Journal of Computational Intelligence in Bioinformatics.
ISSN: 0973-385X Volume 4 Number 1 (2011) pp. 177—183

http://www.ripublication.com/jcib.htm

A Virtual Screening & Molecular Docking Study of Benzothiazoles: Discovery of New Anti-Tubercular Agents

U. Raj[1,*], K. Mishra[2] and S.A.H. Naqvi[2]

[1]Amity Institute of Biotechnology, Amity University, India
[2]BioDiscovery-Solutions for Future, Agra, India
**Corresponding Author E-mail: rajamity1@gmail.com*

Abstract

AKT protein family, of which members are also called protein kinases B (PKnB) play an important role in mammalian cellular signaling. Mycobacterium tuberculosis PKnB is an essential receptor-like protein kinase involved in cell growth control. 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 elucidate the extent of specificity of protein kinase B towards different classes of Benzothiazoles (an anti-tubercular agent). Total numbers of Benzothiazoles were 122 in number and all the selected 122 inhibitors were taken from three different databases on the basis of the structural specificity to the enzyme towards its substrate and inhibitors. The docking result of the study of 122 Benzothiazoles demonstrated that the binding energies were in the range of -8.81 kcal/mol to -3.67 kcal/mol, with 4 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 Benzothiazole 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.

Introduction

The ability of *Mycobacterium tuberculosis*, the pathogen responsible for tuberculosis (TB), to adapt to changing environmental conditions requires an efficient way of sensing and transducing extracellular signals. 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 [1].

M. tuberculosis PknB is a trans-membrane Ser/Thr protein kinase (STPK) highly conserved in Gram-positive bacteria and apparently essential for mycobacterial viability [2]. The crystal structure of the kinase domain of PknB in complex with an ATP analogue [3] and [4] showed a striking conservation of both protein fold and catalytic mechanism between eukaryotic and prokaryotic STPKs. We have previously shown that PknB is regulated by auto-phosphorylation and dephosphorylation by the Ser/Thr protein phosphatase PstP [5] and [6] 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 [7].

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 [8] and [9]. Here we report that benzothiazole, a compound reported as anti-tubercular agent (Ketan, M.; Desai, K.R.; I. J. Chem. 45B, 1762-1766 (2006).), is a PKnB inhibitor capable of preventing mycobacterial cell growth, suggesting that bacterial kinases may also represent a potential target for drug design. The crystal structure of the complex demonstrates that benzothaizole is an ATP-competitive inhibitor of PknB and suggests a mode of regulation of protein kinases in mycobacteria.

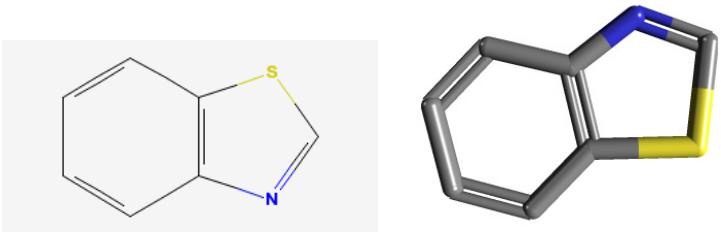
Materials and Methods

In silico screening

122 compounds from different chemical databases were screened, including the PubChem & ChemBank. They were docked into the nucleotide-binding pocket of the *M. tuberculosis* PknB structure (pdb ID 2fum [3]) using the program AutoDock4 [10].

Substrate selection

Firstly top 22 drug structures most 2D-similar to BENZOTHIAZOLE (Fig 1) were chosen based on screening from the ChemBank[11] .The chosen ligands have conformational stability and structural diversity in relation to the bound ligands of the Benzothiazole crystal structure. The ligand structures used in docking were obtained from ChemBank compound database. Ligands were identified as per the pharmacokinetic parameter and solubility. 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).



Synonyms:
BENZOTHIAZOLE
1,3-Benzothiazole
95-16-9
InChI=1/C7H5NS/c1-2-4-7-6(3-1)8-5-9-7/h1-5

Figure 1: Structure of Benzothaizole.

Secondly, 100 molecules, based on structural similarity of Benzothiazole was selected from PubChem[12] and than docking was performed.

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) (Fig 2). The molecules were screened where the structure is similar to C1=CC=C2C(=C1)N=CS2 (of benzothiazole from PubChem) using the Tanimoto metric with a distance of .8.

Docking setup

Docking was performed using Autodock 4, which 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 from Mycobacterium tuberculosis 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. The coordinate of the docked protein along with the ligand was visualized using UCSF chimera (http://www.cgl.ucsf.edu/chimera) within 6.5 Å region.

Results and Discussion

Docking was carried out for PknB with benzothiazole it showed a binding energy of -

8.81 and this value was taken as reference .Benzothiazole is a anti-TB, anti allergic, antimicrobial and fungicidal [14].

On docking of 122 molecules with VAL 95 (as an active site) residue according to the minimum binding energy generated by autodock4 the results show the following compounds:

N- (2,6- diethylphenyl) - 1,3- benzothiazol- 2- amine
2- [(2- chlorobenzyl) thio]- 1,3- benzothiazol- 6- amine
2- hydroxybenzaldehyde 1,3- benzothiazol- 2- ylhydrazone
N- (2,5- dimethylphenyl) - 1,3- benzothiazol- 2- amine
4- [(1,3- benzothiazol- 2- ylthio) methyl]benzonitrile
3- (1,3- benzothiazol- 2- yl) - 1,3- benzoxazol- 2(3H) - one
N- (4,6- dimethylpyrimidin- 2- yl) - 1,3benzothiazol 2- amine
N'- 1,3- benzothiazol- 2- yl- N,N- dimethylsulfamide
N- 1,3- benzothiazol- 2- yl- 2- phenylacetamide

as given in table1.

The corresponding ligands which we hav find out are matched for the Lipinski's Rule of 5 parameter and the results showed that only 9 from the list of 122 compounds follow Lipinski's rule of 5 (table 4).

Our result indicates that the ligand have varied binding pattern with substrate binding site i.e. VAL-95 along with various other active sites (table 4). 9 compounds demonstrate better binding patterns with protein in terms of hydrogen bonds with the various residues of the protein.

In summary, based on the molecular docking we found that compounds N- (2,6- diethylphenyl) - 1,3- benzothiazol- 2- amine, 2- [(2- chlorobenzyl) thio]- 1,3- benzothiazol- 6- amine, 2- hydroxybenzaldehyde 1,3- benzothiazol- 2- ylhydrazone, N- (2,5- dimethylphenyl) - 1,3- benzothiazol- 2- amine, 4- [(1,3- benzothiazol- 2- ylthio) methyl]benzonitrile, 3- (1,3- benzothiazol- 2- yl) - 1,3- benzoxazol- 2(3H) – one, N- (4,6- dimethylpyrimidin- 2- yl) - 1,3benzothiazol 2- amine, N'- 1,3- benzothiazol- 2- yl- N,N- dimethylsulfamide, N- 1,3- benzothiazol- 2- yl- 2- phenylacetamide as of structure similarity from PubChem showed better binding affinities with the active site pocket (comprising of VAL-95) of the PknB enzyme. 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 from ChemBank 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 drug with better specificity and metabolism.

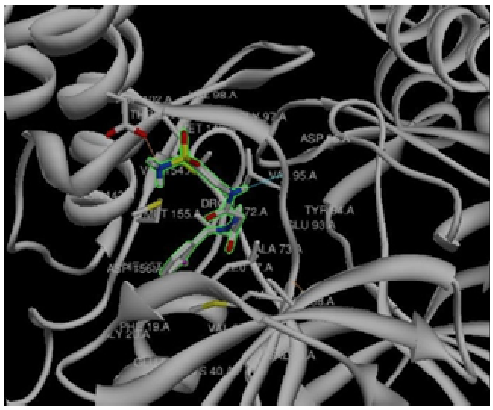


Figure 2a: H-Bond with active site residue.

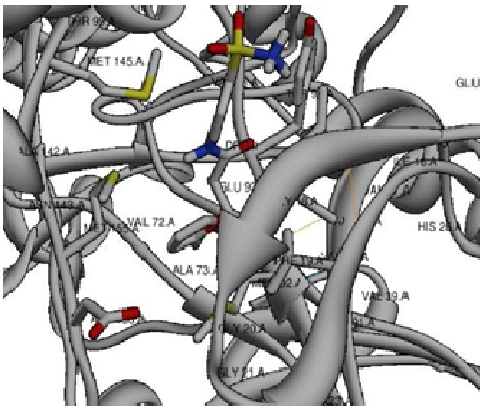


Figure 2b: H-Bond with active site residue.

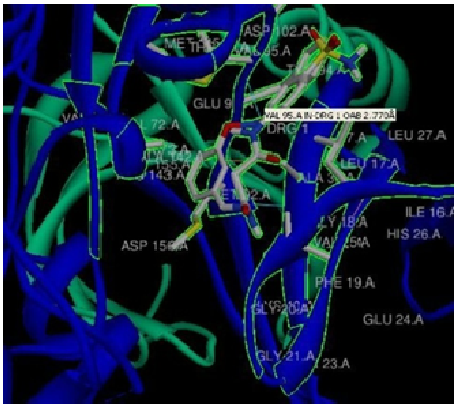


Figure 2c: H-Bond with active site residue.

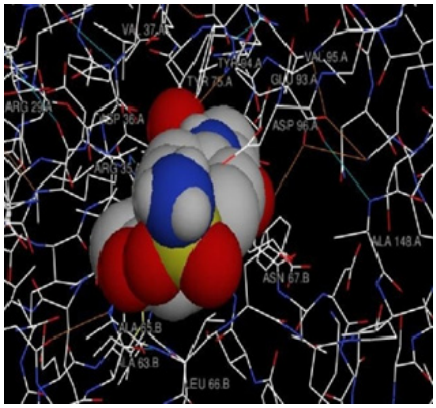


Figure 2d: H-Bond with active site residue.

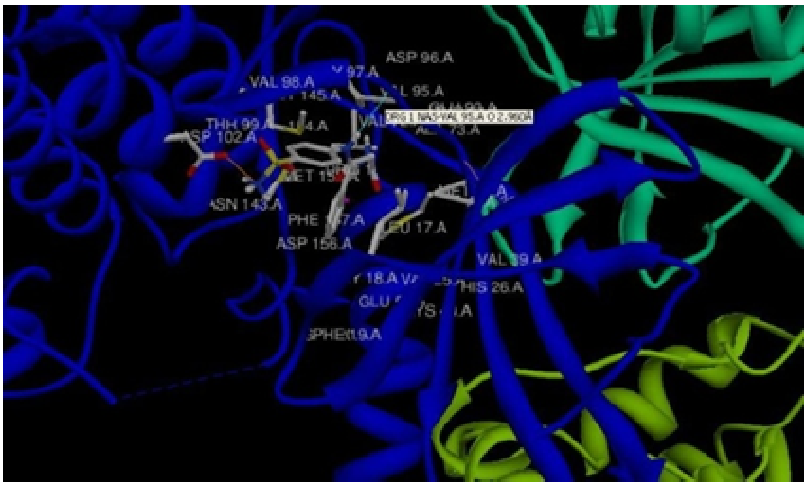


Figure 2e: H-Bond with active site residue.

Table 1: Binding Energy of Compunds obtained from ChemBank and PubChem.

S.No.	Compounds Name	Binding energy MIN	Binding energy MAX
1.	N- (2,6- diethylphenyl) - 1,3- benzothiazol- 2- amine	-8.81	-8.72
2.	2- [(2- chlorobenzyl) thio]- 1,3- benzothiazol- 6- amine	-8.71	-8.64
3.	2- hydroxybenzaldehyde 1,3- benzothiazol- 2- ylhydrazone	-8.61	-8.07
4.	N- (2,5- dimethylphenyl) - 1,3- benzothiazol- 2- amine	-8.52	-8.51
5.	4- [(1,3- benzothiazol- 2- ylthio) methyl]benzonitrile	-8.48	-8.37
6.	3- (1,3- benzothiazol- 2- yl) - 1,3- benzoxazol- 2(3H) - one	-8.34	-8.19
7.	N'- 1,3- benzothiazol- 2- yl- N,N- dimethylsulfamide	-8.32	-8.27
8.	N'- 1,3- benzothiazol- 2- yl- N,N- dimethylsulfamide	-8.06	-8.05
9.	N- 1,3- benzothiazol- 2- yl- 2- phenylacetamide	-8.05	-7.31

Table 4: Hydrogen Bonds and compounds that follow Lipinski's rule of 5.

Componds	Min	Max	Hydrogen Bonds
N- (2,6- diethylphenyl) - 1,3- benzothiazol- 2- amine	-8.81	-8.72	2, 1
2- [(2- chlorobenzyl) thio]- 1,3- benzothiazol- 6- amine	-8.71	-8.64	3, 1
2- hydroxybenzaldehyde 1,3- benzothiazol- 2- ylhydrazone	-8.61	-8.07	4, 2
N- (2,5- dimethylphenyl) - 1,3- benzothiazol- 2- amine	-8.52	-8.51	2, 1
4- [(1,3- benzothiazol- 2- ylthio) methyl]benzonitrile	-8.48	-8.37	3, 0
3- (1,3- benzothiazol- 2- yl) - 1,3- benzoxazol- 2(3H) - one	-8.34	-8.19	3, 0
N'- 1,3- benzothiazol- 2- yl- N,N- dimethylsulfamide	-8.32	-8.27	4, 1
N'- 1,3- benzothiazol- 2- yl- N,N- dimethylsulfamide	-8.06	-8.05	3,1
N- 1,3- benzothiazol- 2- yl- 2- phenylacetamide	-8.05	-7.31	2,1

References

[1] Hunter, T. (2000) Signaling-2000 and beyond. Cell 100, 113– 127.

[2] 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.

[3] Wehenkel, A., Fernandez, P., Bellinzoni, M., Catherinot, V., Barilone, N., Labesse, G., Jackson, M., Alzari, P.M. (2006) The structure of PknB in complex with mitoxantrone, an ATP-competitive inhibitor, suggests a mode of protein kinase regulation in mycobacteria Febs Lett. **580:** 3018-3022

[4] Young, T.A., Delagoutte, B., Endrizzi, J.A., Falick, A.M. and Alber, T. (2003) Structure of Mycobaterium tuberculosis PknB supports a universal activation mechanism for Ser/Thr protein kinases. Nat. Struct. Biol. 10, 168–174.

[5] Boitel, B., Ortiz-Lombardia, M., Duran, R., Pompeo, F., Cole, S.T., Cerven˜ansky, C. and Alzari, P.M. (2003) PknB kinase activity is regulated by phosphorylation in two Thr residues and dephosphorylation by PstP, the

cognate phospho-Ser/Thr phosphatase, in Mycobacterium tuberculosis. Mol. Microbiol. 49, 1493–1508.

[6] Villarino, A., Duran, R., Wehenkel, A., Fernandez, P., England, P., Brodin, P., Cole, S.T., Zimny-Arndt, U., Jungblut, P.R., Cerven˜ansky, C. and Alzari, P.M. (2005) Proteomic identification of M. tuberculosis protein kinase substrates: PknB recruits GarA, a FHA-containing protein, through activation loop-mediated interactions. J. Mol. Biol. 350, 953–963.

[7] Kang, C.M., Abbott, D.W., Park, S.T., Dascher, C.C., Cantley, L.C. and Husson, R.N. (2005) The Mycobacterium tuberculosis serine/threonine kinases PknA and PknB: substrate identification and regulation of cell shape. Genes Dev. 19, 1692–1704.

[8] Cohen, P. (2002) Protein kinases, the major drug targets of the 21st century? Nat. Rev. Drug Discov. 1, 309–316.

[9] Noble, M.E.M., Endicott, J.A. and Johnson, L.N. (2004) Protein kinase inhibitors: insights into drug design from structure. Science 303, 1800–1805.

[10] Morris, G M. *et al.* (1998) Automated docking using a Lamarckian genetic algorithm and empirical binding free energy function. *J. Comp Chem*. 19, 1639-1662.

[11] http://chembank.broadinstitute.org/chemistry/search/execute.htm?id=5434202

[12] http://www.ncbi.nlm.nih.gov/sites/entrez?db=pccompound&term=mitoxantrone

[13] http://chembank.broad.harvard.edu/chemistry/search/execute.htm?id=5376907

[14] Ketan, M.; Desai, K.R.; I. J. Chem. 45B, 1762-1766 (2006).

[15] The structure of PknB in complex with mitoxantrone, an ATP-competitive inhibitor, suggests a mode of protein kinase regulation in mycobacterial.