogues deleting amino group was accomplished by conventional synthetic routes or purchased from Aldrich Company. Heterocyclic analogues, as bioisosteres of methionine, were prepared by following reference procedures (benzisothiazole trione **10**[15], tetrazole **11**[16], pyrazol-3-one **12**[17] and pyrazole **13**, **14**[17]). Amide and hydroxylamine analogues of methionine (**17-30**) were synthesized from N-Boc-protected methionine by anhydride formation with isobutyl chloroformate, amidation by the corresponding amines or hydroxyamine derivatives and followed by Boc-deprotection under the condition of trifluoroacetic acid in anisole.

Biological Results

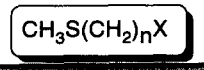
The aminoacylation reaction of the *E. coli* methionyl-tRNA synthetase was carried out as described in reference.[18a] The enzyme inhibition of the synthesized compounds was determined by measuring the decrease of the reaction product, the [^{35}S]methionylated *E. coli* -tRNA^{fMet}, in the presence of 270 μM of each chemical. Their inhibitory activities to the enzyme were depicted in percent compared with the control condition in which the enzyme carried out the reaction in the absence of inhibitors. Methylthioalkyl analogues (**1-14**) including heterocyclic compounds which were designed as bioisosteres of carboxylic acid showed little inhibitory activity (Table 1). This suggests that the interaction of amino group with the enzyme is indispensable for its inhibition. We also examined the activity of the methionine analogues containing different functionality of carboxylic acid such as ester, amide and hydroxamate (Table 2). Hydroxamate of L-methionine (**20**) showed the best inhibitory activity to the enzyme. Structure-activity studies of hydroxamate **20** showed that any modifications decreased their inhibitory ability to the enzyme and can be summerized as follows: 1) The enzyme was very selective only to the L-isomer because the inhibitory activity of the racemic hydroxamate **19** was reduced to the half of L-isomer and the change of chirality to D-form **18** abolished its inhibitory activity. 2) Amino group, nitrogen and oxygen of hydroxylamine in **20** are necessary for interacting with the enzyme because protection of each group (**21-24**) decreased their activities. 3) Methylthioethyl group as side chain of methionine seems to be necessary for inhibitory activity because its modification on sulfur atom (**25**, **26**) or other chain (**27**) show little activities.

The detailed kinetic studies of **20** were examined for explaining its mechanism of enzyme inhibition.[18b] Analysis indicated that while not affecting on the aminoacyl adenylation as the first step in the enzyme reaction, it inhibits the aminoacylation of tRNA^{fMet} in a competitive manner. The estimated K_i was 19.6 μM. This suggest that unlike the mechanism of pseudomonic acid in which it specifically inhibits the formation of the enzyme-isoleucine-AMP complex in isoleucyl-tRNA synthetase[19], it inhibits the transfer of methionine from the complex, after the formation of methionyl adenylate, to tRNA.[20]

Antibacterial activity of methionine hydroxamates (**18-20**) was tested against 10 different bacterial species by the agar dilution method[21] and their minimal inhibitory concentration (MIC) values are represented in Table 3. Similar to the results of the enzyme inhibition, antibacterial activity was also sensitive to the chirality of α-carbon. While D-form didn't show any growth inhibition to the tested bacteria, L-form was active against gram-negative *E.coli* JM 109, *P. vlulganis* 6059 and *C. freundii* 8090.

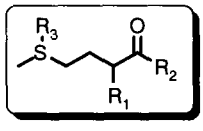
In conclusion, L-methionine hydroxamate **20** is identified as a good inhibitor of *E. coli* methionyl-tRNA synthetase and a lead compound for developing potential antibacterial agent. We are currently exploring the further mechanical study using its analogues to find more potent inhibitor of the enzyme.

Table 1



Compound	n	X	Relative activity
Control			0
1	2	OH	3.2
2	3	OH	0
3	4	OH	0
4	2	CHO	4.7
5	2	COOH	5.9
6	2	CO_2CH_3	3.8
7	2	COCl	14.0
8	2	CN	7.8
9	2	$COCH_2CO_2CH_3$	0.4
10	2		16.3
11	2		16.2
12	2		1.8
13	2		0
14	2		8.3

Table 2



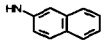
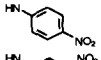
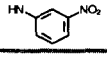
Compound	Chirality	R_1	R_2	R_3	Relative activity (%)
15	L	$NH_3^+Cl^-$	OCH_3		20.4
16	L	NHBoc	OH		11.3
17	L	$NH_3^+Cl^-$	NH_2		17.8
18	D	$NH_3^+CF_3COO^-$	NHOH		0
19	D,L	NH_2	NHOH		45.7
20	L	$NH_3^+CF_3COO^-$	NHOH		100
21	L	$NH_3^+CF_3COO^-$	$NHCH_3OH$		23.1
22	L	$NH_3^+CF_3COO^-$	NHOt-Bu		56.2
23	L	NHBoc	NHOH		11.4
24	L	NHBoc	NHOBoc		0
25	L	$NH_3^+CF_3COO^-$	NHOH	O	8.5
26	L	$NH_3^+CF_3COO^-$	NHOH	O_2	0
27	L	$NH_3^+CF_3COO^-$	$CH(CH_3)_2$		0
28	D,L	$NH_3^+Cl^-$			22.3
29	L	NH_2			33.5
30	L	$NH_3^+CF_3COO^-$			0

Table 3. Antimicrobial activities of methionine hydroxamate

Microorganism	L-Methionine hydroxamate (**20**)	D-Methionine hydroxamate (**21**)	Ampicillin
Staphylococcus aureus 1538p	>128	>128	>128
Enterococcus faecalis 29212	>128	>128	>128
Escherichia coli JM109	1	2	>128
Salmonella typhimurium 14028	>128	>128	>128
Klebsiella pneumoniae 2011E	>128	>128	>128
Proteus vulgaris 6059	2	4	>128
Citrobacter freundii 8090	8	8	>128
Serratia marcescens 1826E	>128	>128	>128
Pseudomonas aeruginose 1912E	>128	>128	>128
Bacillus subtilis ATCC 6633	>128	>128	>128

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18. (a) Aminoacylation reaction was determined by adding 5 nM of *E.coli* MetRS proteins to a mixture of 20 mM HEPES, pH 7.5, 100 μM EDTA, 150 mM NH_4Cl, 1 mg/ml bovine serum albumin (BSA), 2 mM ATP, 4 μM tRNA-fMet, 4 mM $MgCl_2$, 20 μM methionine, 0.2 μM [^{35}S]methionine and synthesized inhibitor. Aliquots were taken from the reaction and mixed with 10% trichloroacetic acid containing 2 mM methionine on Whatman 3 MM filter pad (2.3 cm) to quench the reaction. The aminoacylated tRNA-fMet was quantified by liquid scintillation counter in 5 ml Betafluor. (b) Kinetic analysis for the effect of L-methionine hydroxamate (**20**) on aminoacylation were carried out by varying concentrations of methionine (5-160 μM) and **20** (3.75-240 μM).
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