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Lead compounds and key residues of ribosomal protein S1 in drug-resistant *Mycobacterium tuberculosis*

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ABSTRACT

Ribosomal protein S1 (RpsA) has been identified as a novel target of pyrazinoic acid (POA), which is the active form of pyrazinamide (PZA), in vivo. RpsA plays a crucial role in trans-translation, which is widespread in microbes. In our investigation, we first described the discovery of promising RpsA antagonists for drug-resistant mycobacterium (MtRpsAd438A) and *M. smegmatis*, as well as wild-type *M. tuberculosis*. These antagonists were discovered via structure/ligand-based virtual screening approaches. A total of 21 targeted compounds were selected by virtual screening, combined scores, affinity, similarities and rules for potential as drugs. Next, the affinities of these compounds for three targeted proteins were tested in vitro by applying various technologies, including fluorescence quenching titration (FQT), saturation transfer difference (STD), and chemical shift perturbation (CSP) assays. The results showed that seven compounds had a high affinity for the targeted proteins. Our discovery set the stage for discovering new chemical entities (NCEs) for PZA-resistant tuberculosis and providing key residues for rational drug design to target RpsA.

1. Introduction

Tuberculosis (TB) is an infectious disease caused by the bacillus *Mycobacterium tuberculosis (Mtb)*. TB causes poor health in millions of people each year and is one of the top 10 causes of death worldwide, ranking above HIV/AIDS as one of the leading causes of death from an infectious disease in 2015 according to the WHO Global Tuberculosis Report 2016 [1]. The currently recommended treatment for new cases of drug-susceptible TB is a 6-month regimen of four first-line drugs: isoniazid, rifampicin, ethambutol and pyrazinamide (PZA). PZA plays a unique role in reducing the period of TB treatment from the 9–12 months required prior to its introduction to the current standard of 6 months, which is often referred to as short-course chemotherapy [2]. However, the ability to cure TB caused by multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains of *Mtb* is more difficult, requiring treatment for up to 2 years with more toxic and costly second- and third-line drugs, often with limited success [3]. With the spread of PZA-resistant *Mtb*, there is an urgent need to develop new drugs to shorten the regimen [4,5].

PZA, which is a prodrug, is converted into its active form, pyrazinoic acid (POA), by mycobacterial pyrazinamidase (PZase, encoded by pncA) [6,7]. PZA has been characterized in pyrazinamide-resistant spontaneous mutants and clinical isolates of *Mtb* [8]. Among these mutants, the genes pncA [8–11] and rpsA [12] are most important. PZA-resistant *Mtb* strains have mutations in pncA that prevent the conversion of PZA to POA. Mutations of rpsA, coding for Ribosomal Protein S1 (RpsA), a vital protein that is involved in protein translation and in the ribosome-sparing process of trans-translation, lead to POA inactivation [13]. Therefore, targeting RpsA and the trans-translation mechanism are expected to be important in the design and development of novel drugs to overcome PZA resistance due to drug-resistant RpsA mutations [13–15].

As a target of POA, RpsA plays two important roles in ribosome function. When *Mtb* is living in conditions that enable it to reproduce exponentially, RpsA binds to upstream sequences of mRNA to ensure connectivity to the 30S ribosomal subunit and thus efficient translation. In contrast, in poor conditions, e.g., during starvation, RpsA engages in trans-translation, which “spares” ribosomes by restarting those that “stalled” during the process of decoding mRNA.[15] The binding of POA to RpsA results in the inhibition of trans-translation (Fig. 1

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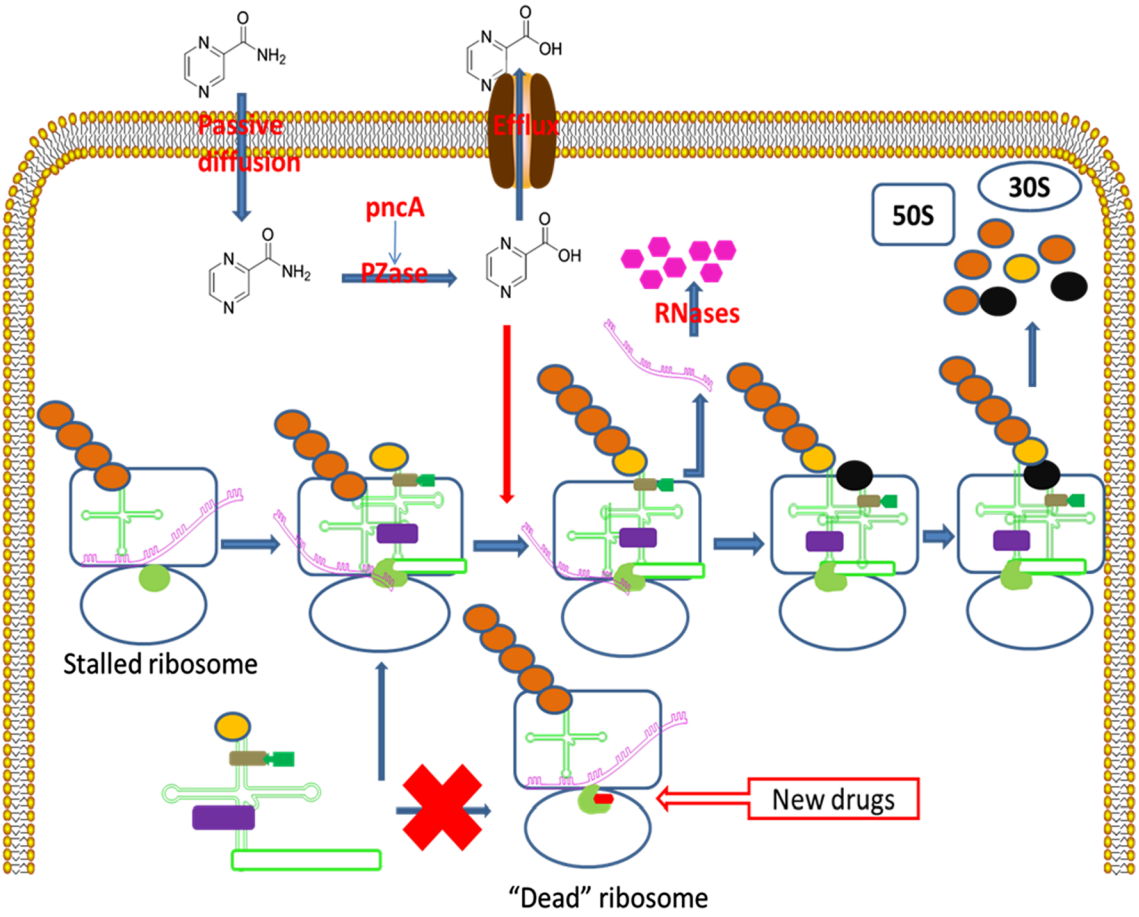

Fig. 1. Diagram of trans-translation and the PZA resistance mechanism.

[7,13–19]).

In 2011, investigators discovered PZA-resistant strains caused by mutations in the wild-type RpsA (MtRpsA) that were the result of a deletion of Ala438 [13] (MtRpsAd438A). Researchers also discovered differences in RpsA that were associated with intrinsic PZA resistance in *M. smegmatis* (MsRpsA) [17]. Previously, we successfully resolved the crystal structures of apo-MtRpsA, MtRpsA with POA, MtRpsAd438A and MsRpsA [14,20]. In this study, we discovered RpsA antagonists by virtual screening of the RpsA protein and the POA ligand and identified the key residues of the binding site. All compounds were tested for their affinities for RpsA in vitro by fluorescence quenching titration, saturation transfer difference (STD) and chemical shift perturbation (CSP) assays.

2. Results

2.1. Virtual screening

POA specifically binds to RpsA and blocks the trans-translation process in *Mtb* and is used to shorten the duration of TB treatment [21]. The crystal structure of the POA-MtRpsA complex has been resolved (PDB code: 4NNI). To obtain targeted RpsA antagonists to block the translation process, we used two virtual screening processes that included protein-based drug design and ligand-based drug design (Fig. 2).

2.1.1. Protein-based virtual screening

2.1.1.1. Identification of key amino acids

2.1.1.1.1. Conserved amino acid analysis. By aligning the 10 amino acid sequences of RpsA from different strains, we identified several conserved key amino acid regions, including 307–313, 317–326, 348–352, and 356–360 (Supplementary Fig. 1). These highly conserved amino acids are closely related to the intrinsic function of RpsA and might be the key amino acids that would need to be blocked.

2.1.1.1.2. Analysis of the crystal complexes. By analyzing the structure of the POA-MtRpsA complexes, Arg357, Phe310, Phe307 and Lys303 were identified as the key amino acids in the interaction. Among these amino acids, Arg357 interacts through hydrogen bonds with the carboxyl O, whereas Lys303 interacts with the N of the pyrazine ring of POA to form a hydrogen bond. In addition, Phe307 and Phe310 form a Π-Π interaction with the pyrazine aromatic ring of POA (Fig. 3a). Among the three protein structures (apo-MtRpsA, MtRpsAd438 and MsRpsA), Arg357 on MtRpsAd438 and MsRpsA is far from the phenylalanines (Phe307, Phe310). This results in the inability of POA to interact with the benzene ring to form the Π-Π interaction while forming hydrogen bonds with Arg357, which significantly reduces the affinity of POA for MtRpsA (Fig. 3d–g). This may be the reason that POA is inactivated in the MtRpsA mutation. This finding suggested that we needed to extend the distance between the pyrazine ring and the carboxyl group in designing a drug that targets the mutant RpsA.

2.1.1.1.3. Molecular dynamics simulation and protein structure comparison analysis. The results of molecular dynamics simulation of the crystal structures show that the pocket for the POA interaction is smaller in the mutated protein and that the guanidine group of Arg357 is rather flexible, suggesting that the binding of small molecules to RpsA by their interaction with Arg357 is a prerequisite for their activity. However, Lys303, Phe310, Phe307 and Arg357 are still the key residues that constitute the binding pocket (Fig. 4). Additionally, other residues, including Glu319, Leu320 and His322, may play prominent roles in the ligand interaction (Fig. 4).