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DESIGN, SYNTHESIS AND ANTI TUBERCULAR ACTIVITY OF NOVEL 2,5- DIARYL-1,3,4-OXADIAZOLE DERIVATIVES

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ABSTRACT

Tuberculosis is the leading cause of death worldwide. With the emergence of powerful strains of causative bacteria *Mycobacterium tuberculosis*, there is a need to proactively discover and develop new leads on existing or newer targets. In this work, we present the newer 2,5-diaryl-1,3,4-Oxadiazole analogues and analyze *in-silico* binding to the shikimate kinase protein. In Mycobacterium tuberculosis shikimate kinase is essential for the viability of the pathogen. The shikimate kinase is involved in the phosphorylation of shikimate to shikimate-3-phosphate utilizing the ATP and forming ADP as a byproduct. The docking studies revealed that these compounds interacted with shikimate kinase enzyme with the residues, through hydrogen bonding of Lys15, Arg 58A, Gly80A, Gly81A, Arg 117A, hydrophobic interaction of Gly79, Gly80A, Gly81A, Arg117A, Asp34 and pi-stacking interaction of Phe49 and Phe57 determined at resolution of 1.8 Å. Targeted molecules were synthesized using reported methods, then purified and characterized using spectroscopic techniques. The compounds, 2-((1H-1,2,4-triazol-1-yl)methyl)-5-(2-bromophenyl)-1,3,4-oxadiazole and 2-((1H-imidazol-1-yl)methyl)-5-(2-bromophenyl)-1,3,4-oxadiazole, showed promising results as they had positive anti tubercular activity.

Keywords: Shikimate kinase, oxadiazole synthesis, 2,5- diaryl-1,3,4-oxadiazole, Tuberculosis.

INTRODUCTION

Drug discovery is a complex process. Despite of many routes of drug discovery most of the clinically used drugs have been discovered accidently. The application of the computational software to the process of drug discovery has indeed been very useful[1]. The advancing field of molecular biology has revealed numerous basic target structures. The structures of the target protein come from nuclear magnetic resonance or crystallography experiments [2]. The Computer Aided Drug Discovery (CADD) is classified into two general categories: structure-based and ligand-based. Structure-based designing relies on the knowledge of the target protein 3D structure to calculate interaction energies for all compounds tested, while ligand-based drug design exploits the knowledge of known active and inactive molecules through chemical similarity searches or construction of predictive, quantitative structure-activity relation (QSAR) models [3]. Tuberculosis (TB) is the leading cause of death worldwide. One third of the world's population is infected with tuberculosis and TB has also been associated with immune-compromising diseases [4]. In general 1 in 10 of these cases the disease becomes active and if not properly treated can prove to be lethal. Tuberculosis is an infectious disease caused due to bacterium *Mycobacterium tuberculosis (M.Tb.).* TB spreads commonly through the air droplet infection which is caused due to sneezing or coughing during the active state of infection [5]. The *M.Tb.*,is an aerobic bacterium having a cell wall which is high in lipid content and mycolic acids. Minimum time therapy required for TB treatment is 6 months which can go up to 12-18 months depending on the tests. Multidrug-resistant tuberculosis (MDR-TB) is a form of TB caused by bacteria that do not respond to, at least, isoniazid and rifampicin, the two most powerful, first-line (or standard)

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anti-TB drugs. The main cause of this is inappropriate treatment and non adherence to the therapy or substandard quality medicines used. Currently there have been rise in cases of MDR and Extensively drug resistant (XDR) cases of Tuberculosis. Apart from XDR cases, Extremely Drug resistant (XXDR) TB have also been reported. With the emergence of powerful strains of causative bacteria *Mycobacterium tuberculosis*, there is a need to proactively discover and develop new leads on existing or newer targets [6].

Targets available in tuberculosis can aim at disrupting the normal functioning of the bacteria. This includes cell wall biosynthesis, amino acid biosynthesis [7], co-factor biosynthesis [8], ATP synthesis [9], etc. Mycobacterium tuberculosis SK is essential for the viability of the pathogen. This is involved in synthesis of essential aromatic amino acids required for the bacteria for survival. Shikimate is an important intermediate for the synthesis of many essential compounds including aromatic amino acid residues, folic acid, and ubiquinone[10]. The shikimate pathway is critical to fungi, algae, bacteria, and higher plants but is not present in mammals. The fact that the enzyme is present only in the bacteria makes it an excellent target in developing new drugs. The shikimate biosynthetic pathway in *Mycobacterium* leads to the generation of chorismate(seen in diagram 1) from erythrose-4-phosphate and phosphoenolpyruvate [11]. Different enzymes that are involved in this pathway can serve as individual targets, amongst them is the enzyme shikimate kinase, which was chosen as the target protein. The shikimate kinase is involved in the phosphorylation of shikimate to shikimate-3-phosphate utilizing the ATP and forming ADP as a by product[12]. Shikimate kinase (SK) (E.C. 2.7.1.71) catalyzes the fifth step in this pathway, where a phosphoryl transfer occurs from adenosine triphosphate (ATP) to shikimate, yielding shikimate-3-phosphate.

The *M.tb* SK belongs to the nucleoside monophosphate (NMP) kinase family. Initially it was classified into three domains: a shikimate binding (SB) domain or NMP binding domain, a LID domain, and a core domain [13]. It was later structurally classified into four domains according to the compartmental changes resulting from ligand binding. These four domains comprise the extended SB domain, the nucleotide binding domain, LID domain, and reduced core domain. The binding of ATP or ADP induces the rotational movement of NB domain toward SB site, whereas binding of shikimate induces rotation of SB domain toward NB site for the proper positioning of shikimate and ATP for phosphorylation [14]. Shikimatekinase (SK, EC 2.7.1.71) catalyses the fifth step of the biosynthetic pathway where shikimate-3-phosphate is formed as the byproduct by the transfer of phophoryl group of adenosine triphosphate (ATP). The enzyme shikimate kinase is an enzyme which is present in several pathogenic bacteria and is absent in humans which makes it a good target. The chorismate formed is an important precursor for the formation of essential aromatic amino acids, ubiquinone and folic acid.

The enzyme consists of three domain [15].

- The CORE domain is a highly conserved phosphate binding loop The nucleotide binding of the ATP or ADP to *MtSK*involves interactions with the P-loop. The P loop tightens around the β phopahtase of ADP resulting to displacement at Gly12 which interacts with the Arg110
- The LID domain closes over bound ATP and bears functionally essential residues. The conserved residue Lys 15, Arg117 and Arg136 on LID domain are the positively charged residues involved in ADP binding and hence may play critical role in stabilization of transition state [16].
- The substrate binding(SB) domain also known as NMP binding domain and is responsible for the recognition and binding of a specific NMP.

The shikimate binding site is characterized by residues Phe49, Phe57, Gly79, GLy80, Gly81. The shikimate makes several key interactions with the binding pocket, first the carboxylate forms hydrogen bonds with Arg136, the carboxylate of Asp34 forms hydrogen bond with shikimate, the amino of Arg58 forms hydrogen bond with the substrate and the Gly80 of the main chain also interacts with the target.

Molecular docking was used to explore the binding conformations of the reported molecules with the target protein. This led to the designing of new molecules based on oxadiazole scaffold, which were docked using the Biopredicta Module in VLife MDS. In silico tools are very useful for the identification of binding sites on proteins [17]. The docking program uses either flexible docking or rigid docking. It is mainly used to study the various binding orientations for a ligand molecule and a receptor molecule. After refining a model, the structure needs to be validated by verifying several properties, such as Ramachandran plot [18]. RMSD values versus template, as well as validating against its natural (co-crystal)or synthetic ligands. Studies were carried out in this data and this led to production of detailed and accurate Psi,Phi Ramachandran plots [18]. It gives us an insight that of the quality of the protein structure which was used for the purpose of analysis was of high quality. Oxadiazole is a cyclic compound having one oxygen and two nitrogen atoms in a five member ring. Oxadiazoles have occupied a specific place in the field of medicinal chemistry due to its wide range of activities. They have been reported widely in literature and posses diverse activities like antimicrobial and ant tubercular activity. We have used the docking to study the interactions of the new 1,3,4-Oxadiazole derivatives with the protein.

MATERIAL AND METHODS

The flow of entire study is well depicted in the figure 1

Docking Study

The computational process of searching for a ligand that is able to fit both geometrically and energetically into the binding site of a protein is called molecular docking [17]. Distinction of good or bad docked conformation is based on scoring or fitness function. Molecular docking was used to explore the binding conformations of the reported molecules with the target protein. This led to the designing of new molecules based on oxadiazole scaffold, which were docked using the Biopredicta Module in VLife MDS. In silico tools are very useful for the identification of binding sites on proteins. The docking program uses either flexible docking or rigid docking. It is mainly used to study the various binding orientations for a ligand molecule and a receptor molecule. After refining a model, the structure needs to be validated by verifying several properties, such as Ramachandran plot, RMSD values versus template, as well as validating against its natural (co-crystal)or synthetic ligands. Studies were carried out in this data and this led to production of detailed and accurate Psi, Phi Ramachandran plots. It gives us an insight that of the quality of the protein structure which was used for the purpose of analysis was of high quality The flow for the docking part followed is is discussed in figure 2 [18].

Different enzymes that are involved in this pathway can serve as individual targets, amongst them is the enzyme shikimate kinase. The docking studies on 260 compounds were performed using Biopredicta Module from Vlife MDS software.The 260 compounds were obtained from literatureand they were docked on target protein shikimate kinase. The interactions of these compounds were observed with the protein and new molecules were designed on basis of interactions observed. The new molecules were further docked on shikimate kinase (PDB code 2YIQ).The docking studies revealed that the conformers of new molecules interacted with shikimate kinase (PDB code 2YIQ) enzyme with the residues, through hydrogen bonding of Arg 58A, Arg 117A, hydrophobic interaction of Gly80A, Gly81A, Arg117A, Asp34 and pi-stacking interaction of Phe49 and Phe57 determined at resolution of 1.8 Å. These interactions were on the lines similar to that of co- crystal ligand, shikimic acid (shikimate).

The dock score and interactions with the residues of the new hypothetical molecules with the target protein were accounted. The hypothetical molecules with the best interactions were taken for synthesis. Shikimate Binding occurs to the cavity 1 (Image 1) consisting of residues Asp 34, Arg58, Gly80 and Arg136 (Image 2). The length of the protein shikimate kinase used is of 166 amino acids at 1.8 Å. The co-crystal ligand and its interactions can be seen in the fig 3.

Biological Activity

Chemicals, strains and media. All the chemicals such as sodium salt XTT, DMSO, sulfanilic acid, sodium nitrate, HCl, NEED and rifampicin, were purchased from Sigma-Aldrich, USA. Dubos medium was purchased from DIFCO, USA. Compounds were dissolved in DMSO (universal Solvent), this solution was used as stock solutions for further antimycobacterial testing.

Microbial strains such as *M.bovis* BCG (*ATCC* 35734) and *Mycobacterium tuberculosisH37Ra* (ATCC 25177) were obtained from AstraZeneca, India. The stock culture was maintained at -80^{o} C and subcultured once in a liquid medium before inoculation into an experimental culture.

Cultures were grown in Dubos media (enrichment media). For antimycobacterial assay, M. pheli medium (minimal essential medium) was used. It contains 0.5 g KH_2PO_4, 0.25 g trisodium citrate, 60 mg $MgSO_4$, 0.5 g aspargine and 2 ml glycerol in distilled water (100 ml) followed by pH adjustment to 6.6.

Antimycobacterium activity

All 10 compounds were screened in vitro against two *Mycobacterium* strains such as *Mycobacterium tuberculosis H37Ra* and *Mycobacterium Bovis BCG*. Both species of *Mycobacterium* were grown in M.pheli medium. Screening of *Mycobacterium tuberculosis H37Ra* was done by using XTT reduction menadione assay and *Mycobacterium Bovis BCG* screening was done by using NR (Nitrate reductase) assay. Briefly 2.5 µL of these inhibitor solutions were added in a total volume of 250 µl of M. pheli medium consisting of bacilli. The incubation was terminated on the 8th day for active and 12 days for dormant MTB culture. The XRMA and NR was then carried out to estimate viable cells present in different wells of the assay plate. The optical density was read on a micro plate reader (Spectramax plus384 plate reader, Molecular Devices Inc) at 470 nm filter for XTT and at 540 nm filter for NR against a blank prepared from cell-free wells. Absorbance given by cells treated with the vehicle alone was taken as 100% cell growth.

Initially primary screening was done at 30, 10 and 3µg/mL. Those compounds which showed 90 percent inhibition of bacilli at 30 µg/mL which were selected for further dose response curve. All experiments were performed in triplicates and the quantitative value was expressed as the average ± standard deviation. MIC and IC_{50} values of selected compound were calculated from their dose response curves by using Origin 6 software. Percent inhibition was calculated by using following formula.

$$\% \text{ Inhibition} = \frac{\text{Cont OD} - \text{Test OD}}{\text{Cont OD} - \text{Blank OD}} \times 100.$$

Blank : Cell free Medium
Control : Medium contains Bacilli along with vehicle

Compound : Medium contains Bacilli along with Drug concentration.

RESULTS AND DISCUSSION

Docking Studies

The shikimate kinase (PDB ID: 2IYQ) was downloaded from the *RCSB* protein data bank. The protein was prepared using vLife engine in the software. This resulted in an optimized protein which gave the results shown below.

Shikimate Binding occurs to the cavity 1 (Image 1) consisting of residues Asp 34, Arg58, Gly80 and Arg136 (Image 2). The length of the protein shikimate kinase used is of 166 amino acids at 1.8 Å. The co-crystal ligand and its interactions can be seen in the fig 4.

The protein analysis data interpretation for the ramachandran plot of proteinshikimate kinase (PDB ID: 2IYQ) from Vlife MDS showed that the protein structure used is of good quality.

The structures of the 10 compounds shown in table 1 were drawn in 2D format (.mol) following which all the 2D structures were converted to 3D structures (.mol2) using VLife engine. The 3D structures were now optimized. The software generated various conformers of optimized 10 compounds. Using these conformers of the 10 compounds batch docking was carried out using GRIP docking (the grid used is shown in image 3) option in the Biopredicta module of the Vlife MDS. The results were obtained ligand wise as function of the dock score. Also the result was analysed by the interactions each conformer of the 10 compounds gave. The dock scores of top five compounds are tabulated in the table below and the interactions of top scoring conformer is depicted in image 4.

The solution of Imidazole (6.8g, 0.1mole) in dry methanol was made. To this solution 5g anhydrous potassium carbonate was added, resulting in a suspension. Ethylbromoacetate (16.7g, 0.1mole) was added to this suspension very slowly with constant stirring. The reaction mixture was then refluxed for about 9 hours, till the completion of reaction which was monitored by TLC, Chloroform: Methanol :: 2:1 mobile phase. After the completion of reaction the reaction mixture was left overnight at RT and filtered. Next day the filtrate was distilled off to obtain Ethyl 2-(1H-imidazol-1-yl)acetate**(1).** Ethyl 2-(1H-imidazol-1-yl)acetate, **(1)**, (15.4g, 0.1 mole) was dissolved in 15 mL methanol and to this solution; 99% of hydrazide hydrate,(7.65g, 0.15 mole) was added slowly under constant stirring. The resultant mixture was refluxed for about 12-14 hours until TLC confirmed the completion of reaction. The completion of the step 2 was confirmed with TLC using Ethyl acetate: hexane :: 2:0.5 mobile phase. The excess of solvent was removed under vaccum to yield 2-(1H-imidazol-1-yl)acetohydrazide, **(2).**

A mixture of (2), (1.91g, 0.01 mole), substituted benzoic acids(0.01 mole) and phosphorous oxychloride (15 mL) was refluxed in an oil bath at 115-120° C until the TLC confirmed the completion of reaction. Then reaction mass was cooled to room temperature and neutralized with ice cold solution of saturated sodium bicarbonate solution. A residue thus obtained was washed thoroughly with cold water, triturated with hexane. The compounds 3a-e were further purified with coloumn chromatography.

The solution of 1*H*-1,2,4-triazole (6.9g, 0.1mole) in dry methanol was made. To this solution 5g anhydrous potassium carbonate was added, resulting in a suspension. Ethylbromoacetate (16.7g, 0.1mole) was added to this suspension very slowly with constant stirring. The reaction mixture was then refluxed for about 14 hours, TLC confirmed the completion of reaction. The completion of the step 1 was confirmed with TLC using Chloroform: Methanol:: 1:1 mobile phase. After the completion of reaction the reaction mixture was left overnight at RT and filtered. The filtrate was distilled to obtain Ethyl 2-(1H-1,2,4-triazol-1-yl)acetate.

Ethyl 2-(1H-1,2,4-triazol-1-yl)acetate, (4), (15.5g, 0.1 mole) was dissolved in 15 mL methanol and to this solution; hydrazide hydrate,(7.65g, 0.15 mole) was added slowly under constant stirring. It was refluxed for about 27 hours until TLC confirmed the completion of reaction. The completion of the step 1 was confirmed with TLC using Ethyl acetate: hexane:: 2:0.5 mobile phase. The excess of solvent was removed under vaccum to yield 2-(1H-1,2,4-triazol-1-yl) acetohydrazide,(5).

A mixture (5), (1.91g, 0.01 mole), substituted benzoic acids(0.01 mole) and phosphorous oxychloride(15 mL) were refluxed in an oil bath at 115-120° C until the TLC confirmed the completion of reaction. Then reaction mass was cooled to room temperature and neutralized with ice cold solution of saturated sodium bicarbonate solution. A residue thus obtained was washed thoroughly with cold water, triturated with hexane. The compounds 3a-e were further purified with coloumn chromatography.

Biological activity

In vitro anti tubercular assay

The results of the *in vitro* anti tubercular assay showed in table 4 indicate that compound 3a, 3c, 3e and 6a are active against the *Mycobacterium tuberculosis* H37Ra and Mycobacterium Bovis *BCG*. Compound 3a and 6a were effective against active *M.Tuberculosis* H37 Ra (table 5). The MIC and IC_{50} values of 3a were found to be 22.58 mcg/mL and 12 mcg/mL while that of 6awas 38.55 mcg/mL and 11.38 respectively.

MTB Shikimate kinase assay

The MTB Shikimate kinase was recombinantly purified and the phosphorylation of Shikimic acid was determined. Since there is no shikimate kinase inhibitor in market, Stauosporine and Rottlerin were used as broad spectrum kinase inhibitors. The assay of the enzyme was

performed either by ESI-LC-MS or by ATP-ADP conversion.

Compounds 3a, 3c, 3e, and 6a were tested for further Shikimate kinase activity. The compounds 6a and 3a showed activity (Figure 3) at 12.5 ug/ml only against whole mycobacteria (MTB H37Ra) in the 2nd in-vitro testing assay done at CDRI, Lucknow. All the compounds were tested for shikimate kinase inhibition and were completely inactive for shikimate kinase inhibition.

Fig 1. A schematic representation of the flow of the study.

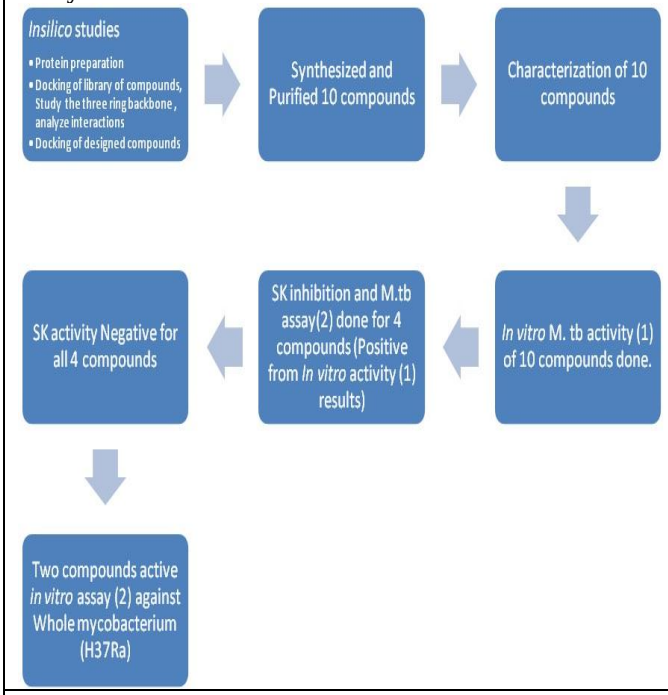


Fig 2. The above chart shows the crux of CADD. Molecular docking was done by selecting the target, shikimate kinase was prepared using VLifeMDS and optimised molecules were docked on the prepared protein

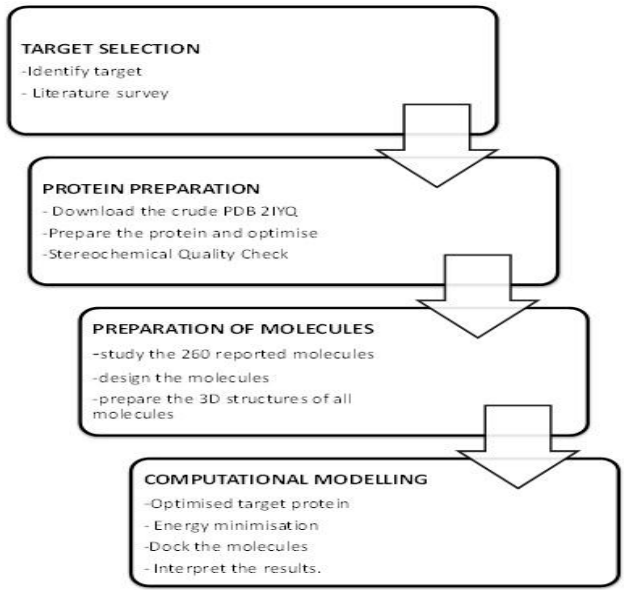


Fig 3. The surface of the optimized shikimate kinase protein and Binding sites the cavity 1 in green(Shikimate binding site) and cavity 3 in yellow.

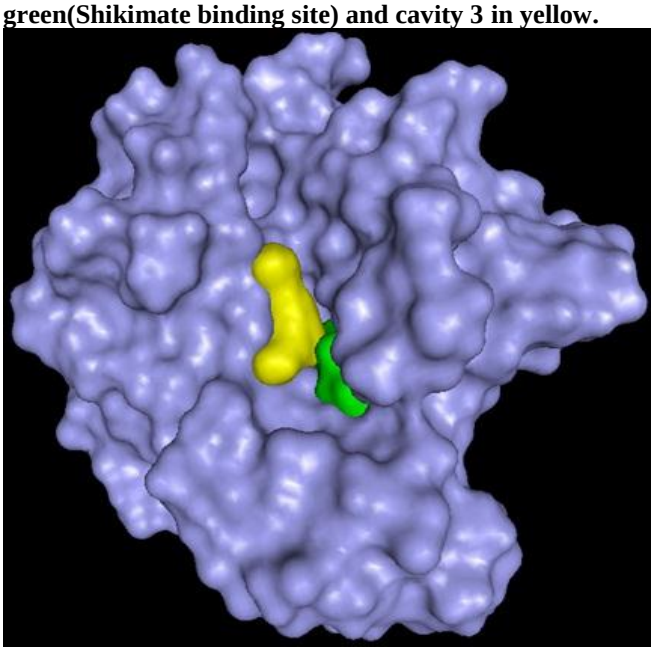


Fig 4. The surface of the optimized shikimate kinase protein and the interactions(H bond in green, Hphobic in light blue and charge in yellow) of the co crystal ligand shikimate.

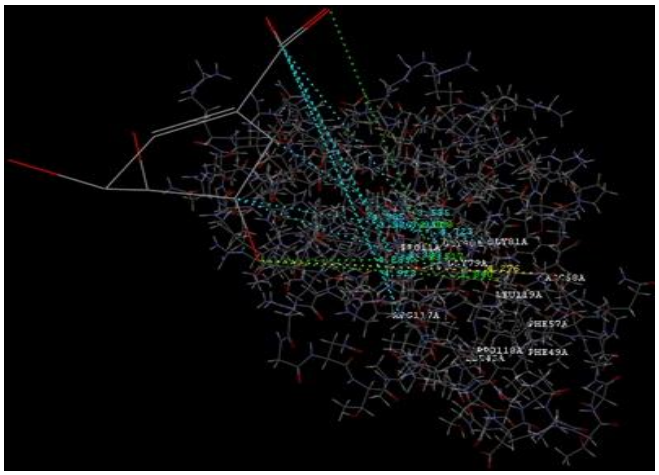


Fig 5. The grid used for GRIP docking of the molecules

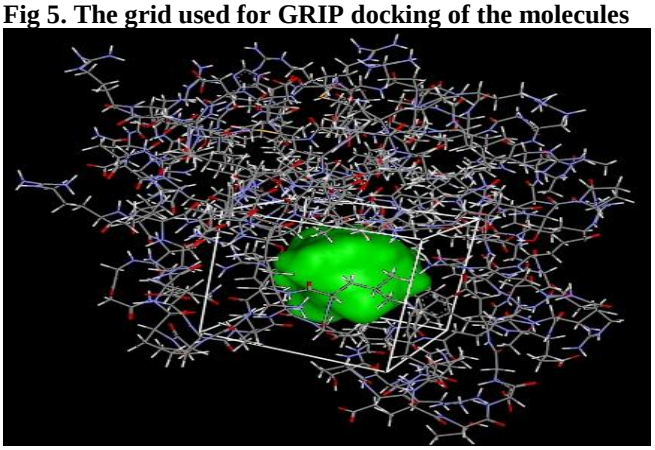
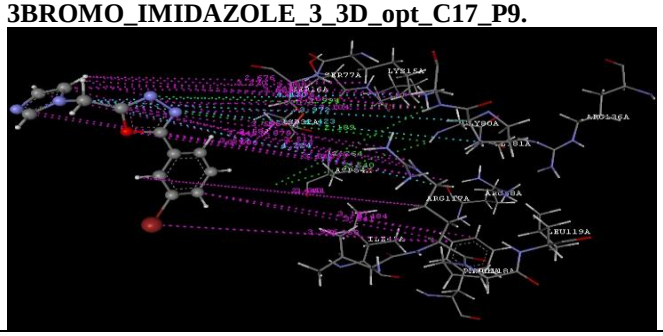


Fig 5. The image above is showing the hydrogen bond interactions with residues Lys 15, Gly 80, hydrophobic interactions with Lys 15, Arg 117 and vanderwals interactions at cavity 1 of protein for 3BROMO_IMIDAZOLE_3_3D_opt_C17_P9.



Graph 1. A plot of percent inhibition versus concentration of compound 6a

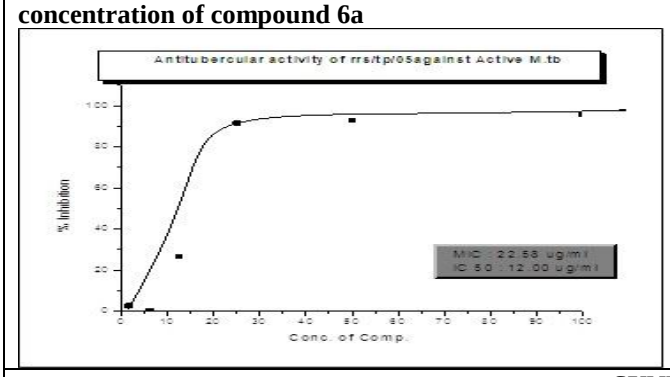
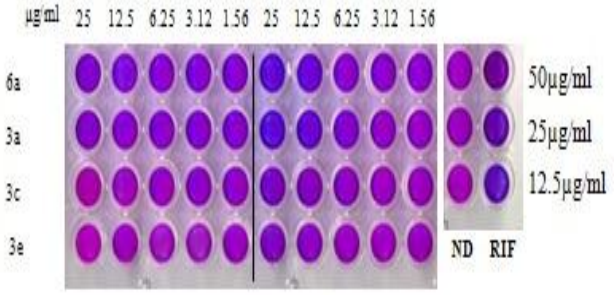
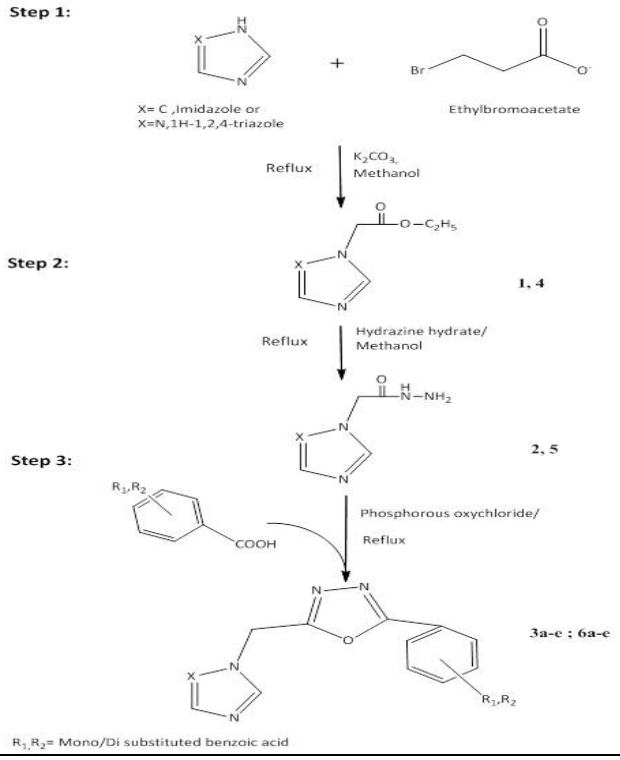


Fig 7. MIC data for four compounds against MTB 37Ra, ND=no drug and RIF=rifampicin



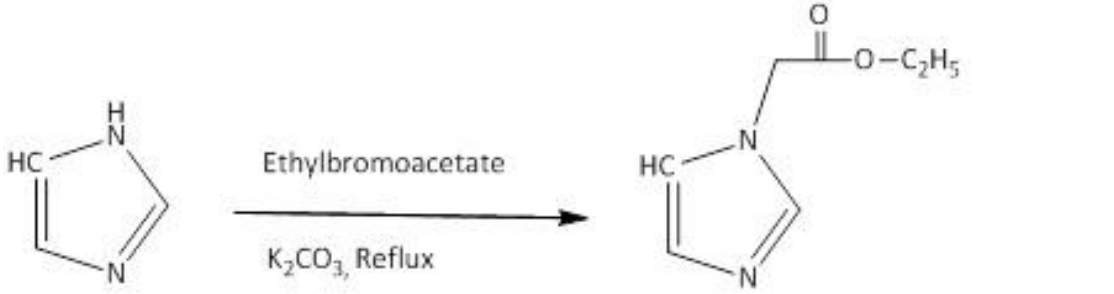
SYNTHESIS

GENERAL SCHEME OF SYNTHESIS



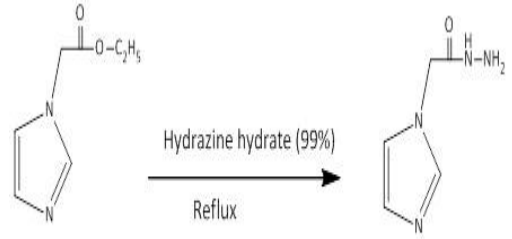
SYNTHESIS OF IMIDAZOLE DERIVATIVES OF 1,3,4- OXADIAZOLE (SCHEME 1)

Step1.a: Formation of ethyl 2-(1H-imidazol-1-yl)acetate from imidazole



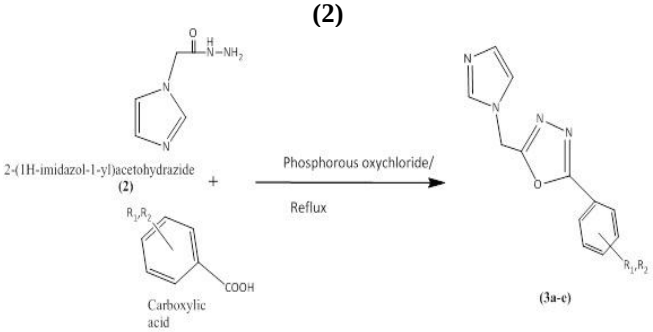
Imidazole — Ethyl 2-(1*H*-imidazol-1-yl)acetate

Step1.2: Conversion of (1) to 2-(1H-imidazol-1-yl)acetohydrazide



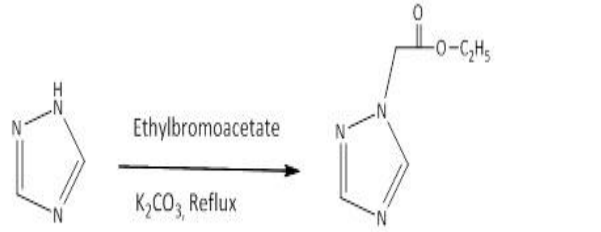
Ethyl 2-(1*H*-imidazol-1-yl)acetate — 2-(1*H*-imidazol-1-yl)acetohydrazide

Step1.3: Formation of 2,5-Diaryl-1,3,4-oxadiazole from (2)



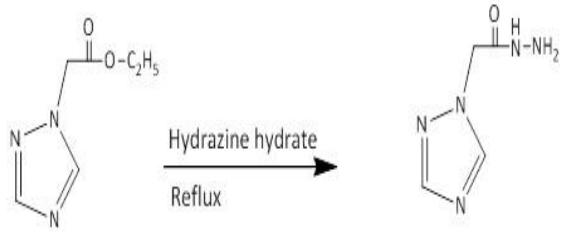
SYNTHESIS SCHEME 2

Step 2.1: Formation of Ethyl 2-(1H-1,2,4-triazol-1-yl)acetate from 1*H*-1,2,4-triazole



1*H*-1,2,4-triazole — Ethyl 2-(1*H*-1,2,4-triazol-1-yl)acetate

Step 2.2: Conversion of Ethyl 2-(1H-1,2,4-triazol-1-yl)acetate to 2-(1*H*-1,2,4-triazol-1-yl) acetohydrazide (5)



ethyl 2-(1*H*-1,2,4-triazol-1-yl)acetate — 2-(1*H*-1,2,4-triazol-1-yl)acetohydrazide

Step2.3: Formation of 2,5-Diaryl-1,3,4-oxadiazole from 2-(1*H*-1,2,4-triazol-1-yl)acetohydrazide

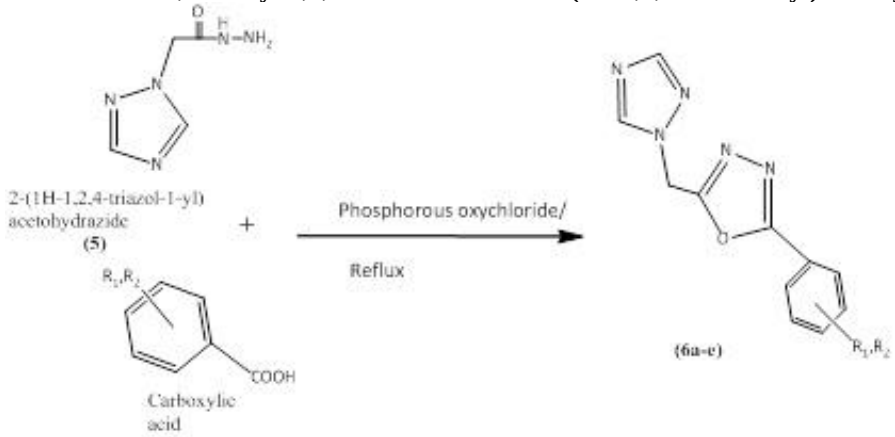


Table 1. Top five scoring compounds and its interactions with the residues of shikimate kinase (PDB ID: 2IYQ)

Rank	Conformer	Dock Score	Compound	Interactions with residues
1	3BROMO_IMIDAZOLE_3_3D_opt_C17_P9	-58.84	**3a**	Hydrogen bond; Lys15, Arg58,Gly80,Gly81,Arg117 Hydrophobic with; Lys15, Gly79, Gly80, Gly81, Arg117 Aromatic ring; Phe49,Phe 57
2	3BROMO_TRIAZOLE_4_3D_opt_C23_P14	-57.71	**6c**	Hydrogen bond; Arg58,Gly81,Arg117 Hydrophobic with; Gly80, Gly81, Arg117
3	4ASA_TRIAZOLE_8_3D_opt_C25_P6	-56.35	**6e**	Hydrogen bond; Lys15, Arg58,Gly81, Arg117 Hydrophobic with; Asp34, Ile45, Arg117
4	3HYDROXY_TRIAZOLE_6_3D_opt_C53_P7	-54.92	**6d**	Hydrogen bond; Lys15, Arg58,Gly80 Hydrophobic with; Asp34, Phe49, Arg117
5	3HYDROXY_IMIDAZOLE_5_3D_opt_C53_P26	-54.02	**3d**	Hydrogen bond; Lys15, Gly80,Arg117 Hydrophobic with; Lys15, Asp34, Ile45,Arg117 Aromatic ring; Phe49,Phe 57

Table 2. The compounds 3a-e along with precursor carboxylic acid used in its synthesis

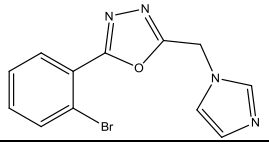
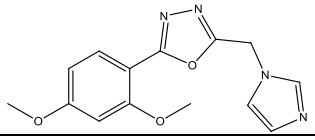
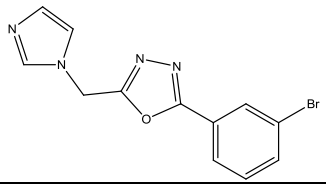
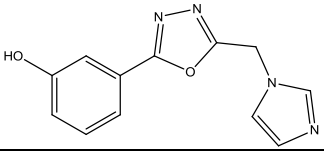
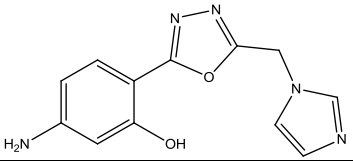
Compound	Ar-COOH	IUPAC name of product
3a	2-Bromo benzoic acid	
3b	2,4-Dimethoxy benzoic acid	
3c	3-Bromo benzoic acid	
3d	3-Hydroxy benzoic acid	
3e	4-Aminosalicylic acid	

Table 3. The compounds 6a-e along with precursor carboxylic acid used in its synthesis

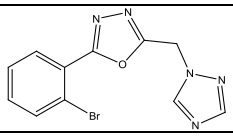
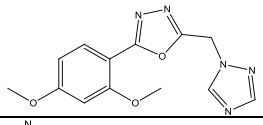
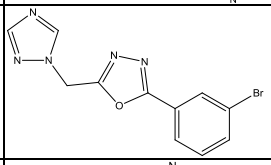
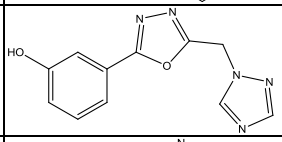
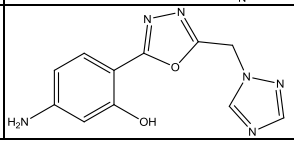
Compound	Ar-COOH	IUPAC name of Product
6a	2-Bromo benzoic acid	
6b	2,4-Dimethoxy benzoic acid	
6c	3-Bromo benzoic acid	
6d	3-Hydroxy benzoic acid	
6e	4-Aminosalicylic acid	

Table 4. *In vitro* anti tubercular activity results of 10 compounds at different concentrations

Compound	*Mycobacterium tuberculosis* H37Ra			*Mycobacterium Bovis BCG*		
	Dormant Stage			Dormant Stage		
	30 mcg/mL	10 mcg/mL	3 mcg/mL	30 mcg/mL	10 mcg/mL	3 mcg/mL
3a	**87.53**	**94.58**	**47.84**	**95.92**	**96.62**	**92.00**
3b	-4.58	9.86	13.71	-82.33	-140.45	-113.95
3c	**84.37**	**76.93**	**33.15**	**95.60**	**95.01**	**84.20**
3d	-8.58	-4.83	-5.02	6.66	3.90	2.88
3e	**87.79**	**75.45**	**9.63**	86.78	-28.39	-0.18
6a	**79.41**	**90.97**	**30.83**	**94.93**	**94.25**	**94.81**
6b	27.53	15.66	20.02	77.52	24.67	24.59
6c	-3.19	32.77	3.73	-13.21	8.65	33.37
6d	-1.73	-4.66	-7.67	-79.30	-106.00	-109.08
6e	67.31	48.83	36.58	74.36	44.48	0.63

Table 5. Biological activity of compounds 6a and 3a against active *M.tb* with MIC and IC $_{50}$

Active *M.Tuberculosis* H37 Ra					
Compound	**100**	**25**	**12.5**	**MIC (ug/ml)**	**IC $_{50}$ (ug/ml)**
6a(rrs/tp/05)	**95.58**	**91.53**	**26.64**	**22.58**	**12**
3a(rrs/tp/06)	**97.42**	**76.72**	**52.42**	**38.55**	**11.38**

CONCLUSION

The compounds were designed *in silico* and were successfully synthesized. They were purified using column chromatography. All the synthesized compounds were characterized using FT-IR and NMR Spectroscopy. The compounds were evaluated for their *in vitro* anti TB activity. The dock score analysis rates compounds 3c, 6c, 6e, 6d, 3d as top five in terms of scoring function compounds. In comparison of results in molecular docking versus anti tubercular activity, compound 3c somewhat performs well in both aspects, the dock rank and the activity against dormant TB strain. However the best biological activity against active anti TB strain was observed for compound 3a and 6a. Even if 3a and 6a are

not in top ten scoring compounds, their *in silico* interactions are along the lines of co crystal ligand shikimate and hence significant. Hence the interpretation of the molecule binding *in silico* should primarily not focus on dock score but the interaction of every conformer of the compound should be analysed.

This study hereby reports compounds 3a and 6a may be further tested for probable in vivo anti-tubercular activities. The compounds showed positive activity against Mycobacterium *in-vitro* which further tested negative in the in-vitro specific shikimate kinase inhibition. Also other molecular targets inside MTB, excluding shikimate kinase for the said compounds could be explored.

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CONFLICT OF INTEREST

No interest

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