Detection of Drug Resistant Mycobacterium Tuberculosis using Molecular Method

Preeti Sharma[1#], Jinendra Singh Chauhan[2], Birjesh Singh[3]
[1#]AISECT University, Bhopal, M.P., India
[2]Govt. BLP PG College, MHOW, Indore, M.P., India
[3]AISECT University, Bhopal, M.P., India

Abstract:- Tuberculosis is commonly known as contagious disease which usually attacks on lungs. Sometimes it affects other parts of body like bones, spine and the bacteria of TB is called mycobacterium tuberculosis. These all symptoms of disease are shows that the patient now highly infected by the disease and he were capable to spread this decease through cold, flue, talking etc. But you couldn't find the TB patient easily and it spread through shaking hands into family members, friends therefore treatment is required. In this category of treatment patient diagnosis based on previous history of TB treatment. DR-TB program often use a combination of the standardized and individualized approaches. Compound which is synthesized and analyzed it found that it given remarkable ability to cure TB, fungal infection and showing great efficiency against the bacterial infection therefore we are mentioned side effects limitations and recommendation about all synthesized compound with comparable study.

Keywords: Tuberculosis, Mycobacterium Tuberculosis, Mechanisms of Resistance, Molecular

I. INTRODUCTION

Mycobacterium tuberculosis infects one third of the world's population. India bears 28.4% of the entire world's tuberculosis burden. Every second an Indian over twenty years of age is infected with M.tuberculosis. There are 14 million estimated tuberculosis cases in India. Of these 3.5 million are sputum positive which means that they spread the disease to at least 10 to 15 people they come in contact with, Every year 2.2 million people contract tuberculosis but only half of them seek medical help. One Indian dies of TB every minute and over a thousand die of TB every day. Thus, about five lakh people die of TB in India every year by a disease that is eminently curable [1, 2].

It is ironic that India with a population lesser than China has double the number of tuberculosis patients. Tuberculosis is a disease that gets no publicity. The sheer lack of glamourlessness of TB in a mediacentric society has celebrities shaking hands with AIDS patients, has managed to push it out from its priority position in our public health agenda [3].

Significant treatment was restricted to the conventional methods until 1942. However, the convincing treatment was established in early 1944, when the „Streptomycin" and „Patricia" was discovered. With amazing speed after the introduction of streptomycin, para-amino salicylic acid (PAS), isoniazid and pyrazinamide were introduced into the clinical practice. With the development of effective surgery and therapeutic drugs, the pulmonary TB was almost eliminated. TB had been transformed from a relentlessly progressive, often fatal illness to an infection which responded to appropriate drugs [4].

Throughout the 20th century, the number of cases and the death rate has declined dramatically in the U.S.A. and Europe. However, since 1985, this decline halted and a progressive rise has been seen with total increase of about 9% per year. This can be due to the initiation of the partnership of TB with HIV which can be evident by the fact that the first reported case of HIV was detected by 1984. In March 1993, the WHO took an unprecedented step and declared tuberculosis as a "Global Emergency". Today, tuberculosis has returned with a new face and the global scourge of Multi-drug resistant TB (MDR-TB) and the recalcitrant nature of persistent infections pose additional challenges to treatment with currently available anti-TB drugs. The situation is exacerbated by the emergence of extensively drug resistant tuberculosis (XDR-TB). Patients with XDR-TB are virtually untreatable and carry rapid and extremely high fatality rate.

Even though there are so many different types of diagnostic tests based on different principles, in general, diagnostic capabilities depend upon the technical expertise involved, geographical region like high endemicity, hot zones etc, type of laboratory involved and so many other factors. It is very important to evaluate the correlation of different diagnostic tests with caution from different geographical regions [5].

The ultimate move to eradicate tuberculosis is the proper diagnosis along with prompt treatment. While the incidence of TB has been rapidly rising, the large outbreak of drug resistant TB contributes more to this pandemic. The size of the recent MDR-TB and the rapidity with which it has propagated has been so alarming that WHO had to declare it as the 3rd emergency. (Resurgence of TB being the 1st and its association with HIV being the 2nd). Moreover, chronic excretors of drug resistant bacilli pose a serious public health problem as they may infect and spread drug resistant TB in the society. To add further, previously treated patients are much more likely to have resistant TB bacilli, and therefore, treatment failure, relapse cases and defaulters should be meticulously investigated for drug resistance. The issue of drug resistance will perhaps be more important for formulating their re-treatment regimens. Hence it is very important to evaluate the "Primary Drug Resistance" and "Acquired Drug Resistance", pattern of individual first line anti -TB drugs and evaluate the Multi-Drug Resistance.

Thus, considering all the facts mentioned above, it has been decided to undertake studies on the bacteriological aspects of Tuberculosis such as characterization of Mycobacteria isolated from the patients of pulmonary tuberculosis, comparison of conventional diagnostic techniques, prevalence of primary and acquired anti-tuberculosis drug resistance and HIV-TB co-infection [6].

II. PREVIOUS WORK

WHO reported that in 2018 there were an estimated 484,000 incident cases of MDR/rifampin-resistant (RR) TB cases, including about 378,000 MDR-TB cases and 214,000 deaths [1]. The average proportion of MDR-TB cases with XDR-TB was 6.2%. The countries accounting for 50% of the global burden of MDR/RR-TB were India (27%), China (14%) and the Russian Federation (9%). Among 24 countries with a high TB or MDR-TB burden and representative data to second-line drugs, the proportion of MDR/RR TB cases with resistance to any FQ including ofloxacin (OFL),

levofloxacin (LFX) and moxifloxacin (MFX) was 20.8%. At the global level, 3.4% of new cases (patients never treated with anti-TB medicines, or treated for < 1 month) and 18% of previously treated cases (patients treated for 1 month in the past) had MDR/RR-TB, with the highest proportion occurring in the former Soviet Union (FSU) countries. In the low incidence countries of the European Economic Area, the MDR-TB was more prevalent among migrants (particularly from the FSU) than the native population [7, 8].

In the last decades, WHO made great efforts to facilitate and improve the treatment of patients with MDR-TB in high burden countries using various actions including the Directly Observed Treatment Strategy (DOTS)-Plus, to stress the use of second-line drugs in low- and middle-income settings, but the cure rate was lower than the WHO 2015 target of at least 75% to 90% [6]. For instance, treatment success for MDR/RR-TB cases started on treatment in 2016 in India, China and Russian Federation was 48%, 52% and 54%, respectively [1].

In 2018, the results from an individual patient data meta-analysis involving 12,030 patients from 25 countries showed that treatment success and death of pulmonary MDR-TB were significantly reduced after the administration of the newer or repurposed drugs linezolid (LZD), later generation FQs, bedaquiline (BDQ), clofazimine (CFZ) and carbapenems [7]. On the basis of this and other studies, in March 2019, WHO released a new drug classification and new recommendations for the treatment of MDR-TB [8–10]. The second-line drugs were reorganized into three groups, including priority drugs [Group A: LFX or MFX, BDQ, LZD], preferentially used drugs [Group B: CFZ, cycloserine (CS) or terizidone (TRD)], and other drugs [Group C: EMB, delamanid (DLM), PZA, imipenem-cilastatin (IPM-CLN) or meropenem (MPM) (administered with clavulanic acid, CLV), AM or streptomycin
(SM), ethionamide (ETO) or protionamide (PTO), para-aminosalicylic acid (PAS)].

In summary, WHO recommended an injection-free therapy (groups A and B drugs) at the initiation of MDR-TB treatment. Group C agents (oral and parenteral) should be administered when groups A and B drugs cannot be used. The commonly used second-line injectable drugs KM and CM were associated with worse outcomes [7], and were no longer recommended for the treatment of MDR-TB; AK and SM may be administered only if drug susceptibility testing (DST) confirms susceptibility.

To further reduce the burden of drug-resistant TB in the near future, in December 2019, WHO also released a Rapid Communication to inform countries and stakeholders that a regimen containing BDQ, pretomanid (PRT, formerly PA-824) and LNZ (BPaL regimen) may be used under operational research conditions conforming to WHO standards for the treatment of XDR-TB patients [11]. This communication was released after a previous announcement of the Global TB Alliance in the second half of 2019, following the decision of the United States Food and Drug Administration (FDA) to administer BPaL (Nix-TB trial by the Global TB Alliance) to adults with pulmonary XDR-TB or intolerant/not responsive MDR-TB [12].

III. COMPLEXITY OF TB GRANULOMAS

Long lasting therapies are also attributable to the complex pathology of TB. In the lungs of patients with active and latent TB, a spectrum of heterogeneous granulomatous lesions coexist, ranging from well-vascularized cellular granulomas, in which a rim of lymphocytes surrounds macrophages and neutrophils, to avascular caseous granulomas, characterized by a necrotic center with a cheese-like aspect (caseum) formed by the lysis of host cells and bacteria [2]. In these lesions, tubercle bacilli range from actively replicating (AR) stages, particularly in cellular granulomas, to dormant, slowly-replicating or NR stages, typical of hypoxic caseous granulomas [3]. In Mtb-infected rabbits, the fraction unbound of a drug penetrates the caseum via passive diffusion, and caseum binding of a drug is proportional to hydrophobicity (cLogP) and aromatic ring count [3].

The current 4-drugs therapeutic regimen (RIF-INH-PZA-EMB) is effective against AR intracellular bacilli in cellular granulomas, while NRextracellular bacilli localized in pH-neutral, caseous granulomas are refractory to drug action [1, 6]. The necrotic center of caseous granulomas contains NR bacilli phenotypically resistant to several drugs (drug-tolerant persisters), with the exception of rifamycins, which are known to sterilize caseum in ex-vivo assays [6]. Spatial and temporal differences in drug distribution and the kinetics of accumulation of drugs in specific lesion compartments may create local windows of monotherapy that increase the risk of the emergence of drug-resistance [7]. This is in keeping with the knowledge that genetically resistant mutants of Mtb may emerge from the persistence phase of some TB drugs, due to hydroxyl radical-mediated genome-wide random mutagenesis [14].

In this view, drug combinations should contain complementary drugs preferentially distributing in lesions in which their most vulnerable target population resides [17]. In the event of caseous granulomas expansion, the necrotic centers fuse with the airway structures of bronchi to form pulmonary cavities in which are found both extracellular bacilli from liquefied caseum and intracellular bacilli derived from the lysis of infected macrophages of the cavity walls. In contact with the atmospheric oxygen, these bacilli rapidly proliferate in the lumen of cavities, and later appear in the sputum of TB patients [17]. Due to high bacterial load in pulmonary cavities, genetically resistant bacilli with chromosomal mutations may be generated, playing an important role in the development of resistance [16]. Noticeably, in comparison with paired sputum isolates, additional resistances were found in Mtb isolates recovered from surgically resected cavities of the same patient [18]. A single founder Mtb strain underwent genetic mutations during treatment, leading to the acquisition of additional drug resistance in di_erent sections of the lung of the same patient, preferentially in the cavity wall [2]. In keeping with this observation, drug-specific gradients in the walls of human pulmonary cavities were reported to be associated with the development of acquired resistance in patients with MDR-TB, due to the low level of some drugs in the cavities centers, where there is a high number of replicating bacilli [43]. In the latter study, spatial heterogeneity of drug concentrations across the pulmonary cavity resulted in the development of mutations in the Mtb genes gyrA (FQ resistance) and gydB (aminoglycoside resistance), consistent with evolution from MDR- to XDR-TB after about five months of therapy [43]. Overall, these observations indicate that acquired Mtb resistance may be related to the formation of drug-penetration gradients in TB lesions generating suboptimal drug concentrations in non-vascularized caseous granulomas and in liquefied caseum in the cavity centers [16].

The constituents of the mycobacterial cell envelope are: the cytoplasmic membrane, the periplasmic space (PS), a network of peptidoglycan (PG), the arabinogalactan (AG), the long-chain mycolic acids (MA) and the capsule, made of a loose matrix of glucans and secreted proteins [44]. As to the first-line TB drugs, the bactericidal agent INH inhibits MA synthesis, while the bacteriostatic EMB inhibits AG synthesis and may sensitize Mtb to other drugs [4].

It is assumed that the innermost hydrophilic layers of PG and AG hinder the penetration of hydrophobic molecules. Instead, in the external part of the envelope, the PG and AG layers are linked to the hydrophobic MA layer, formed by long-chain fatty acids that restrict the penetration of hydrophilic drugs [18]. In principle, more lipophilic drugs, such as rifamycins, macrolides, and some FQs, di_use by passive transport into and through the lipid-rich cell wall [21]. In early studies, mutants defective in the biosynthesis of cell wall components were very useful to demonstrate the role of the cell wall in the intrinsic resistance to drugs. For instance, a mycolate defective Mycobacterium smegmatis mutant showed increased

susceptibility to RIF, chloramphenicol (CF), novobiocin and erythromycin [8]. Also, insertions in genes involved in the mycolate biosynthesis of Mtb (mymA operon) showed enhanced chemical penetration and sensitivity to RIF, INH, PZA and ciprofloxacin [9].
Small hydrophilic drugs traverse the cell wall of bacteria via water-filled porins, without energy consumption. M. tuberculosis encodes at least two porin-like proteins (OmpA, encoded by Rv0899 and Rv1698), but the role of porins in Mtb drug uptake and susceptibility needs to be further investigated [18]. Penetration of hydrophilic _-lactam antibiotics through the mycobacterial cell was about 100 times lower than in the Escherichia coli cell wall [20]. The ff-lactamases, probably in conjunction with slow drug penetration, were shown to be major determinants of Mtb resistance to-lactams [5]. In Mtb, the PG is remodeled by nonclassical l, d-transpeptidases (LDT). The structural basis and the inactivation mechanism of LDT and the active role of carbapenems were investigated, providing a basis for their potential use in inhibiting Mtb [5]. Indeed, the carbapenems IPM-CLN and MPM (both to be used with CLV, available only in formulations combined with amoxicillin) have been listed as add-on drugs in the recent WHO treatment guidelines of MDR/XDR TB [8].
Overall, it is thought that anti-TB drugs have the peculiarity of being more lipophilic than many other antimicrobial agents, likely due to improved penetration through the waxy mycobacterial cell wall [6]. However, the issue is perhaps more complex, since some studies showed that lipophilicity is an important but not exclusive factor of compound permeability [5].

IV. METHODOLOGY

Last few years, progsen had been done in arena of new drug discovery for anti TB, in that maners few new drugs were developed and some others in under progress. Some of them under pre-clinical trial and in early development.
Bedaquilines and its derivatives are approved by several countries and justify that the MDR-TB is so dangerous and critical desease therefore so many trials are going on anti TB for example clojazimine and levofloxacin.
Despite the recent and very advance research against the TB shows that new molecule has been tested for urgent treatment of TB which play an important role against this deadly desease.
WHO introduce new areas of research therefore we are going to setup new imidazole, pyrazole to help in solution of desease in different concentration at low cost
In this present study we are going to find out new drug complex of imidazole and pyrazole, which showing good efficiency at low concentration against the MDR TB virus.
Similar study done by so many researchers and they were concluded that the nitrogen constituent compounds are showing the anti TB properties for example Roh suggested that di nitrobenzyl sulphonyl-1 oxadiazoles and its derivatives has potency against the TB at 0.25µm. Oxazolidinose dem of derivative also useful for that desease but it causes some adverse effect, so that several N-atom containing heterocyclic drugs now synthesized for example imidazole and pyrazole are reported. Some more scientists also found that at low concentration of 5µm TB strain were destroy. Pyrimidine heterocyclic derivatives also tested up to 0.86µm as antiTB reagent. Similar work we introduced in this analysis and find a great efficiency of compound against the TB.

Synthesis- Similar to previous our analysis we are going to synthesized a compound of pyrazole 1-{3-[1-4-chloro-4H-pyrazol-3yl methyl)-piperidine-4-yl amino]-6-fluoro-3H-imidazol-5yl}-ethenone for the synthesis of compound we are using AR gohad of chemical in first step imidazole converted into chloro and azyl derivative by the reaction of chlorination and formylation. (at 60^0C) Step two is reaction of imidazole amine with chloro benzyl derivatives of imidazole in presence of H_2SO_4 to produced additive complex at 65 ± 2^0C.
In step three compound of step two reacted with acetyl flouro pyridine to produce 1-{3[1-4-chloro-4H-pyrazol-3-yl methyl)-piperidine-4yl amino]-6floro-3H-imidazole-5yl}-ethanone.
2. Spectroscopic Analysis- The IR spectra of this molecule suggested that the derivative containg so many groups in the constitutionof its structure. An IR spectrum taken in bucker instrument through FTIR process and found that the IR peaks occurs at different frequency. -C=O 1680cm^{-1}, Ar-CH str-3030cm^{-1}
CN-1592cm^{-1} and for N-H it is at-3215cm^{-1}, 1250 cm^{-1} for C-N. Asymetrically 3020cm^{-1}, C=C at 600cm^{-1}, 1410cm^{-1} for F, 3226 for N-H 1650 for aceto group were found.
The product confirm by mass spectroscopy which done at RGPV-Bhopal pharmacy lab and it found that the maximum abundance occure at 376.12 m/e ratio and the it confirm from its data that the moleculer mass of compound assigned 376.12amu. And moleculer weight is 376.82amu. The following data suggested that the formula of compound as it predict.
Dormancy is not necessary or sufficient for Mtb persistence, indicating that persistence is a phenomenon more complex than dormancy, and that additional characteristics are needed to define the persister phenotypes, which depends on the NR model used [9]. A poor correlation was found between the transcriptomes of class I persisters enriched by cycloserine [1] and class II persisters obtained under hypoxia, the stationary phase or nutrient starvation [4]. On the other hand, persister diversity is expected also from the different host environments in which these specialized cells live, ranging from the intracellular location in the phagosomes to extracellular life in the caseum. In
BDQ-treated guinea pigs, persisting bacilli where located in the acellular rim of necrotic lesions, morphologically similar to human TB lung lesions [2].
The state of non-replication is associated with phenotypic drug-tolerance, but different stresses may induce phenotypically different bacilli. Few compounds were dual active molecules with bactericidal activity against both replicating and NR Mtb. They included RIF, BDQ, PRT and MFX, which target RNA polymerase, ATP synthase, cell wall synthesis/cell respiration, and DNA gyrase, respectively [2]. In BALB/c mice, persisters were eradicated by regimens containing high-dose RIF and BDQ [4]. In BALB/c mice, C3HeB/FeJ caseum-forming mice and athymic nude mice, PRT contributed significantly to the efficacy of BDQ-containing regimens, with either LZN (BPaL regimen) or MFX and PZA (BPaMZ regimen) [5].
Interestingly, RIF-resistant or MXF resistant mutants carrying mutations in rpoB or gyrA genes emerged at high frequency from the persistent phase of Mtb cells exposed to RIF for prolonged periods.
These cells carried elevated levels of the hydroxyl radical, which inflicted genome-wide mutations facilitating resistance to the same, or another, antibiotic [9]. In consideration of the long TB therapy, these observations may have clinical significance in the emergence of drug-resistant mutants if local monotherapy occurs in patients who do not correctly take multi-drug TB therapy.

V. CONCLUSION

Several investigational compounds have the potential for further development as additional parts of the anti-TB regimen, as previously mentioned. Their inclusion in TB therapy will not only lead to shortened treatment by adding synergistic effects (such as griselimycin with RIF and PZA) but can also lead to higher success rates by improving effectivity against resistant Mtb strains (such as nitazoxanide, nitrofuran analog 10n, and isoniazid hydrazides) or preventing certain resistance mechanisms (such as dihydropyridomycin). Further proving the importance of building on investigational compounds is the probability to improve patient adherence by reducing drug complications and adverse effects due to lower cytotoxicity and hepatotoxicity (as indicated by primaquine derivatives and isoniazid hydrazides).

Drug-resistant TB is a significant challenge for the successful control of the disease worldwide. A comprehensive review of clinical, biological and microbiological issues favoring resistance development has been provided, helping in the development of new tools for the rapid diagnosis and treatment of drug-resistant TB. The review was based on the most recent updates on drug resistance mechanisms reported in the literature, and on the international recommendations of WHO to facilitate the clinical and microbiological management of MDR/XDR TB at global level.

References

[1] Abdizadeh T, Kalani MR, Abnous K, Tayarani-Najaran Z, Khashyarmanesh BZ, Abdizadeh R, Ghodsi R, Hadizadeh F (2017) Design, synthesis and biological evaluation of novel coumarin-based benzamides as potent histone deacetylase inhibitors and anticancer agents. Eur J Med Chem 132:42–62

[2] Aguilar-Ayala DA, Cnockaert M, Vandamme P, Palomino JC, Martin A, Gonzalez-Y-Merchand J (2018) Antimicrobial activity against Mycobacterium tuberculosis under in vitro lipid-rich dormancy conditions. J Med Microbiol 67:282–285

[3] Agyeman AA, Ofori-Asenso R (2016) Efficacy and safety profile of linezolid in the treatment of multidrug-resistant (MDR) and extensively drug-resistant (XDR) tuberculosis: a systematic review and meta-analysis. Ann Clin Microbiol Antimicrob 15:41

[4] Aljayyoussi G, Jenkins VA, Sharma R, Ardrey A, Donnellan S, Ward SA, Biagini GA (2017) Pharmacokinetic-pharmacodynamic modelling of intracellular Mycobacterium tuberculosis growth and kill rates is predictive of clinical treatment duration. Sci Rep 7:1–11

[5] Gupta R, Lavollay M, Mainardi J-L, Arthur M, Bishai WR, Lamichhane G (2010) The Mycobacterium tuberculosis protein LdtMt2 is a nonclassical transpeptidase required for virulence and resistance to amoxicillin. Nat Med 16:466–469

[6] Gupta S, Tyagi S, Almeida DV, Maiga MC, Ammerman NC, Bishai WR (2013) Acceleration of tuberculosis treatment by adjunctive therapy with verapamil as an efflux inhibitor. Am J Respir Crit Care Med 188:600–607

[7] Gupta S, Tyagi S, Bishai WR (2015) Verapamil increases the bactericidal activity of bedaquiline against Mycobacterium tuberculosis in a mouse model. Antimicrob Agents Chemother 59:673–676

[8] Gurumurthy M, Verma R, Naftalin CM, Hee KH, Lu Q, Tan KH, Issac S, Lin W, Tan A, Seng K-Y, Lee LS-U, Paton NI (2017) Activity of faropenem with and without rifampicin against Mycobacterium tuberculosis: evaluation in a whole-blood bactericidal activity trial. J Antimicrob Chemother 72:2012–2019

[9] Imperiale BR, Cataldi ÁA, Morcillo NS (2017) In vitro anti-tuberculosis activity of azole drugs against Mycobacterium tuberculosis clinical isolates. Rev Argent Microbiol 49:332–338

[10] Ivanovs A, Salmane-Kulikovska I, Viksna L (2016) The impact of socioeconomic factors on tuberculosis prevalence in Latvia. Age 13:39 Jaiswal I, Jain A, Verma SK, Singh P, Kant S, Singh M (2017) Effect of efflux pump inhibitors on the susceptibility of Mycobacterium tuberculosis to isoniazid. Lung India : official organ of Indian Chest Society 34:499–505

[11] Jang J, Kim R, Woo M, Jeong J, Park DE, Kim G, Delorme V (2017) Efflux attenuates the antibacterial activity of Q203 in Mycobacterium tuberculosis. Antimicrob Agents Chemother 61: e02637–e02616 Jansen RS, Rhee KY (2017) Emerging approaches to tuberculosis drug development: At home in the metabolome. Trends Pharmacol Sci 38:393–405

[12] Modongo C, Sobota RS, Kesenogile B, Ncube R, Sirugo G, Williams SM, Zetola NM (2014) Successful MDR-TB treatment regimens including amikacin are associated with high rates of hearing loss. BMC Infect Dis 14:542–542

[13] Molina-Torres CA, Barba-Marines A, Valles-Guerra O, OcampoCandiani J, Cavazos-Rocha N, Pucci MJ, Castro-Garza J, VeraCabrera L (2014) Intracellular activity of tedizolid phosphate and ACH-702 versus Mycobacterium tuberculosis infected macrophages. Ann Clin Microbiol Antimicrob 13:13

[14] Ramesh R, Shingare RD, Kumar V, Anand ABS, Veeraraghavan S, Viswanadha S, Ummanni R, Gokhale R, Srinivasa Reddy D (2016) Repurposing of a drug scaffold: Identification of novel sila analogues of rimonabant as potent antitubercular agents. Eur J Med Chem 122:723–730

[15] Ramón-García S, González del Río R, Villarejo AS, Sweet GD, Cunningham F, Barros D, Ballell L, Mendoza-Losana A, FerrerBazaga S, Thompson CJ (2016) Repurposing clinically approved cephalosporins for tuberculosis Exploring the potential of adjunct therapy in tuberculosis. Trends Pharmacol Sci 36:506–513

[16] Reddy DS, Hosamani KM, Devarajegowda HC (2015a) Design, synthesis of benzocoumarin-pyrimidine hybrids as novel therapy. Sci Rep 6:34293

[17] Ran K, Gao C, Deng H, Lei Q, You X, Wang N, Shi Y, Liu Z, Wei W, Peng C, Xiong L, Xiao K, Yu L (2016) Identification of novel 2-aminothiazole conjugated nitrofuran as antitubercular and antibacterial agents. Bioorg Med Chem Lett 26:3669–3674

[18] Rayasam GV, Balganesh TS (2015) class of antitubercular agents, their DNA cleavage and X-ray studies. Eur J Med Chem 101:705–715

[19] Walker NF, Clark SO, Oni T, Andreu N, Tezera L, Singh S, Saraiva L, Pedersen B, Kelly DL, Tree JA, D'Armiento JM, Meintjes G, Mauri FA, Williams A, Wilkinson RJ, Friedland JS, Elkington PT (2012) Doxycycline and HIV infection suppress tuberculosis-induced matrix metalloproteinases. Am J Respir Crit Care Med 185:989–997

[20] Walker NF, Stek C, Wasserman S, Wilkinson RJ, Meintjes G (2018) The tuberculosis-associated immune reconstitution inflammatory syndrome: recent advances in clinical and pathogenesis research. Curr Opin HIV AIDS 13:512–521

[21] Wallis RS, Maeurer M, Mwaba P, Chakaya J, Rustomjee R, Migliori GB, Marais B, Schito M, Churchyard G, Swaminathan S, Hoelscher M, Zumla A (2016) Tuberculosis—advances in development of new drugs, treatment regimens, host-directed therapies, and biomarkers. Lancet Infect Dis 16:e34–e46