Recent Progress in the Identification and Development of InhA Direct Inhibitors of *Mycobacterium tuberculosis*

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Abstract: The InhA-related enoyl-ACP reductase, an enzyme involved in fatty acid synthesis, is one of the best validated targets for the development of anti-tubercular agents. However, the majority of isoniazid (INH)-resistant clinical strains are observed mainly due to the emergence of KatG mutants that do not form an INH-NAD adduct. Thus compounds that directly inhibit InhA avoiding activation by KatG would be promising candidates for combating MDR-TB. Herein, some predominant examples of InhA direct inhibitors recently developed are reviewed and special attention is paid to 3D-structures of InhA in drug design process.

Keywords: InhA, Anti-tubercular agents, Isoniazid, KatG, INH-NAD adduct, Indirect inhibitors, Direct inhibitors, Drug design.

1. INTRODUCTION

Tuberculosis (TB), an ancient infectious disease of global influence, re-emerged with the multi-drug resistant strains (MDR-TB) and acquired immune deficiency syndrome (AIDS). According to World Health Organization (WHO), one third of the world's population are infected with *Mycobacterium tuberculosis* (MTB) and 8.2 million new TB cases will occur worldwide up to 2020 [1,2]. Furthermore, it has been more than 40 years since a new anti-tubercular drug was discovered [3]. Short-course chemotherapy for TB-infected patients is based on the combined use of multiple chemotherapeutics including isoniazid (INH), rifampin (RIF), pyrazinamide (PZA), streptomycin (SM) and ethambutol (EMB), over a period of 6-10 months in accordance with WHO's TB treatment guidelines [4]. During the treatment with those drugs, MDR-TB is still grows, although at a slow rate. So there is an urgent need to develop novel anti-tubercular drugs that can be equally effective against MTB and MDR-TB, and shorten the duration of treatment.

Elucidation of the whole genome sequence of MTB [5] will certainly provide new ideas for the discovery of new anti-tubercular drugs. MTB has a unique cell wall comprising three covalently linked macromolecules: mycolic acid, arabinogalactan and peptidoglycan [6-8]. The three polymers in the cell wall provide a thick layer that impedes general antibiotic permeation and protects MTB from drugs. Mycolic acids are the key permeability determinant of the outer cell envelope of MTB [9], which are α-branched β-hydroxylated long chain (C60-C90) fatty acids linking the arabinogalactan to trehalose [10]. The structures, functions and biosynthesis of these complex lipids have been reviewed in detail [11]. The enzymes involved in biosynthesis of cell wall are also excellent sources of molecular targets for discovering anti-tubercular drugs in the absence of homologues in mammalian system.

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2. MTB FATTY ACID BIOSYNTHESIS AND ENOYL REDUCTASE

Unlike other genera, MTB has two fatty acid synthase (FAS) systems: FAS-I and FAS-II. The FAS-I is responsible for the synthesis of fatty acids and the elongation to produce C24-C26 fatty acids, which are used for the production of phospholipids and as primers for complex lipids. The FAS-II system extends these fatty acids up to C56 to make precursors of mycolic acids, which are the essential and specific constituents of MTB [12]. In the FAS synthesis cycle, there are five key enzymes: the 2-trans enoyl acyl carrier protein (ACP) reductase (InhA) that reduces carbon/carbon double bond, the β-ketoacyl ACP synthase (FabH) that catalyzes the first step in elongation *via* condensation as well as KasA and KasB, β-hydroxyacyl dehydrase (DE), and β-ketoacyl reductase (MabA) (Fig. (**1**)).

InhA, encoded by the *Mycobacterium tuberculosis* gene *inhA*, catalyzes the final enzymatic step in the elongation cycle of the FAS-II pathway and was first identified as an effective target by Jacobs and co-workers [13,14]. It is an NADH-dependent reductase that exhibits specificity for long chain enoyl thioester substrates, and its reduction mechanism is described in Fig. (**2**).

InhA has already been known as the target of isoniazid (INH) [14-17], a front-line agent for the treatment of TB, which is active only against sensitive and growing TB but not against multi-drug resistant and resting TB due to selection for KatG mutants. Therefore, it has been proposed that compounds directly inhibiting InhA without requiring activation by KatG would be quite promising as novel drugs for combating MDR-TB. More recently, it has been reported that the broad-spectrum antibacterial inhibitor triclosan (TCN) does indeed target InhA without KatG activation. Based on the mechanism of action of TCN, a novel potent class of InhA direct inhibitors, diphenyl ethers, was designed

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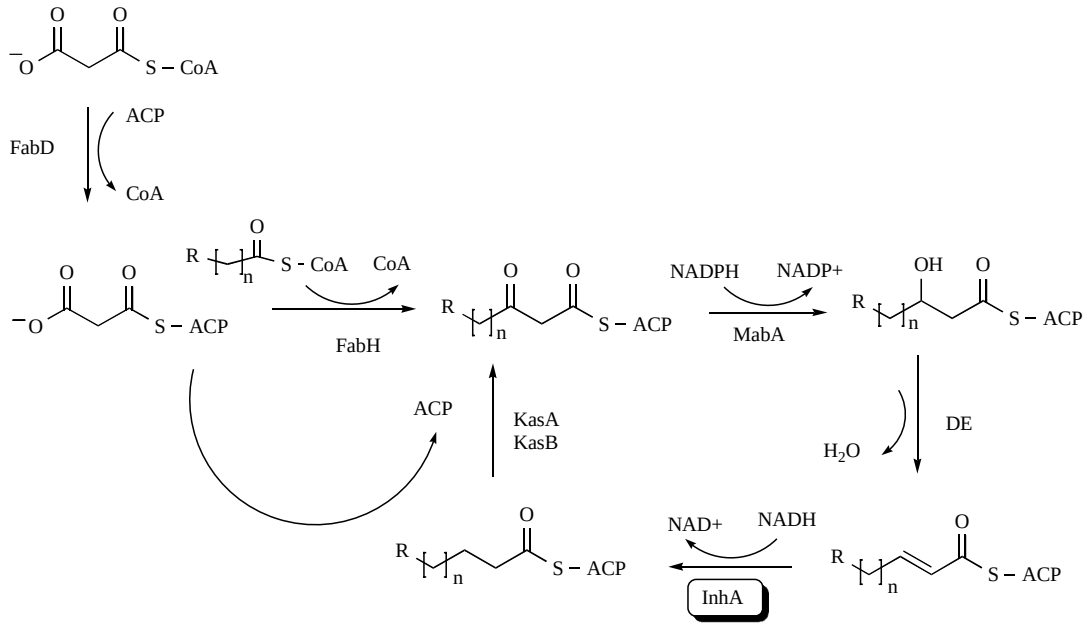

Fig. (1). The process of fatty acids synthesis of TB.

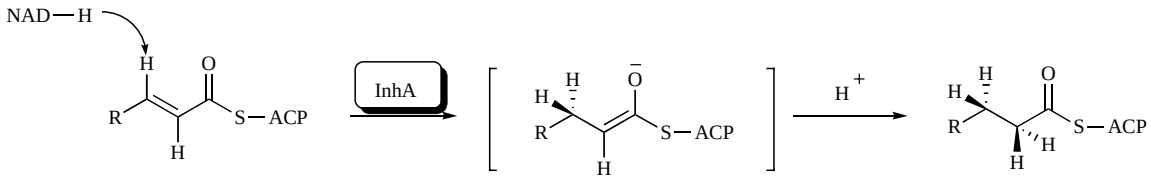

Fig. (2). The reduction mechanism of InhA.

using structure-based drug design [18]. The great success of this approach prompted scientists to apply high-throughput screening technology to discover novel InhA direct inhibitors. Meanwhile, the popularity of InhA as a screening target may derive from the facts that screens may usually lead to hits and at least two commercially useful compounds act by inhibiting it. Based on this basic viewpoint, some novel classes of InhA direct inhibitors have been identified using high-throughput screening strategy [19-22], such as indole-5-amides [19], pyrazole derivatives [19], pyrrolidine carboxamides [20], arylamides [21] and imidazopiperidines [22], etc. Understanding the mechanism of action and resistance of some InhA indirect inhibitors, providing some prominent examples of InhA direct inhibitors recently developed and the application of 3D-structures of InhA in drug design process are the subjects of this review.

3. INDIRECT INHIBITORS OF ENOYL REDUCTASE

3.1. Isoniazid (INH)

INH has been used in TB chemotherapy since its discovery in 1952 [23,24]. As a pro-drug, INH requires activation by KatG [25-30], a catalase-peroxidase enzyme with dual activities of catalase and peroxidase oxidizing INH to an acyl radical binding to position 4 of nicotinamide adenine dinucleotide (NAD) to form an active INH-NAD adduct [31,32] (Fig. (**3**)). Addition of the isonicotinoyl radical to position 4 of the nicotinamide ring can result in two stereoisomers, in which only 4(S) isomers of INH-NAD adduct possesses potent activity [33]. However, the majority of INH-resistant clinical isolates have mutations in KatG [S315T] [34-37], resulting in no formation of INH-NAD adduct. Previous studies show that INH resistance also correlates with mutations in InhA and kasA in MTB [38-43].

The crystal structure of InhA with bound isonicotinic acyl-NADH determined by Sacchettini and co-workers [31] indicates that the NAD part of the adduct is maintained in NADH binding site, compared with the crystal structure of InhA with bound NADH [14]. The only difference is the side chain of Phe^{149} is rotated ~90° to optimize aromatic ring-stacking with the pyridine ring of the isonicotinic acyl group (Fig. (**4**)), which is surrounded by hydrophobic residues (phe^{149}-Trp^{222}). Molecular contacts between INH-NAD adduct and InhA also indicate that the carbonyl oxygen of isonicotinic acyl group forms two hydrogen bonds with the amide nitrogen of nicotinamide ring and the 2'-hydroxyl oxygen of nicotinamide ribose ring. The interactions play a critical role in understanding resistance mechanisms of INH and methodologies of optimizing lead compounds.

3.2. Ethionamide (ETH) and Prothionamide (PTH)

Ethionamide (ETH) and prothionamide (PTH), two INH analogues (Fig. (**5**)), have been widely used in the treatment of MTB along with other front-line drugs [44-46]. Moreover, ETH and PTH also inhibit mycolic acid biosynthesis by

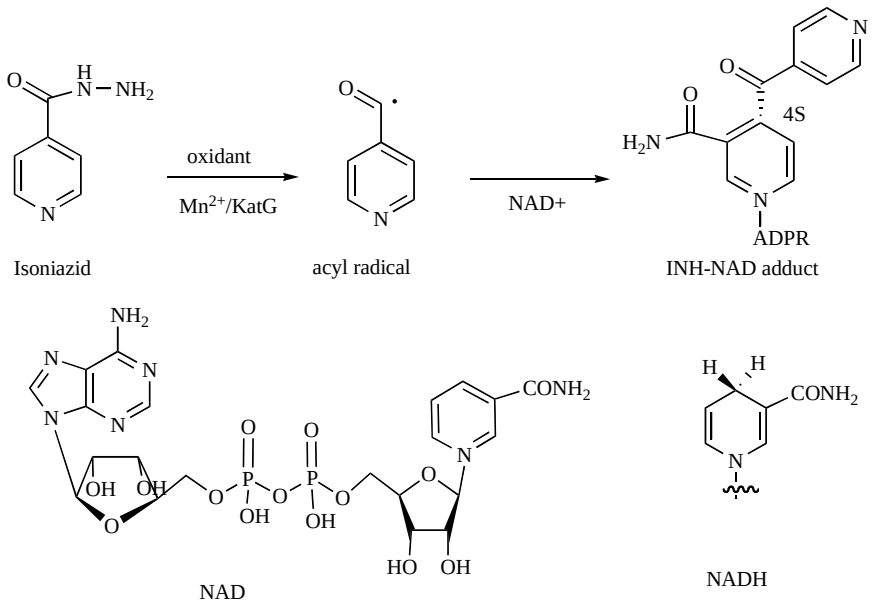

Fig. (3). Schematic representation of INH-NADH adduct formation.

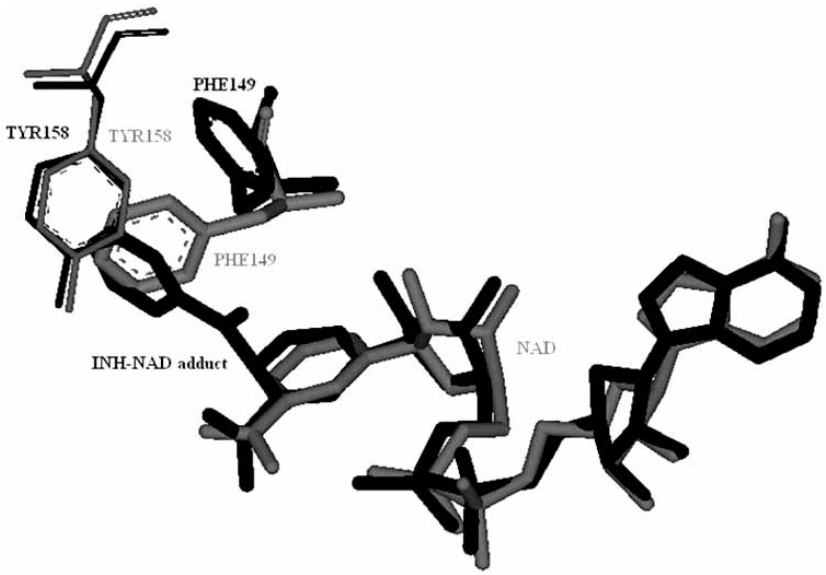

Fig. (4). Conformation comparison of catalytic residue Phe149 before (gray) and after (black) INH-NAD adduct binding to the InhA.

targeting InhA as prodrugs [47,48]. Unlike INH, ETH and PTH require activation by EthA, a flavin-dependent monooxygenase [49-52]. According to the crystal structure of InhA with bound ETH-NAD or PTH-NAD adduct, the ethyl-isonicotinic-acyl or propyl-isonicotinic-acyl moieties are located in a hydrophobic pocket formed by the rearrangement of the side chain of Phe^{149}, and an aromatic ring-stacking interaction with the pyridine ring. Though ETH-NAD adduct is similar to INH-NAD adduct, it is still not clear how ETH and PTH are oxidized by EthA [53].

3.3. NAD Analogues

The disclosure of the mechanism of INH activation brings a new dimension to design of novel inhibitors to mimic INH-NAD adduct, which do not require activation by KatG for combating MDR-TB. Therefore, several studies have been focused on the chemical formation of INH-NAD adduct [54-57]. It was found that the benzoylhydrazine-NAD adduct (BH-NAD) formed *via* the reaction of the activated benzoic acid hydrazide and NAD competes with INH-NAD for binding to InhA [17], prompting scientists to investigate benzoyl and isonicotinoylnicotinamide analogues.

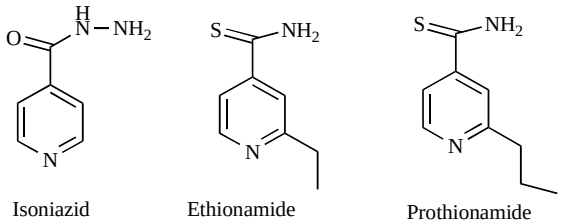

Fig. (5). Indirect small inhibitors of InhA.

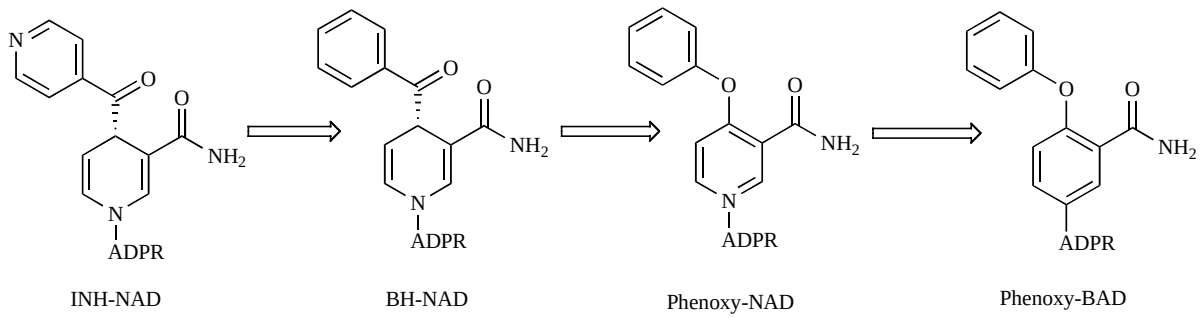

Fig. (6). The process of development of NAD analogues.

However, due to poor stability, three major structures (open keto-amide, cyclized hemiamidal and oxidized hemiamidal) rather than INH-NAD adducts exist under biomimetic conditions [58,59], which have been identified to be unable to inhibit InhA [59,60]. Given the above reasons, Pankiewicz and co-workers redesigned the INH-NAD adduct at position 4, and used 4-phenoxy substitution of NAD instead of INH [61]. The modeling structure resulted in a global minimum with an RMS error of 0.69Å compared to the crystal structure of INH-NAD adduct [61]. Given the glycosidic bond instability of INH-NAD adduct, they also replaced the nitrogen of nicotinamide ring with carbon in previous studies [62,63] to provide 4-phenoxy-substituted BAD analogues. The IC_{50} value is 27 μM, which confirms the idea that simple aromatic mimics of the INH-NAD adduct can also inhibit InhA. Like mycophenolic adenine bis(phosphonate) analogues as inhibitor of inosine monophosphate dehydrogenase (IMPDH) [64], it was supposed that a replacement of the pyrophosphate oxygen of INH-NAD adduct by methylene group would improve the activity and metabolic stability. The process of developing NAD analogues is illustrated in Fig. (**6**). Although these NAD analogues do not need KatG activation, they are not able to overcome the resistance found in in clinical strains caused by InhA (S94A) mutant.

4. DIRECT INHIBITORS OF ENOYL REDUCTASE

4.1. Triclosan (TCN) and Alkyl Diphenyl Ethers

TCN is known to inhibit the synthesis of fatty acids in *Escherichia coli, Staphlococcus aureus* and other bacterium, targeting enoyl-acyl carrier protein reductase (ENR or FabI) [65-69] directly. FabI, a homolog of InhA and a potential target for antibacterial action [70], has been elucidated to be inhibited by diazaborines [71,72], TCN [65-68], diphenyl ethers [73], aminopyridines [74,75], indole naphthyridinones [76-78], thiopyridines [79], 4-pyridones [80] and CG400549 [81]. Previous studies have shown that TCN acts as an uncompetitive and site-directed inhibitor of ecFabI and forms a stable FabI-NAD+-TCN ternary complex through noncovalent interactions with amino acids [69]. Crystal structure of FabI-TCN complex [66,67,69] reveals phenol ring of TCN stacks π-π interaction with the NAD+ nicotinamide ring, while phenolic hydroxyl group of TCN forms two hydrogen bonds with the active amino acid Tyr156 and with the 2'-hydroxyl group of the nicotinamide ribose of the nucleotide. TCN is a picomolar inhibitor (K_i = 7 pM) of the ecFabI by mimicking its natural substructure [66].

Recently, molecular genetic studies have shown that TCN is also an inhibitor of InhA of MTB [16] and *Mycobacterial smegmatis* [82]. It has been validated to be a submicromolar uncompetitive inhibitor (K_i = 0.2 μM) of InhA. In addition to the ecFabI-TCN complex, another molecule of TCN binds to the active site of InhA, which has never been observed in any other ENR-TCN structures [19]. From the thermodynamic perspective, both TCN molecules are located in low energy conformations and exhibit van der Waals interactions with InhA. InhA specifically chooses C16 long chain fatty acyl as its substrates [83], leading to a deeper hydrophobic substrate binding crevice (residues$^{203\text{-}210}$), which explains the reason for the existence of a second TCN occupying substrate binding site. The mode of action of InhA-TCN is quite different from that of INH, not only avoiding KatG activation but also combating InhA (S94A) mutant resistance.

To understand the activity difference (30000 fold) in the affinity of TCN with ecFabI and InhA, Tonge and coworkers also determined the structure of TCN with InhA [84], which only contains one TCN molecule in the active site and is different from Sacchettini's crystal structure [19]. The difference between ecFabI-TCN and InhA-TCN complexes is the ordering of substrate binding loop, which is ordered (residues$^{195\text{-}200}$) when TCN binds to ecFabI and disordered (residues $^{197\text{-}211}$) in the InhA-TCN complex [85]. To cause substrate binding loop of InhA to be ordered and improve the affinity of InhA, Tonge and co-workers superimposed the C16 acyl substrate (C16-NAC) on the InhA-TCN complex. It was found that the substrate binding loop is ordered in the InhA-C16-NAC complex [84]. Thus, a series of diphenyl ethers were designed by replacing chlorine atom on the phenol ring of TCN with various alkyl groups (Fig. (**7**)) guided by structure-based drug design strategies.

Alkyl diphenyl ethers were synthesized and their IC_{50} and K_i values for InhA inhibition were determined (Table **1**). The activity results suggest that 5-octyl-2- phenoxyphenol (8PP) is the most potent compound with a K_i value of 1.1nM for InhA [84]. As the alkyl substituent is lengthened from 2 to 8 carbons, a corresponding decrease in IC_{50} value is observed. However, 14PP is much less potent than 8PP with a K_i value of 30.3nM for InhA. To observe the interactions of the alkyl diphenyl ethers with InhA, 5PP and 8PP were co-crystallized with InhA. Results indicate that they bind in a similar mode compared to TCN, namely, hydrogen bonds with Tyr158 and 2'-hydroxyl group of NAD+ and alkyl substituted phenol

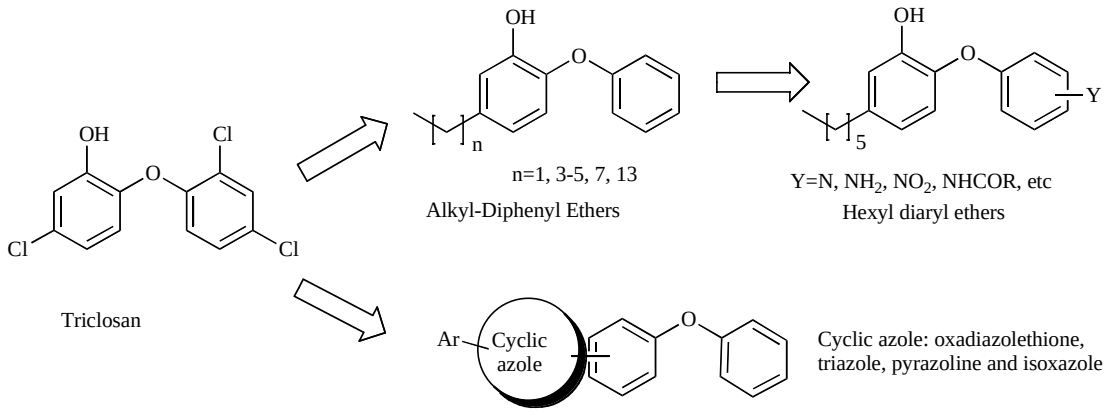

Fig. (7). Triclosan and its derivatives.

Table 1. The InhA and H37Rv Inhibition activity of Alkyl Diphenyl Ethers

Compd. [a]	IC_{50} nM	K_i nM	H37Rv, MIC_{99} μM
2PP	2000 ± 700	- [b]	3.8 ± 0
4PP	80 ± 15	-	-
5PP	17 ± 5	11.8 ± 4.5	1.0 ± 0
6PP	11 ± 1	9.4 ± 0.5	2.1 ± 0.9
8PP	5.0 ± 0.3	1.1 ± 0.2	1.9 ± 0.5
14PP	150 ± 24	30.3 ± 4.7	175

[a]2PP, 4PP, 5PP, 6PP, 8PP and 14PP are the diphenyl ethers with ethyl, butyl, pentyl, hexyl, octyl and tetradecyl group.
[b]Not determined.

ring stacks with the nicotinamide ring of NAD+. The pentyl chain of 5PP is bent and occupies the alkyl chain of C16-NAC site, while octyl chain of 8PP extends through the whole C16-NAC binding site with a linear conformation. The hydrophobic pocket is a major site of interaction for side chains of the alkyl diphenyl ethers binding to InhA according to the studies. The *in vivo* studies showed that 6PP and 8PP exhibit higher affinity for InhA and lower cytotoxicity than TCN, but still show limited bioavailability [86].

To improve bioavailability of alkyl diphenyl ethers, am Ende and co-workers modified the phenyl ring with various heterocycles including nitrogen atom or phenyl ring substituted by nitro, amino, amide and piperazine groups at the *ortho, meta or para* positions [87] (Fig. (**7**)). Most of the derivatives showed significantly improved ClogP values (ClogP < 5). In contrast, activities of the compounds were not as good as the lead compounds. These results indicate that the introduction of a bulky substituent at the *ortho, meta or para* positions of the phenyl ring or the incorporation nitrogen atom into the phenyl ring reduces anti-InhA activity.

In 2008, Suvarna and co-workers also reported a series of novel cyclic azole substituted diphenyl ether derivatives based on TCN skeleton for anti-tubercular activity [88] (Fig. (**7**)). The compounds exhibit high activity against the H37Rv with MIC value of 2.4-3.7μM, which is comparable to the standard drugs such as INH and RIF. Molecular modeling studies show that the cyclic azole substituted derivatives occupy a larger binding domain with ENR than TCN.

4.2. Indole-5-Amides (Genz10850)

Based on the clear understanding of the mechanism of TCN against InhA, to discover more InhA direct inhibitors without the KatG activation, Sacchettini and co-workers have identified two novel series of InhA direct inhibitors using high-throughput screening of ~500000 combinatorial library compounds [19], which are effective against MDR-TB. The representative structures are (4-(9*H*-fluoren-9-yl)piperazin-1-yl)(indolin-5-yl)methanone (Genz10850, (**1**)) and 4-(trifluoromethyl)-2-(4,5-dihydro-4-(2,4-dinitrophenyl) pyrazol-1-yl)pyrimidine (Genz8575, (**2**)) (Fig. (**8**)), with IC_{50} values against InhA 0.16 μM and 0.24 μM, respectively.

The structure-activity relationship of Genz10850 analogues shows that carbon position 2 or 3 of the piperazine ring can not be replaced by other substitutions probably because of the steric clash with the Phe149 of the protein, and that polar substitutions of 2-position of the fluorenyl improve the affinity of InhA, which suggests exposure to solvent and coincidence with one of the chlorine atoms of TCN position. Indole nitrogen forms hydrogen bonds with the phosphate oxygen of NAD+ and can not be alkylated or acylated. Kinetic studies indicate that they are both competitive with the C16-NAC substrate. The crystal structure of InhA-

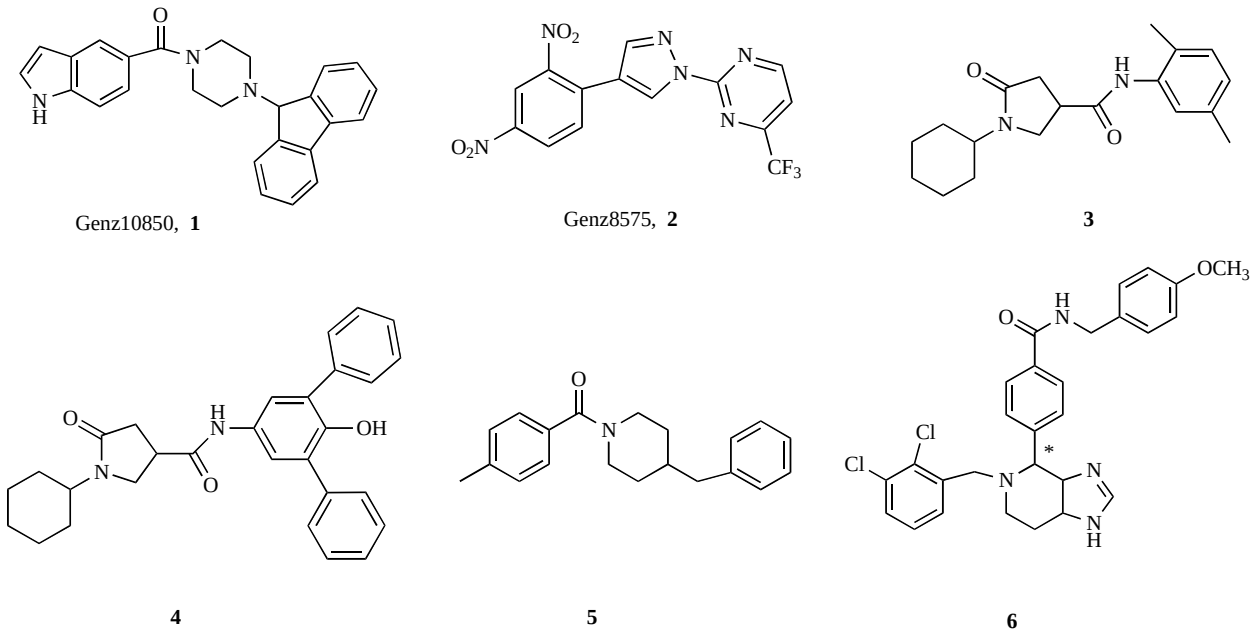

Fig. (8). Representation of Chemical structures of InhA direct inhibitors from high-throughput screening.

Genz10850 (**1**) has been determined, revealing the binding mode of Genz10850 (**1**) to InhA. The piperazine and indole groups of Genz10850 (**1**) overlaid the phenol ring and the dichlorophenyl ring of TCN, respectively. The carbonyl group of the Genz10850 (**1**) forms hydrogen bonds with catalytic residue Tyr158 and 2'-hydroxyl group of NAD+, and the bulky fluorenyl group occupies the long acyl chain of C16-NAC binding site with van der Waals interactions. This hydrophobic interaction is essential for high affinity of InhA based on the existence of a second TCN molecule [19].

4.3. Pyrazoles (Genz8575)

Several analogues of pyrazole were prepared based on the screening results [19], among which, Genz8575 (**2**) displayed 91% inhibition at 40 μM against InhA. Replacing the trifluoromethylpyrimidine group of Genz8575 (**2**) with other substituted aromatic rings causes a significant decrease in activity, suggesting trifluoromethylpyrimidine group is critical to the high affinity of InhA [19]. Unfortunately, there has not been any report in detail on the binding mode of Genz8575 (**2**) to InhA up to now.

4.4. Pyrrolidine Carboxamides

In 2006, He and co-workers also performed a high-throughput screening of a library of 30000 compounds, leading to the identification of pyrrolidine carboxamides compounds (**3**) (Fig. (**8**)) [20]. Due to the importance of the ketopyrrolidine core, lead compound (**3**) was further optimized by replacing the phenyl ring A and cyclohexyl ring C with other groups while keeping ketopyrrolidine core B invariable

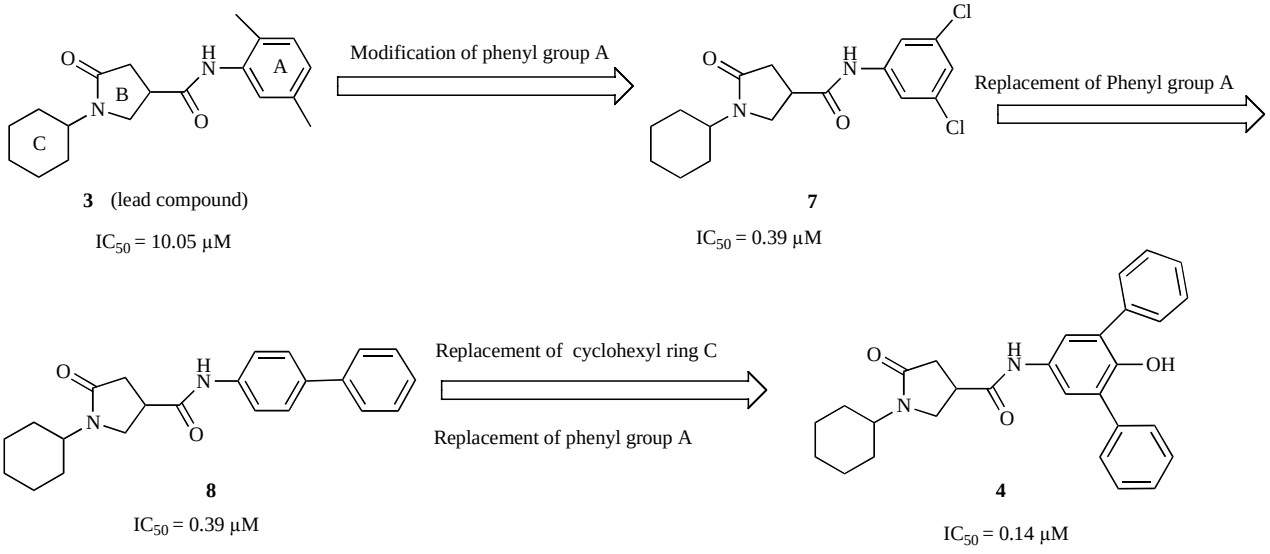

Fig. (9). The discovery process of the most potent pyrrolidine carboxamide compound (**4**).

(Fig. (**9**)). The compounds were optimized by a microtiter synthetic method and in situ screening according to Wu's report [89].

Modification of various substitutions at phenyl ring A of pyrrolidine carboxamides resulted in compounds with significant activity. The most potent compound (**7**), with symmetric 3- and 5-chloro atoms on the phenyl, shows an IC_{50} of 0.39 μM against InhA. The oxygen of the carbonyl group of ketopyrrolidine are observed to form hydrogen bonds with the hydroxyl group of Tyr158 and with 2'-hydroxyl moiety of the nicotinamide in the crystal structure of InhA with bound compound (**7**). The obvious change in InhA is that active residue Tyr158 rotated ~90° to accommodate the inhibitor (Fig. (**10**)). This phenomenon is also observed in other direct inhibitors of InhA, such as TCN and alkyl diphenyl ethers [84].

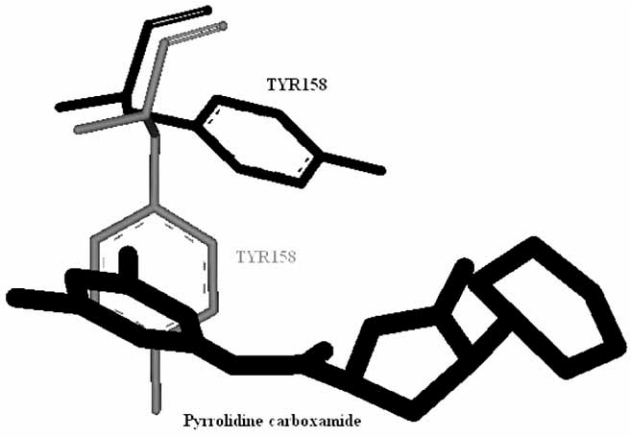

Fig. (10). Conformation comparison of catalytic residue Tyr158 before (gray) and after (black) compound (**7**) binding to the InhA.

Replacing phenyl ring A of pyrrolidine carboxamides with various aromatic and aliphatic rings, indicates that enlarging the aromatic ring can improve the activity due to the hydrophobic interactions. Such compounds may occupy the long alkyl side chain of the C16-NAC binding site and correspond with a second TCN in the active site of InhA.

Based on the derivatization of the phenyl ring A of pyrrolidine carboxamides, to further investigate the function of cyclohexyl ring C, He and co-workers adopted substituted phenyl rings and other saturated rings (smaller or larger than cyclohexyl) as replacements of cyclohexyl. According to the SAR results of the modification of phenyl ring A and cyclohexyl ring C, the most potent compound in this series is compound (**4**) (Fig. (**8**)) with an IC_{50} of 1.14 μM, and its discovery process is depicted in Fig. (**9**). The hydrophobic interactions between the large phenyl ring and the active site of InhA are essential for the activity. Meanwhile, it is suspected that hydroxyl group at the phenyl ring enhances the activity by forming hydrogen bond with the protein and causes a subtle change in the binding site. But, based on the knowledge that polar substitutions at the 2-position of the fluorenyl and one of the chlorine atoms of triclosan is solvent-exposed, we speculate this hydroxyl group is also solvent-exposed.

Pyrrolidine carboxamide classes are racemic and only R-enantiomers are active as potent inhibitors of InhA. The results show that the R-enantiomers exhibits 10-fold higher potency than S-enantiomers. To investigate the difference of the binding mode of enantiomers with InhA, we docked the R- and S- enantiomers of (**7**) into the active site of InhA. It is suggested that the docked pose of R-enantiomer of (**7**) overlaps the biologically active conformation of (**7**) in the X-ray crystal structure well (Fig. (**11A**)) while the S-enantiomer of (**7**) is offset (Fig. (**11B**)).

Unfortunately, pyrrolidine carboxamide classes with good enzyme inhibitory do not exhibit ideal activity against MTB strain H37Rv. The most potent compound has a MIC value of 62.5 μM. The results suggest that pyrrolidine carboxamide compounds show poor membrane permeability. Nevertheless, these newly pyrrolidine carboxamide inhibitors provide a good foundation for the discovery of novel direct inhibitors of InhA.

4.5. Arylamides

Arylamide compounds are the largest class of InhA inhibitors obtained from the high-throughput screening by He and co-workers [21]. A representative compound is compound (**5**) with an IC_{50} value of 5.16 μM (Fig. (**8**)). The structure-activity relationships show meta-phenyl of arylamides with electron-withdrawing substituents or para-benzoyl of arylamides substituted by dimethyl display potent activity for InhA. In the InhA- (**5**) complex, the amide carbonyl group oxygen forms hydrogen bonds with Tyr158 and 2' -hydroxyl of the nicotinamide ribose [21], a conserved feature in all crystal structures of InhA complexed with various direct inhibitors. The unsubstituted phenyl ring locates in the hydrophobic site with van der Waals interactions. This hydrophobic site is so large that additional space is still available to accommodate other hydrophobic groups by analyzing the complex. Using bulky hydrophobic groups instead of the initial phenyl ring, most of the compounds exhibit excellent activity against InhA. Among the analogues, one compound with a fluorenyl group attached to piperazine ring exhibits an IC_{50} of 0.09μM. However, just like pyrrolidine carboxamide series, arylamides do not exhibit good antitubercular activity *in vitro*. Most of the compounds' MIC values are higher than 125 μM, and only one compound displays a MIC value of 62.5μM. These results also indicate that arylamides display poor membrane permeability or are easily extruded by efflux pumps.

4.6. Imidazopiperidines

Researchers at *GlaxoSmithKline* described a new series of non-covalent inhibitors of InhA from screening [22]. Compound (**6**) (Fig. (**8**)) exhibited the most potent anti-InhA activity in this series of imidazopiperidine. Due to the specificity of the imidazopiperidine structure, it is suitable to use solid phase synthesis to carry out the optimization on the carbonyl and piperidine positions according to the previous studies [90,91]. Initial screening results show, (1) substitutions at the carbonyl and piperidine positions are critical to activity; (2) *para*- of benzylamine group with electron-donating group at the carbonyl position and the mono or dichloro benzyl at the piperidine exhibit good activity; (3) re-

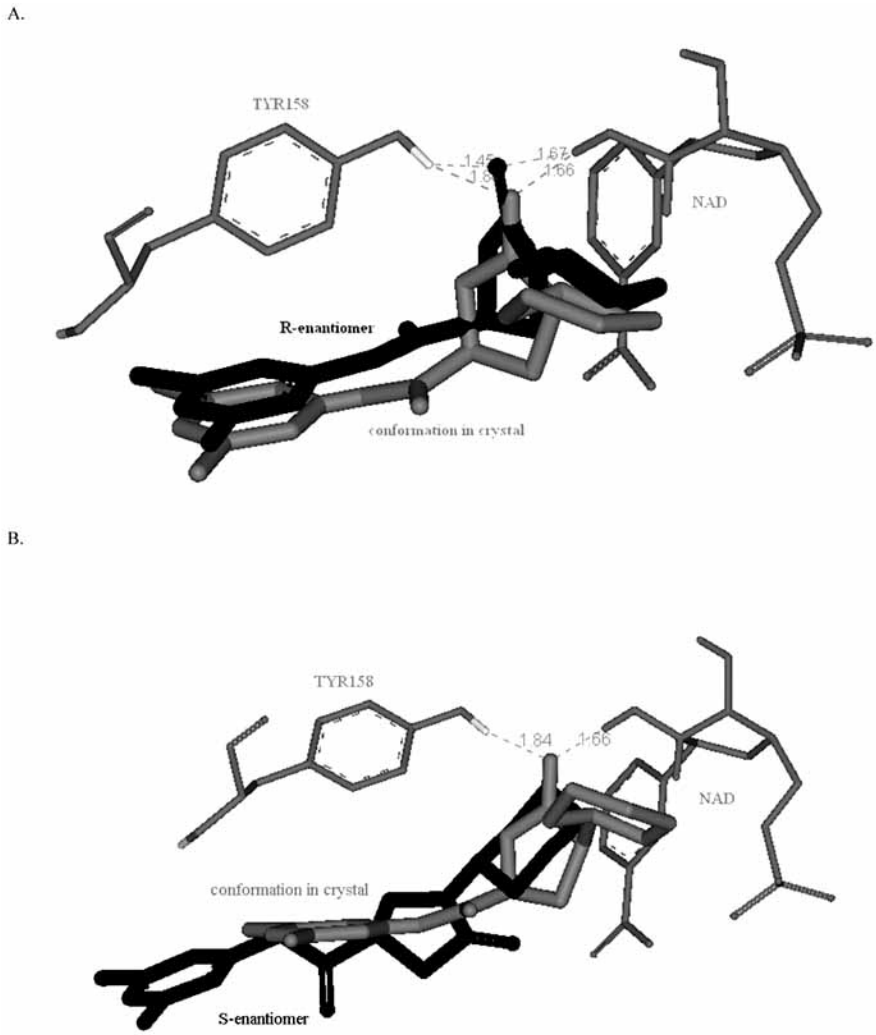

Fig. (11). Conformation comparison of enantiomers of compound (**7**) (**A**) R-enantiomer color by black and the conformation in crystal structure color by gray; (**B**) S-enantiomer color by black and the conformation in crystal structure color by gray.

placing the imidazole group with a phenyl group leads to a complete loss of activity for InhA. The mechanism of action of imidazopiperidines remains unclear, yet it is suggested that a non-specific mechanism may exist, based on the lack of broad-spectrum antibacterial activity compared to TCN. So the next task is to identify the mechanism and to improve the cellular activity for MTB.

5. DISCOVERING NOVEL CLASSES OF INHA DIRECT INHIBITORS WITH VIRTUAL SCREENING

On the basis of many publications about crystal structures of InhA complexed with various inhibitors and the development of computer-aided drug design on InhA, such as *de novo* design of pyrrolidines carboxamides based on CoMFA studies [92] and structure-based design of a tripeptide inhibitor of InhA [93], virtual screening of databases should be useful for discovering novel direct inhibitors of InhA [94]. During our current work on the structure-based drug design of InhA direct inhibitors, the crystal structures (PDB code: 2H7M) with the lowest resolution of 1.62 Å was chosen as the macromolecule [95]. For all docking calculations, we have used GOLD 3.0.1 [96], a powerful docking program using genetic algorithm (GA) method for conformational search.

Lipinski's Rule-of-five [97] was adopted to filter virtual compound library (SPECS. Inc) including a collection of about 230000 chemical compounds. Several known direct inhibitors of InhA were incorporated into the database in random to verify the effectiveness of virtual screening. The top-ranked 100 compounds were selected and analyzed based on the conserved interactions with the active site of InhA. 50% of known inhibitors were positioned in the 100 top scoring compounds, especially the most potent inhibitor 5-octyl-2-phenoxyphenol (8PP) were ranked in top 2. These preliminary results will be considerably researched and the activities of selected compounds against InhA will be evaluated.

6. CONCLUSIONS AND FUTURE DIRECTIONS

MDR-TB brings a great threat to human health. Based on the understanding of the mechanism of the INH resistance, compounds that directly inhibit InhA avoiding activation by KatG would be promising candidates for combating MDR-TB. In this article, several predominant examples of InhA

direct inhibitors were described, as well as the role of the 3D-structures of InhA in drug design process.

Compared to the previous inhibitors, the potency of newly identified direct inhibitors of InhA (IC_{50}) was much higher. The most potent compound had an IC_{50} value of 0.09 μM. Interestingly, we found that all of these InhA direct inhibitors had conserved interactions with the active site of InhA, a hydrogen bond network with the active amino acid Tyr158 and the 2'- hydroxyl group of the nicotinamide ribose of the nucleotide and π-π stacking interaction with the NAD+ nicotinamide ring. These interactions provided opportunities to the further development of structurally novel InhA direct inhibitors. Combined with X-ray crystallography and molecular modeling, it was found that enhancing the hydrophobic interaction of inhibitors with protein was very favorable for the activity. Guided by the structural information, we suppose that using polar group or hydrophilic substituent exposed to bulk solvent may improve the affinity of InhA.

Some direct inhibitors of InhA described in this review exhibit good affinity of InhA, but show poor anti-tubercular activity. Further efforts will be focused on improving membrane permeability and bioavailability of these inhibitors by reasonably chemical modifying. Nevertheless, the examples reviewed in this paper provide a solid foundation for discovery of new candidates overcoming MDR-TB.

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ABBREVIATIONS

AIDS = Acquired immune deficiency syndrome

InhA = 2-Trans enoyl acyl carrier protein reductase (ENR/FabI)

DE = β-hydroxyacyl dehydrase

EMB = Ethambutol

ETH = Ethionamide

FabH = β-ketoacyl ACP synthases

INH = Isoniazid

PZA = Pyrazinamide

PTH = Prothionamide

MDR-TB = Multi-drug resistant tuberculosis

MTB = Mycobacterium tuberculosis

MabA = β-ketoacyl reductase

NAD = Nicotinamide adenine dinucleotide

RIF = Rifampin

SM = Streptomycin

TB = Tuberculosis

TCN = Triclosan

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