



## MOLECULAR DOCKING AND DYNAMIC SIMULATION OF 3-DEHYDROQUINATE DEHYDRATASE FROM *MYCOBACTERIUM TUBERCULOSIS*

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### ABSTRACT

Tuberculosis is the most common cause of death due to an infectious agent known as *Mycobacterium tuberculosis*. Therefore, the aim of this study was to identify novel inhibitors of 3-dehydroquininate dehydratase of Shikimate pathway using both natural and synthetic ligands libraries. Three dimensional structure (3D) of 3-dehydroquininate dehydratase was retrieved from Protein Data Bank (PDB ID: 3N76) and subjected to energy minimization and protein optimization. Virtual screening was performed based on physicochemical properties (Lipinski rule of five) and biological activity. Molecular docking analysis of protein-ligand complex was carried out using AutoDock4.0 for both synthetic and natural ligands (PubChem Database and Zinc Database). Molecular Dynamic (MD) simulation was also performed to determine the stability of protein-ligand complex using CHARMM (Chemistry at Harvard Macromolecular Mechanics) force field in NAMD v2.12 (Nanoscale Molecular Dynamic Program). The results of molecular docking revealed that five molecules had high binding energy (ZINC14981770, ZINC01147665, ZINC22910025, ZINC8442077 and PubChem72341) range between -8.99 and -8.39 kcal/Mol. Leu14, Gly15, Gly18, Glu21, Tyr25, Gln52, Asp54, Gln58, Asp76, Gly79, Ile103 and Arg109 stabilised the protein-ligand complex through the formation of hydrogen bonding. Lastly, the ligands were further screening for pharmacokinetic properties based on Absorption, distribution, metabolism, excretion and toxicity (ADMET) properties. Therefore, these ligands will be recommended for the treatment of tuberculosis after successful *in vitro* and *in vivo* biological assay.

**KEYWORDS:** *Nanoscale Molecular Dynamics (NAMD), Molecular Dynamic (MD) Simulation, Mycobacterium tuberculosis, 3- dehydroquininate dehydratase and Docking*



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## INTRODUCTION

Tuberculosis is the most common cause of death due to an infectious agent known as *Mycobacterium tuberculosis* (MTB). It is a devastating disease which leading to higher morbidity and mortality across the world. The present drugs in existence, play an important role in controlling the disease to the extent that is being done today, although several lacuna attached to it, the most common among them is the emergence of drug resistance making even the frontline drugs inactive. Besides, drugs such as rifampicin have high levels of side effects, devastating to patient compliance. In addition, most of the antitubercular drugs, lack ability to attack the latent stage of the bacillus. Another major problem is the presence of human immunodeficiency virus (HIV) couple with TB co-infection poses a tremendous challenge for antimycobacterial drug discovery<sup>1</sup>. Despite the presence of vaccine, Bacille Calmette-Guerin (BCG) and short-course chemotherapy, tuberculosis responsible for claiming many lives than any other single infectious agent. In recent years the diseases have seen an increased incidence especially in developing countries due to widespread of emergence of multidrug-resistant tuberculosis and co-infection with human immunodeficiency virus. Resistance due to multidrug-resistant tuberculosis (MDR-TB) is a serious threat posing a great challenge globally and impede the tuberculosis control programme. Since the advent of HIV/TB co-infection many people died of tuberculosis<sup>2-5</sup>. Tuberculosis (TB) is the major threat to global health resulting to about 8.6 million people infected in 2012<sup>6</sup>. The World Health Organization (WHO) reported that almost 450, 000 people had multidrug-resistant tuberculosis (MDR-TB) in 2012, in contrast to 62,000 cases in 2011. Of this total, 9.6% cases were found to have extensively drug-resistant tuberculosis (XDR-TB)<sup>6</sup>. Due to rapidly growing MDR-TB and XDR-TB cases, it is necessary for discovery of new and alternative tuberculosis drug that can combat the resistance species of the organism. An alternative and less expensive drug development is very important to completely eradicate the multidrug resistant cases of the tuberculosis. From 1980 to 2004 about 81 million new cases were reported and based on genome sequencing it clearly indicated that many enzymes can be useful as a potential candidate for computational drug design including *3-dehydroquininate dehydratase* in shikimate pathway from *Mycobacterium tuberculosis*. Shikimate pathway enzymes play an indispensable role in the biosynthesis of aromatic amino acids and many other essential compounds in plants, bacteria, fungi and algae, and due to their absence in mammal making them an important target for development of antimicrobial drugs. It is a biosynthetic pathway that starts with condensation of erythrose 4-phosphate and phosphoenolpyruvate to chorismate which can be achieved in seven steps<sup>7</sup>. One of the important enzymes in this pathway is *3-dehydroquininate dehydratase* that catalysing third of the seven steps involved in the pathway. This enzyme responsible for catalysing 3-dehydroquininate to 3-dehydroshikimate by releasing water molecules, thus inhibition of this enzyme is a promising target for the development of antitubercular

drugs. Therefore, the aim of this study was to determine *in silico* docking and molecular dynamic simulation of 3-dehydroquininate dehydratase from *Mycobacterium tuberculosis*.

## METHODOLOGY

### **Protein Optimization and Energy Minimization**

The crystal structure of *3-dehydroquininate dehydratase* was obtained from Protein Data Bank (PDB ID: 3N76) with resolution of 1.9Å and optimized through separating the protein residues and non-protein moieties. To ensure reliable and high quality structure, the protein was subjected to energy minimization with SPDV by adding all missing hydrogens and removal of all water molecules.

### **Binding site prediction**

The active site of the protein was predicted using 3DLigandSite-Ligand binding site prediction Server (<http://www.sbg.bio.ic.ac.uk/3dligandsite/>)<sup>8</sup>, which determined the functional active residues in the target protein that have high capability and potentiality to bind to the ligands.

### **Virtual screening of the ligands**

The compounds with antibacterial activity were obtained from Zinc database and PubChem database public domain through virtual screening using RASPD online tool<sup>9</sup>. The compounds were further filtered for physicochemical properties and drug likeness using Molsoft server based on Lipinski rule of five. Also bioactivity such as GPCR ligand, Ion channel modulator, Kinase inhibitor, nuclear receptor ligand, Protease inhibitor and Enzyme inhibitor of the ligands were determined using Molinspiration online tool and all compounds passed these tests were further used for molecular docking.

### **Molecular docking**

Molecular docking was performed to determine the interaction modes and binding affinities between the modelled structure of *3-dehydroquininate dehydratase* and different ligands using Autodock4.0<sup>10</sup>, which uses a rapid energy evaluation through precalculated grids of affinity potentials with different search algorithms to determine the suitable binding positions for a ligand on a given protein. All torsion angles for each ligand were made to be flexible. The grid maps representing the proteins in the actual docking process were determined using AutoGrid. The dimensions of the grids were set as 60×60× 60 Å, with a spacing of 0.375 Å between the grid points<sup>11</sup>.

### **Determination of absorption, distribution, metabolism, excretion and toxicity (ADMET)**

The pharmacokinetic properties of the ligands were determined based on ADMET properties using AdmetSAR online tool (<http://lmmd.ecust.edu.cn:8000/predict/>)<sup>12</sup>. Also mutagenicity of the ligands was determined based on the Ames toxicity test which predicts the tendency of the ligands to cause mutation to human cells. The Admet assay is necessary in drug discovery and development because it affects the drug levels and exposure of the drug to the tissues and hence affects the performance and pharmacological activity of the ligand as a drug.

### Molecular Dynamic Simulation

The molecular dynamic simulation of protein-ligand complex was performed using NAMD-VMD program<sup>13</sup>. The complex was prepared using and chimera<sup>14</sup> and parameterized using SwissParam servers<sup>15</sup>. The coordinate of all atoms in the complex were generated through protein structure file (PSF) and then solvated with a 10.0 Å of TIP3P water box. Finally, the solvated protein-ligand complex were equilibrated and minimized with 1,000 steps and simulation of the complex was performed at 1,000,000 (2ns) runs. The entire trajectory was saved for further analysis.

## RESULTS AND DISCUSSION

### Protein Optimization and Energy Minimization

The pandemic effect of multi-drug resistance TB (MR-TB) and extensively drug-resistant tuberculosis (XDR-TB) are global threat. Due to some lacuna attached to standard six month treatment for TB, MDR-TB case becomes ineffective, costly and time consuming. Therefore, an alternative and less expensive drug development is very important to completely eradicate the multidrug resistant cases of the tuberculosis. Shikimate pathway plays indispensable role in the synthesis of aromatic compounds and many other essential nutrients in plants and microorganisms (bacteria, fungi and algae) and due to their absences in mammal making them an important target for the development of antimicrobial drugs. In this study 3-dehydroquinase dehydratase from shikimate pathway of *Mycobacterium tuberculosis* H37Rv was obtained from Protein Data Bank (PDB ID: 3N76) with resolution of 1.9Å. The protein was optimized by removing all non-residues moieties and the energy minimization was carried out by adding all missing hydrogens and removal of all water molecules.

### Binding site prediction

The binding site prediction of 3-dehydroquinase dehydratase was performed using 3DLigandSite-Ligand binding site prediction Server, which determines the active site with higher average precision. Leu14, Gly15, Gly18, Glu21, Tyr25, Gln52, Asp54, Gln58, Asp76, Gly79, Ile103 and Arg109 found to be functional active sites of the 3-dehydroquinase dehydratase.

### Virtual screening of the ligands and Molecular docking

The compounds with antibacterial activity were obtained from Zinc database and PubChem database public

domain through virtual screening using the RASPD online tool<sup>9</sup>. The total of nineteen thousand seven hundred compounds (19700 compounds) was obtained and further filtered based on physicochemical properties (molecular and drug likeness) and bioactivity. Three hundred and fifty (350) compounds were selected based on the aforementioned rules and subjected to molecular docking to determine the binding poses between the 3-dehydroquinase dehydratase and ligands using Autodock4.0 (Table 1 and 2). Five compounds showed a promising activity against the 3-dehydroquinase dehydratase and satisfied Lipinski rule of five and possessed drug likeness properties (Table 3). The biological activities, of the compounds include GPCR ligand also known as 7-Transmembrane receptors (7-TM receptors), which are target of majority of all modern medicinal drugs. They are readily accessible to hydrophilic drugs due to their expression on the cell surface which provides selectivity in activating event. Therefore the antagonist and agonist of 7-TM receptors are essential in the treatment of disease in every organ system. In this study, three ligands (ZINC14981770, ZINC22910025 and PubChem72341) are antagonists of GPCR ligand while the remaining ligands are agonist. Other biological activities include Ion channel modulator, Kinase inhibitor, Nuclear receptor ligand, Protease inhibitor and Enzyme inhibitor were determined and all the ligands showed various degree of antagonist and agonist activities (Table 2). The ligands bind to different residues constituting the binding pocket of the protein. The compound with least binding energy was ZINC14981770 with minimum binding affinity of -8.99 kcal/mol and inhibitory constant of 258.55 nM (nanomolar) and formed hydrogen bond with Tyr25 and Asn76 residues, followed by ZINC01147665 (minimum binding affinity of -8.57 kcal/mol and inhibitory constant of 525.12 nM (nanomolar) and formed hydrogen bond with Leu14, Asn76 and Gly78 residues), ZINC22910025 (minimum binding affinity of -8.48 kcal/mol and inhibitory constant of 681.02 nM (nanomolar) formed hydrogen bond with Gly11, Gly15, Asp54, His30 and Asp31) and ZINC8442077 (minimum binding affinity of -8.41 kcal/mol and inhibitory constant of 681.02 nM (nanomolar) and formed hydrogen bond with Asp54 and Gly15), on the other hand natural ligand (PubChem72341) had least binding energy of -8.39 kcal/mol and inhibitory constant of 712.89 nM (nanomolar) and formed hydrogen bond with Glu21, Asp76, and His102 (Table 3: Figure 1).

**Table 1**  
**Physicochemical/molecular and drug likeness properties of ligands with least docking scores**

S/No.	Zinc /PubChem ID	Molecular weight	Number of HBA	Number of HBD	MolLogP	MolLogS (in Log(moles/L))	MolPSA (Å <sup>2</sup> )	MolVol (Å <sup>3</sup> )
	Range value	<=500	<=10	<=5	<5		<140	
1.	ZINC14981770	453.27	6	2	1.94	-1.19	67.70	466.34
2.	ZINC01147665	472.10	6	2	5.70	-8.63	68.22	454.92
3.	ZINC22910025	492.26	6	0	5.42	-5.24	40.24	523.84
4.	ZINC8442077	456.10	8	2	1.98	-5.46	99.21	448.12
5.	PubChem72341	475.28	5	3	4.11	-4.87	58.36	506.73

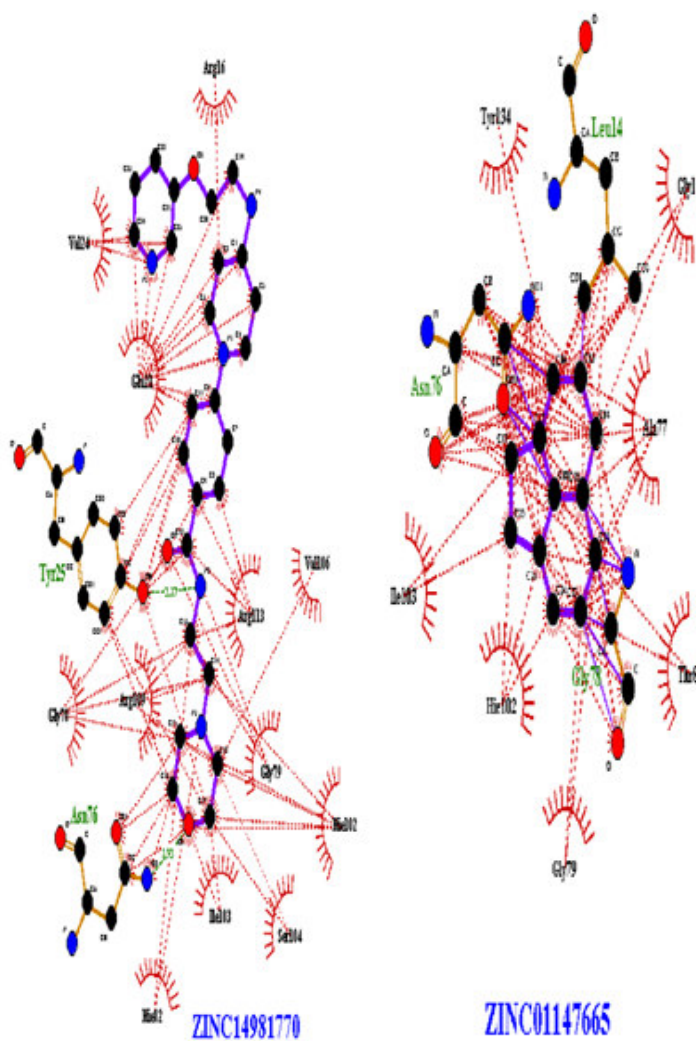
**LogP (octanol/water partition coefficient), LogS (water solubility), Molecular Polar Surface Area (PSA) and Volume**

**Table 2**  
**Biological activity of ligands with least docking score**

S/No.	Zinc /PubChem ID	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
1.	ZINC14981770	0.26	0.23	0.26	-0.17	0.08	0.08
2.	ZINC01147665	-0.57	-0.55	-0.64	-0.70	-0.60	-0.34
3.	ZINC22910025	0.06	-0.29	-0.38	-1.15	-0.39	-0.24
4.	ZINC8442077	-0.89	-1.45	-0.97	-1.52	-1.01	-0.75
5.	PubChem72341	0.36	0.15	-0.13	-0.15	-0.02	0.08

**Table 3**  
**Docking result of 3-dehydroquinate dehydratase with synthetic and natural ligands**

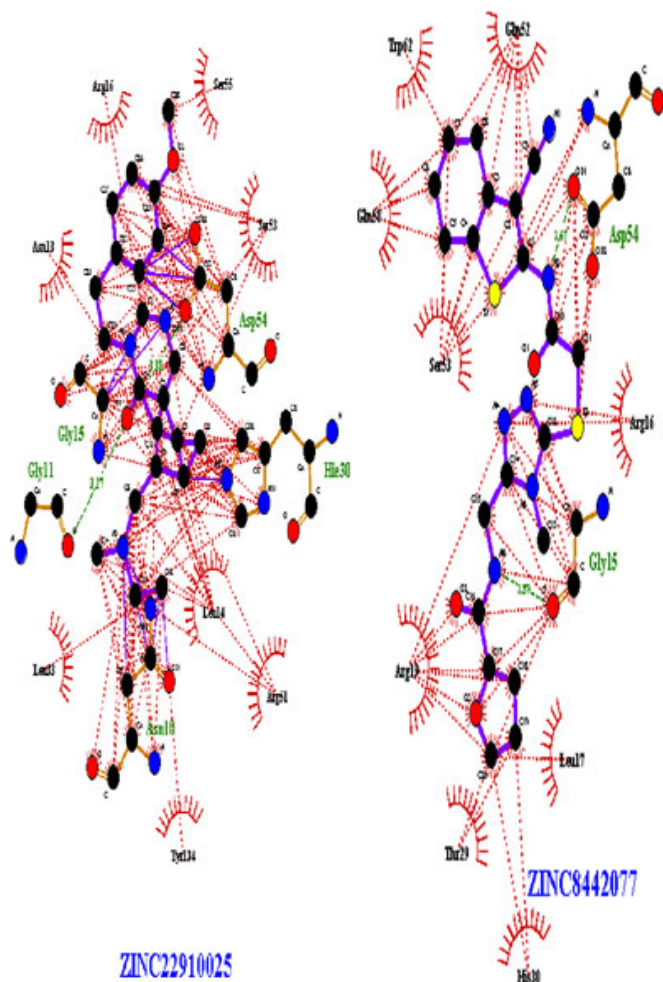
S/No.	Zinc Code	Minimum Free Energy of Binding (kcal/mol)	Inhibition Constant, Ki (nM (nanomolar))	Residues Involved in Hydrogen Bonding	Number Number of Hydrogen Bonding	Residues Involved in hydrophobic interaction
1.	ZINC14981770	-8.99	258.55	Tyr25 and Asn76	2	Arg16, Val24, Glu21, Gly78, Arg109, His82, Ile103, Ser104, Gly79, His102, Arg113, Val106
2.	ZINC01147665	-8.57	525.12	Leu14, Asn76 and Gly78	3	Tyr134, Ile103, His102, Gly79, Thr81, Ala77, Gly11
3.	ZINC22910025	-8.48	604.37	Gly11, Gly15, Asp54, His30 and Asp31	5	Arg16, Asn13, Leu33, Tyr134, Leu14, Arg51, Ser53, Ser55
4.	ZINC8442077	-8.41	681.02	Asp54 and Gly15	2	Gln52, Trp62, Gln58, Ser53, Arg19, Thr29, His30, Leu17, Arg16
5.	PubChem72341	-8.39	712.89	Glu21, Asp76, and His102	3	Arg16, Asn13, Leu14, Gly78, Gly79, Tyr25, Arg113



(a)

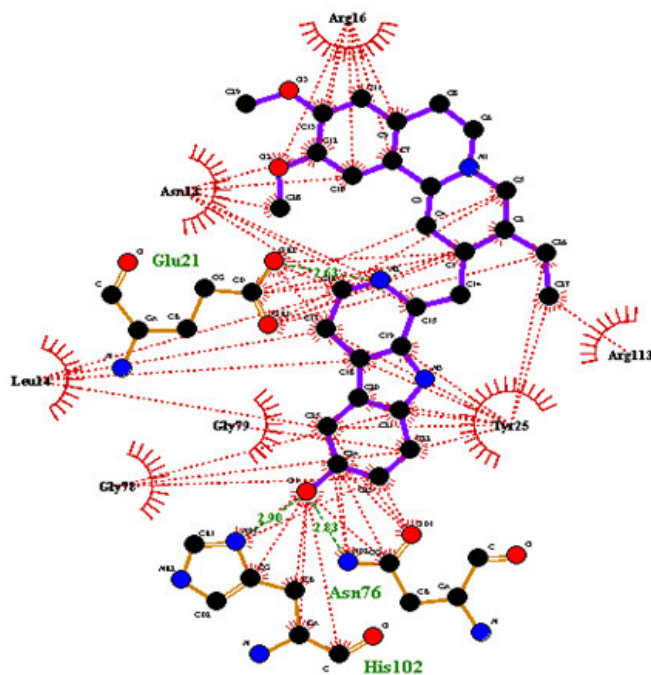
(b)





(c)

(d)



PubChem72341

(e)

Figure 1(a-e)  
Molecular interaction of best five ligands with functional active site of 3-dehydroquinate dehydratase

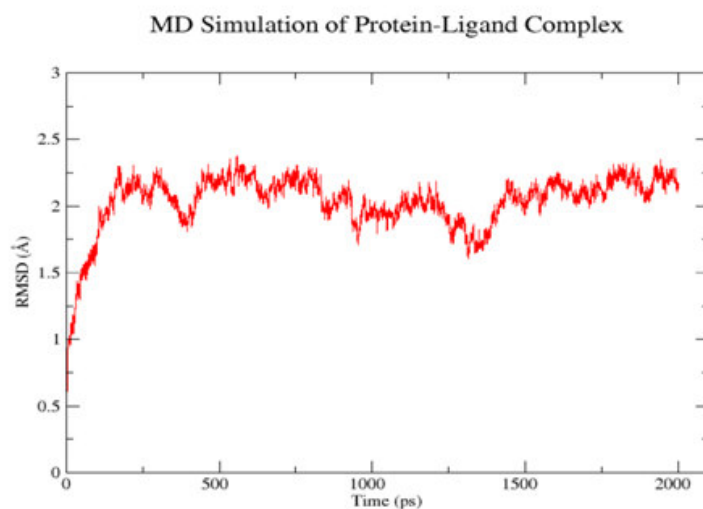
**ADMET properties of ligands**

The ADMET properties of ligands with least docking score were shown in Table 4. The absorption properties of the ligands were tested based on blood brain barrier and human intestinal absorption and all the ligands shown positive to both. Distribution and metabolism were tested based on Cytochrome P450 2D6 (CYP450 2D6) assay and with the exception of ZINC22910025 and PubChem72341, all the remaining ligands were non-inhibitor of the CYP450 2D6 enzymes. Finally, excretion and toxicity assay were determined based on ames toxicity assay, carcinogens assay and biodegradation assay and clearly unravel all the ligands were neither toxic nor carcinogens to human cell (Table 4). The combination of molecular docking, virtual screening and molecular dynamic simulation of 3-dehydroquinate dehydratase performed in this study can

be further evaluated by both in vivo and in vitro biological assay.

**Molecular Dynamic Simulation**

Molecular dynamic simulation was performed to determine the stability or dynamic properties of protein-ligand complex. The result shows that the overall conformation of the complex. The overall system was monitored using root mean square deviation (RMSD) with Phi and Psi value. The protein-ligand complex was simulated for 2000 ps and flexible amino acids residues were found in the active site which gives a reliable conformation of the complex, due to hydrogen bonding and hydrophobic interaction (Figure 2). Thus, the trajectories analysis shows that ligand was stable within the binding pocket of the 3-dehydroquinate dehydratase (Glu21, Tyr25, Asn76 and Ile103).

**Figure2**

**Root mean square deviation of 3-dehydroquinate dehydratase- ZINC14981770 complex runs for 2ns**

**Table 4**  
**ADMET properties of ligands with least docking score**

S/N O.	Zinc /PubChem ID	Absorption			Distribution and Metabolism			Excretion and Toxicity					
		Blood-Brain Barrier	Result	Human Intestinal Absorption	Result	CYP450 2D6 Inhibitor	Result	AMES Toxicity	Result	Carcinogens	Result	Biodegradation	Result
1.	ZINC14981770	0.9366	BBB+	0.9360	HIA+	0.7684	Non-Inhibitor	0.6117	Non Ames toxic	0.8696	Non-carcinogens	0.9722	Not ready biodegradable
2.	ZINC01147665	0.9772	BBB+	0.9853	HIA+	0.8207	Non-Inhibitor	0.6160	Non Ames toxic	0.8117	Non-carcinogens	0.9920	Not ready biodegradable
3.	ZINC22910025	0.8704	BBB+	0.9412	HIA+	0.6153	Inhibitor	0.5856	Non Ames toxic	0.9202	Non-carcinogens	0.5086	Not ready biodegradable
4.	ZINC8442077	0.9134	BBB+	0.9746	HIA+	0.7973	Non-Inhibitor	0.5759	Non Ames toxic	0.7555	Non-carcinogens	0.9870	ready biodegradable
5.	PubChem72341	0.5350	BBB+	0.9754	HIA+	0.9025	Inhibitor	0.7994	Non Ames toxic	0.9188	Non-carcinogens	1.0000	Not ready biodegradable

**Blood-Brain Barrier: BBB+ (positive) and BBB- (negative), Human Intestinal Absorption: HIA+ (positive), HIA-(negative)**

## CONCLUSION

3-dehydroquininate dehydratase is an important enzyme in shikimate pathway which was considered as a best target for drug development against *Mycobacterium tuberculosis*. Leu14, Gly15, Gly18, Glu21, Tyr25, Gln52, Asp54, Gln58, Asp76, Gly79, Ile103 and Arg109 found to be a functional active site of the model protein. Five compounds indicated high activity and great potential to use as anti-tubercular drugs (possessed minimum binding energy/docking score, fulfilled Lipinski rule of five and passed ADMET test). Therefore, these natural and synthetic ligands will be used for the

treatment of tuberculosis after successful pass *in vitro* and *in vivo* evaluations of bioactivity.

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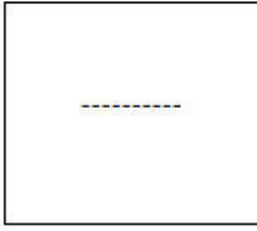
## CONFLICT INTEREST

Conflict of interest declared none.

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