Abstract

Background: Tuberculosis is a well-known airborne disease caused by Mycobacterium tuberculosis. Available treatment regimens were unsuccessful in eradicating the deaths caused by the disease worldwide. Owing to the drawbacks such as prolonged treatment period, side effects, and drug tolerance, there resulted in patient noncompliance. In the current study, we attempted to develop inhibitors against unexplored key target glutamate racemase. Methods: Lead identification was done using thermal shift assay from in-house library; inhibitors were developed by lead derivatization technique and evaluated using various biological assays. Results: In indazole series, compounds 11 (6.32 ± 0.35 µM) and 22 (6.11 ± 0.51 µM) were found to be most promising potent inhibitors among all. These compounds also showed their inhibition on replicating and nonreplicating bacteria. Conclusion: We have developed the novel inhibitors against M. tuberculosis capable of inhibiting active and dormant bacteria, further optimization of inhibitor derivatives can results in better compounds for eradicating tuberculosis.

Keywords: Biofilm, cell wall synthesis, indazole, thermal shift assay, tuberculosis

INTRODUCTION

Tuberculosis (TB) is known to be one of the world’s most fatal diseases. According to the World Health Organization surveillance, every year nearly 10.4 million of new cases and about 1.4 million deaths were recorded despite many available approved drugs. The drawback of available treatment regimens pertaining to its long duration, emergence of drug-tolerant strains, namely, multidrug-resistant made treatment of the disease impossible. This shows the urgency for development of novel drugs targeting unexplored valid targets. It is a popular fact that virulence of organism is due to its complex cell wall. Although there were drugs reported against it, these primarily target polymerization step of cell wall synthesis, whereas the formation of monolayer of peptidoglycan was not exploited. Glutamate racemase (GR) is one of the key enzymes in peptidoglycan synthesis. It racemizes L-glutamate (L-glu) to D-glutamate (D-glu). Moreover, the absence of both enzyme and its product in mammals ensures the host safety. Inhibitors were reported in Staphylococcus aureus and Helicobacter pylori against GR and β-Chloro-D-Alanine against Mycobacterium tuberculosis. There is a need to develop new class of inhibitors to combat TB. In this study, we have used a fluorescence-based biophysical technique, thermal shift assay (TSA) as a screening tool for lead identification from our in-house compound library. This method has gained popularity owing to its advantages of being simple, fast, reliable, and economical.

METHODS

Thermal shift assay

All the reactions were carried out using real-time polymerase chain reaction (PCR) thermal cycler (Bio-Rad, CA). The reaction mixture containing GR enzyme (M. tuberculosis), substrate (D-glu), buffer (50 mM HEPES-Na [pH 7.5] and 100 mM NaCl), and SYPRO orange dye was set with gradient heating of 0.02°C/s from 20 to 80°C. Gradual heating unfolds the protein exposing its polar groups, SYPRO orange dye has tendency to bind to these polar sites of protein. Hence, monitoring the fluorescent (orange dye) readings determines the activity of the enzyme. The enzyme was considered inactive if there was no change in the fluorescence output over the course of heating.

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Glutamate racemase enzyme inhibition assay
The PCR amplified recombinant genes of *M. tuberculosis* H37Rv and *Bacillus subtilis* subsp. *subtilis* 168 GR was expressed in *Escherichia coli* BL21 (DE3) cells with N-terminal histidine tags and purified using Ni ± nitric acetic acid metal-affinity column. The purity was confirmed using sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The protein was stored in aliquots at −80°C.[9,13] The whole assay coupled enzyme assay was performed in triplicates. Reaction mixture of 100 µl contains 100 mM Tris–HCl pH 8.0, 5 mM NAD+, 1 mM D-glu, 10 U/mL L-glutamate dehydrogenase (LDH) (all the chemicals were procured from Sigma), 1 µM *B. subtilis* GR and test compound concentrations (50 µM to 0.5 µM). IC₅₀ was determined by measuring the LDH-mediated NADH (reduced form of nicotinamide adenine dinucleotide) formation at 340 nm using microplate spectrophotometer (Spectromax M4, Molecular devices, USA).[13]

Molecular docking simulations
Molecular docking studies on D-glu bound GR of *M. tuberculosis* (PDB ID: 5HJ7) and *B. subtilis* (PDB ID: 1ZUW) were performed using Schrodinger 9.3. Sitemap was utilized to generate allosteric sites. The preparation of protein, optimization, and grid generation was performed using Protein Preparation Wizard of Schrödinger Suite 9.3, OPLS_2005 (optimized potential for liquid simulations) force field and Glidegrid, respectively.[14] The ligands were energy minimized and docked using Glide XP module. The XP Glide scoring function was used to get the best ranked compounds and the interactions. Molecular dynamics (MD) simulations were run for 10 ns timeframe and calculations were run in Desmond using OPLS_2005 force field to study the stability pattern in solvent system environment. Defining simulation parameters, calculation of root mean square deviation (RMSD), and root mean square fluctuation (RMSF) profiles were carried following the protocol as per reports.[15,16]

**In vitro replicating Mycobacterium tuberculosis assay**
Susceptibility testing of compounds was done against laboratory isolated active (aerobic) *M. tuberculosis* H37Rv culture grown at temperature 37°C having OD₉₀₀ 1.0. Minimum inhibitory concentration (MIC) was determined using microplate alamar blue assay method (MABA).[17] Suspended culture in Middlebrook 7H9 broth (MB) supplemented with 10% oleic acid-albumin-dextrose-catalase (OADC) (HiMedia) was diluted in 1:20 of which 100 µl culture was used as inoculums. Each test compound was diluted in MB by four-fold, the final highest test concentration, diluted serially in sterile 96-well microtiter plates using 100 µl MB. Positive and negative controls along with standards rifampicin (RIF), ethambutol (ETH), and isoniazid (INH) were plated in duplicates and incubated at 37°C for a week. After a week, 30 µl of alamar blue solution was added to each well, and plates were reincubated for 12 h. Color change from blue to pink indicates the growth and the lowest drug concentration at no color change was noted as their individual MIC.

**Nutrient starvation model**
Active *M. tuberculosis* H37Rv laboratory isolated culture was deprived of nutrients (suspended in phosphate buffer saline [PBS]) for 6 weeks to attain dormancy. The test and standards were added to cultures at a concentration of 10 µg/mL and incubated for 1 week. After a week, cell suspensions were diluted by 10-fold with MB (with 10% OADC), 100 µl of each dilution was plated in sterile 48-well plates containing 450 µl of MB in triplicates and incubated at 37°C for 4 weeks. Bacterial growth was counted and most probable number (MPN) values were calculated using standard statistical methods.[18]

**Kill-kinetics determination**
Active *M. tuberculosis* H37Rv laboratory isolated culture with OD₉₀₀ 0.6–1.0 was centrifuged and pellet was diluted to OD₉₀₀ 0.1 with 10% PBS-Tyloxapol. After incubation for 2 weeks, to each tube labeled with the control (dimethyl sulfoxide), inhibitor with test concentrations (5, 10, and 20 µM) were added with 5 mL of PBS-Tyloxapol and 50 µl of starved culture followed by incubation at 37°C. The test compound treated cell suspensions were plated at 0, 7, 14, and 21 day intervals for every concentration. The bacterial count was calculated using standard statistical methods using MPN assay.[19]

**Biofilm assay determination**
Active *M. tuberculosis* H37Rv laboratory isolated culture with OD₉₀₀ between 0.7–1.0 was incubated for 5 weeks in Sautons media (HiMedia) at 37°C to form biofilm. To the matured biofilm test, compound was added at desired concentration (*n* = 4) and sealed. After a week of incubation, Tween-80 (0.1% v/v) was added, swirled for about 15 min. The contents were centrifuged and washed with 5 mL of wash buffer (PBS with 10% glycerol and 0.05% Tween-80) for three times. Obtained pellet was suspended in 5 mL of wash buffer and kept on rocking for 12 h. The persistence of bacteria in the biofilm population was determined by comparing antibiotic-treated plates with positive control plates by MPN assay.[20,21]

**Cytotoxicity determination**
Toxicity profile of all compounds was determined with inhibition assay on mouse macrophage cells (RAW 264.7).[22] Sterile 96-well microtiter plates were incubated with cells and compounds at different concentrations for 48 h at 37°C in triplicates.[23] After the incubation period, 10 µl of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT reagent) (10 mg/mL) was added and reincubated for 3 h. After 3 h, media was removed and 200 µl of DMSO was added to each well. Readings at OD₅₆₀ was recorded using Perkin Elmer Victor X3 microplate reader. The cytotoxicity was calculated as % inhibition at each concentration.
RESULTS

Library screening and lead identification

Medium throughput screening of our in-house compound library with chemically diversified structures employing TSA with GR of *M. tuberculosis* and inhibitory enzyme assay with GR of *B. subtilis* (described in discussion section) was carried out to identify Lead 1 [Figure 1].

Chemical procedures and characterization

The target derivatives were synthesized using protocol depicted in Scheme 1. β-acetylphenylhydrazine, hydroxylamine hydrochloride, hydrochloric acid, and chloral hydrate to get N-acetylaminooisonitrosoacetanilide with good yield. N-acetylaminoisonitrosoacetanilide was further reacted with sulfuric acid to give indazole-3-carboxylic acid. The formed compound was suspended in glacial acetic acid and bromine at cold conditions to result in 5-bromo-1H-indazole-3-carboxylic acid as yellow precipitate with good yield. 5-bromo-1H-indazole-3-carboxylic acid, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDCI) (3 mmol), and hydroxybenzotriazole were added along with different amines procured from commercial sources. The precipitates obtained were collected, extracted with ethyl acetate (15 ml thrice) and water, the organic layers were dried over sodium sulfate and evaporated to get solid which is purified by column chromatography using ethyl acetate: hexane to yield respective amide derivatives 4–24.

Inhibitor identification and characterization by enzyme inhibitory assay, molecular docking, and thermal shift assay

All the synthesized compounds were tested for their inhibitory activity by enzyme assay against GR of *B. Subtilis* following above described protocol and determined their half maximal inhibitory concentration (IC$_{50}$) values [Table 1]. To establish the bridge between the inhibition of GR in both *B. Subtilis* and *M. tuberculosis* by synthesized molecules, we have opted for evaluation using molecular docking and simulation studies. These studies have proved that similar kind of interactions and simulations were shown by inhibitor molecules in both the organisms [Figures 2-6]. Potent inhibitors (based on IC$_{50}$ values) were determined for their mode of inhibition using TSA. Based on the binding abilities with native protein as well as changes in binding with respect to the presence/absence of substrate (D-glu), inhibitor was classified [Figure 7].

Drug susceptibility testing

All the synthesized molecules and standard drugs were tested against replicating *M. tuberculosis* H37Rv strain using MABA assay and the MIC was determined for all compounds Table 1.

Nutrient starved model

Considering the IC$_{50}$ and MIC values, compounds 11 and 22 were selected to determine their inhibitory profile along with

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**Figure 1:** (a) Thermal shift assay plots of a *Mycobacterium tuberculosis* glutamate racemase native, with D-glu and lead 1 (b) represents the plot of derivative fluorescent-based signal against temperature; $T_m$ and $\Delta T_m$ (shift) can be measured from the minimum of the plot (c) Structure of identified lead 1 with $T_m$ and IC$_{50}$ value in *Bacillus subtilis*
standards against nonreplicating/dormant *M. tuberculosis* H37Rv culture [Figure 8].

**Kill-kinetics study**

It is necessary to evaluate the time kill profile compounds at different concentrations against time. Based on the results and inhibitory profiles of all inhibitors, compound 22 was selected for kill-kinetic study [Figure 9].

**Biofilm assay**

To test compounds for their inhibition against drug tolerance, biofilms of *M. tuberculosis* H37Rv were grown and compounds 11 and 22 were tested against persistent bacterial biofilms. Further, they are compared with standard drugs inhibition [Figure 10].

**DISCUSSION**

In the present study, we performed a medium throughput virtual screening using TSA and enzyme assays. A relative analysis of melt temperature ($T_m$) exposed that native protein in the absence of D-glu and ligand showed a melt temperature approximately of 43.1°C, whereas with D-glu showed $T_m$ at 44.3°C approximately. Screened compounds showed a varied range of positive and negative shifts in $T_m$ [Figure 1]. Of the entire compound library, lead 1 showed a significant shift in $T_m$ with native protein at 46.9°C inferring that its stabilizing capability and interactions with protein are much better than D-glu alone as well as than rest of the compound library. Recent reports have stated that it is not possible to obtain catalytically active form of protein in solution state.[9] Therefore, we have considered performing activity assay on GR of *B. subtilis* having 40% and 56% of respective sequence identity and similarity with *M. tuberculosis* and carried out the enzyme inhibitory assay following the recent reports.[13] The screening by enzyme assay also showed good inhibitory activity on GR of *B. subtilis* by lead 1 with an IC$_{50}$ of 19.73 ± 0.65 µM. Hence, we considered lead 1 to optimize further through synthetic strategies (see supplementary).

### Table 1: Synthesized compounds represented with substitutions along with their biological activities

| Compound | R | IC$_{50}$ (µM) | MIC (µM) | Cytotoxicity at 25 µM (percentage of inhibitor) |
|----------|---|----------------|----------|-----------------------------------------------|
| 4        | 4-nitrophenyl | >25 | 69.31 | ND | ND | 30.41 |
| 5        | 3-(trifluoromethyl) phenyl | 11.9±0.24 | 65.15 | 2.05 | 16.23 | 58.11 |
| 6        | 3-chlorophenyl | >25 | 71.45 | ND | ND | 27.98 |
| 7        | 2,6-diethylphenyl | >25 | 67.24 | ND | ND | 60.32 |
| 8        | 2-hydroxy-4-nitrophenyl | >25 | 66.39 | ND | ND | 20.02 |
| 9        | 2-bromophenyl | >25 | 31.67 | ND | ND | 46.61 |
| 10       | Thiazol-2-yl | >25 | 77.41 | ND | ND | 40.72 |
| 11       | Benz[de] thiazol-2-yl | 6.32±0.35 | 67.09 | 67.09 | 67.09 | 37.22 |
| 12       | Pyridin-3-yl | >25 | 78.93 | ND | ND | 49.35 |
| 13       | 4-fluorophenol | 8.11±0.76 | 74.92 | ND | ND | 58.81 |
| 14       | 3-chloro-2-methylphenyl | >25 | 68.79 | ND | ND | 57.23 |
| 15       | Pyrimidin-2-yl | >25 | 39.37 | ND | ND | 79.36 |
| 16       | 5-nitrothiazol-2-yl | 10.96±0.13 | 78.62 | 78.62 | 78.62 | 37.71 |
| 17       | 3,4-dichlorophenyl | >25 | 64.98 | ND | ND | 20.98 |
| 18       | 5-nitrothiazol-2-yl | >25 | 67.82 | ND | ND | 31.82 |
| 19       | 5-chloro-2-methoxyphenyl | >25 | 65.23 | ND | ND | 50.71 |
| 20       | 2,4-dimethylphenyl | >25 | 72.78 | ND | ND | 46.98 |
| 21       | 2,5-dimethylphenyl | >25 | 72.78 | ND | ND | 64.21 |
| 22       | 2-methoxy-4-nitrophenyl | 6.11±0.51 | 63.93 | 63.93 | 63.93 | 60.23 |
| 23       | 5-chloro-2-hydroxyphenyl | >25 | 68.26 | ND | ND | 36.67 |
| 24       | 2-hydroxy-5-methylphenyl | >25 | 72.38 | ND | ND | 54.53 |
| Isoniazid | >25 | 0.72 | 0.72 | 0.72 | ND |
| Ethambutol | >25 | 7.64 | 3.82 | 3.82 | ND |
| Rifampicin | >25 | 0.15 | 0.15 | 0.15 | ND |

ND: Not determined, MIC: Minimum inhibitory concentration, IC$_{50}$: Half maximal inhibitory concentration
We have performed the inhibitory enzyme assay for all the synthesized compounds using GR of *B. subtilis*. The inhibitory activity results of compounds were listed in Table 1. Among all, five compounds have shown considerable inhibitory activity better than lead 1 (IC<sub>50</sub> 19.73 ± 0.65 µM). Compounds 11, 13, and 22 have shown activity < 10 µM. Compound 22 has shown the highest activity among the group with IC<sub>50</sub> of 6.11 ± 0.51 µM. In comparison with lead 1, compound 22 has shown better activity by three times. Log dose–response curve of compound 22 is shown in Figure 2.

Further, to validate the inhibitory activities of synthesized molecules against enzyme in both the organisms, we have employed computational techniques, namely, molecular modeling and dynamics. We analyzed the binding poses of synthesized compounds with the support of molecular docking. We performed docking and MD of molecules (picked based on IC<sub>50</sub> values) in the crystal structures of *M. tuberculosis* and *B. subtilis* GR in complex with D-glutamate (PDB ID: 5HJ7, 1ZUW, respectively) using Glide XP docking and Desmond (Schrodinger). The inhibitors were not docked properly on the substrate binding site (D-glutamate) in both proteins; assuming the inhibitors might be acting through other pocket in enzyme, we have tried docking in allosteric sites generated using sitemap. Based on site-scores, site 1 in both the proteins was selected for further docking studies. Here, we discuss the binding pattern of one of the active compounds 11 (IC<sub>50</sub> 6.32 ± 0.35 µM), the superimposition of both proteins and compound 11 in its binding mode was shown in Figure 3. The 2D-binding orientation of compound
11 within the two proteins binding allosteric sites were represented in Figure 4. The predicted bound conformation of the active compound showed interactions with the side chain of the Glu 153 in both proteins. Apart from this, the compound further stabilized through various hydrophobic and few polar amino acid residue interactions. The compound was very well fit into the allosteric site cavity of the protein with a docking scores of -3.578 kcal/mol and -4.892 kcal/mol in 5HJ7 and 1ZUW, respectively. Thus, molecular docking was a great help in supporting the confirmation of inhibitory activity in both the organisms. Compound 11 in 5HJ7 and 1ZUW protein complexes were subjected to a 10 ns simulation. The RMSD analysis plots for the protein-ligand complexes have been carried out so as to measure the distance between atoms during simulation [Figure 5]. RMSD for 1ZUW, Cα and ligand were within the average of ~1.7 Å and ~1.0 Å, and for 5HJ7 both Cα and ligand were within average of ~1.5 Å and ~ 1.1 Å, respectively, during 10 ns simulation trajectory. The RMSF analysis shows the fluctuation range undergone by every residue in the protein during simulation. RMSF plot for compound 11 in both protein complexes was shown in [Figure 6]. Figure shows least fluctuations of the ligand.
In dormant model, *M. tuberculosis* cultures were starved of nutrients in PBS for 6 weeks. After 6 weeks, the culture was treated with the synthesized test compounds and standard drugs at a concentration of 10 µg/mL. Considering the IC₅₀ and MIC values of the synthesized compounds 11 and 22 were selected for testing in nonreplicating *M. tuberculosis* H37Rv culture assays. INH, RIF, and moxifloxacin (MOXI) were selected for standard comparison. INH, RIF, and MOXI have shown an inhibition of 1, 1.8, and 2.2 log reduction, respectively, compared with control [Figure 8]. Test compounds 11 and 22 have shown a log reduction of 1.4 and 1.3, respectively. This indicates that test compounds were showing better activities than INH on dormant culture.

Kill kinetics is a kind for determining the pattern or type of kill an individual antagonist is following like bactericidal or bacteriostatic. A compound is said to be bactericidal if the minimum bactericidal concentration (MBC) of a compound is less than fourfold of their respective MIC value. Likewise, if the MBC is greater than fourfold of their MBC, then the compound is termed as bacteriostatic. We have evaluated compounds at different concentrations on bacteria obtained after 2 weeks of nutrient starvation at 0, 7, 14, and 21 days after drug treatment. Compounds 11 and 22 showing good inhibition in above study on nutrient starved culture have shown MBC more than fourfold of their MIC values indicating that they are bacteriostatic. The kinetic graph of compound 22 was shown in Figure 9.

Biofilms are formed by the persistent bacteria where many standard drugs such as INH and RIF have failed to show their activity. Hence, we tested our compounds for their action against such biofilm-forming persistent bacteria. Compounds 11 and 22 were >idoglycan/cell wall synthesis of bacteria. Of all molecules in series, compounds 11 and 22 were represented to be most actives in series, equipotent to standard drugs against replicating and nonreplicating bacteria. As the push for novel antitubercular drugs is growing in today’s scenario, this chemical class of compounds can be suit% for further drug development studies.

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**Conflicts of interest**  
There are no conflicts of interest.

**References**

1. World Health Organization. Global Tuberculosis Report; 2016.
2. Singh R, Rajni, Meena A, Meena LS. Multidrug resistant and extensively drug resistant TB: A nuisance to medical science. J Bacteriol Parasit
2011:2:105.
3. Velayati AA, Farnia P, Masjedi MR. The totally drug resistant tuberculosis (TDR-TB). Int J Clin Exp Med 2013;6:307-9.
4. Favrot L, Ronning DR. Targeting the mycobacterial envelope for tuberculosis drug development. Expert Rev Anti Infect Ther 2012;10:1023-36.
5. Israyilova A, Buroni S, Forneris F, Scoffone VC, Shixaliyev NQ, Riccardi G, et al. Biochemical characterization of glutamate racemase as a new candidate drug target against Burkholderia cenocepacia infections. PLoS One 2016;11:e0167350.
6. Fisher SL. Glutamate racemase as a target for drug discovery. Microb Biotechnol 2008;1:345-60.
7. Ruzheinikov SN, Taal MA, Sedelnikova SE, Baker PJ, Rice DW. Substrate-induced conformational changes in Bacillus subtilis glutamate racemase and their implications for drug discovery. Structure 2005;13:1707-13.
8. Geng B, Basarab G, Comita-Prevoir J, Gowravaram M, Hill P, Kiely A, et al. Potent and selective inhibitors of Helicobacter pylori glutamate racemase (MurI): Pyridodiazepine amines. Bioorg Med Chem Lett 2009;19:930-6.
9. Prosser GA, Rodenburg A, Khoury H, de Chiara C, Howell S, Snijders AP, et al. Exploring the structure of glutamate racemase from Mycobacterium tuberculosis as a template for anti-myobacterial drug discovery. Antimicrob Agents Chemother 2016;60:6091-9.
10. Ciulli A. Biophysical screening for the discovery of small-molecule ligands. Methods Mol Biol 2013;1008:357-88.
11. Rudolf'AF, Skovgaard T, Knapp S, Jensen LJ, Berthelsen J. A comparison of protein kinases inhibitor screening methods using both enzymatic activity and binding affinity determination. PLoS One 2014;9:e98800.
12. Venkatraman J, Bhat J, Solapurude SM, Sandesh J, Sarkar D, Aishwarya S, et al. Screening, identification, and characterization of mechanistically diverse inhibitors of the Mycobacterium tuberculosis enzyme, pantothenate kinase (CoaA). J Biomol Screen 2012;17:293-302.
13. Poen S, Nakatani Y, Opel-Reading HK, Lassé M, Dobson RC, Krause KL, et al. Exploring the structure of glutamate racemase from Mycobacterium tuberculosis as a template for anti-mycobacterial drug discovery. Biochem J 2016;473:1267-80.
14. Schrödinger Suite 2012 Protein Preparation Wizard; Epik, v2.2; Impact, v5.7; Prime v2.3. New York: Schrödinger, LLC; 2012.
15. Kräutler V, van Gunsteren WF, Hünenberger PH. A fast SHAKE algorithm to solve distance constraint equations for small molecules in molecular dynamics simulations. J Comput Chem 2001;22:501-8.
16. Suryadevapara Y, Yogeeswarip, Soni V, Devi PB, Nandicoori VK, Sriram D, et al. Computational sampling and simulation based assessment of novel Mycobacterium tuberculosis glutamine synthetase inhibitors: Study involving structure based drug design and free energy perturbation. Curr Top Med Chem 2016;16:978-95.
17. Reck F, Alm R, Brassil P, Newman J, Dejonge B, Eyermann CJ, et al. Novel N-linked aminopiperidine inhibitors of bacterial topoisomerase type II: Broad-spectrum antibacterial agents with reduced hERG activity. J Med Chem 2011;54:7834-47.
18. Betts JC, Lukey PT, Robb LC, McAdam RA, Duncan K. Evaluation of a nutrient starvation model of Mycobacterium tuberculosis persistence by gene and protein expression profiling. Mol Microbiol 2002;43:717-31.
19. Parish T, Stoker NG. Mycobacteria Protoc 1998. p. 269-79.
20. Kulka K, Hatfull G, Ojha AK. Growth of Mycobacterium tuberculosis biofilms. J Vis Exp 2012. pii: 3820.
21. Ojha AK, Baughn AD, Sambandan D, Hsu T, Trivelli X, Guerardel Y, et al. Growth of Mycobacterium tuberculosis biofilms containing free mycolic acids and harbouring drug-tolerant bacteria. Mol Microbiol 2008;69:164-74.
22. Reck F, Alm RA, Brassil P, Newman JV, Ciaccio P, McNulty J, et al. Novel N-linked aminopiperidine inhibitors of bacterial topoisomerase type II with reduced pK(a): Antibacterial agents with an improved safety profile. J Med Chem 2012;55:6916-33.
23. Charfison PS, Grillot AL, Grossman TH, Parsons JD, Badia M, Bellon S, et al. Novel dual-targeting benzimidazole urea inhibitors of DNA gyrase and topoisomerase IV possessing potent antibacterial activity: Intelligent design and evolution through the judicious use of structure-guided design and structure-activity relationships. J Med Chem 2008;51:5243-63.