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Review article

# Novel compounds targeting InhA for TB therapy

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ABSTRACT

Tuberculosis (TB) is described as lethal disease in the world. Resistant to TB drugs is the main reason to have unfavourable outcomes in the treatment of TB. Therefore, new agents to replace existing drugs are urgently needed. Previous reports suggested that InhA inhibitors, an enoyl-ACP-reductase, might provide auspicious candidates which can be developed into novel antitubercular agents. In this review, we explain the role of InhA in the resistance of isoniazid. Furthermore, five classes of InhA inhibitors, which display novel binding modes and deliver evidence of their prosperous target engagement, have been debated.


**Contents**



## Introduction

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (MTB), still remains the foremost cause of morbidity and mortality which accounts for nearly 9 million infections and 1.5 million deaths [1,2]. Short course chemotherapy (SCC), which is considered the backbone of anti-tubercular chemotherapy, includes not only initial phase for two months but also continuation phase of treatment for four months. The initial phase of anti-tuberculosis treatment consists of isoniazid (INH), rifampicin (RIF), pyrazinamide (PZA), and ethambutol (EMB), followed by INH and RIF for the continuation phase of four months [3,4]. However, based on the guidelines of TB treatment, relapses and failures could be reduced by replacement of a regimen including RIF throughout the 2 month period with a regimen based on just 6 months of RIF [5].

Although the most of TB instances are treatable, however, the current complex and long regimen of TB agents can lead to poor adherence with adverse side effects, therefore giving suboptimal therapy responses [4]. Even though many endeavours have been carried out to make the duration of treatment of drug-susceptible TB more shorter and to enhance the outcomes for MDR-TB

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therapies, the mortality rates are still high [6]. Thus, development of new antitubercular agents, which comprise novel chemical scaffolds with distinctive inhibitory binding modes and new modes of action, are urgently needed [7].

The poorly permeable and very complex cell envelope of mycobacteria are considered as a main barrier in the conveyance of TB agents to their action site. The essential and major components of the mycobacterial cell envelope are a-branched, b-hydroxylated fatty acids with chains of carbon atoms between 60 and 90 in length. Two elongation systems comprise fatty acid synthase type I (FAS-I) and fatty acid synthase type II (FAS-II) control the mycobacterial cell envelope synthesis. FAS-I enzyme is only used by eukaryotic cells to synthesize fatty acids, thus the FAS-II enzymes are viable targets for drug development. InhA, which is an enoyl-acyl carrier protein (ACP) reductase of the FAS-II system, catalyses NADH-dependent reduction of the double bond at position two of growing fatty acid chains that are associated with ACP [8,9]. In 1995, the first crystal structure of InhA has been published. Indeed, InhA is a clinically validated target due to the success of INH in treating patients with TB [10]. In this review our aim is to the exploitation of InhA inhibitors that display solid evidence of successful target engagement, and have activity *in silico*.

## Role of InhA in the isoniazid resistance

Besides the main contribution to INH resistance is katG, InhA mutations confer low level resistance to INH (less than 10%) on *M. tuberculosis*. It has been reported that in a genetic loci mutations contain contiguous ORFs (mabA and InhA) which are responsible not only for resistance to INH but also for ethionamide (ETH) in *M. tuberculosis*. The analysis of DNA sequence of INHR and ETHR revealed that *M. smegmatis* and *M. bovis* BCG displayed a single base alteration at the 94th codon of the InhA coding region, resulting in replacement of a Ser to Ala (S94A). Furthermore, it has been shown that InhA mutation alone was adequate to confer INH resistance in a successive experiment by the identical group [11]. Mutations in C(-15)T, T(-8)G/A, A(-16)G, which are considered as regulatory regions of InhA, lead to low-level INH resistance and over-expression of InhA protein [12,13]. Although S94A substitution resulted in a conformational alteration in the InhA upon binding with both INH and NADH complex, however, no clinical importance has been found for this substitution. The other mutations, which occur in the structural region, have been reported to be more clinically noteworthy as they have been associated with INH resistance [12,14]. Based on molecular data on clinical isolates, *M. tuberculosis* does not mainly depend on the mutation in the binding site for adduct formation, but rather with overproduction of the InhA enzyme by acquisition of mutations in the promoter region [15–21]. Unissa, Selvakumar [22] reported that the occurrence of mutations only in the promoter region of InhA rather than in structural region.

## InhA inhibitors

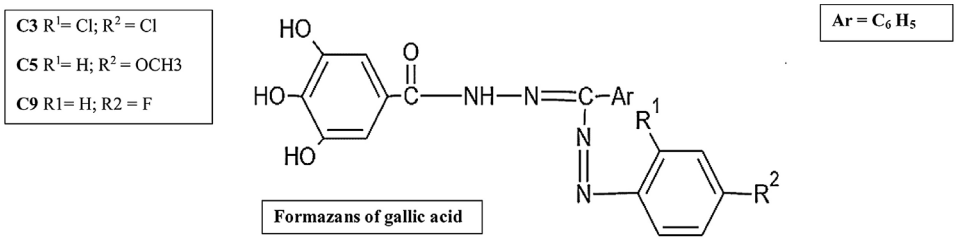
INH, which has been widely used as anti-TB therapy ever since, was discovered in 1952 [23]. Described as a prodrug, INH is transformed *in vivo* into the reactive isonicotinyl acyl radical by the mycobacterial catalase-peroxidase enzyme KatG, which can lead to form a covalent adduct with the co-factor nicotinamide adenine dinucleotide (NAD) of INH [8].

Interestingly, 40–95% of *M. tuberculosis* INH-resistant has displayed mutations in katG which lead to diminished INH activation. Thus, the dependency on the activation of KatG for INH-mediated killing is considered as the main clinical frailty associated with the use of INH. Even though mutations have been detected in the InhA promoter region, however, these only lead to produce low level of INH resistance [24,25].

Diverse categories of direct InhA inhibitors have been found, however, the progression of these inhibitors toward the clinic has been restricted. This is due to a variety of reasons such as poor pharmacokinetic properties, a limited therapeutic selectivity window and a propensity for strong enzymatic inhibition not translating into whole-cell activity [7].

### *Gallic acid formazans*

Considered as antioxidant, gallic acid appears to have several biological activities including antimycobacterial, antityrosinase, antimicrobial, analgesic, and anticancer. Formazans have shown a board spectrum of biological activities such as antibacterial, antimycobacterial, anticonvulsant, antifungal, and anti-




| Compound | Antimycobacterial activity MIC (μM) | | | Enzyme inhibitory potency | | Cytotoxicity[b] (% inhibition) | |
|---|---|---|---|---|---|---|---|
| | MDR- TB | | *M. tuberculosis* | % Inhibition of | | At 100 μM | At 50 μM |
| | HR[c] | HRS[d] | H37RV | InhA at 10 μM[a] | IC50 | | |
| **C3** | 0.33 μM | 1.34 μM | 1.752 μM | 73.18 ± 1.65 | 5.22 ± 0.36 | 21.54 | 12.08 |
| **C5** | 1.9 μM | 1.9 μM | 1.921 μM | 69.34 ± 2.14 | 5.79 ± 0.22 | 15.02 | 4.51 |
| **C9** | 0.49 μM | 0.96 μM | 1.979 μM | 87.12 ± 1.64 | 3.42 ± 0.48 | 23.68 | 11.85 |

[a] *M.tuberculosis* InhA enzyme inhibition activity
[b] Against RAW 264.7 cells.
[c] HR: Isoniazid, Rifampicin-resistant strain
[d] HRS: Isoniazid, Rifampicin, Streptomycin-resistant strain

**Fig. 1.** Chemical structures and activities of the Formazans of gallic acid- based inhibitors of InhA, *M. tuberculosis* and MDR-TB,compounds C3–C5–C9.

inflammatory [26–31]. Additional attentive literature studies for functional groups revealed that the hydrazone moiety ($R^1R^2C{=}N{-}NH$) is common amid most of the effective antitubercular drugs [32].

A series of gallic acid formazans has been designed and docked in the InhA active site. Nine compounds, which exhibit good G-score, have been identified as InhA inhibitors. Therefore, docking has a significant role to filter out those candidates with lower G-score and hence inferior antitubercular activities, *in silico* [33].

The compounds C3, C5 and C9 (Fig. 1), which displayed good G-score in docking study, were found to be the most promising MIC (<2 μM) against *M. tuberculosis* H37Rv. In order to scrutinize the activity of C1–C9 compounds against *M. tuberculosis*, two MDR strains have been used including HR (Isoniazid, Rifampicin-resistant strain), and HRS (Isoniazid, Rifampicin, Streptomycin-resistant strain). Linezolid has been used as the reference compound. The compounds C3, C5, and C9 exhibited activity against both HR and HRS resistant strains. In HR resistant strain, the MIC of compounds C3 (0.33 μM), C5 (1.9 μM), and C9 (0.49 μM) appeared to be lower than linezolid (3.70 μM). In HRS resistant strain, the MIC of the compounds C3 (1.34 μM), C5 (1.9 μM), and C9 (0.96 μM) were noticed to be more active than the standard linezolid (3.70 μM). Thus, most of the compounds displayed very good activity against multi-drug resistant strains. These are very promising results for the development of novel effective agents against the growing number of MDR strains of *M. tuberculosis* [33].

The mode of action of these compounds as InhA inhibitors has been established by the InhA enzyme inhibition assay. The compounds C2–C9 demonstrated good inhibition of InhA in the range of 57.12 ± 1.12 to 87.12 ± 1.64% at 10 μM. The compounds C3, C5 and C9 were found to be the most promising InhA inhibitors with IC50 values of 5.22 ± 0.36, 5.79 ± 0.22 and 3.42 ± 0.48 μM, respectively. Moreover, the compounds C3, C5 and C9, which showed insignificant activity against gram positive and gram negative bacteria and negligible cytotoxicity against RAW 264.7 cell line, appeared to be effective against intracellular mycobacteria with $IC_{50}$ values of 0.35 ± 0.02, 0.56 ± 0.04 and 0.62 ± 0.02, correspondingly [33]. Negligible toxicity, at concentrations (50 and 100 μM), was detected for the compounds C3 (12.08 with 21.54% inhibition), C5 (4.51 with 15.02% inhibition) and C9 (11.85 with 23.68% inhibition). Therefore, these compounds were specific and selective in their mode of action against *M. tuberculosis*. The molecular modelling tool has been used for the prediction of ADME of these compounds. The ADME analysis of all compounds revealed that C3, C5 and C9 compounds possess potential to be used as oral candidates. The results noticeably indicated that C3, C5 and C9 formazans appeared to be good inhibitors of InhA and specifically effective against sensitive and resistant strains of *M. tuberculosis* [33].

Visual inspection of C2–C9 compounds demonstrated that all the ligands might bind into InhA active site, leading to occupy positions very close to native ligand in the crystal structure. The compounds C2–C9, which were found to be buried into the InhA hydrophobic pocket, were detected to form contacts essentially with Tyr158-Ile215-Met155-Leu218-Pro156-Ala157-Met199 and Met161 [33].

The hydrogen bonding network between Tyr158 and NAD+ cofactor, appeared to be a conserved feature amid all the lnhA-inhibitor complexes, served as the key feature governing the orientation of the compound within the active site, has been identified so far [34,35].

The protein-ligand interaction unveiled that all the compounds excluding compound C1 formed hydrogen interactions between Tyr158 and NAD+. The carbonyl oxygen atom of the compounds C2–C9 was comprised in forming a hydrogen bond with Tyr158. The compounds C2–C9 displayed higher G-scores and oriented in a different manner as compared to the compound C1. The hydrogen bonding appeared to help in good binding of the compounds C2–C9 with the active site of the InhA, making them more potent. Therefore, the compounds C2–C9 showed great inhibition of InhA, *in silico*. The stability of a protein-ligand complex is due to the formation of H-bonds with other amino acids by the phenolic hydroxyl groups [33].

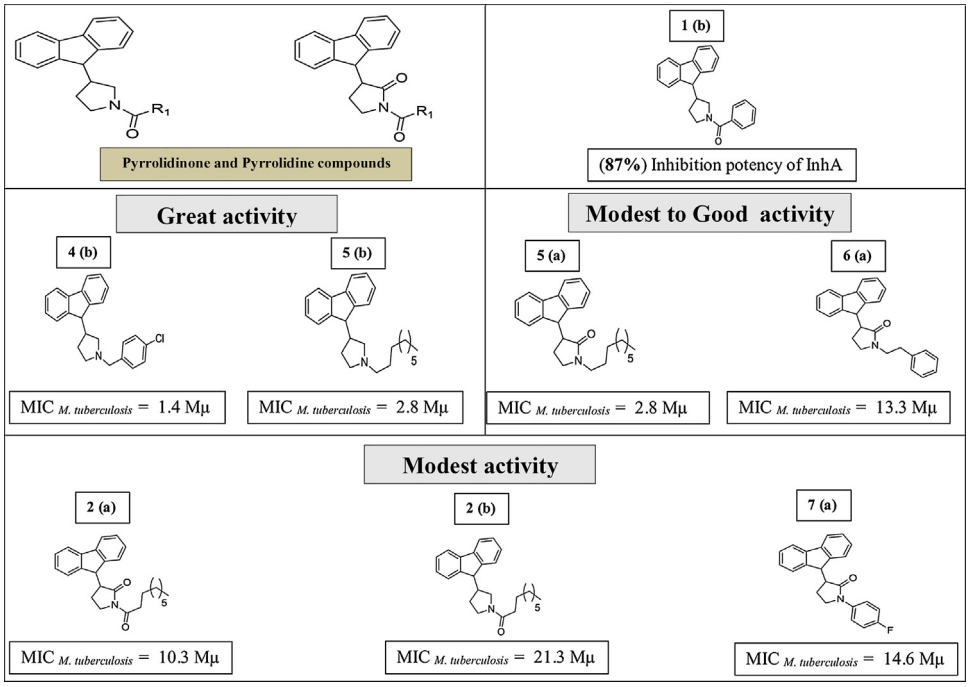



**Fig. 2.** Chemical structures of potent Pyrrolidinone and Pyrrolidine inhibitors of InhA and *M. tuberculosis* with their activities.

*Pyrrolidinone and pyrrolidine derivatives*

Diverse categories of compounds have been found to inhibit InhA enzyme directly. Genz-10850, known as GEQ, was identified by using high-throughput screening (HTS), displayed a great inhibitor of InhA enzyme but a poor inhibitor of *M. tuberculosis* growth [36,37]. Of late, it has been reported that the patterns of substitution on the fluorenyl moiety of GEQ analogues lead to enhance their effectiveness as inhibitors of InhA enzyme and *M. tuberculosis.* Moreover, many of the compounds appeared to have great activity against *M. tuberculosis* growth particularly once associated with efflux pump drugs [38,39].

A series of GEQ analogues bearing pyrrolidinone or pyrrolidine cores have been synthesized and evaluated as inhibitors of InhA enzyme (Fig. 2). GEQ and Triclosan have been used as positive control. All candidate compounds, which were divided into three groups, have been assessed at 50 μM concentration [40]. In the first group, an acyl group is linked to the amino atom of the pyrrolidinone or pyrrolidine frame with the aim to promote an H-bond between the carbonyl group and the Tyr 158 which is considered as a key interaction residue of InhA enzyme. Compounds 1a, 2a and 2b do not have a significant activity against InhA. Compound 1b appeared to have the greatest inhibition potency (87%) but relatively less than GEQ [40].

Regarding second and third group where pyrrolidinone and the pyrrolidine series bearing no amide function, the inhibition activities of these compounds have significantly reduced [40]. This due to the carbonyl group of the amide, which plays an important role in the complex formation between the protein and the inhibitor, is far away from the hydroxyl group of Tyr 158 disfavouring therefore vital hydrogen bonds [41].

The compounds have been divided in the three groups for the inhibition results against InhA. Compound 1b, which was efficient as InhA inhibitor, showed no activity against *M. tuberculosis*. Only 2a and 2b of the four N-substituted acyl compounds displayed modest antitubercular activities. In the pyrrolidinone series, Compounds 3a, 4a bearing benzyl substituents displayed poor MICs (>15 μM). Compounds 5a and 6a with long-chains showed modest to good activity against *M. tuberculosis* with MIC values of 13.3 and 2.8 μM. Compound 7a bearing a 4-F-benzene moiety appeared to have moderately active with MIC 14.6 μM. In the pyrrolidine series and as a general trend, compounds 3b–8b were found to have higher activities, except compound 6b. Compounds 4b and 5b exhibited great activity against *M. tuberculosis* with MIC values of 1.4 and 2.8 μM, correspondingly [40].

A panel of *M. tuberculosis* mutants bearing diverse mutations in genes encoding for drug targets (dprE1, mmpL3, pyrG, Rv2466c, Rv3405c, and ethA) has been used to explore the mode of action of compound 4b. Decaprenyl-phosphoribose-20-epimerase (DprE1), which is considered as the target of benzothiazinones, appeared to be a key enzyme in the cell wall biosynthesis [42,43]. Required for the transport of mycolic acids in the form of trehalose monomycolates (TMM) to the outer membrane or periplasmic space of *M. tuberculosis*, MmpL3 is the cellular target of some antitubercular agents including adamantyl urea AU1235 [44,45], indolecarboxamides [46], tetrahydropyrazolo[1,5-*a*]pyrimidine-3-carboxamides THPPs [47], and 1,5-diarylpyrrole derivative BM212, an asymmetrical diamine SQ109 [48]. PyrG, which is the cellular target of thiophenecarboxamide derivatives, plays a significant role in the conversion of UTP to CTP that is described as a vital step in the pyrimidine metabolic pathway in a diversity of bacteria. Of late, it has been reported that Rv2466c is able to activate the thienopyrimidine derivative TP053 in its reduced form [49]. Rv3405c is a DNA binding protein TetR-type transcriptional repressor of Rv3406 [50,51]. Rv3406 appeared to decarboxylate the Ty38c carboxyquinoxaline into its inactive keto metabolite [51].

Finally, compound 4b has been examined against resistant isolate to first and second-line TB drugs. It has been reported that all the *M. tuberculosis* resistant mutants used are sensitive to compound 4b, indicating that this compound has another mode of action different from that scrutinized [42,44,49,51–53].

Cytotoxicities of diverse compounds have been assessed *via* measuring the LC50. All tested pyrrolidine or pyrrolidinone compounds, excluding compound 6b with moderate cytotoxicity (LD50 $^{1}/_{4}$ 18.9 μM), presented no strong cytotoxicities and particularly for compound 4b (LD50 $^{1}/_{4}$ 35 μM) [40]. To investigate the effect of 4b compound on mycobacterial lipids, metabolic labelling of non-virulent *M. tuberculosis* H37Ra strain has been grown in the presence of two different concentrations of this compound. 14C acetate was added as the tracer to the *M. tuberculosis* and lipids/fatty acids were extracted and analysed by TLC [40].

The presence of compound 4b (42 μM and 84 μM) in the culture medium led to produce 43%, and 48% inhibition of the growth of the tested *M. tuberculosis*, correspondingly. Treatment by the compound 4b caused diminution of trehalose dimycolates, but accretion of trehalose monomycolates, while treatment with isoniazid led to complete reduction of both trehalose dimycolates and trehalose monomycolates. Compound 4b appeared to influence the phosphatidylinositol and phosphatidylinositol mannosides pool, and the amount of phosphatidylethanolamine. Analysis of fatty/mycolic acids demonstrated that compound 4b caused reduction mainly in cell wall bound mycolic acids [40]. These results suggest that compound 4b does not affect InhA enzyme, but it rather targets transport of molecules of trehalose monomycolates through the plasma membrane catalysed by MmpL3 protein [48,54].

Recently it has been reported that the proton motive force (PMF) required for the activity of RND transporters appears to be collapsed by MmpL3 inhibitors. Several studies proved that AU1235, THPP-2 and indole carboxamide 2418 vanish only Δ pH part of PMF while SQ109 and BM212 distort transmembrane proton concentration gradient (Δ pH) and electrical charge gradient (Δψ) of PMF simultaneously [55,56]. Therefore, the effect of compound 4b on lipids and mycolic acids of *M. smegmatis* with the impacts of AU1235 dissipating ΔpH and carbonyl cyanide mchlorophenyl hydrazone (CCCP) interrupting ΔpH and Δψ of PMF have been compared. Adding compound AU1235 in final concentration 6.2 μM led to the 60% inhibition of growth. Furthermore, 2, 5 and 10 μM of CCCP resulted in 0.2%, 9%, and 66% inhibition of growth, respectively. Addition of 16.8 μM compound 4b caused 57% growth inhibition. In the presence of the examined compounds, the lipid and fatty acid profiles of *M. smegmatis* showed that the mechanism of action of 4b compound resembles the inhibitory impact of AU1235 rather than CCCP [40].

*Diphenyl ether derivatives*

Lead optimisation is described as a significant task in drug discovery campaigns. A promising drug candidates is based on the balance between physicochemical and biological properties, therefore, many medicinal chemistry experts have been delayed by its complexity [57]. Of late, advances in computational medicinal chemistry led to contribute to the discovery of new drugs. Many computerised methods have been utilised for *in silico* molecular design including LEA3D, TOPAS, CoG, and Flux. While the methods used to improve the properties of compounds during lead optimisation, the process still depends mainly on the medical chemists experience that often help to generate theories regarding structural moieties [58–61].

The concept of MMP, which can describe the behaviour of small substituents in terms of their molecular properties, has been

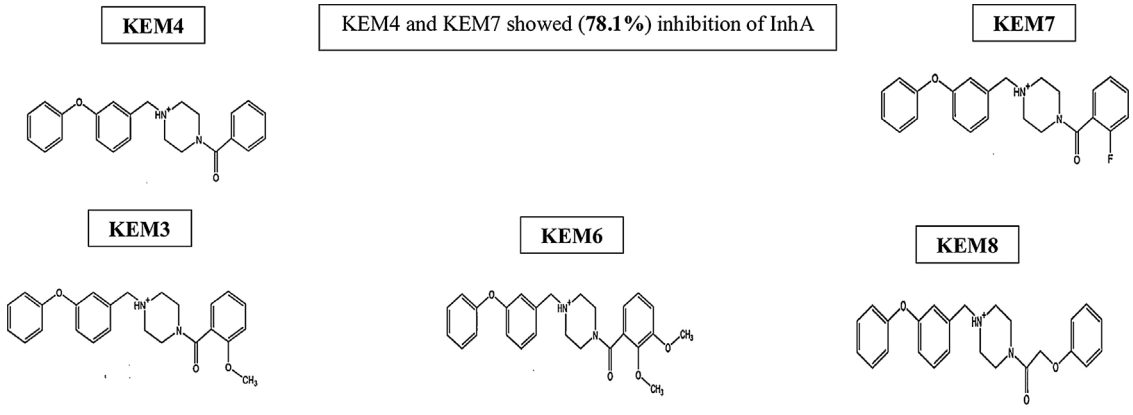
introduced by [13], Leach, Jones [62]. Moreover, MMPs are easily accessible and intuitive due to their small chemical modifications as well as systematic methodology [63]. Many fruitful studies using the method of MMP reported and suggested that this method appears to help not only structure-based drug design but also rational lead optimisation [64,65]. *In silico* combination of the classical matched molecular pair (MMP) analysis and structure-based drug screening (SBDS) resulted in acquisition of structure-activity relationships (SAR) data using *M. tuberculosis* InhA (mtInhA) inhibitors. The 10 KES4 analogues (Fig. 3) (KEM1-KEM10) displayed inhibitory effects on the growth of *M. smegmatis* and InhA of *M. tuberculosis*. While the phenyl group (KEM4) as well as the 2-fluorobenzyl group (KEM7) substituents for the 2-furyl group in KES4 lead to good inhibitory activity (78.1%) against the InhA enzyme, the phenylacetyl group (KEM5), the 2,3-dimethoxybenzyl group (KEM6), and the phenoxyacetyl group (KEM8) substituents are unfavourable [66]. Enhancement in the inhibitory activity of InhA is due to the high affinity of diphenyl ether atoms with nicotinamide adenine dinucleotide (NAD+) and fitting benzene moiety to the hydrophobic pocket surrounding Leu218 [67,68].

The bulky 2, 3-dimethoxybenzyl group appears to reduce the affinity between KEM6 and InhA. The linear elongation of the phenylacetyl group (KEM5) and the phenoxyacetyl group (KEM8) at the 2-furyl group position in KES4 may lead to contribute the opposite orientation compared with the other analogues of KES4 that displayed high inhibitory activity against the InhA enzyme. Moreover, the carbonylation at the 3-phenoxybenzyl linker appeared to have low inhibitory impact against the InhA enzyme, and it is proposed that Phe149 is preferred to Pro193 as a target interaction residue [66]. KEM10 compound showed superior inhibitory effects against the InhA enzyme compared to KEM8 because in KEM10 the 4-methoxy group is prophesied to be near the hydrophobic residue (Leu217) that might regulate the orientation of KEM10. Based on the cytotoxicity assays, the addition of a 4-methoxy group to the phenoxy methyl ligand (KEM8) might result in cytotoxicity in mammalian cells. Compounds (KEM1-KEM10) have not shown any considerable toxic effects on cultured SH-SY5Y cells, however, only KEM10 compound displayed a fragile toxic effect [66]. All the compounds (KEM1-KEM10) either weakly or strongly inhibited the growth of *M. smegmatis*. KEM3, KEM4, KEM6, KEM7, and KEM8 compounds showed moderate inhibitory impacts against *M. smegmatis*. The $IC_{50}$ value of compound KEM7 is 17.3 μM which is close to the $IC_{50}$ value of isoniazid (9.8 μM) [66].

A study regarding the mycobacteria cell division revealed the close similarity between *M. smegmatis* and *M. tuberculosis* [69]. Therefore, it is anticipated that the candidate compounds will display equivalent antibacterial effects against *M. tuberculosis*. However, the inhibitory rate of InhA and growth of model mycobacteria have not been correlated for some compounds including KES4, KEM1, KEM6, KEM7, KEM8, and KEM9. This outcome is due to the low permeability of the *M. tuberculosis* cell wall which comprises mycolic acid, and drug efficiency that is directly influenced by physicochemical properties such as hydrophobicity parameter (logP), and the total polar surface area (TPSA) [70,71]. Based on poly-pharmacological studies, the appropriate modulation of these biological activities is required to improve drug potency. The mentioned compounds might have numerous biological activities to be useful not only for the enzymatic study of InhA but also for the development of new anti-TB medications.

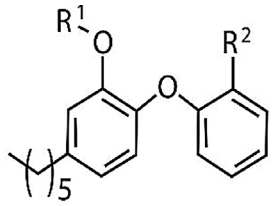
SB-PT052 and SB-PT068, which were both delivered interaction point (IP) in Self-Micro Emulsifying Drug Delivery System (SMEDDS) in an acute *Burkholderia pseudomallei* murine infection model, are considered as lipophilic diphenyl ethers that target the enoyl-ACP reductase FabI1 in *Burkholderia pseudomallei*. While SB-PT052 significantly decreased the bacterial load in the lung (−0.6 log10 CFU/ml) and in the spleen (−1.1 log10 CFU/ml), SB-PT068 only considerably diminished the bacteria load in the spleen (−1.3 log10 CFU/ml) [72,73]. SB-PT070 with an ortho methyl group, and SB-PT091with an ortho substituted chlorine have been identified based on drug-protein interaction kinetics. Compounds SB-PT070 and SB-PT091 appear to have activity against both the InhA enzyme and *M. tuberculosis* H37Rv with MIC values of 3.13 and 1.57 μg/mL, respectively [74].

Formulations of both SB-PT070 and SB-PT091 (Fig. 4) compounds have been developed to enhance the solubility and the efficacy in murine infection models. SB-PT070 and SB-PT091 displayed marginal efficiency as indicated by a 0.78 log10 and a 0.2 log10 decrease in spleen bacterial load at a therapeutic dose of 25 mg/kg and 50 mg/kg, respectively. RIF given alone at a

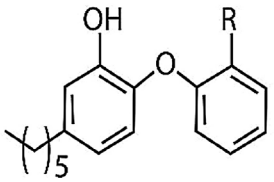



| Compound | Activity against *M. smegmatis* (IC50) value |
|---|---|
| **KEM3** | 25.7 μM |
| **KEM4** | 22.9 μM |
| **KEM6** | 28.1 μM |
| **KEM7** | 17.3 μM |
| **KEM8** | 21.4 μM |

**Fig. 3.** Chemical structures of diphenyl ether derivatives with their activities as potent inhibitors of InhA.




**SB-PT091** $R_1$= H; $R_2$ = Cl




**SB-PT070** R=CH3/ Me

| Compound | Activity against *M. tuberculosis in vivo* | Synergistic Effects with RIF *M. tuberculosis* in *vivo* | *In vitro* activity against *M. tuberculosis* H37Rv MIC (µg/mL) |
|---|---|---|---|
| **SB-PT070 (R*=CH3/Me)** | 0.2 log10 | 2.2 log10 | 3.13 µg/mL |
| **SB-PT091** | 0.78 log10 | 1.9 log10 | 1.57 µg/mL |

R* (where **R = CH3** ) SB-PT070 tested against *M. tuberculosis in vitro*
In the case (**R= Me**) SB-PT070 exaimed against *M. tuberculosis in vivo*

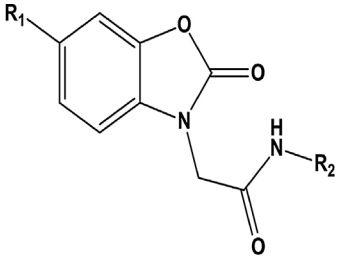
**Fig. 4.** Potent *in vitro* and *in vivo* antitubercular activity of diphenyl ether derivatives.

therapeutic dose of 10 mg/kg diminished the bacterial load by almost 0.5 log10 and 2 log10 in the lung and spleen, respectively. While both SB-PT070 and RIF demonstrated an additional 1.7 log10 decrease of bacterial load in the spleen, SB-PT091 and RIF had a further 1.4 log10 reduction of bacterial load in the spleen compared to RIF alone. Although, there was depletion in the bacterial burden in the lungs for each treatment group, however, the decrease was not statistically important compared to untreated groups. Notably, co-treatment of the InhA inhibitors (diphenyl ether class) enhanced treatment efficiency over RIF itself in rapid model of *M. tuberculosis*. This result indicates that the diphenyl class lead compounds appeared to have potential as members in combinatorial regimens, and might afford therapy options against clinical isolates with different drug susceptibility profiles and avert RIF resistance in bacteria from emerging [75].

*Benzo[d]oxazol-2(3H)-ones derivatives*

Considered as "privileged scaffolds", benzo[d]oxazol-2(3H)-ones are very advantageous for the drug discovery to imitate a catechol moiety or a phenol in a metabolically stable template [76]. Benzo[d]oxazol-2(3H)-ones derivatives appear to have antifungal, anti-inflammatory, anticonvulsant, antioxidant, HIV-1 reverse transcriptase, antitumor, antibacterial, analgesic activity and normolipenic agents [76–89].

Since the electronic characteristic of nitrogen atom is pivotal for the biological activities, thus, the functionalization of nitrogen atom is of interest. Of late, it has been reported that benzoxazol-2(3H)-ones and benzothiazole-2(3H)-ones alkylation conferred the intermediates, which have been found to be used in pharmacotherapy area for their anti-cocaine activity, as these substituted




| Compound | % of InhA inhibition at 10 µM (IC50 in µM)[a] | MIC (µM)[b] | | Cytotoxicity[c] (% inhibtion) MTB |
|---|---|---|---|---|
| | | MTB | XDR-MTB | |
| **28** (R1= H, R2= 5-Nitrothiazol-2-yl) | 48.15±1.34 | 78.05 | NT | 44.62 |
| **29** (R1= Chloro, R2= 5-Nitrothiazol-2-yl) | 72.60±2.73 | 17.61 | 17.61 | 50.64 |
| **30** (R1= Nitro, R2= 5-Nitrothiazol-2-yl) | 82.43±0.83 | 17.11 | 17.11 | 43.79 |
| **32** (R1= Chloro, R2= 2-Benzothiazolyl) | 58.16±0.72 | 17.37 | 34.74 | 52.52 |
| **33** (R1= Nitro, R2= 2-Benzothiazolyl) | 60.80±0.88 | 33.75 | NT | 56.07 |

[a] MTB InhA enzyme inhibition activity
[b] *In vitro* activity against MTB H37Rv
[c] Against RAW 264.7 cells

**Fig. 5.** Chemical structure and activities of the best Benzo[d]oxazol-2(3H)-ones derivatives-based inhibitors of InhA and *M. tuberculosis*.

heterocycles interrelate with signal receptors. Therefore, several approaches including two steps cyclization of ohydroxybenzoic acids, cyclization of arenecarbohydroxamic acid, photochemical rearrangements of 1,2-benisoxazolinones, cyclization of azidoformates, Hofmann rearrangement of amides, carbonylation of o-substituted aryl azides using Rhodium catalyst have been developed for the benzoxazol-2(3H)-one derivatives synthesis [90–93].

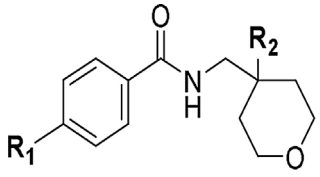
A series of twenty seven substituted 2-(2-oxobenzo[d]oxazol-3(2H)-yl)acetamide derivatives (Fig. 5) have been assessed in vitro for the inhibition of InhA at 10 μM. all synthesized compounds (excluding 16) exhibited significant inhibition percentage ranging from 26.12 to 82.43% at 10 μM. The first subcategory of compounds includes 8, 11, 14, 17, 20, 23, 28, 31 and 34 compound with H atom at R1 and different substituted aryl/heteroaryl groups at R2 position. The docking score has been found to be in the range of −6.87 to −7.59 kcal/mol for this subset of compounds. All compounds (excluding compound 14) appeared to be associated with hydrogen bonding interactions with the Tyr158 side chain and NAD+. In most of the first subset of compounds the carbonyl oxygen atom on benzo[d]oxazol-2(3H)-one ring interrelated with the Tyr158 side chain [94]. While compound 23 appeared to be comparatively least active, compound 28 showed the most selective inhibitor of InhA. For the most active compound, the bound conformation of compound 28 displayed carbonyl oxygen atom of benzo[d]oxazol-2(3H)-one ring forming hydrogen bond with the Tyr158 side chain and ribose hydroxyl group of NAD+, which has been detected in the crystal ligand and also well correlated with the inhibitory activity of InhA *in vitro*. Regarding compound 23, binding analysis demonstrated that this compound failed to provide any hydrogen bonding interaction with the Tyr158 side chain of the enzyme and therefore the inactivity of the compound might be correlated [94].

The second subset of compounds including (9, 12, 15, 18, 21, 24, 29, 32 and 35) showed more inhibitory than previous one. Compounds 29 and 32, which displayed docking scores of −7.12 and −7.073 kcal/mol respectively, appeared to be effective inhibitors of InhA with considerable activity in the range of 28.16–72.60% inhibition at 10 μM. The observed potency of both compounds (29 and 32) might be due to the wide hydrophobic interactions prophesied to be established with the side chains of Met199-Leu218-Met155-Pro156-Ala157-Ile202-Met103-Phe149 and Met161 along with hydrogen bonding interactions with Tyr158 side chain as well as with the ribose hydroxyl group of NAD+ [94]. On the other hand binding analysis of one of the less active derivative (compound 9) revealed hydrophobic interactions amid the phenyl ring and some hydrophobic amino acid residues. Moreover, it exhibited hydrogen bonding interaction amid carbonyl oxygen atom of 6- chlorobenzo[d]oxazol-2(3H)-one ring and Tyr158 side chain. However, the orientation in the active site cavity of target enzyme (InhA) derived the chloro group away from the cavity, which could be the reason for its lesser inhibition of InhA enzyme [94].

In the third subset (10, 13, 16, 19, 22, 25, 30, 33 and 36), the compounds 30, 33 with docking score in the range of 7.11 to −7.48 kcal/mol appeared to be the most promising compounds showing adequate inhibitory activity of InhA *in vitro*. Analysis of both compounds 30, 33 in the binding site demonstrated that the carbonyl oxygen atom on 6-nitrobenzo[d]oxazol-2(3H)-one ring has been included in hydrogen bonding interactions with Tyr158 side chain residue as well as with ribose hydroxyl group of NAD+. Compound (16) from this subset exhibited activity less than whole series of compounds with 15.63% inhibition at 10 μM concentration. *In silico* analysis of compound (9) revealed that the molecule oriented in a diverse mode than that of other active derivatives and failed to produce any interaction with Tyr158 as well as with $NAD^+$ resulting in less inhibition of InhA enzyme [94].

All the synthesized compounds showed activity against *M. tuberculosis* H37Rv with MIC ranging from 15.04 to 82.58 μM. Six compounds (25, 29–32 and 36) inhibited *M. tuberculosis* H37Rv with MIC of <20 μM. Compound 30 appeared to be the most effective compound against *M. tuberculosis* InhA enzyme with $IC_{50}$ 5.12 ± 0.44 μM and inhibited with MIC of 17.11 μM *M. tuberculosis* H37Rv and was non-cytotoxic at 100 μM. The percentage inhibition of cells was in range of 1.61–43.54% at 50 μM, and 12.08–6.10% at 100 μM. Furthermore, All the compounds showed mycobacterial kill against the XDR strain (resistant to Isoniazid, Rifampicin, Ofloxacin and Kanamycin) and five compounds (20, 25, 29–30 and 36) exhibited XDR-TB MIC of <20 μM [94].

Efflux-pump proteins, that are associated with MDR-TB, belong to the ATP-binding cassette (ABC) transporters and major facilitator superfamily (MFS) proteins, or antibiotic-modifying



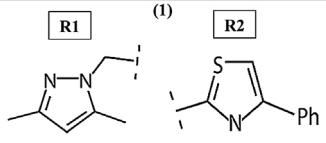
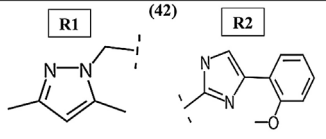

| Compound | InhA IC50 (μM) | MIC (μM) | Cell tox HepG2 TOX50 (μM) |
|---|---|---|---|
| (1)   | 0.02 | 11.7 | 100 |
| (42)   | 0.036 | 5 | 50.1 |

**Fig. 6.** Chemical structure and antimycobacterial activities of tetrahydropyran derivatives.

and degrading enzymes, and small multidrug resistance family (SMR) [95–97]. Efflux-mediated antimicrobial resistance in *M. tuberculosis* might be due to one or more efflux pumps running individually or in coordination [98]. The synthesized compounds have been screened in the presence of efflux pump inhibitors verapamil (50 μg/mL) and piperine (8 μg/mL) [99]. The MIC of the synthesized compounds reduced from 2 to 10 fold when compared to the absence of verapamil and piperine. Compound 30 displayed remarkable potency against *M. tuberculosis* (MIC = 8.55 μM) in the presence of verapamil and piperine and its *M. tuberculosis* InhA $IC_{50}$ was very good correlated [94].

*Tetrahydropyran derivatives*

Tetrahydropyran derivatives were discovered through high-throughput screening (HTS) to find novel inhibitor of InhA enzyme [100,101]. Compound 1 had good InhA inhibitory potency ($IC_{50}$ = 0.020 μM), moderate antimycobacterial activity (MIC = 11.7 μM), and killed *M. tuberculosis* inside macrophages. Moreover, compound 1 exhibited modest hERG inhibition with an $IC_{50}$ of 50.1 μM, and low cytotoxicity against the HepG2 human cell line. Compound 1 has been examined against seven clinical isolates with characterized mutations either in katG or at position 15 upstream of the transcriptional start site of InhA. Mutations in the katG (codon 315) and the InhA promoter are proven isoniazid-resistant [102,103]. Tetrahydropyran compound 1 (Fig. 6) exhibited a small rising change in MIC against isoniazid resistance, but a more important shift was detected in the strains which overexpressed InhA. Isoniazid MIC increased in paired clinical isolates with mutations in katG (codon 315). The drug susceptibility analysis of these isolates proved that strains with katG mutation displayed a great level of resistance to isoniazid (MIC > 25 μg/mL) while susceptibility to compound 1 was close to wild-type (MIC = 15.6-62.5 μM) [101].

The overall binding mode of compound 1 is close to pyrrolidine compound in the crystal structure [104]. The pyrazole on the left-hand side of compound 1 accumulates against the pyrazole 2-nitrogen provides a H-bond interaction with the 2′-hydroxyl group of NAD, and the pyridine portion of the NAD [101]. The InhA substrate-binding loop, that comprises helix α6, is well-organized in the structure, and the N-terminal end forms part of the pyrazole binding pocket. The methylene linker on the left-hand side of the molecule inserts a curl into the ligand which is essential to retain its shape complementarity with the binding site [101].

The compound 1 central amide has water-bridged interactions with the protein, comprising the backbone carbonyl of Met98. The tetrahydropyran indicates of the binding site. The right-hand side of the molecule appears to be in a deep pocket in the protein which is bound on one side *via* the C-terminal end of ordered helix α6 [101].

A series of 18 analogues have been synthesized and assessed. According to the SAR data produced, rings C and D can be altered in further optimization efforts. In this series the best compound 42 (Fig. 6) showed InhA inhibitory potency ($IC_{50}$ = 40 nM), antimycobacterial potency comparable to compound 1 (MIC = 5 μM), and low cytotoxicity [101].

## Concluding remarks

The complications in translating potent on-target activity into antitubercular action with the demanding challenges of growing and continuous TB drug resistance stand in the way of prosperous antitubercular agents design. Isoniazid, which is considered as one of the first antitubercular agents, remains the most given drug for prophylaxis and TB treatment. Resistance to isoniazid described as one of the hallmarks of MDR clinical strains. The compounds discussed in this article comprise InhA inhibitors which have new binding modes of action, display solid evidence of successful target engagement with activity *in silico*.

For the predictable future, *M. tuberculosis* appears to be treated with multidrug combinations. However, the emerging data support the fact that InhA inhibitor is a considerable choice to attain new drugs for use in drug combinations in future treatments. We do hope that this communication will encourage the research community in both academia and industry to target InhA with novel agent discovery methods.

## Conflict of interest

The authors confirm that this article content has no conflict of interest.

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