IDENTIFICATION OF NEW COMPOUNDS AGAINST ISONIAZID TARGET OF MYCOBACTERIUM TUBERCULOSIS

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Summary

The preferred antitubercular drug isoniazid specifically targets a long-chain enoyl-acyl carrier protein reductase (InhA), an enzyme involved in the type II fatty acid biosynthesis pathway of M. tuberculosis. This review deals with the most recent findings on the antimycobacterial compounds reported showing highest inhibitory activity against Mycobacterium tuberculosis. Knowledge of the precise structures and mechanisms of action of these drugs provides insights into designing new drugs that can overcome drug resistance

Keywords: Mycobacterium tuberculosis; InhA inhibitor; enoyl acyl carrier protein reductase; Tuberculosis.

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Introduction

Tuberculosis (TB) is responsible for more than 1.6 million deaths per annum with 8.8 million new cases being reported each year. These numbers make TB one of the leading infectious causes of death, eclipsed only by AIDS. In addition, according to the World Health Organization, the number of multi-drug-resistant and extensively drug-resistant TB cases is growing with almost a half million new cases being reported each year. Therefore, there is an urgent need to develop novel TB chemotherapeutic agents [1].

Tuberculosis (TB) is a chronic infectious disease caused by mycobacteria of the 'tuberculosis complex', including Mycobacterium bovis, Mycobacterium africanum, and mainly Mycobacterium tuberculosis. TB now kills more adults than all other infectious diseases combined. Active TB is usually treated with isoniazid in association with one or more other anti-TB drugs but multi-drug resistant TB (MDR-TB) and very recently extensively drug resistant TB (XDR-TB) has become a serious and unsolved public health problem [2–7].

Isoniazid (isonicotinic acid hydrazide, INH) discovered in 1952 is still the most important drug for treatment of TB. It is a prodrug which requires metabolic oxidation by the M. tuberculosis enzyme, catalase-peroxidase katG [8, 9].

Mechanism:
The INH-NAD(P) adducts inhibit two enzymes involved in the fatty acid biosynthetic pathway of M. tuberculosis [10–13], NAD-dependent enoyl-acyl carrier protein reductase (enoyl-ACP reductase, InhA) and NAD(P)-dependent b-keto-ACP reductase (mycolic acid biosynthesis A, MabA) [14].

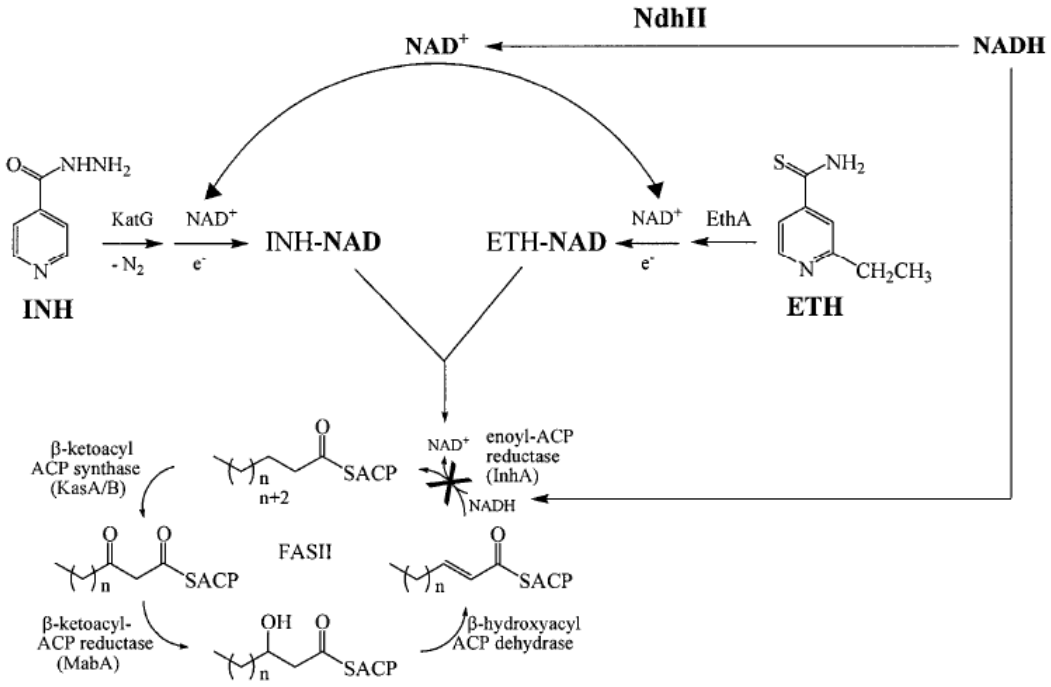


FIG. 1. **Proposed mechanism of action of INH and ETH**.

INH and ETH are both prodrugs that are activated by the catalase-peroxidase KatG or the monooxygenase EthA, respectively. The activated forms react with NAD_ to form an INH-NAD or ETH-NAD adduct. These adducts inhibit the common target InhA, the NADH-dependent enoyl-ACP reductase of the fatty acid synthase type II system, resulting in mycolic acid biosynthesis inhibition and cell lysis. Resistance to INH or ETH is associated with recessive mutations in the genes encoding the activators of the drugs, katG and ethA, respectively, which prevent drug activation. Coresistance to INH and ETH is associated with dominant mutations in the gene encoding the common target of the drugs, inhA, which result in target amplification or target modification. A novel mechanism of coresistance to INH and ETH is by recessive mutations in ndh, which increase the NADH intracellular concentration and cause resistance by competitively inhibiting the binding of the INH-NAD or ETH-NAD adduct to InhA [15].

The current front-line treatment strategy utilizes isoniazid (INH), a pro-drug which inhibits the synthesis of mycolic acids that are essential components required for the integrity of the bacterial cell wall [16]. INH inhibits InhA, the FabI enoyl reductase (ENR) in the fatty acid synthesis (FAS-II) pathway. However, before INH can inhibit InhA, it must be activated by KatG, a catalaseperoxidase enzyme. The activated form of INH then reacts with NAD+ to form the INH-NAD adduct (Scheme 1) [17–21]. A significant number of the strains resistant to INH arise from mutations in KatG [22–25]. Therefore, the development of an InhA inhibitor which can bypass this initial activation step should have activity against INH resistant strains of Mycobacterium tuberculosis (MTB).

Tuberculosis (TB) is the leading cause of morbidity and mortality among the infectious diseases. The World Health Organization (WHO) has estimated that one-third of the world's population, nearly 2 billion people, mostly in the developing countries [26] have been infected with Mycobacterium tuberculosis, the causative agent of TB. Among the infected individuals 8 million develop active TB and nearly 2 million people die from the disease annually [27]. In recent years, the pandemic of AIDS has had a major impact on the worldwide TB problem. On one hand, HIV infection is the most potent risk factor for converting latent TB into the active, transmissible form, thus fueling the spread of TB; on the other hand, TB bacteria can accelerate the progress of AIDS infection. One-third of the increase in the incidence of TB in the past 5 years can be attributed to co-infection with HIV [16]. This situation has been further exacerbated by the emergence of multidrug-resistant tuberculosis (MDR-TB) strains that are resistant to some or most current anti-TB drugs [17]. Over the decade, it is estimated that as many as 50 million people worldwide have been infected with MDRTB strains. According to WHO, from 2002 to 2020, there will be about 1 billion more people newly infected with TB and approximately 36 million deaths if the worldwide ravage of tuberculosis is left unchecked [28].

Despite the increasing worldwide incidence of TB and its alarming threat toward the public health, no novel antituberculosis drugs have been introduced into clinical practice over the past 4 decades. The impact of ever-increasing drug resistance, the serious side effects of some current anti-TB drugs, and the lack of efficacy of current treatments in immunodepressed patients, combine to make the development of new antimycobacterial agents an urgent priority.

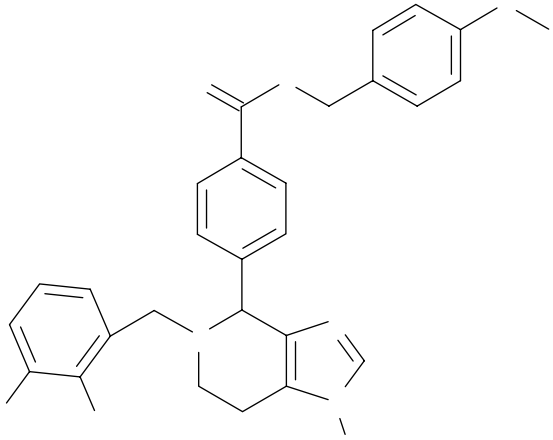
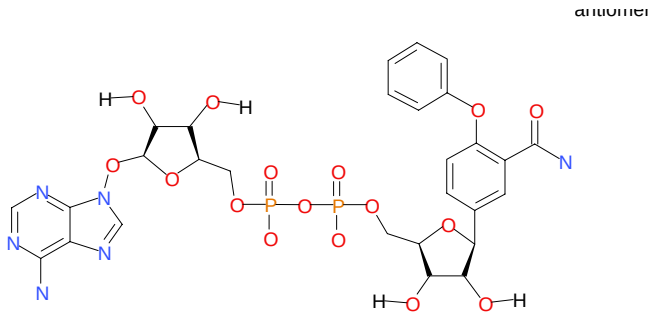
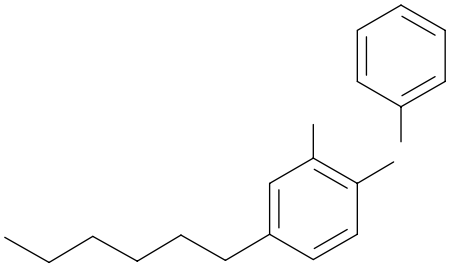
The enzymes involved in the bacterial fatty acid biosynthetic pathway, the fatty acid synthase system, are attractive targets for the design of new antibacterial agents [29-32]. Fatty acid biosynthesis in bacteria is catalyzed by a set of distinct, monofunctional enzymes collectively known as the type II FAS (FASII). These enzymes differ significantly from the type I FAS (FASI) in mammalians, in which all of the enzymatic activities are encoded in one or two multifunctional polypeptides. This distinctive difference in the FAS molecular organization between most bacteria and mammals makes possible the design of specific inhibitors of increased selectivity and lower toxicity. M. tuberculosis contains unique signature fatty acids, the mycolic acids, that are unusually long chain R-alkyl, alpha-hydroxy fatty acids of 60-90 carbons [33]. The TB-specific drugs isoniazid (isonicotinic acid hydrazide (INH)) and ethionamide have been shown to target the synthesis of these mycolic acids, which are central constituents of the mycobacterial cell wall.

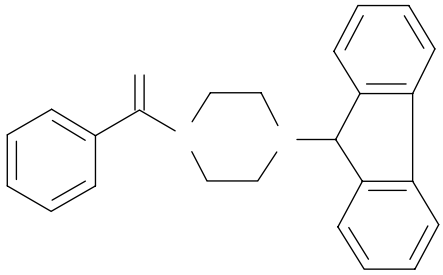
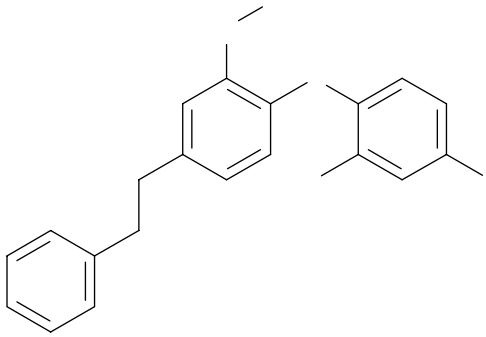
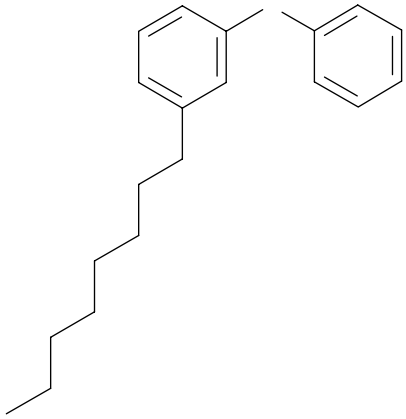
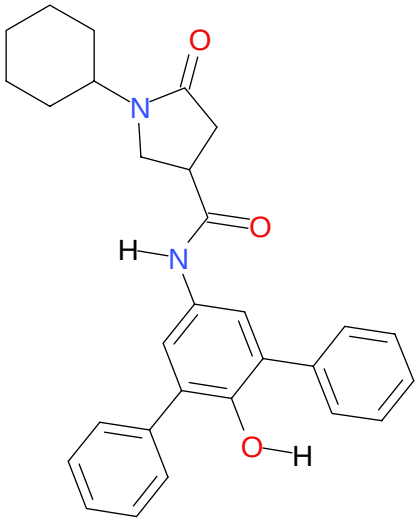
The biosynthesis of mycolic acids is achieved by the FAS in M. tuberculosis. Unlike other bacteria, M. tuberculosis is unique in that it possesses both type I and type II fatty acid biosynthetic pathways. FASI in M. tuberculosis is responsible for generation of the shorter saturated alkyl chain fatty acids, including the 24-carbon R-branch of mycolic acids. Some of the products from the FASI system, such as the C16-C26 fatty acid products, are later transferred to the FASII system, where they are further elongated to up to C56, forming the meromycolate chain that serves as the precursor for the final mycolic acids.

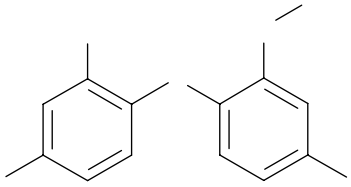
Among the enzymes involved in FASII, the NADH-dependent enoyl-ACP reductase encoded by the Mycobacterium gene inhA is a key catalyst in mycolic acid biosynthesis. Studies over the years have established that InhA is the primary molecular target of INH [34] the drug that for the past 40 years has been, and continues to be, the frontline agent for the treatment of TB. As a prodrug, INH must first be activated by KatG, a catalaseperoxidase that oxidizes INH to an acyl radical that binds covalently to NADH, the cosubstrate for InhA [35]. The INH-NADH adduct then functions as a potent inhibitor of InhA. The requirement for INH activation opened a backdoor for the development of drug resistance by M. tuberculosis. Indeed, KatG-associated mutations account for 50% of the INH-resistant clinical isolates

[36]. Thus, direct InhA inhibitors that avoid this activation requirement would be promising candidates for the development of novel antitubercular agents. Other than the well-known diazoborines [37] and triclosan [38], both of which are nonselective and relatively weak agents, two series of InhA direct inhibitors, pyrazole derivatives 1 (Genz 8575) [4] and indole-5-amides 2 (Genz 10850) [39], have been reported to have both in vivo and in vitro activity.

Reported highest activity molecules against InhA from different journals:

Name as reported in reference	Structure	IC50 (uM)	Reference
Compound 9b (Enantiomer 2)		0.08	40
Compound 3		27	41
Compound 19		0.011	42

Compound ID p2		0.09	43
Compound 25 (Page No: 243, Table No: 2)		0.021	44
8PP		0.005	45
Compound p64		0.14	46

Triclosan		3	47

New Targets for the Next Generation of Antimycobacterial drugs

While most antimicrobial drugs in current clinical use inhibit essential processes such as protein or cell wall biosynthesis, many of these drugs are also bacteriostatic, which may contribute to development of resistance. One way of developing novel antibacterial drugs with minimal potential for resistance development could be to target bactericidal functions of bacterial proteins (eg. essential enoyl-ACP reductase FabI required for fatty acid biosynthesis) [48]. Targeting of essential proteins must take into consideration the structural constraints within substrate binding and catalytic domains and the mitigating effects of mutations on enzyme function. Alternatively, targeting virulence factors [49] or host-microbial response pathways might lead to rapid clearance of infecting organisms.

Bacterial infections such as tuberculosis are difficult to treat due to dormant bacteria that are 50-fold resistant to antibacterial drugs that target growth and division. Mycobacterium tuberculosis is 8-fold more sensitive to ATP synthesis inhibitors than standard anti-TB drugs, since they reduce ATP synthesis while adjusting to the hypoxic conditions while establishing an infection. Disruption of the PMF (proton motive force), by specific inhibitors, which is necessary for ATP generation, is also bactericidal [50]. TMC207 is a novel anti-mycobacterial drug belonging to the diarylquinoline class of compounds and is an ATP synthase inhibitor [51].

New Strategies for Antibacterial Drug Discovery

Most of the current antibacterial drugs were discovered between 1940 and 1980 by traditional approaches which are now saturated, and the emergence of drug resistance as well as the emergence of new pathogens calls for new strategies in antibacterial drug discovery due to the inadequacies of screening libraries for novel antibacterial compounds as described in this section [52]. Antibacterial drugs have unique physicochemical properties which are dependent on their spectrum of activity [53]. Natural products are proposed to be an optimal antibacterial drug screening library as they have optimal cellular penetration and privileged structures to interact with finite structural spaces in protein folds [54]. Identifying feasible drug targets given the vast microbial genomics information by comparing different pathogen genomes and narrowing the targets based on essentiality, novelty of target or mechanism, absence of human homolog and low likelihood of resistance development is a failed strategy, and requires a chemically diverse compound collection. Improving the quality of synthetic libraries by using core scaffolds to introduce natural product like characteristics could be a way to generate a chemically diverse compound collection. Comparative bacterial genomics has yielded knowledge of previously unknown biosynthetic pathways absent in humans which can be specifically targeted to discover antibacterial drugs for a specific microbe [55].

Conclusion

The number of new antibacterial medicines entering the clinic has been declining for years, while the emergence of drug resistance and especially multi-drug resistance continues to rise at an alarming rate. The more traditional approaches of generating new derivatives of old

drugs or finding new ecosystems to mine for natural products are giving way to more innovative non-traditional strategies to develop next generation drugs. The future does not seem bleak as several promising antibacterial drugs with novel mechanisms of action are in development and new types of targets (Type III secretion systems) have emerged. The scourge of drug resistance in microbes will have to be fully understood and the choice of "good targets", both new and old will be vital to the discovery of new antibacterial drugs as we progress forward into the 21st century.

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