Chem Biol Drug Des 2016; **87:** 537–550

Review

A Note on Derivatives of Isoniazid, Rifampicin, and Pyrazinamide Showing Activity Against Resistant *Mycobacterium tuberculosis*

Ameeruddin Nusrath Unissa[1], Luke Elizabeth Hanna[2,*] and Soumya Swaminathan[3]

[1]Centre for Biomedical Informatics, National Institute for Research in Tuberculosis, Chennai, Tamil Nadu 600 031, India
[2]Scientist D, Division of Clinical Research, National Institute for Research in Tuberculosis, Chennai, Tamil Nadu 600 031, India
[3]Director General, Indian Council of Medical Research, Ansari Nagar, New Delhi 110 029, India
**Corresponding author: Luke Elizabth Hanna, hanna@trcchennai.in*

Drug-resistant tuberculosis (DR-TB) is a serious problem that impedes the success of the TB control program. Of note, multidrug-resistant (MDR)-TB and extensively drug-resistant (XDR)-TB have certainly complicated the scenario. One of the possible strategies to overcome drug resistance in an economic and simple manner would involve modification of existing anti-TB drugs to obtain derivatives that can work on resistant TB bacilli. These may have improved half-life and increased bioavailability, be more efficacious, and serve as cost-effective alternatives, as compared to new drugs identified through conventional methods of drug discovery and development. Although extensive literature is available on the activity of various derivatives of first-line drugs (isoniazid, rifampicin and pyrazinamide) on drug-susceptible *Mycobacterium tuberculosis* (MTB), reports on the activity of derivatives on resistant MTB are very limited, to our knowledge. In light of this, the present review aims to provide a concise report on the derivatives of first-line drugs that have the potential to overcome the resistance to the parental drug and could thus serve as effective alternatives.

Key words: derivatives, drug resistance, INH, MTB, PZA, RIF

Despite the fact that tuberculosis (TB) is a preventable, treatable, and curable disease, it still remains as one of the world's major cause of illness and death. It was estimated in 2014 that 9.6 million people were infected and 1.5 million died from TB. TB is a leading killer of human immunodeficiency virus (HIV)-positive people causing one-fourth of all HIV-related deaths[a].

Short course chemotherapy (SCC) the backbone of antitubercular chemotherapy, consists of an initial intensive phase of treatment for 2 months with a regimen comprising of isoniazid (INH), rifampicin (RIF), pyrazinamide (PZA), and ethambutol (EMB), followed by a choice of several options, including INH and RIF, for the continuation phase of 4 months (or alternatively, 6 months of INH and EMB or thiacetazone) (1). The current 6–9 months of treatment for TB is not only lengthy but also gives rise to a number of side-effects that result in poor adherence to therapy, which in turn eventually leads to drug resistance.

The emergence of multidrug-resistant tuberculosis (MDR-TB), defined as infection with TB strains that are resistant to the two most potent anti-TB drugs, *viz.* INH and RIF, and extensively drug-resistant TB (XDR-TB), defined as infection with MDR-TB isolates that are also resistant to second-line anti-TB drugs such as fluoroquinolones and at least one of the injectable drugs such as capreomycin, kanamycin, or amikacin, has worsened the global TB scenario considerably[b]. Globally, 480 000 people are estimated to have developed MDR-TB, and about 170 000 have died due to MDR-TB in 2014. It is estimated that about 9.6% of MDR-TB cases had XDR-TB[a].

Drug resistance poses a potential threat to the TB control program. It requires a longer duration of therapy with close monitoring and specialized treatment facilities; patients remain infectious for longer periods, and drug-resistant (DR)-TB causes accelerated disease leading to substantial mortality. The average treatment duration for DR-TB is 2 years, as compared to 6 months for drug-susceptible TB. Treatment employs second-line medications which are less effective and very expensive, display cross-resistance, exhibit high toxicity profiles, and are not readily available (2,3). Hence, there is a lack of efficient drugs for the treatment of MDR-TB, XDR-TB, and HIV-TB. New and better drugs that are not only active against drug-resistant TB but also more importantly able to shorten the period of therapy are the need of the hour. Simpler regimens consisting of well-tolerated drugs, also appropriate for combined HIV-TB treatment, are required for the success of the TB control program.

 doi: 10.1111/cbdd.12684

It is encouraging to note that several new drugs designed with a view to overcome the problem of drug resistance as well as to shorten the duration of treatment are currently in trial, after a huge gap of 40 years. Two major approaches could be used to develop novel drugs: (i) An existing drug could be chemically modified to improve its pharmacokinetic and pharmacodynamic properties leading to the generation of novel analogues or derivatives, or (ii) new lead molecules could be discovered either through random screening or based on a detailed knowledge of a specific target by rational design. Modification of existing drugs to improve their properties remains a cost-effective alternative to than searching for a new lead (4). An interesting example for a modified derivative of an existing drug is the close homologue of the INH metabolite, N2-acetylisoniazid, which demonstrated an unexpectedly higher level of protection in MTB-infected mice. The investigators on the study state that such close structural congeners of metabolites of INH could serve as significant leads for antitubercular drug discovery and studies aimed at exploring the mechanism of action of INH (5). Thus, the scope of this article was to review existing literature on the feasibility and utility of derivatives designed from existing first-line drugs, as potent alternatives for overcoming the problem of resistance to the parental drug.

Isoniazid

INH, chemically known as isonicotinic acid hydrazide, is a synthetic drug first described in 1912 and used as an anti-TB drug since 1952 (Table 1, Number (No.) 1). Ever since, it has remained a front-line antitubercular drug and a choice of chemoprophylaxis for the treatment of TB (6). INH is exclusively selective and specific to *Mycobacterium tuberculosis* (MTB) and other mycobacteria, particularly the MTB complex (MTBC) (7). It is bactericidal at minimum inhibitory concentration (MIC) levels of 0.02–0.2 μg/mL for susceptible strains of MTB and *M. bovis* (8).

Derivatives of Isoniazid

INH is a principle component in the current 6-month SCC. Its high bactericidal activity, low cost, high bioavailability, excellent intracellular penetration, and narrow spectrum of action make it an almost ideal antimicrobial agent. It has a simple structure, containing a pyridine ring and a hydrazide group, and both moieties are essential for optimal activity against MTB.

Many derivatives of INH have shown activity against TB strains. There have been studies in which the antimycobacterial pharmacophore moiety of INH has been introduced into various kinds of molecules. Several types of INH derivatives have been reported and classified based on their structure, their lipophilicity has been calculated, and structure–activity relationships have been described. Numerous Schiff bases, hydrazones, hydrazides, and metal complexes of INH imparted very good activity. About 510 derivatives of INH have been identified with improved activity against drug-susceptible MTB as well as MDR-TB strains (9). Lourenco *et al.* (10) evaluated *in vitro* antibacterial activity against MTB-H37Rv, using many nicotinic acid and INH analogues; many of the analogues that contained the nitro group exhibited better activity than INH itself suggesting that this class of compounds could be a good starting point for the development of new lead molecules for the treatment of MDR-TB.

Isoniazid Derivatives Potent Against Resistant MTB

Isonicotinohydrazides

Maccari *et al.* (11) carried out a study to evaluate the *in vitro* antimycobacterial activity of isonicotinohydrazides (two compounds), cyanoborane adducts (three compounds), and the parental isonicotinohydrazone (ISNE). They found that ISNE and hydrazides displayed good antimycobacterial properties with rather low cytotoxicity. Hydrazides **2a**, **2d**, and **2e** showed the most appreciable *in vitro* activity, being more effective than parental INH in a macrophage model. Compounds **1b**, **1c**, and **1k** exhibited activity against **INH-resistant (INH^R)** MTB strains at a lower concentration (11), and the activity of the compounds **2a**, **2b**, and **2c** is listed in Table 1. The substitution of fluorine in the benzene ring of these compounds appears to enhance their activity significantly.

Hydrazones

In a trail study that used *N′*-(1-alkyl-2,3-dihydro-2-oxo-1H-3-indolyliden)-4-pyridine carboxylic acid hydrazide derivatives as an alternative to INH, it was found that hydrazones derived from 1-alkylisatin and INH were potent against bovine- and human-sensitive and resistant strains of MTB (12). The potent activity of compound (C) **3a** (numbered as same in Table 1) with reference to structure–activity relationship (SAR) might be attributed to lack of substitution at R group compared to **3d**, **3f**, and **3g** (numbering as **3b**–**d** in Table 1) compounds containing propyl, benzyl, and acetyl groups, respectively.

Hydrazinecarbothioamides

A novel INH derivative 2-isonicotinoyl-*N*-[2-(trifluoromethyl) phenyl] hydrazinecarbothioamide (Table 1 No. **4**) was found to be the most potent against MTB-H37Rv and INH^R MTB strains. The derivative was synthesized by reacting INH with the potassium salt of substituted phenyl thiocarbamate. Substitution in compound No. **4** with strongly deactivating electron withdrawing groups like trifluoromethyl groups in the phenyl ring resulted in excellent antimycobacterial activity (13).

Table 1: INH and its derivatives

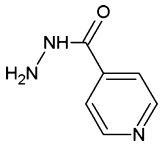
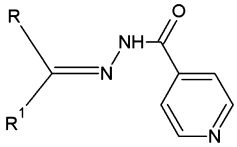
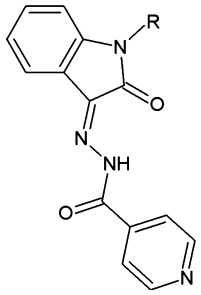
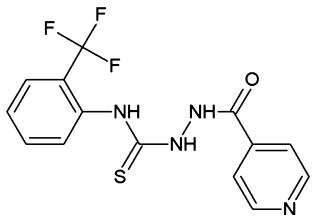
C. no	Structure and IUPAC name of INH compounds	*In vitro* activity against MTB-H37Rv MIC = μg/mL; D	*In vitro* activity against INH^R MTB MIC = μg/mL; D
1	 4-pyridinecarboxylic acid hydrazide	0.02–0.2	–
2	 Parental compound		
2a	R = H; R^1=2-F-C_6H_5 *N′*-[(1*Z*)-1-(2-fluorophenyl)ethylidene]isonicotinohydrazide (11)	0.4	>1.6
2b	R = H; R^1= 3-F-C_6H_5 *N′*-[(1*Z*)-1-(3-fluorophenyl)ethylidene] isonicotinohydrazide (11)	0.8	>1.6
2c	R = CH_3; R^1 = 4-F-C_6H_5 *N′*-[(1*Z*)-1-(4-fluorophenyl)ethylidene] isonicotinohydrazide (11)	0.2	>1.6
3	 Parental compound		
3a	R = H N′-[(3E)-2-oxo-1,2-dihydro-3H-indol-3-ylidene]pyridine-4-carbohydrazide (12)	2.7	2.7
3b	R = C_3H_5 N′-[(3Z)-2-oxo-1-propyl-1,2-dihydro-3H-indol-3-ylidene] pyridine-4-carbohydrazide (12)	3.1	3.1
3c	R = $C_6H_5CH_2$ *N′*-[(3*Z*)-1-(2-methylphenyl)-2-oxo-1,2-dihydro-3*H*-indol-3-ylidene] pyridine-4-carbohydrazide (12)	3.5	3.5
3d	R = CH_2OH *N′*-[(3*Z*)-1-acetyl-2-oxo-1,2-dihydro-3*H*-indol-3-ylidene] pyridine-4-carbohydrazide (12)	3.0	3.0
4	 2-(pyridin-4-ylcarbonyl)-*N*-[2-(trifluoromethyl)phenyl]hydrazinecarbothioamide (13)	0.58[a]	0.58–10.88[a]

Table 1: continued

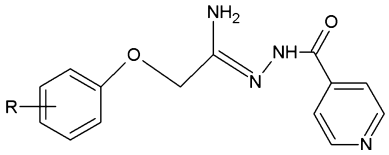
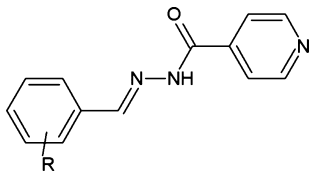
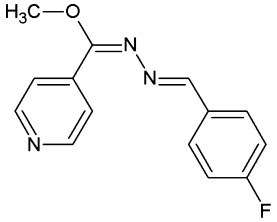
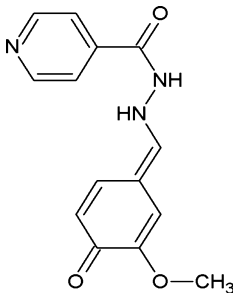
C. no	Structure and IUPAC name of INH compounds	*In vitro* activity against MTB-H37Rv MIC = μg/mL; D	*In vitro* activity against INH^R MTB MIC = μg/mL; D
5			
	Parental compound		
5a	R = 4-CH_3 (1*E*)-*N*′-isonicotinoyl-2-(4-methylphenoxy)ethanehydrazonamide (14)	6.25	50
5b	R = 4-Cl (1*E*)-2-(4-chlorophenoxy)-*N*′-isonicotinoylethanehydrazonamide (14)	3.12	100
5c	R = 3-Cl **(**1*E*)-2-(3-chlorophenoxy)-*N*′-isonicotinoylethanehydrazonamide (14)	3.12	12.5
5d	R = 4-NO_2 (1*E*)-*N*′-isonicotinoyl-2-(4-nitrophenoxy)ethanehydrazonamide (14)	6.25	50
6			
	Parental compound		
6a	R = H *N*′-[(1*E*)-phenylmethylene]isonicotinohydrazide (15)	4.44[a]	8.88[a]
6b	R = 3-F *N*′-[(1E)-(3-fluorophenyl)methylene] isonicotinohydrazide (15)	4.11[a]	8.22[a]
6c	R = 2-Cl *N*′-[(1*E*)-(2-chlorophenyl)methylene] isonicotinohydrazide (15)	1.92[a]	7.70[a]
6d	R = 4-Cl *N*′-[(1*E*)-(4-chlorophenyl)methylene] isonicotinohydrazide (15)	3.85[a]	7.70[a]
6e	R = 2-Br *N*′-[(1*E*)-(2-bromophenyl)methylene] isonicotinohydrazide (15)	3.29[a]	6.58[a]
6f	R = 4-OH *N*′-[(1*E*)-(4-hydroxyphenyl)methylene] isonicotinohydrazide (15)	2.07[a]	8.29 [a]
6g	R = 2-OMe *N*′-[(1*E*)-(2-methoxyphenyl)methylene] isonicotinohydrazide (15)	0.98[a]	7.83 [a]
6h	R = 3-OMe *N*′-[(1*E*)-(3-methoxyphenyl)methylene] isonicotinohydrazide (15)	1.96[a]	7.83 [a]
6i	R = 4-OMe *N*′-[(1E)-(4 methoxyphenyl)methylene] isonicotinohydrazide (15)	1.96[a]	7.83 [a]
6j	R = 2-OEt *N*′-[(1E)-(2-ethoxyphenyl)methylene] isonicotinohydrazide (15)	1.86[a]	7.43 [a]
6k	R = 3-OEt *N*′-[(1E)-(3-ethoxyphenyl)methylene] isonicotinohydrazide (15)	3.71[a]	7.43 [a]
6l	R = 3-NO_2 *N*′-[(1E)-(3-nitrophenyl)methylene] isonicotinohydrazide (15)	7.40[a]	7.40 [a]
6m	R = *N*′-[(1*E*)-(3-cyanophenyl)methylene] isonicotinohydrazide (15) R = 3-CN	3.99[a]	7.99 [a]
7		NA 54.35[b]	NA 57.07 [b]

Table 1: continued

C. no	Structure and IUPAC name of INH compounds	*In vitro* activity against MTB-H37Rv MIC = μg/mL; D	*In vitro* activity against INH^R MTB MIC = μg/mL; D
	N-[(4-fluorophenyl)methylideneamino]-1- methoxy-1-pyridin-4-ylmethanimine (16,17)		
8		0.03–0.05 54.11[b]	>0.75 57.78[b]
	N-[(E)-(3-methoxy-4-oxo-1-cyclohexa-2,5, dienylidene) methyl]pyridine-4-Carbohydrazide (16,18,19)		

R = substitution site, D = docking score, NA = not available.
[a]μM/mL.
[b]kcal/mol.

Isonicotinylhydrazones

Yet, another study evaluated the anti-TB activity of six compounds against 16 strains of MTB isolated from clinical specimens and five reference strains, including four MDR strains. These compounds (Table 1 No. 5**a**–**e**) were found to be significantly more effective against INH^R MTB ATCC 35822, in combination with INH. Lipophilicity of hydrazones was not the critical factor affecting their potency and combination with INH, *para* aminosalicylic acid, RIF, and EMB showed synergic interactions. The ability of these compounds to specifically inhibit the growth of MTB, their high selectivity index, and ability to enhance the activity of standard drugs holds promise for future drug development (14).

N-*acylhydrazones*

Coelho *et al.*'s study (15) evaluated the *in vitro* antibacterial activity of a series of 23 *N*-acylhydrazones derived from INH against one INH-susceptible (INH^S) strain and three INH^R clinical isolates. Interestingly, 13 derivatives (Table 1, labeled as **6a**–**m**) of acylhydrazones showed good activity against the INH^R strain carrying the S315T mutation. Of the 13 derivatives, three (1,12,19) of them also showed activity against another INH^R strain (Table 1, labeled as **6b**, **6h**, **6m**). This finding is significant given the fact that S315T is the most frequently detected mutation in INH^R clinical isolates globally. While four derivatives showed antibacterial activity against the INH^S strain, one derivative (Table 1, No. **6g**) was more active than INH itself.

Failures in the classical methods have led to the evolution of target-based screening methods. Recent advancements in the field of computational biology coupled with genome sequencing have paved way for rational drug designing that has made the traditional tedious drug discovery process simpler and economic. Notably, the development of softwares and tools for molecular modeling, docking, and dynamic studies has made lead screening easier. Further, computational approaches have also aided in understanding the mechanism of action and development of resistance to existing drugs. We undertook a study (16), in which derivatives of INH with proven activity (Table 1, Nos. **7**, **8**) (17–19) were taken from NCBI and ChemSpider databases and docked with the wild-type and mutated forms of KatG using *in silico* tools. Compound No. **7** is a halogenated derivative of INH (17), while compound No. **8** contains INH Schiff base (18,19). Docking results suggested good binding of the INH derivatives with mutant forms of KatG (S315T, S315R, S315I, S315N, and N138S), indicating the possibility of using these derivatives as leads for developing anti-TB agents that can be used to overcome INH resistance.

Although many derivatives or analogues of INH have been synthesized and tested for their efficacy against H37Rv and INH^R MTB, very few of these compounds have actually gone further up the pipeline of the drug discovery process. Albeit rigorous research along this path is underway, refined derivatives with distinct features may be available in the future for tackling INH^R strains of MTB.

Rifampicin

RIF was introduced as an anti-TB drug in 1972. It is a semisynthetic derivative of rifamycin and extremely effective against MTB. It has an MIC of 0.1–0.2 μg/mL (20) and

is highly active against mycobacteria because it diffuses rapidly across the hydrophobic cell envelope. Due to its potent bactericidal action, RIF, along with INH, forms the backbone of SCC for TB (21). Although rare, resistance to RIF has been slowly increasing because of its widespread use (Table 2 No. **1**).

Rifampicin Derivatives Effective Against Resistant MTB

The family of rifamycin comprises of rifampicin or rifampin (RIF), rifalazil (KRM-1648), rifabutin (RBT), rifapentine (RPT), and CGP-7040.

Rifalazil

It is a new semisynthetic RIF, with a long half-life and greater activity than RIF and RBT against MTB both *in vitro* and *in vivo* (22). A study undertaken by Moghazeh (23) evaluated the activity of RIF, KRM-1648 (Table 2 No. **2**), and RPT against 24 **RIF-resistant (RIF^R)** clinical isolates of MTB with characterized *rpoB* gene mutations, to correlate levels of resistance with specific *rpoB* genotypes. The results showed that KRM-1648 was more active *in vitro* than RIF and RPT.

Previously, KRM-1648 was tested in a clinical trial on pulmonary patients with TB (24). However, due to severe side-effects during the trial phase and occurrence of cross-resistance to all rifamycins, the use of KRM-1648 was terminated.

Rifabutin (RBT)

RBT is a semisynthetic spiro-piperidyl derivative of RIF, which is more active than RIF against slow-growing mycobacteria, including MTB and members of the *Mycobacterium avium–intracellulare* complex, both *in vitro* and *in vivo*. Although RBT is thought to retain some activity against a small proportion of RIF^R TB strains, precise studies on the impact of specific *rpoB* mutations on RBT susceptibility in light of RBT exposure are scarce. Therefore, the accuracy of rapid *rpoB* genotyping and identification of patients who may benefit from RBT need to be determined (25).

In a pilot study (26), the relationship between resistance to RBT and RIF was evaluated in 41 RIF^R isolates of MTB. It was found that 35 isolates with RIF MIC $\geq$ 32 mg/L were also resistant to RBT, while six isolates with MIC of 2–16 mg/L to RIF were susceptible to RBT (Table 2 No. **3**). Susceptibility to RIF was influenced by substitutions such as Asp516Val, Asp516Tyr, Leu533Pro, and double substitutions such as Met515Ile and Leu533Pro, but not to RBT. All isolates with mutations at codons 531 and 526, except one isolate with a His526Cys substitution, exhibited resistance to both compounds. RBT is rarely used for DR-TB due to cross-resistance, but RBT has been part of successful regimens to treat XDR-TB (25).

Rifapentine (RPT)

RPT, the cyclopentyl derivative of RIF, is currently approved for intermittent dosing in the treatment of TB. RPT has the potential to further shorten the duration of TB treatment when given in higher doses. There is a significant amount of interest to establish the maximum tolerated dose of this drug, and a number of clinical trials have been planned or are underway to examine the safety, pharmacokinetics, and efficacy of higher than standard doses of RPT in first-line TB treatment (27). Higher doses have recently been shown to have higher bactericidal activity (28). Because of the greater potency of RPT against MTB and its longer half-life as compared to that of RIF, RPT is considered an attractive candidate for shortening or simplifying therapy (29).

Two methods have been developed for testing the susceptibility of MTB to RPT (Table 2 No. **4**), and a critical concentration of 0.5 μg/mL of RPT has been proposed for both methods (30), as it provides a reliable means of distinguishing between susceptible and resistant MTB isolates in which resistance can be mediated by both RIF and RPT.

CGP-7040

Activity of CGP-7040, a long-lasting derivative of RIF (10-fold that of RIF), was compared with that of RIF *in vitro* against RIF^S and RIF^R strains of MTB and *M. avium/intracellulare/scrofulaceum* (MAIS) complex (Table 2, No. **5**). The compound was found to be active against susceptible strains of MTB at MIC 4–8 times lower than that of RIF. CGP-7040 was also more active than RBT and RIF against *M. avium*. In addition, the compound was found to be considerably more stable than RIF (31–33).

The use of rifamycins for the effective treatment of resistant TB is still a matter of debate due to the levels of resistance and cross-resistance, and extensive clinical and molecular research in this line is warranted.

Pyrazinamide

The use of PZA as an anti-TB drug began in 1980 (Table 3, No. **1**). It is an important sterilizing drug and played a unique role in shortening anti-TB therapy from a period of 9–12 months to 6 months, as it kills a population of semi-dormant tubercle bacilli residing in an acidic environment that is not suitable for the action of other TB drugs (20).

Pyrazinamide Derivatives Effective Against Resistant MTB

PZA remains an important compound for the treatment of TB with the unique property of being active even in an acidic environment, *that is,* on semi-dormant bacilli. As in the case of INH, derivatives of PZA have been synthesized and tested on drug-susceptible MTB, but there are few

Table 2: RIF and its derivatives

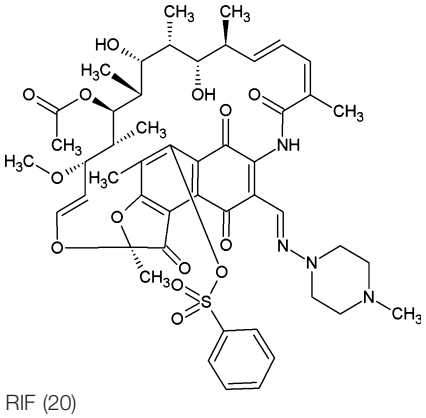
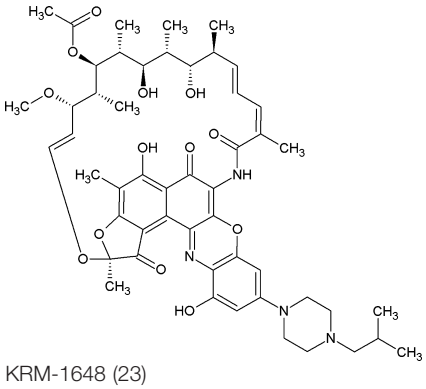
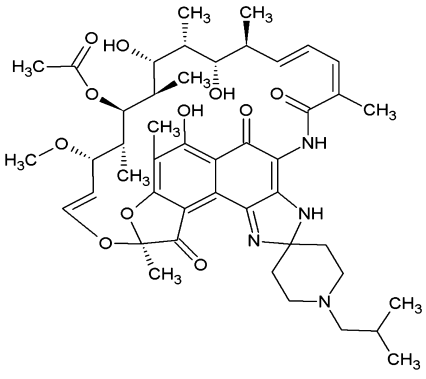
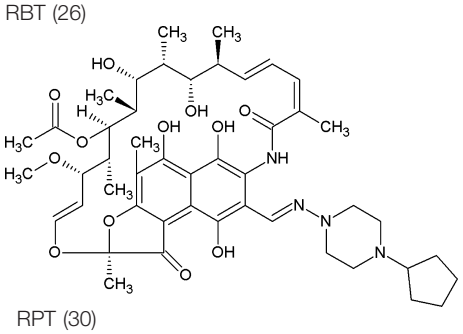
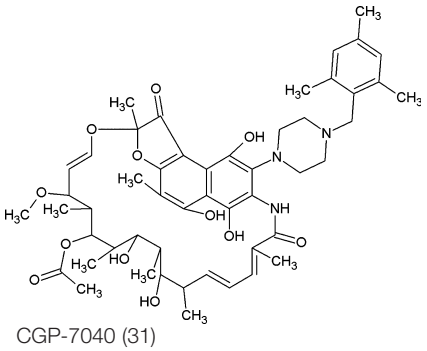
C. no.	Structure and chemical name of RIF compounds	*In vitro* activity against MTB-H37Rv-MIC = μg/mL	*In vitro* activity against RIF^R MTB MIC = μg/mL
1	 RIF (20)	0.1–0.2	
2	 KRM-1648 (23)	<1	4 – >32
3	 RBT (26)	0.06–0.5	≤0.5
4	 RPT (30)	0.03–0.12	≥8.0

Table 2: continued

C. no.	Structure and chemical name of RIF compounds	*In vitro* activity against MTB-H37Rv-MIC = μg/mL	*In vitro* activity against RIF^R MTB MIC = μg/mL
5	 CGP-7040 (31)	0.08	0.3

R = substitution site.

studies that have tested these compounds on **PZA-resistant (PZA^R)** strains, these are described below.

5-Chloro-pyrazinamide

A synthetic analogue of PZA, 5-chloro-pyrazinamide (5-Cl-PZA), was shown to possess activity against MTB (34). The mode of action of 5-Cl-PZA is thought to be on type I fatty acid synthase (FAS-I) (35) and was therefore different from that of PZA. Further evidence for its differential mode of action was that PZA^R MTB strains without PZase activity were still susceptible to 5-Cl-PZA, suggesting that the action of 5-Cl-PZA was independent of PZase activity. It remains to be determined whether 5-Cl-PZA also needs to be metabolized to its active form for killing activity. Another significant difference was that while PZA was only active against MTB with no activity on *M. smegmatis* (MIC = 2000 μg/mL), 5-Cl-PZA was active against both MTB and *M. smegmatis* (MIC = 32 μg/mL) (34) (Table 3, No. **2**). In contrast, another study reported that the MIC of 5-Cl-PZA was between 12.5 and 25 μg/mL against MTB and at doses up to 150 mg/kg was not active in a chronic murine TB model (36).

Esters of pyrazinoic acid

A collection of esters of POA and 5-substituted POA derivatives have been reported to possess enhanced *in vitro* activity against both PZA^S and PZA^R MTB (Table 3, No. **3a**–**d**), as well as against the naturally occurring PZA^R *M. bovis*, *M. kansasii*, and *M. avium* isolates (37,38). The observation that PZA^R strains of MTB were still susceptible to POA prompted efforts to develop POA precursors as anti-TB drugs. Esters of POA were found to have greater anti-TB activity than POA *in vitro*. This indicates that increasing the lipophilicity of POA by converting POA to esters would increase its anti-TB activity and that MTB must contain some esterase activity that can break down POA esters to POA in tubercle bacilli. The mode of action of POA esters is thought to be same as that of PZA and POA (37,38). POA esters **4c** and **4d** (Table 3, No. **3b**, **3c**) were shown to be active against PZA^S and PZA^R MTB isolates. The propyl ester **4d** (**3c**) compared to **4c** (**3b**) was well tolerated when given orally daily at 450 mg/kg for 10 days (38), while the tolyl ester **4h** (**3d**) had good tolerability compared to **4a** (**3a**).

Yet, another study reported that POA and its n-propyl ester inhibit FAS-I in replicating tubercle bacilli (39). Despite the improved anti-TB activity of POA esters *in vitro*, efficacy studies in mice have so far failed to demonstrate any favorable activity against MTB, presumably due to instability of the POA esters *in vivo* (40).

Aminomethylene amides

Aminomethylene amides of PZA have been demonstrated to have an activity equal to or greater than that of PZA. Strikingly, the newer class of aminomethylene amides was found to be active against a PZA^R strain with MIC comparable to that of susceptible strains. Infected macrophages were treated with MTB-H37Rv, for a period of 8 days in the presence of these compounds, and it was observed that there was a decline in the number of colony-forming units (CFU) by one, two, or more logs of viable counts after 8 days of cultivation, demonstrating that the compounds were bactericidal in macrophages. In combination with RIF, compound No. **4** at pH 5.5 (Table 3, No. **4a**) was found to be profoundly effective at reducing the bacterial load. Most significantly, these new agents (Table 3, No. **4a**–**f**) retain activity against PZA^R organisms *in vitro*. Hence, the authors strongly suggest that this line of compounds holds promise for the development of new bactericidal drugs effective against intracellular and extracellular MTB (41). The effect of structure on the activity of PZA analogues is based on effect of aminomethylene amide which has pH-independent activity as well as activity in infected cultured human monocyte-derived macrophages.

Table 3: PZA and its derivatives

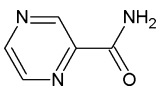
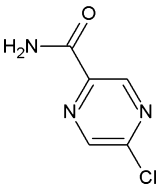
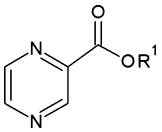
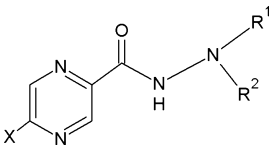
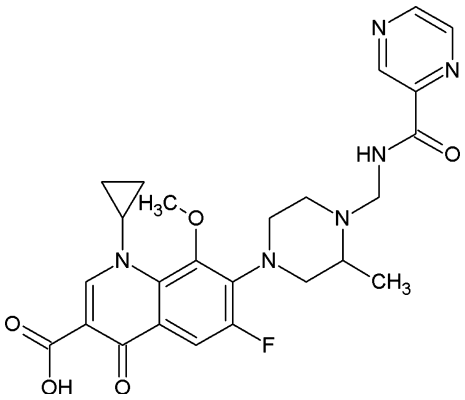
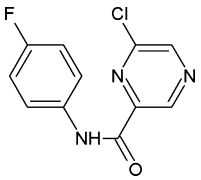
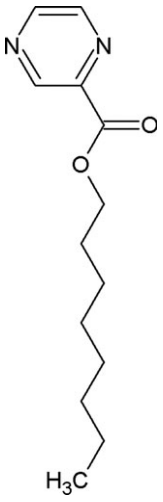
C. no.	Structure and IUPAC name of PZA compounds	*In vitro* activity against MTB-H37Rv MIC = μg/mL; D	*In vitro* activity against PZA-resistant MTB MIC = μg/mL; D
1	 Pyrazine -2-carboxylic acid (20)	8–60	–
2	 5-chloro-pyrazinamide (34)	16	32
3	 Parental Compound		
3a	R = 2, 2, 2-Trifluoroethyl 2, 2, 2- Trifluoroethyl pyrazinoate (38)	25	25
3b	R = Allyl (C_3H_2) Allyl pyrazine-2-carboxylate (38)	≤3.12	6.25
3c	R = Propyl (C_3H_3) Propyl pyrazine-2-carboxylate (38)	≤3.12	6.25
3d	R = 4 -Tolyl ($C_6H_5CH_3$) 4 -Tolyl pyrazinoate (38)	12.5	12.5
4	 Parental Compound		
4a	X = H; R^1 = $(CH_2)_{4;}$ R^2 = Nil *N*-(Pyrrolidin-1-ylmethyl)pyrazine-2-carboxamide (41)	50	100
4b	X = H; R^1 = $C_3H_{7;}$ R^2 = C_3H_7 *N*-(Dipropylaminomethyl)pyrazine-2-carboxamide (41)	50	100
4c	X = H; R^1 = $(CH_2)_{5;}$ R^2 = Nil *N*-(Piperidin-1-ylmethyl)pyrazine-2-carboxamide (41)	25	100
4d	X = H; R^1 = $[(CH_2)_2]_2N\text{-}CH_{3;}$ R^2 = Nil *N*-(Piperazin-1-ylmethyl)pyrazine-2-carboxamide (41)	100	>100
4e	X = H; R^1 = $CH_{3;}$ R^2 = $CH_2C_6H_5$ *N*-(*N*′-Benzyl, *N*′-methylaminomethyl)pyrazine-2-Carboxamide (41)	50	100
4f	X = CH_3; R^1 = $[(CH_2)_2]_2O$ *N*-(Pyrrolidin-1-ylmethyl)-5-methylpyrazine-2-Carboxamide (41)	100	100
4g	X = CH_3; R^1 = $C_3H_{7;}$ R^2 = C_3H_7 *N*-(Morpholin-4-ylmethyl)-5-methylpyrazine-2-carboxamide (41)	100	100
4h	X = CH_3; R^1 = $C_2H_{5;}$ R^2 = C_2H_5 *N*-(Diethylaminomethyl)-5-methylpyrazine-2-carboxamide (41)	100	100
4i	X = CH_3; R^1 = $C_3H_{7;}$ R^2 = C_3H_7 *N*-(Dipropylaminomethyl)-5-methylpyrazine-2-carboxamide (41)	50	100

Table 3: continued

C. no.	Structure and IUPAC name of PZA compounds	*In vitro* activity against MTB-H37Rv MIC = μg/mL; D	*In vitro* activity against PZA-resistant MTB MIC = μg/mL; D
4j	X = CH_3 R^1 = $(CH_2)_2]_2N$-CH_3 R^2 = Nil *N*-(Piperazin-1-ylmethyl)-5-methylpyrazine-2-Carboxamide (41)	50	100
5	 1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-7-(3-methyl-4-((pyrazine-2 carboxamido) methyl)piperazin-1-yl)-4-oxoquinoline-3-carboxylic acid (42)	0.39	0.2
6	 6-chloro-*N*-(4-fluorophenyl) pyrazine-2- carboxamide (43) (45)	6.25 52.87[a]	NA 55.63[a]
7	 Pyrazinoic acid n-octyl ester (44,45)	100 55.04[a]	NA 57.79[a]

R = substitution site, D = docking score, NA = not available, e = activity at pH 5.5.
[a]kcal/mol.

Mannich bases

In an investigation, PZA derivatives were synthesized based on Mannich bases and evaluated them for antimycobacterial activity *in vitro* and *in vivo* against MTB-H37Rv. Compound No. **5** in Table 3 was found to be the most active compound *in vitro* against drug-susceptible MTB

and MDR-TB. Further, it was suggested that the enhanced activity might be due to the inhibition of both enzymes FASI and DNA gyrase of MTB (42).

Chlorinated N-*phenylpyrazine-2-carboxamides and Pyrazinoic acid n-octyl ester*

Compound No. **6** in Table 3 manifested the highest activity against MTB-H37Rv in a study conducted on 16 PZA analogues with the -CONH- linker connecting the pyrazine and benzene rings (43). Another compound (No. **7** in Table 3) was not only bacteriostatic but also bactericidal against MTB-H37Rv and two other species of mycobacteria *in vitro* under conditions in which PZA showed no or little activity (44).

On account of the profound *in vitro* activity of the above two compounds against susceptible MTB, we investigated their effect on resistant MTB using an *in silico* approach (45). In this study, PZA and its derivatives were docked with modeled clinical mutants (Asp8Gly, Lys96Thr, Ser104Arg, Cys138Tyr, and Cys138Ser) of PZase known to exhibit PZA resistance. The analysis showed that the above two compounds had good binding affinity to the mutant forms of PZase.

However, till date, several derivatives of PZA have been synthesized and tested for their efficacy against H37Rv and PZA^R strains, but with rather low success rate.

Concluding Remarks

Development of effective drugs to control various forms of DR-TB is the need of the hour. Unfortunately, during the last 40 years, there has been no new drug for the treatment of TB (46,47). However, there has been enhanced research in this line in recent years (48,49). Development of new drugs from existing ones is a viable approach that focuses on already known TB drug targets and limits the complexity revolving around the complex drug discovery pathways. These may have improved half-life and increased bioavailability, be more efficacious, and serve as cost-effective alternatives. It can be hoped that new drugs will be synthesized in future from the existing TB drugs, curtailing much time and endeavor on bringing out a new medicine from the laboratory to market (50–52). A number of known drugs are being currently investigated for their simplification, and improvements in the current TB drug regimen are being investigated (47). However, cross-resistance limits the applicability of the redevelopment approach of existing drugs as seen in the case of new rifamycin derivatives (25).

Presently, there is a new wave of interest in the research and development of new lead molecules that has been coupled to the availability of the genome sequence of MTB (53) and clinical isolates of MTB (54). The accelerated advancement in drug developing strategies is owing to drug resistance that has developed against many of the currently used anti-TB drugs, resulting in the requirement of longer periods of therapy and displaying higher levels of toxicity. Many researchers have synthesized various analogues of anti-TB agents and tested them for activity against DR-MTB strains to circumvent the problem. As a result of consistent research in this area, certain derivatives of the parental drug are currently in clinical trials against resistant TB. In addition, it is expected, from the advancements made in these areas, that a joint venture led by both academicians and industrialists would be set up to generate novel derivatives to successfully treat resistant strains of MTB. Thus, a considerable amount of effort is still needed for the development and evaluation of novel drugs against MDR-, XDR-, and HIV-TB.

Acknowledgments

Dr. A. Nusrath Unissa receives financial support for post-doctoral fellowship from the Indian Council of Medical Research.

Transparency Declarations

None to declare.

References

1. World Health Organization (2010) Treatment of Tuberculosis Guidelines, 4th edn. Geneva: World Health Organization.
2. Prasad R. (2005) MDR TB current status. Indian J Tub;52:121–131.
3. Paramasivan C.N. (1998) Overview on drug resistant Tuberculosis in India. Indian J Tub;45:73–81.
4. Asif M. (2013) Study of some analogue of currently clinically used antimycobacterial agents. IJEI;1:33–44.
5. Hearn M.J., Cynamon M.H. (2003) *In vitro* and *in vivo* activities of acylated derivatives of isoniazid against *Mycobacterium tuberculosis*. Drug Des Discov;18:103–108.
6. Meyer H., Malley J. (1912) Hydrazine derivatives of pyridine-carboxylic acids. Monastsh;33:393–414.
7. Robitzek E.H., Selikoff I.J. (1952) Hydrazine derivatives of isonicotinic acid (*Rimifon marsilid*) in the treatment of active progressive caseous-pneumonic tuberculosis; a preliminary report. Am Rev Tuberc;65:402–428.
8. Blanchard J.S. (1996) Molecular mechanisms of drug resistance in *Mycobacterium tuberculosis*. Annu Rev Biochem;65:215–239.
9. Vinsova J., Imramovsky A., Jampilek J., Monreal J.F., Dolezal Martin M. (2008) Recent advances on isoniazide derivatives. Anti-Infect. Agents Med Chem;7:12–31.

10. Lourenco M.C., Ferreira Mde L., de Souza M.V., Peralta M.A., Vasconcelos T.R., Henriques M.G. (2008) Synthesis and antimycobacterial activity of (E)-N′ (monosubstituted-benzylidene) isonicotinohydrazide derivatives. Eur J Med Chem;43:1344–1347.
11. Maccari R., Ottana R., Monforte F., Vigorita M.G. (2002) *In vitro* antimycobacterial activities of 2′-monosubstituted isonicotinohydrazides and their cyanoborane adducts. Antimicrob Agents Chemother;46:294–299.
12. Aboul-Fadl T., Mohammed F.A., Hassan E.A. (2003) Synthesis, antitubercular activity and pharmacokinetic studies of some Schiff bases derived from 1-alkylisatin and isonicotinic acid hydrazide (INH). Arch Pharm Res;26:778–784.
13. Sriram D., Yogeeswari P., Yelamanchili Priya D. (2009) Antimycobacterial activity of novel(substituted)-2- isonicotinoylhydrazinocarbothioamide endowed with high activity towards isoniazid resistant tuberculosis. Biomed Pharmacother;63:36–39.
14. De Logu A., Onnis V., Saddi B., Congiu C., Schivo M.L., Cocco M.T. (2002) J Antimicrob Chemother;49:275.
15. Coelho T.S., Cantos J.B., Bispo M.L.F., Gonçalves R.S.B., Lima C.H.S., da Silva P.E.A., Souza M.V. (2012) *In vitro* anti-mycobacterial activity of (E) – N′ – (monosubstituted-benzylidene) isonicotinohydrazide derivatives against isoniazid- resistant strains. Infect Dis Rep;4:e13.
16. Nusrath Unissa A., Hassan S., Selvakumar N. (2012) Elucidating isoniazid resistance in *Mycobacterium tuberculosis* using molecular docking approach. Int J Pharma Biosci;3:314–326.
17. Vigorita M.G., Ottanà R., Zappalà C., Maccari R., Pizzimenti F.C., Gabbrielli G. (1994) Halogenated isoniazid derivatives as possible antimycobacterial and anti-HIV agents-III. Farmaco;9:775–781.
18. Hearn M.J. (2001) Antimycobacterial Compounds and Method for Making the Same. International Patent Application WO 02/43668.
19. Hearn M.J., Cynamon M.H. (2004) Design and synthesis of antituberculars: preparation and evaluation against *Mycobacterium tuberculosis* of an isoniazid Schiff base. J Anti Chem;53:185–191.
20. Heifets L.B. (1994) Antimycobacterial infection. Semin Respir Infect;9:84–103.
21. Stratton M.A., Reed M.T. (1986) Short-course drug therapy for tuberculosis. Clin Pharm;5:977–987.
22. Shoen C.M., DeStefano M.S., Cynamon M.H. (2000) Durable cure for tuberculosis: rifalazil in combination with isoniazid in a murine model of *Mycobacterium tuberculosis* infection. Clin Infect Dis;30:S288–S290.
23. Moghazeh S.L., Pan X.S., Arain T.M., Stover C.K., Musser J.M. (1996) Comparative antimycrobial activities of rifampin, rifapentine, and KRM-1648 against a collection of rifampin-resistant *Mycobacterium tuberculosis* isolates with known rpoB mutations. Antimicrob Agents Chemother;40:2655–2657.
24. Hidaka T. (1999) Current status and perspectives on the development of rifamycin derivative antibiotics. Kekkaku;4:53–61.
25. Dooley K.E., Mitnick C.D., Ann DeGroote M., Obuku E., Belitsky V., Hamilton C.D. (2012) Efficacy subgroup, RESIST-TB. Old drugs, new purpose: retooling existing drugs for optimized treatment of resistant tuberculosis. Clin Infect Dis;55:572–581.
26. Cavusoglu C., Karaca Derici Y., Bilgic A. (2004) *In vitro* activity of rifabutin against rifampicin resistant *Mycobacterium tuberculosis* isolates with known rpoB mutations. Clin Microb Infect;10:662–665.
27. Ginsberg A.M. (2010) Drugs in development for tuberculosis. Drugs;70:2201–2214.
28. Diacon A.H., Patientia R.F., Venter A., Van Helden P.D., Smith P.J., McIlleron H., Maritz J.S., Donald P.R. (2007) Early bactericidal activity of high-dose rifampin in patients with pulmonary tuberculosis evidenced by positive sputum smears. Antimicrob Agents Chemother;51:2994–2996.
29. Keung A.C., Owens R.C. Jr, Eller M.G., Weir S.J., Nicolau D.P., Nightingale C.H. (1999) Pharmacokinetics of rifapentine in subjects seropositive for the human immunodeficiency virus: a phase I study. Antimicrob Agents Chemother;43:1230–1233.
30. Heifets L., Sanchez T., Vanderkolk J., Pham V. (1999) Development of rifapentine susceptibility tests for *Mycobacterium tuberculosis*. Antimicrob Agents Chemother;43:25–28.
31. Dickinson J.M., Mitchison D.A. (1987) *In vitro* activity of new rifamycins against rifampicin resistant *Mycobacterium tuberculosis* and MAIS-complex *Mycobacteria*. Tubercle;68:177–182.
32. Dickinson J.M., Mitchison D.A. (1987) *In vitro* observations on the suitability of new rifamycins for the intermittent chemotherapy of tuberculosis. Tubercle;68:183–193.
33. Heifets L.B., Lindholm-Levy P.J., Flory M.A. (1990) Bactericidal activity *in vitro* of various rifamycins against *Mycobacterium avium* and *Mycobacterium tuberculosis*. Am J Respir Critic Care Med;141:626–630.
34. Cynamon M.H., Speirs R.J., Welch J.T. (1998) *In vitro* antimycobacterial activity of 5-Chloropyrazinamide. Antimicrob Agents Chemother;42:462–463.
35. Boshoff H.I., Mizrahi V., Barry C.E. III (2002) Effects of pyrazinamide on fatty acid synthesis by whole mycobacterial cells and purified fatty acid synthase I. J Bacteriol;184:2167–2172.
36. Ahmad Z., Tyagi S., Minkowski A., Almeida D., Nuermberger E.L., Peck K.M. (2012) Activity of 5-chloro-pyrazinamide in mice infected with *Mycobacterium tuberculosis* or *Mycobacterium bovis*. Ind J Med Res;136:808–814.
37. Cynamon M.H., Gimi R., Gyenes F., Sharpe C.A., Bergmann K.R., Han H.J. (1995) Pyrazinoic acid esters with broad spectrum *in vitro* antimycobacterial activity. J Med Chem;38:3902–3907.

38. Cynamon M.H., Klemens S.P., Chou T.S. (1992) Antimycobacterial activity of a series of pyrazinoic acid esters. J Med Chem;35:1212–1215.
39. Zimhony O., Vilcheze C., Arai M., Welch J.T., Jacobs W.R. Jr (2007) Pyrazinoic acid and its n-propyl ester inhibit fatty acid synthase type I in replicating tubercle bacilli. Antimicrob Agents Chemother;51:752e4.
40. Zhang Y., Mitchison D. (2003) The curious characteristics of pyrazinamide: a review. Int J Tuberc Lung Dis;7:6–21.
41. Chung W.J., Kornilov A., Brodsky B.H., Higgins M., Sanchez T., Heifets L.B., Cynamon M.H., Welch J. (2008) Inhibition of *M. tuberculosis*, *in vitro*, in monocytes and in mice by aminomethylene pyrazinamide analogs. Tuberculosis;88:410–419.
42. Sriram D., Yogeeswari P., Reddy S.P. (2006) Synthesis of pyrazinamide Mannich bases and its antitubercular properties. Bioorg Med Chem Lett;16:2113–2116.
43. Dolezal M., Zitko J., Osicka Z., Kunes J., Vejsova M., Buchta V., Dohnal J., Jampilek J., Kralova K. (2010) Synthesis, antimycobacterial, antifungal and photosynthesis-inhibiting activity of chlorinated N-phenylpyrazine-2-carboxamides. Molecules;15:8567–8581.
44. Yamamoto S., Toida I., Watanabe N., Ura T. (1995) *In vitro* antimycobacterial activities of pyrazinamide analogs. Antimicrob Agents Chemother;39:2088–2091.
45. Nusrath Unissa A., Sudha S., Selvakumar N. (2011) Elucidating pyrazinamide resistance in *Mycobacterium tuberculosis* by molecular docking. Int J App Biol Pharm Technol;2:19–29.
46. Richard O'Brien J., Paul Nunn P. (2001) The need for new drugs against tuberculosis. Am J Respir Crit Care Med;162:1055–1058.
47. Lienhardt C., Vernon A., Raviglione M.C. (2010) New drugs and new regimens for the treatment of tuberculosis: review of the drug development pipeline and implications for national programmes. Curr Opin Pulm Med;16:186–193.
48. Zhang Y., Post-Martens K., Denkin S. (2006) New drug candidates and therapeutic targets for tuberculosis therapy. Drug Discov Today;11:1–2.
49. Lilienkampf A., Pieroni M., Wan B., Wang Y., Franzblau S.G., Kozikowski A.P. (2010) Rational design of 5-phenyl-3- isoxazolecarboxylic acid ethyl esters as growth inhibitors of *Mycobacterium tuberculosis* a potent and selective series for further drug development. J Med Chem;53:678–688.
50. Duncan K. (2003) Progress in TB drug development and what is still needed. Tuberculosis;83:201–207.
51. Nusrath Unissa A. (2011) Rational drug designing strategies for *Mycobacterium tuberculosis*. Int J Pharma Biol Sci;1:534–555.
52. Bavesh Kana D., Mizrahi V. (2004) Molecular genetics of *Mycobacterium tuberculosis* in relation to the discovery of novel drugs and vaccines. Tuberculosis;84:45–75.
53. Cole S.T., Brosch R., Parkhill J., Garnier T., Churcher C., Harris D., Gordon S.V. *et al.* (1998) Deciphering the biology of *Mycobacterium tuberculosis* from the complete genome sequence. Nature;393:537–544.
54. Zhang H., Li D., Zhao L., Fleming J., Lin N., Wang T., Liu Z. *et al.* (2013) Genome sequencing of 161 *Mycobacterium tuberculosis* isolates from China identifies genes and intergenic regions associated with drug resistance. Nat Genet;45:1255–1260.

Author Information

Dr. Ameeruddin Nusrath Unissa was born in Chennai, Tamil Nadu, India. She obtained her graduation in the field of biochemistry and postgraduation in biotechnology from University of Madras, India, 1999 and 2001, respectively. She completed Ph.D in the interdisciplinary area of biotechnology–microbiology in the year 2010. She worked as lecturer for biotechnology in couple of colleges from 2001 to 2003. She obtained the GATE award in 2003. She also qualified Indian Council of Medical Research (ICMR)-JRF award in the year 2003, then as ICMR-SRF during 2007, at National Institute for Research in Tuberculosis, (NIRT) Chennai. She was awarded as ICMR—Postdoctoral Fellowship (PDF) in 2013. Currently, she is working in the molecular analysis of isoniazid and resistance in *Mycobacterium tuberculosis* and *in silico* analysis of rifampicin, bedaquiline, and HIV resistance, in the Division of Biomedical Informatics at NIRT. She has undertaken a number of research studies on TB drug resistance and published in peer-reviewed journals.

Dr. Luke Elizabeth Hanna was born in Alleppey, Kerala, India. She did her graduation in the field of zoology and postgraduation in biomedical genetics from University of Madras, 1991 and 1993, respectively. She obtained Ph.D in the area of basic medical sciences in the year 2000. Her postdoctoral research includes the field of molecular virology in relation to HIV, from Albert Einstein College of Medicine, New York, USA in the year 2007. From research assistant, she promoted as Scientist B, C, and D in the respective years 2002, 2008, and 2015 after completing her doctorate degree, at National Institute for Research in Tuberculosis (NIRT), Chennai. Therefore, she had 20 years of working experience in the broad area of immunology of infectious diseases including tuberculosis, HIV, and lymphatic filariasis. She is the Chief Laboratory Liaison for WHO HIV Drug Resistance Network activities at TRC, supervisor, and coordinator for activities relating to the Virology Quality Assurance Program (NIH) at NIRT, and laboratory resource person for the second-line ART and early infant diagnosis programs of NACO. She is the principal investigator for the ICMR Biomedical Informatics Project at NIRT and a recognized guide for Ph.D. students registered with the Madras University.

Dr. Soumya Swaminathan was born in Kerala, India. She holds M.B.B.S. from Armed Forces Medical College,

India, and M. D. from All India Institute of Medical Sciences, along with a Diplomate of National Board from National Board of Examinations. She subsequently held Postdoctoral Medical Fellowship in Pediatric Pulmonology at the Children's Hospital Los Angeles, Keck School of Medicine at the University of Southern California. Then, she joined as a research fellow (Registrar) in the Department of Pediatric Respiratory Diseases, University of Leicester, UK, during 1989–1990. She served as adjunct associate clinical professor, at Tufts University School of Medicine. She joined as senior research officer, Cardiopulmonary Medicine Unit, during 1992 at NIRT. She then promoted as assistant director till 1997, as deputy director from 1997 to 2002, deputy director Sr. Grade till 2008, and Scientist 'G' from May 2008 onwards. She took over the charge of Head of the Division HIV/AIDS in 2000. She served as the director of NIRT from 2012 to 2015. She is currently positioned as secretary, Department of Health Research—Ministry of Health and Family Welfare, Government of India, and Director General of Indian Council of Medical Research—the apex body in India for the formulation, coordination, and promotion of biomedical research. She is the recipient of several prestigious awards, holding membership in many scientific committees and attended several national and international conferences, and author of numerous scientific publications to her credit. She is a renowned Indian pediatrician and clinical scientist known for her works in tuberculosis.

Notes

[a]World Health Organization. (2015) Media centre, Fact sheet N′104. updated March 2015. http://www.who.int/mediacentre/factsheets/fs104/en/ accessed on 19-05-15.
[b]World Health Organization. (2015) Tuberculosis, extensively drug-resistant tuberculosis. http://www.who.int/tb/challenges/mdr/xdr/en/. accessed on 30-06-15.