

Identification of Novel Inhibitors of Methionyl-tRNA Synthetase by Virtual Screening

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ABSTRACT

Background: New antibacterial agents that act via novel mechanisms are needed to combat bacterial resistance. Although previous studies have demonstrated that inhibition of bacterial methionyl-tRNA synthetase (MetRS) can result in potent antibacterial activity, these agents lose significant potency in serum. Hence, there is a need to identify new classes of MetRS inhibitors that have structural features more amenable to systemic treatment.

Methods: Crystallization of MetRS-ligand complexes were conducted in standard hanging drop chambers. Conditions for crystallization were identified by Trius' high-throughput structural biology system that allows low volume scanning of conditions. Using the structural information for MetRS-ligand complexes, pharmacophore models were designed to search large commercial libraries of small molecule compounds. Compounds that fit the model were purchased and MetRS activity was measured by monitoring the incorporation of radiolabelled methionine into tRNA.

Results: Based on the solved structures of multiple ligand-*S. aureus* MetRS complexes, we constructed a Catalyst® pharmacophore that was then used to screen a large commercial library. The top scoring ligands were then docked into SaMetRS using LigandFit® in flexible mode. A set of 32 high-scoring MetRS ligands were selected for screening against *S. aureus* MetRS at 100 µM. The IC₅₀ was determined for the 17 compounds that demonstrated >70% inhibition. Six new series of active compounds were identified with several compounds demonstrating an IC₅₀ <10 µM.

Conclusions: Using a structure-based drug design approach, we have identified novel MetRS inhibitors. These are new starting points for structure-guided optimization.

MATERIALS AND METHODS

Multiple potent small molecule MetRS inhibitors have been disclosed (Fig 1).¹⁻⁴ Several constructs of the *S. aureus* MetRS enzyme were surveyed for crystallization behavior using the Fluidigm Topaz® microfluidics system. Diffraction-quality crystals were grown with a single truncation of the enzyme. An initial structure was determined by molecular replacement, to 1.7Å, using a search model derived from the coordinates of the *Thermus aquaticus* MetRS (PDB ID 1A8H).

Virtual screening was conducted in two phases. In the first phase a Catalyst® pharmacophore was constructed from the ligand-MetRS structure using the Structure-Based Focusing tools in Cerius2 (Accelrys). This pharmacophore was used to search commercial compound databases.

The hits from the catalyst search were then docked into the *S. aureus* MetRS structure using the LigFit® program (Accelrys) in flexible mode. The results from this docking were scored and the top scoring compounds selected after visual inspection of the "docked" structure.

Enzymatic inhibition was determined by measuring the inhibition of the incorporation of radioactive methionine into protein.

Figure 1: Structures of known MetRS inhibitors

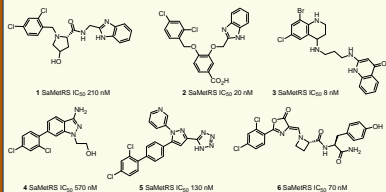


Figure 2: Overview of VHTS Process

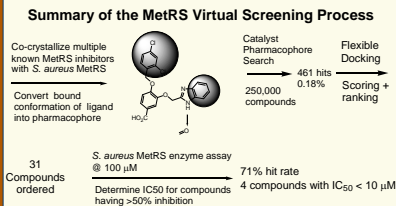


Figure 3: Structure of compound 2-*S. aureus* MetRS complex



Figure 4: Catalyst® Pharmacophore *S. aureus* MetRS Model

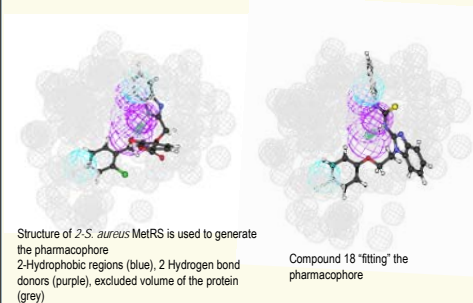


Figure 5: Docked Fit of 22 Bound to *S. aureus* MetRS

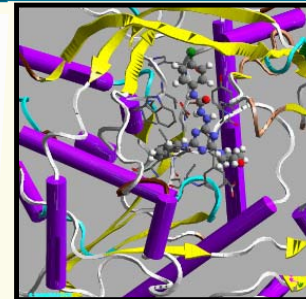


Table 1: *S. aureus* MetRS inhibition of the vHTS hits

ID	MOLSTRUCTURE	% of control @ 100 µM	SaMetRS IC ₅₀ µM	ID	MOLSTRUCTURE	% of control @ 100 µM	SaMetRS IC ₅₀ µM
7		13.99	4.80	18		19.93	6.3
8		16.02	46.30	19		16.95	5.7
9		13.38	46.50	20		22.15	47% inh @ 100 µM
10		39.02	40.90	21		45.32	8.3
11		22.96	23.60	22		19.39	34% inh @ 40 µM
12		27.86	41.70	23		24.14	52% inh @ 40 µM
13		8.87	94.90	24		17.66	56
14		17.49	47% inh @ 100 µM	25		34.40	25.3
15		7.85	11.50	26		32.93	86.8
16		47.54	35.6	27		8.94	35% inh @ 40 µM
17		18.23	45% inh @ 100 µM	28		19.99	26.6

RESULTS

- Figure 2 provides an overview of the virtual screening process. Overall 70% of the compounds ordered were inhibitors of *S. aureus* MetRS at 100 µM. This result is contrasted to the 0.05% hit rate from traditional HTS
- Figure 3 shows the structure of compound 2 bound to *S. aureus* MetRS
- Figure 4 shows the Catalyst pharmacophore that was developed using the structure of 2 bound to MetRS. This pharmacophore consists of 2 hydrophobic regions (blue spheres), 2 hydrogen bond donors (purple spheres) and multiple excluded volumes (grey spheres) that reflect the protein structure
- Figure 5 shows the "docked" structure of compound 22 bound to *S. aureus* MetRS. Notice that all 4 pharmacophoric points are satisfied
- Figure 6 shows the inhibition data for the 22/31 compounds that displayed >50% inhibition at 100 µM
- Overall, these results demonstrate that multiple new series of active compounds were identified with several compounds demonstrating an IC₅₀ <10 µM. These hits are significantly different from the known compounds and in many cases are missing the dihalophenyl and benzimidazole groups present in the older inhibitors

SUMMARY & CONCLUSIONS

- The use of structural information from ligand-protein complexes is very productive in virtual screening. In the case of *S. aureus* MetRS, this strategy yielded a 13% hit rate of inhibitors with potency of 10 mM or less.
- S. aureus* MetRS is an attractive antibacterial target because there are numerous starting scaffolds for optimization
- The results of this study indicate that it will be possible to design more drug-like *S. aureus* MetRS inhibitors as multiple compounds in this study lack both the di-halogenated phenyl ring and the benzimidazole/quinoline functionalities of earlier compounds

LITERATURE CITED

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