Accepted Manuscript

Synthesis of novel 1,2,3-triazole derivatives of isoniazid and their *in vitro* and *in vivo* antimycobacterial activity evaluation

Deepak Kumar, Beena, Garima Khare, Saqib Kidwai, Anil K. Tyagi, Ramandeep Singh, Diwan S. Rawat

PII: S0223-5234(14)00418-8

DOI: 10.1016/j.ejmech.2014.05.005

Reference: EJMECH 6960

To appear in: *European Journal of Medicinal Chemistry*

Received Date: 30 December 2013

Revised Date: 1 April 2014

Accepted Date: 1 May 2014

Please cite this article as: D. Kumar, Beena, G. Khare, S. Kidwai, A.K. Tyagi, R. Singh, D.S Rawat, Synthesis of novel 1,2,3-triazole derivatives of isoniazid and their *in vitro* and *in vivo* antimycobacterial activity evaluation, *European Journal of Medicinal Chemistry* (2014), doi: 10.1016/j.ejmech.2014.05.005.

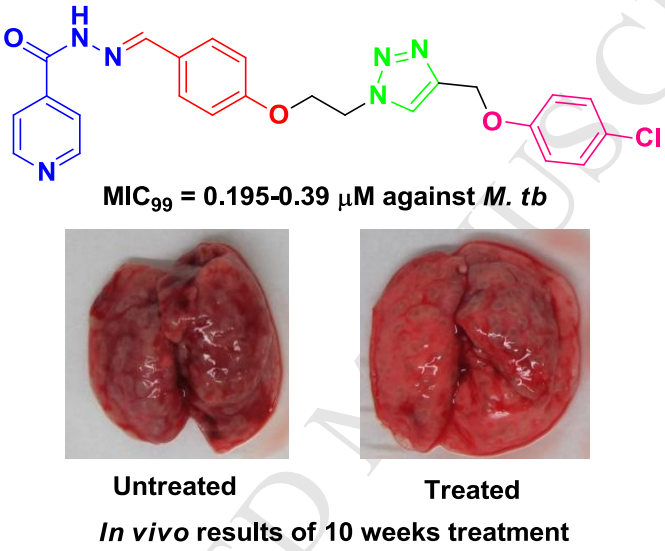
Highlights

- *In vitro* antituberculosis activity of novel 1,2,3-triazole derivatives of isoniazid was reported against *M. tuberculosis* H37Rv.
- Compounds exhibited potent activity with MIC ranging from 0.195 to 1.56 μM *in vitro*.
- One compound when tested *in vivo*, showed significant activity.
- Compounds were nontoxic against THP-1 cell line even at 50 μM concentration.

Synthesis of novel 1,2,3-triazole derivatives of isoniazid and their *in vitro* and *in vivo* antimycobacterial activity evaluation

Deepak Kumar[a], Beena[a], Garima Khare[b], Saqib Kidwai[c], Anil K. Tyagi[b], Ramandeep Singh[c], Diwan S Rawat[a]*

Graphical abstract



Synthesis of novel 1,2,3-triazole derivatives of isoniazid and their *in vitro* and *in vivo* antimycobacterial activity evaluation

Deepak Kumar[a], Beena[a], Garima Khare[b], Saqib Kidwai[c,] Anil K. Tyagi[b], Ramandeep Singh[c], Diwan S Rawat[a]*

[a] Department of Chemistry, University of Delhi, Delhi-110007, India

[b]Department of Biochemistry, University of Delhi, South Campus, New Delhi-110021, India

[c]Translational Health Science and Technology Institute, Vaccine and Infectious Disease Research Centre, Gurgaon, 122016, India

Abstract

We report herein the synthesis and antimycobacterial activity of 1,2,3-triazole derivatives of isoniazid. Most of the compounds exhibited potent activity against *Mycobacterium tuberculosis* H37Rv strain with MIC_{99} values ranging from 0.195 to 1.56 μM *in vitro*. One compound showed better *in vitro* activity than the reference, whereas five compounds were equally potent to the reference compound isoniazid. The cytotoxicity of these compounds was studied against THP-1 cell line and no toxicity was observed even at 50 μM concentration. The compound with most potent *in vitro* activity was evaluated for *in vivo* in murine model of tuberculosis and significantly reduced bacillary load in both lungs and spleen at 10 weeks post-treatment. However this clearance effect was more pronounced in the case of spleen. Molecular docking and molecular dynamics simulations have been performed using two targets 2IDZ 1 (wild type Enoyl-acyl-carrier-protein reductase) and 4DQU 2 (mutant type Enoyl-acyl-carrier-protein reductase) to study the binding orientation and stability of the compound **47**. Docking studies proved compound **47** fit well into the binding pocket of both the targets. Molecular dynamic simulations concluded that the highest active compound **47** in complex with 4DQU was more stable when compared to the 2IDZ. We believe that further optimization of these molecules may lead to potent anti-tubercular agents.

Key Words: antimycobacterial, isoniazid, MDR-TB, TDR-TB, LL-3858, *in vivo*.

*Corresponding author:

E. Mail: dsrawat@chemistry.du.ac.in

Phone No: 91-11-27667465

Fax No: 91-11-27667501

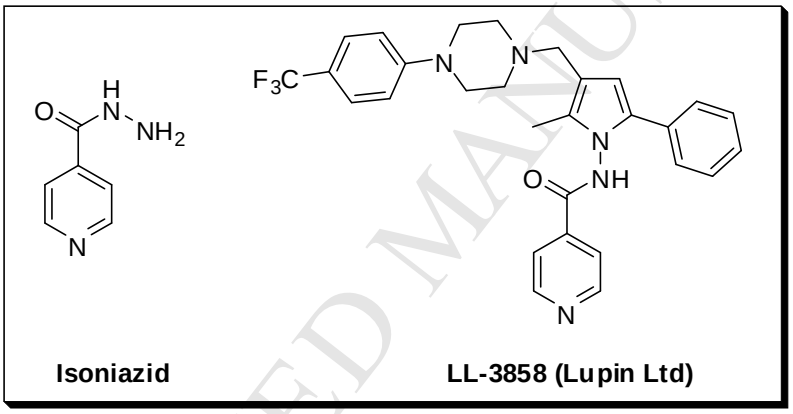
1. Introduction

Tuberculosis (TB), one of the leading infectious diseases, still remains a major global health problem [1]. The number of people infected with TB and the deaths reported every year is enormously high. In 2010, WHO reported 9.4 million new TB cases and 1.7 million deaths due to tuberculosis [2]. In terms of number of TB cases, India alone is responsible for one-fifth of the global TB incidences [3]. It is anticipated that by 2020, one billion people may be newly infected and over 30 million may die due to TB. The situation becomes more severe to people with human immunodeficiency virus (HIV) infection [4,5]. TB in most instances is a curable disease and more than 90% of people with drug-susceptible TB can be cured within six months using combinations of first-line drugs. However, the emergence of multi drug resistant (MDR) and extensively drug resistant (XDR) tuberculosis has worsen the situation and its treatment is more challenging [1,6]. It requires the use of second-line drugs that are more costly, cause more severe side effects, and must be taken up for two years [7]. Recently, a more dangerous and completely incurable form of tuberculosis, totally drug resistance tuberculosis (TDR) has also been reported in Italy, Iran and more recently in India [8-12]. TDR-TB strain has been shown to be resistant to all the first line, second line and third line anti-TB drugs. Therefore, there is an urgent need to develop novel anti-TB agents, which are synthetically feasible, have fewer side effects and shorter duration of treatment. Although, several new compounds are currently in different stages of clinical trials [13-17], only one new drug bedaquiline has been recently approved by FDA for its use in drug resistant tuberculosis [18-20].

Isonicotinic acid hydrazide (Isoniazid or INH), a first-line anti-TB drug is one of the most effective agents used for the treatment of *Mycobacterium tuberculosis* infection since 1952 [21,22]. It is a pro-drug and gets activated by a catalase-peroxidase enzyme known as KatG in mycobacteria. The activated form reacts non-enzymatically with coenzymes, NAD^+ and $NADP^+$ to form isonicotinoyl-NAD(P) complex [23-25], which binds with the enoyl-acyl carrier protein (ACP) reductase InhA. The enzyme, InhA helps in the synthesis of mycolic acid. Thus isoniazid inhibits the synthesis of mycolic acid, required for the mycobacterial cell wall. Unfortunately, bacterial strains resistant to INH are becoming common and with the emergence of multi-drug resistant tuberculosis especially among persons with HIV infection, even combination therapy is also no longer successful. Last few years have witnessed the synthesis of a number of lipophilic analogues of isoniazid (INH) and some of them exhibited

good *in vitro* antimycobacterial activity [26-33]. It has been suggested that the increased lipophilicity of INH derivatives may facilitate diffusion through bio-membranes, therefore enhancing the antimycobacterial activity. Moreover, by functionalising the hydrazine group of isoniazid and retaining its activity can avoid the toxicity and other severe problems related to the inactivation of isoniazid by the enzyme *N*-acetyltransferase-2.

LL-3858 is an isoniazid derivative and is developed by Lupin Limited. It has shown bactericidal activity against both drug sensitive and multidrug resistant TB [34]. Phase I clinical trial studies of LL-3858 have shown the potential of treating pulmonary tuberculosis patients effectively [35]. The mechanism of action of LL-3858 is not yet known. Currently this compound is in the initial stage of phase II clinical trial for the treatment of tuberculosis [36].



The 1,2,3-triazoles are known for several biological activities such as antibacterial, antiviral, anti-inflammatory, analgesic, anticancer, anticonvulsant and anti-platelet [37-46]. Triazoles also have shown very promising anti-TB activity and antifungal activity *via* inhibiting the cell wall synthesis [47,48]. Recently, the concept of hybrid molecules has been the most interesting topic in medicinal chemistry, where two or more pharmacophores are linked covalently resulting into one molecule [49-51]. The two units of final molecule may act on different targets to exert dual drug action or one part can counterbalance the side effects caused by another part. As discussed both isoniazid and triazoles act by similar mechanism *i.e.* inhibition of cell wall synthesis and both are very potent anti-TB agents, thus keeping these things in our mind and in continuation of our efforts towards the synthesis of novel biological important molecules [52-64], we proposed to link isoniazid and triazole entities together and synthesized novel 1,2,3-triazoles derivatives of isoniazid. These two scaffolds were joined covalently into one single derivative in anticipation that the hybrid may

show potent anti-TB activity. All the synthesized compounds were evaluated for their *in vitro* antituberculosis activity against *M. tuberculosis* H37Rv.

2. Chemistry

For the synthesis of the title compounds, firstly different benzaldehydes having triazole moiety (**6-22**, **34-38**) were synthesized as outlined in scheme 1 and 2. The 4-hydroxybenzaldehyde (**1**) was treated with 1,2-dibromoethane or 1,3-dibromopropane to get the compounds **2** and **3** as depicted in scheme 1. The bromo group of compounds **2** and **3** was then converted into azido group (**4** and **5**) by reaction with sodium azide in the presence of K_2CO_3 as base and dimethylformamide as solvent. The cycloaddition of azide group and terminal alkyne *via* click reaction gave different triazole based compounds (**6-22**). Additionally, benzaldehydes having isomeric triazole linkage (**34-38**) were also synthesized by different route as shown in scheme 2. The 4-hydroxybenzaldehyde (**1**) was treated with propargyl bromide to obtain compound **23** [65]. Also the alkyl halides **29-33**, were converted to alkyl azides (**34-38**) in presence of K_2CO_3 using dimethylformamide as solvent. The cycloaddition of these alkyl azides (**34-38**) and terminal alkyne (**23**) *via* click reaction gave triazole based compounds (**34-38**). These compounds (**6-22** and **34-38**) were then condensed with isoniazid to get the desired compounds (**39-55** and **56-60**) in good yields as depicted in scheme 3.

All the new compounds were characterized by their IR, ^{13}C NMR and mass spectral data analysis. The formation of compound **39** was confirmed by the IR spectrum, which showed peaks at 3222 and 1675 cm^{-1} due to NH and C=O groups, respectively. In the ^{1}H NMR of compound **39**, a triplet at δ 0.88 was assigned to the terminal methyl protons of *n*-butyl group. The sextet and quintet at δ 1.57 and 2.26 respectively were assigned to the two internal methylene protons of *n*-butyl group. The CH_2 protons of *n*-butyl group directly attached to the triazole ring appeared as triplet at δ 2.55. The two triplets at δ 4.01 and 4.47 were assigned to the protons of CH_2 protons attached to oxygen and nitrogen atoms in the ethylene linker, respectively. Peak at δ 8.38 was assigned to the N=CH proton. Proton of triazole ring was observed at δ 7.87. All aromatic protons appeared in the region of δ 6.99 to 8.76. The NH proton of the amide group appeared at δ 11.93. The ^{13}C NMR spectra showed peaks at δ 13.62, due to the terminal methyl carbon of *n*-butyl group, whereas peaks at δ 22.27, 27.08 and 29.45 were assigned to the methylene carbons of *n*-butyl group. The methylene carbons, NCH_2 and OCH_2 of the ethylene linker appeared at δ 46.26, 64.70,

respectively. The carbon of N=CH linkage appeared at δ 148.86. The most downfield peak at δ 161.42 was assigned to the carbonyl carbon of amide linkage. The remaining nine peaks at 114.87, 121.52, 121.94, 126.72, 128.93, 140.61, 146.77, 150.32 and 160.18 were due to the rest of the carbons of phenyl, pyridine and triazole nucleus. Further structure of compound **39** was confirmed by the mass spectral analysis which showed peaks at 393.4422 and 415.3933 due to $(M + H)^+$ and $(M + Na)^+$, respectively.

<Insert Scheme 1 here>

<Insert Scheme 2 here>

<Insert Scheme 3 here>

3. Biological Activity

3.1. *In vitro* anti-tuberculosis assay

A stock culture of *M. tb* H37Rv (ATCC 27294) was grown to Abs_{600nm} of 0.2 in Middlebrook 7H9 broth (Difco) supplemented with 0.05% Tween 80, 0.2% glycerol and albumin/NaCl/glucose (ADS) complex. The culture was diluted 1:1000 in 7H9-based medium before aliquoting 50 µL into each well of a 96-well plate. Compounds were dissolved in DMSO (Sigma) to make stock solutions of 50 mM. Compounds (100 µL solution) were added to the first row of the 96-well plate at a final concentration 100 µM. 2-fold serial dilutions were made and 5 dilutions of each compound (50 µM-0.195 µM) were tested for anti-mycobacterial activity. The compounds were diluted 1:1 by addition of 50 µL of 1:1000 diluted cultures. Row 6 and 12 of the 96-well plates were no compounds control. The plates were incubated at 37 °C and the MIC_{99} values were read macroscopically using an inverted plate reader after 14 days. MIC_{99} is defined as the minimum inhibitory concentration of the compound required for 99% inhibition of bacterial growth. Each measurement was repeated thrice.

3.2. *In vivo* anti-tuberculosis assay

The most active compound **47** from the series was selected for *in vivo* antituberculosis activity evaluation. For activity evaluation, pathogen-free Balb/c mice of either sex (25-30 g) were procured from the Division of Laboratory Animals, Central Drug Research Institute, Lucknow, India. The animals were maintained in a BSLIII animal facility at University of

Delhi South Campus, New Delhi and routinely cared according to the guidelines of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals), India. All the experimental protocols included in this study were reviewed and approved by the Institutional Animal Ethics Committee (Ref No. 1/IAEC/AKT/Biochem/UDSC/14.10.2011).

Mice were challenged with *M. tb.* H37Rv bacilli by respiratory route in inhalation chamber (Glascol Inc., USA) pre-calibrated to deliver approximately 1000 bacilli per animal in the lung by using frozen stocks of *M. tb.* H37Rv with their CFU pre-determined. Mice were euthanized on the day after infection to determine the number of CFU implanted in the lungs. Following two weeks of infection, mice were divided into different groups − untreated, DMSO, isoniazid (25 mg/kg), rifampicin (10 mg/kg), compound **47** (25 mg/kg, 50 mg/kg, 100 mg/kg) and therapy was initiated.

All drugs were administered once daily, five days per week, in a maximum volume of 0.175 mL by oral gavage. Before initiating the chemotherapy, the infection in the animals was verified by euthanizing a group of animals (N = 5) at 2 weeks post infection followed by pathological observation and enumeration of the bacillary load in lungs and spleen. Mice (N = 5 each group) were euthanized by CO_2 asphyxiation after three weeks, six weeks and ten weeks time points post therapy and monitored for gross pathological observations and bacillary load. For bacterial enumeration, mice were dissected and lungs and spleens were aseptically removed and homogenized in saline. Appropriate dilutions of the homogenates were plated in duplicates onto MB7H11 agar and the plates were incubated at 37 °C for 3–4 weeks followed by enumeration of colonies. The results were expressed as $\log_{10}$ CFU per organ.

At 3 weeks time point, a bacillary load of 6.38 and 4.19 $\log_{10}$ CFU was measured in the lungs and spleens of untreated animals, respectively. The disease continued to persist and at 6 weeks post infection also, a bacillary load of 5.91 and 3.68 $\log_{10}$ was recorded in the lungs and spleens of untreated animals, respectively. At 10 weeks post infection, the disease further progressed with a bacillary load of 6.04 and 4.02 $\log_{10}$ in the lungs and spleens of untreated animals, respectively.

3.3. In Silico Studies

Selected proteins 2IDZ and 4DQU was obtained from the Protein Data Bank (www.rcsb.org) and further modified using Schrodinger's Glide docking protocol. For Glide

v5.8 calculations, both the complexes were imported to Maestro v9.3. Using Protein Preparation Wizard (PPW), included biological unit and assigned bond orders, created zero order bonds to metals, created disulfide bonds, converted any selenomethionines to methionines, deleted water molecules beyond 5 Å from hetero groups, generated metal binding states, added missing hydrogens, filled any missing side chains and loops and capped the termini making use of the prime module integrated within protein preparation wizard (PPW). Under review and modify tab of PPW, apart from Aricept, all the co-crystallized ligands were identified and generated states. Under the refine tab of PPW, optimization of the H-bond network was carried out to fix the overlapping hydrogens and the most likely positions of hydroxyl and thiol hydrogen atoms, protonation states and tautomers of 'His' residues, and Chi 'flip' assignments for 'Asn', 'Gln' and 'His' residues were selected by the protein assignment script shipped by Schrodinger. The pH range was set to 7.0 and the protein was minimized by applying OPLS 2005 force field. Restrained minimizations were performed until the average root mean square deviation (RMSD) of the non-hydrogen atoms converged to 0.3Å. Location and surface area of active site/subsites of the targets were predicted using Sitemap module integrated within Schrödinger software. Compound **47** was also refined to its minimized state. Proteins and compound **47** refined was used for XP docking, which is a flexible ligand docking protocol.

Desmond v3.1 Package was used to study the thermodynamic stability of the ligand-receptor complex. Predefined TIP4P water model was used to simulate water molecules using OPLS_2005 force field. Orthorhombic periodic boundary conditions were set up to specify the shape and size of the repeating unit. In order to neutralize the system electrically, Na^+ ions were added to balance the system charge and were placed randomly in the solvated system. After building the solvated system containing protein in complex with the ligand, the system was minimized and relaxed using default protocol integrated within Desmond. Molecular dynamic simulations were carried out with the periodic boundary conditions in the NPT ensemble. The temperature and pressure were kept at 300 K and 1 atmospheric pressure using Nose-Hoover temperature coupling and isotropic scaling, and the operation was followed by running the 10 ns NPT production simulation and saving the configurations thus obtained with 5ps intervals with a time step of 4.8 ps. All the MD runs were performed on a Dell workstation with 8 processors, each of which is Intel(R) Xeon(R) CPU E5607@2.27 GHz, with 16 GB DDR RAM. Desmond v3.1 integrated within Schrodinger was compiled and run under Linux CentOS 6.1 operating system.

4. Results and Discussion

All the isoniazid-triazoles conjugates (**39**-**60**) were tested for their *in vitro* anti-mycobacterial activity against *M. tuberculosis* H37Rv. Isoniazid was used as a reference drug. Table 1 and 2 shows the minimum concentration for all the derivatives that inhibited the growth of 99% of the input cells (MIC_{99}). Results show that most of the compounds exhibited excellent activity with MIC_{99} values ranging from 0.39 to 1.56 μM. Seven compounds **43**-**46**, **49, 58** and **59** were found to be potent as standard compound isoniazid (MIC_{99} = 0.39 μM). Compound **47** was the most active derivative (MIC_{99} = 0.195-0.39 μM), being about two fold more effective than isoniazid. The lipophilicity (ClogP values) of the isoniazid-triazole conjugates was also calculated and it was remarkably higher than the isoniazid which is very important in terms of permeability through biomembranes.

<Insert Table 1 here>

<Insert Table 2 here>

After 3 weeks of treatment with isoniazid, CFU in the lungs of infected animals was reduced by 1.22 log_{10} while after 6 weeks it showed a further CFU reduction in the bacillary load by 2.59 log_{10} and a furthermore reduction of 3.38 log_{10} at 10 weeks when compared with the animals in the untreated group (Fig. 1A). In spleens also, isoniazid treatment resulted in a sharp reduction of bacillary load with a 2.66 log_{10} reduction at 3 weeks when compared with the animals in the untreated group (Fig. 1B). At 6 weeks and 10 weeks time point, no bacilli were recovered from the spleens. Whereas, after 3 weeks of treatment with rifampicin, the CFU in the lungs of infected animals was reduced by 1.93 log_{10} while at 6 weeks it showed a further CFU reduction in the bacillary load by 2.8 log_{10} when compared with the animals in the DMSO group. After 10 weeks of rifampicin administration, no bacilli were recovered from the lungs of the infected animals (Fig. 1A). In spleens also, rifampicin treatment resulted in a marked reduction in the bacillary load with a 1.76 log_{10} and 3.31 log_{10} reduction at 3 weeks and 6 weeks, respectively when compared with the animals in the DMSO group. At 10 weeks time point, no bacilli were recovered from the spleens (Fig. 1B).

The evaluation of the efficacy of compound **47** was carried out at 25 mg/kg, 50 mg/kg and 100 mg/kg concentrations. Compound **47**, when administered at a low concentration of 25 mg/kg demonstrated no effect on the multiplication of bacilli in lungs at 3 weeks and 6 weeks time point as was evident from a comparable bacillary load in the lungs of compound

47 treated animals and DMSO treated animals. An extension of the time period up to 10 weeks also did not make any further improvement in the bacillary burden present in the lungs of compound **47** treated animals in comparison to DMSO treated animals at this concentration (Fig. 1A). An increase in the concentration of compound **47** to 50 mg/kg also showed no significant improvement in the control of pulmonary bacillary load at all the time points evaluated. Even when compound **47** was given at a higher dose of 100 mg/kg for 3 weeks, there was no significant reduction in the bacillary load in the lungs when compared with the DMSO treated animals, however, extending the time of therapy to 6 weeks and 10 weeks displayed a 0.82 $\log_{10}$ CFU and 0.91 $\log_{10}$ CFU reduction in the bacillary load in the lungs of the animals when compared with the DMSO treated animals (Fig. 1A).

<Insert Fig. 1 here>

<Insert Fig. 2 here>

The efficacy of compound **47** was also examined in terms of its ability to reduce the splenic bacillary load (Fig. 1B). The observations were also made in order to assess the role of compound **47** in curtailing the hematogenous spread of the bacilli. It was observed that at 25 mg/Kg concentration of compound **47**, the compound was unable to exert any influence on the replication of splenic bacilli of the animals treated with the compound when compared with the DMSO treated animals at 3 weeks and 6 weeks time points. Extension of the time period up to 10 weeks exhibited improvement in the efficacy of the compound and splenic bacillary load was reduced by 1.5 $\log_{10}$ CFU in comparison to the bacillary load in DMSO treated animals. However, when the concentration of compound **47** was increased to 50 mg/kg, a substantial therapeutic effect was observed which was evident by a significant reduction in the splenic bacillary load. At this concentration of compound **47**, at the end of 3 weeks of chemotherapy, a reduction of 1.08 $\log_{10}$ CFU was observed in the splenic bacillary load when compared with the DMSO treated animals. The extension of chemotherapy up to 6 weeks resulted in a significantly higher reduction (2.15 $\log_{10}$ CFU) in the bacillary load. On further extension of therapy period to 10 weeks, a very significant reduction in the bacillary load (3.31 $\log_{10}$ CFU) in the spleens was observed when compared with the DMSO treated animals (Fig. 1B). The efficacy of compound **47** was even more pronounced at a concentration of 100 mg/kg. When animals were administered 100 mg/kg compound **47** for 3 weeks, the splenic bacillary load exhibited a reduction of 1.46 $\log_{10}$ when compared with that of the DMSO treated animals. Further continuation of the chemotherapy up to 6 weeks and

10 weeks resulted in a more prominent reduction in the bacillary load by 2.58 $\log_{10}$ in the spleens of the animals treated with compound **47** when compared with the DMSO treated animals (Fig. 1B).

All the compounds were further examined for toxicity in THP-1 cell line. The compounds were found to be non-toxic upto a concentration of 50 μM the highest concentration tested (Table 1). It was not possible to test toxicity at higher concentrations due to solubility limitations.

Furthermore, compound **47** was docked into both wild type InhA (2IDZ 1) and mutant type InhA (4DQU 2) for identifying the binding orientation and stability of the compound **47** [66, 67]. Docking was performed using Glide module present in the Maestro 6.5 3 [68]. Binding pockets of both the proteins are almost similar, but Asp 148 of 2IDZ is changed to Gly 148 in 4DQU (Fig. 3). Docking scores of -7.14 and -9.48 were obtained for compound **47** against 2IDZ and 4DQU proteins. Compound **47** fit well into the binding pockets of both the targets. Stability in the binding pockets is due to the presence of hydrophobic amino acids in the pocket. The 2IDZ protein contains Ile 95, Val 65, Leu 63, Ile 122, Phe 97, Ile 16, Ile 21, Met 147, Phe 149, Met 155, Ala 191, Tyr 158, Pro 193 and Trp 222 in the binding pocket, and 4DQU contains Ile 16, Phe 41, Ile 122, Leu 63, Val 65, Ile 95, Phe 97, Met 147, Pro 193 and Ile 194.

Acquired complexes were redirected to molecular dynamic simulations of 10 ns. Predefined TIP4P water model [69] was used to simulate water molecules using OPLS_2005 force field [70,71]. RMSD (Root mean square deviation) of 2IDZ complex with compound **47** was in between 1.5 to 3.5 Å throughout the simulation (Fig. 4). There was fluctuation between the 2.5 to 4.5 ns and after 5 ns it got stabilized. Interesting results were observed in the case of 4DQU complex with compound **47**. This complex was more stable when compared to the initial one. RMSD of this complex was in between 1.5 to 2 Å. RMSF (Root mean square fluctuation) was also analyzed for the both the complexes and there was no much difference in the fluctuations of amino acids during the run (Fig. 5). Fluctuations of the amino acids in complexes were coinciding throughout the simulation showing meagre difference. Superimposed RMSD and RMSF results confirmed the stability of the resistant target when compared with the wild type. These theoretical studies may be useful to give insights into the activity of compound **47** against resistant targets.

<Insert Fig. 3 here>

<Insert Fig. 4 here>

<Insert Fig. 5 here>

5. Conclusions

A series of 22 isoniazid-triazole conjugates were synthesized and evaluated for their *in vitro* and *in vivo* antitubercular activity. Most of the compounds exhibited potent activity and one compound exhibited better activity than isoniazid *in vitro*. When tested for *in vivo* activity this compound exhibited mild activity both in case of lung as well as spleen. The influence of the compound on the replication of the pathogen was far superior in the case of spleen as compared to the influence on the lung. We do not have clear explanation for this observation at present however it might suggest that the bio-availability of these compounds could be the possible reason for the better activity profile in the case of spleen. It is not unlikely that this difference could stem from the fact that the required concentration of the compound could be more easily available in the blood stream as compared to pulmonary tissue thereby leading to a more pronounced effect on the control of bacillary load in spleens as the bacteria residing in spleens results from the hematogenous spread. However, translation of this speculation into real evidence would require further experiments. In terms of toxicity, all the compounds were found to be nontoxic upto 50 μM concentration against THP-1 cell line. Docking and molecular dynamics simulations results indicated that compound **47** showed important amino acids interactions with both wild type and mutant type proteins, and displayed significant stability in the binding pocket, which may be considered as a positive sign for compound **47** in terms of activity against INH-resistant strains of *M. tuberculosis*.

From the activity and toxicity results, it can be concluded that these compounds exhibited potent activity in *in vitro* but unfortunately mild activity in *in vivo* and are safe in terms of toxicity profile and hence can be used as lead molecule for further optimisation. The major problem faced during performing the experiments was the solubility of these compounds therefore efforts are being made to improve the solubility of these compounds so that the real therapeutic potential of the compounds can be evaluated.

6. Experimental Protocols

All the chemicals were purchased from Sigma-Aldrich. Solvents used for the chemical synthesis were acquired from commercial sources, were of analytical grade and used without further purification. Thin layer chromatography (Merck Kiesel 60 F254, 0.2 mm thickness) was used to monitor the progress of the reactions and the compounds were purified by silica gel (60-120 mesh) column chromatography. Melting points were recorded on EZ-Melt automated melting point apparatus, Stanford Research Systems and are uncorrected. IR spectra were recorded on Perkin-elmer FT-IR spectrophotometer using KBr pellets or as film in chloroform and the values were expressed in cm^{-1}. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on Jeol ECX spectrospin instrument using $CDCl_3$ or DMSO-d_6 as solvent and TMS as internal reference. The chemical shift values were expressed on δ scale and the coupling constant (*J*) in Hz. Mass data were recorded in Jeol-Accu TOF JMS-T100LC mass spectrometer.

*6.1. Typical procedure for the synthesis of 4-(2-bromoethoxy)benzaldehyde (**2**) and related compound (**3**):* A mixture of 4-hydroxybenzaldehyde (**1**, 5.0 g, 0.041 mol), 1,2-dibromoethane (23.0 g, 0.122 mol) and K_2CO_3 (16.9 g, 0.122 mol) in dry DMF (80 mL) was stirred at 35-40 ^{o}C for 8-10 h. After completion of the reaction, cold water was added to the reaction mixture and the compound was extracted with $CHCl_3$ (3 x 50 mL). The organic layer was washed with cold water to remove DMF. The organic phase was dried over Na_2SO_4 and the excess solvent was removed under reduced pressure. The crude product was purified by column chromatography using EtOAc:hexane as eluent to obtain compound **2**.

*6.2. Typical procedure for the synthesis of 4-(2-azidoethoxy)benzaldehyde (**4**) and related compound (**5**):* To a stirred solution of compound **2** (6.0 g, 0.026 mol) and K_2CO_3 (10.8 g, 0.078 mol) in DMF (50 mL), NaN_3 (5.1 g, 0.078 mol) was added slowly. The reaction mixture was stirred at 50 °C for 4-5 h. After cooling down to room temperature, water was added to dissolve excess NaN_3 and the solution was extracted with $CHCl_3$ (3 x 50 mL). The combined organic layer was washed several times with water to remove DMF and then dried over Na_2SO_4. Excess solvent was distilled off under reduced pressure to get compound **4** in quantitative yield.

*6.3. Typical procedure for the synthesis of 4-(2-(4-butyl-1H-1,2,3-triazol-1-yl)ethoxy) benzaldehyde (**6**) and related compounds (**7-22**):* 4-(2-Azidoethoxy)benzaldehyde (**4**, 500 mg, 2.6 mmol) and 1-butyne (170 mg, 3.1 mmol) were dissolved in 20 mL of *t*-BuOH at room temperature. To this, a solution of $CuSO_4.5H_2O$ (130 mg, 0.52 mmol) and sodium

ascorbate (207 mg, 1.0 mmol) in 20 mL of water was added. The reaction mixture was stirred at 35-40 °C for 4-5 h. After completion, the reaction mixture was extracted with EtOAc (3 x 40 mL). The organic layer was dried over Na_2SO_4 and the excess solvent was removed under reduced pressure. Crude product was purified by column chromatography to get the desired compound **6**.

*6.3.1. 4-(2-(4-Butyl-1H-1,2,3-triazol-1-yl)ethoxy)benzaldehyde (**6**).* Yield 85%; IR (film, cm^{-1}): 2957, 2937, 2871, 2860, 2741, 1690, 1602, 1580, 1312, 1254, 1217, 1162, 1045; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.92 (t, *J* = 7.3 Hz, 3H, $CH_2CH_2CH_2CH_3$), 1.37 (sextet, *J* = 7.3 Hz, 2H, $CH_2CH_2CH_2CH_3$), 1.64 (quintet, *J* = 7.3 Hz, 2H, $CH_2CH_2CH_2CH_3$), 2.71 (t, *J* = 7.3 Hz, 2H, $CH_2CH_2CH_2CH_3$), 4.45 (t, *J* = 5.8 Hz, 2H, NCH_2), 4.76 (t, *J* = 5.1 Hz, 2H, OCH_2), 6.97 (d, *J* = 8.8 Hz, 2H, Ar*H*), 7.44 (s, 1H), 7.83 (d, *J* = 8.8 Hz, 2H, Ar*H*), 9.89 (s, 1H, *CHO*).

*6.3.2. 4-(2-(4-(1-Hydroxycyclohexyl)-1H-1,2,3-triazol-1-yl)ethoxy)benzaldehyde (**7**).* Yield 78%; IR (film, cm^{-1}): 3394, 2929, 2854, 1680, 1601, 1254, 1161, 1053, 907; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.18 (s, 1H), 1.26-1.32 (m, 1H), 1.47-1.49 (m, 2H), 1.54-1.56 (m, 1H), 1.66-1.69 (m, 2H), 1.82 (s, 2H), 1.89-1.91 (m, 2H), 2.32 (brs, 1H, *OH*), 4.39 (t, *J* = 5.1 Hz, 2H, NCH_2), 4.71 (t, *J* = 5.1 Hz, 2H, OCH_2), 6.90 (d, *J* = 8.0 Hz, 2H, Ar*H*), 7.59 (s, 1H), 7.76 (d, *J* = 8.8 Hz, 2H, Ar*H*), 9.81 (s, 1H, *CHO*).

*6.3.3. 4-(2-(4-(Hydroxymethyl)-1H-1,2,3-triazol-1-yl)ethoxy)benzaldehyde (**8**).* Yield 74%; IR (film, cm^{-1}): 3364, 2925, 2853, 1677, 1600, 1578, 1508, 1311, 1252, 1162, 1042; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.37 (brs, 1H, CH_2OH), 4.46 (t, *J* = 5.1 Hz, 2H, NCH_2), 4.81 (t, *J* = 5.1 Hz, 4H), 6.98 (d, *J* = 8.8 Hz, 2H, Ar*H*), 7.32 (s, 1H), 7.84 (d, *J* = 8.8 Hz, 2H, Ar*H*), 9.89 (s, 1H, *CHO*).

*6.3.4. (1-(2-(4-Formylphenoxy)ethyl)-1H-1,2,3-triazol-4-yl)methyl propionate (**9**).* Yield 86%; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.12 (t, *J* = 7.3 Hz, 3H, $COCH_2CH_3$), 2.34 (q, *J* = 7.3 Hz, 2H, $COCH_2CH_3$), 4.46 (t, *J* = 5.1 Hz, 2H, NCH_2), 4.81 (t, *J* = 5.1 Hz, 2H, OCH_2), 5.22 (s, 2H, CH_2OCO), 6.98 (d, *J* = 8.8 Hz, 2H, Ar*H*), 7.80 (s, 1H), 7.84 (d, *J* = 8.8 Hz, 2H, Ar*H*), 9.89 (s, 1H, *CHO*).

*6.3.5. 4-(2-(4-(Pyridin-2-yl)-1H-1,2,3-triazol-1-yl)ethoxy)benzaldehyde (**10**).* Yield 65%; ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.51 (t, *J* = 5.1 Hz, 2H, NCH_2), 4.88 (t, *J* = 5.1 Hz, 2H, OCH_2), 7.01 (d, *J* = 8.8 Hz, 2H, Ar*H*), 7.23-7.25 (m, 1H, Ar*H*), 7.76-7.81 (m, 1H, Ar*H*), 7.83 (d, *J* =

8.8 Hz, 2H, Ar*H*), 8.18 (d, *J* = 7.3 Hz, 1H, Ar*H*), 8.35 (s, 1H), 8.59 (d, *J* = 5.1 Hz, 1H, Ar*H*), 9.88 (s, 1H, *CHO*).

*6.3.6. 4-(2-(4-(Phenoxymethyl)-1H-1,2,3-triazol-1-yl)ethoxy)benzaldehyde (**12**).* Yield 70%; ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.45 (t, *J* = 5.1 Hz, 2H, N*CH*$_2$), 4.80 (t, *J* = 5.1 Hz, 2H, O*CH*$_2$), 5.22 (s, 2H, *CH*$_2$OPh), 6.94-6.99 (m, 5H, Ar*H*), 7.26-7.30 (m, 2H, Ar*H*), 7.79 (s, 1H), 7.82 (d, *J* = 9.8 Hz, 2H, Ar*H*), 9.88 (s, 1H, *CHO*).

*6.3.7. 4-(2-(4-(p-Tolyloxymethyl)-1H-1,2,3-triazol-1-yl)ethoxy)benzaldehyde (**13**).* Yield 78%; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.28 (s, 3H, *CH*$_3$), 4.45 (t, *J* = 5.1 Hz, 2H, N*CH*$_2$), 4.80 (t, *J* = 5.1 Hz, 2H, O*CH*$_2$), 5.19 (s, 2H, *CH*$_2$OPh), 6.87 (d, *J* = 8.8 Hz, 2H, Ar*H*), 6.95 (d, *J* = 8.8 Hz, 2H, Ar*H*), 7.07 (d, *J* = 8.8 Hz, 2H, Ar*H*), 7.78 (s, 1H), 7.82 (d, *J* = 8.8 Hz, 2H, Ar*H*), 9.88 (s, 1H, *CHO*).

*6.3.8. 4-(2-(4-((4-Chlorophenoxy)methyl)-1H-1,2,3-triazol-1-yl)ethoxy)benzaldehyde (**14**).* Yield 75%; ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.45 (t, *J* = 5.1 Hz, 2H, N*CH*$_2$), 4.81 (t, *J* = 5.1 Hz, 2H, O*CH*$_2$), 5.19 (s, 2H, *CH*$_2$OPh), 6.90 (d, *J* = 9.5 Hz, 2H, Ar*H*), 6.94 (d, *J* = 8.8 Hz, 2H, Ar*H*), 7.22 (d, *J* = 8.8 Hz, 2H, Ar*H*), 7.79 (s, 1H), 7.82 (d, *J* = 8.8 Hz, 2H, Ar*H*), 9.88 (s, 1H, *CHO*).

*6.3.9. 4-(3-(4-Propyl-1H-1,2,3-triazol-1-yl)propoxy)benzaldehyde (**15**).* Yield 85%; IR (film, cm^{-1}): 3137, 3076, 2960, 2933, 2873, 1688, 1601, 1578, 1510, 1313, 1257, 1161, 1049; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.94 (t, *J* = 7.3 Hz, 3H, CH_2CH_2*CH*$_3$), 1.66 (sextet, *J* = 7.3 Hz, 2H, CH_2*CH*$_2$$CH_3$), 2.43 (quintet, *J* = 5.9 Hz, 2H, OCH_2*CH*$_2$$CH_2N$), 2.68 (t, *J* = 7.3 Hz, 2H, *CH*$_2$$CH_2CH_3$), 4.05 (t, *J* = 5.9 Hz, 2H, N*CH*$_2$), 4.56 (t, *J* = 6.6 Hz, 2H, O*CH*$_2$), 6.98 (d, *J* = 8.8 Hz, 2H, Ar*H*), 7.27 (s, 1H), 7.84 (d, *J* = 8.8 Hz, 2H, Ar*H*), 9.89 (s, 1H, *CHO*).

*6.3.10. 4-(3-(4-Butyl-1H-1,2,3-triazol-1-yl)propoxy)benzaldehyde (**16**).* Yield 80%; IR (film, cm^{-1}): 2950, 1685, 1603, 1251, 1041, 806; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.91 (t, *J* = 7.3 Hz, 3H, $CH_2CH_2CH_2$*CH*$_3$), 1.35 (sextet, *J* = 7.3 Hz, 2H, CH_2CH_2*CH*$_2$$CH_3$), 1,62 (quintet, *J* = 7.3 Hz, 2H, CH_2*CH*$_2$$CH_2CH_3$), 2.43 (quintet, *J* = 6.6 Hz, 2H, OCH_2*CH*$_2$$CH_2N$), 2.43 (t, *J* = 6.6 Hz, 2H, *CH*$_2$$CH_2CH_2CH_3$), 4.03 (t, *J* = 5.9 Hz, 2H, N*CH*$_2$), 4.56 (t, *J* = 6.6 Hz, 2H, O*CH*$_2$), 6.98 (d, *J* = 8.8 Hz, 2H, Ar*H*), 7.27 (s, 1H), 7.84 (d, *J* = 8.8 Hz, 2H, ArH), 9.89 (s, 1H, *CHO*).

*6.3.11. 4-(3-(4-(1-Hydroxycyclohexyl)-1H-1,2,3-triazol-1-yl)propoxy)benzaldehyde (**17**).* Yield 75%; IR (film, cm^{-1}): 3394, 2933, 2857, 1686, 1600, 1577, 1509, 1256, 1218, 1160,

1047; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.31-1.36 (m, 1H), 1.50-1.55 (m, 2H), 1.59-1.64 (m, 1H), 1.67-1.78 (m, 2H), 1.82-1.86 (m, 2H), 1.92-1.95 (m, 2H), 2.04 (s, 1H, *OH*), 2.45 (quintet, *J* = 5.9 Hz, 2H, $OCH_2CH_2CH_2N$), 4.07 (t, *J* = 5.1 Hz, 2H, NCH_2), 4.57 (t, *J* = 6.6 Hz, 2H, OCH_2), 6.98 (d, *J* = 8.8 Hz, 2H, Ar*H*), 7.45 (s, 1H), 7.83 (d, *J* = 8.0 Hz, 2H, Ar*H*), 9.88 (s, 1H, *CHO*).

*6.3.12. 4-(3-(4-(Hydroxymethyl)-1H-1,2,3-triazol-1-yl)propoxy)benzaldehyde (**18**).* Yield 77%; IR (film, cm^{-1}): 3370, 2926, 2853, 1686, 1601, 1577, 1509, 1313, 1258, 1218, 1161, 1045; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.10 (s, 1H, *OH*), 2.45 (quintet, *J* = 6.6 Hz, 2H, $OCH_2CH_2CH_2N$), 4.07 (t, *J* = 5.9 Hz, 2H, NCH_2), 4.60 (t, *J* = 6.6 Hz, 2H, OCH_2), 4.78 (s, 2H, CH_2OH), 6.98 (d, *J* = 8.8 Hz, 2H, Ar*H*), 7.56 (s, 1H), 7.83 (d, *J* = 8.8 Hz, 2H, Ar*H*), 9.89 (s, 1H, *CHO*).

*6.3.13. (1-(3-(4-Formylphenoxy)propyl)-1H-1,2,3-triazol-4-yl)methyl propionate (**19**).* Yield 79%; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.12 (t, *J* = 5.8 Hz, 3H, $COCH_2CH_3$), 2.34 (quintet, *J* = 7.3 Hz, 2H, $OCH_2CH_2CH_2N$), 2.45 (q, *J* = 5.8 Hz, 2H, OCH_2CH_3), 4.07 (t, *J* = 5.8 Hz, 2H, NCH_2), 4.59 (t, *J* = 6.6 Hz, 2H, OCH_2), 5.21 (s, 2H, CH_2OCO), 6.98 (d, *J* = 8.8 Hz, 2H, Ar*H*), 7.61 (s, 1H), 7.84 (d, *J* = 8.0 Hz, 2H, Ar*H*), 9.89 (s, 1H, *CHO*).

*6.3.14. 4-(3-(4-(Phenoxymethyl)-1H-1,2,3-triazol-1-yl)propoxy)benzaldehyde (**20**).* Yield 74%; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.45 (quintet, *J* = 5.6 Hz, 2H, $OCH_2CH_2CH_2N$), 4.06 (t, *J* = 5.1 Hz, 2H, NCH_2), 4.60 (t, *J* = 6.6 Hz, 2H, OCH_2), 5.21 (s, 2H, CH_2OPh), 6.96 (d, *J* = 8 Hz, 5H, Ar*H*), 7.29 (d, *J* = 7.3 Hz, 2H, Ar*H*), 7.62 (s, 1H), 7.82 (d, *J* = 8.8 Hz, 2H, Ar*H*), 9.88 (s, 1H, *CHO*).

*6.3.15. 4-(3-(4-(p-Tolyloxymethyl)-1H-1,2,3-triazol-1-yl)propoxy)benzaldehyde (**21**).* Yield 76%; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.28 (s, 3H, CH_3), 2.45 (quintet, *J* = 6.6 Hz, 2H, $OCH_2CH_2CH_2N$), 4.06 (t, *J* = 5.9 Hz, 2H, NCH_2), 4.60 (t, *J* = 6.6 Hz, 2H, OCH_2), 5.17 (s, 2H, CH_2OPh), 6.85 (d, *J* = 8.8 Hz, 2H, Ar*H*), 6.96 (d, *J* = 8.8 Hz, 2H, Ar*H*), 7.07 (d, *J* = 8.8 Hz, 2H, Ar*H*), 7.61 (s, 1H), 7.82 (d, *J* = 8.8 Hz, 2H, Ar*H*), 9.88 (s, 1H, *CHO*).

*6.3.16. 4-(3-(4-((4-Chlorophenoxy)methyl)-1H-1,2,3-triazol-1-yl)propoxy)benzaldehyde (**22**).* Yield 72%; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.46 (quintet, *J* = 5.9 Hz, 2H, $OCH_2CH_2CH_2N$), 4.05 (t, *J* = 5.9 Hz, 2H, NCH_2), 4.61 (t, *J* = 6.6 Hz, 2H, OCH_2), 5.17 (s, 2H, CH_2OPh), 6.89 (d, *J* = 9.5 Hz, 2H, Ar*H*), 6.95 (d, *J* = 8.8 Hz, 2H, Ar*H*), 7.22 (d, *J* = 8.8 Hz, 2H, Ar*H*), 7.61 (s, 1H), 7.83 (d, *J* = 8.8 Hz, 2H, Ar*H*), 9.89 (s, 1H, *CHO*).

*6.4. Procedure for synthesis of 4-(prop-2-ynyloxy)benzaldehyde (**23**):* To a mixture of *para*-hydroxybenzaldehyde (**1**, 5.0 g, 0.041 mol) and K_2CO_3 (16.9 g, 0.122 mol) in dry DMF (25 mL), a solution of propargyl bromide (5.2 g, 0.049 mol) in DMF (50 mL) was added dropwise. The reaction mixture was stirred at room temperature for 10-12 h. Progress of the reaction was monitored by thin layer chromatography. After completion, the reaction mixture was poured into ice cold water and extracted with chloroform (3 x 50 mL). The combined organic layer was washed with cold water to remove DMF and dried over anhydrous Na_2SO_4. Excess solvent was removed under reduced pressure and crude product thus obtained was purified over silica gel column using EtOAc/Hexane as an eluent to afford compound **23**.

*6.5. Typical procedure for the synthesis of 1-azidopropane (**29**) and related compounds (**30**-**33**):* Sodium azide (792 mg, 0.012 mol) was added to a solution of 1-bromopropane (**24**, 500 mg, 0.004 mol) and K_2CO_3 (1.68 g, 0.012 mol) in dry DMF (15 mL). The mixture was stirred for 2-3 h at 50 ^oC. After cooling down to room temperature, water was added to dissolve excess NaN_3 and compound was extracted with $CHCl_3$ (3 x 50 mL). The organic layer was washed with water. The resulting organic layer was dried over Na_2SO_4 and solvent was removed under vacuum to get compound **29**.

*6.6. Typical procedure for the synthesis of 4-((1-propyl-1H-1,2,3-triazol-4-yl) methoxy)benzaldehyde (**34**) and related compounds (**35**-**38**):* 4-(Prop-2-ynyloxy)benzaldehyde (**23**, 500 mg, 3.12 mmol) and 1-azidopropane (**29**, 265 mg, 3.12 mmol) were dissolved in 20 mL of *t*-BuOH at room temperature. To this, a solution of $CuSO_4.5H_2O$ (156 mg, 0.62 mmol) and sodium ascorbate (248 mg, 1.24 mmol) in 20 ml of water was added. The reaction mixture was stirred at 35-40 °C for 4-5 h. After completion, the reaction mixture was extracted with EtOAc (3 x 40 mL). Organic layer was dried over Na_2SO_4 and the excess solvent was removed under reduced pressure. Crude product was purified by column chromatography to get the pure compound **34**.

*6.6.1. 4-((1-Propyl-1H-1,2,3-triazol-4-yl)methoxy)benzaldehyde (**34**).* Yield 80%; ^1H NMR (400 MHz, $CDCl_3$): δ 0.97 (t, *J* = 7.3 Hz, 3H, $NCH_2CH_2\mathit{CH_3}$), 1.96 (sextet, *J* = 7.3 Hz, 2H, $NCH_2\mathit{CH_2}CH_3$), 4.34 (t, *J* = 7.3 Hz, 2H, $N\mathit{CH_2}$), 5.30 (s, 2H, $\mathit{OCH_2}$), 7.11 (d, *J* = 8.8 Hz, 2H, Ar*H*), 7.63 (s, 1H), 7.84 (d, *J* = 8.8 Hz, 2H, Ar*H*), 9.89 (s, 1H, *CHO*).

*6.6.2. 4-((1-iso-Propyl-1H-1,2,3-triazol-4-yl)methoxy)benzaldehyde (**35**).* Yield 82%; IR (film, cm^{-1}): 2982, 2928, 2852, 1689, 1600, 1578, 1508, 1311, 1247, 1215, 1161, 1110; ^1H

NMR (400 MHz, $CDCl_3$): δ 1.60 (d, *J* = 6.6 Hz, 6H, CH(*CH*$_3$)$_2$), 4.85 (septet, *J* = 6.6 Hz, 1H, *CH*(CH$_3$)$_2$), 5.29 (s, 2H, O*CH*$_2$), 7.12 (d, *J* = 8.8 Hz, 2H, Ar*H*), 7.67 (s, 1H), 7.84 (d, *J* = 8.8 Hz, 2H, Ar*H*), 9.89 (s, 1H, *CHO*).

*6.6.3. 4-((1-Butyl-1H-1,2,3-triazol-4-yl)methoxy)benzaldehyde (**36**).* Yield 85%; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.95 (t, *J* = 7.3 Hz, 3H, NCH$_2$CH$_2$CH$_2$*CH*$_3$), 1.36 (sextet, *J* = 7.3 Hz, 2H, NCH$_2$*CH*$_2$*CH*$_2$CH$_3$), 1.90 (quintet, *J* = 7.3 Hz, 2H, NCH$_2$*CH*$_2$CH$_2$CH$_3$), 4.37 (t, *J* = 7.3 Hz, 2H, N*CH*$_2$), 5.30 (s, 2H, O*CH*$_2$), 7.11 (d, *J* = 8.8 Hz, 2H, Ar*H*), 7.63 (s, 1H), 7.84 (d, *J* = 8.8 Hz, 2H, Ar*H*), 9.89 (s, 1H, *CHO*).

*6.6.4. 4-((1-Pentyl-1H-1,2,3-triazol-4-yl)methoxy)benzaldehyde (**37**).* Yield 75%; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.89 (t, *J* = 7.3 Hz, 3H, CH$_2$CH$_2$CH$_2$CH$_2$*CH*$_3$), 1.32-1.35 (m, 4H), 1.92 (quintet, *J* = 7.3 Hz, 2H, NCH$_2$*CH*$_2$CH$_2$CH$_2$CH$_3$), 4.36 (t, *J* = 7.3 Hz, 2H, N*CH*$_2$), 5.30 (s, 2H, O*CH*$_2$), 7.11 (d, *J* = 8.8 Hz, 2H, Ar*H*), 7.62 (s, 1H), 7.84 (d, *J* = 8.8 Hz, 2H, Ar*H*), 9.89 (s, 1H, *CHO*).

*6.6.5. 4-((1-Benzyl-1H-1,2,3-triazol-4-yl)methoxy)benzaldehyde (**38**).* Yield 90%; ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.19 (s, 2H, O*CH*$_2$), 5.47 (s, 2H, N*CH*$_2$Ph), 7.01 (d, *J* = 8.8 Hz, 2H, Ar*H*), 7.19-7.22 (m, 2H, Ar*H*), 7.29-7.33 (m, 3H, Ar*H*), 7.48 (s, 1H), 7.75 (d, *J* = 8.8 Hz, 2H, Ar*H*), 9.81 (s, 1H, *CHO*).

*6.7. Typical procedure for the synthesis of (E)-N'-(4-(2-(4-butyl-1H-1,2,3-triazol-1-yl) ethoxy)benzylidene)isonicotinohydrazide (**39**) and related compounds (**40-60**):* A mixture of isoniazid (200 mg, 1.45 mmol) and 4-(2-(4-butyl-1H-1,2,3-triazol-1-yl)ethoxy)benzaldehyde (**6**, 360 mg, 1.31 mmol) in EtOH/H_2O was stirred at room temperature for 4-6 h. After completion of the reaction, separated solid was filtered and washed with cold EtOH to obtain the pure compound **39**.

*6.7.1. (E)-N'-(4-(2-(4-Butyl-1H-1,2,3-triazol-1-yl)ethoxy)benzylidene)isonicotinohydrazide (**39**):* Yield 80%; mp 187-190 ^{o}C; IR (KBr, cm^{-1}): 3222, 3046, 2955, 2875, 1675, 1608, 1559, 1511, 1286, 1259, 1174, 1048, 947, 825; ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.88 (t, *J* = 7.3 Hz, 3H, CH$_2$CH$_2$CH$_2$*CH*$_3$), 1.57 (sextet, *J* = 7.3 Hz, 2H, CH$_2$CH$_2$*CH*$_2$CH$_3$), 2.26 (quintet, *J* = 6.5 Hz, 2H, CH$_2$*CH*$_2$CH$_2$CH$_3$), 2.55 (t, *J* = 7.3 Hz, 2H, *CH*$_2$CH$_2$CH$_2$CH$_3$), 4.01 (t, *J* = 6.5 Hz, 2H, N*CH*$_2$), 4.47 (t, *J* = 6.5 Hz, 2H, O*CH*$_2$) 6.99 (d, *J* = 8.7 Hz, 2H, Ar*H*), 7.67 (d, *J* = 8.7 Hz, 2H, Ar*H*), 7.79-7.80 (m, 2H, Ar*H*), 7.87 (s, 1H), 8.38 (s, 1H, N=*CH*), 8.76 (dd, *J* = 1.4, 4.3 Hz, 2H, Ar*H*), 11.93 (s, 1H, CO*NH*); ^{13}C NMR (100 MHz, DMSO-d_6): δ 13.62, 22.27,

27.08, 29.45, 46.26, 64.70, 114.87, 121.52, 121.94, 126.72, 128.93, 140.61, 146.77, 148.86, 150.32, 160.18, 161.42; ESI-HRMS (*m/z*) calculated for $C_{21}H_{24}N_6O_2$: 392.1961, found: 393.4422 $(M + H)^+$, 415.3933 $(M + Na)^+$; Anal. calcd. for $C_{21}H_{24}N_6O_2$: C, 64.27; H, 6.16; N, 21.41; O, 8.15, found: C, 64.33; H, 6.23; N, 21.57; O, 8.28.

*6.7.2. (E)-N'-(4-(2-(4-(1-Hydroxycyclohexyl)-1H-1,2,3-triazol-1-yl)ethoxy)benzylidene) isonicotinohydrazide (**40**).* Yield 55%; mp 205-207 ^oC; IR (KBr, cm^{-1}): 3294, 3107, 2939, 1668, 1610, 1536, 1508, 1423, 1311, 1279, 1251, 1169, 1040, 971, 840; ^1H NMR (400 MHz, DMSO-d_6): δ 1.23-1.27 (m, 1H), 1.37-1.40 (m, 2H), 1.48 (brs, 1H), 1.62-1.69 (m, 4H), 1.81-1.84 (m, 2H), 4.45 (t, *J* = 5.1 Hz, 2H, N*CH*$_2$), 4.73 (t, *J* = 5.1 Hz, 2H, O*CH*$_2$), 4.85 (s, 1H, *OH*), 7.01 (d, *J* = 8.7 Hz, 2H, Ar*H*), 7.67 (d, *J* = 8.7 Hz, 2H, Ar*H*), 7.79-7.80 (m, 2H, Ar*H*), 7.94 (s, 1H), 8.38 (s, 1H, N=*CH*), 8.77 (brs, 2H, Ar*H*), 11.94 (s, 1H, CO*NH*); ^{13}C NMR (100 MHz, DMSO-d_6): δ 21.67, 25.25, 37.83, 48.83, 66.42, 67.99, 115.0, 121.51, 127.08, 128.95, 140.57, 148.73, 150.32, 155.89, 159.69, 161.45; ESI-HRMS (*m/z*) calculated for $C_{23}H_{26}N_6O_3$: 434.2066, found: 435.2711 (M + H)+; Anal. calcd. for $C_{23}H_{26}N_6O_3$: C, 63.58; H, 6.03; N, 19.34; O, 11.05, found: C, 63.63; H, 6.15; N, 19.50; O, 11.17.

*6.7.3. (E)-N'-(4-(2-(4-(Hydroxymethyl)-1H-1,2,3-triazol-1-yl)ethoxy)benzylidene) isonicotinohydrazide (**41**).* Yield 50%; mp 215-217 ^oC; IR (KBr, cm^{-1}): 3400, 3197, 3144, 2962, 2852, 1654, 1609, 1574, 1574, 1514, 1367, 1303, 1254, 1174, 1058, 1017, 918, 830; ^1H NMR (400 MHz, DMSO-d_6): δ 4.45 (t, *J* = 5.1 Hz, 2H, N*CH*$_2$), 4.51 (d, *J* = 5.1 Hz, 2H, O*CH*$_2$), 4.76 (t, *J* = 5.1 Hz, 2H, *CH*$_2$OH), 5.18 (t, *J* = 5.1 Hz, 1H, *OH*), 7.00 (d, *J* = 8.7 Hz, 2H, Ar*H*), 7.67 (d, *J* = 8.7 Hz, 2H, Ar*H*), 7.79-7.80 (m, 2H), 8.04 (s, 1H), 8.38 (s, 1H, N=*CH*), 8.75-8.77 (m, 2H, Ar*H*), 11.94 (s, 1H, CO*NH*); ESI-HRMS (*m/z*) calculated for $C_{18}H_{18}N_6O_3$: 366.1440, found: 367.2901 $(M + H)^+$; Anal. calcd. for $C_{18}H_{18}N_6O_3$: C, 59.01; H, 4.95; N, 22.94; O, 13.10, found: C, 59.11; H, 5.05; N, 22.87; O, 13.18.

*6.7.4. (E)-(1-(2-(4-((2-Isonicotinoylhydrazono)methyl)phenoxy)ethyl)-1H-1,2,3-triazol-4-yl)methylpropionate (**42**).* Yield 62%; mp 180-181 ^oC; IR (KBr, cm^{-1}): 3233, 3132, 3077, 1743, 1678, 1611, 1553, 1513, 1351, 1288, 1244, 1168, 1052, 840, 823; ^1H NMR (400 MHz, DMSO-d_6): δ 1.00 (t, *J* = 7.6 Hz, 3H, COCH$_2$*CH*$_3$), 2.30 (q, *J* = 8.0 Hz, 2H, CO*CH*$_2$CH$_3$), 4.45 (t, *J* = 5.1 Hz, 2H, N*CH*$_2$), 4.78 (t, *J* = 5.1 Hz, 2H, O*CH*$_2$), 5.11 (s, 2H, *CH*$_2$OCO), 7.00 (d, *J* = 8.7 Hz, 2H, Ar*H*), 7.66 (d, *J* = 8.7 Hz, 2H, Ar*H*), 7.79 (dd, *J* = 2.2, 4.3 Hz, 2H, Ar*H*), 8.22 (s, 1H), 8.38 (s, 1H, N=*CH*), 8.75-8.77 (m, 2H, Ar*H*), 11.94 (s, 1H, CO*NH*); ESI-HRMS (*m/z*) calculated for $C_{21}H_{22}N_6O_4$: 422.1703, found: 423.4100 $(M + H)^+$; Anal. calcd. for

$C_{21}H_{22}N_6O_4$: C, 59.71; H, 5.25; N, 19.89; O, 15.15, found: C, 59.83; H, 5.31; N, 19.88; O, 15.20.

*6.7.5. (E)-N'-(4-(2-(4-(Pyridin-2-yl)-1H-1,2,3-triazol-1-yl)ethoxy)benzylidene)isonicotino hydrazide (**43**).* Yield 55%; mp 212-214 ^{o}C; IR (KBr, cm^{-1}): 3305, 3130, 2940, 1661, 1605, 1543, 1510, 1477, 1381, 1264, 1169, 1045, 1030, 828; ^{1}H NMR (400 MHz, DMSO-d_6): δ 4.53 (t, *J* = 5.1 Hz, 2H, NCH_2), 4.86 (t, *J* = 5.1 Hz, 2H, OCH_2), 7.03 (d, *J* = 8.7 Hz, 2H, Ar*H*), 7.31-7.34 (m, 1H, Ar*H*), 7.66 (d, *J* = 8.7 Hz, 2H, Ar*H*), 7.78-7.79 (m, 2H, Ar*H*), 7.85-7.90 (m, 1H, Ar*H*), 8.01-8.03 (m, 1H, Ar*H*), 8.37 (s, 1H), 8.58 (m, 1H, Ar*H*), 8.68 (s, 1H, N=*CH*), 8.75-8.76 (m, 2H, Ar*H*), 11.93 (s, 1H, CO*NH*); ESI-HRMS (*m/z*) calculated for $C_{22}H_{19}N_7O_2$: 413.1600, found: 414.4650 $(M + H)^+$, 436.4455 $(M + Na)^+$; Anal. calcd. for $C_{22}H_{19}N_7O_2$: C, 63.91; H, 4.63; N, 23.72; O, 7.74, found: C, 64.11; H, 4.76; N, 23.88; O, 7.83.

*6.7.6. (E)-N'-(4-(2-(4-Phenyl-1H-1,2,3-triazol-1-yl)ethoxy)benzylidene)isonicotinohydrazide (**44**).* Yield 75%; mp 235-237 ^{o}C; IR (KBr, cm^{-1}): 3307, 3091, 2941, 1662, 1606, 1542, 1510, 1405, 1381, 1317, 1264, 1169, 1047, 1029, 828; ^{1}H NMR (400 MHz, DMSO-d_6): δ 4.51 (t, *J* = 5.1 Hz, 2H, NCH_2), 4.82 (t, *J* = 5.1 Hz, 2H, OCH_2), 7.04 (d, *J* = 8.7 Hz, 2H, Ar*H*), 7.30-7.33 (m, 1H, Ar*H*), 7.41-7.45 (m, 2H, Ar*H*), 7.67 (d, *J* = 8.7 Hz, 2H, Ar*H*), 7.78 (d, *J* = 5.8 Hz, 2H, Ar*H*), 7.84 (d, *J* = 7.3 Hz, 2H, Ar*H*), 8.37 (s, 1H), 8.65 (s, 1H, N=*CH*), 8.75 (d, *J* = 5.8 Hz, 2H, Ar*H*), 11.93 (s, 1H, CO*NH*); ^{13}C NMR (100 MHz, DMSO-d_6): δ 49.29, 66.33, 115.10, 121.61, 122.05, 125.25, 127.16, 127.99, 129.02, 130.76, 140.66, 146.49, 148.85, 150.39, 159.75, 161.58; ESI-MS (*m/z*) calculated for $C_{23}H_{20}N_6O_2$: 412.16, found: 413.22 $(M + H)^+$; Anal. calcd. for $C_{23}H_{20}N_6O_2$: C, 66.98; H, 4.89; N, 20.38; O, 7.76, found: C, 67.12; H, 4.98; N, 20.44; O, 7.89.

*6.7.7. (E)-N'-(4-(2-(4-(Phenoxymethyl)-1H-1,2,3-triazol-1-yl)ethoxy)benzylidene)isonicotino hydrazide (**45**).* Yield 80%; mp 199-201 ^{o}C; IR (KBr, cm^{-1}): 3278, 3044, 2936, 1665, 1607, 1543, 1511, 1497, 1287, 1247, 1172, 1054, 970, 822, ; ^{1}H NMR (400 MHz, DMSO-d_6): δ 4.46 (t, *J* = 5.1 Hz, 2H, NCH_2), 4.80 (t, *J* = 5.1 Hz, 2H, OCH_2), 5.18 (s, 2H, CH_2OPh), 6.93 (t, *J* = 7.3 Hz, 1H, Ar*H*), 6.99-7.03 (m, 4H, Ar*H*), 7.28 (t, *J* = 8.0 Hz, 2H, Ar*H*), 7.67 (d, *J* = 8.7 Hz, 2H, Ar*H*), 7.79-7.81 (m, 2H, Ar*H*) 8.31 (s, 1H), 8.39 (s, 1H, N=*CH*), 8.76-8.77 (m, 2H, Ar*H*), 11.97 (s, 1H, CO*NH*); ^{13}C NMR (100 MHz, DMSO-d_6): δ 49.06, 60.92, 66.32, 114.67, 115.0, 120.84, 121.56, 125.17, 127.13, 128.96, 129.53, 140.60, 142.83, 148.76, 150.33, 158.04, 159.62, 161.50; ESI-HRMS (*m/z*) calculated for $C_{24}H_{22}N_6O_3$: 442.1753,

found: 443.2369 $(M + H)^+$, 465.1719 $(M + Na)^+$; Anal. calcd. for $C_{24}H_{22}N_6O_3$: C, 65.15; H, 5.01; N, 18.99; O, 10.85, found: C, 65.23; H, 5.17; N, 19.11; O, 10.99.

6.7.8. *(E)-N'-(4-(2-(4-(p-Tolyloxymethyl)-1H-1,2,3-triazol-1-yl)ethoxy)benzylidene) isonicotino hydrazide* ***(46)***. Yield 75%; mp 174-176 ^oC; IR (KBr, cm^{-1}): 3222, 3050, 2963, 1654, 1608, 1575, 1552, 1509, 1456, 1383, 1301, 1253, 1230, 1178, 1051, 842, 813; ^1H NMR (400 MHz, DMSO-d_6): δ 2.21 (s, 3H, CH_3), 4.46 (t, J = 5.1 Hz, 2H, NCH_2), 4.79 (t, J = 5.1 Hz, 2H, OCH_2), 5.08 (s, 2H, CH_2OPh), 6.90 (d, J = 8.7 Hz, 2H, ArH), 7.0 (d, J = 8.7 Hz, 2H, ArH), 7.07 (d, J = 8.7 Hz, 2H, ArH), 7.67 (d, J = 8.7 Hz, 2H, ArH), 7.80-7.81 (m, 2H, ArH), 8.29 (s, 1H), 8.39 (s, 1H, N=CH), 8.77 (brs, 2H, ArH), 11.96 (s, 1H, CONH); ^{13}C NMR (100 MHz, DMSO-d_6): 20.08, 49.03, 61.01, 66.32, 114.54, 115.01, 121.52, 125.06, 127.13, 128.94, 129.50, 129.84, 140.60, 142.95, 148.75, 150.32, 155.92, 159.62, 161.47; ESI-HRMS (m/z) calculated for $C_{25}H_{24}N_6O_3$: 456.1910, found: 457.3754 $(M + H)^+$, 479.3582 $(M + Na)^+$; Anal. calcd. for $C_{25}H_{24}N_6O_3$: C, 65.78; H, 5.30; N, 18.41; O, 10.51, found C, 65.86; H, 5.41; N, 18.53; O, 10.41.

6.7.9. *(E)-N'-(4-(2-(4-((4-Chlorophenoxy)methyl)-1H-1,2,3-triazol-1-yl)ethoxy)benzylidene) isonicotinohydrazide* ***(47)***. Yield 82%; mp 190-192 ^oC; IR (KBr, cm^{-1}): 3301, 3127, 3072, 2932, 1666, 1608, 1542, 1510, 1489, 1285, 1249, 1208, 1050, 911, 839; ^1H NMR (400 MHz, DMSO-d_6): δ 4.46 (t, J = 5.1 Hz, 2H, NCH_2), 4.80 (t, J = 5.1 Hz, 2H, OCH_2), 5.14 (s, 2H, CH_2OPh), 7.0 (d, J = 8.7 Hz, 2H, ArH), 7.05 (dd, J = 2.2, 6.5 Hz, 2H, ArH), 7.31 (dd, J = 2.2, 6.5 Hz, 2H, ArH), 7.67 (d, J = 8.7 Hz, 2H, ArH), 7.80 (d, J = 5.8 Hz, 2H, ArH), 8.31 (s, 1H), 8.38 (s, 1H, N=CH), 8.76 (d, J = 5.8 Hz, 2H, ArH), 11.96 (s, 1H, CONH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 49.09, 61.31, 66.31, 114.97, 116.48, 121.56, 124.61, 125.32, 127.14, 128.96, 129.25, 140.59, 142.49, 148.76, 150.32, 156.84, 159.60, 161.50; ESI-HRMS (m/z) calculated for $C_{24}H_{21}ClN_6O_3$: 476.1364, found: 477.1584 $(M + H)^+$, 499.1008 $(M + Na)^+$; Anal. calcd. for $C_{24}H_{21}ClN_6O_3$: C, 60.44; H, 4.44; Cl, 7.43; N, 17.62; O, 10.06, found: C, 60.56; H, 4.51; Cl, 7.59; N, 17.82; O, 10.23.

6.7.10. *(E)-N'-(4-(3-(4-Propyl-1H-1,2,3-triazol-1yl)propoxy)benzylidene)isonicotino hydrazide* ***(48)***. Yield 85%; mp 188-190 ^oC; IR (KBr, cm^{-1}): 3222, 3046, 2956, 2933, 1675, 1609, 1560, 1511, 1286, 1258, 1174, 1144, 1048, 947, 825; ^1H NMR (400 MHz, DMSO-d_6): δ 0.88 (t, J = 7.3 Hz, 3H, $CH_2CH_2CH_3$), 1.57 (sextet, J = 7.3 Hz, 2H, $CH_2CH_2CH_3$), 2.26 (quintet, J = 6.5 Hz, 2H, $OCH_2CH_2CH_2N$), 2.56 (t, J = 7.3 Hz, 2H, $CH_2CH_2CH_3$), 4.01 (t, J = 5.8 Hz, 2H, OCH_2), 4.48 (t, J = 6.5 Hz, 2H, NCH_2), 7.00 (d, J = 8.7 Hz, 2H, ArH), 7.68 (d, J

= 8.7 Hz, 2H, Ar*H*), 7.80-7.81 (m, 2H, Ar*H*), 7.88 (s, 1H), 8.39 (s, 1H, N=*CH*), 8.76-8.77 (m, 2H, Ar*H*), 11.95 (s, 1H, CO*NH*); ^{13}C NMR (100 MHz, DMSO-d_6): δ 13.61, 22.26, 27.08, 29.44, 46.24, 64.69, 114.86, 121.51, 121.93, 126.70, 128.91, 140.59, 146.75, 148.82, 150.31, 160.16, 161.39; ESI-HRMS (*m/z*) calculated for $C_{21}H_{24}N_6O_2$: 392.1961, found: 393.4258 (M + H)$^+$, 415.3584 (M + Na)$^+$; Anal. calcd. for $C_{21}H_{24}N_6O_2$: C, 64.27; H, 6.16; N, 21.41; O, 8.15, found: C, 64.39; H, 6.23; N, 21.62; O, 8.28.

*6.7.11. (E)-N'-(4-(3-(4-Butyl-1H-1,2,3-triazol-1-yl)propoxy)benzylidene)isonicotinohydrazide (**49**).* Yield 82%; mp 191-192 ^{o}C; IR (KBr, cm^{-1}): 3294, 3129, 2932, 1663, 1609, 1535, 1511, 1306, 1278, 1255, 1172, 1055, 962, 841, 817; ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.87 (t, *J* = 7.3 Hz, 3H, $CH_2CH_2CH_2CH_3$), 1.29 (sextet, *J* = 7.3 Hz, 2H, $CH_2CH_2CH_2CH_3$), 1.54 (quintet, *J* = 7.3 Hz, 2H, $CH_2CH_2CH_2CH_3$), 2.27 (quintet, *J* = 6.5 Hz, 2H, $OCH_2CH_2CH_2N$), 2.58 (t, *J* = 7.3 Hz, 2H, $CH_2CH_2CH_2CH_3$), 4.01 (t, *J* = 6.5 Hz, 2H, OCH_2), 4.47 (t, *J* = 6.5 Hz, 2H, NCH_2), 7.00 (d, *J* = 8.7 Hz, 2H, Ar*H*), 7.68 (d, *J* = 8.7 Hz, 2H, Ar*H*), 7.79-7.81 (m, 2H, Ar*H*), 7.87 (s, 1H), 8.39 (s, 1H, N=*CH*), 8.76-8.77 (m, 2H, Ar*H*), 11.93 (s, 1H, CO*NH*); ^{13}C NMR (100 MHz, DMSO-d_6): δ 13.71, 21.68, 24.71, 29.46, 31.13, 46.26, 64.69, 114.86, 121.53, 121.89, 126.73, 128.93, 140.60, 146.92, 148.84, 150.32, 160.18, 161.41; ESI-HRMS (*m/z*) calculated for $C_{22}H_{26}N_6O_2$: 406.2117, found: 407.1853 (M + H)$^+$, 429.0985 (M + Na)$^+$; Anal. calcd. for $C_{22}H_{26}N_6O_2$: C, 65.01; H, 6.45; N, 20.68; O, 7.87, found: C, 65.14; H, 6.61; N, 20.75; O, 7.90.

*6.7.12. (E)-N'-(4-(3-(4-(1-Hydroxycyclohexyl)-1H-1,2,3-triazol-1-yl)propoxy)benzylidene) isonicotinohydrazide (**50**).* Yield 62%; mp 185-187 ^{o}C; IR (KBr, cm^{-1}): 3449, 3199, 3199, 2930, 2854, 1669, 1602, 1516, 1302, 1257, 1237, 1172, 1065, 1049, 1002, 833; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.23-1.26 (m, 1H), 1.38 (brs, 2H), 1.46 (brs, 1H), 1.62-1.68 (m, 4H), 1.82-1.87 (m, 2H), 2.26-2.29 (m, 2H), 3.97-4.03 (m, 2H), 4.47-4.51 (m, 2H), 4.84 (brs, 1H, *OH*), 7.0 (d, *J* = 8.0 Hz, 2H, Ar*H*), 7.68 (d, *J* = 8.0 Hz, 2H, Ar*H*), 7.80 (d, *J* = 5.1 Hz, 2H, Ar*H*), 7.92 (s, 1H), 8.39 (s, 1H, N=*CH*), 8.76 (brs, 2H, Ar*H*), 11.95 (s, 1H, CO*NH*); ^{13}C NMR (100 MHz, DMSO-d_6): δ 21.74, 25.29, 29.50, 37.85, 46.32, 64.78, 67.99, 114.90, 121.18, 121.55, 126.74, 128.97, 140.63, 148.89, 150.34, 155.74, 160.21, 161.46; ESI-MS (*m/z*) calculated for $C_{24}H_{28}N_6O_3$: 448.22, found: 449.27 (M + H)$^+$; Anal. calcd. for $C_{24}H_{28}N_6O_3$: C, 64.27; H, 6.29; N, 18.74; O, 10.70, found: C, 64.35; H, 6.31; N, 18.91; O, 10.66.

*6.7.13. (E)-N'-(4-(3-(4-(Hydroxymethyl)-1H-1,2,3-triazol-1-yl)propoxy)benzylidene) isonicotinohydrazide (**51**).* Yield 58%; mp 225-228 ^{o}C; IR (KBr, cm^{-1}): 3265, 3144, 3083, 2953, 2914, 1666, 1605, 1557, 1516, 1407, 1367, 1287, 1258, 1175, 1007, 956, 827; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.28 (quintet, *J* = 5.8 Hz, 2H, OCH$_2$*CH$_2$*CH$_2$N), 4.01 (t, *J* = 5.8 Hz, 2H, O*CH$_2$*), 4.49-4.53 (m, 4H) 5.17 (brs, 1H, *OH*), 7.0 (d, *J* = 8.7 Hz, 2H, Ar*H*), 7.67 (d, *J* = 8.7 Hz, 2H, Ar*H*), 7.80 (d, *J* = 5.8 Hz, 2H, Ar*H*), 8.01 (s, 1H), 8.39 (s, 1H, N=*CH*), 8.76 (brs, 2H, Ar*H*), 11.94 (s, 1H, CO*NH*); ^{13}C NMR (100 MHz, DMSO-d_6): δ 29.47, 46.32, 55.06, 64.68, 114.88, 121.50, 122.82, 126.73, 128.92, 140.61, 148.03, 148.86, 150.31, 160.17, 161.40; ESI-HRMS (*m/z*) calculated for C$_{19}$H$_{20}$N$_6$O$_3$: 380.1597, found: 381.5581 (M + H)$^+$, 403.5226 (M + Na)$^+$; Anal. calcd. for C$_{19}$H$_{20}$N$_6$O$_3$: C, 59.99; H, 5.30; N, 22.09; O, 12.62, found: C, 60.15; H, 5.51; N, 22.24; O, 12.77.

*6.7.14. (E)-(1-(3-(4-((2-Isonicotinoylhydrazono)methyl)phenoxy)propyl)-1H-1,2,3-triazol-4-yl)methylpropionate (**52**).* Yield 66%; mp 162-163 ^{o}C; IR (KBr, cm^{-1}): 3286, 3139, 2938, 1735, 1665, 1604, 1542, 1512, 1349, 1289, 1255, 1171, 1054, 951, 836; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.00 (t, *J* = 7.6 Hz, 3H, COCH$_2$*CH$_3$*), 2.28-2.33 (m, 4H), 4.03 (t, *J* = 5.8 Hz, 2H, O*CH$_2$*), 4.54 (t, *J* = 6.5 Hz, 2H, N*CH$_2$*), 5.11 (s, 2H, *CH$_2$*OCO), 6.99 (d, *J* = 8.7 Hz, 2H, Ar*H*), 7.68 (d, *J* = 8.7 Hz, 2H, Ar*H*), 7.79-7.81 (m, 2H, Ar*H*), 8.19 (s, 1H), 8.39 (s, 1H, N=*CH*), 8.76-8.77 (m, 2H, Ar*H*), 11.94 (s, 1H, CO*NH*); ^{13}C NMR (100 MHz, DMSO-d_6): δ 8.89, 26.68, 29.40, 46.63, 57.10, 64.73, 114.83, 121.52, 124.83, 126.74, 128.91, 140.59, 142.02, 148.86, 150.30, 160.16, 161.43, 173.39; ESI-HRMS (*m/z*) calculated for C$_{22}$H$_{24}$N$_6$O$_4$: 436.1859, found: 437.3393 (M + H)$^+$, 459.2805 (M + Na)$^+$; Anal. calcd. for C$_{22}$H$_{24}$N$_6$O$_4$: C, 60.54; H, 5.54; N, 19.25; O, 14.66, found: C, 60.71; H, 5.66; N, 19.19; O, 14.73.

*6.7.15. (E)-N'-(4-(3-(4-(Phenoxymethyl)-1H-1,2,3-triazol-1-yl)propoxy)benzylidene) isonicotino hydrazide (**53**).* Yield 80%; mp 186-188 ^{o}C; IR (KBr, cm^{-1}): 3286, 3095, 2962, 2873, 1661, 1611, 1541, 1510, 1375, 1264, 1284, 1171, 1037, 841, 822; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.27 (quintet, *J* = 6.5 Hz, 2H, OCH$_2$*CH$_2$*CH$_2$N), 4.0 (t, *J* = 5.8 Hz, 2H, O*CH$_2$*), 4.52 (t, *J* = 6.5 Hz, 2H, N*CH$_2$*), 5.09 (s, 2H, *CH$_2$*OPh), 6.90 (t, *J* = 7.3 Hz, 1H, Ar*H*), 6.97 (t, *J* = 8.7 Hz, 4H, Ar*H*), 7.23-7.27 (m, 2H, Ar*H*), 7.65 (d, *J* = 8.7 Hz, 2H, Ar*H*), 7.78 (d, *J* = 5.8 Hz, 2H, Ar*H*), 8.24 (s, 1H), 8.36 (s, 1H, N=*CH*), 8.73-8.74 (m, 2H, Ar*H*), 11.92 (s, 1H, CO*NH*); ^{13}C NMR (100 MHz, DMSO-d_6): δ 29.38, 46.59, 60.99, 64.72, 114.64, 114.85, 120.80, 121.48, 124.61, 126.71, 128.89, 129.48, 140.59, 142.75, 148.83, 150.29, 158.02,

160.13, 161.38; Anal. calcd. for $C_{25}H_{24}N_6O_3$: C, 65.78; H, 5.30; N, 18.41; O, 10.51, found: C, 65.66; H, 5.55; N, 18.33; O, 10.47.

*6.7.16. (E)-N'-(4-(3-(4-(p-Tolyloxymethyl)-1H-1,2,3-triazol-1-yl)propoxy)benzylidene) isonicotinohydrazide (**54**).* Yield 72%; mp 199-200 ^{o}C; IR (KBr, cm^{-1}): 3291, 3128, 3095, 2926, 2874, 1662, 1611, 1541, 1509, 1265, 1234, 1170, 1021, 841, 822; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.21 (s, 3H, CH_3), 2.30 (quintet, J = 5.8 Hz, 2H, OCH$_2$$CH_2CH_2$N), 4.02 (t, J = 5.8 Hz, 2H, OCH_2), 4.55 (t, J = 5.8 Hz, 2H, NCH_2), 5.07 (s, 2H, CH_2OPh), 6.90 (d, J = 8.7 Hz, 2H, ArH), 6.99 (d, J = 8.7 Hz, 2H, ArH), 7.07 (d, J = 8.0 Hz, 2H, ArH), 7.67 (d, J = 8.7 Hz, 2H, ArH), 7.80 (d, J = 5.1 Hz, 2H, ArH), 8.26 (s, 1H), 8.39 (s, 1H, N=CH), 8.76 (brs, 2H, ArH), 11.95 (s, 1H, CONH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 20.08, 29.40, 46.61, 61.07, 64.72, 114.51, 114.87, 121.53, 124.58, 126.72, 128.92, 129.48, 129.83, 140.65, 142.90, 148.84, 150.32, 155.91, 160.16, 161.41; ESI-HRMS (*m/z*) calculated for $C_{26}H_{26}N_6O_3$: 470.2066, found: 471.2482 (M + H)$^{+}$, 493.1452 (M + Na)$^{+}$; Anal. calcd. for $C_{26}H_{26}N_6O_3$: C, 66.37; H, 5.57; N, 17.86; O, 10.20, found: C, 66.48; H, 5.49; N, 18.01; O, 10.32.

*6.7.17. (E)-N'-(4-(3-(4-((4-Chlorophenoxy)methyl)-1H-1,2,3-triazol-1-yl)propoxy) benzylidene)isonicotinohydrazide (**55**).* Yield 82%; mp 182-184 ^{o}C; IR (KBr, cm^{-1}): 3291, 3095, 2928, 2874, 1661, 1611, 1540, 1510, 1492, 1283, 1264, 1236, 1172, 1018, 825; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.30 (quintet, J = 6.5 Hz, 2H, OCH$_2$$CH_2CH_2$N), 4.02 (t, J = 5.8 Hz, 2H, OCH_2), 4.55 (t, J = 6.5 Hz, 2H, NCH_2), 5.13 (s, 2H, CH_2OPh), 6.98 (d, J = 8.7 Hz, 2H, ArH), 7.05 (d, J = 8.7 Hz, 2H, ArH), 7.31 (d, J = 8.7 Hz, 2H, ArH), 7.67 (d, J = 8.7 Hz, 2H, ArH), 7.81 (d, J = 5.8 Hz, 2H, ArH), 8.28 (s, 1H), 8.39 (s, 1H, N=CH), 8.77 (d, J = 5.8 Hz, 2H, ArH), 11.96 (s, 1H, CONH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 29.37, 46.61, 61.39, 64.70, 114.83, 116.48, 121.48, 124.53, 124.74, 126.71, 128.88, 129.22, 140.58, 142.39, 148.81, 150.28, 156.84, 160.12, 161.37; ESI-HRMS (*m/z*) calculated for $C_{25}H_{23}ClN_6O_3$: 490.1520, found: 491.3194 (M + H)$^{+}$, 513.2664 (M + Na)$^{+}$; Anal. calcd. for $C_{25}H_{23}ClN_6O_3$: C, 61.16; H, 4.72; Cl, 7.22; N, 17.12; O, 9.78, found: C, 61.28; H, 4.66; Cl, 7.07; N, 17.27; O, 9.89.

*6.7.18. (E)-N'-(4-((1-Propyl-1H-1,2,3-triazol-4-yl)methoxy)benzylidene)isonicotinohydrazide (**56**).* Yield 68%; mp 195-197 ^{o}C; IR (KBr, cm^{-1}): 3258, 3157, 2935, 1656, 1607, 1542, 1510, 1289, 1253, 1172, 1053, 1007, 844; ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.82 (t, J = 7.3 Hz, 3H, CH$_2$CH$_2$$CH_3$), 1.81 (sextet, J = 7.3 Hz, 2H, CH$_2$$CH_2CH_3$), 4.31 (t, J = 7.3 Hz, 2H, CH_2CH$_2$CH$_3$), 5.19 (s, 2H, OCH_2), 7.12 (d, J = 8.7 Hz, 2H, ArH), 7.68 (d, J = 8.7 Hz, 2H,

Ar*H*), 7.79 (d, *J* = 8.7 Hz, 2H, Ar*H*), 8.23 (s, 1H, Ar*H*), 8.39 (s, 1H, N=C*H*), 8.76 (d, *J* = 5.8 Hz, 2H, Ar*H*), 11.93 (s, 1H, CO*NH*); ^{13}C NMR (100 MHz, DMSO-d_6): δ 10.78, 23.14, 50.96, 61.28, 115.13, 121.49, 124.51, 126.85, 128.89, 140.58, 142.29, 148.84, 150.29, 159.84, 161.39; Anal. calcd. for $C_{19}H_{20}N_6O_2$: C, 62.62; H, 5.53; N, 23.06; O, 8.78, found: C, 62.77; H, 5.46; N, 23.20; O, 8.84.

*6.7.19. (E)-N'-(4-((1-Iso-propyl-1H-1,2,3-triazol-4-yl)methoxy)benzylidene)isonicotino hydrazide (**57**).* Yield 74%; mp 186-187 ^{o}C; IR (KBr, cm^{-1}): 3151, 2982, 2852, 1664, 1598, 1513, 1308, 1240, 1176, 1061, 1031, 1005, 824; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.48 (d, *J* = 6.5 Hz, 6H, CH(*CH*$_3$)$_2$), 4.81 (septet, *J* = 6.5 Hz, 1H, *CH*(CH$_3$)$_2$), 5.18 (s, 2H, O*CH*$_2$), 7.13 (d, *J* = 8.7 Hz, 2H, Ar*H*), 7.69 (d, 2H, *J* = 8.7 Hz, Ar*H*), 7.80 (d, *J* = 5.8 Hz, 2H, Ar*H*), 8.31 (s, 1H, Ar*H*), 8.40 (s, 1H, N=C*H*), 8.76 (d, *J* = 5.8 Hz, 2H, Ar*H*), 11.94 (s, 1H, CO*NH*); ESI-MS (*m/z*) calculated for $C_{19}H_{20}N_6O_2$: 364.16, found: 365.21 $(M + H)^+$; Anal. calcd. for $C_{19}H_{20}N_6O_2$: C, 62.62; H, 5.53; N, 23.06; O, 8.78, found: C, 62.86; H, 5.66; N, 23.19; O, 8.69.

*6.7.20. (E)-N'-(4-((1-Butyl-1H-1,2,3-triazol-4-yl)methoxy)benzylidene)isonicotinohydrazide (**58**).* Yield 65%; mp 205-207 ^{o}C; IR (KBr, cm^{-1}): 3250, 3159, 3047, 2960, 2935, 2873, 1661, 1607, 1544, 1511, 1467, 1288, 1252, 1174, 1005, 844; ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.87 (t, *J* = 7.3 Hz, 3H, CH$_2$CH$_2$CH$_2$*CH*$_3$), 1.23 (sextet, *J* = 7.3 Hz, 2H, CH$_2$CH$_2$*CH*$_2$CH$_3$), 1.79 (quintet, *J* = 7.3 Hz, 2H, CH$_2$*CH*$_2$CH$_2$CH$_3$), 4.35 (t, *J* = 7.3 Hz, 2H, *CH*$_2$CH$_2$CH$_2$CH$_3$), 5.19 (s, 2H, O*CH*$_2$), 7.13 (d, *J* = 8.7 Hz, 2H, Ar*H*), 7.69 (d, *J* = 8.7 Hz, 2H, Ar*H*), 7.79-7.81 (m, 2H, Ar*H*), 8.24 (s, 1H), 8.40 (s, 1H, N=*CH*), 8.76-8.77 (m, 2H, Ar*H*), 11.94 (s, 1H, CO*NH*); ^{13}C NMR (100 MHz, DMSO-d_6): δ 13.28, 19.07, 31.71, 49.10, 61.28, 115.14, 121.51, 124.52, 126.86, 128.90, 140.58, 142.30, 148.84, 150.31, 159.85, 161.40; ESI-HRMS (*m/z*) calculated for $C_{20}H_{22}N_6O_2$: 378.1804, found: 379.1872 $(M + H)^+$, 401.1347 $(M + Na)^+$; Anal. calcd. for $C_{20}H_{22}N_6O_2$: C, 63.48; H, 5.86; N, 22.21; O, 8.46, found: C, 63.63; H, 5.91; N, 22.34; O, 8.55.

*6.7.21. (E)-N'-(4-((1-Pentyl-1H-1,2,3-triazol-4-yl)methoxy)benzylidene)isonicotinohydrazide (**59**).* Yield 64%; mp 208-210 ^{o}C; IR (KBr, cm^{-1}): 3248, 3157, 2958, 1660, 1607, 1542, 1511, 1290, 1254, 1174, 1054, 1006, 845; ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.83 (t, *J* = 7.3 Hz, 3H, CH$_2$CH$_2$CH$_2$CH$_2$*CH*$_3$), 1.16-1.21 (m, 2H), 1.23-1.31 (m, 2H), 1.79 (quintet, *J* = 7.3 Hz, 2H, CH$_2$*CH*$_2$CH$_2$CH$_2$CH$_3$), 4.34 (t, *J* = 7.3 Hz, 2H, *CH*$_2$CH$_2$CH$_2$CH$_2$CH$_3$), 5.19 (s, 2H, O*CH*$_2$), 7.12 (d, *J* = 8.7 Hz, 2H, Ar*H*), 7.68 (d, *J* = 8.7 Hz, 2H, Ar*H*), 7.79-7.81 (m, 2H,

Ar*H*), 8.24 (s, 1H, Ar*H*), 8.39 (s, 1H, N=C*H*), 8.75-8.77 (m, 2H, Ar*H*), 11.94 (s, 1H, CO*NH*); ^{13}C NMR (100 MHz, DMSO-d_6): δ 13.77, 21.50, 28.00, 29.39, 49.36, 61.28, 115.14, 121.50, 124.51, 126.86, 128.89, 140.58, 142.29, 148.83, 150.31, 159.84, 161.39; ESI-MS (*m/z*) calculated for $C_{21}H_{24}N_6O_2$: 392.19, found: 393.25 $(M + H)^+$; Anal. calcd. for $C_{21}H_{24}N_6O_2$: C, 64.27; H, 6.16; N, 21.41; O, 8.15, found: C, 64.35; H, 6.32; N, 21.65; O, 8.28.

*6.7.22. (E)-N'-(4-((1-Benzyl-1H-1,2,3-triazol-4-yl)methoxy)benzylidene)isonicotinohydrazide (**60**).* Yield 84%; mp 205-208 °C; IR (KBr, cm^{-1}): 3270, 3032, 2958, 1655, 1607, 1547, 1513, 1454, 1408, 1290, 1252, 1175, 1051, 812; ^{1}H NMR (400 MHz, DMSO-d_6): δ 5.19 (s, 2H, OCH_2), 5.60 (s, 2H, NCH_2Ph), 7.11 (d, *J* = 8.7 Hz, 2H, Ar*H*), 7.29-7.33 (m, 3H, Ar*H*), 7.34-7.38 (m, 2H, Ar*H*), 8.79-8.80 (m, 2H, Ar*H*), 7.68 (d, *J* = 8.7 Hz, 2H, Ar*H*), 8.29 (s, 1H, Ar*H*), 8.39 (s, 1H, N=C*H*), 8.75-8.77 (m, 2H, Ar*H*), 11.94 (s, 1H, CO*NH*); ^{13}C NMR (100 MHz, DMSO-d_6): δ 52.88, 61.22, 115.16, 121.53, 124.85, 126.89, 127.99, 128.20, 128.81, 128.92, 136.0, 140.60, 142.73, 148.86, 150.32, 159.84, 161.43; ESI-HRMS (*m/z*) calculated for $C_{23}H_{20}N_6O_2$: 412.1648, found: 413.4110 $(M + H)^+$, 435.3596 $(M + Na)^+$; Anal. calcd. for $C_{23}H_{20}N_6O_2$: C, 66.98; H, 4.89; N, 20.38; O, 7.76, found: C, 66.83; H, 4.81; N, 20.47; O, 7.88.

Acknowledgements

D.S.R. thanks Council of Scientific and Industrial Research (CSIR) [No. 02(0049)/12/EMR-II] New Delhi, India for financial support. A.K.T. is thankful to the Department of Biotechnology, Ministry of Science and Technology, Government of India for financial support. D.K. and Beena are thankful to CSIR for the award of junior and senior research fellowship. Authors are also thankful to CIF-USIC, University of Delhi, Delhi for NMR spectral data and RSIC, CDRI, Lucknow for mass data.

References

[1] Beena, D.S. Rawat, Med. Res. Rev. 33 (2013) 693–764.

[2] http://whqlibdoc.who.int/publications/2011/9789241564380_eng.pdf.

[3] http://whoindia.org/en/Section3/Section123.htm.

[4] R.A.M. Breen, L. Swaden, J. Ballinger, M.C.I. Lipman, Drugs 66 (2006) 2299−2308.

[5] S. Swaminathan, C. Padmapriyadarsini, G. Narendran, Clin. Infect. Dis. 50 (2010) 1377−1386.

[6] M.R. Masjedi, P. Farnia, S. Sorooch, M.V. Pooramiri, S.D. Mansoori, A.Z. Zarifi, V.A. Akbar, S. Hoffner, Clin. Infect. Dis. 43 (2006) 841−847.

[7] A.S. Albanna, D. Menzies, Drugs 71 (2011) 815−825.

[8] G.B. Migliori, G. De Iaco, G. Besozzi, R. Centis, D.M. Cirillo, Euro. Surveill. 12 (2007) E070517.1.

[9] A.A. Velayati, M.R. Masjedi, P. Farnia, P. Tabarsi, J. Ghanavi, A.H. ZiaZarifi, Chest 136 (2009) 420−425.

[10] K. Rowland, Nature News (2012).

[11] S. Loewenberg, Lancet 379 (2012) 205.

[12] A.S. Fauci, J. Infect. Dis. 197 (2008) 1493-1498.

[13] P. Ahirrao, Mini-Rev. Med. Chem. 8 (2008) 1441−1451.

[14] http://www.newtbdrugs.org/pipeline.php.

[15] B. Villemagne, C. Crauste, M. Flipo, A.R. Baulard, B. Deprez, N. Willand, Eur. J. Med. Chem. 51 (2012) 1−16.

[16] A.M. Ginsberg, Tuberculosis 90 (2010) 162−167.

[17] S.T. Cole, G. Riccardi, Curr. Opin. Microbiol. 14 (2011) 570−576.

[18] A.H. Diacon, P.R. Donald, A. Pym, M. Grobusch, R.F. Patientia, R. Mahanyele, N. Bantubani, R. Narasimooloo, T. De Marez, R. van Heeswijk, N. Lounis, P. Meyvisch, K. Andries, D.F. McNeeley, Antimicrob. Agents Chemother. 56 (2012) 3271−3276.

[19] R. Mahajan, Int. J. App. Basic Med. Res. 3 (2013) 1-2.

[20] M.S. Butler, M.A. Blaskovich, M.A. Cooper, J. Antibiot. 66 (2013) 571–591.

[21] H.A. Offe, W. Siefken, G. Domagk, Naturwissenschaften 39 (1952) 118.

[22] E. Grunberg, R.J. Schnitzer, Proc. SOCE. xp. Biol. Med. 84 (1953) 220.

[23] Y. Zhang, B. Heym, B. Allen, D. Young, S. Cole, Nature 358 (1992) 591−593.

[24] X. Zhao, H. Yu, S. Yu, F. Wang, J.C. Sacchettini, R.S. Magliozzo, Biochemistry 45 (2006) 4131−4140.

[25] M. Nguyen, C. Claparols, J. Bernadou, B. Meunier, Chem. Bio. Chem. 2 (2001) 877−883.

[26] R. Maccari, R. Ottana, F. Monforte, M.G. Vigorita, Antimicrob. Agents Chemother. 46 (2002) 294–299.

[27] T.H. Manjashetty, P. Yogeeswari, D. Sriram, Bioorg. Med. Chem. Lett. 21 (2011) 2125–2128.

[28] A.V. Ramani, A. Monika, V.L. Indira, G. Karyavardhi, J. Venkatesh, V.U. Jeankumar, T.H. Manjashetty, P. Yogeeswari, D. Sriram, Bioorg. Med. Chem. Lett. 22 (2012) 2764–2767.

[29] M.J. Hearn, M.H. Cynamon, M.F. Chen, R. Coppins, J. Davis, H.J. Kang, A. Noble, B.T. Sekine, M.S. Terrot, D. Trombino, M. Thai, E.R. Webster, R. Wilson, Eur. J. Med. Chem. 44 (2009) 4169–4178.

[30] E. Vavrikova, S. Polanc, M. Kocevar, K. Horvati, S. Bosze, J. Stolarikova, K. Vavrova, J. Vinsova, Eur. J. Med. Chem. 46 (2011) 4937–4945.

[31] E. Vavrikova, S. Polanc, M. Kocevar, J. Kosmrlj, K. Horvati, S. Bosze, J. Stolarikova, A. Imramovsky, J. Vinsova, Eur. J. Med. Chem. 46 (2011) 5902–5909.

[32] N. Boechat, V.F. Ferreira, S.B. Ferreira, M.L.G. Ferreira, F.C. Silva, M.M. Bastos, M. S. Costa, M.C.S. Lourenco, A.C. Pinto, A.U. Krettli, A.C. Aguiar, B.M. Teixeira, N.V. Silva, P.R.C. Martins, F.A.F.M. Bezerra, A.L.S. Camilo, G.P. Silva, C.C.P. Costa, J. Med. Chem. 54 (2011) 5988–5999.

[33] P. De, G.K. Yoya, P. Constant, F. B. Belval, H. Duran, N. Saffon, M. Daffe, M. Baltas, J. Med. Chem. 54 (2011) 1449–1461.

[34] S.K. Arora, N. Sinha, S. Jain, R.S. Upadhayaya, G. Jain, S. Ajay, R.K. Sinha, International patent 2004 WO/2004/026828.

[35] http://www.ctri.nic.in/Clinicaltrials/pmaindet2.php?trialid=922.

[36] http://www.newtbdrugs.org/project.php?id=155

[37] G. Biagi, G. Dell'Omodarme, M. Ferretti, I. Giorgi, O. Livi, V. Scartoni, Farmaco 45 (1990) 1181−1192.

[38] K. Dabak, O. Sezer, A. Akar, O. Anac, Eur. J. Med. Chem. 38 (2003) 215−218.

[39] E. Banfi, G. Scialino, D. Zampieri, M.G. Mamolo, L. Vio, M. Ferrone, M. Fermeglia, M.S. Paneni, S. Pricl, J. Antimicrob. Chemother. 58 (2006) 76−84.

[40] B.S. Holla, M. Mahalinga, M.S. Karthikeyan, B. Poojary, P.M. Akberali, N.S. Kumari, Eur. J. Med. Chem. 40 (2005) 1173−1178.

[41] A.J. Srivastava, S. Swarup, V.K. Saxena, B.L. Chowdhury, J. Indian Chem. Soc. 68 (1991) 103−104.

[42] R.J. Singh, Rasayan J. Chem. 2 (2009) 706−708.

[43] M.J. Genin, D.A. Allwine, D.J. Anderson, M.R. Barbachyn, D.E. Emmert, S.A. Garmon, D.R. Graber, K.C. Grega, J.B. Hester, D.K. Hutchinson, J. Morris, R.J. Reischer, C.W. Ford, G.E. Zurenco, J.C. Hamel, R.D. Schaadt, D. Stapertand, B.H. Yagi, J. Med. Chem. 43 (2000) 953−970.

[44] Z.Y. Zhang, Y. Liu, S. Yang, Pharm. Sinica 26 (1991) 809−814.

[45] A.C. Cunha, J.M. Figueiredo, J.L.M. Tributino, A.L.P. Miranda, H.C. Castro, R.B. Zingali, C.A.M. Fraga, M.C.B.V. de Souza, V.F. Ferreira, E.J. Barreiro, Bioorg. Med. Chem. 11 (2003) 2051−2059.

[46] Q. Zhang, Y. Peng, X.I. Wang, S.M. Keenan, S. Arora, W.J. Welsh, J. Med. Chem. 50 (2007) 749−754.

[47] K.H. Bechet, W. Draber, K. Regal, Drugs Germ. 15 (1972) 79−82.

[48] M.D. Kakwani, N.H.P. Desai, A.C. Lele, M. Ray, M.G.R. Rajan, M.S. Degani, Bioorg. Med. Chem. Lett. 21 (2011) 6523–6526.

[49] B. Meunier, Acc. Chem. Res. 41 (2008) 69–77.

[50] V. Pokrovskaya, T. Baasov, Expert Opin. Drug Discov. 5 (2010) 883–902.

[51] L.F. Tietze, H.P. Bell, S. Chandrasekhar, Angew. Chem. Int. Ed. 42 (2003) 3996–4028.

[52] M.C. Joshi, G.S. Bisht, D.S. Rawat, Bioorg. Med. Chem. Lett. 17 (2007) 3226−3230.

[53] H. Atheaya, S.I. Khan, R. Mamgain, D.S. Rawat, Bioorg. Med. Chem. Lett. 18 (2008) 1446−1449.

[54] Beena, N. Kumar, R.K. Rohilla, N. Roy, D.S. Rawat, Bioorg. Med. Chem. Lett. 19 (2009) 1396−1398.

[55] N. Kumar, S.I. Khan, Beena, G. Rajalakshmi, P. Kumaradhas, D.S. Rawat, Bioorg. Med. Chem. 17 (2009) 5632−5638.

[56] S. Manohar, S.I. Khan, D.S. Rawat, Bioorg. Med. Chem. Lett. 20 (2010) 322−325.

[57] D. Kumar, S. Joshi, R.K. Rohilla, N. Roy, D.S. Rawat, Bioorg. Med. Chem. Lett. 20 (2010) 893−895.

[58] D. Kumar, R.K. Rohilla, N. Roy, D.S. Rawat, Chem. Biol. Int. 1 (2011) 263–278.

[59] N. Kumar, S.I. Khan, H. Atheaya, R. Mamgain, D.S. Rawat, Eur. J. Med. Chem. 46 (2011) 2816−2827.

[60] M. Sharma, P. Joshi, N. Kumar, S. Joshi, R.K. Rohilla, N. Roy, D.S. Rawat, Eur. J. Med. Chem. 46 (2011) 480−487.

[61] S. Manohar, U.C. Rajesh, S.I. Khan, B.L. Tekwani, D.S. Rawat, ACS Med. Chem. Lett. 3 (2012) 555−559.

[62] Beena, S. Joshi, N. Kumar, S. Kidwai, R. Singh, D.S. Rawat, Arch. Pharm. Chem. Life Sci. 345 (2012) 896–901.

[63] Beena, D. Kumar, D.S. Rawat. Bioorg. Med. Chem. Lett. 23 (2013) 641–645.

[64] D. Kumar, K.K. Raj, M. Bailey, T. Alling, T. Parish, D.S. Rawat, Bioorg. Med. Chem. Lett. 23 (2013) 1365−1369.

[65] M.J. Khoshkholgh, S. Balalaie, R. Gleiter, F. Rominger, Tetrahedron 6 (2008) 10924–10929.

[66] M.V.B. Dias, I.B. Vasconcelos, A.M.X. Prado, V. Fadel, L.A. Basso, W.F. de Azevedo, D.S. Santos, J. Structural. Biol. 159 (2007) 369–380.

[67] R.C. Hartkoorn, C. Sala, J. Neres, F. Pojer, S. Magnet, R. Mukherjee, S. Uplekar, S. Boy-Rottger, K.H. Altmann, S.T. Cole, EMBO Mol. Med. 4 (2012) 1032–1042.

[68] Maestro, version 9.6, Schrödinger LLC, New York, 2014.

[69] M. Harel, D.M. Quinn, H.K. Nair, I. Silman, J.L. Sussman, J. Am. Chem. Soc. 118 (1996) 2340.

[70] G. Kryger, I. Silman, J.L. Sussman, Structure 7 (1999) 297.

[71] F.C. Bernstein, T.F. Koetzle, G.J Williams, E.F. Meyer Jr, M.D. Brice, J.R. Rodgers, O. Kennard, T. Shimanouchi, M. Tasumi, J. Mol. Biol. 12 (1977) 535.

Table 1: *In vitro* antituberculosis activity of isoniazid-triazole conjugates (39-55)

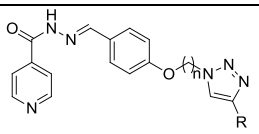
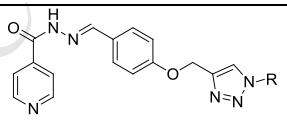
Compd			MIC_{99} (µM)	THP-1 Cytotoxicity (µM)	ClogP
	R	n			
39	*n*-Bu	2	0.78	>50	2.949
40	Cyclohexan-1-ol	2	0.39-0.78	>50	2.076
41	CH_2OH	2	0.78	>50	0.055
42	CH_2OCOEt	2	0.78-1.56	>50	1.440
43	2-Pyridyl	2	0.39	>50	2.077
44	C_6H_5	2	0.39	>50	3.191
45	$CH_2OC_6H_5$	2	0.39	>50	2.780
46	$CH_2OC_6H_4$-4-Me	2	0.39	>50	3.279
47	$CH_2OC_6H_4$-4-Cl	2	0.195-0.39	>50	3.633
48	*n*-Pr	3	0.78	>50	2.731
49	*n*-Bu	3	0.39	>50	3.260
50	Cyclohexan-1-ol	3	0.78	>50	2.387
51	CH_2OH	3	0.78	>50	0.366
52	CH_2OCOEt	3	0.78	>50	1.539
53	$CH_2OC_6H_5$	3	0.39-0.78	>50	3.091
54	$CH_2OC_6H_4$-4-Me	3	0.78	>50	3.590
55	$CH_2OC_6H_4$-4-Cl	3	0.78	>50	3.944
Isoniazid			0.39		-0.668

Table 2: *In vitro* antituberculosis activity of isoniazid-triazole conjugates (56-60)

Compd		MIC_{99} (µM)	THP-1 Cytotoxicity (µM)	ClogP
	R			
56	*n*-Pr	0.78	>50	1.844
57	*i*-Pr	0.78	>50	1.624
58	*n*-Bu	0.39	>50	2.373
59	*n*-Pentyl	0.39	>50	2.902
60	Benzyl	0.78	>50	2.554

Isoniazid	0.39	-0.668

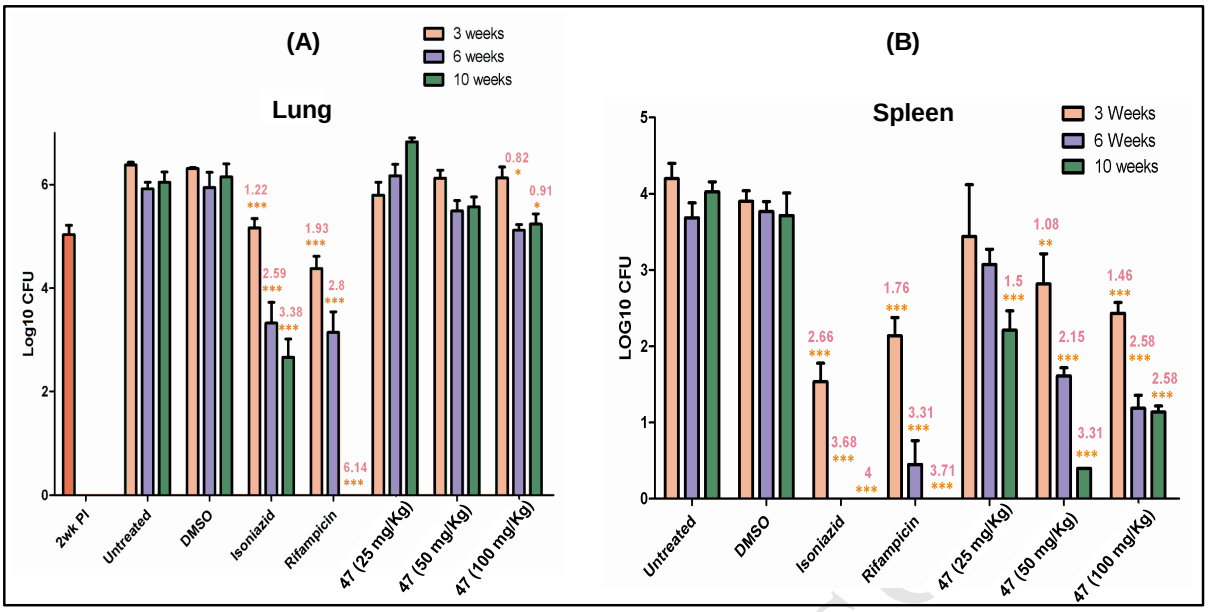


Fig. 1: Bacterial enumeration in lungs (A) and spleens (B) of animals belonging to different groups was carried out at various time points following infection. Bacillary load was measured as described in the section biological activity. Various groups are indicated. PI represents post infection. Data was analyzed by two-way analysis of variance (ANOVA) with the Bonferroni multiple comparision test (*p<0.001, **p<0.01 and *p<0.05. The numbers in red represent the log_{10} CFU value by which a reduction in the particular case was observed when compared with the CFU value in the case of control animals.**

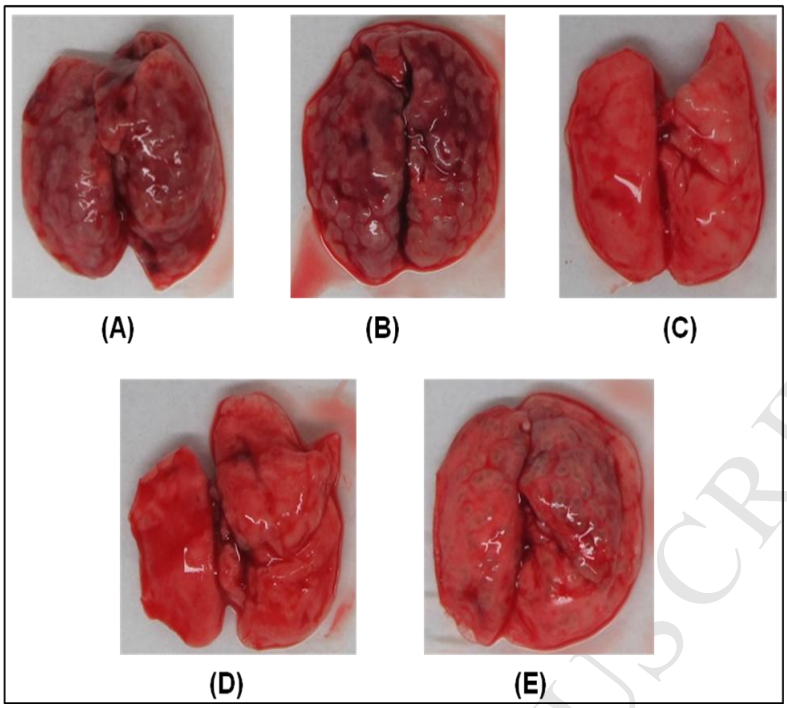


Fig. 2: Lungs of infected mice after treatment for 10 weeks either untreated or treated with different compounds: (A) Untreated, (B) DMSO, (C) Isoniazid, (D) Rifampicin and (E) Compound 47 (100 mg/kg).

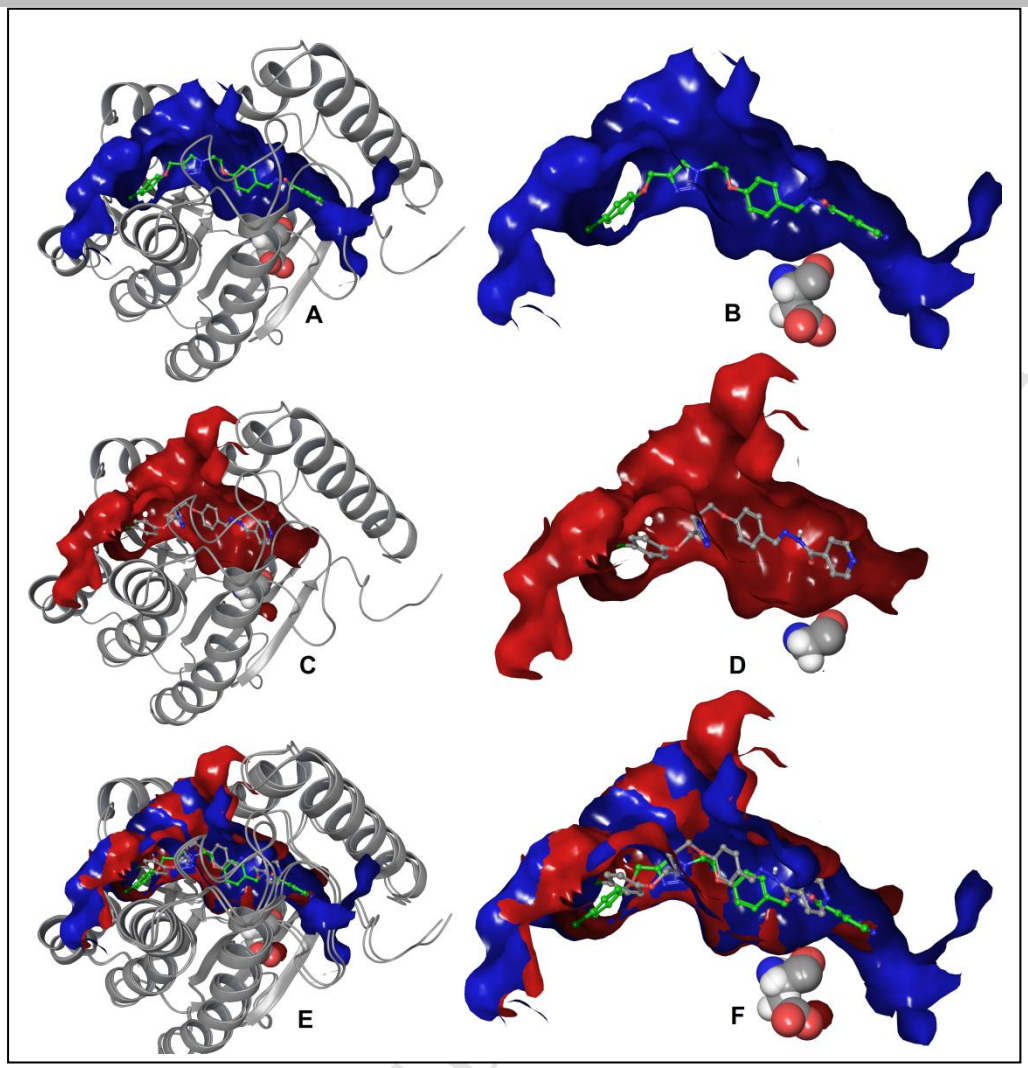


Fig. 3: (A and B): Wild type protein 2IDZ in cartoon with binding pocket in blue colour and compound 47 in green colour. Asp 148 shown in cpk format outside binding pocket. (C and D): Resistant protein 4DQU in cartoon with binding pocket in red colour along with compound 47 in grey colour. Gly 148 shown in cpk format outside binding pocket. (E and F): Superimposed structures of 2IDZ and 4DQU in complex with compound 47 along with its binding pockets.

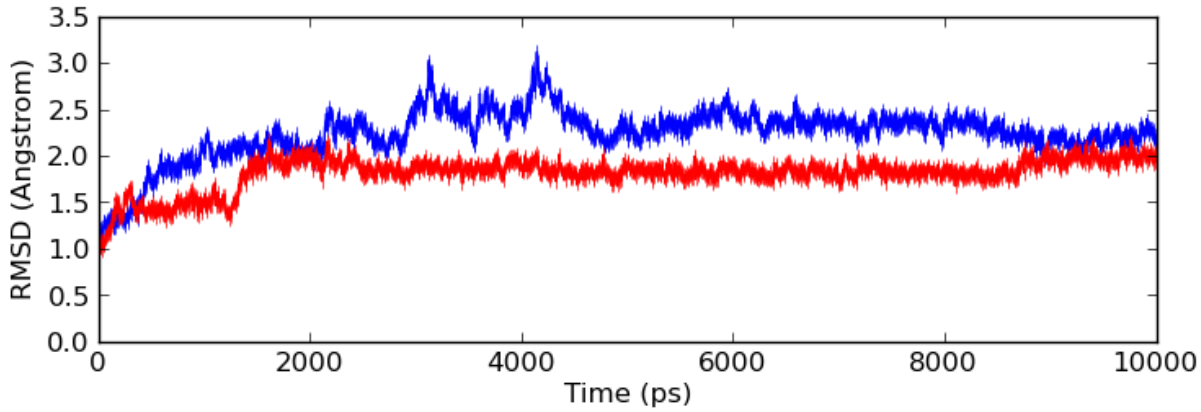


Fig. 4: RMSD observations during molecular dynamic simulations of wild type and resistant type proteins

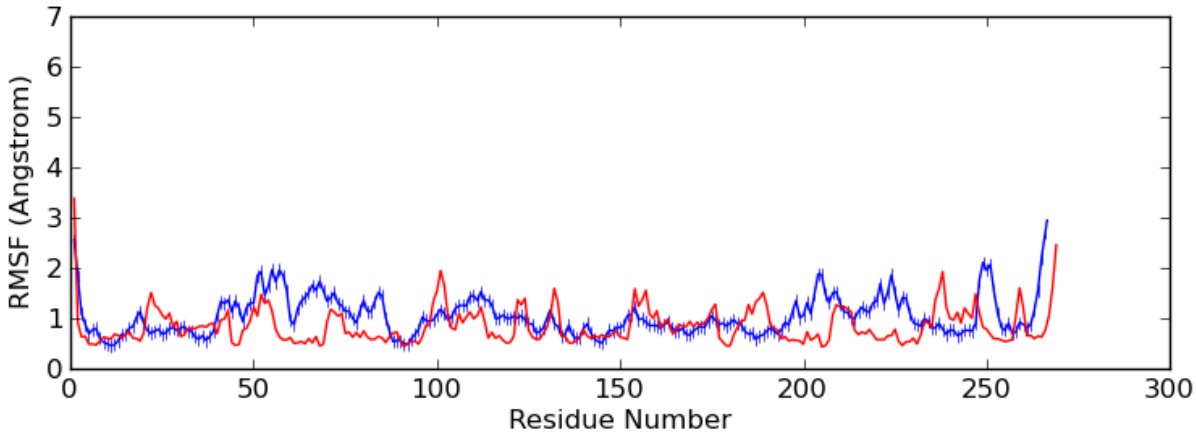
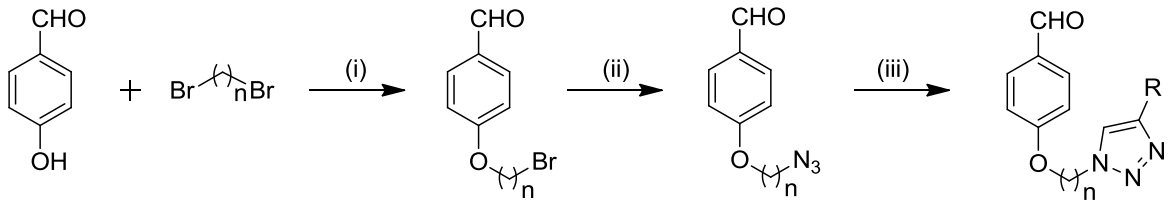
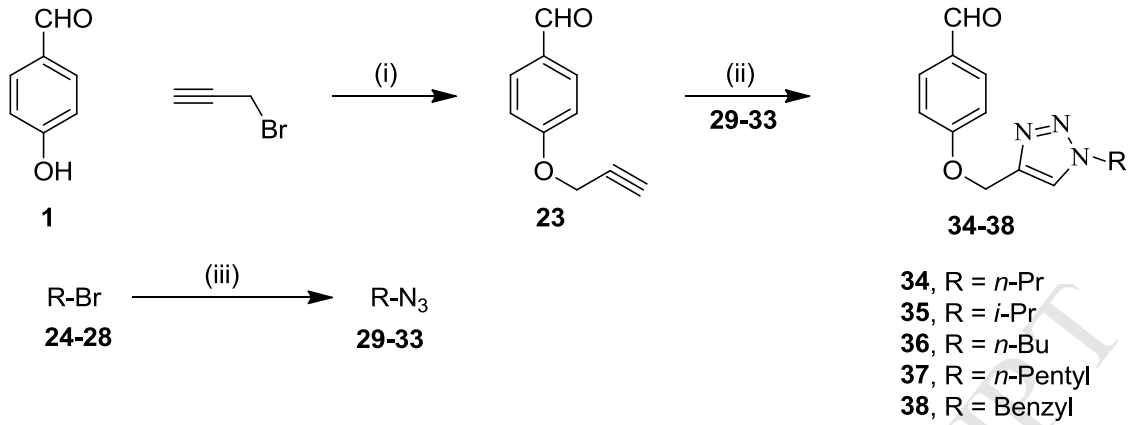


Fig. 5: RMSF observations during molecular dynamic simulations of wild type and resistant type proteins

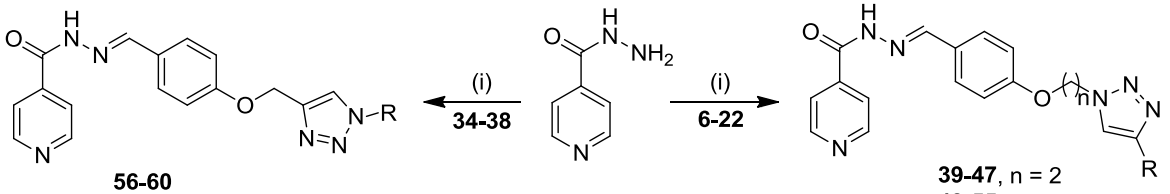


6, R = *n*-Bu	**15**, R = *n*-Pr
7, R = Cyclohexan-1-ol	**16**, R = *n*-Bu
8, R = CH_2OH	**17**, R = Cyclohexan-1-ol
9, R = CH_2OCOEt	**18**, R = CH_2OH
10, R = 2-Pyridyl	**19**, R = CH_2OCOEt
11, R = C_6H_5	**20**, R = $CH_2OC_6H_5$
12, R = $CH_2OC_6H_5$	**21**, R = $CH_2OC_6H_4$-4-Me
13, R = $CH_2OC_6H_4$-4-Me	**22**, R = $CH_2OC_6H_4$-4-Cl
14, R = $CH_2OC_6H_4$-4-Cl	

Scheme 1: Reagents and Conditions: (i) K_2CO_3, Dry DMF, RT, 8-10 hr; (ii) K_2CO_3, NaN_3, Dry DMF, 50 ^oC, 4-5 hr; (iii) Terminal alkynes, Sodium ascorbate, $CuSO_4.5H_2O$, t-BuOH:H_2O (1:1), RT, 2-5 hr



Scheme 2: Reagents and Conditions: (i) K_2CO_3, Dry DMF, RT, 8-10 h; (ii) RN_3, Sodium ascorbate, $CuSO_4.5H_2O$, t-BuOH:H_2O (1:1), RT, 2-5 h; (iii) K_2CO_3, NaN_3, Dry DMF, 50 ^oC, 4-5 h



56-60

56, R = *n*-Pr
57, R = *i*-Pr
58, R = *n*-Bu
59, R = *n*-Pentyl
60, R = Benzyl

39-47, n = 2
48-55, n = 3

39, R = *n*-Bu
40, R = Cyclohexan-1-ol
41, R = CH_2OH
42, R = CH_2OCOEt
43, R = 2-Pyridyl
44, R = C_6H_5
45, R = $CH_2OC_6H_5$
46, R = $CH_2OC_6H_4$-4-Me
47, R = $CH_2OC_6H_4$-4-Cl

48, R = *n*-Pr
49, R = *n*-Bu
50, R = Cyclohexan-1-ol
51, R = CH_2OH
52, R = CH_2OCOEt
53, R = $CH_2OC_6H_5$
54, R = $CH_2OC_6H_4$-4-Me
55, R = $CH_2OC_6H_4$-4-Cl

Scheme 3: Reagents and Conditions: (i) $EtOH/H_2O$