Bulletin of Advanced Scientific Research 01 [03] (2015) e-ISSN: 2454-3691
www.asdpub.com/index.php/basr

Review Article

Study of Some Analogue of Currently Cilinically used Pyrazinamide Compounds

Mohammad Asif

Department of Pharmacy, GRD (PG) Institute of Management & Technology, 248009, Dehradun, (Uttarakhand), India

***Corresponding Author**
Mohammad Asif
Department of Pharmacy,
GRD (PG) Institute of Management & Technology,
248009, Dehradun, (Uttarakhand), India
E-mail: aasif321@gmail.com

Keywords:
Antitubercular,
Multidrug resistance,
Mycobacterium

Abstract

There has been considerable interest in the development of new compounds with anti mycobacterial activity particularly against multidrug resistance (MDR) and extensively drug resistant (XDR) tuberculosis (TB) because *mycobacterium* species have developed resistant against currently used drugs. The currently use anti-TB agents having toxic effect and long period of therapy. Therefore, many researchers have synthesized various analougues of currently used anti-TB agents for antiTB activity. These observations have been guiding for the development of new agents that possess potent antiTB activity with minimum side effects or effective against MDR, XDR *mycobacterium*, and also in patient co-infected with HIV/AIDS.

1. Introduction

In particular, the emergence of multidrug resistant (MDR) and extensive drug resistance (XDR) has become a serious problem in the treatment of bacterial diseases. Tuberculosis (TB) is a chronic infectious disease caused by *Mycobacterium* of the "tuberculosis complex", including primarily *M. tuberculosis.* Other species *M. bovis, M. africanum*, *M. canetti* and *M. microti* can also cause TB, these species do not usually infect healthy adults. TB is an airborne communicable disease caused by transmission of aerosolized droplets of *M. tuberculosis* (1-5). *Mycobacterium* belongs to the order actinomycetales, family *Mycobacteriaceae*. Several species, including *M. tuberculosis*, *M. bovis*, *M. africanum*, *M. microti*, *M. canetti, M. kansasii*, *M. avium,* and *M. leprae*, are intracellular pathogens of higher vertebrates. The *M. tuberculosis* complex includes three other TB-causing mycobacteria: *M. bovis, M. africanum and M. microti*. The first two only very rarely cause disease in immuno competent people. On the other hand although *M. microti* is not usually pathogenic, it is possible that the prevalence of *M. microti* infections has been under estimated. Other Known pathogenic mycobacteria include *M. leprae, M. avium* and *M. kansasii* (6,7). The last two are part of the non *tuberculous mycobacterium* (NTM) group. The NTM cause neither TB nor leprosy, but they do cause pulmonary diseases resembling TB. TB requires much longer periods of treatment to entirely eliminate *mycobacterium* from the body (8). Therefore, recently attention has focused on the treatment of TB the development of new compounds to deal with against MDR and XDR resistant *mycobacterium* species has become one of the most important areas of anti-TB research today (9-12). Therefore, recent efforts have been directed toward exploring new, potent anti-TB agents with low toxicity profiles when compared with anti-TB agents currently in the market (13,14). Although, there is an increasing resistance to antimicrobial drugs, to overcome the development of drug resistance it is necessary to synthesize new antibacterial agents that possessing different chemical properties as well as less toxic effect from those of used commonly.

2. Tuberculosis an Overview

Tuberculosis (TB) is one of the oldest and most pervasive, respiratory transmitted diseases in history. According World Health Organization (15) report, TB has spread to every corner of the globe. As much as one-third of the world's population is currently infected, more than any other infectious disease (16). It was estimated that nearly 1 billion more people will be infected with TB in the next 20 years (17). The occurrence of TB is linked to dense population, poor sanitation and poor nutrition (18). Direct Observed Treatment, short-course (DOTS) strategy, constitutes the cornerstone of the current protocol for control of TB. However, the three key drugs, isoniazide, pyrazinamide and rifampicin, used in the regimen are potentially hepatotoxic and may lead to drug associated hepatitis. Despite the undoubted success of DOTS strategy, the emergence of MDR resistant strains, recurrently isolated from patient's sputum, darken the future (19). The increase in TB incidence during recent years is largely due to the prevalence of TB is synergy with Human Immunodeficiency Virus (HIV) epidemic, which augments the risk of developing the disease 100-fold and also the emergence of MDR-TB strains. In addition to this, the increase in *M. tuberculosis* strains resistant to front line antiTB drugs such as rifampin and INH has further complicated the problem, which clearly indicates the need for more effective drugs for the efficient management of TB (20-22).

2.1 Need of new Antitubercular Drugs:

A new TB treatment should offer following three improvements over the existing regimens: shorten the total duration of treatment and/or significantly reduce the number of doses, improve the treatment of MDR-TB and XDR-TB, provide a more effective treatment of latent TB infection (23,24). In order to analyse useful to group drug candidates currently in two main categories: 1) Novel chemical entities and 2) Compounds originating from existing families of drugs, where innovative chemistry is used to optimise the compounds. Drug resistance (MDR and XDR) by *M. tuberculosis* is an important obstacle for the treatment and control of TB. The MDR-TB refers to simultaneous resistance to at least two or more of the five first-line anti-TB drugs (isoniazid, rifampicin, pyrazinamide, ethambutol, and streptomycin) (25). Treatment for MDR-TB is long lasting, less effective, costly, and poorly tolerated (26-29). The XDR-TB by definition is resistance to at least isoniazid and rifampicin in addition to any quinolone and at least

one injectable second-line agent (capreomycin, amikacin, kanamycin). The principles of treatment for MDR-TB and XDR-TB are the same. The main difference is that XDR-TB is associated with a much higher mortality rate than MDR-TB, because of reduced number of effective treatment options (30-32). Hence there is an urgent need for novel drugs that are active against *M. tuberculosis* in order to shorten the duration of TB therapy.

2.2 Current chemotherapy of Tuberculosis

Current chemotherapy of TB are mainly depends on first-line anti-TB drugs, which include streptomycin, isoniazid, rifampicin, ethambutol and pyrazinamide, they more effective and less toxic as compare to second-line anti-TB drugs. Second line anti-TB drugs are 5 or 6 classeses of drugs. Second-line drug may be less effective than the first-line drugs or it may have toxic side-effects or. These comprise of different classes namely, aminoglycosides (amikacin, kanamycin), polypeptides (capreomycin, viomycin), fluoroquinolones (ciprofloxacin, moxifloxacin, *etc*), thioamides: (ethionamides, prothioamide), cycloserine and *p*-aminosalicylic acid (1,33).

2.2 Toxic Effects of Currenly Used Antitubercular Agents:

The currently available key medications (first line) used in the regimen are show serious side effects like severe damage to the eighth cranial nerve, inducing irreversible impairment of auditory function, hypersensitivity reactions (streptomycin), potentially hepatotoxic and may lead to drug associated hepatitis (isoniazide, pyrazinamide and rifampicin (rifampicin, rifabutin, rifapentine) and thrombocytopenic purpura (rifampicin) (34-37). Second line anti-tb drugs are more toxic than first line drugs, **a**mikacin and kanamycin causes kidney damage as well as hearing loss, viomycin and capreomycin causes nephrotoxicity and eighth cranial nerve toxicity. Fluoroquinolones (ciprofloxacin, moxifloxacin, ofloxacin (levofloxacin, the chiral form of ofloxacin is more effective), gatifloxacin, trovafloxacin, enofloxacin and sparfloxacin etc). Fluoroquinolones are increasingly contraindicated for patients due to growing prevalence of antibiotic resistance. Ethionamid and prothionamide (structural analogues of isoniazid) causes adverse effects are g.i.t. disorders (anorexia, salivation, nausea, abdominal pain, and diarrhea), mental disturbances (depression, anxiety, psychosis, dizziness, drowsiness, and headache) and hypersensitivity (38-40). Cycloserine causes side effects of this drug are mainly CNS manifestations such as headache, irritability, depression, convulsions. Para amino salicyclic acid causes g.i.t. problems including anorexia, nausea, epigastric pain, abdominal distress, diarrhea, ulcers and hypersensitivity (41,42).

3. Compounds Originating From Existing Drug Pyrazinmide

The five first-line drugs for treatment are highly effective and the rate of severe adverse reactions is low and six classes of second line drugs, it may be less effective than the first-line drugs or it may have more toxic side-effects (43-49). However, unpleasant side effects, relatively long duration of treatment and non-compliance to treatment regimen are drawbacks. Such non-adherence with the course of treatment leads to treatment failure and the development of drug resistance. The second line drugs used for MDR-TB are more expensive, less effective and more toxic than the five drug standard regimen. The goal now is to develop bactericidal drugs in a cost-effective manner, which efficaciously treats infectious MDR/XDR strains of *M. tuberculosis* and latent infections with shortened treatment periods as well as reduced frequency of dosage. Some of recently discovered first line drug analogues as anti-Tb agents are discussed below (50-52).

3.1 Pyrazinamide (Aldinamide):

Pyrazinamide (**1**) is the synthetic pyrazine analog of nicotinamide. Pyrazinamide exhibits bactericidal activity *in vitro* only at a slightly acidic pH. Activity at acid pH is ideal, since *M. tuberculosis* resides in an acidic phagosome within the macrophage. Tubercle bacilli within monocytes *in vitro* are inhibited or killed by the drug at a concentration of 12.5µg/ml. Resistance develops rapidly if PZA is used alone. The target of pyrazinamide appears to be the mycobacterial fatty acid synthase I gene involved in mycolic acid biosynthesis (53-57).

Pyrazine analog of nicotinamide and mechanism suspected to be similar to isoniazid based mostly on structural similarity, not direct evidence. It also has to be metabolically activated. PZA-resistant strains of M. tuberculosis have a mutation in the hydrolase gene. Pyrazinamide (**I**) is indicated for the initial treatment of active tuberculosis in adults and children when combined with other anti-TB agents. PZA is an important sterilising tuberculosis drug that helps to shorten the duration of current chemotherapy regimens for tuberculosis. It is unique among antituberculosis drugs in having no genomic site of action and having greater bactericidal activity as bacillary metabolism slows down; it is remarkably effective in human disease. PZA is an important component in the intensive phase of short-course treatment of TB owing to its sterilising activity, ability to work acidic environments (in macrophages), and excellent synergy with RIF (58,59). Pyrazinamide appears to kill at least 95% of the semi-dormant bacterial population persisting in a low-pH environment since its activity is present only in the acidic environment found in active inflammation (60). The development of a new drug with a similar mode of activity might be very fruitful, especially if there were no need for an acid environment (61). Objectives for Understanding TB drug development are: (*i*) to shorten the total duration of effective treatment and/or significantly reduce the total number of doses needed to be taken under directly observed treatment, short-course (DOTS) supervision, (*ii*) to improve the treatment of MDR-TB, which cannot be treated with INH and RMP, and (*iii*) to provide a more effective treatment of latent TB infection (LTBI). Genomics, the systematic identification of all of the genes in a cell through deoxyribonucleic acid (DNA) sequencing and bioinformatic analysis, also offers great potential in terms of drug target discovery and development of new antibacterial agents, and the recently sequenced genome of *M. tuberculosis* should provide a number of new targets for novel drugs.

Pyrazinamide is well absorbed from the gastrointestinal tract and widely distributed throughout the body. The drug is excreted primarily by renal glomerular filtration. Pyrazinamide is distributed widely-including to the CNS, lungs, and liver after oral administration. Penetration of the drug into the CSF is excellent. Pyrazinamide is hydrolyzed to pyrazinoic acid and subsequently hydroxylated to 5-hydroxypyrazinoic acid, the major excretory product. Pyrazinamide has become an important component of short-term (6-month) multiple-drug therapy of tuberculosis.

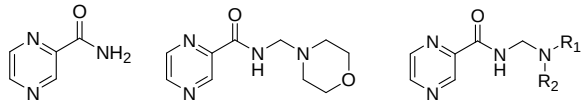
3.2 Untoward Effects:

Injury to the liver is the most serious side effect of pyrazinamide such as hepatic disease, jaundice, hepatic necrosis. Elevations of plasma alanine and aspartate aminotransferases are the earliest abnormalities produced by the drug. The drug inhibits excretion of urate, resulting in hyperuricemia in nearly all patients; acute episodes of gout have occurred. Other untoward effects that have been observed with pyrazinamide are arthralgias, anorexia, nausea and vomiting, dysuria, malaise, and fever. While some international organizations recommend the use of pyrazinamide in pregnancy, this is not the case in the United States because of inadequate data on teratogenicity (62-67).

3.3 Modifications of PZA structure:

The PZA and pyrazinoic acid (POA) as potential analeptic drugs and intermediate compounds on the pathway of aminopyrazine synthesis (68), but the anti-TB activity of PZA was reported later, in 1952. The use of nicotinamide-related compounds for the therapy of TB followed the unexpected observation that nicotinamide was effective for the treatment of murine TB. The initial information that nicotinamide possessed modest anti-TB activity stimulated the evaluation of other

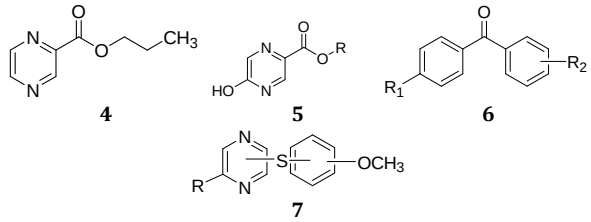
derivatives. This observation led to the subsequent discovery of not only PZA but also INH (69) and ethionamide. Of many nicotinamide analogues that were subsequently evaluated for anti-TB activity, only PZA was active *in vivo* (70-72). In the same year (73) the synthesis of 49 PZA derivatives and 14 related compounds was reported, and their anti-TB activities in the *in vivo* murine system were briefly referred to, but no information on their *in vitro* anti-TB activities was provided. It was soon shown that an enzyme called nicotinamidase inside the mycobacterium hydrolyzed nicotinamide and pyrazinamide to the corresponding carboxylic acids, and these carboxylic acids were the actual active compounds (74). However, nicotinic and pyrazinoic acids did not demonstrate activity due to their poor penetration into mycobacterial cells. PZA is still unusual because of its narrow spectrum of activity. Several aminomethylene analogues/prodrugs of PZA were prepared and evaluated including morphazinamide (**2**), which is a morpholine derivative, Mannich base of PZA (75); the lack of any demonstrable improvement over PZA *in vivo* led to its abandonment. Surprisingly, it was found that amides of 5-substituted pyrazinoic acid displayed good activity against *M. avium*, PZA-resistant *M. tuberculosis*, and phagocytized *M. tuberculosis*, and these compounds also showed activity over a broad range of pH values (76). *M. avium* became a serious cause of disseminated infection among patients with AIDS. Novel aminomethylene analogues of PZA (**3**) were tested (77). The activity of *N*-(pyrrolidin-1-ylmethyl)pyrazine-2- carboxamide was higher than that of PZA when administered with rifalazil or RIF in a mouse model of infection. The activity of these analogues against PZA-resistant strains suggests that development of the second generation PZA analogues may be especially fruitful.



1 **2** **3**

Fig. 1: Structures of Pyridazine and aminomethylene analogues of Pyridazine

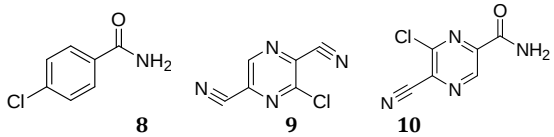
PZA exhibits remarkably stringent structure-activity relationships (SAR) demonstrating an absolute requirement for the pyrazine nucleus and the carboxamide moiety in position 2 for the activity. The modifications of the pyrazine ring with pyridazine and/or pyrimidine were not successful (78). It can be concluded that any isosteric replacement of carbon by nitrogen or shift of nitrogen to another position results in the loss of antimycobacterial activity. Also *ortho*-condensation of the aromatic/heteroaromatic ring with pyrazine nucleus did not lead to active compounds. Similarly, the substitution of the carboxamide group in thioamide, *N*-methyl, *N*-acetyl, hydrazide (analogy with INH), nitrile, tetrazole and free carboxylic acid provided compounds that were completely inactive *in vivo* (73). One of the effective methods that can lead to new drug discovery is the bioisosteric replacement of a functional group. Numerous functional groups have been reported as bioisosteric replacements for the carboxylic acid functionality (79). The prodrug approach in series of PZA derivatives is already very hopeful. Substituted pyrazinecarboxylic acid esters have been previously reported to have *in vitro* activity against *M. avium* and *M. kansasii* as well as *M. tuberculosis*. Modification of both the pyrazine nucleus and the ester functionality was successful in expanding the anti-TB activity associated with PZA to include *M. avium* and *M. kansasii*, organisms usually not susceptible to pyrazinamide. In an attempt to understand the relationship between the activities of the esters and the needed biostability, quantitative structure-activity relationships (QSAR) were found (80). While POA cannot pass through mycobacterial cell walls due to its low lipophilicity, the esterification of POA is a suitable approach to increase the likelihood of its penetration into the resistant mycobacteria (81,82). Thus series of POA esters were prepared and evaluated. PZA-resistant isolates became susceptible *in vitro* to pyrazinoic acid, and *n*-propyl pyrazinoate was the most promising candidate (**4**). Esters of POA appeared to circumvent the requirement for activation by mycobacterial amidase. The MICs of *n*-propyl pyrazinoate for *M. tuberculosis* isolates were lower than those of pyrazinoic acid. This may lead to a candidate compound with enhanced activity against both PZA-susceptible and PZA-resistant *M. tuberculosis* isolates suitable for clinical development (82,83). However, efficacy studies in mice failed to show any anti-TB activity likely due to poor stability of the esters in plasma. Another series of more lipophilic ester prodrugs (*i.e.* tetradecyl ester) were found to be active in concentrations 10-fold lower than those needed for PZA to kill sensitive *M. tuberculosis* and also have suitable stability in the presence of plasma (84). These relationships are consistent with the observation that *tert*-butyl 5-chloropyrazine-2-carboxylate and 2-methyldecyl 5-chloropyrazine-2-carboxylate are 100-fold more active than PZA against *M. tuberculosis* and exhibit serum stability 900-1000 times greater than the lead compounds in the series. Some 5-hydroxypyrazine-2-carboxylic acid derivatives (**5**) are up to 1000-fold more active against *M. tuberculosis* and other *Mycobacterium* strains than existing antituberculous agents (85); synthesis of compounds **5** is important, because it is a building block for the synthesis of new anti-TB agents. 5-Hydroxypyrazine-2-carboxylic acid can be produced microbiologically by whole-cell biotransformation of 2-cyanopyrazine (86). Various substituted analogues of PZA with bioisosteric replacements of the carboxylic acid functionality were prepared. Thus 5-aroylpyrazine-2-carboxylic acid **6**, (87) and arylsulfanyl pyrazinecarboxylic acid (**7**), (88) derivatives were investigated as potential anti-TB agents.



4 **5** **6** **7**

Fig. 2 Structures of pyrazine-2-carboxylic acid derivatives.

In order to find more active PZA derivatives, various PZA analogues were synthesized and assayed against *M. tuberculosis* (89). In these experiments, four compounds showed high levels of anti-TB activities, not only bacteriostatic but also bactericidal, against *M. tuberculosis* as well as *M. avium* complex (MAC). These compounds, namely, pyrazinoic acid pivaloyloxymethyl ester, pyrazinoic acid *n*-octyl ester, pyrazinethiocarboxamide and *N*-hydroxymethylpyrazinethiocarboxamide, may warrant further examinations. 5-Chloropyrazine-2-carboxamide (**8**) showed excellent *in vitro* activity against PZA-resistant strains of *M. tuberculosis* (90). Therefore FAS I and/or FAS II were proposed as a target of this compound, *i.e.* this compound possesses a different mechanism of action (91). Due to this fact 3-chloropyrazine-2,5-dicarbonitrile (**9**), and 6-chloro-5-cyanopyrazine-2-carboxamide (**10**), (92,93) and their derivatives were synthesized, and their noteworthy anti-TB activities were reported recently.



8 **9** **10**

Fig. 3 Structures of chloropyrazine derivatives.

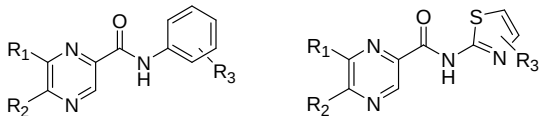
3.4 Anti-*Mycobacterium tuberculosis* bioassays

Several *in vitro* bioassays have been developed to evaluate antitubercular activity of chemical compounds. In most of these methods, *Mycobacterium* is cultured in various types of broth- and agar-based media. But, the main problems are long growth time (several weeks) and its pathogenicity, hence containment facilities are required.

The common conditions for the anti-TB susceptibility evaluation are influenced by acid-base properties of medium (94). For nearly two decades the radiometric BACTEC 460 TB System provided the most rapid method for antimicrobial susceptibility testing (95).

***3.5 In vitro* bioassays**

The primary screening was conducted at 6.25μg/mL against *M. tuberculosis* H37Rv (ATCC 27294) in BACTEC 12B medium using the Microplate Alamar Blue Assay (MABA). In general, compounds effecting >90% inhibition in the primary screening (MIC <6.25μg/mL) were further evaluated. The initial screening was conducted against *M. tuberculosis* H37Rv (ATCC 27294) in BACTEC 12B medium using the MABA (96). The following was synthesized in preference: (*i*) compounds with lipophilic and/or electron-withdrawing substituents on the benzene moiety (R_3), (*ii*) compounds with hydrophilic and/or electron-donating groups on the benzene part of the molecule (R_3), and finally (*iii*) compounds with a lipophilic alkyl chain (R_2), *i.e. tert*-butyl (-$C(CH_3)_3$) and/or halogen (chlorine) substitution (R_1) on the pyrazine nucleus (97).



11 **12**

Fig. 4 Structure of substituted *N*-phenylpyrazine-2-carboxamides (11) and substituted *N*-(thiazol-2-yl)pyrazine-2-carboxamides (12).

4. Discussions

In the recent past, drug discovery efforts shifted towards the drug design based on docking studies. These docking computational techniques allow investigating the possible binding modes of a substrate to a given receptor, enzyme or another binding site and consequently determining and identifying the precise or different mechanism of action of both PZA and its derivatives, e.g. 5-chloropyrazine-2-carboxamide (**8**) and similar compounds. Therefore the priority is to isolate the enzymes (from *M. tuberculosis* and subsequently from cells of other *Mycobacterial* strains) responsible for metabolization/activation of PZA and other enzymes that can be influenced by POA generation, e.g. FAS I, FAS II, etc. After isolation and determination of 3D structure by X-Ray structural analysis, it will be needed to crystallize enzymes with PZA and other PZA derivatives, determine 3D structures of these complexes and develop 3D-pharmacophore for systematic virtual screening based on this process. Then it will be possible to evaluate all PZA-like derivatives based on their virtual binding simulation and to carry out their evaluation using a large set of distance-based topological indices. In addition, various molecular descriptors can be used. As most pyrazine derivatives seem to be prodrugs and their activity is strongly dependent on the rate of hydrolysis to POA, further *in vitro* experiments with isolated enzymes should be focused on determination of the hydrolysis rate. Also compounds of interest will be subject to *in vivo* studies for determination of their efficacy against murine *M. tuberculosis* (98-100). PZA is a cornerstone drug of current TB therapy and emerged as an important building block for regimens with promise to shorten TB treatment. PZA is a prodrug which must be activated by the *M. tuberculosis* enzyme pyrazinamidase within the bacterium in order to exert its anti-TB activity. The project was focused on discovery of PZA analogues with PZA-like efficiency characteristics along with improved potency and increased safety. The main task was search for new anti-TB pyrazines-structure analogues of PZA (101-103). Biological evaluation comprised of anti-TB activity screening as the main task. The compounds relieved into level 2 testing underwent MIC and CC50 determination followed by Selectivity Index calculation (SI, ratio of measured CC50 to MIC). To be relieved to level 3 (*in vivo* screening) the compound had to exhibit SI>10. The *in vivo* screening of compound **1** was not finished yet. The results of this project could be a very good starting point for the advanced drug design and development of new anti-TB agents based on pyrazine. In view of the persistent drug-resistant TB problem of currently used anti-TB drugs, it is important that new anti-TB molecules or drugs should address different targets, as those of currently used drugs including the shortening of TB therapy. The unique structure of the mycobacterial cell wall makes it a useful target for drug development (104,105). The identification of novel target sites will also be needed to circumvent the problems associated with the increasing occurrence of MDR and XDR strains (106-108). Many unique metabolic processes occur during the biosynthesis of mycobacterial cell wall components. For example, one of these attractive targets for the rational design of new antitubercular agents are the mycolic acids, the major components of the cell wall of *M. tuberculosis* (109-112). From the chemotherapeutic point of view, there are two sources of new chemical entities. The first is the extraordinary diversity provided by new molecules. The second results from the design of new or the modernization of synthetic transformations.

5. Conclusion

Tuberculosis remains a leading infectious killer worldwide. Development of new anti-TB drugs is the need to control TB. In the last fourty years no new compound has been brought to the market for the treatment of TB. However, in recent years there is an enhanced activity in the research and development of new drugs for TB. Some compounds are presently in clinical development, while others are being investigated pre-clinically in an attempt to explore new molecules for the target based treatment of TB. Simultaneously some new targets are being identified and validated for their practical usefulness. This is mainly due to the lack of new drugs, particularly effective against the MDR and XDR strains, and patients co-infected with HIV/AIDS. Therefore, there is an urgent need of new anti-TB drugs with lesser side-effects, improved pharmacokinetics and effective against MDR and XDR bacterial strains with reduce the overall duration of treatment. Precisely, it is imperative to develop smart new drugs that inhibit novel targets that are structurally and functionally different from those currently known. Medicinal chemists will be interested to working on new compounds. In view of above facts, various new drugs will be synthesized in the future for development of new effective molecule.

References

[1] Kamal A, Azeeza S, Malik MS, Shaik AA, Rao MV. Efforts Towards the Development of New Antitubercular Agents: Potential for Thiolactomycin Based Compounds. *J Pharm Pharmaceut Sci*, 2008, 11 (2): 56s-80s.

[2] Cole ST, Alzari PM. Towards new tuberculosis drugs. *Biochemical Society Transactions*, 2007, 35(5): 1321-1324.

[3] Dye C. Global epidemiology of tuberculosis. *Lancet*, 2006, 367(9514): 938-40.

[4] Zhang Y, Post-Martens K, Denkin S. New drug candidates and therapeutic targets for tuberculosis therapy. *Drug Discovery Today*, 2006, 11(1), 21-27.

[5] Duncan K. Identification and validation of novel drug targets in tuberculosis. *Current Pharm Design*, 2004, 10(26), 3185-94.

[6] Nahid P, Pai M, Hopewell PC. Advances in the diagnosis and treatment of tuberculosis. *Proc Am Thorac Soc,* 2006, 3: 103-10.

[7] Nguyen L, Thompson CJ. Foundations of antibiotic resistance in bacterial physiology: the mycobacterial paradigm. *Trends Microbiol,* 2006, 14: 304-12.

[8] Tripathi RP, Tewari N, Dwivedi N, Tiwari VK. Fighting tuberculosis: An old disease with new challenges. *Med Res Rev.* 2005, 25: 93-131.

[9] Amalio T, Michael I. Drug-Resistant Tuberculosis: What Do We Do Now? *Drugs,* 2000, 59: 171-179.

[10] Espinal MA. The global situation of MDR-TB. *Tuberculosis,* 2003, 83: 44-51.

[11] Flynn JL, Chan J. Tuberculosis: latency and reactivation. Infect Immun, 2001, 69, 4195-4201.

[12] Frieden TR, Sterling TR, Munsiff SS, Watt CJ, Dye C. Tuberculosis. *Lancet*. 2003, *362,* 887–899.

[13] Mayekar NA, Yathirajan SH, Narayana B, Sarojini BK, Suchetha KN. Synthesis and antimicrobial studies on new substituted 1,3,4-oxadiazole derivatives bearing 6-bromonaphthalene moiety. *Int J Chem*, 2010, 2(1): 38–54.

[14] Surendra SB, Arya A, Sudhir KS, Vinita C, Rama PT. Synthesis and antitubercular activity of 5-benzyl-3-phenyl dihydroisoxa-zole. *Int J Drug Des Discov,* 2010, 1(1):11–18.

[15] *Global Tuberculosis Control*: *WHO Report 2010* (2010). pp.1–42, WHO Press, ISBN 978-92-4-156406-9, Geneva, [cited 2011 July22], Available form: http://whqlibdoc. who.int/publications/2010/ 9789241564069_eng.pdf.

[16] Okada M, Kobayashi K. Recent progress in mycobacteriology, *Kekkaku*, 2007, 82(10): 783-799.

[17] World Health Organisation, Tuberculosis, Fact Sheet No. 104, 2007. http:// www.who.int/mediacentre/factsheets/who104/en/index.html/.

[18] Dony JF, Ahmad J, Khen TY. Epidemiology of tuberculosis and leprosy, Sabah, Malaysia. *Tuberculosis*, 2004, 84: 8-18.

[19] Omar A, Ahmed MA. Synthesis of Some New 3H-quinazolin-4-One Derivatives as Potential Antitubercular Agents: *World App Sci J,* 2008, 5 (1): 94-99.

[20] Sunduru N, Sharma M, Chauhan PMS. Recent advances in the design and synthesis of Heterocycles as anti-tubercular agents. *Fut. Med. Chem.*, 2010, 2(9): 1469-1500.

[21] Vinšová J, Krátký M. Tuberculosis-The development of new MDR-TB drugs, In: *Drug-Resistant Tuberculosis, Causes, Diagnosis and Treatments*, Nguy, S. & K'ung, Z. (Eds.), 2010, pp.59-141, Nova Science Publishers, ISBN 978-1-60876-055-8, New York.

[22] Wade MM, Zhang Y. Mechanism of drug resistance in *Mycobacterium tuberculosis*. *Frontiers in Bioscience*, 2004, 9(1), 975-994.

[23] Global Alliance for TBdrug development. Tuberculosis. Scientific blueprint for tuberculosis drug development. Tuberculosis (Edinb), 2001, 81 Suppl 1, 1-52.

[24] O'Brien RJ, Nunn PP. The need for new drugs against tuberculosis. Obstacles, opportunities, and next steps. *Am J Respir Crit Care Med,* 2001, 163, 1055-1058.

[25] Barry CE, Slayden RA, Sampson AE, Lee RE. Use of genomics and combinatorial chemistry in the development of new antimycobacterial drugs. *Biochem & Pharmacol*, 2000, 59: 221-231.

[26] Global Tuberculosis Control: Surveillance, Planning, Financing WHO Report, 2008; 51-54.

[27] Rivers EC, Mancera RL. New anti-tuberculosis drugs in clinical trials with novel mechanisms of action. *Drug Discovery Today,* 2008, 13, 1090-1098.

[28] Sarkar S, Suresh MR. An overview of tuberculosis chemotherapy-a literature review. *J Pharm and Pharm Sci*, 2001, 14(2), 148-161.

[29] Shi R, Sugawara I. Development of new anti-tuberculosis drug candidates. *The Tohoku J exper med,* 2010, 22(2), 97-106.

[30] Ruiz-Serrano MJ, Alcala L, Martinez L, Diaz M, Marin M, Gonzalez-Abad MJ, Bouza E. *In vitro* activities of six fluoroquinolones against 250 clinical isolates of Mycobacterium tuberculosis susceptible or resistant to first-line antituberculosis drugs. *Antimicrob Agents Chemother,* 2000, 44, 2567-2568.

[31] Smith CV, Sharma V, Sacchettini JC. TB drug discovery: Addressing issues of persistence and resistance. *Tuberculosis*. 2004, 84, 45–55.

[32] Russell DG. *Mycobacterium tuberculosis*: here today, and here tomorrow. *Nat Rev Mol Cell Biol,* 2001, 2, 569-577.

[33] Center for Disease Control. "Emergence of Mycobacterium tuberculosis with Extensive Resistance to Second-Line Drugs—Worldwide, 2000–2004". 2006, 55: 301–305.

[34] Rajini RS, Aparna P, Sasikala CH, Ramana, CH V. Microbial metabolism of pyrazines. *Critical Rev Microbiol*, 2011, 37(2), 99-112.

[35] Palomino JC, Ramos DF, da Silva PA. New anti-tuberculosis drugs: strategies, sources and new molecules. *Curr Med Chem*, 2009, 16(15): 1898-1904.

[36] Mdluli K, Spigelman M. Novel targets for tuberculosis drug discovery. *Current Opinion Pharmacol,* 2006, 6(5): 459-467.

[37] Glickman SW, Rasiel EB., Hamilton CD, Kubataev A, Schulman KA. Medicine. A portfolio model of drug development for tuberculosis. *Science,* 2006, 311, 1246-1247.

[38] Awwadi FF, Landee CHP, Turnbull MM, Twamley B, Wells BM. Low-dimensional quantum magnetic systems: Synthesis, structure and magnetic behavior of (2,5-dimethylpyrazine)copper(ii) chloride and synthesis and magnetic behavior of bis (2,6-dimethylpyrazine)copper(ii) chloride. *Polyhedron*, 2005, 24(16-17): 2153-2159.

[39] Banfi E, Mamolo MG, Zampieri D, Vio L, Bragadin CM. Antimycobacterial activity of *N*1-{1-[3-aryl-1-(pyridin-2-,3- or 4-yl)-3-oxo] propyl}-2-pyridinecarboxamidrazones. *J Antimicrob Chemother,* 2001, 48(5), 705-707.

[40] Gezginci MH, Martin AR, Franzblau SG. Antimycobacterial Activity of Substituted Isosteres of Pyridine- and Pyrazinecarboxylic Acids. *J. Med. Chem,* 2001, 44(10), 1560-1563.

[41] Janin YL Antituberculosis drugs: Ten years of research. *Bioorg. Med. Chem*. 2007, 15: 2479–2513.

[42] Rieder HL, Arnadottir T, Trebucq A, Enarson DA. Tuberculosis treatment: dangerous regimens? *Inter J Tuberculosis & Lung Dis.* 2001, 5: 1–3.

[43] Yamaguchi T. The relationship between the chemical structures of dihydropyrazine derivatives and DNA strand-breakage activity. *Chemical Pharmaceutical Bulletin*, 2007, 55(4), 532-536.

[44] Wade MM, Zhang Y. Effects of weak acids, UV and proton motive inhibitors on pyrazinamide activity against *Mycobacterium tuberculosis in vitro*. *J Antimicro Chemother*, 2006, 58(5), 936-941.

[45] WHO. Global tuberculosis control-surveillance, planning, financing (Geneva: World Health Organization), 2005.

[46] Vargas F, Rivas C, Diaz Y, Fuentes A. Photodegradation pathways and the *in vitro* phototoxicity of pyrazinamide, a phototoxic antitubercular drug. *J Photochem & Photobiol B- Biology*, 2003, 72(1-3), 87-94.

[47] Wagner R, Czerny M, Bielohradsky J, Grosch W. Structure-odour-activity relationships of alkylpyrazines. *Zeitschrift für Lebensmitteluntersuchung und-Forschung A*, 1999, 208(5-6): 308-316.

[48] Zhang Y, Scorpio A, Nikaido H, Sun Z. Role of acid pH and deficient efflux of pyrazinoic acid in unique susceptibility of *M. tuberculosis* to pyrazinamide. *J Bacteriol*, 1999, 181(7):2044–2049.

[49] Woolfson A, Rothschild M. Speculating about pyrazines. *Proceedings of the Royal Society of London Series B-Biological Sciences*, 1990, 242(1304), 113-119.

[50] Mantu D, Luca MC, Moldoveanu C, Zbancioc G, Mangalagiu II. Synthesis and antituberculosis activity of some new pyridazine derivatives. Part II. *Eur J Med Chem,* 2010, 45(11): 5164-5168.

[51] Müller R, Rappert S. Pyrazines: Occurrence, formation and biodegradation. *Applied Microbiol & Biotechnol*, 2010, 85(5): 1315-1320.

[52] Ngo SC, Zimhony O, Chung WJ, Sayahi H, Jacobs WR Jr, Welch JT. Inhibition of isolated *Mycobacterium tuberculosis* fatty acid synthase I by pyrazinamide analogs. *Antimicro Agents & Chemother*, 2007, 51(7): 2430-2435.

[53] Somoskovi A, Parsons LM, Salfinger M. The molecular basis of resistance to Isoniazid, Rifampin, and Pyrazinamide in *Mycobacterium tuberculosis*. *Respir*. *Res*. 2001, *2*: 164–168.

[54] Zimhony O, Cox JS, Welch JT, Vilcheze C, Jacobs WR, Jr. Pyrazinamide inhibits the eukaryotic-like fatty acid synthetase I (FASI) of *Mycobacterium tuberculosis. Nat. Med.,* 2000, *6:* 1043-1047.

[55] Scorpio A, Zhang Y. Mutations in PncA, a gene encoding pyrazinamidase/nicotinamidase, cause resistance to the antituberculous drug pyrazinamide in tubercle bacillus. *Nature Medicine*, 1996, 2(6), 662-667.

[56] Steele MA, Des Prez, RM. The role of pyrazinamide in tuberculosis chemotherapy. *Chest*, 1988, 94(4), 845-850.

[57] Tarnok I, Pechmann H, Krallmann Wenzel, U, Rohrscheidt E, Tarnok Z. Nicotinamidase and the so-called pyrazinamidase in mycobacteria; the simultaneous occurrence of both activities. *Zentralblatt für Bakteriologie*, 1979, 244(2-3), 302–308.

[58] Mitchison DA. The action of antituberculosis drugs in short-course chemotherapy. *Tubercle*, 1985, 66(3): 219-225.

[59] Zhang Y, Mitchison DA. The curious characteristics of pyrazinamide: A review. *Inter J Tuberculosis & Lung Dis*, 2003, 7(1), 6-21.

[60] Heifets L, Lindholm-Levy, P. Pyrazinamide sterilizing activity *in vitro* against semidormant *Mycobacterium tuberculosis* bacterial populations. *Am Rev Resp Dis*, 1992, 145(5), 1223–1225.

[61] Aldrich CC, Bosshoff HI, Remmel RP. Antitubercular agents, In: *Burger's Medicinal Chemistry, Drug Discovery and Development*, vol. 7 *Antiinfectives*, Abraham, Understanding Tuberculosi-D. J. & Rotella, D. P. (Eds.), 7th ed., 2010, pp.713–812, Wiley, ISBN 978-0-470-27815-4, New York.

[62] Grosset J, Truffot-Pernot C, Lacroix C, Ji B. Antagonism between isoniazid and the combination pyrazinamide-rifampin against tuberculosis infection in mice. *Antimicrob Agents Chemother*, 1992, 36, 548-551.

[63] Maga JA. Pyrazine update. *Food Reviews International*, 1992, 8(4), 479-558.

[64] Edelman MJ, Meyers FJ, Grennan T, Lauder J, Doroshow J. Phase II trial of pyrazine diazohydroxide in androgen-independent prostate cancer. *Investigational New Drugs*, 1998, 16(2): 179-182.

[65] McCullough KJ. Pyrazines and related ring structures. In: *Rodd's Chemistry of Carbon Compounds*, Coffey, S. & Ansell, M. F. (Eds.), 1989, pp.294-296, Elsevier Science, ISBN 0444421831, New York

[66] Ambrogi V, Cozzi P, Sanjust P, Bertone L, Lovisolo PP, Briaticovangosa G, Angelucci R. Anti-lipolytic activity of a series of pyrazine-*N*-oxides. *Eur J Med Chem*, 1980, 15(2), 157-163.

[67] Grosset J. The sterilizing value of rifampicin and pyrazinamide in experimental short-course chemotherapy. *Bulletin of the International Union against Tuberculosis*, 1978, 53(1), 5-12.

[68] Hall SA, Spoerri PE. Syntheses in the pyrazine series. II. Preparation and properties of aminopyrazine. *J Am Chem Soc*, 1940, 62(3):664-665.

[69] Vinšová J, Imramovský A, Jampílek J, Monreal-Férriz J, Doležal M. Recent advances on isoniazide derivatives. *Anti-Infective Agents in Med Chem*, 2008, 7(1): 12-31.

[70] Yeager RL, Munroe WGC, Dessau FI. Pyrazinamide (aldinamide) in the treatment of pulmonary tuberculosis. *Am Rev Tuberculosis*, 1952, 65(5): 523–545.

[71] Malone L, Schurr A, Lindh H, McKenzie D, Kiser JS, Williams JH. The effect of pyrazinamide (aldinamide) on experimental tuberculosis in mice. *Am Rev Tuberculosis*, 1952, 65(5), 511–518.

[72] Solotorovsky M, Gregory FJ, Ironson EJ, Bugie EJ, O'Neill RC, Pfister K. Pyrazinoic acid amide; an agent active against experimental murine tuberculosis. *Proceed Soc for Exp Biol & Med*, 1952, 79(4), 563-565.

[73] Kushner S, Dalalian H, Sanjurjo JL, Bach FL Jr, Safir SR, Smith VKJr, Williams JH. Experimental chemotherapy of tuberculosis. 2. The synthesis of pyrazinamides and related compounds. *J Am Chem Soc*, 1952, 74(14), 3617–3621.

[74] Konno K, Feldmann FM, McDermott W. Pyrazinamide susceptibility and amidase activity of tubercle bacilli. *Am Rev Resp Dis*, 1967, 95(3): 461-469.

[75] Felder E, Tiepolo U. *Preparation from pyrazinamide, morpholine, and formalin*, 1962, DE 1129492.

[76] Heifets L, Iseman MD, Crowle AJ, Lindholm-Levy PJ. Pyrazinamide is not active *in vitro* against *Mycobacterium avium* complex. *Am Rev Resp Dis*, 1986, 134(6): 1287-1288,

[77] Chung WJ, Kornilov A, Brodsky BH, Higgins M, Sanchez T, Heifets LB, Cynamon M, Welch J. Inhibition of *M. tuberculosis in vitro* in monocytes and in mice by aminomethylene pyrazinamide analogs. *Tuberculosis*, 2008, 88(5): 410-419.

[78] Rodriguez JC, Ruiz M, Climent A, Royo G. In vitro activity of four fluoroquinolones against *Mycobacterium tuberculosis*. *Int J Antimicrob Agents*, 2001, 17, 229-231.

[79] Thornber CW. Isosterism and molecular modification in drug design. *Chem Soc Rev*, 1979, 8(4): 563-580.

[80] Fernandes JPS, Pasqualoto KFM, Felli VMA, Ferreira EI, Brandt CA. QSAR Modeling of a set of pyrazinoate esters as antituberculosis prodrugs. *Archiv der Pharmazie–Chem Life Sci*, 2010, 343(2), 91-97.

[81] Cynamon MH, Gimi R, Gyenes F, Sharpe CA, Bergmann KE, Han HJ, Gregor LB, Rapolu R, Luciano G, Welch JT. Pyrazinoic acid esters with broad spectrum *in vitro* antimycobacterial activity. *J Med Chem*, 1995, 38(20), 3902–3907.

[82] Cynamon MH, Klemens SP, Chou TS, Gimi RH, Welch JT. Antimycobacterial activity of a series of pyrazinoic acid-esters. *J Med Chem*, 1992, 35(7), 1212-1215.

[83] Speirs RJ, Welch JT, Cynamon MH. Activity of *n*-propyl pyrazinoate against pyrazinamide-resistant *Mycobacterium tuberculosis*: Investigations into mechanism of action of and mechanism of resistance to pyrazinamide. *Antimicrob Agents & Chemother*, 1995, 39(6): 1269-1271.

[84] Simoes MF, Valente E, Gómez JRM, Anes E, Constantino L. Lipophilic pyrazinoic acid amide and ester prodrugs stability, activation and activity against *M. tuberculosis*. *Eur J Pharm Sci*, 2009, 37(3-4), 257-263.

[85] Bergmann K E, Cynamon MH, Welch JT. Quantitative structure-activity relationships for the *in vitro* antimycobacterial activity of pyrazinoic acid esters. *J Med Chem*, 1996, 39(17): 3394-3400.

[86] Wieser M, Heinzmann K, Kiener A. Bioconversion of 2-cyanopyrazine to 5-hydroxypyrazine-2-carboxylic acid with *Agrobacterium* sp. DSM 6336. *App Microbiol & Biotechnol*, 1997, 48(2), 174-176.

[87] Doležal M, Jampílek J, Osička Z, Kuneš J, Buchta V, Víchová P. Substituted 5-aroylpyrazine-2-carboxylic acid derivatives: Synthesis and biological activity. *Farmaco*, 2003, 58(11): 1105-1111.

[88] Jampílek J, Doležal M, Buchta V. Antimicrobial evaluation of some arylsulfanylpyrazine carboxylic acid derivatives. *Med Chem*, 2007, 3(3): 277-280,

[89] Yamamoto S, Toida I, Watanabe N, Ura T. *In vitro* antimycobacterial activities of pyrazinamide analogs. *Antimicrob Agents & Chemother*, 1995, 39(9), 2088-2091.

[90] Cynamon MH, Speirs RJ, Welch JT. *In vitro* antimycobacterial activity of 5-chloropyrazinamide. *Antimicrob Agents & Chemother*, 1998, 42(2), 462-463.

[91] Boshoff HI, Mizrahi V, Barry CE. Effects of pyrazinamide on fatty acid synthesis by whole mycobacterial cells and purified fatty acid synthase I. *J Bacteriol*, 2002,184(8): 2167-2172.

[92] Dlabal K, Palat K, Lycka A, Odlerova Z. Synthesis and proton and carbon-13 NMR spectra of sulfur derivatives of pyrazine derived from amidation product of 2-chloropyrazine and 6-chloro-2-pyrazinecarbonitrile. Tuberculostatic activity. *Collection of Czechoslovak Chemical Communications*, 1990, 55(10), 2493-2501.

[93] Zitko J, Dolezal M, Svobodová M, Vejsová M, Kuneš J, Kučera R, Jílek P. Synthesis and antimycobacterial properties of *N*-substituted 6-amino-5- cyanopyrazine-2-carboxamides. *Bioorg & Med Chem*, 2011, 19(4): 1471-1476.

[94] McDermott W, Tompsett R. Activation of pyrazinamide and nicotinamide in acidic environment *in vitro*. *Am Rev Tuberculosis*, 1954, 70(4): 748-754.

[95] Siddiqi SH. Antimicrobial susceptibility testing: Radiometric (BACTEC) tests for slowly growing mycobacteria, In: *Clinical Microbiology Procedure Handbook*, Isenberg, H. D. (Ed.), 1992, pp.14-25.

[96] Collins LA, Franzblau SG. Microplate alamar blue assay versus BACTEC 460 system for high-throughput screening of compounds against *Mycobacterium tuberculosis* and *Mycobacterium avium*. *Antimicrob Agents & Chemother*, 1997, 41(5), 1004–1009.

[97] Topliss JG, Costello RJ. Chance correlations in structure–activity studies using multiple regression analysis. *J Med Chem*, 1972, 15(10), 1066-1068.

[98] Dolezal M, Cmedlová P, Palek L, Vinšová J, Kuneš J, Buchta V, Jampílek J, Kráľová K. Synthesis and antimycobacterial evaluation of substituted pyrazinecarboxamides. *Eur J Med Chem*, 2008, 43(5), 1105-1113.

[99] Doležal M, Zitko J, Kešetovičová D, Kuneš J, Svobodová M. Substituted *N*-phenylpyrazine-2-carboxamides: Synthesis and antimycobacterial evaluation. *Molecules*, 2009, 14(10): 4180–4189.

[100] Doležal M, Zitko J, Osička Z, Kuneš J, Buchta V, Vejsová M, Dohnal J, Jampílek J, Kráľová K. Synthesis, antimycobacterial, antifungal and photosynthesisinhibiting activity of chlorinated *N*-phenylpyrazine-2-carboxamides. *Molecules*, 2010, 15(12): 8567–8581.

[101] Dolezal M, Kralova K. Synthesis and evaluation of pyrazine derivatives with herbicidal activity, In: *Herbicides, Theory and Applications*, Soloneski, S. & Larramendy, M. L. (Eds.), 2011, pp.581-610.

[102] Doležal M, Palek L, Vinšová J, Buchta V, Jampílek J, Kráľová K. Substituted pyrazinecarboxamides: Synthesis and biological evaluation. *Molecules*, 2006, 11(4), 242-256.

[103] Doležal M, Tůmová L, Kešetovičová D, Tůma J, Králová, K. Substituted *N*-phenylpyrazine-2-carboxamides, their synthesis and evaluation as herbicides and abiotic elicitors. *Molecules*, 2007, 12(12), 2589-2598.

[104] Slayden RA, Lee RE, Armour JW, Cooper AM, Orme IM, Brennan P J, Besra GS. Antimycobacterial action of thiolactomycin: An inhibitor of fatty acid and mycolic acid synthesis. *Antimicro Agents Chemother,* 1992, 40: 2813-2819.

[105] Tomioka H, Namba K. Development of antitubercular drugs: current status and future prospects, *Kekkaku*, 2006, 81(12): 753-774.

[106] Corbett EL, Watt CJ, Walker N, Maher D, Williams BG, Raviglione MC, Dye C. The growing burden of tuberculosis: global trends and interactions with the HIV epidemic. *Arch Intern Med*, 2003, 163, 1009-1021.

[107] Garay SM. In Tuberculosis, W. N. a. G. Rom, S.M., ed. (Philadelphia: Lippincott Williams & Wilkins), 2004, 345-394.

[108] Zhang Y. The magic bullets and tuberculosis drug targets. *Annu Rev Pharmacol Toxicol*, 2005, 45: 529-564.

[109] Gundersen LL, Meyer JN, Spilsberg B. Synthesis and antimycobacterial activity of 6-arylpurines: The requirements for the *N*-9 substituent in active antimycobacterial purines. *J Med Chem*, 2002, 45: 1383-1386.

[110] Jones PB, Parrish NM, Houston TA, Stapon A, Bansal NP, Dick JD, Townsend CA. A new class of antituberculosis agents. *J Med Chem*, 2000, 43, 3304-3314.

[111] Pasquato KFM, Ferreira EI. An approach for the rational design of new antitubercular agents. *Curr Drug Targets*. 2001, 2: 427-437.

[112] Tangalapally RP, Yendapally R, Lee RE., Lenaerts AJM, Lee RE. Synthesis and Evaluation of Cyclic Secondary Amine Substituted Phenyl and Benzyl Nitrofuranyl Amides as Novel Antituberculosis Agents. *J Med Chem,* 2005, 48: 8261-8269.