

# STRUCTURE-GUIDED DESIGN OF PYRIMIDINE ANTIBACTERIALS TARGETING METHIONYL-tRNA SYNTHETASE (MetRS)

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## ABSTRACT

**Background:** Enzyme-inhibitor co-crystal structures are of great utility in the discovery of new classes of antibacterial agents. Although structures of compounds bound to Gram-negative MetRS are known, there is significant sequence diversity between the Gram-negative and Gram-positive enzymes. Consequently, the structures of compounds bound to a Gram-positive MetRS ortholog are critical in the design of potent inhibitors with Gram-positive spectrum.

**Methods:** Multiple constructs of four Gram-positive orthologs were screened for crystallization using the Fluidigm Topaz instrument. The best construct, a truncated variant of the *S. aureus* enzyme, yielded crystals that allowed for 1.8Å structure determination by molecular replacement. Once enabled, subsequent iterations of structure-based design were conducted.

**Results:** Analysis of the structures of inhibitors bound to *S. aureus* MetRS identified several key interactions that provide enzymatic potency, including binding to Asp-51, and interactions with two distinct hydrophobic pockets. Initial structures also revealed an adjacent pocket, formed by the interface of two sub-domains of the enzyme, representing opportunities for additional interactions. Through several rounds of synthesis, testing, crystallography and design, we advanced a 100 micromolar MetRS virtual screening hit with no antibacterial activity to a series of pyrimidines with highly potent enzymatic ( $IC_{50}$  1 nM) and antibacterial activity (*S. aureus* MIC 1 µg/mL). Further structure-based optimization provided compounds with increased solubility and no increase in MIC values in the presence of serum. In addition, we were able to rationally design compounds with novel interactions in the "interface cleft", including a hydrogen bond with Tyr-235.

**Conclusions:** Iterative cycles of structure based drug design yielded compounds with both improved potency and drug properties including reduced serum binding. Continued use of structure-guided optimization provides a method to improve *in vivo* properties.

## MATERIALS AND METHODS

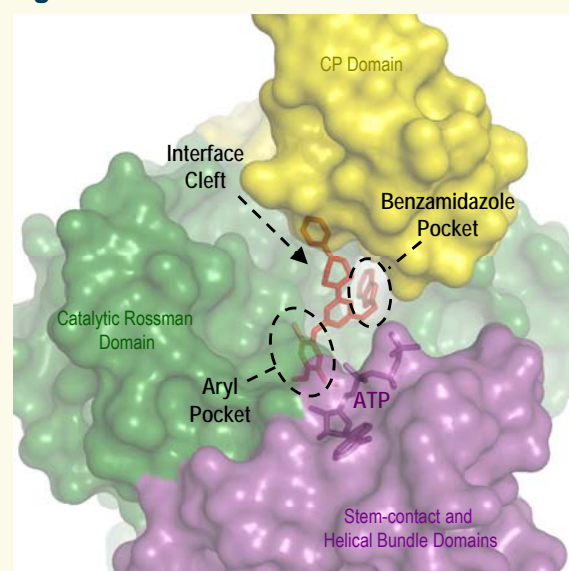
**Protein Preparation and Crystallization:** Four full-length Gram-positive MetRS orthologs were cloned into a modified pET vector encoding an N-terminal TEV protease-cleavable affinity tag. Several C-terminal truncations of each were generated through the introduction of stop codons at different sites, roughly guided by sequence alignments with previously crystallized MetRS orthologs. Each of these constructs were expressed, purified, and subjected to nanovolume crystallization trials with a Fluidigm Topaz crystallization system. Although preliminary crystallization hits were obtained with several constructs, a single *S. aureus* construct gave crystals that diffracted to high resolution (1.8Å).

## MATERIALS AND METHODS

**Structure Determination and Co-crystallography:** The structure of *Thermus thermophilus* MetRS (PDB ID 1A8H; 39% sequence identity) was used as a Molecular Replacement search model. Although apparent solutions were found using both PHASER and MOLREP, subsequent refinement with REFMAC5 and manual model building in COOT did not result in a meaningful lowering of  $R_{Free}$ . Inspection of the available MetRS structures suggested that subtle differences in the orientation of several portions of secondary structure could be responsible. Therefore, the initial molecular replacement hit was broken into eight independent fragments, with boundaries determined with the TLS Motion Determination Server (1), and then subjected to rigid body refinement. This novel combination of TLSMD and rigid body refinement improved the model such that the structure of the enzyme could be built and fully refined (final  $R_{Work}$ =17.7%,  $R_{Free}$ =21.9%). Subsequent co-crystal structures were solved, in the same space group (P2<sub>1</sub>2<sub>1</sub>1, 1 copy per A.S.U.), by routine molecular replacement.

## RESULTS

### Fig 1. Structure of *S.aureus* MetRS

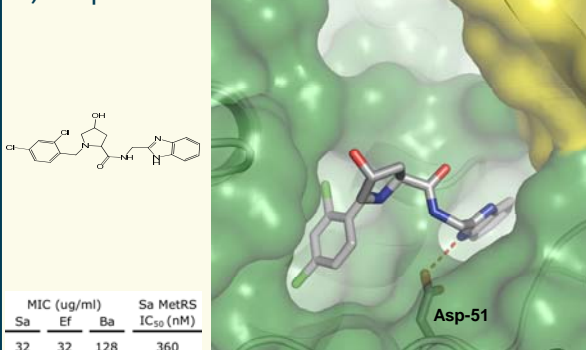


The domains and inhibitor binding pockets of MetRS. The initial structure(s) revealed binding pockets exploited by previously described inhibitors (the Aryl and Benzamidazole sites), and also a new potential site (the interface cleft). The more advanced Compound 4 is shown (in red) to illustrate the three pockets.

## RESULTS

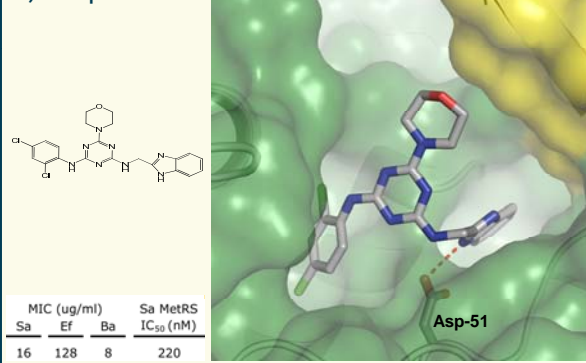
### Fig 2. Compound Progression: A Key Subset of MetRS-Inhibitor Co-crystal Structures

#### A) Compound 1



The structure of Compound 1, a member of an early Proline-based series, revealed two major binding pockets, in addition to the core Hydrogen bond with the catalytic Asp-51. The Aryl pocket, which overlaps with the Methionine-binding site, is more highly conserved than the Benzamidazole pocket. Mutations in the Benzamidazole pocket have been shown to be responsible for resistance (2). Several weak inhibitors, identified by virtual screening, suggested possible modifications of the core scaffold and dichlorophenyl group (3). (For MICs tables: Sa = *S. aureus*; Ef = *E. faecalis*; Ba = *B. anthracis*)

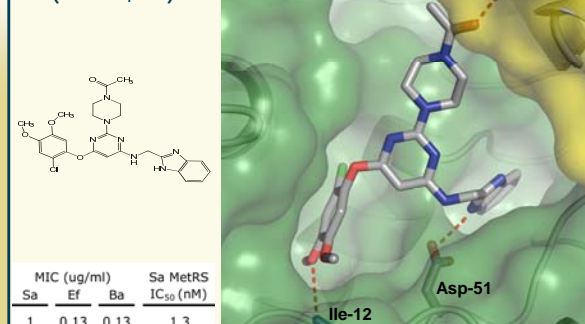
#### B) Compound 2



The Triazine represented an excellent scaffold for positioning groups in the appropriate binding pockets, and suggested the possibility of exploiting the interface cleft (here with a Morpholino group). None of the Nitrogens of the Triazine were found to participate in Hydrogen bonding, so Pyrimidine and Pyridine scaffolds were explored.

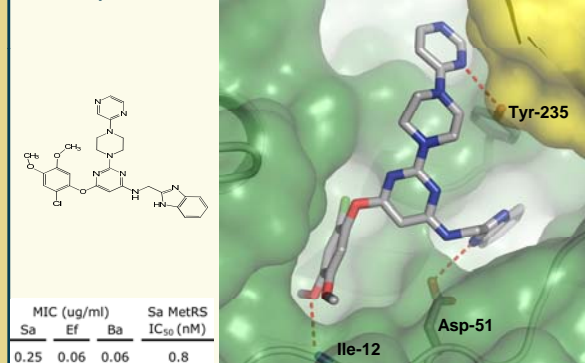
## RESULTS

#### C) Compound 3 (Rx-100,472)



The structure of Compound 3 illustrates several improvements including a 2-Chloro-3,4-dimethoxyphenyl group in the Aryl pocket that gave improved solubility, and increased potency (in part due to a new H-bond with the amide of Ile-12). Further, the switch to the Pyrimidine scaffold gave increased potency and a large improvement in MICs. A Hydrogen bond between the Acetyl group and the amide of Tyr-134 also contributes to potency.

#### D) Compound 4



Compound 4 was a successful attempt to rationally design additional interactions in the interface cleft. A second pyrimidine ring gave both Van der Waals interactions and an additional H-bond with a rearranged Tyr-235. These interactions increased potency as evidenced by lower MICs compared to previous compounds (e.g., compound 3).

## SUMMARY & DISCUSSION

- ◆ Truncation scanning yielded a construct that was amenable to routine, high-resolution co-crystal structure determination, thus enabling structure-based drug design for Gram-positive MetRS
- ◆ Once SBDD-enabled, information from virtual screening and previous scaffolds was synthesized and a new series of inhibitors was designed and optimized with respect to both potency and properties
- ◆ X-ray structures of MetRS revealed a novel pocket, at the interface of the catalytic and CP domains, that was exploited in compound design for additional potency
- ◆ Further potency gains, particularly those achieved in the interface cleft, may permit the design of compounds that do not rely on interactions in the Benzamidazole pocket, a less conserved region that may be susceptible to mutations leading to resistance

## LITERATURE CITED

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