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*Research Article*

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**ABSTRACT**

**The biosynthetic pathway of riboflavin is an essential one for *Mycobacterium tuberculosis*. The inhibitors of the enzymes which are involved in this pathway are not likely to interfere with the enzymes of the mammalian metabolism. So these enzymes could be considered as attractive targets for the development of new drugs against *M. tuberculosis*. The present study focuses on the enzyme Lumazine synthase (LS) which catalyzes the penultimate step in the riboflavin biosynthesis pathway. The main objective is to search for an inhibitor of LS by virtual screening method. The binding energy of 11 already reported inhibitors of LS were compared with that of the 100 new experimental ligands using AutoDock. In-silico ADMET, study was also performed to know their unique drug properties. From all the in-silico study, Quinapril proved to be the potent inhibitor of *M. tuberculosis* LS. It showed better binding energy than any other ligands from set one. It was not previously known for its anti-TB activity so it could be a novel inhibitor of *M. tuberculosis*.**

**Keywords- Autodock; Drug-design; In-silico; Lumazine Synthase; Tuberculosis; Virtual screening; Quinapril.**

## INTRODUCTION

*Mycobacterium tuberculosis* is the main causative agent of Tuberculosis (TB) in humans (Kumar et al., 2007). It is the most frequently occurring infectious disease in the world and also considered as one of the major cause of morbidity, disability and death globally (Tomioka and Namba, 2006). WHO reported that in 2011 almost 8.7 million people were infected with TB and 1.4 million lives were claimed by TB (WHO, Global tuberculosis control 2012). In case of India, WHO reported 300,000 deaths (excluding HIV) in the year 2011 because of tuberculosis (WHO Tuberculosis country profiles 2012). The available treatment of TB requires many months of therapy with more than one drug. This extensive course of treatment not only results in poor compliance but also helps in the development of multidrug-resistant (MDR) *M. tuberculosis* strains, which would spread the infection in much of the world (Sassetti et al., 2003). In 2011, there were an estimated 3,10,000 cases of MDR-TB, and almost 60% of these cases were in India, China and the Russian Federation (WHO Global tuberculosis control 2012). There are antibiotics that are effective in treating mycobacterial infections but these drugs target a small number of essential functions in human. Therefore identification of such pathway is very much crucial that absent in our body but essential for bacteria. The extensive studies of these pathways give us new avenues for the rational design of more effective anti-mycobacterial drugs that could be active even against MDR strains (Sassetti et al., 2003).

Genomic analysis studies have suggested that the riboflavin (Vitamin B2) biosynthesis pathway is essential in *M. tuberculosis* (Cole et al., 2001). It is the direct precursor of redox enzyme cofactors flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD), which are essential for multiple cell physiology. That is why this pathway is regarded as a rich resource for therapeutic targets for broad spectrum antibiotics (Long et al., 2010). *M. tuberculosis* is absolutely dependent on the endogenous synthesis of riboflavin because it is unable to take up the vitamin from the environment. On the other hand the enzymes involved in this pathway are not present in the human or any other animals which makes them promising candidates for the inhibition of bacterial growth (Morgunova et al., 2006). Lumazine synthase (LS) and riboflavin synthase are two enzymes in the riboflavin biosynthesis pathway **(Figure 1)**. LS catalyzes the condensation of 5-amino-6-D-ribitylamino-2,4-(1H,3H)-pyrimidinedione (**A**) with 3,4-dihydroxybutanone 4-phosphate (**B**) to afford 6,7-dimethyl-8-D-ribityllumazine (**C**) ( Neuberger and Bacher , 1986 ; Kis et al . , 1 9 9 5 ) . Riboflavin synthase catalyzes a

mechanistically unusual dismutation of two molecules of **C** to form one molecule of riboflavin (**D**) and one molecule of **A (**Plaut and Harvey, 1971; Bacher et al., 1996; Illarionov et al., 2001). LS could be an attractive target for the design and synthesis of new antibiotics.

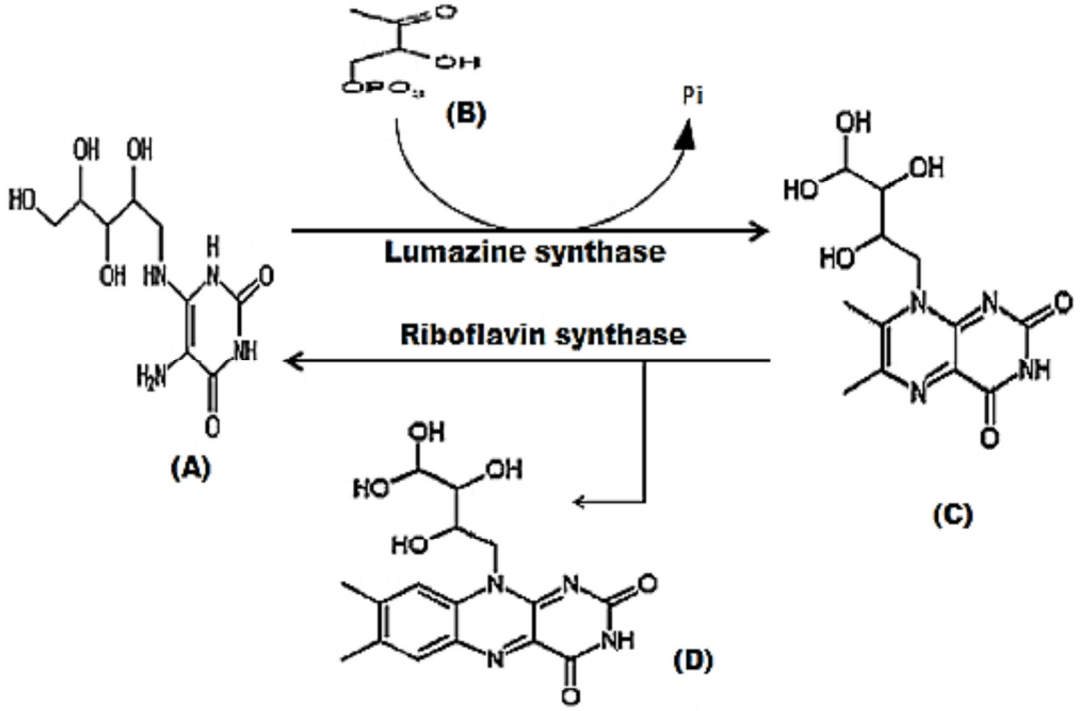


**Figure.1** A schematic diagram of terminal reactions that are catalyzed by lumazine synthase and riboflavin synthase in the pathway of riboflavin biosynthesis. (**A**) 5-amino-6-ribitylamino-2,4(1*H*,3*H*)-pyrimidinedione; (**B**) 3,4-dihydroxy-2-butanone 4-phosphate; (**C**) 6,7-dimethyl-8-ribityllumazine; and (**D**) riboflavin

So far various pyrimidine or purine ring containing compounds were reported as potent inhibitors of LS (Persson et al., 1999; Gerhardt et al., 2002; Morgunova et al., 2005; Morgunova et al., 2006; Zhang, Y. et al., 2008; Zhang, X. et al., 2008). These compounds were almost identical to substrate of LS (designated as A in **figure1**) and inhibit the activity of the enzyme in competitive mechanism. The pyrimidine or purine ring takes part in the bond formation that helps the inhibitors to bind at the active site of LS. But it is not known that the ligand that does not have either pyrimidine or purine ring can also bind with LS. In order to quest for new structural entities this study we include 100 ligands that don't have either pyrimidine or purine ring in their structure. They are also not yet known for their anti-TB effect. These 100 ligands were virtually screened for their binding ability with *M. tuberculosis* LS by computational docking method.

## MATERIALS AND METHODS

### Protein 3D structure preparation

The 3D structure of LS from *Mycobacterium tuberculosis* solved by X-ray crystallography at 1.90Å (2C94) was retrieved from the Protein Data Bank (http://www.pdb.org). All the water molecules, ligand and potassium ion were removed from protein structure using Arguslab® software and modified structure of LS was saved as .pdb file extension for further docking studies.

### Preparations of ligands

Two sets of ligand molecules were prepared. One is designated as 'reference ligands' which were extracted from PDB. These ligands contain pyrimidine or purine ring and were reported as inhibitors of LS of different microorganisms in previous works. This set of ligand was prepared to compare their binding energy with that of the ligands of experimental set. ***(See Table 1 Supplimentary material)***.

The second set is designated as 'experimental ligands' which were derived from DrugBank database (http://www.drugbank.ca/). In a previous work N-[2,4-Dioxo-6-D-ribitylamino- 1 , 2 , 3 , 4 - tetrahydropyrimidin - 5 -

yl]oxalamic acid derivatives were reported as potent inhibitors of *M. tuberculosis* LS (ZhangY et al., 2008). So we started our ligand search using oxalamic acid derivatives which do not have either pyrimidine or purine ring. Only one ligand (**DB**02784**)** with our choice was found. By searching similar structures in the database a set of 100 ligand molecules were prepared. Some of them were approved drugs for other diseases and some were experimental drug molecules which yet to receive the approval. ***(See Table 2 Supplimentary material)***

**Docking of ligands**

The reference ligands as well as experimental ligands were docked into the binding site of the *M. tuberculosis* LS structure using software AutoDock4.2. AutoDock combines an empirical free energy force field with a Lamarckian Genetic Algorithm, providing fast prediction of bound conformations with predicted free energies of association (Morris et al., 2009). The polar hydrogen atoms were added to the LS structure and its non-polar hydrogen atoms were merged. Kollman charges were assigned and solvation parameters were added to this enzyme molecule. For the ligands, non-polar hydrogen atoms were merged with Gasteiger charges assigned. All rotatable bonds of ligands were set to be rotatable. The 40 x 40 x 40 dimension of grid box size and 0.375 Å grid spacing was placed around the catalytic triad to cover the entire enzyme active site mentioned by Zhang, Y. et al., (2008) and accommodate ligands to move freely. Clustering histogram analyses were performed after the docking searches. The best conformations were chosen from the lowest docked energy that populated in the highest number of molecules in a particular cluster with not more than 2.0 Å root-mean-square- deviation (rmsd).

**In-silico ADMET study**

After oral administration, a drug travels through different environment inside the body before reaches the target site. The ADME (absorption, distribution, metabolism and elimination) study measures how well a drug is making this journey. Another important aspect along with ADME that needs to be measured is its toxicity (ADMET). The ideal oral drug or 'drug-like' compound will be rapidly and completely absorbed from the alimentary canal. Then it will move directly to its site of action and specifically bind to it, may be a substrate for the liver enzymes and transporters that clear alien compounds from the body, but in an entirely predictable fashion and neither it will induce nor inhibit their activity. Consequently, there is no risk that breakdown of this ideal compound will give rise to any toxic metabolites and every chance that the compound will have an appropriate half-life, passing gradually through the kidneys without harming them (Hodgson, 2001). The ADMET properties of experimental ligands could be evaluated in-silico for their drug-likeness. This approach in association with docking study helps in selecting potent lead molecule which can be further carried through the drug discovery and development cycle (Bharath et al., 2011). The most important study of "Lipinski's rule of five" which points out some critical properties must be considered for compounds with oral delivery (Lipinski et al., 2001). The servers like OSIRIS Property Explorer (http://www.organic-chemistry.org/prog/peo/), Drug-Likeness and molecular property prediction by MOLSOFT (http://molsoft.com/mprop/), Mobyle@RPBS v1.0.4 (http://mobyle.rpbs.univ-paris-diderot.fr:8080/cgi-bin/portal.py?form=PASS#forms::FAF-Drugs2), Molinspiration (http://www.molinspiration.com/cgi-bin/properties) and ALOGPS 2.1 (http://www.vcclab.org/lab/alogps/) were used here to evaluate ADMET study.

**RESULTS**

**Results obtained from Docking with reference ligands**

We perform docking between *M. tuberculosis* LS and 11 different reference ligands using AutoDock 4.2. **Table 1** shows the estimated binding energy and inhibition constant of each ligand. It could be seen that Ref1 had produced the best docking result in this set of ligands. The docked conformation of Ref1 in LS is shown in **figure 2**. Six hydrogen bonds could be seen among ligand Ref3 and the residues Asn261B (two), Arg275B, Ala59A, Ile60A, and Gln86A of LS respectively.

**Table1: Docking results of reference ligands with *Mycobacterium tuberculosis* Lumazine synthase**. Ligand code was given to each ligand as the rank according to its docking score in descending order. Ligand name, Estimated Free energy of binding and Estimated Inhibition Constant ($K_i$) were also given in this table

| Ligand Code | Ligand | Estimated Free energy of binding (Kcal/mol) | Estimated Inhibition Constant, $K_i$ (nM) |
|---|---|---|---|
| Ref1 | 3-(1,3,7-trihydro-9-d-ribityl-2,6,8-purinetrione- 7-yl) 1,1 difluoropentane-1-phosphate | -10.84 | 7.85 |
| Ref2 | 3-(1,3,7-trihydro-9-d-ribityl-2,6,8-purinetrione- 7-yl ) hexane 1-phosphate | -10.52 | 19.38 |
| Ref3 | 4-(6-chloro-2,4-dioxo-1,2,3,4-tetrahydropyrimidin- 5-yl) butyl phosphate | -10.49 | 10.15 |
| Ref4 | 3-{2,6,8-trioxo-9-[(2r,3s,4r)-2,3,4,5-tetrahydroxypentyl]-1,2,3,6,8,9-hexahydro-7h-purin-7-yl}propyl dihydrogen phosphate | -10.41 | 23.28 |
| Ref5 | 4-{2,6,8-trioxo-9-[(2r,3s,4r)-2,3,4,5-tetrahydroxypentyl]-1,2,3,6,8,9-hexahydro-7h-purin-7-yl} butyl dihydrogen phosphate | -10.30 | 28.35 |
| Ref6 | 5-nitro-6-ribityl-amino-2,4(1h,3h)-pyrimidinedione | -9.26 | 163.56 |
| Ref7 | 3-(1,3,7-trihydro-9-d-ribityl-2,6,8-purinetrione- 7-yl) | -8.80 | 351.84 |
| Ref8 | 5-nitroso-6-ribityl-amino-2,4(1H,3H)-pyrimidinedione | -8.77 | 372.83 |
| Ref9 | 1-deoxy-1-{[(5S)-2,6-dioxo-5-(propanoylamino)-1,2,5,6-tetrahydropyrimidin-4-yl]amino}-D-ribitol | -8.51 | 575.67 |
| Ref10 | 3-(1,3,7-trihydro-9-d-ribityl-2,6,8-purinetrione- 7-yl) pentane 1-phosphate | -8.46 | 687.47 |
| Ref11 | 5-(6-d-ribitylamino-2,4(1h,3h)pyrimidinedione-5-yl) pentyl-1-phosphonic acid | -7.78 | 1970 |

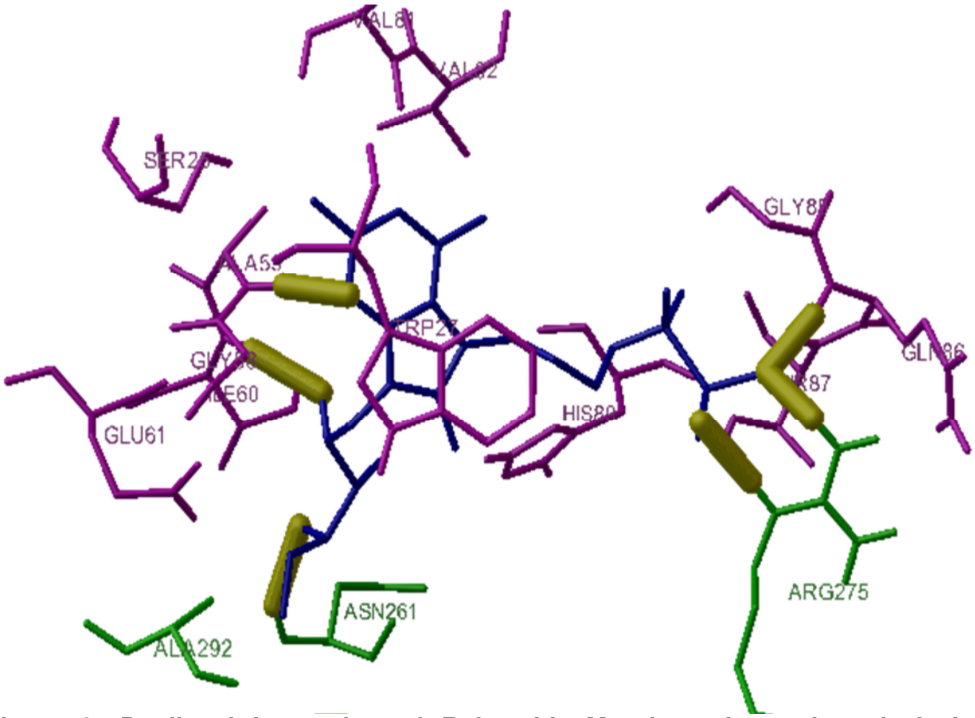


**Figure 2: Predicted interaction of Ref1 with *Mycobacterium tuberculosis* Lumazine synthase in AutoDock.** Hydrogen bonds (shown in yellow) can be seen between ligands (shown in blue) and *Mycobacterium tuberculosis* Lumazine synthase (two chains are shown in pink and green colour respectively). Ref1 forms six hydrogen bonds with LS. The models were visualized in AutoDock 4.2.

**Results obtained from Docking with experimental ligands**

The experimental ligand molecules from DrugBank database were docked into the active site of the *Mycobacterium tuberculosis* LS. Among those ligand molecules only 10 best docking scores were presented in **Table 2**. Ex1 is showing the maximum binding energy as well as the lowest $K_i$ value. All these ten ligands produce much better binding energy with LS than experimental ligand molecules. **Figure 3** showed the interactions between LS and Ex1. Here four hydrogen bonds could be seen within the active site of LS at the residues Lys285, Ala59, Ile83 and His89 and ligand.

**Table2** Docking results of ten experimental ligands with *Mycobacterium tuberculosis* Lumazine synthase. Ligand code was given to each ligand as the rank according to its docking score in descending order. Ligand name, Estimated Free energy of binding and Estimated Inhibition Constant ($K_i$) were also given in this table.

| Ligand Code | DrugBank ID | Estimated Free energy of binding (Kcal/mol) | Estimated Inhibition Constant, $K_i$ (nM) |
|---|---|---|---|
| **Ex1(Quinapril)** | **DB00881** | **-11.21** | **3.50** |
| Ex2 | DB08187 | -10.62 | 16.43 |
| Ex3 | DB04709 | -10.42 | 23.11 |
| Ex4 | DB08289 | -10.41 | 23.26 |
| Ex5 | DB06858 | -10.39 | 24.06 |
| Ex6 | DB04092 | -10.36 | 25.53 |
| Ex7 | DB07090 | -10.33 | 26.88 |
| Ex8 | DB02628 | -10.23 | 31.55 |
| Ex9 | DB07188 | -10.21 | 33.02 |
| Ex10 | DB07222 | -10.17 | 35.30 |

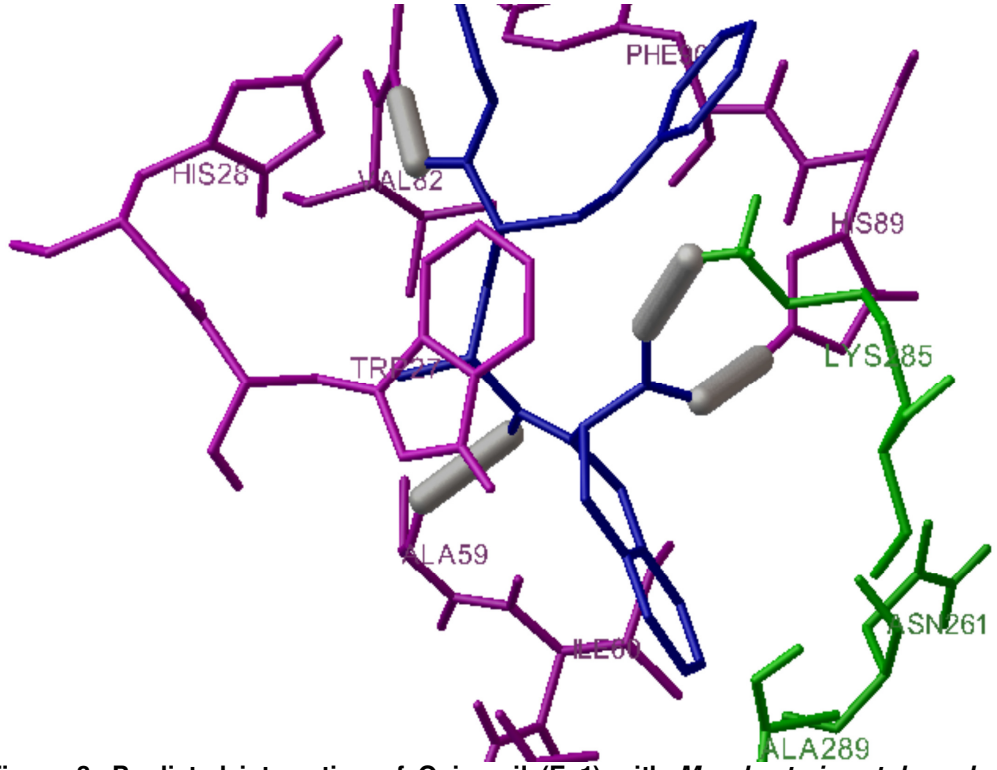


**Figure 3: Predicted interaction of Quinapril (Ex1) with *Mycobacterium tuberculosis* Lumazine synthase in AutoDock.** Hydrogen bonding (shown in white) illustration between ligands (shown in Blue) and *Mycobacterium tuberculosis* Lumazine synthase (two chains are shown in pink and green colour respectively). Ex1 produces four hydrogen bonds with LS. The models were visualized in AutoDock 4.2.

**Results obtained from *in-silico* ADMET study**

The *in silico* ADMET study of Ex1 is presented in Table 3. The study was done in various servers to find logP, solubility, molecular weight, topological polar surface area, number of rotatable bonds, Number of Hydrogen bond Donor, Number of Hydrogen bond acceptor, volume, Lipensky violation, Oral Bioavailability, Number of stereo centers, Mutagenic and Tumorigenic risk, irritating effects, reproductive effects and drug-likeness score to assure experimental ligands' drug-likeness behaviour. The Ex1 showed good result. It was not violating the "Lipinski's rule of five". The drug score of Ex1 was 0.96.

**Table3**: The result of in-silico ADMET study of ligand Ex1

| Ligand code | In-silico ADMET result | |
|---|---|---|
| Ex1 | LogP | 2.35 |
| | Solubility in mg/L | 3.81 |
| | Molecular Weight | 438.52 |
| | topological Polar Surface Area | 95.94 |
| | Rotatable bonds | 10 |
| | Number of Hydrogen bond Donor | 2 |
| | Number of Hydrogen bond acceptor | 7 |
| | Volume | 411.364 |
| | Lipensky violation | 0 |
| | Oral Bioavailability (by Veber Rule) | Good |
| | Oral Bioavailability (by Egan Rule) | Good |
| | Number of stereo centers | 3 |
| | Mutagenic Risk/ Tumorigenic Risk/ Irritating Effects/ Reproductive effects | No |
| | **Druglikeness score** | **0.96** |

## DISCUSSION

Zhang, Y. et al., (2008) mentioned that the active site of *Mycobacterium tuberculosis* Lumazine Synthase (LS) is located at the interface between two neighboring subunits in the pentameric assembly. The active site is consists of the residues from three *β*-loops (residues 26-28, 58-61, 81-87) of one subunit as well as residues 128-141 and residue Asn114 of the neighboring subunit. So this was the region where grid was placed in the AutoDock. According to Morgunova et al. [8] 3-(1,3,7-trihydro-9-d-ribityl-2,6,8-purinetrione-7-yl) 1,1 difluoropentane-1-phosphate (which is Ref1 in our study) showed the lowest free energy of binding than other compounds. In our study it also showed the lowest estimated free energy of binding in reference ligand set (-10.84 Kcal/mol). Among the interactions predicted by AutoDock for Ref1 (**Figure 2**) five hydrogen bonds were also visible in the interactions reported in the PDB ID: 2C94. So it is evident that the prediction made by this docking software is quite similar with the reported information.

In the experimental ligand set Quinapril (Ex1) showed the maximum estimated binding energy. It is an approved drug in USA and Canada. It is a non-sulphhydryl monoethyl ester that belongs to the angiotensin-converting enzyme (ACE) inhibitor class of medications. It is metabolized to quinaprilat (quinapril diacid) following oral administration. Quinaprilat is a competitive inhibitor of ACE, the enzyme responsible for the conversion of angiotensin I (ATI) to angiotensin II (ATII). ATII regulates blood pressure and is a key component of the renin-angiotensin-aldosterone system (RAAS). Quinapril may be used to treat essential hypertension and congestive heart failure. Quinaprilat is eliminated primarily by renal excretion, up to 96% of an IV dose. Elimination half-life is 2-3 hours but due to slow dissociation from tissue ACE, once daily dosing is sufficient for effective ACE inhibition. Overdose may lead to severe hypotension. $LD_{50}$ of quinapril is 1739 mg/kg (orally in mice). The most common adverse effects observed in controlled clinical trials were dizziness, cough, chest pain, dyspnea, fatigue, and nausea/vomiting. But its anti-TB property was not known at all. In this current study quinapril showed better binding energy than all the ligands. So it could be said that it might have some sort of affinity for the active site of *M. tuberculosis* LS. From its in-silico ADMET study it could be seen that it produced drug-likeness score 0.96 and it didn't violate the Lipenski rule. It also has no risk of mutagenicity, tumorgenicity, irritating effect and reproductive effect. So this study first time predicts the potential of quinapril as a lead compound for anti-TB drugs [23-25].

Analysis of these of molecular docking and in-silico ADMET results we successfully establish that ligand that does not have either pyrimidine or purine ring can also bind with *Mycobacterium tuberculosis* Lumazine Synthase (LS). Considering all the results it could be concluded that Quinapril (Ex1) stands out to be a putative novel inhibitor of LS among all the other inhibitors. Now further in-vitro and in-vivo tests are needed to formulate its doses.

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## REFERENCE

Bacher A., Eberhardt S. and Richter G., 1996. In: Escherichia coli and Salmonella: Cellular and Molecular Biology; Neidhardt F.C., Curtis R.III, Ingraham J.L., Lin E.C.C., Low K.B., Magasanik B., Reznikoff W.S., Riley M., Schaechter M. and Umbarger, H.E., Ed., ASM Press, Washington DC, 657-664.

Begg E.J., Robson R.A. Bailey R.R. Lynn K.L. Frank G.J. and Olson S.C., 1990. The pharmacokinetics and pharmacodynamics of quinapril and quinaprilat in renal impairment. Br. J. Clin. Pharmacol. 30: 213-220.

Bharath E.N., Manjula S.N. and Vijaychand A., 2011. In Silico Drug Design tool for overcoming the innovation deficit in the drug discovery process. Int. J. Pharm. and Pharm. Sci. 3: 8-12.

Cole T.S., Eiglmeier K., Parkhill J., James K.D., Thomson N.R., Wheeler P.R., Honore N., Garnier T., Churcher C., Harris D., Mungall K., Basham D., Brown D., Chillingworth T., Connor R., Davies R.M., Devlin K., Duthoy S., Feltwell T., Fraser A., Hamlin N., Holroyd S., Jagels K., Lacroix C., Maclean J., Moule S., Murphy L., Oliver K., Quail M.A., Rajandream M.A., Rutherford K.M., Rutter S., Seeger K., Simmonds M., Skelton J., Squares R., Squares S., Stevens K., Taylor K., Whitehead S., Woodward J.R. and Barrel B.G., 2001. Massive gene decay in the leprosy bacillus. Nature 409: 1007-1011.

Gerhardt S., Haase I., Steinbacher S., Kaiser J.T., Cushman M., Bacher A., Huber R. and Fischer M., 2002. The structural basis of riboflavin binding to Schizosaccharomyces pombe 6,7-dimethyl-8-ribityllumazine synthase. J. Mol. Biol. 318: 1317-1329.

Hodgson J., 2001. ADMET— turning chemicals into drugs. Nature Biotech.19: 722-726.

Illarionov B., Kemter K., Eberhardt S., Richter G., Cushman M. and Bacher A., 2001. Riboflavin synthase of Escherichia coli Effect of single amino acid substitutions on reaction rate and ligand binding properties. J. Biol. Chem. 276: 11524-11530.

Kieback A.G., Felix S.B. and Reffelmann T., 2009. Quinaprilat: a review of its pharmacokinetics, pharmacodynamics, toxicological data and clinical application. Expert Opin. Drug Metab. Toxicol. 5: 1337-1347.

Kis K., Volk R. and Bacher A., 1995. Biosynthesis of riboflavin Studies on the reaction mechanism of 6,7-dimethyl-8-ribityllumazine synthase. Biochem. 34, 2883–2892.

Kumar V. Abbas A.K., Fausto N. and Mitchell R.N., 2007. Robbins Basic Pathology, 8th ed. Saunders Elsevier 516–522.

Lipinski C.A., Lombardo F., Dominy B.W. and Feeney P.J., 2001. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv. Drug. Del. Rev. 46: 3–26.

Long Q,; Ji L., Wang H. and Xie J., 2010. Riboflavin Biosynthetic and Regulatory Factors as Potential Novel Anti-Infective Drug Targets. Chem. Biol. Drug. Des. 75: 339–347.

Morgunova E., Illarionov B., Sambaiah T., Haase I., Bacher A., Cushman M. Fischer M. and Ladenstein R., 2006. Structural and thermodynamic insights into the binding mode of five novel inhibitors of lumazine synthase from Mycobacterium tuberculosis. FEBS J. 273: 4790-4804.

Morgunova E., Meining W., Illarionov B., Haase I., Kaiser J.T., Cushman M., Bacher A., Huber R. and Fischer M., 2005. Crystal structure of lumazine synthase from Mycobacterium tuberculosis as a target for rational drug design: binding mode of a new class of purinetrione inhibitors. Biochem. 44: 2746-2758.

Morris G.M., Huey R., Lindstrom W., Sanner M.F., Belew R.K., Goodsell D.S. and Olson A.J., 2009. AutoDock4 and AutoDock Tools 4: Automated docking with selective receptor flexibility. J. Comput. Chem.30: 2785-2791.

Neuberger G. and Bacher A., 1986. Biosynthesis of riboflavin Enzymatic formation of 6,7-dimethyl-8-ribityllumazine by heavy riboflavin synthase from Bacillus subtilis. Biochem. Biophys. Res. Commun. 139: 1111–1116.

Persson K., Schneider G., Jordan D.B., Viitanen P.V. and Sandalova T. 1999. Crystal structure analysis of a pentameric fungal and an icosahedral plant lumazine synthase reveals the structural basis for differences in assembly. Protein Sci. 8: 2355–2365.

Plaut G.W.E. and Harvey R.A., 1971. In: Methods in Enzymology; McCormick D.B. and Wright L.D. Ed., Academic Press, New York, Vol. 18B, 515–538.

Sassetti C.M., Boyd D.H. and Rubin E.J., 2003. Genes required for mycobacterial growth defined by high density mutagenesis. Mol. Microbiol., 48: 77–84.

Tomioka H. and Namba K., 2006. Development of antituberculous drugs: current status and future prospects. *Kekkaku* 81: 753-774.

Wadworth A.N. and Brogden R.N., 1991. Quinapril A review of its pharmacological properties, and therapeutic efficacy in cardiovascular disorders. Drugs. 4: 1378-1399.

World Health Organisation. WHO Global tuberculosis control 2012. http://www.who.int/tb/en/. (Accessed on February 5, 2013)

World Health Organisation. WHO Tuberculosis country profiles 2012. http://www.who.int/tb/country/en/index.html. (Accessed on February 5, 2013)

Zhang X., Meining W. Cushman M. Haase I. Fischer M., Bacher A. and Ladenstein R., 2008. A structure-based model of the reaction catalyzed by lumazine synthase from Aquifex aeolicus. J. Mol. Biol. 328: 167-182.

Zhang Y., Illarionov B., Morgunova E., Jin G., Bacher A., Fischer M., Ladenstein R. and Cushman M., 2008. A New Series of N-[2,4-Dioxo-6-d-ribitylamino-1,2,3,4-tetrahydropyrimidin-5-yl]oxalamic Acid Derivatives as Inhibitors of Lumazine Synthase and Riboflavin Synthase: Design, Synthesis, Biochemical Evaluation, Crystallography, and Mechanistic Implications. J. Org. Chem. 73: 2715–2724.