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Potent and selective inhibitors of bacterial methionyl tRNA synthetase derived from an oxazolone–dipeptide scaffold

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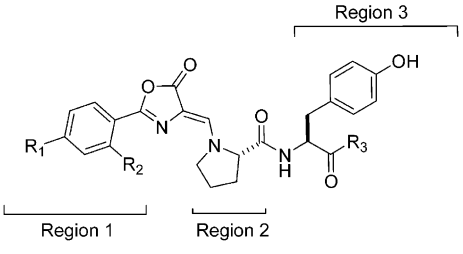
This communication is dedicated to the memory of David L. Coffen

Abstract—The preparation and structure–activity relationships (SARs) of potent and selective small molecule inhibitors of bacterial methionyl-tRNA synthetase (MetRS) derived from an oxazolone–dipeptide scaffold are described. Examples combine *Staphylococcus aureus* MetRS (SaMetRS) potency with selectivity over human MetRS. As a result of the SAR expansion compound **14a** was identified, as a potent SaMetRS inhibitor ($IC_{50} = 18$ nM) having moderate inhibition of MetRS derived from *Enterococci faecalis* ($IC_{50} = 3.51$ μM).

The increasing frequency of bacterial resistance and the associated rise in healthcare costs to successfully treat patients with infections due to resistant bacteria is providing renewed impetus for the pursuit of antimicrobial agents with novel modes of action. Methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin resistant enterococci (VRE) are two members of an increasingly long list of resistant Gram-positive pathogens.[1] We have screened a number of our small molecule libraries against such bacterial targets. One campaign targeted bacterial amino acyl–tRNA synthetases, which were available for screening at Cubist Pharmaceuticals, Inc. These enzymes are essential for bacterial growth, being responsible for the charging of tRNA molecules with the cognate amino acids required in an essential step of protein synthesis.[2] The clinical antibiotic mupirocin (pseudomonic acid), a topically active agent for the eradication of nasal carriage of MRSA,[3] is an inhibitor of bacterial isoleucyl tRNA synthetase.[4] This paper describes work and early SAR investigations of a novel series of potent and selective small molecule inhibitors of methionyl tRNA synthetase (MetRS) derived from a high-throughput screening hit.

High-throughput screening identified two oxazolone–dipeptides (**1a** and **b**) as low micromolar inhibitors of MetRS from *Staphylococcus aureus* (SaMetRS). Significantly, follow-up screening of **1a** and **b** showed them to be selective for SaMetRS versus human MetRS (hMetRS).[5] In the case of **1a** the selectivity for SaMetRS was more than 100-fold (Table 1). Thus, these novel low molecular weight hits represented a promising starting point for SAR expansion.

Three regions of structural diversity were explored simultaneously in the first phase of SAR determination as shown in Figure 1. As illustrated in Scheme 1, reaction of the aromatic carboxylic acids **2** (activated as the acid chlorides) with glycine provided glycine amides **3**. Cyclization of **3** with a tri-substituted orthoformate in acetic anhydride at elevated temperature provided the



1a $R_1 = R_2 = Cl$, $R_3 = NH_2$ SaMetRS $IC_{50} = 0.68$ μM
1b $R_1 = Br$, $R_2 = H$, $R_3 = NH_2$ SaMetRS $IC_{50} = 1.1$ μM

Figure 1. HTS hits from small molecule library screening.

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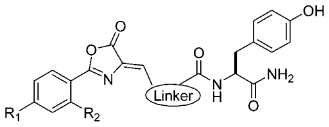
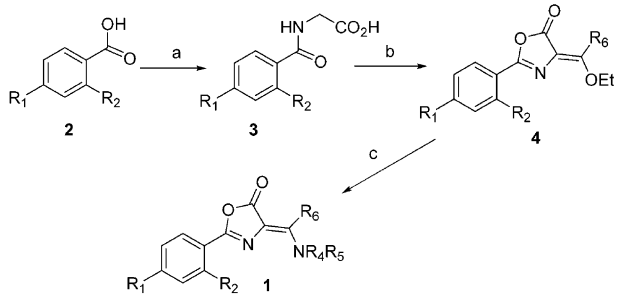
Table 1. Inhibition of MetRS by compounds with linker modifications



Compd	R_1	R_2	Linker	IC_{50} (μM)		
				SaMetRS	EfMetRS	hMetRS
1a	Cl	Cl		0.68	14.0	>100
1b	Br	H		1.1	48.0	5%@10 μM
6a	Cl	Cl		0.07	0.73	>100
6b	Br	H		0.51	1.4	>100
7a	Cl	Cl		73%@10 μM	60%@10 μM	88%@10 μM
7b	Br	H		55%@10 μM	7%@10 μM	19%@10 μM
8a	Cl	Cl		43%@10 μM	2%@10 μM	20%@10 μM
8b	Br	H		39%@10 μM	ia	23%@10 μM
9a	Cl	Cl		3%@10 μM	5%@10 μM	5%@10 μM
9b	Br	H		1%@10 μM	7%@10 μM	6%@10 μM



Scheme 1. (a) Oxalyl chloride, triethylamine, CH_2Cl_2, then glycine; (b) $R_6C(OEt)_3$, Ac_2O, reflux; (c) THF, DMSO, R_4R_5NH, 50 °C.

key intermediate substituted 4-ethoxy-5-oxazolones **4**.[6] The oxazolones **4** were then treated with a series of dipeptides, amino acids and amino acid amides to provide **1** in good yields.[7]

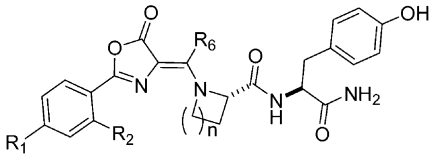
Key structural features were readily determined. In Region 1 only 2,4-dichloro- or 4-bromo phenyl substiutents were tolerated. Of the more than 100 dipeptides used in the libraries the preferred Region 2 amino acid was the cyclic amino acid L-proline. When the tyrosine in Region 3 was replaced with tryptophan or phenylalanine a substantial loss in activity was observed. Additionally, the importance of the C-terminal of the initial hits **1a** and **b** was confirmed when the corresponding acids were screened (**1c**, $R_1 = R_2 = Cl$, $R_3 = OH$; SaMetRS $IC_{50} = 3.1$ μM and **1d**, $R_1 = Br$, $R_2 = H$, $R_3 = OH$; SaMetRS $IC_{50} = 9.5$ μM).

In the next phase of SAR expansion alternatives to L-proline in Region 2 were explored. Four building blocks were prepared using standard peptide coupling methodology. Region 1 was limited to two variants (2,4-dichloro- and 4-bromophenyl) and Region 3 was fixed as an L-tyrosine amide. The four L-tyrosine amides were prepared from L-azetidine carboxylic acid, pipecolic, nipecotic, and isonipecotic acids.

In order to provide an initial assessment of the Gram-positive enzymatic spectrum for this and subsequent hit expansion libraries, screening was extended to include the MetRS from *Enterococcus faecalis* (EfMetRS). The results are presented in Table 1. Ring expansion to a 6-membered ring resulted in a substantial loss of activity against SaMetRS and ring contraction resulted in a 5–10-fold decrease in IC_{50} against SaMetRS with both L-azetidine compounds (**6a** and **6b**) displaying moderate activity for EfMetRS. In addition no significant activity against hMetRS was observed for the L-azetidine and L-proline analogues and we therefore concentrated our attention on additional structural variants containing these amino acids.

In the final phase of SAR expansion, the effect of the exomethylene ($R_6 = H$) was investigated. A series of 4-ethoxy-5-oxazolones **4** were prepared, maintaining the 4-bromo and 2,4-dichlorophenyl substitution pattern in Region 1 while the R_6-substituent was varied in size (methyl, ethyl, *n*-propyl) and electronic character (phenyl). Both L-azetidine and L-proline dipeptide amides were

Table 2. Inhibition of MetRS by L-azetidine and L-proline substituted at R_6



Compd	R_1	R_2	R_6	n	IC_{50} (μM) SaMetRS	EfMetRS	hMEtRS
10a	Cl	Cl	Me	2	0.34	6.63	>100
10b	Br	H	Me	2	1.67	>100	>100
11a	Cl	Cl	Et	2	0.18	1.28	>100
11b	Br	H	Et	2	0.85	5.93	>100
12a	Cl	Cl	*n*Pr	2	0.17	99.0	>100
12b	Br	H	*n*Pr	2	0.76	3.11	>100
13a	Cl	Cl	Ph	2	0.43	27.4	>100
13b	Br	H	Ph	2	0.81	6.14	>100
14a	Cl	Cl	Et	1	0.018	3.51	>100
14b	Br	H	Et	1	0.17	99	>100
15a	Cl	Cl	*n*Pr	1	0.04	3.52	>100
15b	Br	H	*n*Pr	1	0.087	>100	>100

retained. The screening results for this small array of compounds are shown in Table 2.

Generally increasing the size of the R_6 substituent is tolerated with all analogues showing comparable or improved potency against SaMetRS and no activity at hMetRS. Where Region 2 was set at L-proline both the 2,4-dichlorophenyl and the 4-bromophenyl analogues are most potent when the R_6 substituent is an *n*-propyl group **12a**, SaMetRS $IC_{50}=170$ nM and **12b**, SaMetRS $IC_{50}=760$ nM. However, **13b** ($R_6=Ph$) has a comparable IC_{50} to **12b** ($IC_{50}=810$ nM) suggesting that the 4-bromophenyl analogue is more tolerant of an aromatic ring at R_6 than is the case with corresponding 2,4-dichlorophenyl analogue (**13a**, $IC_{50}=430$ nM). In this case there is a more than a two-fold loss in activity.

The most potent SaMetRS inhibitors were found when Region 2 was the L-azetidine linker. This holds true for both the 2,4-dichloro- and the 4-bromophenyl analogues. However, the 2,4-dichlorophenyl is present in the two most potent analogues, **14a** and **15a**. Both compounds have IC_{50}'s below 50 nM.

While none of the analogues in this third set showed sub-micromolar EfMetRS inhibition the observed SAR shows that when Region 2 is L-proline an R_6 substituent the size of an ethyl group confers maximal EfMetRS inhibition (**11a**, EfMetRS $IC_{50}=1.3$ μM). However, when Region 2 is L-azetidine no substituent larger than $R_6=H$ is tolerated since **6a** remains the most potent inhibitor of the set (EfMetRS $IC_{50}=730$ nM, Table 2).

The most active SaMetRS inhibitor identified in this SAR exploration was **14a** (SaMetRS $IC_{50}=18$ nM). The compound had low micromolar activity at EfMetRS and was devoid of activity at hMetRS.

In conclusion, novel low molecular weight high-throughput screening hits **1a** and **b** were the starting point for a three-phase SAR expansion. Successive improvements in activity against SaMetRS led to **14a**, which had an IC_{50} of 18 nM against SaMetRS. This was a 40-fold improvement in IC_{50} from the original hit **1a**. A 4-fold improvement against the bacterial MetRS enzyme from the Gram-positive pathogen *Enterococuss faecalis* was also obtained for **14a**. In addition small modifications to the structures of **1a** and **b** were shown to elicit changes in inhibition at the two enzyme targets and as such may provide additional insight into the putative binding pockets of MetRS derived from *S. aureus* and *E. faecalis*. Disappointingly compounds from this series lacked cellular activity and were not pursued further. However, **14a** and the series from which it was obtained represent a new class of potent and selective inhibitors of *S. aureus* methionyl tRNA synthetase and may provide the basis for the design of therapeutically useful antibacterials.

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