

Targeting and Synthesizing new Anti-Tuberculosis Agents for use as Drugs

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Abstract

Tuberculosis (TB) is a deadly contagious disease that is caused by a bacterium called *Mycobacterium tuberculosis*. More than sixty years ago, the introduction of the first anti-TB drugs for the treatment of TB (streptomycin (STR), p-aminosalicylic acid (PAS), isoniazid (INH) and then later ethambutol (EMB) and rifampicin (RIF)) gave optimism to the medical community, and it was believed that the disease would be completely eradicated soon. Recently, highly drug-resistant forms of TB have emerged worldwide. The prolonged use of classical drugs developed a growing resistance and these drugs have gradually become less effective and incapable to meet the challenges, especially those of multi drug resistant (MDR)-TB, Extensively drug resistant (XDR)-TB, and HIV-TB co-infections. This paper attempts to bring out the review of anti-TB drugs, and presents a novel method of synthesizing new anti-tuberculosis drugs and potential compounds to overcome the bacterial resistance and combat the re-emergence of tuberculosis.

Keywords: Tuberculosis, Multi drug resistant (MDR)-TB, Extensively drug resistant (XDR)-TB.

1. Introduction

Tuberculosis or **TB** is a common and often deadly contagious disease, ranking second amongst infectious diseases after HIV-AIDS. It is caused by various strains of mycobacteria, usually by a Gram-positive bacterium, *Mycobacterium tuberculosis*. This bacterium was first identified in 1882 by the German scientist Robert Koch

(1843-1910).^[1,2] Tuberculosis is primarily an illness of the respiratory system usually attacking the lungs but can also affect other parts of body such as the brain, stomach, bones, skin, intestine, liver, kidneys, spinal cord and breasts. It is spread through the air by coughing and sneezing of infectious person. Most infections in humans result in an asymptomatic, latent infection, and about one in ten latent infections eventually progresses into an active disease. Normally, the symptoms of active tuberculosis are chronic cough (for more than two weeks), coughing up of blood, weight loss, fever, pain in the chest and sweating at night^[3,4,5]. The disease occurs more frequently in children and in people with a weak immunity system such as pregnant women and people infected by the Human Immunodeficiency Virus (HIV).

New infections caused by *M. tuberculosis* are registered to occur at a rate of about one per second.^[6] As a result of this TB stroke back! In 1993, the return of tuberculosis was declared as a global emergency by World Health Organization (WHO) and compared it to a hypothetic third world war with 9 million new TB cases and 2 million deaths reported each year^[7,8]. It is estimated that one-third of the world's population is already infected by *M. tuberculosis* and 30 million people will die in the next 10 years^[9]. According to the 13th annual tuberculosis report of the WHO published on **World TB Day, March 24, 2009** – there were an estimated 9.27 million new cases of tuberculosis worldwide. In addition to this frightening statistics, resistant TB has started to develop, also called as Multidrug resistant tuberculosis (MDR-TB). It occurs in presence of partially suppressive drug concentrations that enable replication of bacteria, formation of mutants and overgrowth of wild type strains of mutants.^[10] According to some recent reports an increase of up to 500,000 new cases of Multidrug resistant TB (MDR-TB) and 40,000 new cases of Extensively drug resistant TB (XDR-TB) could be expected.^[11] In 2010, 292,972 cases were reported for MDR-TB. It is estimated that about 40% of the Indian population is infected with *M. Tuberculosis* majority of whom have latent TB^[15]. The current front line therapy for tuberculosis consists of administering three or more different drugs (usually isoniazid, rifampicin, pyrazinamide and ethambutol) over an extended period of time of around 6 to 8 months of daily treatment. XDR-TB is resistant to both isoniazid and rifampin and to any fluoroquinolone drug and at least one of three second-line injectable drugs (amikacin, kanamycin, or capreomycin). Consequently, problems due to MDR-TB arise and it is necessary to develop new therapeutic agents in order to treat drug resistant forms of tuberculosis. Hence searching for new compounds, which would combine a specific activity against a broad spectrum of bacteria seems to be a promising way to overcome that problem.

2. Materials and Methodology

Intensive literature survey was carried out on the subject. we identified and arrived at some 'minimum common bioactive substructures' (MCBS), responsible for describing a diverse set of compounds, suitable to be labeled as potent candidates for previously unexplored anti-tuberculosis compounds. It was carried out by the given procedure. A database of 847 compounds with known minimum inhibitory concentration (MICs)

against *Mycobacterium Tuberculosis* was created, using ISIS Base.^[12] For the purpose of analysis, the database has been divided into three different classes- actives ($MIC \leq 4 \mu\text{g/mL}$), moderately actives ($4 \mu\text{g/mL} < MIC \leq 32 \mu\text{g/mL}$), in-actives ($MIC > 32 \mu\text{g/mL}$). With respect to this criterion, there are 232 active compounds, with 334 moderately actives and 106 in-actives; the rest were excluded because of the non availability of precise MIC values. The data of compounds with known minimum inhibitory concentration (MICs) is gathered and is presented in the table 1.

Table 1: Summary of Different Classes of Chemical Compounds in Database.

Sr. No	Class	Total No.	Average MIC ($\mu\text{g/mL}$)	Max-Min MIC ($\mu\text{g/mL}$)	% of Active, Moderate, Inactive
1	Imidazole	122	8.67	0.39-64	67.2,32,0.8
2	Pyrazinoic acids	5	8.74	6.2-12.51	0,100,0
3	Pyridines	304	29.51	0.05-367.6	30.9,51.0,18.1
4	Isonicotinic acid hydrazides	73	13.83	0.05-118.6	46.6,41.1,12.3
5	Thioamides	43	9.66	0.15-110.6	41.9,53.5,4.7
6	Quinoxalines	22	3.86	0.39-6.25	54.5,45.5,0.0
7	Quinolones	19	23.47	0.2-69	52.6,21.1,26.3
8	Pyrroles	43	27.92	0.04-250	44.2,46.5,9.3

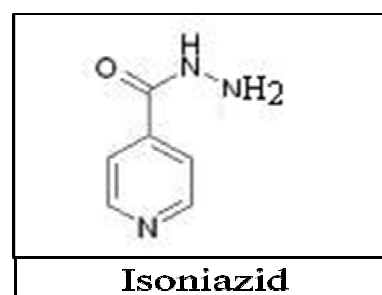
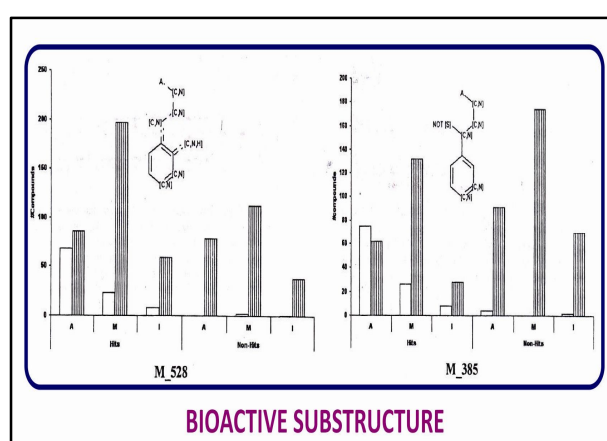


Fig. a: Relative similarity between the bioactive motif and Isoniazid

The compounds were subjected to clustering using Distill (Tripos). Distill is a hierarchical clustering tool, which classifies compounds according to their common substructures. It creates a structure based dendrogram, with each node of it representing a substructure. It helped to relate the components of a structure (i.e., atoms, bonds and connectivity) to the biological activity^[12,13]. Patterns in substructural fragments which will be relevant to biological activity were identified, using a QSAR technique. HQSAR analysis was used in generation of substructural fragments of each compound and correlating it with available biological data.^[12,14] Thus a 'bioactive motif' (a design of compound which would be most effective against *M. tuberculosis*) was identified.

2.1 Chemistry and spectral analysis

Isoniazid ($C_6H_7N_3O$) was selected as the starting compound. 0.001 M (0.137 g) Isoniazid was dissolved in 10 ml Methyl alcohol. 0.001 M 'R' was dissolved in 10 ml methyl alcohol (where 'R' = Ethyl acetate, Phthalic acid, Benzoic acid, Acetic acid, Methyl acetate, Glycine, o-Amino benzoic acid, Oxalic acid). Isoniazid and the other reactants were dissolved separately in solvent in equal concentrations. The Isoniazid solution and 'R' solution prepared as above were mixed together and placed on a water bath maintained at 60°C. They were refluxed for about 5 hours until the product was formed and the solvent was evaporated. Melting points of the products were determined on a Buchi apparatus and are uncorrected & recorded. The spectral data of IR and NMR of the products are gathered and presented. Shimadzu FT IR-8400 and Varian Mercury 300 Hz Spectrometer models were used for analysis.

2.2 Biological test

Newly synthesized product was assayed *in vitro* for anti tubercular activity. Evaluation of the products for their *in vitro* antitubercular activity against *Mycobacterium Tuberculosis* H₃₇Rv using Microplate Alamar Blue Assay (MABA) biological test was done. This methodology is nontoxic, uses a thermally-stable reagent and shows good correlation with proportional and BACTEC radiometric methods. Briefly, 200 µL of sterile deionized water was added to all outer-perimeter wells of sterile well plates to minimize evaporation of the medium in the test wells during incubation. The final drug concentrations tested were 0.01 to 20.0 µL/mL. Plates were covered and sealed with parafilm and incubated at 37°C for five days. After this time, 25 µL of a freshly prepared 1:1 mixture of Alamar Blue (Accumed International, Westlake Ohio) reagent and 10% tween 80 was added to the plate and incubated for 24 h. A blue color in the well was interpreted as no bacterial growth, and a pink color was scored as growth. The MIC (Minimal Inhibition Concentration) was defined as the lowest drug concentration, which prevented a color change from blue to pink. The results obtained are given below in a table 2.

3. Results and Discussion

The results obtained by MABA test and respective MICs of the products are given in table 2. A schematic representation is as follows

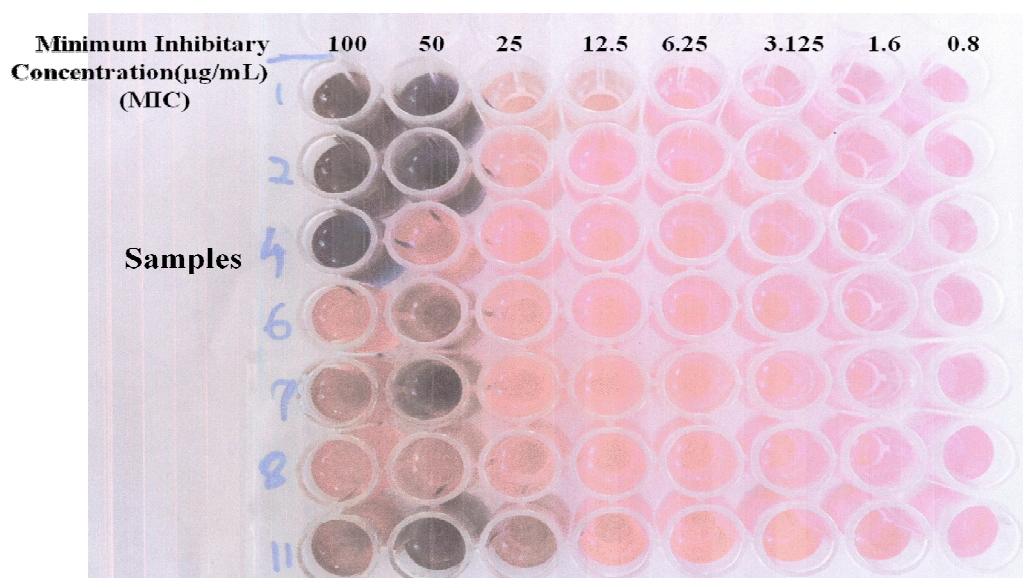
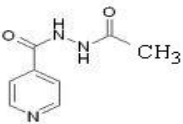
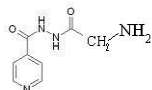
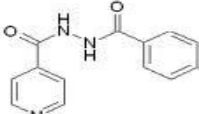
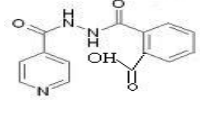
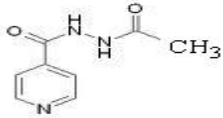
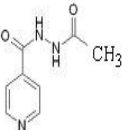
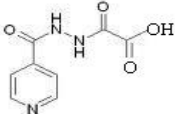
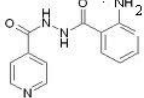


Fig. b: MABA test and respective MICs of the products.

Table 2: Product obtained, Melting point and Minimum Inhibitory Concentration.

Sr No	PRODUCTS	M.P.(°C)	MIC (mg/L)	Sr No	PRODUCTS	M.P. (°C)	MIC(mg/L)
1	ISONIAZID + ACETIC ACID → 	87	50	5	ISONIAZID + GLYCINE → 	95	50
2	ISONIAZID + BENZOIC ACID → 	89	50	6	ISONIAZID + PHTHALIC ACID → 	143	25

3	ISONIAZID ETHYL ACETATE + 276 → 	100	7	ISONIAZID METHYL ACETATE + 165 → 	50
4	ISONIAZID OXALIC ACID + 212 → 	50	8	ISONIAZID +O-AMINO BENZOIC ACID + 137 → 	25

All the synthesized derivatives were evaluated for anti mycobacterial activity against *M. Tuberculosis* H₃₇Rv. The results of anti mycobacterial activities with standard drug isoniazid exhibited stronger anti-mycobacterial activity. The compounds have shown good potency and significant *in vitro* activity against *M. Tuberculosis*. The novel synthetic antitubercular molecule will cost much less so that all governments may guarantee access of all the diseased population.

4. Conclusion

The prolonged use of classical drugs developed a growing resistance and these drugs have gradually become less effective and incapable to meet the challenges, especially those of MDR-TB, XDR-TB, and HIV-TB co-infections. Such MDR-TB is difficult and expensive to treat and is not always curable. Several nicotinic and isoniazid derivatives, and evaluated for their *in vitro* antibacterial activity against *Mycobacterium tuberculosis* H37Rv using the Alamar Blue susceptibility test and the activity expressed as the minimum inhibitory concentration (MIC) in mg/mL. The compound exhibited the best result when compared with first line drugs such as isoniazid (INH) and rifampicin (RIP). Therefore this class of compounds could be a good starting point to develop new lead compounds in the treatment of multi-drug resistant tuberculosis. It suggests that this class of compounds may be selectively targeted to *M. tuberculosis* growth.

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