

Discovery and SAR of a Novel Series of Pyrimidine Antibacterials Targeting Methionyl-tRNA Synthetase

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ABSTRACT

Background: Bacterial resistance to current Gram-positive antibacterial agents defines a clear medical need for novel drugs that inhibit novel targets. Previous efforts have demonstrated that potent antibacterial agents can be discovered by targeting methionyl-tRNA synthetase (MetRS) but these agents were tightly serum bound and consequently lost significant potency in serum. Consequently, new antibacterial series targeting MetRS are desired to treat systemic infections.

Methods: Compounds were prepared by synthetic routes that utilize simple pyrimidine starting materials. MICs were determined against *Staphylococcus aureus*, *Streptococcus pneumoniae* and *Enterococcus faecalis* and *Bacillus anthracis* using CLSI guidelines. Enzymatic MetRS inhibition was determined by measuring the incorporation of radiolabeled methionine into tRNA. Structure activity relationships (SAR) were developed and new compounds were designed, guided by structural information from multiple ligand-protein complexes.

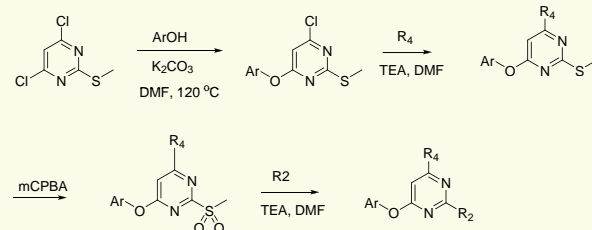
Results: Starting with a virtual screening hit with modest high micromolar activity, a pyrimidine-based series of compounds were designed that are highly potent MetRS inhibitors with good antibacterial activity versus Gram-positive pathogens (MIC < 2 µg/mL). Further rounds of structure-based drug design yielded compounds possessing good antibacterial potency with or without serum. Rx-100472 exemplifies this series: *S. aureus* (Smith) MIC 1 µg/mL (+/- 20% serum); *S. aureus* MetRS IC₅₀ 1.2 nM.

Conclusions: Structure-based drug design yielded a novel highly potent antibacterial series with Gram-positive spectrum. Due to their good antibacterial activity in serum, multiple compounds were selected for further *in vitro* and *in vivo* characterization.

METHODS

Pyrimidine analogs were prepared using the four step procedure outlined in Figure 1. The enzymatic activity of these compounds was determined by measuring the incorporation of radiolabeled methionine into methionyl-tRNA as previously described.¹ MICs were determined using CLSI guidelines.

Figure 1: Synthetic Route to Pyrimidine Analogs



RESULTS

Table 1: Triazine Analogs of the vHTS Hit

Cmpd	n	Ar	X	<i>S. aureus</i>	<i>B. anthracis</i>	SaMetRS
1	1	C ₆ H ₅	NH	>50,000*		
2	1	4-ClC ₆ H ₄	NH	32	8	8,700
3	1	2,4-Cl ₂ C ₆ H ₃	NH	16	8	222
4	0	2,4-Cl ₂ C ₆ H ₃	NH	>128	>128	1A
5	1	2-CH ₃ ,4-MeOC ₆ H ₃	NH	>128	>128	1,600
6	1	2,4-Cl ₂ C ₆ H ₃	O	>128	64	14

Starting with a triazine identified by virtual HTS,² analogs were prepared incorporating a hydrophobic aromatic and a benzimidazole group. This led to the discovery of 3 (IC₅₀ 222 nM). The structure of 3 bound to *S. aureus* MetRS suggested changing X from NH to O resulting in 6 (IC₅₀ 14 nM).

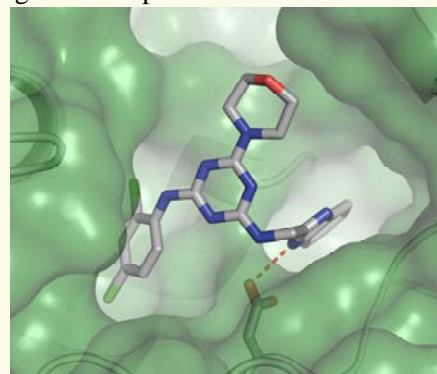
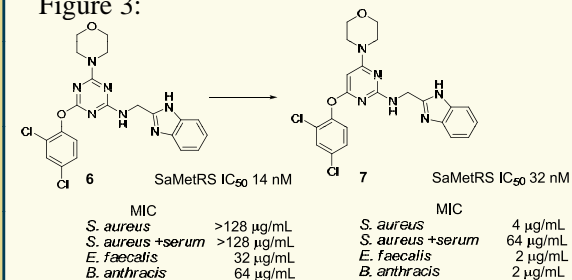
Figure 2: Cmpd 3 bound *S. aureus* MetRS

Figure 3:



While 6 has potent MetRS inhibition, it had little antibacterial activity. This led to the exploration of a pyrimidine scaffold. Pyrimidine 7 has enzymatic potency (IC₅₀ 32 nM) similar to 6 with good antibacterial activity against Gram-positive pathogens.

RESULTS

Table 2: SAR of Pyrimidine MetRS Inhibitors

Cmpd	Ar	R2	R4	MIC (µg/mL)				IC ₅₀ * (nM)
				<i>S. aureus</i>	<i>S. aureus</i> + 20% serum	<i>B. anthracis</i>	<i>E. faecalis</i>	
8	4-ClC ₆ H ₄	NMe2	NMe2	128	>128	4	8	78
9	4-ClC ₆ H ₄	NMe2	NMe2	16	128	32	16	10
10	2,4-Cl ₂ C ₆ H ₃	NMe2	NMe2	>128		16	8	777
11	2,4-Cl ₂ C ₆ H ₃	NMe2	NMe2	8	32	2	1	78
12	2-CH ₃ ,4-MeOC ₆ H ₃	NMe2	NMe2	8	128	2	2	3
13	2-CH ₃ ,4-MeOC ₆ H ₃	NMe2	NMe2	2	8	0.5	2	2.8
14	2-CH ₃ ,4-MeOC ₆ H ₃	NMe2	NMe2	2	8	0.5	2	2.4
15	2-CH ₃ ,4-MeOC ₆ H ₃	NMe2	NMe2	1	1	0.125	0.125	1.3
16	2-CH ₃ ,4-MeOC ₆ H ₃	NMe2	NMe2	2	2	0.25	0.25	1.4
17	2-CH ₃ ,4-MeOC ₆ H ₃	NMe2	NMe2	0.5	2	0.25	0.25	1
18	2-CH ₃ ,4-MeOC ₆ H ₃	NMe2	NMe2	0.125	1	0.06	0.06	2.8
19	2-CH ₃ ,4-MeOC ₆ H ₃	NMe2	NMe2	0.25	1	0.06	0.06	0.8
20	2-CH ₃ ,4-MeOC ₆ H ₃	NMe2	NMe2	0.25	1	0.06	0.125	2

**S. aureus* MetRS assay uses 1-5 nM of enzyme; all IC₅₀s below 10 nM are at the limit of the assay

SAR Notes

A set of pyrimidine analogs were prepared with the goal of increasing the antibacterial potency, especially in the presence of serum (Table 2).

The aminomethylene-benzimidazole group is preferred at R4. As shown by sets 8-9, 10-11, 12-13

Incorporation of a 2-chloro-4-methoxyphenyl group increases antibacterial activity in serum. See compounds 12-14

Based on the structures of 13 and 14 bound to *S. aureus* MetRS, a second methoxy group was added to increase solubility and to restrict rotation of the 4-methoxy group that observed in the bound conformation.

The resulting compounds 15 (Rx-100,472) and 16 have excellent antibacterial potency that is not affected by the presence of serum.

Exploration of the R2 group led to compounds 17-20 that have additional interactions with the enzyme, providing more antibacterial potency

RESULTS

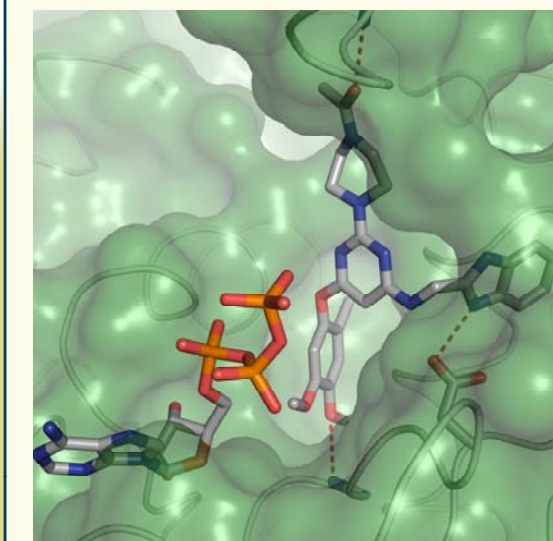
Figure 4: Cmpd 15 bound to *S. aureus* MetRS

Figure 4 shows the structure of 15 bound to *S. aureus* MetRS illustrating the additional hydrogen bonding interaction of the 4-methoxy group.

SUMMARY

Structure-Based Drug Design generated the pyrimidine series of highly potent MetRS inhibitors with Gram-positive antibacterial spectrum.

Structural information was used to guide the optimization of a hit compound with weak MetRS inhibition into a series of antibacterial agents with good potency and spectrum.

Unlike previous series of MetRS-based antibacterial agents, multiple members of the pyrimidine series retain their antibacterial potency in the presence of serum.

LITERATURE CITED

- 1) R. Macarron, L. Mensah, C. Cid, C. Carranza, N. Benson, A. Pope, E. Diez; *Anal. Biochem.* **2000**, *284*, 183
- 2) J. Finn, M. Stidham, M. Hilgers, Kedar GC. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 3932-3937

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