Development of new antitubercular drugs containing benz-fused ring system

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ABSTRACT
Complicated composition of the mycobacterial cell wall, therapy of tuberculosis is very difficult, because Mycobacterium tuberculosis makes many antibiotics and drugs ineffective and hinders the entry of drugs. Now, tuberculosis is still the second most imperative infectious disease worldwide. The most important reason for this is drug resistant, persistent or latent infection and synergism of tuberculosis with HIV. Furthermore various chemical entities containing drugs has come in the treatment of tuberculosis. These drugs are mainly containing different heterocyclic moiety both in fused or substituted form. In present review we discussed brief introduction of tuberculosis followed the substituted benz-fused compounds including currently used drugs and newly developed molecules which are effective against tuberculosis.

KEYWORDS
Drug-resistant tuberculosis; Anti-TB drugs; Resistant bacteria.

INTRODUCTION
The history of TB changed dramatically after the introduction of anti-TB agents. Anti-TB drug treatment started in 1944, when streptomycin and paraaminosalicylic acid were discovered. The combined therapy was more effective in the TB treatment that consisted of a long course of both drugs. In 1952, a third drug, isoniazid, was introduced, greatly improving the efficacy of treatment. In 1960, ethambutol substituted paraaminosalicylic acid, and the treatment course was reduced to 18 months. In the 1970s, with the introduction of rifampicin into the combination, treatment was shortened to just nine months. In 1980, pyrazinamide was introduced into the anti-TB treatment, which could be reduced further to only six months. Soon after the introduction of the first anti-TB drugs, drug resistant bacilli started to emerge, but the launch of both combination therapy and new and more effective drugs seemed to be enough to control the disease. However, TB unexpectedly re-emerged in the '80s, and in the following years there was an important increase in the incidence of multiple-, and extensively drug resistant strains. Since 1970, no new drug has been discovered for anti-TB treatment, which today seems insufficient to confront the disease. Fortunately, research efforts have been accomplished and today there is a wide range of new molecules with promising anti-TB activity. The modernization of synthetic transformations, although many compounds are in clinical trials, it is astonishing that with this background, there have been no new drugs registered to treat TB in the last 40 years[7,8,21]. This
reflects the inherent difficulties in discovery and clinical testing of new agents and the lack of pharmaceutical industry research in this area.

TB is one of the oldest and most pervasive diseases in history[19,20]. According to WHO report, TB has spread to every corner of the globe. As much as one-third of the world’s population is currently infected and more than 5000 people die from TB every day[57]. It is estimated that between 2002 and 2020, approximately 1000 million people will be newly infected, over 150 million people will develop diseases and 36 million will die of TB if proper control measures are not established[10].

The Directly Observed Treatment, short-course (DOTS) strategy, constitutes the cornerstone of the current protocol for control of TB. However, the three key drugs, isoniazid, pyrazinamide and rifampicin, used in the regimen are potentially hepatotoxic and may lead to drug associated hepatitis[166]. Despite the undoubted success of DOTS strategy, the emergence of multi drug resistant strains (MDR-TB), recurrently isolated from patient’s sputum, darken the future. Furthermore, one of the main causes for the prevalence of TB is synergy with Human Immunodeficiency Virus (HIV) epidemic where 31% of new TB cases were attributable to HIV co-infection[28]. From the chemotherapeutic point of view, there are two sources of new chemical entities.

NEW POTENTIAL ANTI-TUBERCULAR AGENTS

Tuberculosis (TB) is a chronic infectious disease caused by M. tuberculosis. The term MDR-TB is used to describe strains that are resistant to two or more of the five first-line anti-TB drugs. Treatment regimen of tuberculosis comprises five first line anti-TB drugs namely isoniazid, rifampicin, pyrazinamide, streptomycin and ethambutol followed by second line anti-TB drugs namely fluoroquinolones and one of the injectable aminoglycosides. Besides the traditional anti-TB drugs available commercially, several new heterocycles were synthesized in recent past. The new potential anti-TB agents have been classified according to their chemical entities. In an effort to studied more effective therapies, molecules that can be effective against MTB and MDR-TB. In an effort to developed new and more effective anti-TB agents. The new potential antitubercular agents are classified on the basis of their chemical entities. Benz-fused derivatives have shown interesting biological properties and some of them are known for their anti-TB activity[32-34]. Hence, for the purpose of obtaining new and more potent anti-TB molecule that can improve the current chemotherapeutic anti-TB treatment.

ANTITUBERCULAR AGENT WITH BENZ-FUSED RING SYSTEM

A series of 2,6-diaryl piperidin-4-ones and tetrahydropyridin-4-ol based benzimidazole and O-arylsulfonyl derivatives were screened for their anti-TB activity, three compounds (1) have shown equal potency of MIC 16 µg/mL against M. tuberculosis H37Rv, which are one-fold more potent than of the rifampicin[2]. In an effort to increase the potency of piperidones, a series of spiro-piperidin-4-ones as anti-TB agent, compound (2) showed promising in vitro potency of MIC 0.07 µg/mL.
and 0.16 μg/mL against *M. tuberculosis* and MDR-TB respectively and also showed in vivo potency by decreasing the bacterial load in lung and spleen tissues with 1.30 and 3.73-log10 protections respectively, which is comparable to isoniazid[2].

In a series of chalcones, compound 3 has shown MIC of 6.8 μg/mL against *M. tuberculosis* H37Rv[49]. A number of three hybrids of pyrazine and two of them showed promising anti-TB activity. Compound 4 showed MIC of 0.78, 0.1 μg/mL respectively, against *M. tuberculosis* H37Rv and also showed good activity against atypical strains of *M. tuberculosis*[14]. In a different approach, a series of 1,4-substituted piperazine/homopiperazines, compound 5 showed MIC of 62.5 μM[11,27].

Clofazimine (9) is a fat-soluble riminophenazine dye used in combination with rifampicin and dapsone as multidrug therapy for the treatment of leprosy. On this basis and to minimize the side-effects and to improve the anti-TB activity of Clofazimine[60], developed 3-(2,4-dichloroanilino)-10-(2,4-dichlorophenyl)-2,10-dihydro-2-(2,2,6,6-tetramethylpiperid-4-ylimino)phenazine (B4128) (10), which posses a similar mode of action of Clofazimine[53]. A series of phthalimido- and naphthalimido-linked phenazines were found two compounds (11a and 11b) with a potency of MIC 1 μg/mL against *M. tuberculosis* H37Rv. These compounds exhibited potent activity against resistant strains of *Mycobacterium*[36]. Whereas in a series of phenazine carboxamides, compounds 12a and 12b showed excellent activity against *M. tuberculosis* H37Rv with a MIC of 0.19 μg/L and also against drug-resistant strains of *M. tuberculosis*[18], validating them as future anti-TB drugs.
Phenothiazines have been reported for their anti-TB activity, and the phenothiazine drug chlorpromazine (13a) is reported to have been successfully used as anti-TB agent. In this concern, a series of phenothiazines were synthesized for anti-TB agents. Three compounds (13b-d) exhibited promising activity with a mean MIC of 2.13 µg/mL. Whereas quaternized CPZ, triflupromazine (14a) and promethazine (14b) derivatives inhibited non-replicating *M. tuberculosis* at concentrations equal to or double their MICs against the actively growing strain. All the active compounds (14c-f) were non-toxic toward Vero cells (IC50 > 128 µM).

The benzyl or substituted benzyl groups, an electron-withdrawing substituent on the phenothiazine ring improved the potency. Commonly the optimum anti-TB structures possessed *N*-(4- or 3-chlorobenzyl) substituent on triflupromazine. While a macro lactone (15) derived from benzo[a]phenazine exhibited best potency against *M. tuberculosis* H37Rv with a MIC 0.62 µg/mL, which is better than that of Rifampacin. In search of potential anti-TB agents, pyridazinoindole analogues were screened for inhibition of the growth of *M. tuberculosis*. The most active compound (16) exhibited a MIC of 0.42 µg/mL against *M. tuberculosis* H37Rv. In the series 2-aryl-3,4-dihydro-2H-thieno[3,2-b]indoles, compound 17 was found to be the most active compound with MIC of 0.4 µg/mL against *M. tuberculosis* and MDR-TB. A series of pyrrolo[1,2-a]quinoxaline-2- or -4-carboxylic acid hydrazides and one compound (18) showed an interesting activity at 6.25 µg/mL against *M. tuberculosis* H37Rv, with a 100% inhibition. Compound 19 inhibited 80% at a concentration of 6.25 µM. Enamine-containing analogues of heteroarylquinones showed promising activity with a MIC in the range 6.25-0.1 µg/mL against *M. tuberculosis* H37Rv and molecule (20) with a MIC 0.39 µg/mL.

A series of benzothiadiazine 1,1-dioxide derivatives were evaluated against *M. tuberculosis*, *M. avium* and *M. intracellulare*. Of these, compound 21 showed best potency of MIC 0.5 µg/mL against *M. tuberculosis* H37Rv and 0.5-2 µg/mL against resistant strains. However, the in-vivo testing in a mouse model of TB infection did not show significant anti-TB activity, probably due to its poor bioavailability.
trophuran, 5-nitrothiophene and arylfuran coupled benzothiadiazines were evaluated, these compounds exhibited moderate anti-TB activity. The most active compound (22) displayed a MIC of 1 μg/mL against *M. tuberculosis* H37Rv\(^{[35]}\). A number of fifteen 2-amino-6-methyl-4-aryl-8-(E)-arylmethylidene]-5,6,7,8-tetrahydro-4H-pyran[3,2-c]pyridine-3-carbonitriles were evaluated for their anti-TB activity. Among all, compound 23 was found to be the most potent compound with MIC value is 0.43 μM against *M. tuberculosis* and MDR-TB, being 100 times more active than isoniazid against MDR-TB\(^{[47]}\).

In search of novel anti-TB agents, tetrahydro-indazole based compounds were evaluated their efficiency. Among all, three compounds 24a-c have shown MIC in the range 1.7-1.9 μM against *M. tuberculosis* H37Rv. These compounds also displayed any toxicity against VERO cells up to the concentration 128 μM\(^{[24]}\). Compound 1,3-benzothiazin-4-ones kills *M. tuberculosis* by blocking arabinan synthesis. The most advanced compound (25), was found to a candidate for inclusion in combination therapies for both MDR and XDR-TB\(^{[52]}\). The 1,2,4-Triazolo[1,5-a]pyrimidine-6-carboxylic acid derivatives and one compound (26) has shown 92% growth inhibition of *M. tuberculosis* H37Rv at 6.25 μg/mL concentration\(^{[1]}\). A novel series of spiro-pyrrolothiazoles were evaluated for their anti-TB activity. Among all, the best potency was displayed by compound 27 with a MIC of 0.6 μM against MTB and MDR-TB\(^{[38]}\). 3-amino-imidazo[1,2-alpyridines as a novel class of *M. tuberculosis* glutamine synthetase inhibitors. The most active compound (28) showed an inhibition of IC50 = 0.38 ± 0.02 μM\(^{[55]}\).

Compound 29 showed moderate activity of >32 μg/mL against *Mycobacterium*\(^{[17]}\). In the same direction, compound 5-[(E)-2-(6-methoxy-4-quinolinyl)ethyl]-3-isoxazolcarboxylic acid butyl ester (30) showed the best activity against *M. tuberculosis* H37Rv with a MIC 1.8 μM. Both these compounds showed almost equal potency with standard drugs isoniazid and refampin\(^{[58]}\). While in another series, (R)-methyl 2-(5-((2-methylbenzo[d]thiazol-5-yloxy)methyl)isoxazole-3-carboxamido)-2-phenylacetate (31) has shown less activity with a MIC 1.4 μM\(^{[29]}\) in comparison to compound 30. A series of thiazolylhydrazone derivatives, compound 32 showed best inhibition of 89% at a concentration of >6.25 μg/mL\(^{[75]}\). A series of clubbed [1,2,3] triazoles with fluoro-benzimidazole, two compounds (33a and 33b) have
shown almost equal MIC of 0.34 µM and 0.32 µM respectively, against *M. tuberculosis* H37Rv\[^{20}\].

A series of novel 2-[4-(1H-[1,2,4]-triazol-1-yl)phenyl]-1-substituted-4,6-difluoro-1H-benzo[d]imidazole derivatives were exhibited anti-TB activity against *M. tuberculosis* H37Rv, two compounds 34a and 34b have shown preeminent activity of MIC 0.36 µg/mL and 0.58 µg/mL respectively\[^{31}\].

In spirooxindole derivatives, two compounds (35a and 35b) showed best MIC of 0.05 µg/mL against *M. tuberculosis* H37Rv, which is comparable to isoniazid and Rifampacin. A series of hydrazone and 3-nitrovinylanalogs of indole-3-carboxaldehydes and related compounds and found an active molecule (36), which exhibited 91% inhibition at <6.25 µg/mL against *M. tuberculosis* H37Rv\[^{68}\]. While *N*-Hydroxythiosemicarbazides (37a and 37b) showed poor potency of MIC 62.04, 24.58 µM respectively\[^{38}\]. Compound 38 showed moderate activity of MIC 2 µg/mL against *M. tuberculosis* H37Rv\[^{79}\].

A series of 2-(hydrazinocarbonyl)-3-aryl-1H-indole-5-sulfonamides were evaluated as inhibitors of two β-carbolic anhydrases from *M. tuberculosis*, Rv1284 and Rv3273. The compound (39) showed excellent nanomolar inhibitory activity, with several subnanomolar inhibitors being detected. The activity profile confirmed that the Rv1284 and Rv3273 have potential for developing anti-TB agents\[^{25}\].

A series of 2-alkylsulfanyl benzimidazoles were tested against *M. tuberculosis*. The values of MIC were within the range 4-125 µM/L in comparison to that of isoniazid having a MIC of 4 µM/L. The most active compounds were 40a and 40b exhibited a MIC of 4 µM/L\[^{41}\]. In view of the good activity of (40a), the benzene ring of benzimidazole was further substituted by methyl group at the 5-position to see the change of activity. The most active compound (41) showed same potency of MIC 4 µM/L\[^{43}\]. In another effort, a series of substituted 2-polyfluoroalkyl and 2-nitrobenzylsulphanyl benzimidazoles were evaluated for their activity against *Mycobacterium* strains. Compound sulphanyl benzimidazole (42) exhibited best potency of MIC in the range 2-32 µM/L against *M. tuberculosis*. While MIC values against *M. kansasii* and *M. avium* exceeded that of isoniazid. The SAR of this series confirmed that the 3,5-dinitro compounds were several times more effective against *M. tuberculosis* and *M. kansasii* than the respective isomeric 2,4-dinitro derivatives\[^{40}\].

Benzoxazoles can be considered as structural bioisosters of nucleotides such as adenine and guanine, which allow them to interact with the biopolymers of a living system. They are also identified for their anti-TB activity. With this interest a number of 30,2-substi-
tuted 5,7-di-tert-butylbenzoxazoles were evaluated for their anti-TB potency. Of these, 5,7-ditet-butyl-2-styrylbenzoxazole (43) showed MIC of 3.13 µg/mL against *M. tuberculosis* H37Rv and also found least toxic[77]. In the similar way, a series of 2-benzylsulfanyl derivatives of benzoxazole and benzothiazole were evaluated for their in vitro anti-TB activity against *M. tuberculosis* and non-tuberculous mycobacteria. The substances bearing two nitro groups (44a-d) or a thioamide group (44e-h) exhibited appreciable anti-TB activity of MIC in the range 2-8 µM/L against *M. tuberculosis* and exhibited great activity particularly against non-tuberculous strains[48]. In a series of (1,1-dioxido-3-oxo-1,2-benzisothiazol-2(3H)-yl)methyl N,N-disubstituted dithiocarbamates and (1,1-dioxido-3-oxo-1,2-benzisothiazol-2(3H)-yl)methyl O-alkyl dithiocarbonates, three compounds (45a-c) showed equal potency of MIC 0.78 µg/mL against *M. tuberculosis* H37Rv[26] and benzothiolone derivatives 46a-c exhibited poor activity of MIC 50 µg/mL[44].

A series of 3-aryl substituted-2-[1H(2H)]benzotriazol-1-(2)-yl]acrylonitriles (47a and 47b) were showed 98, 99% inhibition and a MIC of 6.25 µg/mL and 12.5 µg/mL respectively against *M. tuberculosis*[61]. Some benzotriazole series compound (36) showed moderate anti-TB activity. Also the 1-Benzotriazole derivatives were more active than 2-isomers. Conversion of the cyano group into carboxamido or carboxylic group also produced loss of activity which indicates that an increase of the hydrophilic properties is not profitable for the activity[61].

Recently, purine analogues are possessing anti-TB activity. In this perception, 9-benzylpurines with a variety of substituents at 2, 6 or 8 positions were found as good anti-TB agents. High activity was exhibited by 9-benzylpurines carrying a phenyl ethynyl, transstyril or aryl substituents at the 6th position and generally chlorine at the 2nd position. The most active compounds 49a and 49b showed a MIC of 3.13 and 0.78 µg/mL respectively, against *M. tuberculosis* H37Rv[3]. In continuation a series of 6-arylpurines having a variety of substituents in the 9 position and screened against *M. tuberculosis* H37Rv. The most active compound of the series was again found to be same 9-benzyl-2-chloro-6-(2-furyl)purine (49b) having a MIC of 0.78 µg/mL. This compound was active against several singly drug-resistant strains of *M. tuberculosis*[3]. Eleven analogues of 9-sulphonated/sulphenylated 6-mercaptopurines[63], out of them six exhibited MIC in the range of 0.39-0.78 µg/mL. The most potent compound (168) (MIC=0.39 µg/mL) exhibited good activity against MDR-TB strains of *M. tuberculosis*. 

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A series of 9-aryl-, 9-arylsulfonyl- and 9-benzyl-6-(2-furyl)purines were screened for their anti-TB activity against *M. tuberculosis* H37Rv. Among all, 2-chloro-6-(2-furyl)-9-(4-methoxyphenylmethyl)-9H-purine (50) exhibited best potency of MIC 0.39 µg/mL[4]. In a purine derivatives, 9-(ethylcarboxymethyl)-6-(dodecylthio)-9H-purine (51) showed MIC of 0.78 µg/mL[56]. In the analogues of agelasine E (52), one derivative (52a) showed promising anti-TB activity with MIC of 1.56µg/mL against *M. tuberculosis* H37Rv[41]. 6-(2-furyl)-9-(p-methoxybenzyl)purines carrying a variety of substituents in the 2- or 8-position was identifying a more potent molecule (53, MIC=0.20 µg/mL)[13]. The purine derivatives was found a more potent molecule (54) of above all series, which has shown an IC90 of <0.20 µg/mL against *M. tuberculosis* H37Rv[12].

In search of new anti-TB purine type analogues, a series of 1-[1-(4-hydroxybutyl)-1,2,3-triazol-(4 and 5)-ylmethyl]-1Hpyrazolo[3,4-d]pyrimidines, all of them were inactive but one compound (55) has shown MIC of 12.5 µg/mL[54].
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TB activity with MICs down to $\leq 2 \mu g/mL$. Among all, one compound (58) has shown MIC=0.5-1 $\mu g/mL$ against *M. tuberculosis* H37Rv. A homologous series of three pyrazolopyrimidine analogues (59a-c) were evaluated as lumazine synthase inhibitors. All three compounds were potent inhibitors of the lumazine synthases of *M. tuberculosis* with constants in inhibition in the low nanomolar to subnanomolar range. In a series of Thieno[2,3-d]pyrimidin-4-one, two compounds (60a and 60b) have shown moderate potency of 5 $\mu M/L$ against *M. tuberculosis* and *M. avium*, which is equal to that of rifampicin.

**DISCUSSION**

Anti-TB drugs have traditionally been identified by their ability to suppress or kill replicating cultures of bacteria in vitro. The weak sterilizing property of available TB drugs is one of the major drawbacks for TB chemotherapy. Thus, although achieving a clinical cure, the current TB chemotherapy does not achieve a bacteriological cure since the therapy cannot completely eradicate all bacilli in the lesions. HIV has dramatically increased the risk of developing active TB and HIV co-infection makes TB more difficult to diagnose and treat due to interactions and side-effects. The increasing emergence of MDR-TB and the recalcitrant nature of persistent infections pose additional challenges to treatment with conventional anti-TB drugs. Although TB can be cured with current drugs treatment is complex and long-lasting. As emphasized, a new TB treatment should offer at least one of the following three improvements over the existing regimens: shorten the total duration of treatment and/or significantly reduce the number of doses needed to be taken, improve the treatment of MDR-TB and provide a more effective treatment of latent TB infection.

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