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Review Article

Unraveling the EthR Puzzle: Exploring Novel Inhibitors in the Fight against Tuberculosis from a Medicinal Chemistry Vistas

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Abstract

Tuberculosis stands as a menacing infectious disease that has instilled worldwide apprehension owing to its profound impact on the well-being of afflicted individuals. Ethionamide is the second line drug therapy for active multidrug resistant tuberculosis. Functional impairment of EthA enzyme which is responsible for the activation of ETH (Ethionamide) result in drug resistance in *Mtb*. To combat against Ethionamide resistance and boost up its activity against *Mtb* in second line therapy of tuberculosis, various types of ETH boosters have been designed and/or developed by different strategies. These Ethionamide promoters work by upregulating the action of EthA enzyme. The gene responsible for downregulation of EthA enzyme is EthR gene and the process is called as ETH repression which is the cause of drug resistance. So, to get prevail over this Ethionamide resistance and to enhance ETH activation in *Mtb* infected cell, EthR inhibitors are designed and developed to upregulate the EthA enzyme. EthR inhibitors demonstrated the desired interaction into its DNA recognition site that results into inhibition of EthR activation.

Keywords: *Ethionamide repressor (EthR); Ethionamide (ETH); Ethionamide activator; Mycobacterium tuberculosis (Mtb)*

Abbreviations

ETH: Ethionamide; EthA: Ethionamide Activator; *Mtb*: *Mycobacterium tuberculosis*; MDR-TB: Multidrug-Resistant Tuberculosis; SMARt: Small Molecule Aborting Resistance

Introduction

Worldwide tuberculosis represents a major public health issue and prime leading causes of death from an infectious agent, *Mycobacterium tuberculosis* (*Mtb*). About one third of the world population is being infected by the *Mtb* and susceptible to develop the disease. *Mycobacteria* are probably the most successful microorganisms to parasite animals and humans [1]. An emergence of the multidrug-resistant tuberculosis (MDR-TB) poses a major threat to global tuberculosis (TB) control programmers. Globally, the estimated proportion of new TB cases with MDR/RR-TB were 3.6% in 2021. Ethionamide is currently used only as a secondary agent, always in

combination with other antitubercular agents namely isoniazid, ethambutol, pyrazinamide and/or rifampin and usually for multidrug resistant mycobacterial infections or in situations where first line agents are contraindicated.

Ethionamide has been classified as a second-line drug by the World Health Organization (WHO) and currently has its greatest use in the management of drug-resistant TB. The mechanism of action of Ethionamide is activation of prodrug by activator gene/enzyme EthA followed by inhibition of cell wall formation of *Mtb* cells [2] as shown in figure 1. EthR inhibits the Ethionamide activator EthA that leads to inhibition of prodrug activation ultimately causing resistance [3], so to enhance the antitubercular potential of Ethionamide, development of repressor inhibitors is the prime requirement in the arena of the antitubercular drug discovery.

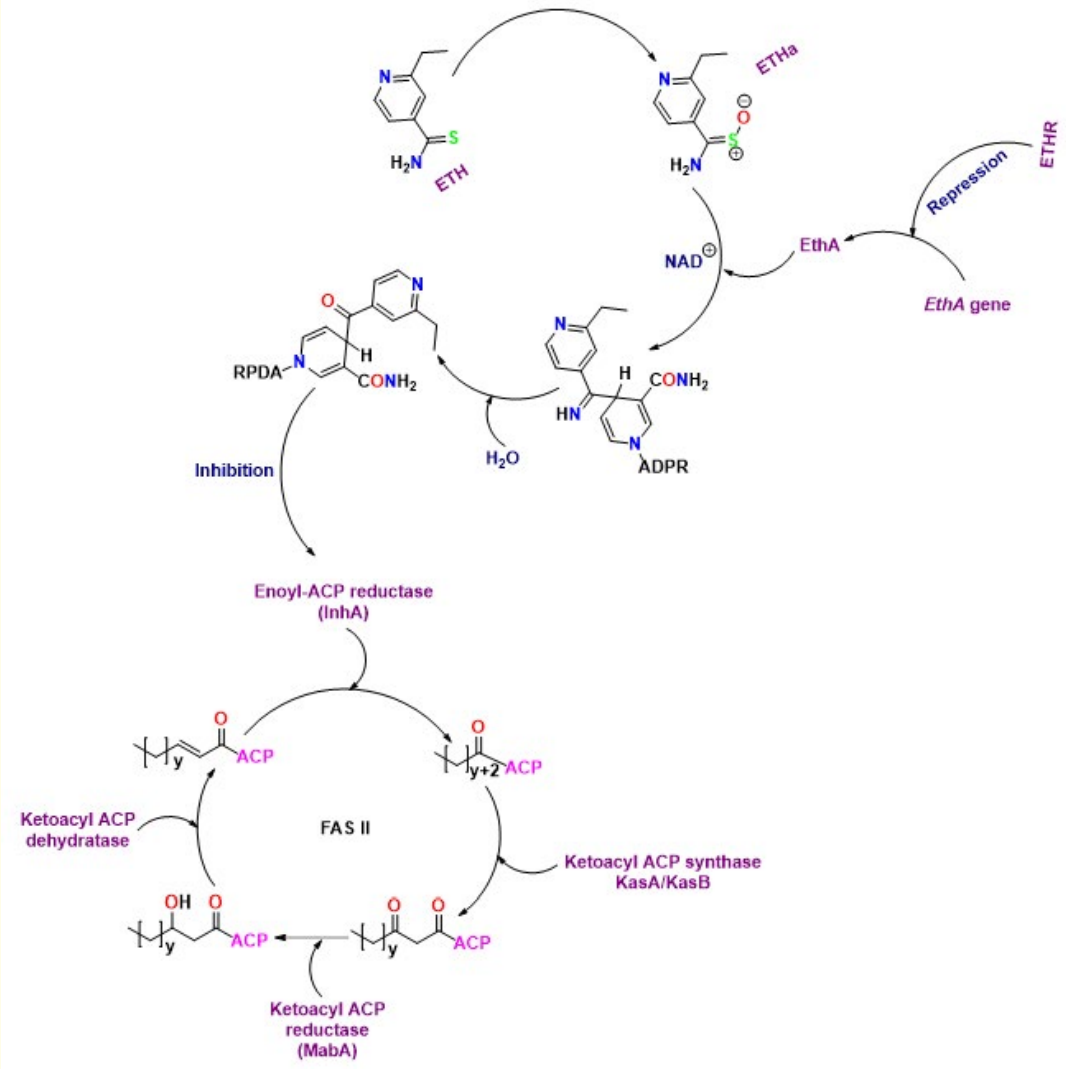


Figure 1: *Mechanism of action of Ethionamide.*

Ethionamide is one of the antitubercular prodrug which is transformed into its bioactive form by the action of enzyme monooxygenase EthA inside the mycobacterial cell. Ethionamide an analog of thioamide class is currently employed for the treatment of MDR-TB in combination with isoniazid [4]. Based on the molecular basis, isoniazid and Ethionamide are potent anti-tubercular drugs structurally similar to the isonicotinic acid [5]. Cloning experiment in *Mtb* (*M. smegmatis* and *M. bovis* BCG) demonstrated by DeBarber., *et al.* proved that protein EthA which is responsible for activation of Ethionamide is flavoprotein containing FAD molecule as prosthetic group [6].

Ethionamide (ETH) is activated by an enzyme EthA which is a membrane associated flavoprotein constituting FAD molecule as prosthetic group proved to be responsible for Ethionamide activation by oxidizing Ethionamide to the corresponding S-oxide (intermediate I) bearing similar biological potential. The S-oxide further undergoes oxidation reaction with EthA to form sulfinic acid intermediate which is considered to be in the doubly oxidized unstable form. The non-toxic sulfoxide form of Ethionamide (ETHa) is further triggered to incept an inactive intermediate II that is further converted to an intermediate III demonstrating action similar to isoniazid on InhA. Furthermore, intermediate III generates NADH covalent bonding at active site InhA crystal structure leading to inhibition of enoyl acyl ACP reductase which is pivotal for mycolic acid biosynthesis hence, mycolic acid biosynthesis is the prime target for Ethionamide inside the *Mtb* cell membrane [7].

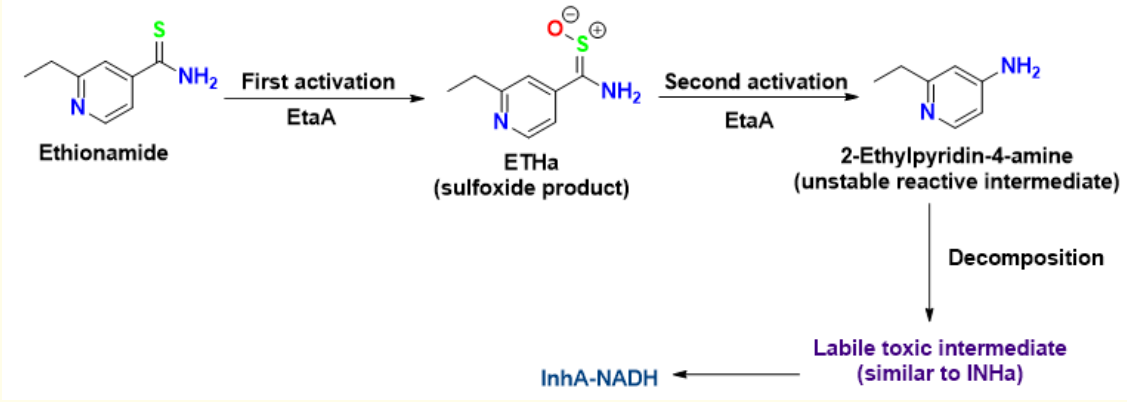


Figure 2: *Steps of Ethionamide (ETH) metabolism.*

Wang., *et al.* have proved that Ethionamide forms covalent adduct with NAD to form ETH-NAD that inhibits InhA protein by binding on its surface of active site resulting a complex having molecular weight corresponding to the INH-NAD covalent adduct. Enzyme inhibitory studies revealed that Ethionamide demonstrated potent antitubercular activity similar to Isoniazid with K_i value of 7 ± 5 nM. NAD molecule forms covalent binding with nicotinamide ring at 4th place and portrayed its binding in the active cavity of the InhA that mimicked the binding of isoniazid [8].

Ethionamide particularly targets InhA protein that is amenable for the mycolic acid biosynthesis shows 24% sequence homology with several flavin containing monooxygenase in protein sequence data.

Based on a comparison of protein crystal structure sequences, the TetR/CamR repressor protein family includes EthR. As like other TetR/CamR family of repressor proteins, EthR is a homodimer that binds to its operator in different fashion consisting 55 bp. The EthR operator gene is over four times longer than that of the majority of other TetR/CamR family members. Plasmon resonance studies demonstrated EthR octamerization while binding to DNA site at 1.7 Å resolution [9]. Numerous crystal structures of proteins belong to

TetR/CamR family, constitute two domains namely N and C-terminals from which N-terminal contains three helical bundles that makes up a DNA binding site by helix turn helix fashion [10]. C terminal domain that is bigger in size which creates small molecule binding domain with constitutes of 4 - 9 helices [11]. The detail understanding of crystal structure of EthR suggested that it encompasses hydrophobic as well as hydrophilic region. The small lipophilic compounds that potentially possess hydrophobic and the polar hydrogen bonding capabilities can induce inactive conformation of EthR that leads to inhibition of repressor effect.

Dover., *et al*. documented the theory that an EthR crystal structure has a hydrophobic drug-binding pocket. The helices 4, 5, 7 and 8 in the crystal structure produce a tiny tunnel like cavity known as a hydrophobic tunnel with a length of 20 Å. The opening of the tunnel is controlled by the loop joined by helices 4 and 5, which exhibits flexibility that signifies contribution of helices 5 and 7 producing side chains by aromatic residues. The hydrophobic tunnel comprising of some uncharged polar amino acid residues Asn179, Asn176 and Thr149. The wide range of ligand molecules that may reside in this hydrophobic tunnel include 1, 4-dioxane or hexadecyl octanoate.

The structural conformation of EthR DNA binding domain is not totally identical to the conformation of other known domain topology of TetR/CamR family. It shares a surface area with the TetR family and has four patches that are known to function as DNA-binding domains. Highly similar N-terminal DNA-binding domains that are coupled to ligand binding domains that appear to be non-homologous are shared by QacR.

Frenois., *et al*. has described the crystal structure of EthR by correlating it with whole helical structure of TetR family members. The hexadecyl octanoate (HexOc) bounded crystal structure of EthR was compared with the ligand bounded crystal structure of QacR, which revealed a similarity in both ligand bounded crystal structures. In ligand bounded form of crystal structure, there is an increase in on center spacing between DNA recognition helices (helix 3 and 3') from 37 to 48 Å in QacR while similarly in case of EthR structure there is separation of 52 Å. Binding of ligand in the crystal structure of EthR, showed a translation and rotation of DNA binding domain by virtue of 5 helix additional turn formation that leads to movement of helix 6.

Helix number	Amino acid residues involved in ligand binding domain
α 4	Leu76, Leu87
α 5	Met102, Trp103, Gly106, Ile107, Phe110, Phe114
α 6	Thr121, Gly124, Gln125, Arg128
α 7	Trp138, Met142, Trp145, Tyr148, Thr149, Val152
α 8	Asn176, Asn179, Glu180, Leu183, Phe184, Phe187
α 9	Trp207

Table 1: *Amino acid residues belonging to the helices in the dimerization/ligand binding domain of the HexOc binding site.*

Ligand when bound to this DNA binding domain triggers conformational change in EthR protein crystal structure which is incompatible with EthR function that leads to EthA de-repression consequently escalated sensitivity to Ethionamide.

Resistance of Ethionamide

Mutation in the bacterial gene encoding enzyme is responsible for resistance to antibiotic. Resistance to this type of antibiotics are produced by two mechanisms i.e. bacterial cell membrane alteration and drug active efflux from bacterial cell. The challenge spotted for the treatment of TB is majorly the resistance to the available therapy further resistance is bifurcated into two major classes namely co-resistance and cross resistance which go hand in hand with a slight alteration in its mechanism.

Co-resistance act by four different mechanisms towards Ethionamide namely:

a. Overexpression of the InhA target by increasing the expression of the gene encoding in the target enzyme.
b. Preventing adduct binding to target enzyme by alteration of the target enzyme (Target modification) that has developed resistance to antibiotics [12].
c. Preventing activation of prodrug and adduct formation by alterations of an activation mechanism (Activator modification).
d. Impermeability of the mycobacterial cell to the antibiotic due to deregulation of enzyme NADH dehydrogenase II via *ndh* gene mutation [13].

Cross-resistance: Ethionamide (ETH) resistance has revealed cross resistance to other thiocarbamide drugs (link to EtaA activation) which indicated that EtaA displayed an exceptional broad prodrug acceptance.

Two types of mutation are responsible for acquired Ethionamide resistance that are:

- A genetic change in the mabA-inhA operon's promoter region at the 8th position that causes InhA to be overexpressed.
- EthA genetic mutation, resulting in inhibition of monooxygenase EthA [14].

Mutation in the EthA gene leads to the Ethionamide resistance which results in the functional impairment of EthA enzyme in the bioactivation pathway of Ethionamide drug. The resistance of *Mycobacteria* to Ethionamide has been attributed to low production of Ethionamide (ETH) activator protein EthA forcing an excessive dosage of ETH, frequently associated with side effects. So, to overcome the above stated problems and also to increase the therapeutic efficacy of Ethionamide, drug like inhibitors of EthR have been reported to enhance the *in vitro* bioactivation of Ethionamide.

EthR inhibitors

Ethionamide repression (EthR) inhibitors are compounds designed to target EthR, a regulatory protein found in *Mycobacterium tuberculosis*. EthR plays crucial role in the resistance of tuberculosis to Ethionamide. These inhibitors are developed to block the activity of EthR, thereby preventing its ability to repress the expression of genes involved in Ethionamide resistance. By inhibiting EthR, these compounds enhance the effectiveness of Ethionamide and contribute to more successful tuberculosis treatment by overcoming drug resistance. Development of EthR inhibitors is a crucial step in the ongoing efforts to combat tuberculosis and reduce its global impact.

Prevet., *et al*. documented new EthR inhibitors by employing fragment based screening of about nine hundred sixty fragments from the library. For structure-based optimization using X-ray crystallography, twelve fragments that might stabilize the EthR protein in a dose dependent manner were used. Further five hit molecules complexed with EthR which are similar to SMARt-420 in which the tropinone scaffold resided in the hydrophilic region developing interactions with Asp168 and Glu170. The carbonyl functionality and the tertiary amine on tropinone ring pointing towards Glu70 and Asp168. In the XRD study of EthR co-crystallized with BDM14272, (add picture PDB 6HRW) tertiary amine functionality forms hydrogen bonding with side chain of Asp168 side chain, aromatic ring established hydrophobic contacts with Ser134, Ile164, Leu167, Met177, Trp100 and Thr138. The phenyl ring forms π-π stacking interaction with Trp100 in T-shaped manner and lastly the bicyclic moiety form bonding with Val73, Met74, Trp100, Phe126, Ile113 and Ala130 discretely.

Compound 1 comprising of tropinone core divulged improvement of properties like solubility and other physicochemical effects. Compound 1 was found to be the most potent on dose dependent assay demonstrating pIC_{50} value 6.0 but was spotted to be totally inactive when tested in a mammalian reporter assay at 30 μM concentration. The replacement of ethylene group in the tropinone core

propylene bridge (Compound 2) preserved the potency while reduction in the chain length is not acceptable. Side chain comprising two carbon atom attach at beta carbon of carbonyl compound found to be responsible for improved affinity towards EthR protein. Replacement of 4-chlorophenyl ring with another substituted phenyl ring by introducing chlorine atom at meta position gave the more active compound 3, moreover substituents at ortho position affect the biological potential by virtue of steric hindrance. Introduction of bulkier atom/groups such as methyl or Br (Compound 4) may increase the activity while electron withdrawing nature of the substituent is not considered. Due to lack of bacterial membrane permeability, these compounds are not found as much effective as to enhance the ETH activity. The trifluoromethyl derivative, which had a high ligand efficiency index, was more potent than the hit and was equally effective as compounds 2, 3 and 4 [15].

Villemagne., *et al*. primarily reported development of oxadiazole analogs by fragment based EthR inhibitors. The analogs were identified by combination of analytical techniques namely X-Ray and SPR diffraction which are further evaluated for its corresponding *in vitro* anti-tubercular activity. Combination of spectral methods led to identification of new chemotype of EthR ligand. Further hit to ligand optimization was done employing fragment budding, merging and linking approaches from compound 5.

Compound 5 was developed by structure optimization through fragment growing strategy from chemotype BDM41906. In XRD study of ligand compound 5 with EthR protein showed that oxygen and nitrogen atoms of amide developed hydrogen bond interactions with side chain of Asn176 and Asn179. Methyl thiazole motif of compound 5 resided into the region comprising hydrophobic residues and side group of Tyr148 structure. Substitution on 5^{th} and 2^{nd} positions of thiazole ring occupied pockets D2 and D2'. Other five membered heterocycles replaced in place of thiazole ring was detrimental to stabilize physicochemical parameters. Compound 5 optimized by fragment-based approach from reference compound was able to boost *in vitro* anti-tubercular activity for ten times of ETH at low nanomolar concentration. In the structure of BDM71339 (Compound 6), a thiazole ring was substituted by cyclopropyl and an oxadiazole ring. Subtle modification by replacement of 2-methylthiazole of reference compound by 2-cyclopropyloxadiazole gives potent compound with better microsomal stability (Compound 6) and higher ETH activity (*In vitro* study) at lower nanomolar concentrations.

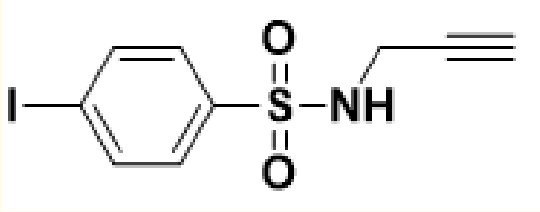


Figure 3: *Structure of BDM41906.*

Villemagne., *et al*. also demonstrated that co-encapsulation of compound 5 and 6 with ETH in pCD, leads to increase the solubility of boosters. Enhancing effect of developed compounds was found to be equivalent to the compound BDM41906 [16].

In order to modify the binding affinity and facilitate the chemical synthesis of compounds, road and colleagues found allosteric inhibitors that specifically target the lipophilic binding region of transcriptional factor EthR. The lipophilic allosteric binding site of EthR was targeted using a structure fragment based strategy. In total, 86 fragment hits, four fragments occupied the central pocket of EthR and bind to Asn179. This series of compounds form key hydrogen bond interactions in a hydrophobic area of EthR with Asn176 and Asn179. Among these series of compounds, fragment hit 4 establishes H-bonding and also π-stacking interactions. Fragment hit 1 resided in the hydrophobic cavity at two positions, alterations in the conformation of side chains of Phe184 and Gln125. As the two molecules of fragment hit 1 together could span the entire binding channel, the fragment-linking approach was identified as the most efficient perspective.

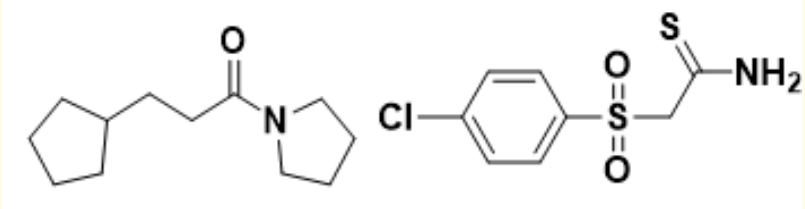


Figure 4: *Structures of fragment hit 1 and 4.*

Nikiforov., *et al*. reported the development of fragment-sized EthR ligands previously identified by Surade., *et al*. for enhancing the Ethionamide activity. Molecular docking studies of this identified lipophilic chemical entity named as fragment 1 with EthR revealed that it can be a good starting point for design of further derivatives containing H bond donor/acceptor group with scaffold. Compounds derived from fragment 1 by substituting hydrogen bond donor or acceptor moieties in the parent scaffold revealed that carbamate 2 exhibit a good Ethionamide boosting effect. Expanding the ring size of cyclopentyl group of fragment 1 gave 4-fold potent compound, additional propylene linker (7) gave approximately seven fold increase in MEC over fragment hit 1. Structure of compound containing Cyclohexyl ring and linker as propylene moiety gave about seven fold improved MEC value (0.4 ± 0.2 μM) over starting fragment 1 while further addition of methylene unit to propylene linker led to decrease potency by four folds. Cyclohexyl ring is essential for activity, adamantly group along with attached methylene unit linked to carbonyl carbon of pyrrolidine amide caused thirty fold increase in ETH enhancing activity. Increasing ring size of pyrrolidine ring decreases activity while decreasing ring size results in increase of ETH improving ability. Decreasing ring size of cyclopentyl found to be beneficial for activity. Linker of three carbon atoms length found to be optimal (8) in carbamate series for Ethionamide boost up effect. 7 and 8 has MEC 400 nM ($1/10^{th}$ of its MIC in presence of ETH) with tenfold upliftment in activity of ETH and seven fold improvement upon starting fragment one. 7 and 8 exhibited significant intrinsic bactericidal activity in the absence of Ethionamide [17].

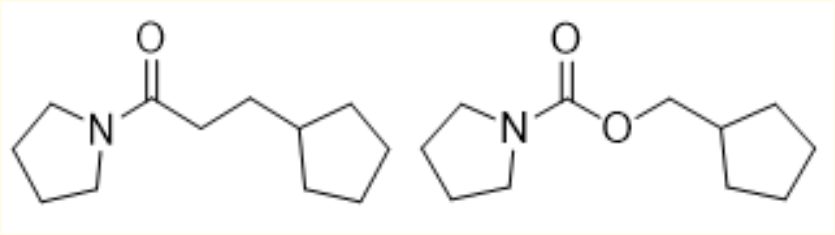


Figure 5: *Structures of fragment 1 and carbamate 2.*

Fragment based screening library of 1250 fragments was done against EthR, out of which 86 fragment compounds were recognized using fluorescence based thermal shift. From the X ray crystallographic analysis, two chemical hits 1 and 2 were shown to bind twice to EthR monomer together, they completely filled the hydrophobic cavity of the EthR monomer and serve as appealing starting spots for fragment integrating to produce stronger EthR ligands. Three likely combinations of merging two neighboring fragment units were discovered through analysis of the X-ray crystal structures of these fragments. The newly synthesized 9 had higher binding affinity than their parent fragment hits followed by subsequent SAR exploration of 9 by introducing chemical moieties like nitrile (10) or ethyl ester (11) groups gave the tenfold improved activity in SPR functional assay. 10 revealed a five-fold decrease in KD by ITC and significant 10-fold upgradation employing the SPR functional assay (KD = 1 μM by ITC and IC_{50} = 3 μM). Based on SPR assay, 11 demonstrated

comparable affinity (IC_{50} = 3 µM), despite of having an ethyl ester functionality as opposed to the nitrile group of 10. Compounds were made by the strategy of merging one molecule of fragment hit 1 with two molecules of fragment hit 2 representing the potency of 10. However, none of merged compounds were capable to pass through the mycobacterial cell envelope which results into neutralizing effect of Ethionamide [18].

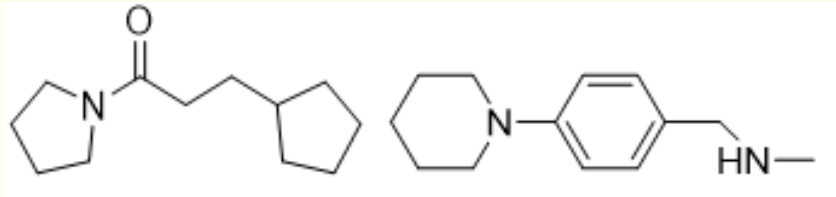


Figure 6: *Structures of fragment 1 and 2.*

Flippo., *et al.* discovered EthR inhibitors by using high throughput screening of 14640-compound library whole mycobacteria phenotypic assay on M. *smegmatis*. A new chemical class of N-phenylphenoxyacetamide motif was identified from the screening. In X-ray diffraction study, the co-crystallized structure of EthR protein with N-phenylphenoxy acetamide derivative was spotted to be stable and revealed that N-phenyloxy acetamide moiety is the key pharmacophore in designed molecules. The amide bridge of ligand forms hydrogen bond with Asn179 and Asn176. O-methylphenoxy motif occupied upper hydrophobic pocket which is generated by Trp207, Thr149, Leu87, Gly106 (HTF motif), while on the flip side benzotriazole functionality faces towards Phe110 and Phe114. Aromatic ring of the benzotriazole ring lies on small hydrophobic pocket of Trp138 and Phe187. From the screening of synthesized 960-member focused library, 14 and 15 found to be most active and potent Ethionamide promoter *in vitro* with EC_{50} values of 0.21 and 0.34 µM. Inh > 50% at 10 µM in presence of 0.1 µg/ml sub active dose of Ethionamide. Finally, the EthR co-crystallized with 14 divulge increased active surface due to additional hydrophobic contacts with Met102 and Val152 (aliphatic side chain) and Trp103 and Tyr148 (aromatic side chain) which results increased activity than 14.

On the basis of discovery of locus named as ethR2 and ethA2 which are similar to ethA and ethR gene, Blondiaux., *et al*. have produced the drug-like molecule (16) known as SMARt (Small Molecule Aborting Resistance) from which spiroisoxazoline scaffold was spotted as highly effective to improvement in Ethionamide activity independently of EthR but abolished the ability of compound to inhibit EthR activity. It causes *MTB* to use alternate bioactivation route of Ethionamide. 16 significantly increases the expression of a collection of distant ethA genes. The inhibitory activity of 16 towards EthR DNA interaction is quantified by expression of SEAP (Secreted Alkaline Phosphatase) reporter gene (SEAP assay) and results stated the inhibition of SEAP production by 16. MIC of Ethionamide decreases from 2 to 0.25 µg/ml thus, decreases resistance of ETH in *Mtb*. EthA mutation leading ETH resistant strains develops sensitivity when co-administered with SMARt-420 [19].

Baulard group developed a pharmacophore based on the aforementioned inklings consisting two hydrophobic terminals separated by 4-6 Å and hydrogen bonding characteristic that interacted with vital hydrophilic amino acids Asn176 and Asn179. Surface Plasmon Resonance assay technique was employed to screen a library of one hundred thirty compounds for an extent to which they inhibited EthR-DNA interaction. Two categories of compounds bearing 1, 2, 4-oxadiazole and 1, 2, 4-triazole compounds showed encouraging activity in inhibiting EthR- DNA interaction. BMD-14500 (18) exhibited more than 50% EthR-DNA interaction inhibitory activity that was crystallized with EthR which showed that the molecule was encapsulated above small molecule active cavity in C terminal domain of EthR. The compound was positioned exactly at the same place where dioxane was located in the hydrophobic pocket of the crystal structure. The two hydrophobic pockets made by Phe184, Leu183, Trp145, Trp138, Met142, Trp207, Leu87, Trp103, and Ile107 were occupied by

the thienyl and piperidine moieties. Encouraged by the crystallographic study, the same compound was subjected for ETH boosting effect at the concentration of 20 nM along with 2 µg/mL Ethionamide using radial diffusion experiments on *M. tuberculosis* bacteria H37Rv. This confirmed an enhancing effect against resistant strains in which even at 4 µg/mL concentration of Ethionamide alone failed to show growth inhibition of organism [20].

Benoit., *et al*. further extended work which led to identification of new leads BDM 31343 and BDM 31381 as EthR-DNA interaction inhibitors, fifty eight thiophene containing 1,2,4-oxadiazole compounds were synthesized and screened for Ethionamide advancing effect at sub-active dose. Both BDM 31343 and BDM 31381 exhibited EC_{50} values of 1.5 and 0.1 µM in Ethionamide boosting study while in SPR study, BDM 31381 showed IC_{50} value of 0.5 µM. In an approach to synthesize new compounds, five and six membered aromatic rings were investigated for Ethionamide elevating effects, elongation of spacer was detrimental for enhancing activity. Numerous compounds containing fluorine and small sized heterocyclic ring such as pyrrolidine were also reported with more potent intracellular penetration. It was substantially more effective to introduce aliphatic chains or rings that fit the ligand binding domain's pocket. Both compounds BDM31343 and BDM31381 were H-bonded to Asn179, according to the X-ray crystal structure of EthR complexes. The Phe114, Trp138, Trp145, and Phe184 aromatic residues are positioned in a 3-5Å sphere around the thienyl or phenyl substituents of the two boosters near the bottom of the ligand binding domain, which is rich in aromatic residues.

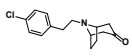
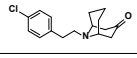
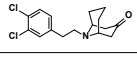
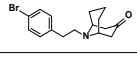
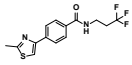
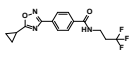
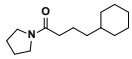
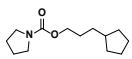
Based on the SAR, analogs of thiophen-2-yl-1, 2, 4-oxadiazole demonstrated tenfold escalation in Ethionamide activity. A fluorinated 19 was more stable and potent than thienoacetyl while also having better solubility. When methyl groups were replaced with smaller substituents, like fluorine atoms, the activity increased in comparison to the parent hydrogenated compounds. Trifluoroalkyl moiety is strongly responsible for potency based on findings *ex vivo* assay study because introduction of fluorine substituents (19) possibly facilitated cell penetration and enhance physicochemical properties but fails to improve protein ligand interaction. The replacement by pyrrolidine was well tolerated in 19. 20 with *R* configuration demonstrated encouraging potential than *S* configuration, keeping the same fluorinated chain, replacement of piperidine by a pyrrolidine provided the stable compound along enhanced solubility and potency [21].

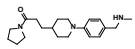
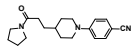
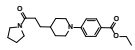
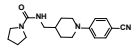
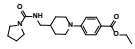
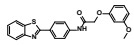
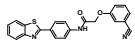
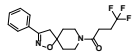
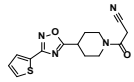
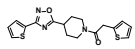
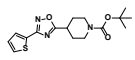
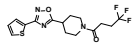
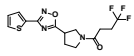
A thorough *in silico* structure-based screening methodology was divulged by Tatum., *et al*. identified novel EthR inhibitor scaffolds that were biologically and physically characterized using a thermal shift experiment using the DSF or Thermofluor assay technique. Further virtual screening was also performed, in which all binding poses of 40919 novel compounds were filtered after docking and 284 potential ligand poses were inspected. Eighty five hit ligands were brought forward for biophysical screening from these results. Growth inhibition assays were performed on 20 biophysical hits which revealed that five amongst the twenty biophysical hits showed best results in *in vitro* study with EC_{50} value 34 µM of best novel scaffold. In XRD study with resolution ranging from 2.1 to 1.4 Å, the co-crystallization of EthR protein with four new drug candidates showed that the binding of ligands resulted good inhibition of EthR. Lead compound, 21 (N-butyl-4-methyl-1-piperidine carboxamide) in XRD co-crystallized with EthR protein structure at 2.1 to 1.4 Å resolution retained hydrogen bond and hydrophobic interactions with Asn179, Asn176 and Phe110 and Trp145. 21 containing central carbonyl flanked by substituents viz. 1,2-oxazole moiety and 1H,4H,5H,6H-pyrrolo group on other side of carbonyl carbon which showed hydrogen bond interaction at two different sites of protein structure i.e. Asn179 and Thr149 to carbonyl group to ligand compound. Ligand containing the aliphatic chain with iso-oxazole ring is located in hydrophobic pocket created by amino acid Phe184, Trp138 and Leu183. In second orientation, pyrrolo ring system form hydrophobic stacked with Phe110. Furan moiety of 22 sustained lipophilic interactions with Phe110 and hydrophobic interaction of 2-methylcyclopropyl moiety with Phe184, Phe114 and Leu183. 23 co-crystallization with EthR structure was determined. Hydroxyl group formed hydrogen bond with Thr149 and carbonyl group of carboxamide with Asn176.

1, 3-benzodioxole motif formed hydrophobic interaction in hydrophobic pocket containing group residues Leu183, Phe184 and Phe114. These four lead compounds had crucial groups for molecular binding of EthR protein crystal structure which will be considered as basic ligand structure to optimize ligands with new chemical scaffolds [22].

Kumar and Elma have undertaken *in silico* methodologies for the identification of novel antitubercular drug like molecules for the inhibition of Ethionamide repressor gene. Identification of about fifty antitubercular drug molecules employing *in silico* methods such as molecular docking and dynamics followed by drug-likeness assessment and ADMET study, prophecy of metabolism on P450 site. Molecular docking study of fifty ligands were performed individually against the InhA and EthR receptor proteins by using the DockThor server for the initial docking evaluation and shows the lower affinity score. Drug Compounds 24, 25, 26, 27 showed cytochrome metabolism for CYP2C8, CYP2A6, CYP2D6, CYP2C19, CYP2B6, CYP1A2, CYP2E1, CYP2C9 isoforms that displayed the drug likeliness property. The four potential drug candidates that are 3-[3-(4-Fluorophenyl)-1,2,4-oxadiazol-5-yl]-N-(2-methylphenyl)piperidine-1-carboxamide (C22) (24); Maritinone (C38) (25); 3-Methyl-benzofuran-2-carboxylic acid pyridin-4-ylamide (C21) (26) and 5-(4-Ethyl-phenyl)-2-(1H-tetrazol-5-ylmethyl)-2H-tetrazole (C29) (27) obeyed the Lipinski's rule of five without disregarding any of its parameters, they followed the Ghose, Veber, Egan, and Muegge rules as well. Furthermore, they exhibited positive ADMET properties and displayed good intestinal absorption and oral bioavailability. These four compounds have the ability of Caco-2 cell line penetration and 24 and 27 showed the negative results of hepatotoxicity and carcinogenicity study. Molecular dynamics simulation revealed that 24 and 27 demonstrated residential stability in the active site of the protein in the overall 100 ns trajectory. The best four compounds were examined for analyzing pharmacokinetics and pharmacodynamics properties and they showed promising results in drug-likeness and ADMET profiling [23].

Grau., *et al*. revealed the enhancement of antitubercular activity of the second line drug. *In vitro* growth inhibitory assay stated escalation in the GI_{50} Ethionamide, isoxyl, and thiacetazone by addition of 2-PEB by upregulating the function of Ethionamide activator EthA respectively.

Sr. no.	Compound no. in original paper	Chemical structure	EC_{50} (µM)	IC_{50} (µM)	Reference
1	BDM 14272		n/a	6.0	[15]
2	Compound 9		n/a	9.8	
3	Compound 22		n/a	6.0	
4	Compound 28		n/a	6.1	
5	Compound 3 (BDM43266)		n/a	0.10	[16]
6	Compound 27 (BDM71339)		n/a	0.072	
7	Compound 14		0.4 ± 0.5	n/a	[17]
8	Compound 28		0.4 ± 0.2	n/a	

9	Compound 5		n/a	35	[18]
10	Compound 15		n/a	3	
11	Compound 16		n/a	3	
12	Compound 17		n/a	4	
13	Compound 18		n/a	2	
14	Compound 4		0.21	n/a	[21]
15	Compound 5		0.34	n/a	
16	SMARt-420 (BDM41420)		n/a	10	[19]
17 (a)	BDM31343		1.5	0.9	[20]
17 (b)	BDM31381		n/a	n/a	
18	BDM14500		n/a	38	
19	Compound 46		0.1	1.6	[21]
20	Compound 55		0.4	2.0	

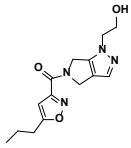
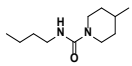
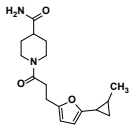
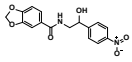
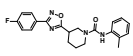
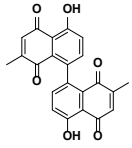
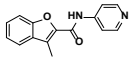
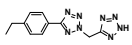
21	Compound 10		n/a	n/a	[22]
21	Compound 25		760 nM/0.76µM	n/a	
22	Compound 42		> 90 µM	n/a	
23	Compound 57		n/a	n/a	
24	Compound 22		n/a	n/a	[23]
25	Compound 38		n/a	n/a	
26	Compound 21		n/a	n/a	
27	Compound 29		n/a	n/a	

Table 2: *List of most active compounds as EthR inhibitors.*

Conclusion

Tuberculosis a lung associated infection cause due to *Mycobacterium tuberculosis* which is most lethal infectious disease spotted after cancer. The incurability of this disease has installed a fear in humans. Ethionamide belong to the second line antitubercular prodrug

that which is activated by mycobacterial monooxygenase EthA, the production of which is controlled by the transcriptional repressor EthR. From the established line of treatment Ethionamide and its corresponding combinations have developed resistance. This review provides insights into the medicinal chemistry aspects of the EthR and its inhibitors since decades. Elaboration of various strategies for the development of EthR inhibitors such as fragment based screening, fragment merging approach pharmacophore based screening, *in* silico structure based screening, and HTS methods along with the activity were documented.

Conflict of Interest

The authors declared that there existed no clash on authorship.

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