

Metabolism of the Antituberculosis Drug Ethionamide

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Abstract: Ethionamide (ETH) is an important second-line antituberculosis drug used for the treatment of patients infected with multidrug-resistant *Mycobacterium*. Although ETH is a structural analogue of isoniazid (INH), both are pro-drugs that need to be activated by mycobacterial enzymes to exert their antimicrobial activity. ETH mechanism of action is thought to be identical to INH although the pathway of activation is distinct from that of INH. ETH is activated by an EthA enzyme, leading to the formation of an S-oxide metabolite that has considerably better activity than the parent drug. This review comprehensively examines the aspects related with the metabolism of ETH since its discovery up to today.

Keywords: Ethionamide, ETH-SO, EthA, EthR, ETH-NAD, metabolism, tuberculosis, mycobacterium tuberculosis, multidrug resistance.

INTRODUCTION

Tuberculosis is an infectious disease caused by the bacillus *M. tuberculosis* and is still a world health problem of staggering proportions. About one-third of the world's population is infected with *M. tuberculosis* without exhibiting symptoms of the disease. This airborne disease typically affects the lungs, but can also affect other sites of the body. Although only a relatively small proportion of people infected with *M. tuberculosis* will go on to develop tuberculosis disease, in the case of people infected with the human immunodeficiency virus (HIV) the probability of developing tuberculosis is rather high. Tuberculosis is generally thought to develop in an individual as a consequence of one of three processes: progression of primary infection, exogenous reinfection, or endogenous reactivation [1-3].

In 1993, the World Health Organization (WHO) declared tuberculosis a global public health emergency. According to the 2011 WHO report [1, 2], there were 8.8 million incident cases of tuberculosis in 2010, 1.1 million deaths from tuberculosis among HIV-negative people and an additional 0.35 million deaths from HIV-associated tuberculosis. In the same year, there were 5.7 million notifications of new and recurrent cases of tuberculosis. A large effort has been put forward in several countries to fight against this disease and the estimated investment for treatment in 97 countries with 92% of the world's tuberculosis is expected to be USD 4.4 billion in 2012 [1]. Tuberculosis is spread from person to person through the air. When a person with lung tuberculosis coughs, sneezes or spits, the tuberculosis germs propel into the air. A person needs to inhale only few of these germs to become infected [1].

The first line drugs normally employed to treat tuberculosis drug resistance are isoniazid (INH), ethambutol, pyrazinamide and rifampin [3]. When the infection is unresponsive to the treatment with first line drugs, second line drugs come into play. Because of the emergence of multidrug-resistant (MDR) strains of *M. tuberculosis*, second-line drugs such as ethionamide (ETH) have become increasingly important as replacements for INH and rifampin, although ETH is relatively weak tuberculosis drug with usual

treatments that can last over 2 years with several side effects [4], as opposed to 6–9 months with INH and rifampin-containing regimens [5].

ETH is a prodrug that must undergo metabolic activation to exert its cytotoxic effects. ETH (**1**, Fig. (1)) was first used in the chemotherapy of tuberculosis in the early 1950's. The feature that distinguishes structurally and classifies this drug is the presence of a thioamide function. It was recognized that S-oxygenation of the thiourea moiety of ETH was involved in the bioactivation of a hepatotoxic metabolite [6], in a similar manner to what had been shown earlier for thiocarbamides [7]. This S-oxygenation also determines the therapeutic efficacy of ETH. Ten years later ETH propyl analogue, prothionamide, PTA (**2**, Fig. (1)), was introduced into clinical treatment because it was shown *in vitro* to be as active as ETH against *M. tuberculosis* in contrast to the experimental tuberculosis in the mouse, but appeared to be better tolerated by patients [8, 9].

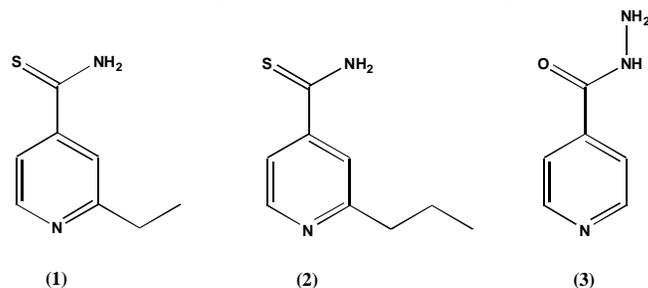


Fig. (1). Chemical structure of ETH (**1**), PTA (**2**) and INH (**3**). These pro-drugs have similar structures but INH is activated by a catalase-peroxidase, whereas ETH and PTA are activated by a flavin-dependent monooxygenase (FMO).

These drugs have been used in the treatment of mycobacterial infections of *M. leprae* and *M. avium* [10, 11]. They are the most frequently used drugs for the treatment of drug-resistant tuberculosis and, therefore, are becoming increasingly relevant as the number of MDR and extensively drug-resistant cases is increasing worldwide [12, 13]. ETH and PTA are structurally similar to INH (**3**, Fig. (1)), which has been successful in the treatment of tuberculosis infection in the past. INH is an antibacterial drug that has been used to both prevent and treat tuberculosis since 1952. INH should be part of the treatment for all persons with tuberculosis unless there is a compelling reason to omit it, such as allergy to the pharmacologic agent. There are currently ten drugs with FDA approval for the

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treatment of tuberculosis and the first-line agents that form a four-drug regimen for treatment of active tuberculosis are INH, rifampin, ethambutol and pyrazinamide [14].

However, in recent history, poor compliance with the prolonged and complicated chemotherapeutic regimens currently used to treat tuberculosis, in conjunction with the advent of the AIDS epidemic and the increased mobility of human populations, has led to the emerging of numerous MDR *M. tuberculosis* strains [15, 16]. Resistance to frontline therapeutics, such as INH, results in the treatment of patients with "second-line" agents that are less effective and/or more toxic [17]. One of the most effective in the treatment of tuberculosis is ETH.

For 25 years, the main research involving ETH was focused on the type of metabolites formed [18-28], starting, thus, the study of ETH metabolism. During this period the principal metabolite identified was ethionamide sulphoxide (**4**, Fig. (2)). In recent years, the studies involving the pharmacokinetics (PK) of ETH are more complete and accurate. As an example, a study of the absorption and elimination of ETH in twelve healthy, adult male volunteers after administration of oral tablets and rectal suppositories can be mentioned [29]. Treatments compared 250-mg ETH tablets and a placebo suppository to a 500 mg ETH suppository and two placebo tablets, given 7 days apart. In this study, the area under serum concentration-time curve was used to determine the relative bioavailability of drug from preparations. The relative bioavailability after rectal administration was 57.3% of that after oral administration. The maximum concentration after rectal administration was 33% of that after oral administration.

Aclair *et al.* reported the effect of food, orange juice, or antacids on the absorption of a single oral 500-mg dose of ETH in healthy volunteers, including the bioequivalence assessment, and determined the ETH population PK parameters [30]. The PK behaviour of ETH was not significantly modified by the different conditions studied. Mean ratios for area under curve (AUC) ranged from 0.91 to 0.96 for the orange juice, food, and antacid treatments, indicating a minimal effect on the relative bioavailability. ETH can be administered with food if tolerance is an issue [30].

The PK parameters of ETH were also determined following oral doses. ETH PK parameters were independent of age, weight, height, gender, and creatinine clearance [31]. Tuberculosis patients appeared to have lower volumes of distribution (3.22 L/Kg) and clearance values (1.88 L/h/Kg) compared to healthy volunteers (Table 1). Therefore, this resulted in lower AUC values (3.95 mcg h/mL) in the tuberculosis patients. ETH displayed a short elimination half-life (1.94 h).

In the second-line therapy for drug-resistant tuberculosis, ETH should be administered with other agents due to the rapid resistance development when the drug is used as monotherapy. The PK parameters of ETH are given in Table 1. PK parameters differed between healthy subjects and tuberculosis patients, resulting in a lower AUC for tuberculosis patients, possibly due to a decrease in bioavailability [13, 31].

Table 1. PK Parameters of ETH in Humans

Half-life (h)	AUC (mg.h/L)	C _{max} (µg/mL)	Volume of distribution (L/Kg)	PK methodology
1.94	7.67	2.16	93.5 (sugar coated tablets)	Mean PK for 250 mg oral dose using film-coated tablets in healthy adults (FDA label)

FROM THE PRINCIPAL METABOLITE TO THE UNDERSTANDING OF ETH METABOLISM

Activation of ETH

The link between the ETH-resistant tuberculosis and mutations in the Rv3855 and Rv3854c genes clearly indicates that the oxidation of ETH-SO catalyzed by FAD-containing enzyme is critical for the activation of the drug to its cytotoxic metabolite [32, 33]. This provided information on the catalytic and physical properties of this enzyme, and demonstrated that the catalyzes in the activation of ETH occurs in two steps rather than in one step. [17].

As a result of the oxidation of ETH by EtaA the S-oxide is formed (**4**, Fig. (2)) [17, 33], which is a metabolite that is known to retain the full antituberculosis activity of ETH [33]. Further oxidation of the S-oxide then produces the unstable sulfinic acid and, eventually, the 2-ethyl-4-carboxamidopyridine metabolite that has no antimycobacterial activity [17, 34]. Therefore, the active metabolite of ETH lies between the S-oxide and 2-ethyl-4-carboxamidopyridine and might be the sulfinic acid or a product of its decomposition [35]. However, the sulfinic acid has not been isolated yet. The activation of ETH by the *M. tuberculosis* EtaA was also extended to other related compounds [36], such as thiacetozone, PTA and isoxy (4,4'-diisoamyloxydiphenyl-thiourea) [37-39]. Magic angle spinning NMR studies in living mycobacterial cells on the EtaA-dependent oxidation of ETH have shown that the formation of the S-oxide and 4-pyridylmethanol metabolites are formed primarily outside the cells, and the accumulation of a unidentified activated intermediate formed inside the cells [40, 41].

ETH Metabolites

ETH, like INH, is thought to be a prodrug that must be converted to its active form by the bacterial cell. *In vivo* mammalian and bacterial studies suggested that the first metabolite of ETH is the thioamide S-oxide (ETH-SO, **4**, Fig. (2)), which retains the biological activity of the parent drug [17, 33, 42]. Other metabolites identified in the whole cell bacterial systems include the ETH nitrile (**5**, Fig. (2)), ETH amide (**6**, Fig. (2)) and ETH alcohol (**7**, Fig. (2)) [17, 33].

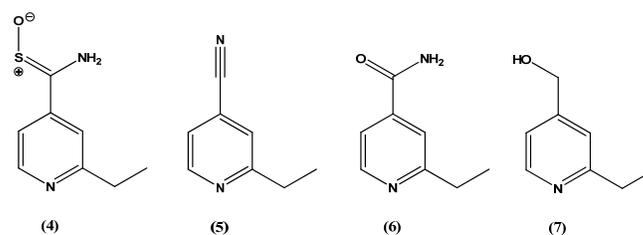


Fig. (2). Structures of metabolites of the ETH: (4), ETH S-oxide; (5), 2-ethyl-4-cyanopyridine; (6), 2-ethyl-4-amidopyridine; and (7), 2-ethyl-4-hydroxymethylpyridine.

De Barber *et al.* postulated that ETH is activated via the corresponding S-oxide (**4**) to a sulfinate that can form an analogous aldehyde equivalent (an imine) through a radical intermediate (see Fig. (3)). Hydrolysis of this imine could be followed by reduction of the resulting aldehyde to the metabolite alcohol, confirmed and identified as **7** by chromatography with a synthetic characterized alcohol standard [33].

A wide variety of toxic thiocarbonyl-containing compounds have been shown to be converted to the corresponding S-oxides during *in vivo* metabolism by mammals supporting this intermediate oxidation in the metabolic activation of ETH [43].

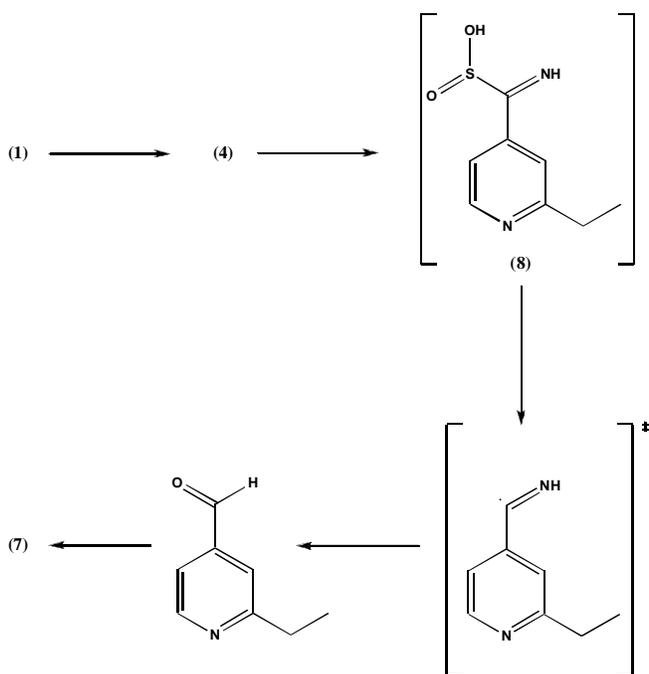


Fig. (3). Proposed activation process for the thioamide oxidation of ETH [33]. *In vivo* production of (2-ethyl-pyridin-4-yl) methanol (7) from ETH by whole cells of *M. tuberculosis*.

ETH-SO is not very reactive and, thus, it can be stored and physically characterized. The properties of ETH-SO, along with the observation that it is as lethal to the tuberculosis bacilli as ETH, suggested that ETH-SO itself requires further activation to a final cytotoxic species [32]. Incubation of authentic ETH-SO with ETH under turnover conditions shows, indeed, that it is further metabolized by the enzyme to another metabolite [9]. Chromatographic data of this second metabolite with synthesized standards identified it as 2-ethyl-4-amidopyridine (6, Fig. (2)). This second metabolite is thus a product of the enzymatic action of ETH on ETH-SO (1→4) and not a branching product stemming from an alternative reaction with ETH [17]. However, 6 is likely not the actual enzymatic product of ETH and ETH-SO but rather is a more stable molecule resulting from the decomposition of this putative cytotoxic metabolite, possibly a sulfinic acid specie (8, Fig. (4)).

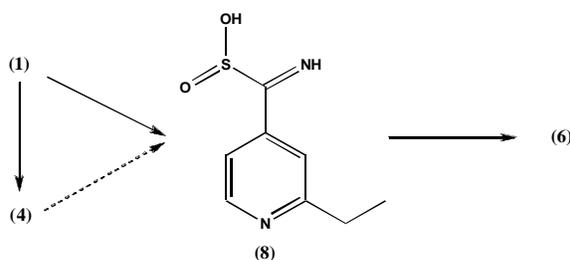


Fig. (4). Possible formation of ETH amide (6) via a sulfinic acid (8). The ETH amide has no antitubercular activity.

Cell incubations of *M. tuberculosis* with radiolabelled ETH have indicated that ETH-SO and ETH nitrile (5) are initially formed and accumulated to a maximum concentration over a couple of hours [33]. Following this early concentration increase, ETH-SO and 5 concentrations begin to decrease, and an increase in the ETH amide (6) and ETH alcohol (7) amounts are observed [33].

ETH Metabolism

To understand the ETH metabolism it is essential to cite the work of Johnsson *et al.*, who suggested that this drug could be metabolized to a higher S-oxide capable of undergoing addition reactions to the nucleophilic protein side chain [44]. The experiments suggested for the first time a molecular mechanism of action, where ETH was activated by unidentified mycobacterial enzymes [44]. After that, two works were reported and explained the ETH activation [32, 33]. Firstly, it was demonstrated that overproduction of Rv3855 (EthR), a putative regulatory protein from *M. tuberculosis*, confers ETH resistance whereas overproduction of an adjacent, clustered monooxygenase (Rv3855c, EthA) confers ETH hypersensitivity [33]. The production of EthA appears to be negatively regulated by EthR and correlates directly with [¹⁴C]-ETH metabolism, suggesting that EthA is the activating enzyme responsible for thioamide oxidation and subsequent toxicity [33].

In another work, it was reported the identification of an activator for ETH. The ETH activator Rv3854c (EthA) was found to be homologous to various monooxygenases and induced ETH sensitivity when overexpressed in mycobacteria [32]. Also, the neighbouring open reading frame (Rv3855), which was found homologous to transcriptional repressors of the TetR family, led to ETH resistance when overexpressed. In addition, chromosomal inactivation of this gene by transposon led to ETH hypersensitivity. These data suggested that Rv3855 (EthR), regulates the production of EthA, which subsequently activates the pro-drug ETH [32].

After the identification of the ability of EthA-enzyme to activate the drug, it was reported that mutations within EthA structural genes were associated with relatively high levels of ETH resistance, i.e. approximately 76% of isolates resistant to > 50 µg/mL of ETH had such mutations [45].

Vannelli *et al.* reported that the enzyme EthA (Rv3854c code) is a flavoprotein containing a single FAD group that catalyzes the NADPH- and O₂-dependent monooxygenation of ETH to the corresponding S-oxide (4) [17]. The S-oxide which has a similar biological activity as ETH, is further oxidized by EthA to 2-ethyl-4-amidopyridine (6). This flavoenzyme also oxidizes thiacetazone, thioacetamide, and isothionicotinamide, and thus, is probably responsible, as suggested by the observation of crossover resistance, for the oxidative activation of other thioamide antituberculosis drugs [17]. It was discovered that purified EthA displayed a remarkably low activity with ETH.

Typical components of the mycobacterial cell wall are mycolic acids. It has been shown that the ability of mycobacteria to synthesize the so-called wax ester micolic acids relies on a Baeyer-Villiger oxidation step. Hinted by the presence of a Baeyer-Villiger monooxygenase sequence motif in the EthA sequence, it was discovered that the enzyme converts a wide range of ketones to the corresponding esters or lactones via a Baeyer-Villiger reaction, indicating that EthA represents a Baeyer-Villiger monooxygenase [42]. EthA is also able to catalyze enantioselective sulfoxidation of methyl-*p*-tolylsulfide [42]. Redox monitoring of the flavin cofactor during turnover of phenylacetone indicates that a step in the reductive half-reaction is limiting the rate of catalysis.

Vilchève *et al.* proposed the first mechanism of action of ETH, that is a drug activated by the monooxygenase EthA. The activated forms react with nicotinamide adenine dinucleotide (NAD⁺) to form an ETH-NAD adduct (Fig. (5)) [46]. This adduct inhibited the common target InhA, the NAD-dependent enoyl-ACP reductase of the fatty acid synthase type II system, resulting in the inhibition of mycolic acid biosynthesis and cell lysis. The resistance to ETH is associated with recessive mutations in the genes encoding the activator of the drug, EthA, which prevents drug activation. ETH resistance is associated with dominant mutations in the gene encoding the common target of the drug, InhA, which result in target amplification or target modification [46].

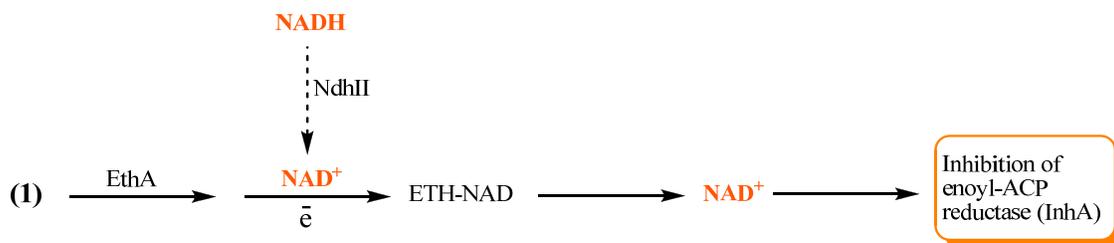


Fig. (5). Mechanism of action of ETH [46].

Hanoulle *et al.* showed that the intracellular metabolism of ETH strictly depends on the presence of the monooxygenase (EthA) and that EthA-dependent activation of ETH is coupled to a precise molecular sorting mechanism of the ETH metabolites [40]. The authors reported that substantial amounts of the ETH-treated mycobacteria produced the previously identified ETH metabolite 2-ethyl-4-hydroxymethylpyridine (7, Fig. (2)), which is present exclusively outside of the bacteria.

The appearance of new data about the type of metabolite formed can be clarified by other research works involving the metabolism of thioamides [47, 48]. Thioamide could be metabolized to support bacterial growth but the mechanism of nitrogen release was not established [47]. In another study, a bacterium was isolated for its ability to grow on thioacetamide as its sole nitrogen source. A pure culture was obtained, determined to be a *Ralstonia pickettii* strain, and its mechanism of thioacetamide and thiobenzamide metabolism was elucidated. It was proposed that *R. pickettii* degrades thioamides via a mechanism involving consecutive oxygenations of the thioamide sulphur atom [33]. Using a cell-based activation method, it was demonstrated that thioamides (as ETH) formed covalent adducts with NAD and that these adducts were tightly binding inhibitors of *M. tuberculosis* and *M. leprae* InhA [34]. The crystal structures of the inhibited *M. leprae* and *M. tuberculosis* InhA complexes provided the molecular details of target-drug interactions. ETH-NAD showed monomolar inhibitor concentration (K_i) against *M. tuberculosis* InhA. Nishida *et al.* further determined the IC_{50} values from the dose-response plots of enzyme fractional activity as a function of the K_i values [35].

In Fig. (6) two plausible mechanisms are represented for the activation of ETH. Both routes will lead to the observed ETH-NAD adduct, retaining a tetrahedral carbon at position 4 of the nicotinamide ring [37].

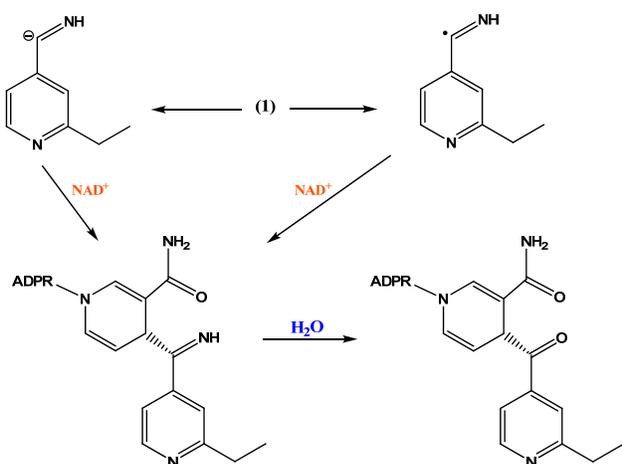


Fig. (6). Possible reaction mechanisms for the activation of ETH and the formation of ETH-NAD [37].

Dover *et al.* reported that EthA, a common activator of thiocarbamide-containing drugs, acts on different mycobacterial targets [38]. This study indicated that EthA is the activator of three antituberculosis drugs: ETH, thiacetazone (8, TAC, Fig. (7)) and isoxyl (9, ISO, Fig. (7)).

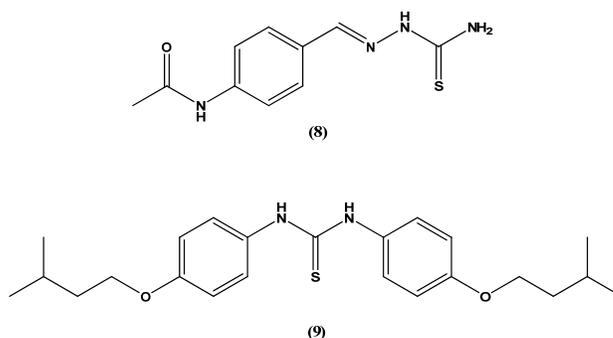


Fig. (7). Structures of the thiocarbamide-containing antituberculosis drugs: 8, thiacetazone (TAC); and 9, isoxyl (ISO).

Once activated, ETH inhibits mycolic acid biosynthesis by targeting InhA, which is also the primary target of activated INH (3, Fig. (1)). These aspects are represented in detail in Fig. (8) [38].

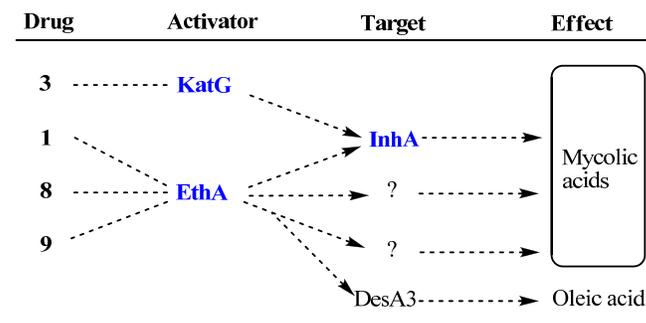


Fig. (8). Summary of the activation mechanism of the four antituberculosis drugs. Prodrugs get into tubercle bacilli by passive diffusion, where they are activated by specific proteins to a range of reactive radicals or species [38]. DesA3 (Rv3229c) from *M. tuberculosis* is a membrane-bound stearyl coenzyme A Δ^9 desaturase that reacts with the oxidoreductase Rv3230c to produce oleic acid.

Mycothioliol (MSH) is the glutathione analogue for mycobacteria. Mutations in MSH biosynthetic genes have been associated with resistance to INH and ETH in mycobacteria. The construction of independent null deletion mutants for all four genes involved in the MSH biosynthesis pathway (MshA, MshB, MshC and MshD) showed different resistance to INH and ETH [49, 50].

One of the most pronounced characteristics observed in the first MSH mutant isolated from *Streptomyces* strains was its >25-fold increase in resistance to INH (3) [51]. The MshB-deficient mutant,

which makes 5 to 10% of normal MSH levels, has been reported to be only slightly more resistant to INH than the wild type in a disk assay [52, 53], suggesting that moderate levels of MSH are sufficient to confer normal sensitivity to **3**. Also, enhanced resistance to ETH is another characteristic of the MshA-, MshB-, and MshD-deficient mutants, with complete resistance at 50 $\mu\text{g/mL}$ [52, 53]. The high levels of resistance of the MSH mutants to **3** and ETH suggest that MSH is involved in the activation of these drugs, because both **3** and ETH are prodrugs which need to be activated intracellularly and whose reactive groups must be unmasked before inhibiting their target [54]. Recently, it was demonstrated that mutations in MSH biosynthesis genes may contribute to INH or ETH resistance across mycobacterial species, because these mutants showed low-level resistance to INH but were highly resistant to ETH [55]. The MshA, MshC, and MshB single deletion mutants were resistant to ETH, indicating that ETH resistance is modulate by the level of Msh in *M. smegmatis*. Surprisingly, the MsdD deletion mutant lacked Msh production but was sensitive to both INH and ETH [56].

It is important to note that in the second-line therapy for drug-resistant tuberculosis, ETH should be administered with other drugs to avoid rapid development of resistance when the drug is used as monotherapy. The use of ETH and PTA (**2**, Fig. (1)) is increasing in MDR resistant strains *M. tuberculosis*. ETH is used interchangeably with **2** [57].

With the integration of EthR, which controls the resistance of *M. tuberculosis* to ETH, into a synthetic mammalian gene network, Weber *et al.* have been able to identify the licensed food additive 2-phenylethyl-butyrate as a potent inhibitor of EthR in *M. tuberculosis*, which dramatically increases the sensitivity of this pathogen to the last-line-of-defense drug ETH and potentially to other EthA-dependent compounds [58]. More recently, drug-like inhibitors of EthR were identified to boost the bioactivation of ETH [59]. These compounds were screened for their capacity to inhibit EthR-DNA interaction by cocrystallization with EthR. One of these analogs enabled the preparation of a substantially reduced dose of ETH in order to lessen the capacity of mycobacterial load in an efficient manner as the conventional higher-dose used in the treatment [59]. Again, it was concluded that thiocarbamide-containing drugs, as ETH, were activated by the mycobacterial monooxygenase EthA, and the production of which was controlled by the transcriptional repressor EthR.

The mammalian FMOs (EC 1.14.13.8), a superfamily of flavo-protein monooxygenases active towards a wide range of xenobiotics, catalyse the oxidative metabolism of bioactive thiourea-based drugs [36, 60-63], and FMO1 and FMO3 have been shown *in vitro* to catalyse the oxygenation of thiacetazone (**8**, TAC, Fig. (8)), forming the same products as EtaA [62]. TAC and ETH act against mycobacteria in the lung, and were investigated for the ability of human FMO2.1 to catalyse the oxygenation of these antituberculosis drugs [34]. This protein catalyzes oxygenation of both TAC and ETH, forming the same metabolites as those produced by human FMO1 and FMO3, and by the mycobacterial enzyme EthA. These results provide a potential explanation for some of the observed inter-individual differences in the efficacy and response to TAC and ETH, and have implications for the treatment of tuberculosis in sub-Saharan Africa and in individuals of recent African descent [34].

By studying the mutations of enzymes associated to ETH metabolism we can understand the resistance of the drug. Various mechanisms involved in mycothiol biosynthesis were reported, including mutations altering EthA/EthR, InhA and its promoter, the NADH dehydrogenase encoded by *ndh*, and the MshA enzyme. However, the mechanism of resistance to ETH remained unknown for 19% of the ETH^r (clinical isolates: 47 ETH-resistant), highlighting the complexity of the mechanisms of ETH resistance in *M. tuberculosis* [64].

Carette *et al.* demonstrated that the inhibitory effect of highly diverse specific ligands of EthR can be mimicked by the mutation G106W [65]. This explains how two small cyclic molecules, such as dioxane [66], induced the same conformational state as the one induced with hexadecyl-octanoate [67, 68]. These results suggested that exploitation of deeper positions of this ligand pocket to increase their affinity/specificity would be successful as long as these molecules interact with the upper region of the surrounding glycine 106 [65]. Engohang-Ndong *et al.* reported that EthR, which encodes a repressor belonging to the TetR/CamR family of transcriptional regulators, negatively regulates the expression of EthA [69]. At this point, it is possible to propose the *cycle* for metabolism of ETH (Fig. (9)), according to its active form (ETH-SO), its activator (EthA) and the repressor implicated in drug resistance in mycobacteria (EthR).

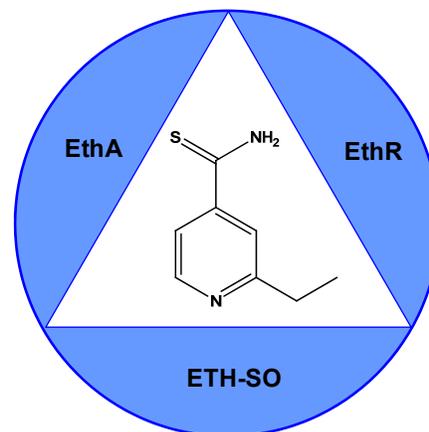


Fig. (9). The ETH cycle proposed.

A biophysical study of ETH was presented recently involving ETH. The interaction between ETH and serum albumin was investigated employing different spectroscopic techniques [70]. The results revealed that the secondary structure of the protein was affected upon interaction with the drug. The fluorescence results indicated the presence of static quenching mechanism in the binding of ETH to the protein. Based on spectral data it was concluded that the ETH bound to side I of the protein, which is located in the hydrophobic pocket of subdomain IIA [70].

After ionization, compounds with functional thioamide group exist under tautomeric equilibria [71] and, despite of that, it has been possible to develop new ETH derivatives, denominated by oxazolinyll (**10**, Fig. (10)) and N-hydroxy-alkyl (**11**, Fig. (10)) [72]. This is an important step to fight the MDR-tuberculosis and the valuable contribution of new derivatives of ETH can help to understand better the metabolism of the parental drug. Other advantages can be the association of ETH with other drugs, such as gatifloxacin, which in combination with ETH has great promise for the treatment of tuberculosis [73].

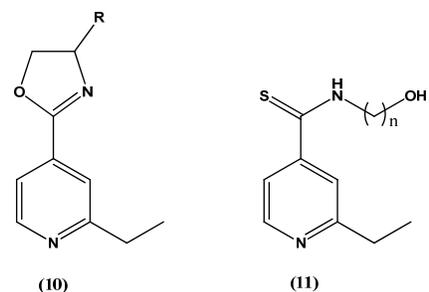


Fig. (10). New ETH derivatives: **10**, R=H or CH₃; and **11**, n=3-5.

Site of Activation

The ultimate site of action of ETH is the same as the one of INH. Gene array studies have shown that both ETH and INH induce similar patterns of protein expression [74], and mutations in the *InhA* gene give rise to resistance to both ETH and INH [45, 75]. In addition, it has also been shown that both activated ETH and PTA inhibit *EtaA* [37], and that the basis for the inhibition of *InhA* by ETH and PTA yields *ETA-NADH* and *PTA-NADH* adducts, respectively, which are similar to the adduct obtained with INH [37]. The formation mechanism of the activated ETH molecule is possible due to the formation of the *S*-oxide preceded by hydrolysis of the putative sulfinic acid to give the amide [35]. This is explained by the fact that sulfinic acid is a good leaving group and can be displaced by nucleophiles, whereas NAD^+ is an electron deficient ring and is highly unlikely to act as a nucleophile. Therefore, the sulfinic acid might undergo homolytic cleavage to give the carbon radical, as addition to the NAD^+ ring followed by hydrolysis of the imine function [35]. Recently, by using high throughput microarray data and interactome network, Chen *et al.* have shown that the active drug-treated networks are associated with the trigger of fatty acid metabolism and synthesis, as well as to NADH -related processes, and that the efflux pumps processes appear to be the major mechanisms of resistance [76].

CONCLUSION

Global tuberculosis epidemic has been complicated by the recent worldwide emergence of MDR strains of *M. tuberculosis*. Recent attempts to potentiate the existing antimycobacterial activities, such as ETH and other drugs, have increased the possibility for targeting resistance which might offer a shortcut to broaden the chemotherapeutic options for tuberculosis treatment, thus decelerating the dissemination of drug resistant forms. ETH plays a central role in the treatment of patients that exhibit resistance to the front-line drugs. This anti-tuberculosis drug is known to strongly inhibit mycolic acid synthesis in *M. tuberculosis*. As a result of the second oxidative cycle, the oxidation of thioamide groups in ETH produces sulfoxides which are converted to sulfinic acid. The sulfinic acids can decompose and in some cases react with NAD^+ to generate adducts that reversibly inhibit cell wall synthesis in *M. tuberculosis*. In addition, it may serve as electrophilic agent that modifies proteins in either the mycobacterium or in the host tissue if produced by host FMO enzymes. This makes ETH a molecule of great interest still to fight against *M. tuberculosis*, which can even be further explored in the future by combining it with other therapeutic approaches.

CONFLICT OF INTEREST

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

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