Pergamon

Bioorganic & Medicinal Chemistry Letters 11 (2001) 961–964

BIOORGANIC &
MEDICINAL
CHEMISTRY
LETTERS

Ester and Hydroxamate Analogues of Methionyl and Isoleucyl Adenylates as Inhibitors of Methionyl-tRNA and Isoleucyl-tRNA Synthetases

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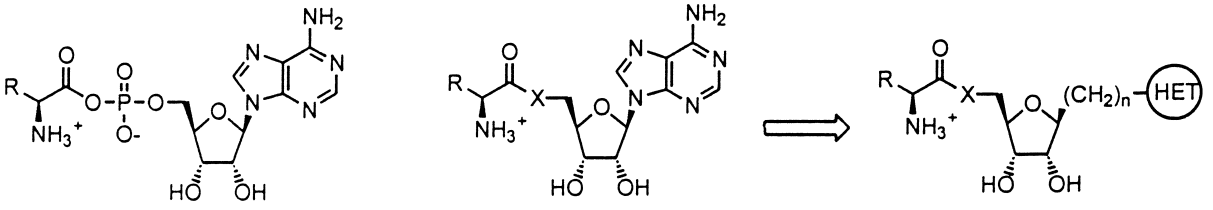
Received 24 November 2000; accepted 31 January 2001

Abstract—The structure–activity relationship on a series of ester and hydroxamate analogues of methionyl and isoleucyl adenylate has been investigated through introducing linkers between the 1′-position of ribose and adenine surrogates as methionyl-tRNA, and isoleucyl-tRNA synthetase inhibitors, respectively. The results indicate that ester analogue **23** was found to be a potent inhibitor of *Escherichia coli* methionyl-tRNA synthetase, and its interaction with the active site was proposed by a molecular modeling study. © 2001 Elsevier Science Ltd. All rights reserved.

Introduction

Aminoacyl-tRNA synthetases (aaRSs) catalyze the transfer of specific amino acids to their corresponding tRNAs to form aminoacyl-tRNAs, which are used for protein synthesis.[1,2] Since the aminoacylation reaction is essential in all living organisms, these enzymes have attracted much attention as promising antibacterial targets to overcome the resistance problem caused by mainline antibiotics.[3,4] Obviously, the selective inhibition of pathogen synthetases to the human cell counterpart is an important issue when considered as a drug candidate. Aminoacyl adenylates, a mixed anhydride intermediate generated during the reaction, have long been recognized as the lead compounds in finding potent and selective inhibitors, because they are known to bind more tightly to the enzyme than the substrates, amino acid and ATP, generally by two or three orders of magnitude. Most aminoacyl adenylate analogues, reported as enzyme inhibitors, have been designed with a similar strategy, in which they ensure both tight binding and stability by replacing the labile anhydride bond of aminoacyl-AMP with stable non-hydrolyzable bioisosteres. Among them, sulfamate bioisostere has been



Aminoacyl adenylate

1 R=Met X=O **3** R=Ile X=O
2 R=Met X=N-OH **4** R=Ile X=N-OH

n = 1,2

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PII: S0960-894X(01)00095-6

the most successful in terms of binding affinity to a variety of aaRSs, such as AlaRS,[5] ArgRS,[6] HisRS,[6] IleRS,[7] ProRS,[8] SerRS,[9] ThrRS,[6] and TyrRS.[10]

An extensive SAR study on sulfamate analogues of isoleucyl adenylate demonstrated that the binding region of adenine moiety in *Escherichia coli* isoleucyl-tRNA synthetase (IleRS) contained a wide hydrophobic pocket large enough to afford three linear aromatic rings.[3]

We recently reported that ester **1** and hydroxamate analogue **2** of methionyl adenylate, as another stable analogue of aminoacyl adenylate, were potent inhibitors of methionyl tRNA synthetases (MetRS) isolated from *E. coli, M. tuberculosis, S. cerevisiae* and human.[11] As part of our continuing effort to find potent and pathogen-selective inhibitors of MetRS and IleRS as potential antibacterial agents, we herein describe extensive SARs of ester and hydroxamate analogues of methionyl and isoleucyl adenylates, in which heterocycles as adenine surrogates are separated from ribose by one or two carbon units.

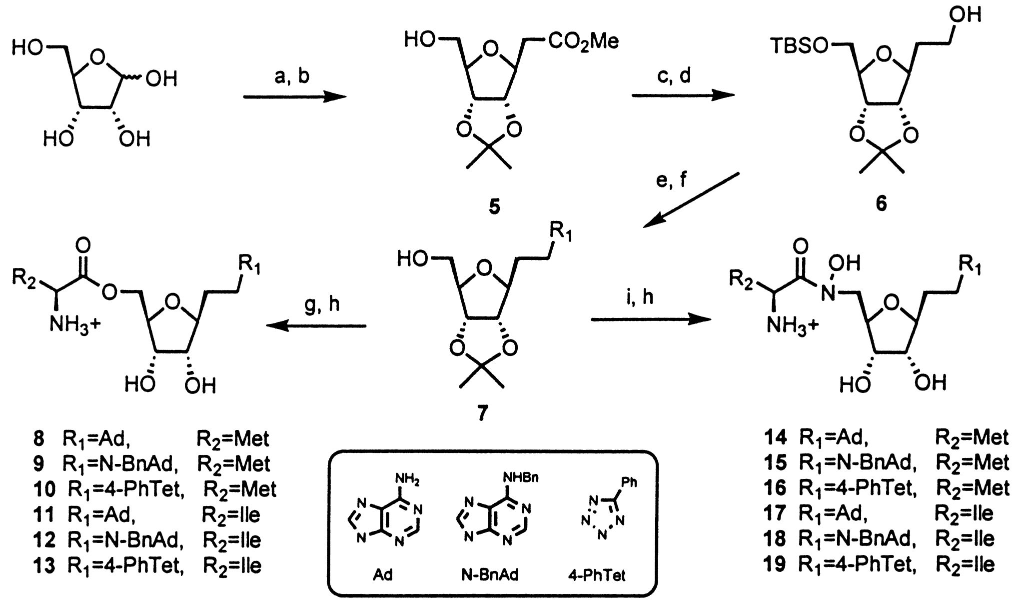
Synthesis

Ester and hydroxamate analogues of isoleucyl adenylate, **3** and **4**, were prepared from 2′,3′-isopropylidene adenosine by following the previous procedure.[11] Syntheses of two-carbon-elongated analogues are outlined in Scheme 1. Methyl 3,6-anhydro-2-deoxy-4,5-*O*-isopropylidene-D-allo-heptonate (**5**) as a starting material was prepared from D-ribose by a literature procedure.[12] Primary alcohol of **5** was protected with the TBS group, and its ester was reduced into corresponding alcohol **6** by $LiAlH_4$. The Mitsunobu reaction of **6** with heterocycles, including adenine, *N*-benzyladenine and 4-phenyltetrazole, followed by deprotection of the TBS group provided key intermediates **7**. For the syntheses of ester analogues, alcohols **7** were esterified with *N*-Boc methionine, or *N*-Boc isoleucine, and then deprotected under acidic conditions, to give **8**–**13**, respectively. For the syntheses of hydroxamate analogues, alcohols **7** were condensed with *N*-Boc methionine hydroxamate or *N*-Boc isoleucine hydroxamate by Mitsunobu reaction, and then deprotected to afford final compounds **14**–**19**, respectively. Syntheses of one-carbon-elongated analogues are described in Scheme 2. Alcohol **6** was dehydrated into alkene **20** by mesylation and subsequent base-mediated elimination. Ozonolysis, followed by $NaBH_4$ reduction of double bond **20**, provided the corresponding alcohol **21**, whose hydroxyl was substituted by heterocycles under Mitsunobu conditions, and then deprotected to give **22** as key intermediates. By following the same sequence described in Scheme 1, alcohols **22** were converted into ester analogues **23**–**26** and hydroxamate analogues **27**–**30**, respectively.

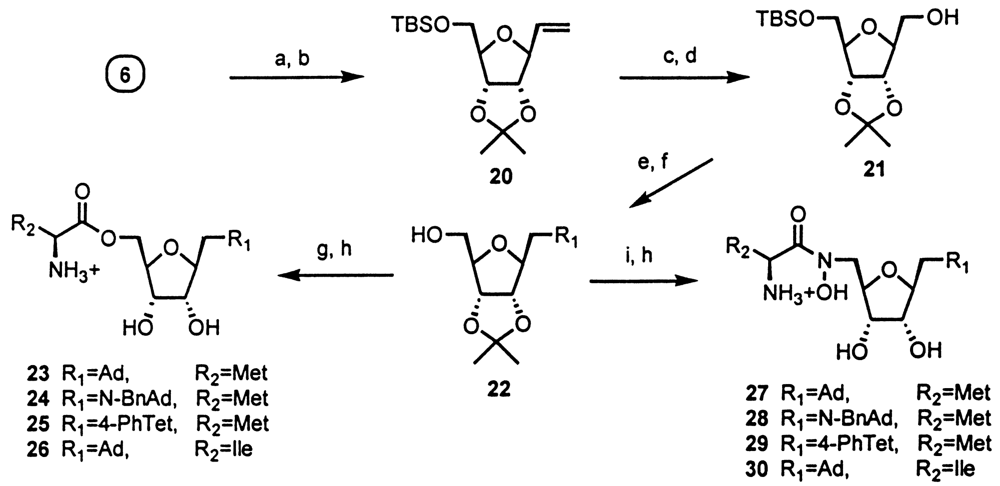
Biological Results and Discussion

Synthesized methionyl and isoleucyl adenylate analogues were evaluated as inhibitors of *E. coli* MetRS and IleRS, respectively, which are mechanistically regarded as class I type with similar structural folds and sequence motifs.[13] Inhibitory activities were determined by measuring the decrease of the aminoacylation product, the [^{35}S]methionyl *E. coli*-tRNA^{Met} or [^{3}H]isoleucyl *E. coli*-tRNA^{Ile}, in the presence of different chemical concentrations.

Ester and hydroxamate analogues of methionyl adenylate, **1** and **2**, as standards were examined with $IC_{50} = 5.0$ and $27\,\mu M$, respectively.[11] The introduction of one carbon (ester: **23** with $IC_{50} = 3.6\,\mu M$; hydro-



Scheme 1. Synthesis of two-carbon linker analogues. Reagents and conditions: (a) H_2SO_4, acetone, 87%; (b) Ph_3PCHCO_2Me, MeCN, 85%; (c) TBSCl, imidazole, THF, 97%; (d) $LiAlH_4$, THF, 77%; (e) heterocycle, DEAD, PPh_3, THF, 60–98%; (f) Bu_4NF, THF, 70–98%; (g) $RCH(NHBoc)CO_2H$, DCC, DMAP, CH_2Cl_2, 70–95%; (h) TFA, anisole, 70–95%; (i) $RCH(NHBoc)CONHOPMB$, PPh_3, THF, 58–90%.



Scheme 2. Synthesis of one-carbon linker analogues. Reagents and conditions: (a) MsCl, NEt_3, CH_2Cl_2, 95%; (b) *t*-BuOK, THF, 50%; (c) O_3, CH_2Cl_2 (d) $NaBH_4$, 70% in two steps; (e) heterocycle, DEAD, PPh_3, THF, 60–94% (f) Bu_4NF, 87–96%; (g) RCH(NHBoc)CO_2H, DCC, DMAP, CH_2Cl_2, 63–93%; (h) TFA, anisole, 75–98%; (i) RCH(NHBoc)CONHOPMB, PPh_3, THF, 60–75%.

xamate: **27** with $IC_{50} = 26\,\mu M$) or two carbon (ester: **8** with $IC_{50} = 8.3\,\mu M$, hydroxamate: **14** with $IC_{50} = 56\,\mu M$) as a linker between 1′-position of ribose and adenine did not significantly affect the inhibitory activity of the parent compounds, **1** and **2**. However, the order of inhibitory activities was consistently expressed as one-carbon linker ≥ parent > two-carbon linker in both the ester (**23** ≥ **1** > **8**) and hydroxamte (**27** ≥ **2** > **14**) template. Particularly ester **23** is found to be the most potent one in this series. The SAR analysis revealed that the catalytic pocket of *E. coli* MetRS to bind the adenine moiety of methionyl adenylate would be large enough to afford structural variations. It appears that one-carbon linker is the optimal length for inhibition. Replacement of the adenine group with *N*-benzyladenine and 4-phenyltetrazole, as more lipophilic and bulky, did not improve their inhibitory activities in both ester (one-carbon linker: **24** and **25**; two-carbon linker: **9** and **10**) and hydroxamate analogues (one-carbon linker: **28** and **29**; two-carbon linker: **15** and **16**). However, these analogues also represented a similar relationship, as shown in the adenine series: ester analogues with one-carbon linker, **24** and **25** were found to be the most active in each series (**24** > **9**, **15**, **28** and **25** > **10**, **16**, **29**), respectively (Table 1).

Table 1. Enzyme inhibitory activities of target compounds

MetRS inhibitors	*E. coli* MetRS (IC_{50}, μM)	IleRS inhibitors	*E. coli* IleRS (IC_{50}, μM)
1	5.0	**3**	183
2	27	**4**	181
8	8.3	**11**	> 128
9	33.2	**12**	84
10	> 256	**13**	63.6
14	56	**17**	> 128
15	23	**18**	> 128
16	34	**19**	> 128
23	3.6	**26**	> 128
24	16.5	**30**	> 128
25	12.2		
27	26		
28	63		
29	17		

In isoleucyl adenylate analogues, ester **3** and hydroxamate **4** analogues unexpectedly showed poor inhibitory activity to *E. coli* IleRS with $IC_{50} = 183$ and $181\,\mu M$, respectively. The result was in strong contrast to sulfamate analogues of isoleucyl adenylate previously reported, which represented the nanomolar range of enzyme inhibitory activity.[3] Structural modification on the adenine part, as MRS inhibitors did above, also had no influence on their inhibitory activities. According to the SAR of sulfamate analogues of isoleucyl adenylate, their inhibitory activities to *E. coli* IleRS improved as the adenine moiety was removed from ribose, and replaced by more lipophilic aromatic rings. The result indicated that *E. coli* IleRS had a wide hydrophobic pocket on the adenine binding site, which was enough to interact with a long and lipophilic side chain. This finding was also confirmed from two inhibitors, **12** and **13**, with two-carbon linkers and a more lipophilic adenine surrogate, which displayed better activity than the parent compounds, **3** and **4**, as compared to the sulfamate analogues.

A recent report on the X-ray crystal structure of *E. coli* MetRS[14] prompted us to direct a receptor-guided approach for investigating MetRS inhibitors. Based on the data, we proposed a model of the active site docked

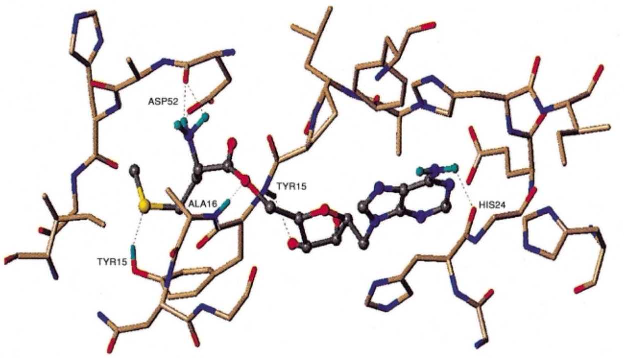


Figure 1. Proposed model of *E. coli* MetRS active site docked with compound **23**.

with a potent MetRS inhibitor **23** by molecular modeling (Fig. 1). The docking conformation of **23** into the active site of MetRS was obtained from energy minimization, followed by an alignment procedure on the reported X-ray structure of aminoacyl adenylate bound into aminoacyl-tRNA synthetases using GSAP.[15] We referred to a previous model of the active site proposed by the site-directed mutagenesis of the methionine binding site.[16] The docking study was performed using the DOCK procedure of the Sybyl 6.6. **23** was rigidly docked into the binding site using graphical manipulation with continuous energy monitoring. The manually docked local energy minimized receptor–ligand complex was subjected to an additional conjugate gradient minimization using the minimization criteria. The result is represented in Figure 1. In this model, two essential interactions in methionine were examined as reported previously.[16] While the sulfur atom interacted with phenolic hydroxyl of Tyr15 by hydrogen bonding, the ammonium group coordinated with both carbonyl and carboxylate of Asp52 by dipole-ionic and ionic interaction. We also found two hydrogen bonds in adenosine, whose 3′-OH and 6-NH_2 interacted with the NH of Tyr15 and carbonyl of His24, respectively. Further examination indicated that the enzyme contained a deep and narrow hydrophobic pocket around the binding site of the adenine base in order to afford binding of synthesized MetRS inhibitors with methylene and ethylene linkers. Although this model did not consider the participation of water during the ligand–receptor interaction, due to the lack of its X-ray structure, it will be helpful for our continuing receptor-guided investigation into MetRS inhibitors.

In summary, the SAR on a series of ester and hydroxamate analogues of methionyl and isoleucyl adenylate has been investigated through adenine surrogates bearing carbon linkers between them and the 1′-position of ribose. In methionyl adenylate analogues, ester analogues with a one-carbon linker appeared to be optimal for the *E. coli* MetRS inhibitory activity, and **23** emerged as a potent candidate. SARs revealed that the adenine binding site of *E. coli* MetRS would be a flexible hydrophobic pocket large enough to afford bulky adenine surrogates with one- or two-carbon linkers, whose existence was examined by a modeling study. A model of the *E. coli* MetRS active site derived from the X-ray structure of the enzyme and inhibitor **23**, was proposed in order to explain the ligand–enzyme interaction. It will be helpful in the further study of the receptor-guided inhibitor design.

Acknowledgements

This work was supported in part by a grant (HMP-00-CH-15-0014) from the Ministry of Health & Welfare, R.O.K. the Brain Korea 21 Project to J. Lee, as well as a grant from the National Creative Research Initiatives to S. Kim.

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