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Original article

Design, synthesis, molecular docking and 3D-QSAR studies of potent inhibitors of enoyl-acyl carrier protein reductase as potential antimycobacterial agents

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ABSTRACT

In order to develop a lead antimycobacterium tuberculosis compound, a series of 52, novel pyrrole hydrazine derivatives have been synthesized and screened which target the essential enoyl-ACP reductase. The binding mode of the compounds at the active site of enoyl-ACP reductase was explored using surflex-docking method. The binding model suggests one or two hydrogen bonding interactions between pyrrole hydrazones and InhA enzyme. Highly active compound **5r** (MIC 0.2 μg/mL) showed hydrogen bonding interactions with Tyr158 and NAD^+ in the same manner as those of ligands PT70 and triclosan. The CoMFA and CoMSIA models generated with database alignment were the best in terms of overall statistics. The predictive ability of the CoMFA and CoMSIA models was determined using a test set of 13 compounds, which gave predictive correlation coefficients (r^2_{pred}) of 0.896 and 0.930, respectively.

1. Introduction

Tuberculosis (TB), is a major chronic infectious diseases caused by *Mycobacterium tuberculosis* (*M. tuberculosis*) and to a lesser degree by *Mycobacterium bovis* and *Mycobacterium Africanum*, affects nearly 32% of the World's population with about 9.4 million Worldwide and 1.6–2.4 million cases alone in India [1,2]. The disease has been the leading cause of morbidity and mortality among the infectious diseases. To address these issues, research and developmental activities to develop novel and potent new chemical entities are necessary.

InhA, the enoyl acyl carrier protein reductase (ENR) from *M. tuberculosis,* is one of the key enzymes involved in mycobacterial fatty acid elongation cycle, which has been validated as an effective antimicrobial target. Inhibition of mycolic acid biosynthesis is the first event detected in *M. tuberculosis* treated with isoniazid (INH) [3], and numerous observations indicate that INH treatment causes extensive damage to the envelope organization, such as loss of acid-fast property [4], release of abnormal amount of proteins into the culture media and altered ultrastructure [5]. As a prodrug, INH must first be activated by KatG, a catalase-peroxidase that oxidizes INH to an acyl-radical which then forms a covalent adduct (INH-NAD) with NAD^+, the co-substrate for InhA. The INH-NAD adduct then functions as a potent inhibitor of InhA [6].

Triclosan is present in a wide variety of consumer products, such as mouthwashes, toothpaste, and hand soaps [7], and is a widely used broad-spectrum biocide [8], despite its intravenous toxicity and biocidal component warranting against its systemic use [9]. Its active site entry results in the reordering of amino acids, making it a slow and tight-binding inhibitor [10] with a long residence time that is correlated with its *in vivo* activity [11].

Three-dimensional quantitative structure activity relationships (3D QSAR) methods, such as comparative molecular field analysis (CoMFA) [12] have been successfully applied to guide the design of new bioactive molecules [13]. The study has extended CoMFA and the more recently introduced comparative molecular similarity indices analysis (CoMSIA) [14,15] and other 3D QSAR

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methodologies to potent antitubercular agents to gain better insights as to how steric, electrostatic, hydrophobic, and hydrogen-bonding interactions influence their activity, and thus derive the predictive 3D QSAR models for designing and forecasting the activity of InhA inhibitors of this class.

In the present study, design, synthesis, computation and evaluation of potential InhA inhibitors having pyrrole as the core structure are attempted. Other chemotherapeutically-active groups into the structure are introduced, with a hope to impart synergism to the target compounds (Fig. 1). Hydrazones, characterized by the presence of –NH–N=C– group, play an important role to exhibit antimicrobial activity [16]. Substituted carbohydrazone moiety has been found to be a good pharmacophore group for many antituberculosis active compounds [17–20]. However, some widely used antitubercular drugs such as thioacetazone, phtivazid, salinazid, verazide and opiniazide are also known to contain this group (Fig. 2).

As early as 1953, it was reported that some pyrrole derivatives showed *in vitro* antitubercular activity [21,22], but this keen observation was not given concerted follow-up at that time. More recently, major work has resumed on antitubercular drug design using pyrroles as templates for synthesis [23,24], including well-designed molecular modeling studies in conjunction with laboratory experiments [25–28]. Biava and co-workers have reported several 1,5-diarylpyrrole derivatives with very good activity against MTB (BM 212), On the basis of work by Deidda et al. [29], Lupin has synthesized a series of pyrrole compounds, one of which (LL3858) is currently in clinical development for the treatment of TB [30].

A database search shows that several pyrrole hydrazone derivatives have been developed against *M. tuberculosis* [23,24,31]. The main objective of this study is to design novel pyrrole hydrazones as specific inhibitors of *M. tuberculosis* and to further explore these entities as potential and novel antitubercular lead candidates. Earlier, we have reported antimycobacterial activity of pyrroles [24,27,28]. In continuation of these studies, the present investigation deals with the development of potent antitubercular compounds with selective inhibition of enoyl acyl carrier protein reductase. Interest for this study stems from the presence of aryloxy moiety in many of the compounds, a common structural feature found in potent and potentially useful InhA inhibitors, such as 5-hexyl-2-(2-methylphenoxy)phenol (PT70 or TCU), 5-chloro-2-(2,4-dichloro phenoxy) phenol (TCL), a ligand in the published X-ray structure of InhA enzyme. In addition to 3D QSAR analyses, docking simulations were performed using the only published X-ray crystallographic structure of *M. tuberculosis* InhA (ENR) complexed with 5-hexyl-2-(2-methylphenoxy)phenol (PT70) and co-factor nicotinamide adenine dinucleotide (NAD^+) (2X22 PDB) together with the available pharmacophore to explore the binding modes of these compounds at the InhA active site.

2. Molecular modeling/docking studies

The 3D structures were generated using SYBYL package (Tripos Associates, St. Louis, MO, USA) [32]. Using the standard bond lengths and bond angles, geometry optimization was carried out with the help of standard Tripos force field [33] with a distance dependent-dielectric function, energy gradient of 0.001 kcal/mol and MMFF94 as the electrostatics. Conformational analyses of all the 52 compounds were performed using a repeated molecular dynamics-based simulated annealing approach as implemented in Sybyl-X 2.0. The molecule was heated up to 1000 K within 2000 fs, held at this temperature for 2000 fs and annealed to 0 K for 10,000 fs using an exponential annealing function. By applying this procedure, a total of 100 conformations were sampled out during the 100 cycles to account for conformational flexibility to find the most likely conformations occurring most often in the resulting pool. All the conformations were then minimized with Tripos force field and atomic charges were calculated using the MMFF94 (Merck Molecular Force Field) method.

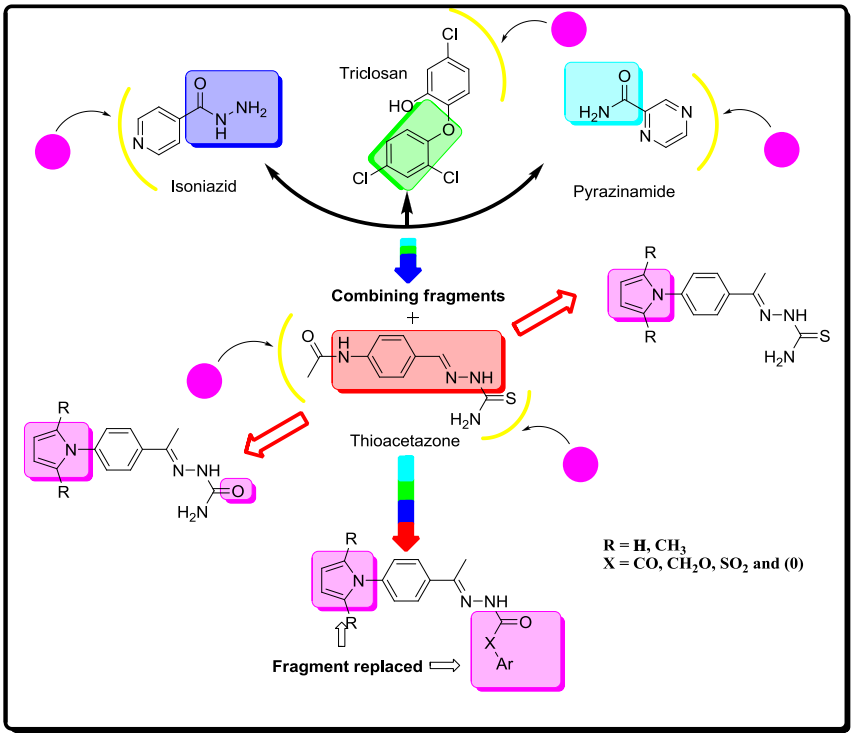

Fig. 1. Design concept for pyrrole hydrazone derivatives.