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ANTI- MYCOBACTERIAL SCREENING METHODS FOR NEW COMPOUNDS IDENTIFICATION

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ABSTRACT
Tuberculosis (TB) is one of the oldest diseases and leading to cause a big number of deaths every year. The emergence of multidrug-resistant strains of *Mycobacterium tuberculosis* underscores the need of continuous developments on new and efficient methods to determine the drug susceptibility of clinical isolates of *M.tuberculosis* in the search for novel antimicrobial agents. Development of new drugs has become an immediate necessary to stop the prevalence of the Tuberculosis. As the present drugs were expressing complexity, toxicity to host and sensitivity towards the multidrug resistance TB, there is a need to develop or design newer drugs to treat TB in a short period and affordable. In the present study we tried to evaluate different methods for testing new synthetic compounds against Mycobacteria activity. Minimum inhibitory concentrations (MIC), Proportion method and MABA in liquid method were used to determine the potency of new antimicrobial agents according to RNTCP guidelines. A total of 101 compounds were screened and 28 compounds were identified in expressing their Anti-mycobacterial activity for both laboratory strain (H37Rv) and clinical isolate of MTB and MDR-TB, by using different analysis techniques (Spectrophotometer, Calorimeter, Turbidometer). In this study we screened newer synthetic compounds which may be helpful in the development of new TB drugs more rapidly to patients. We would like to conclude that, rapid method of detection of drug activity was noted by MABA in liquid media and we are able to differentiate within 5 days of time which was a rapid method for identification.

KEYWORDS: Proportion based, MIC, Mycobacterium tuberculosis, MDR-TB.

INTRODUCTION
Tuberculosis is the one of the leading cause of death worldwide. In spite of many efforts made to control tuberculosis it has still become major public health concern as it is one of the leading causes of death in the developing countries. (WHO) *Mycobacterium tuberculosis* (MTB) which is responsible for the cause of TB which commonly affects lungs (pulmonary) and other parts of the body (extra pulmonary).[1] In 2015 estimated to be, 10.4 million people developed TB and 1.8 million died from the disease out of these 0.4 million people were co infected with HIV.[3] Deaths rates are high developing countries due tuberculosis infections. *Mycobacterium tuberculosis* is a small, aerobic, non motile bacillus has an outer membrane lipid bilayer acid fast bacilli.[2] Which divides approximately for every 16 to 20 hours, which is an extremely slow rate compared with other bacteria.[4] Treatment process with antibiotics to kill the bacteria will takes a longer time nearly 6-8 month. Effective TB treatment may be difficult, due to the unusual structure and chemical composition of the Mycobacterial cell wall,[2] which hinders the entry of drugs and makes many antibiotics ineffective. The approach to chemotherapy for tuberculosis will be very different from that for other bacterial infections. The bacteria have a long generation time and a capacity for dormancy is more, when its low metabolic activity makes it a difficult therapeutic target.[5,6,7] The site of, *M. tuberculosis* may be located in pulmonary cavities, empyema pus, or solid caseous material, where penetration of antibiotics will be difficult and the pH is sufficiently low to inhibit the activity of most antibiotics.[8,9] A part of that treatment of first line, period is longer and used a wide number of drugs which included of a specific regimen of isoniazid (INH), rifampicin (RIF), pyrazinamide (PZA), and ethambutol (EMB) combination. The rate at which resistance emerges differs for all of the antituberculosis agents, being highest for ethambutol and lowest for rifampin and quinolones. As per reports the risks of mutation for most of the antibiotics used in tuberculosis treatment have been defined previously;[10] for rifampin, isoniazid,

streptomycin, and ethambutol, they are 3.32×10^{-9}, 2.56×10^{-8}, 2.29×10^{-8}, and 1.0×10^{-7} mutations per bacterium per cell division, respectively. The mutation rate, rather than the mutation frequency, is the most reliable measure, as it records the risk of mutation per cell division rather than the proportion of mutant cells which leads to appearance of mutant drug-resistant strains MDR TB (Multi Drug Resistant), first line drugs Isoniazid and Rifampicin referred to as MDR TB (Millard, James). Clinical Strains resistant to at least Rifampicin and Isoniazid in addition to being resistant to one of the fluoroquinolones;[11] as well as resistant to at least one of the second line injectable of TB drugs Amikacin, Kanamycin or Capreomycin referred as XDR TB.[12] First line drugs of mycobacterium used are Isoniazid, Rifampicin, Pyrazinamide, Ethambutol and Streptomycin which have the greatest activity against TB bacteria and they are core to any TB drug treatment program as RNTCP.[13] According to WHO 2016 report, in 2015, an estimated 480 000 people developed multidrug-resistant TB (MDR-TB).

There is an urgent need to develop new drugs which should show more effectiveness with minimal side effects and be affordable for treatment of TB. The foremost stage for Anti-mycobacterial drug detection involves the assessment of potency of compound against mycobacterium in vitro conditions. This can be possible by determining the Minimum Inhibitory Concentrations (MICs), which plays a significant role in the screening process. Drug Susceptibility testing (DST) for *Mycobacterium* is considered to be highly important for therapy guidance and surveillance of drug resistance.[14] Drug Susceptibility Tests were generally performed based on the absolute proportion method developed at national institute at public health and the environment. This method involves a concentration series of compounds under study were distributed in different tubes containing liquid media middle brooks 7H9. A large number of drug susceptibility assays were determined to identify cell viability like BACT[15] but in the present study Proportion method, MIC method and Alamar blue reagent (MABA) were evaluated using turbidometery, calorimetric and spectrometer by plotting growth curve at different OD values and determining the cell growth is used as it is rapid, less expensive and also provides high throughput. The present study involves the Anti-mycobacterial drug screening activity of new synthetic compounds using different methods against control (H37Rv) and clinical isolate identified in the patient's sample.

MATERIALS AND METHODS

The evaluation of different anti tuberculosis testing methods was carried out using some synthetic compounds and natural compounds.

Source of Mycobacterial strains and preparation of inoculums

Mycobacterium tuberculosis (H37Rv) as control and clinical isolates isolated from the patient's sputum sample approached District Tuberculosis Centre, MGMH, Warangal were included in the study. The clinical isolates were identified as *M. tuberculosis* based on AFB staining, conventional, Biochemical, DST and phenotypic methods of which 1 MTB and other MDRTB were studied. Further clinical isolates were preserved at -20ºC in liquid broth containing 10% glycerol; retrieve of the culture was carried by sub culturing on sterile L-J medium and incubated for a period of 8 weeks. 4th week culture were used to prepare the Mycobacterial suspension in middle brook media, Mycobacterial suspension were prepared according to McFarland turbidity method. The bacterial suspension was homogenized by vortex shakeup and the turbidity was adjusted in agreement with tube according to McFarland no. 1 scale (3.2×106 cfu/ mL). The inoculum was prepared diluting the bacterial suspension in the proportion of 1:20 in Middle Brook 7H9 broth medium. This diluted suspension (100 µL) is used to inoculate for screening the drug activity.[16]

Preparation of media and inoculation: Mineral salt solution: (Potassium dihydrogen Phosphate (0.15%). Magnesium Sulfate (0.015%), Magnesium Citrate (0.0375%), L-Asparagine (0.45%), Glycerol (0.75%), dissolve the ingredients by heating and autoclave at 121ºc for 25 minutes to sterilise this solution keep indefinitely and may be stored in suitable amounts.

Malachite Green Solution: Distilled water (100ml), Malachite green (4gms). Prepared a 2%solution of malachite green in sterile water with sterile precautions by dissolving the dye in the incubator for 1-2 her this solution can be stored indefinitely and should be shaken be for us. After preparation of malachite green is added to the mineral salt solution.

Egg suspension: Fresh egg solution (15-20egg were used) was added to the media and mixed them. According to the procedure, And poured the breeze bottles, put in to the Inspissator and observed the media bottles for solidification with (70-80c) temperature. Then Preserved at - 4ºC. The media was allowed to incubate for eight weeks after inoculation, as the mycobacterium grows slow when compared with other bacteria.[17]

Middle Brook Media (Liquid media): For screening the new compounds a loop of *M.TB* retrieved from LJ media was inoculated in Middle brook 7H9 Broth purchased from Himedia labroorys pvl, Ltds Mumbai (Ammonium Middle brook 7H9: Middle brook 7H9 Broth purchased from Himedia, Mumbai (Ammonium sulphate 0.05%, Disodium phosphate 0.25%, Monopotassium phosphate 0.1%, Sodium citrate 0.01%, Magnesium sulphate 0.005%, Calcium chloride 0.00005%, Zinc sulphate 0.0001%, Copper sulphate

0.0001%, Ferric ammonium citrate 0.004%, L-Glutamic acid 0.05%,Pyridoxine 0.0001%, Biotin 0.00005%) is a liquid growth medium especially used for cultivation of *Mycobacterium tuberculosis*.

Glycerol: Glycerol (2ml) added to the media, dissolves the ingredients by heating and autoclave at 121ºc for 15 minutes to sterilise this solution keep indefinitely cool it.

OADC: bovine Albumin Fraction v (2.50gm), Dextrose (1.00), Catalyse (0.002gm), Oleic acid (0.025gm), sodium chloride (0.425gm), distilled water (50ml).The OADC were added to the cool medium And poured the test tube sunder aseptic condition. For screening the new compounds a loop of *M. Tuberculosis* diluted with 2ml of distilled water retrieved from LJ media was inoculated in Middle brook 7H9 media liquid growth medium especially used for cultivation of *Mycobacterium tuberculosis*. Cultures were read using spectrophotometer at 450nm wave lenght from the day 3 after inoculation and for every alternate day up to 21 days.[18]

Preparation of Stock solution for screening Antimycobacterial activity: The new compounds were dissolved in suitable solvents such as Dimethyl sulphoxide (DMSO), Deuterated chloroform ($CDCl_3$) and stock solution of concentration 1mg/ml was prepared. Isoniazid was used as the drug control for the compounds. 50 mg of isoniazid was prepared by adding 5 mL sterile distilled water. 0.5 mL from the stock solution is taken and 24.5 mL of distilled water is added and 0.1 mL of the drug is added to the media (conc. 0.2 µg/mL).The stock solutions were successively diluted to half (8 times) to obtain 9 concentrations, ranging from 1000 to 4µg/ml. Compounds (1 mg) are soluble in 1 mL of the DMSO (con. 1 mg/mL). After dilutions the final and different subsequent concentrations of synthetic compounds used for screening are proportion based were as 1000 µg/mL/, 500 µg/mL, 250 µg/mL, 125 µg/ mL, 62 mg/mL, 32µg/mL, 16µg/mL, 8 µg/mL, 4µg/mL.[18]

Inoculation of new drugs and Mycobacterium: Specific concentrations of new compounds were added individually to the freshly prepared sterile media and mixed well. A loop of diluted Mycobacterial subculture and control were inoculated into sterile medium separately in respective tubes under aseptic conditions and mixed properly using Vortex. After inoculation, the culture tubes were allowed to incubate at 37ºC over the growth period.[19]

Drug screening

A total of 64 synthetic and 37 natural compounds were screened against three strains, MTB, MDRTB and H37Rv.

Drug Susceptibility Tests: There are three general methods used for determining drug susceptibility of mycobacterium: the proportion method, absolute concentration method (MIC method) and the resistance ratio method. When properly standardized and performed, all three methods have been shown to be equally satisfactory. In India, proportion method is advised since large numbers of laboratories have standardized this method for DST.[20]

Determination by Proportion method: Drug susceptibility testing is one of the most difficult procedures to perform and standardize in the mycobacteriology laboratory. Proficiency in susceptibility tests demands an understanding of the origin of drug resistance, variation in stability of drugs subjected to different conditions of filtration, heat or storage, the alteration in the activity of certain drugs when incorporated into different kinds of media, the type of susceptibility test performed, the reading and reporting of test results and the criteria of resistance

Determination of MIC by micro dilution method:

MICs of new compounds against *M. tuberculosis* and H37Rv were determined in Middle brook7H9 broth using the standard micro dilution method,[21,27] Minimum inhibitory concentrations (MIC) are important in diagnostic laboratories to confirm resistance of microorganisms to an antimicrobial agent and also to determine the potency of new antimicrobial agents. MIC is the lowest concentration of an antimicrobial activity which inhibits the visible growth of a microorganism after overnight incubation. It is the most basic laboratory measurement of the activity of an antimicrobial agent against an organism. The compounds were dissolved in DMF (1.25 mg/mL) and used as a stock solution. Concentrations ranging from 1 to 1000 µg/mL were used to assess the effectiveness of the compounds. Micro titer tubes were incubated at 37 °C for 72 h, and the growth inhibition was recorded for 14 and 21 days respectively. The MIC value represents the lowest dilution of the compound at which no bacterial growth was detected.[23,24,25]

Evaluation of antimycobacterial activity using Alamar Blue reagent

M.TB growth was identified spectrophotometrically and to check the cell viability and proliferation after 21days of growth period, Resazurin dye (alamar blue) (0.01%) indicator was added and incubated for about 48hrs. The MABA system is a simple, rapid, low-cost, high-throughput system does not require high expenditure instrumentation and the mycobacterial growth can be measured by a visible color change[26,27,23] Further colour change from blue to pink indicates sensitivity towards drug and no change in the colour indicates the drug activity.

RESULTS

The Antimycobacterial activity was quantitatively determined using the Resazurin dye in order to reveal the Minimal Inhibitory Concentrations (MIC). A total of 101

compounds were screened for Antimycobacterial activity in liquid media and its MICs were determined against laboratory strain H37Rv and strain isolated from the patient's sample. Minimum Inhibitory Concentration of each synthetic compound is represented as the lowest concentration of the drug which shows activity against the two strains used for the study. Out of 86 new synthetic compounds, 20 24 Compounds exhibited their potential Antimycobacterial activity for both strains at all dilutions ranging from 4µM- 1000µM and 28 compounds exhibited their sensitivity towards both the strains at all dilutions. Thus the minimum inhibitory concentrations for compounds showing activity can be 4µM. For 4 synthetic compounds (SSCP 23, SSCP 24, SSCP 26 and SSCP 29) which exhibited Antimycobacterial activity at concentrations 500µM and 1000µM, the MIC may be 500 µM. From the present study we identified 3 compounds (SSCP10, SSCP11 and SSCP12) which exhibited their Antimycobacterial activity for both the strains at 1000 µM concentration. The present study also showed 4 compounds (SSCP37, SSCP38, SSCP39 and SSCP54) exhibiting activity only towards the laboratory strain H37Rv.And the present study we identified 1compound (SSCP81) which is exhibited their Antimycobacterial activity for H37RV and both the strains at 1000µl concentration. We identified exhibiting activity the laboratory strain H37Rv, MTB MDRTB. And the present study we identified 5 compounds (SSCP94, SSCP97, SSCP98, SSCP99, and SSCP100) which is exhibited their Antimycobacterial activity for H37RV and both the strains at 250µl concentration.

NATURAL COMPOUNDS

S. No	Compound names	Year of working	Type of compounds	Total no of screening samples	Result	MABA DYE
1	SSCP 1	2015	Natural	12	4 active	DONE
2	SSCP 2	2015	Natural	4	active	NOT DONE
3	SSCP 3	2015	Natural	11	6 active	DONE
4	SSCP 4	2015	Natural	10	No active	NOT DONE

S.no	Compound names	Year of working	Type of compounds	Total no of screening samples	Result	MABA DYE
1	SSCP 5	2015	Synthetic	27	4 active	DONE
2	SSCP 6	2015	Synthetic	6	No activity	DONE
3	SSCP 7	2017	Synthetic	8	No activity	NOT DONE
4	SSCP 8	2017	Synthetic	6	1 active	NOT DONE
5	SSCP 9	2017	Synthetic	6	6 active	NOT DONE
6	SSCP 10	2018	Synthetic	5	2 active	NOT DONE
7	SSCP11	2018	Synthetic	6	5 active	NOT DONE

DISCUSSION

Mycobacterium tuberculosis plays an important role in drug resistance and spreads a wide range of drug resistance infections in the community based on the pervious studies conducted at sri Shivani college of pharmacy we tried to establish a simple and cost effective diagnostic testing protocols for first line drug resistance and new drug susceptibility testes. From decades there has been considerable interest in the generation of new drugs to control pathogenic microorganism and their drug resistance development. Many components of products have been shown to be specially targeted against resistant pathogenic bacteria. The emergence of multidrug resistant strain of many pathogens is serious threat and makes chemotherapy more difficult Levy, Stuart B; moreover the current cost of most of the chemotherapeutic agents is unbearable to the public especially in developing countries like India. The raise of multi-drug-resistant strains of *M. TB is* makes the discovery of new synthetic compounds a main concern, and the existing condition even necessitates the re-engineering and relocation of some aged drug families to attain effective control. Successful treatment for any bacterial infection is done only with the discovery and introduction of non toxic antibiotic over a certain period of time. Standard treatment for drug sensitive tuberculosis involves the intake of four first line drugs for 2months and later continued the use of two first line drugs for further four months[14] but in case of Multi drug resistant tuberculosis there is a need in the intake of drugs for a period of approximately 24 months. This has made difficult to continue the treatment for such a long period of time in the resource constrained countries. Early and rapid drug invention for TB is majorly dependent on the assessment of the effectiveness of compounds which can be evaluated using MIC. Alamar Blue involved MIC determination is widely used as the standard method in TB drug discovery and requires at least 1week to develop the results.[22] Therefore we had made an attempt of screening new compounds and method evaluations for testing the drug resistance in resource settings which may be made to direct towards the development of effective, non toxic drug for treatment of Mycobacterial infectious disease Tuberculosis so that it might contribute to reduce the duration of course of treatment for tuberculosis.

CONCLUSION

As there is urgent need to develop more effective and less toxic antibiotics to control the prevalence of

multidrug resistant tuberculosis, in the present study we tried to provide helpful information regarding the antimycobacterial activity DST testing method for new compounds. This study might lead to the development of some valuable compounds that has to be used to formulate new, different and more potent antimycobacterial drugs. Further studies are needed to identify the biologically active compounds and to evaluate the efficiency of the compound against pathogenic microorganisms associated with various human diseases using simple and cost effective drug testing methods.

REFERENCES

1. Gerald L. Mandell, John E. Bennett, Raphael (2010). Mandell, Douglas, and Bennett's principles and practice of infectious diseases. Chapter 250. ISBN 978-0-443-06839-3.
2. Bansal-Mutalik, Ritu & Nikaido, Hiroshi. (2014). Mycobacterial outer membrane is a lipid bilayer and the inner membrane is unusually rich in diacyl phosphatidylinositol dimannosides. Proceedings of the National Academy of Sciences of the United States of of America. 111.10.1073/pnas.1403078111.
3. WHO Tuberculosis Fact sheet Reviewed March 2017.
4. Anandakumar S, Shanmughavel P (2008) Computational Annotation for Hypothetical Proteins of *Mycobacterium Tuberculosis*. J Comput Sci Syst Biol, 1: 050-062.
5. **David, H. L.** 1970. Probability distribution of drug-resistant mutants in unselected populations of *Mycobacterium tuberculosis*. Appl. Microbiol., **20:** 810–814.
6. **Elliott, A. M., S. E. Berning, M. D. Iseman, and C. A. Peloquin.** 1995. Failure of drug penetration and acquisition of drug resistance in chronic tuberculous empyema. Tuber. Lung Dis., **76:** 463–467.
7. **Iseman, M. D., and L. A. Madsen.** 1991. Chronic tuberculous empyema with bronchopleural fistula resulting in treatment failure and progressive drug resistance. Chest, **100:** 124–127.
8. **McKinney, J. D., K. Honer zu Bentrup, E. J. Munoz-Elias, A. Miczak, B. Chen, W. T. Chan, D. Swenson, J. C. Sacchettini, W. R. Jacobs, Jr., and D. G. Russell.** 2000. Persistence of *Mycobacterium tuberculosis* in macrophages and mice requires the glyoxylate shunt enzyme isocitrate lyase. Nature, **406:** 735–738.
9. **Pablos-Mendez, A.** 2000. Working alliance for TB drug development, Cape Town, South Africa, February 8th, 2000. Int. J. Tuberc. Lung Dis., **4:** 489–490.
10. **Wayne, L. G.** 1994. Dormancy of *Mycobacterium tuberculosis* and latency of disease. Eur. J. Clin. Microbiol. Infect. Dis., **13:** 908–914.
11. Konstantinos A (2010). "Testing for tuberculosis". Australian Prescriber., 33(1): 12–18. Archived from the original on 4 August 2010.
12. *Woodring JH, Vandiviere HM, Fried AM, Dillon ML, Williams TD, Melvin IG* the radiographic features of pulmonary tuberculosis. *AJR Am J Roentgenol, 1986 Mar; 146(3): 497-506.*
13. Millard, James; Ugarte-Gil, Cesar; Moore, David A. J. (2015-02-26). "Multidrug resistant tuberculosis". BMJ., 350: h882. ISSN 1756-1833.
14. WHO MDR TB Factsheet 2016. Diagnosis and notification of multi drug resistant TB.
15. Basic TB Facts CDC. 13 March 2012. Archived from the original on 6 February 2016.
16. Longo, Fausci; et al. (2012). Harrison's Principles of Internal Medicine (18th ed.). New York: McGraw Hill. pp. Chapter 165: Tuberculosis.
17. *WHO*. October 2015. Archived from the original on 23 August 2012. Retrieved 11 February 2016.
18. Elbir H, Abdel-Muhsin AM, Babiker A (February 2008). "A one-step DNA PCR-based method for the detection of Mycobacterium tuberculosis complex grown on Löwenstein–Jensen media". Am. J. Trop. Med. Hyg., 78(2): 316–7. PMID 18256436.
19. *Krysl J, Korzeniewska-Kosela M, Müller NL, FitzGerald JM* Radiologic features of pulmonary tuberculosis: an assessment of 188 cases *Can Assoc Radiol J., 1994 Apr; 45(2): 101-7.*
20. Levy, Stuart B; Marshall, Bonnie 2004 **Antibacterial resistance worldwide: causes, challenges and responses Nature Medicine; New York** 10 Suppl 1.12s S122-S129.
21. Thulasi W, Maria M.H, Manuel Marine A, Gonzalo C, Esther P, Ana Isabel A. P, Maria T. F, Pedro A. T, Landys L. Q, Ben G, Julia R, Yan L, Selin S. K, David L., Nicholas C, Carl N and Alfonso M.L. . 2015, Identification of Novel Anti-mycobacterial Compounds by Screening a Pharmaceutical Small-Molecule Library against Nonreplicating Mycobacterium tuberculosis ACS Infect. Dis1, 580−585.
22. Aziz, M., K. Ryszewska, L. Blanc, V. Vincent, H. Getahun, A. Wright, P. Nunn, and M. Raviglione. 2007. Expanding culture and drug susceptibility testing capacity in tuberculosis diagnostic services: the new challenge. Int. J. Tuberc. Lung Dis., 11: 247–250. 2. Bemer, P., F. Palicova, S. Rusch.
23. Arain, T. M., A. E. Resconi, M. J. Hickey, and C. K. Stover. 1996. Bioluminescence screening in vitro (Bio-Siv) assays for high-volume antimycobacterial drug discovery. 17.Antimicrob. Agents Chemother., 40: 1536-1541.
24. Chung, G. A. C., Z. Aktar, S. Jackson, and K. Duncan. 1995. High-throughput screen for detecting antimycobacterial agents. Antimicrob. Agents Chemother., 39: 2235–2238.
25. Singh R, Dar SA, Sharma P. Antibacterial activity and toxicological evaluation of semipurified hexane extract of Urtica dioica leaves. Res J Med Plants, 2012; 6(2): 123-135.

26. Joseph mcfarland, m.d. jama. 1907; xlix(14): 1176-1178. the nephelometer: an instrument for estimating the number of bacteria in suspensions used for calculating the opsonic index and for vaccines.
27. Franzblau SG, Witzig RS, McLaughlin JC, Torres P, Madico G, Hernandez A, Degnan MT, Cook MB, Quenzer VK, Ferguson RM, Gilman RH. 1998. Rapid, low-technology MIC determination with clinical Mycobacterium tuberculosis isolates by using the microplate Alamar Blue assay. J. Clin. Microbiol, 36: 362–366.
28. Sreevalli Sharma, a Ekaterina Gelman, b Chandan Narayan, a Deepa Bhattacharjee, a Vijayashree Achar, a Vaishali Humnabadkar, a V. Balasubramanian, a Vasanthi Ramachandran, a Neeraj Dhar, b Neela Dinesha Simple and Rapid Method To Determine Antimycobacterial Potency of Compounds by Using Autoluminescent Mycobacterium tuberculosis. Antimicrobial Agents and Chemotherapy, October 2014; 58(10): 5801–5808.
29. Delogu G, Sali M, Fadda G (2013) The biology of Mycobacterium tuberculosis infection. Mediterr J Hematol, Infect Dis, p 5.
30. Zhang Y, Chen C, Liu J, Deng H, Pan A, Zhang L, Zhao X, Huang M, Lu B, Dong H, Du P, Chen W, Wan K (2011) Complete genome sequences of Mycobacterium tuberculosis strains CCDC5079 and CCDC5080, which belong to the Beijing family. J Bacteriol, 193: 5591.
31. Vanderberg D, Zoellner KR, Ogunrombi MO, Malan SF, TerreBlanche G, Castagnoli N, Bergh JJ, Petzer JP (2007) Inhibition of monoamine oxidase B by selected benzimidazole and caffeine analogues. Bioorg Med Chem, 15: 3692–3702.
32. Collins LS, 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: 1004–1009.
33. Franzblau SG, Witzig RS, McLaughlin JC, Torres P, Madico G, Hernandez A, Degnan MT, Cook MB, Quenzer VK, Ferguson RM, Gilman RH. Rapid, low-technology MIC determination with clinical Mycobacterium tuberculosis isolates by using the microplate Alamar Blue assay. J. Clin. Microbiol., 1998; 36: 362–366.