

STRUCTURE-BASED DESIGN OF NOVEL 7-SUBSTITUTED DIAMINOQUINAZOLINE ANTIBACTERIAL AGENTS TARGETING DIHYDROFOLATE REDUCTASE (DHFR)

M. HILGERS, T. LAM, X. LI, J. ZHANG, Z. CHEN, M. TRZOSS, C. CREIGHTON, L. KOHEN, M. CUNNINGHAM, B. KWAN, K. NELSON, M. STIDHAM, V. BROWN-DRIVER, K. J. SHAW, J. FINN
Trius Therapeutics Inc., San Diego, CA

ABSTRACT

Background: Multidrug-resistant gram-positive pathogens are a major cause of morbidity and mortality. Trimethoprim (TMP), a safe and frequently prescribed antibiotic, has become less effective due to the emergence of TMP-resistant forms of DHFR, its molecular target. Here we report a structure-guided effort to design next-generation DHFR inhibitors demonstrating increased potency and activity against both TMP-susceptible and -resistant strains.

Methods: Crystallization conditions were identified for *S. aureus* DHFR such that high-resolution (1.7 Å) inhibitor-bound co-crystal structures could be rapidly determined. This enabled an iterative optimization process of co-crystallization, structure-determination, characterization, and new compound synthesis.

Results: Enzymatic potency for a series of 7-Aryl-2,4-diaminoquinazolines was quickly improved >10-fold against *S. aureus* DHFR. Structures of these inhibitors bound to DHFR, presented here, revealed that potency gains were achieved through active site interactions adjacent to the NADP⁺ sub-site, or through interactions with residues involved in binding the aminophenyl portion of the folate substrate. The design of compounds that bridge both regions led to a second series, the 7-[Benzimidazol-1-yl]-2,4-diaminoquinazolines. In addition to a further 10-fold gain in potency, this second series displayed impressive selectivity (as large as 200,000-fold) due to active site differences between the human and *S. aureus* enzymes.

Conclusions: A detailed molecular understanding of the binding of two series of 7-substituted diaminoquinazolines led to the design of DHFR agents with good gram-positive spectrum, excellent selectivity, and activity against susceptible and TMP-resistant strains.

MATERIALS AND METHODS

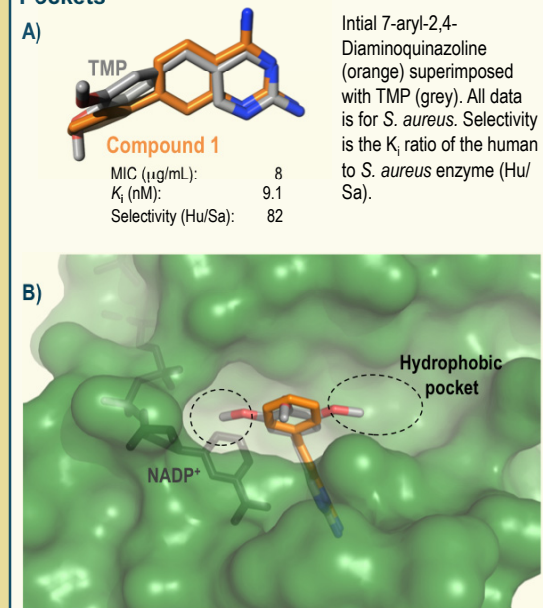
Protein Preparation and Crystallization: The gene for *S. aureus* DHFR was amplified from genomic DNA, cloned into pET28 (Novagen), and expressed in BL21-AI (Invitrogen). The resulting construct, containing a C-terminal 6XHis tag, was purified with Nickel-chelating resin, and screened against several commercial sparse matrix screens (Qiagen, Hampton Research). One of the resulting crystallization leads was optimized such that high-resolution data sets (~1.7Å) could be collected with a standard in-house rotating anode X-ray source. Although the crystallization conditions identified were novel (and the resulting crystals diffracted to higher resolution), the space group is the same as that previously reported for *S. aureus* (P6₂22 with similar cell parameters).¹

MATERIALS AND METHODS

Structure Determination and Co-crystallography: Because no *S. aureus* DHFR structures had been deposited in the PDB at the time, the initial structure was solved by molecular replacement with Phaser, using an *E. coli* structure (PDB identifier 1RA3) as the search model. Subsequent structures were solved using this initial structure. All structures were refined with Refmac5, resulting in good stereochemistry and statistics (e.g., $R_{work} = 0.19$, $R_{free} = 0.21$). Complexes were prepared through co-concentration of protein, NADP⁺ and inhibitor, followed by co-crystallization; all crystallized in the same space group with similar cell dimensions.

RESULTS

Fig 1. 7-aryl-2,4-Diaminoquinazolines Present Opportunities for Accessing Key Binding Pockets

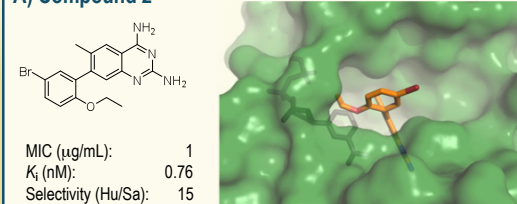


- ◆ Superposition as in (A), but with protein depicted as a solvent accessible surface.
- ◆ Substitutions of the 7-aryl would allow for the design of interactions with binding pockets that are conserved, and known to be important for the potency of diaminopyrimidines such as TMP and Iclaprim.^{1,2}

RESULTS

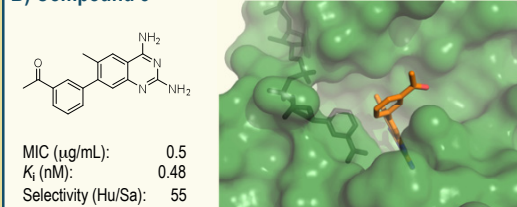
Fig 2. The 7-aryl-2,4-diaminoquinazoline Series: Representative Crystal Structures Illustrate Compound Progression

A) Compound 2



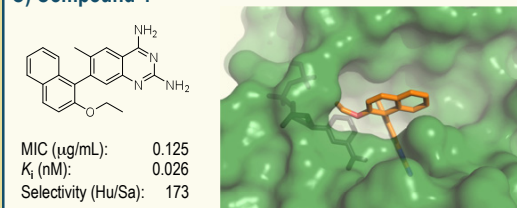
Compound 2 showed how potency could be achieved through interactions in the cavity adjacent to the NADP⁺ binding site. Several inhibitors in this series were found to pack against the nicotinamide ring.

B) Compound 3



Some substituents led to binding of the hydrophobic pocket. However, predicting the orientation, whether toward the NADP⁺ or hydrophobic pockets, was difficult for smaller groups.

C) Compound 4

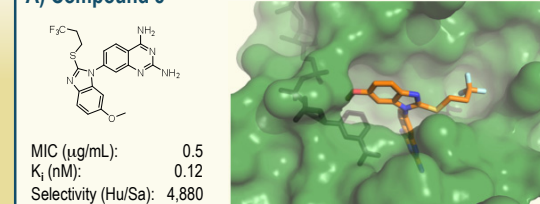


Additional inhibitors were designed to bind both pockets such that large gains in potency were possible. However, selectivity was only modestly improved.

RESULTS

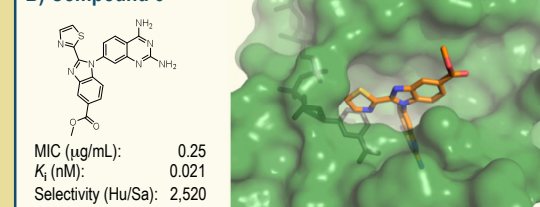
Fig 3. The 7-[Benzimidazol-1-yl]-2,4-diaminoquinazolines Series: Improved Potency and Selectivity

A) Compound 5



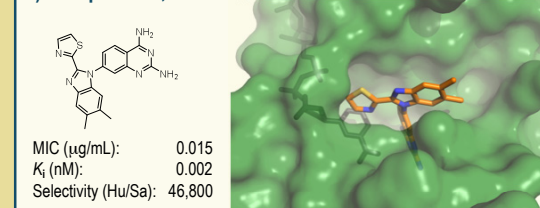
Inspired by larger members of the 7-aryl series, multi-ring heterocycles were explored. Compound 5 had excellent contacts with the hydrophobic pocket, but interactions with the NADP⁺ pocket were not optimized.

B) Compound 6



Benzimidazoles substituted at the 2-position with small heterocycles resulted in a 180° rotation of the scaffold such that tight interactions were achieved adjacent to the NADP⁺. However, further design was required to optimize interactions with the hydrophobic pocket.

C) Compound 7, Rx-101005



The dimethylbenzimidazole increased potency through additional contacts with the bottom of the hydrophobic pocket. It also more rigidly directs the thiazole into the NADP⁺ pocket. This likely causes steric clashes with the smaller pocket of the human enzyme, yielding enhanced selectivity (see poster F1-836).

SUMMARY AND DISCUSSION

- ◆ The 7-aryl-2,4-diaminoquinazoline scaffold uniquely accesses binding pockets of DHFR, previously explored with 5-benzyl-2,4-diaminopyrimidines, providing a new class of antibacterial.
- ◆ Substitutions of the aryl group of the 7-aryl-2,4-diaminoquinazolines allowed for the exploration of two adjacent sub-pockets known to impart potency. These substituents led to >20-fold gains in enzyme potency through Van der Waals contacts with the enzyme, as well as improvement in MICs.
- ◆ The 7-aryl-2,4-diaminoquinazoline series suggested the design of multi-ring systems that better exploited interactions in the key binding pockets. Of these, the 7-[Benzimidazol-1-yl]-2,4-diaminoquinazolines showed particular promise.
- ◆ 7-[Benzimidazol-1-yl]-2,4-diaminoquinazolines with heterocycles at the 2-position of the benzimidazole imparted both great potency and selectivity. Relative to our initial 7-aryl series, enzyme potency is improved nearly 400-fold, and selectivity by more than 500-fold.

LITERATURE CITED

- 1) Dale G.E., et al. (1997) Journal of Molecular Biology 266, 23-30
- 2) Oefner, C. et al. (2009) Journal of Antimicrobial Chemotherapy 63, 687-698