Molecular Modeling study of Protein Kinase PKnB from Mycobacterium Tuberculosis with derivatives of 1, 3, 4-Thiadiazoles

V. C. Gupta [(1)], J. S. Tanwar [(2)],K. Mishra [(3)], S.A.H. Naqvi [(3)]*

(1) *SRM University, Chennai, India*

(2) *Jamia Hamdard, Hamdard University (New Delhi, IN);*

*Corresponding Author: [(3)] *BioDiscovery-Solutions for future, Agra, India*

E-mail- contactme.asif@gmail.com

ABSTRACT

Tuberculosis causes more than two million deaths per year. Faced with this global threat it is crucial to better understand the physiology of the causative organism, Mycobacterium Tuberculosis, in order to develop efficient therapeutic strategies. PKnB from Mycobacterium tuberculosis is a crucial receptor-like protein kinase involved in signal transduction. M. tuberculosis PKnB is a trans-membrane Ser/Thr protein kinase (STPK) highly conserved in Gram-positive bacteria and apparently essential for Mycobacterial viability. We have attempted with the help of virtual screening and docking approach to expound the extent of specificity of protein kinase B towards different classes of Thiadiazoles (an anti-tubercular agent). The selected Thiadiazoles were selected on the basis of the structural specificity to the enzyme towards its substrate and inhibitors. Total number of Thiadiazoles were 5000 in number with the minimum binding energy of -10.46 kcal/mol with 10 molecules showing hydrogen bonds with the active site residue. The protein kinase B peptide contains two types of structural elements (Valine 95, Arginine 97) and basic residue ring constituted of glycine rich residue. The structure of the protein-ligand complex reveals that Thiadiazoles partially occupies the adenine-binding pocket in PKnB, providing a framework for the design of compounds with potential therapeutic applications. The study provides hints for the future design of new derivatives with higher potency and specificity.

Keywords: Protein Kinase PKnB, Mycobacterium Tuberculosis, 1, 3, 4- Thiadiazdole, Drug Discovery, Molecular Docking, AutoDock4.

INTRODUCTION

The knack of Mycobacterium tuberculosis, pathogen liable for Tuberculosis (TB), to adapt to the changing environmental conditions requires an efficient way of sensing and transducing extracellular signals [1]. One of the mechanisms used in Mycobacteria to assure a tight regulation of cell growth and division involves the reversible phosphorylation on serine/threonine residues, a well-established process for eukaryotic signaling networks [2]. M. tuberculosis PknB is a trans-membrane Ser/Thr protein kinase (STPK) highly conserved in Gram-positive bacteria and apparently essential for mycobacterial viability [3]. The crystal structure of the kinase domain of PknB in complex with an ATP analogue [4] and [5] showed a striking conservation of both protein fold and catalytic mechanism between eukaryotic and prokaryotic STPKs. Earlier it was shown that PKnB is regulated by auto-phosphorylation and dephosphorylation by the Ser/Thr protein phosphatase [6] and [7] and recent work showed that PKnB is predominantly expressed during exponential growth, where its over expression causes morphological changes linked to defects in cell wall synthesis and cell division [8].

Aberrant kinase activity is implicated in numerous human diseases and, not surprisingly, protein kinases

represent today one of the most important groups of drug targets [9] and [10]. Here we report that thiadiazole, a compound reported as anti-microbial [10-13] and anti-tubercular agent [14-18] is a PKnB inhibitor capable of preventing mycobacterial cell growth, suggesting that bacterial kinases may also represent a potential target for drug design and derivatives of Thiazdiazoles exhibit a property of inhibiting mycobacterial growth. The structure of the protein-ligand complex shows that Thiadiazole derivatives bound to the structural element (VAL 95) of PKnB from MTB partially occupies the adenine-binding pocket in PKnB and is an ATP-competitive inhibitor of PKnB and suggests a mode of regulation of protein kinases in mycobacteria.

2. MATERIALS AND METHODS

2.1. In silico screening

5000 compounds from different chemical databases were screened, including the PubChem & ChemBank compound datbases. They were docked into the nucleotide-binding pocket comprising of VAL 95(active site residue) of the *M. tuberculosis* PKnB structure (PDB ID. 2fum [1]) using the program AutoDock4 [19].

2.2 Substrate selection

4000 structures most 2D-similar to Thiadiazole and 1, 3, 4- Thiadiazole (Fig 1) were chosen based on screening from the ChemBank[20] .The chosen ligands have conformational stability and structural diversity in relation to the bound ligands to the crystal structure. The ligand structures used in docking were obtained from ChemBank compound database [21]. Ligands were identified as per the pharmacokinetic parameter and solubility. The molecules were searched by similarity search compound collection, by similarity to a structure (may be specified *via* a SMILES string, or drawn with JME Molecular Editor). The molecules were screened where the structure is similar to C1=NN=CS1 (of Thiadiazole from PubChem) using the Tanimoto metric with a distance of .8.

The active site i.e. VAL 95 in the protein interacts with ligands of the substrate and gives rise to the catalytic activity to test ligands that helps in determining the binding pattern of the ligands to the active site of PKnB (PDB; 2FUM).

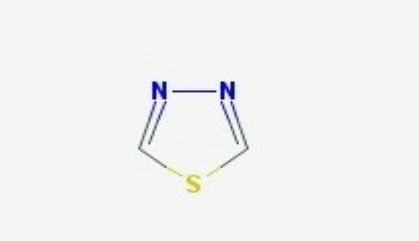


Fig 1: Structure of 1, 3, 4 Thiadiazole

In Last, 1000 molecules, based on structural similarity of 1, 3, 4- Thiadiazole was selected from PubChem and then docking was performed.

2.3. Docking setup

Docking was performed using Autodock 4, a novel and robust automated docking method that predicts the bound conformations of flexible ligands to macromolecular targets. Interestingly, this method applies a Lamarckian model of genetics, in which environmental adaptations of an individual's phenotype are reverse transcribed into its genotype and become heritable traits (*sic*). We have considered Lamarckian genetic algorithm, search method which can handle ligands with more degrees of freedom than the Monte Carlo simulated annealing and traditional genetic algorithm method used in earlier versions of AUTODOCK, and that the Lamarckian genetic algorithm is the most efficient, reliable, and successful. AutoDock 4, combines energy evaluation through grids of affinity potential employing various search algorithms to find the suitable binding position for a ligand on a given protein (Morris et al., 1998). While docking, polar hydrogen's were added to ligands using the hydrogen's module in Autodock tool and thereafter, Kollman united atom partial charges were assigned (La Motta *et al.*, 2007). Docking of PKnB to ligands was carried out using LGA with standard docking protocol on the basis a population size of 150 randomly placed individuals; a maximum number of 2.5 * 107 energy evaluations, a mutation rate of 0.02, a crossover rate of 0.80 and an elitism value of 1. Fifteen independent docking runs were carried out for each ligand and results were clustered according to the 1.0 Å rmsd criteria. The grid maps representing the proteins were calculated using auto grid and grid size was set to 60*60*60 points with grid spacing of 0.375 Å.

RESULTS AND DISCUSSION

Docking of 5000 molecules was carried out for PKnB which showed a binding energy of -10.46. The docking results were interpreted according to the .pdb

file. We have used the co-ordinates of the minimum energy run created in the .dlg which was determined using the rmsd table created in the .dlg file itself (Fig-2).

RMSD TABLE

Rank	Sub-Rank	Run	Binding Energy	Cluster RMSD	Reference RMSD	Grep Pattern
1	1	9	-10.46	0.00	254.49	RANKING
1	2	7	-9.44	0.43	254.48	RANKING
1	3	2	-9.28	1.32	254.59	RANKING
1	4	10	-8.59	1.59	253.87	RANKING
1	5	3	-8.47	1.59	254.74	RANKING
2	1	1	-8.13	0.00	251.59	RANKING
3	1	4	-7.95	0.00	251.21	RANKING
3	2	6	-7.92	0.99	251.36	RANKING
3	3	5	-7.89	1.66	251.87	RANKING
3	4	8	-7.79	1.35	251.88	RANKING

Fig 2: Rmsd table of the DLG file generated

On docking of 5000 molecules with VAL 95 (active site) residue according to the minimum binding energy generated by AutoDock 4 the results shows that 5 molecules showed best results (Table-1).

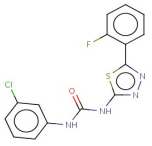
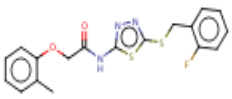
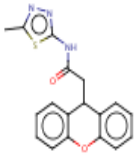
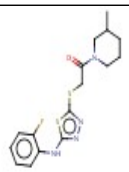
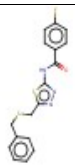
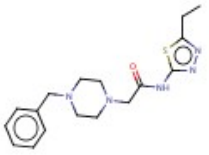
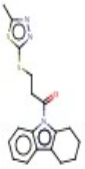
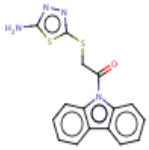
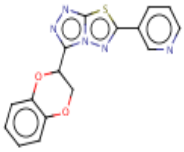
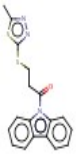
2,5-bis[(4-bromobenzyl)thio]-1,3,4-thiadiazole	
N- {5- [(2- fluorobenzyl) thio]- 1,3,4- thiadiazol- 2- yl}- 2- (2- methylphenoxy) acetamide	
N- (5- methyl- 1,3,4- thiadiazol- 2- yl) - 2- (9H- xanthen- 9- yl) acetamide	
N- (2- fluorophenyl) - 5- {[2- (3- methylpiperidin- 1- yl) - 2- oxoethyl]thio}- 1,3,4- thiadiazol- 2- amine	
N- {5- [(benzylthio) methyl]- 1,3,4- thiadiazol- 2- yl}- 4- fluorobenzamide	
2- (4- benzylpiperazin- 1- yl) - N- (5- ethyl- 1,3,4- thiadiazol- 2- yl) acetamide	
9- {3- [(5- methyl- 1,3,4- thiadiazol- 2- yl) thio]propanoyl}- 2,3,4,9- tetrahydro- 1H- carbazole	
5- {[2- (9H- carbazol- 9- yl) - 2- oxoethyl]thio}- 1,3,4- thiadiazol- 2- amine	
3- (2,3- dihydro- 1,4- benzodioxin- 2- yl) - 6- pyridin- 3- yl[1,2,4]triazolo[3,4- b] [1,3,4]thiadiazole	
9- {3- [(5- methyl- 1,3,4- thiadiazol- 2- yl) thio]propanoyl}- 9H- carbazole	

Table 1- Molecules showed best docking results

The molecules that gave best binding energies (Table-2) are then looked for Lipinski's Rule of 5 parameter and the results showed that all 10 of them follow Lipinski's rule of 5. After that they were tested for structure analysis by the visualization tool. The coordinate of the docked protein along with the ligand was visualized using UCSF chimera [22] within 6.5 Å region. The protein-ligand complex showed H -bond with the active site residue (Table- 3).

Our result indicates that the ligand have varied binding pattern with substrate binding site i.e. VAL-95 along with various other active sites. 10 compounds demonstrate better binding patterns with protein in terms of hydrogen bonds with the various residues of the protein. The docked confirmation of all the ligands is shown in Fig 2.

In summary, based on the molecular docking we found that compounds from Table 1 as of structure similarity of 1 3 4 Thiadiazole showed better binding affinities with the active site pocket (comprising of VAL-95) of the PKnB enzyme(Fig- 3a, b, c, d, e). Fig 4 shows the docked confirmation of 2,5-bis[(4-bromo benzyl)thio]-1,3,4-thiadiazole. Our study gives an idea about the interaction between the active site residues and the substrate which is explained on the basis of size & hydrophobicity of the binding pocket. The molecules that showed less binding energy and showed better interactions with protein are not yet tested in the laboratory and the autoflourescence data for these molecules is not available. The extent of the work stretches to the in-silico approach for determining the binding mode. Further there is need to generate in vitro and in-vivo activity of the generated data to synthesize and test so to design drugs with better specificity and metabolism.

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FIGURES

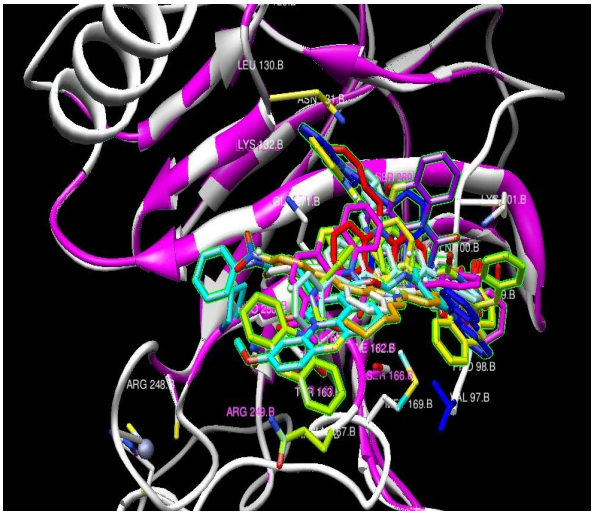

Fig: 2- Docked conformation of all the ligands, visualized by Chimera

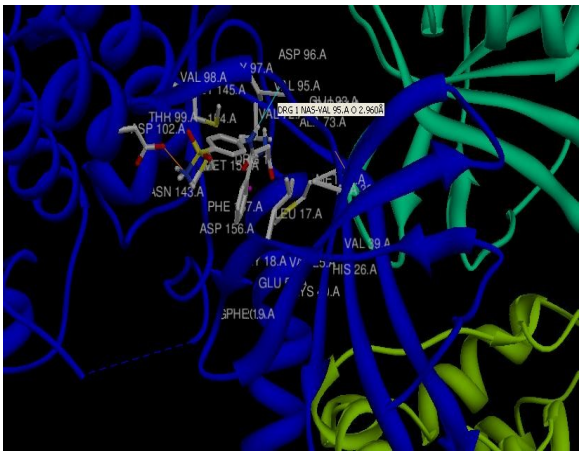

Fig: 2a- H-Bond with active site residue

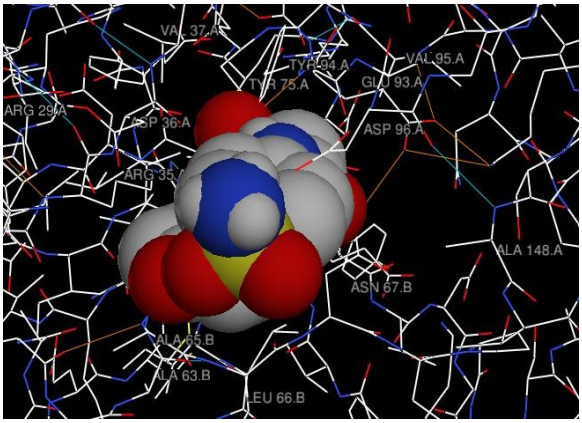

Fig: 2b- H-Bond with active site residue

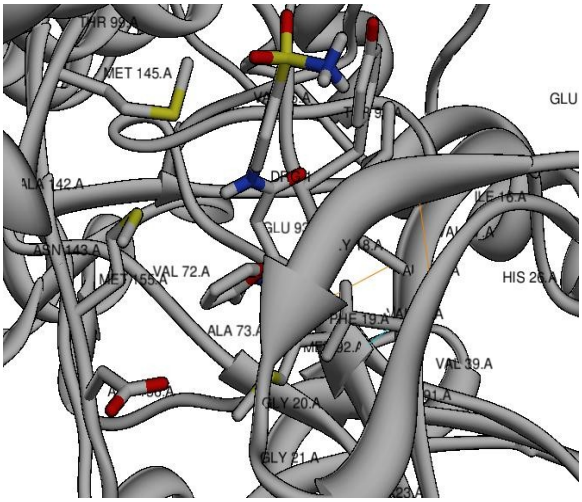

Fig: 2c- H-Bond with active site residue

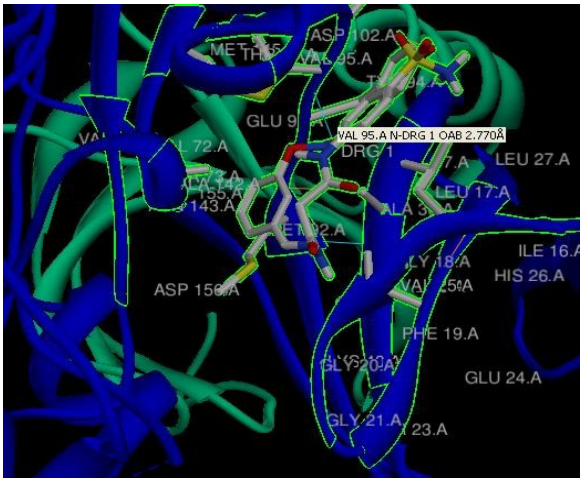

Fig: 2d- H-Bond with active site residue

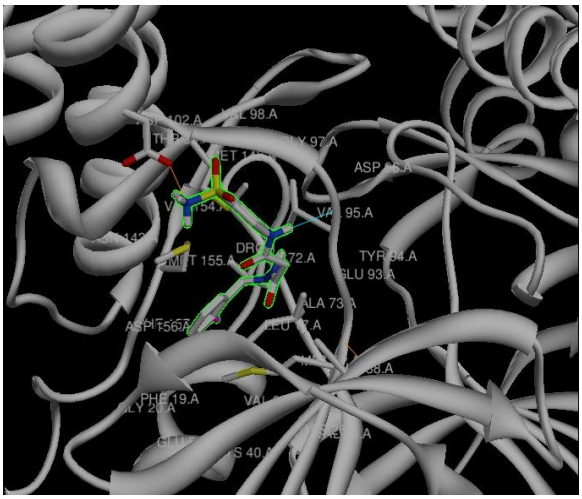

Fig: 2e- H-Bond with active site residue

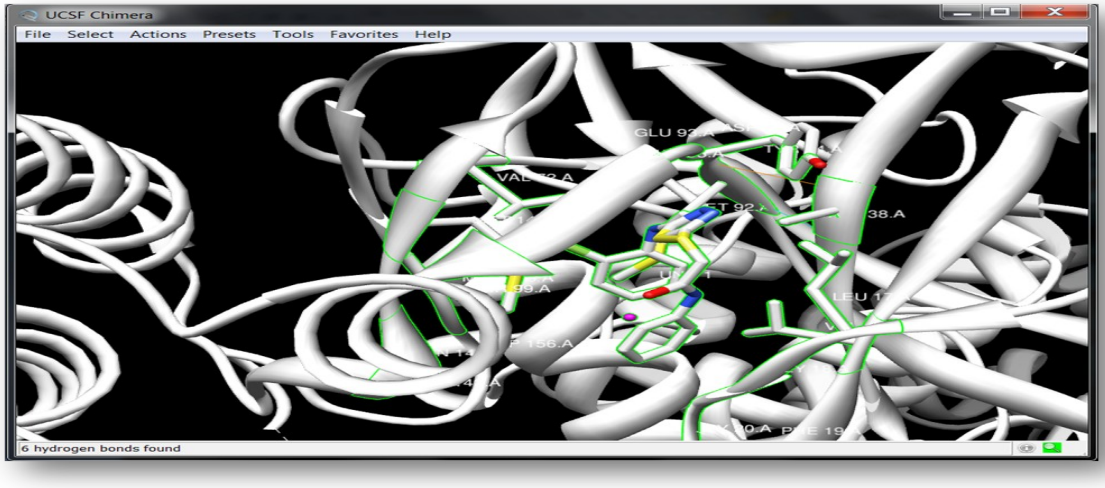

Fig: 4- Docked confirmation of 2,5-bis[(4-bromo benzyl)thio]-1,3,4-thiadiazole

TABLES

TABLE- 2 BINDING ENERGIES OF TOP 10 MOLECULES

S. No.	COMPOUNDS NAME	BINDING ENERGY (kcal.mol) (MIN)	BINDING ENERGY (kcal.mol) (MAX)
1.	2,5-bis[(4-bromobenzyl)thio]-1,3,4-thiadiazole	-10.46	-7.79
2.	N- {5- [(2- fluorobenzyl) thio]- 1,3,4-thiadiazol- 2- yl}- 2- (2- methylphenoxy) acetamide	-9.57	-7.43

3.	N- (5- methyl- 1,3,4- thiadiazol- 2- yl) - 2- (9H- xanthen- 9- yl) acetamide	-9.35	-9.01
4.	N- (2- fluorophenyl) - 5- {[2- (3- methylpiperidin- 1- yl) - 2- oxoethyl]thio}- 1,3,4- thiadiazol- 2- amine	-9.28	6.34
5.	N- {5- [(benzylthio) methyl]- 1,3,4- thiadiazol- 2- yl}- 4- fluorobenzamide	-9.00	-8.88
6.	2- (4- benzylpiperazin- 1- yl) - N- (5- ethyl- 1,3,4- thiadiazol- 2- yl) acetamide	-9.00	-8.02
7.	9- {3- [(5- methyl- 1,3,4- thiadiazol- 2- yl) thio]propanoyl}- 2,3,4,9- tetrahydro- 1H- carbazole	-8.96	-6.53
8.	5- {[2- (9H- carbazol- 9- yl) - 2- oxoethyl]thio}- 1,3,4- thiadiazol- 2- amine	-8.88	-5.22
9.	3- (2,3- dihydro- 1,4- benzodioxin- 2- yl) - 6- pyridin- 3- yl[1,2,4]triazolo[3,4- b] [1,3,4]thiadiazole	-8.86	-7.46
10.	9- {3- [(5- methyl- 1,3,4- thiadiazol- 2- yl) thio]propanoyl}- 9H- carbazole	-8.79	-8.11

TABLE- 3 H- BOND INFORMATION OF TOP 10 MOLECULES

COMPONDS	MIN	MAX	HYDROGEN BONDS BLUE-ORGANGE
2,5-bis[(4-bromobenzyl)thio]-1,3,4- thiadiazole	-10.46	-7.79	1, 3
N- {5- [(2- fluorobenzyl) thio]- 1,3,4- thiadiazol- 2- yl}- 2- (2- methylphenoxy) acetamide	-9.57	-7.43	2, 1
N- (5- methyl- 1,3,4- thiadiazol- 2- yl) - 2- (9H- xanthen- 9- yl) acetamide	-9.35	-9.01	0, 2
N- (2- fluorophenyl) - 5- {[2- (3- methylpiperidin- 1- yl) - 2- oxoethyl]thio}- 1,3,4- thiadiazol- 2- amine	-9.28	6.34	1, 1
N- {5- [(benzylthio) methyl]- 1,3,4-	-9.00	-8.88	2, 1

thiadiazol- 2- yl}- 4- fluorobenzamide			
2- (4- benzylpiperazin- 1- yl) - N- (5- ethyl- 1,3,4- thiadiazol- 2- yl) acetamide	-9.00	-8.02	2, 3
9- {3- [(5- methyl- 1,3,4- thiadiazol- 2- yl) thio]propanoyl}- 2,3,4,9- tetrahydro- 1H- carbazole	-8.96	-6.53	2, 4
5- {[2- (9H- carbazol- 9- yl) - 2- oxoethyl]thio}- 1,3,4- thiadiazol- 2- amine	-8.88	-5.22	3,2
3- (2,3- dihydro- 1,4- benzodioxin- 2- yl) - 6- pyridin- 3- yl[1,2,4]triazolo[3,4- b] [1,3,4]thiadiazole	-8.86	-7.46	1,2
9- {3- [(5- methyl- 1,3,4- thiadiazol- 2- yl) thio]propanoyl}- 9H- carbazole	-8.79	-8.11	2, 0

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