International Journal of Chemical Studies

P-ISSN2349–8528
E-ISSN 2321–4902
IJCS 2016; 4(5): 10-16

Received: 03-07-2016
Accepted: 04-08-2016

Jagbir Singh
Pharmaceutical Chemistry Laboratory, Department of Pharmaceutical Sciences, Maharishi Dayanand University, Rohtak. Haryana, India

Rani Mansuri
Pharmacoinformatics Laboratory, Department of Pharmacoinformatics, National Institute of Pharmaceutical Education and Research, Hajipur, Bihar, India

Mahesh Kumar
Pharmaceutical Chemistry Laboratory, Department of Pharmaceutical Sciences, Maharishi Dayanand University, Rohtak. Haryana, India

Ganesh Chandra Sahoo
Biomedical Research Department, Rajendra Memorial Research Institute of Medical Sciences, Bihar, India

Correspondence
Ganesh Chandra Sahoo
Biomedical Research Department, Rajendra Memorial Research Institute of Medical Sciences, Bihar, India

Docking studies with drug bank compounds and inhibitor identification for an excellent drug target serine/Threonine protein kinase A of *Mycobacterium tuberculosis*

Jagbir Singh, Rani Mansuri, Mahesh Kumar and Ganesh Chandra Sahoo

Abstract
The phosphorylation of Serine/Threonine protein kinaseA (PknA) enzyme plays an important role in cellular signaling transduction in *Mycobacterium tuberculosis* (*Mtb*) and has very less homology with humans. Hence, use of PknA as drug target may be good approach towards drug designing. Crystal structure of PknA was used for structure based inhibitor identification. Approved compounds from Drug Bank were docked using Glide (Schrodinger 9.2) with PknA and ten approved compounds, showed good interaction within the active pocket of target protein. Compounds DB00349 (Clobazam), DB00348 (Nitisinone), DB04552 (Niflumic Acid), DB00821 (Carprofen), DB00643 (Mebendazole), DB08621 (Thiamphenicol), DB00498 (Phenindione), DB00744 (Zileuton), DB00897 (Triazolam) and DB01123 (Proflavine) showed the highest XP glide score and good interaction with PknA protein. Hence, these selected compounds have been or being used as medicinal compounds for humans. These may also be tested and used as combination drug with present antitubercular drugs to kill the Mtb at faster rate. These compounds may also be used as template for further ligand based drug designing and combinatorial compound library designing.

Keywords: STPKs, PknA, Mtb, drug bank, docking, Schrodinger

1. Introduction
According to the WHO reports tuberculosis (TB) still in the South East Asia is one of the serious public health alarms. In 2014, globally 1.5 million were killed by TB. Approximately, 140 000 children were died from TB [1].
Resistance for standard anti-tubercular drugs is growing very fast where multidrug resistance is a major concern. Disease caused by resistant bacteria fails to conventional, first-line treatment [1]. Resistance to the most common antitubercular drug, INH found frequently among clinical isolates [2]. Point mutations in rifamycins were found in *rpoB* gene and found responsible for rifamycins resistance. These mutation predominantly noticed in more than 90% of rifampin-resistant clinical isolates [3]. Pyrazinamide (PZA) drug has also acquired resistance. Gene which causes resistance has been recognized as *pncA* gene [4]. Importantly, mutations in the form of point mutations (deletion, insertion) were found [5, 6]. The proteins responsible for ethambutol resistance against in *M. tuberculosis* (*Mtb*) due to the *EmbA* and *EmbB* proteins which have been proved to play important role during arabinogalactan synthesis in the formation of hexaarabinofuranoside motif [7] whereas *EmbC* is found to involve in lipoarabinomannan synthesis [8].
Multidrug resistant TB (MDR-TB) is being treated by using second-line drugs but there is no satisfactory results have been found. Hence, identification of any novel drug target and drug candidate becomes important.
Protein kinases have a vast family and these are key enzymes of important biochemical pathways like cell differentiation, and cell division in many of organisms. In the *Mtb*, STPKs (Serine/Threonine protein kinases) participate in essential role in survival of *Mtb* by promoting the cellular growth. The STPKs are found in both the soluble and transmembrane form [9]. Serine threonine protein kinaseA functions as transmembrane receptor within the mycobacterial cell. Morphological changes related to cell division are regulated by Magnesium/manganese dependent autophosphorylating ability of protein kinaseA (PknA). Inhibition of this enzyme may lead to the death of *Mtb* [9].

Thakur *et al*. have defined that PknA is importantly, transphosphorylate the ligase mMurD enzyme (mycobacterial UDP-N-acetylmuramoyl-L-alanine: D-glutamate ligase) which is responsible for peptidoglycan biosynthesis crucial for connecting UDP-N-acetylmuramoyl-L-alanine and D-glutamate [10].

There is very less homology between the STPKs of MTB and humans. Since a good drug target enzyme should have crucial and unique role in any important biochemical pathway and also should have either no very less identity with humans. Hence and pknA protein can be used as drug target for antitubercular drug designing to overcome the problem of drug resistance [11, 12]**.** Crystal *3-D* structure of of PknA[13] and PknB [14, 15] of *Mtb* has been developed which enhances the informative knowledge about the catalytic domain for phosphorylation and mechanism of phosphorylation of STPKA.

In this paper, Serine/Threonine-protein kinaseA, PknA (STPK A) of *Mtb* was selected as drug target for structure based drug designing. PknA protein has been used for docking studies with Drug Bank compounds using Schrodinger9.2. Ten compounds identified may be used as combination drugs with antitubercular drugs and may be helpful in killing the *Mtb* by inhibiting the PknA protein. Hence, these compounds may be tested as combination drug and can further be modified and optimized.

2. Methods and materials

2.1 Compound library and Protein Preparation

Compound library was designed by collecting 1991 approved compounds from Drug Bank*5.0* (http://www.drugbank.ca/). Drug Bank is a unique online bioinformatics and cheminformatics database that provides the complete information about the compound approved by FDA and compounds under investigation too. The database also provides pharmacological and pharmaceutical details along the chemistry of the compounds [16]. Lig Prep module of Schrodinger9.2 was used for the preparation of compounds for the docking studies [17]. PknA crystal structure PDB Id: 4OW8 was retrieved from PDB site (http://www.rcsb.org/pdb/home/home.do). The protein structure was prepared using Protein Preparation Wizard module of Schrodinger9.2 [18].

2.2 Binding site

The binding sites were predicted from Site Map module of Schrodinger 9.2. Site Map searches binding pockets based upon the novel search quality of binding sites and generates informative data about the binding sites. A Site Map calculation begins with identifying large or small cavities within the surface of a protein or on the surface, capable of providing surface for any ligand rotation and binding with receptor. It generates the contour maps and hydrophobic and hydrophilic maps of the binding pockets identified [19, 20]. Binding site was selected base on the Site Score, size, Dsocre and volume of the cavity identified. The binding site selected for docking studies was covering the active residues (AA 137 – AA149) identified from Prosite pattern scan and functionally crucial amino acids THR172, THR 174 and THR 180 [13].

2.3 Docking studies

Since docking is a program to analyze the interacting affinity for the target protein and the orientation of the compounds (small molecules) within the possible active site of the protein (macromolecule). So the docking was performed using Glide module of Schrodinger 9.2. Glide provides good accuracy in scoring of docking results. Accuracy and precision in the results increases as the scoring moves from High Throughput Virtual screening (HTVS) to the Extra Precision (XP) [21]**.** Glide performs ligand database screening and docking with good accuracy. Schrödinger's proprietary Glide Score was selected as scoring function. Glide provides the information in terms of glide score (docking score) and interaction score of interacting residues with the ligands. Interacting residues were identified for each compound which showed better affinity towards target protein.

3. Results and Discussion

3.1 Compound Library and Protein Preparation

The Drug Bank approved compounds were curated and library was developed. Total of 1991 compounds were collected in the library. Lig Prep module was used to minimize conformers by applying OPLS force field. Since the crystal structure of PknA of *Mtb* is available at PDB, thus the crystal structure PDB Id: 4OW8 was retrieved and prepared for docking studies using Schrodinger 9.2

3.2 Binding site

The first site with greatest volume (893.47 $\acute{A}^3$) and size (357) scored the good Site Score (1.03) more than 1.0 is found the promise able binding site. The good Dscore (0.94) suggested the druggability of the selected binding site. Hence, the selected binding site has better druggability for binding with the compounds and selected as binding site for docking. Predicted by sitemap was chosen to generate grid of Protein crystal structure (PDB Id: 4OW8) and XYZ coordinates were extended to the 12 Å in each dimension. The better part of the binding site selected was that the binding site covered occupied the experimentally identified crucial amino acids (Thr172, Thr174 and Thr180) (Fig.1) for the phosphorylation of the PknA protein of *Mtb* [13]**.** Hence the active grid was created upon binding site described above for the docking studies of compound library.

3.2 Docking studies

Docking studies of PknA of *Mtb* was done using the Glide module of Schrodinger9.2. Compounds from Drug Bank were curated and corrected for the missing atoms manually according the literature of concerned compound. Compounds were screened by High Throughput method and top scored fifty compounds with glide score more than -10.0 Kcal/mol were again rescored using XP docking to get accuracy within the results and finally only ten Drug Bank compounds with XP glide score more than -10.0 were selected such as DB00349 ((Clobaza) highest Glide score -11.8 Kcal/mol), DB00348 (Nitisinone), DB04552 (Niflumic Acid), DB00821 (Carprofen), DB00643 (Mebendazole) DB08621 (Thiamphenicol), DB00498 (Phenindione), DB00744 (Zileuton), DB00897 (Triazolam) and DB01123 (Proflavine) Clobazam as given in Table.1. Interestingly all the ten compounds were binding in the activating loop (AA162-AA180) where the phosphorylation of threonine residues (Thr172, Thr174 and Thr180) occurs. Thus, these inhibitors may block the phosphorylation of PknA and ultimately the process of cell division may hamper.

The compounds formed the hydrogen bonds with amino acids of kinase domain of PknA as shown in Table.2. The all compounds interacted in a similar manner as shown in Fig.2. Since all the selected compounds already have medicinal

effects, these may have antitubercular effect also. The compound DB00897 (Triazolam) which has been withdrawn from market, showed good affinity for PknA may be modified to minimize problems of adverse effects. If these compounds found active against *Mtb*, cost and time for vivo trial investigation will be reduced.

Hence, these selected compounds may be used as template for further modification to develop the novel compounds. Also these may be used as combination drug with antitubercular drugs to the kill or suppress the *Mtb* bacteria at faster rate.

4. Conclusion

Protein kinases have a vast family and these are key enzymes of important biochemical pathways like cell differentiation, and cell division in many of organisms. STPKs (Serine/Threonine protein kinases) participate in essential role in survival of *Mycobacterium tuberculosis* by promoting the cellular growth. PknA functions as transmembrane receptor with the kinase domain located within the mycobacterial cell. Morphological changes related to cell division are regulated by Magnesium/manganese dependent autophosphorylating ability of protein kinaseA (PknA). So the inhibition of the PknA protein will kill the *Mtb*. Hence the Crystal structure was used for docking with approved compounds of Drug Bank. Compounds DB00349, DB00348, DB04552, DB00821, DB00643, DB08621, DB00498, DB00744, DB00897, and DB01123 scored the good dock score. Hence, these approved compounds may be having the binding affinity for PknA. These may act as new template for ligand based combinatorial drug designing and these may also be used as combination drugs with antitubercular drugs.

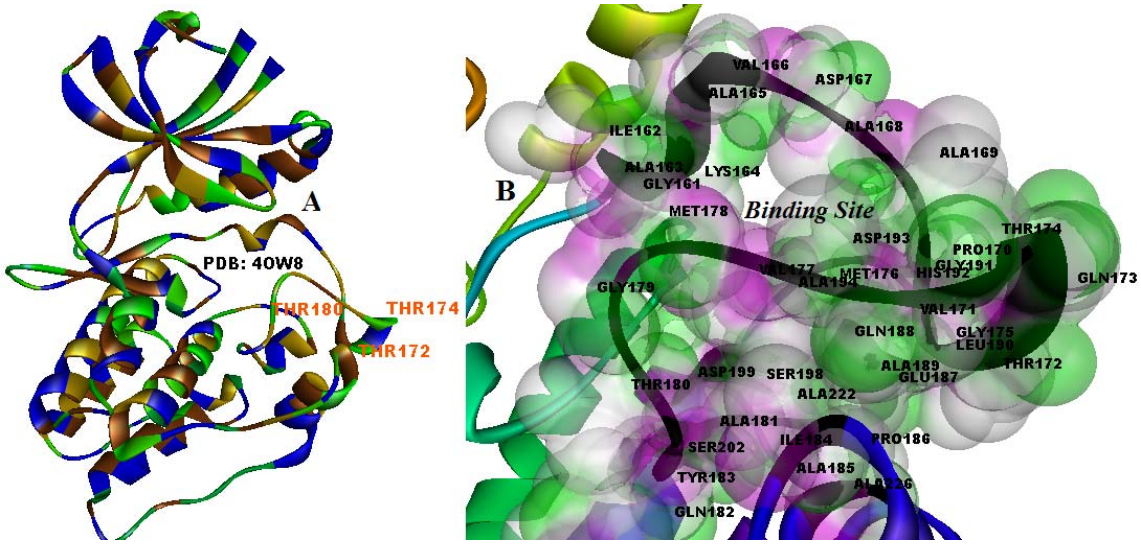


Fig 1 A: PDB selected for docking studies and the important amino acids highlighted**. B:** Binding site amino acids (Met 24, Phe48, Phe54, Arg57, His139-Lys143, Gly161-Ala194, Ser19-Asp199, Ser202, Ala222, Ala226) shown within the binding site in vander-wall surface view (Hydrogen bond donor/acceptor type)

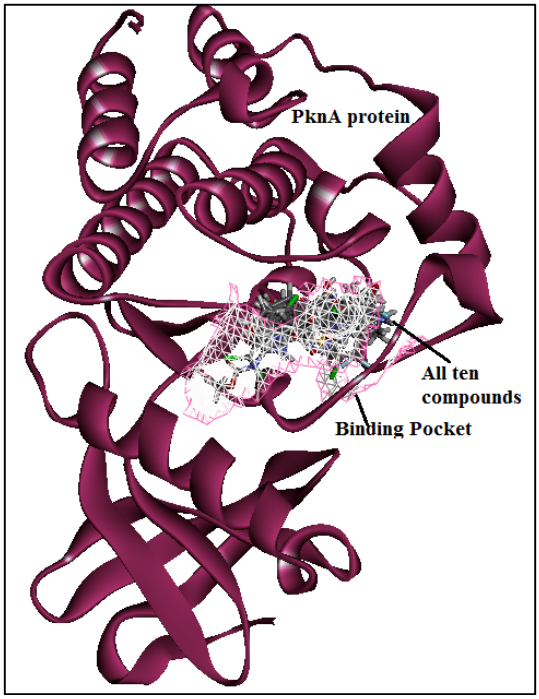
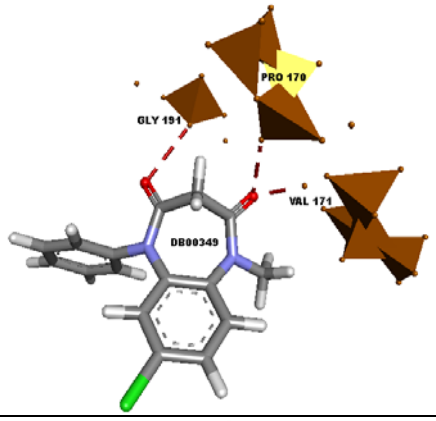
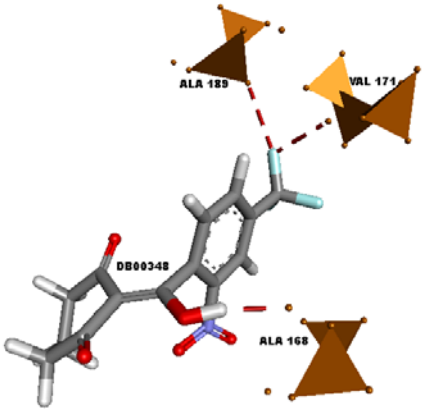
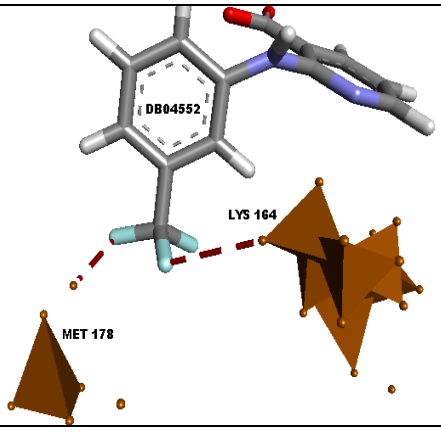


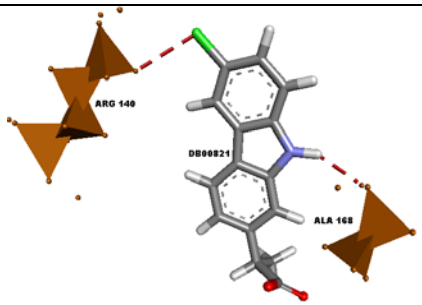
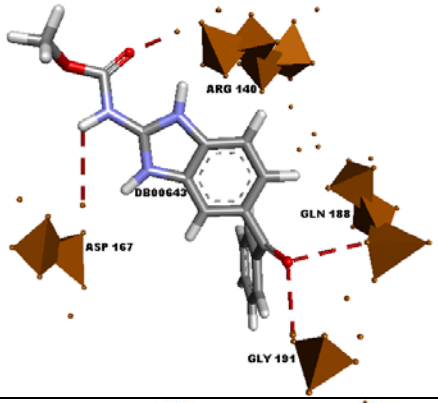
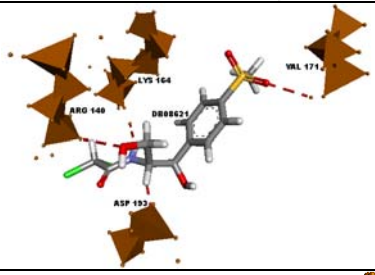
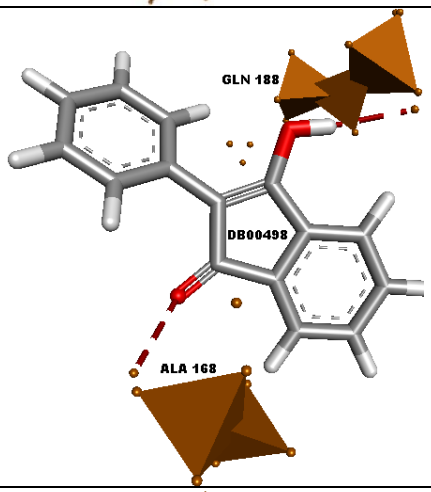
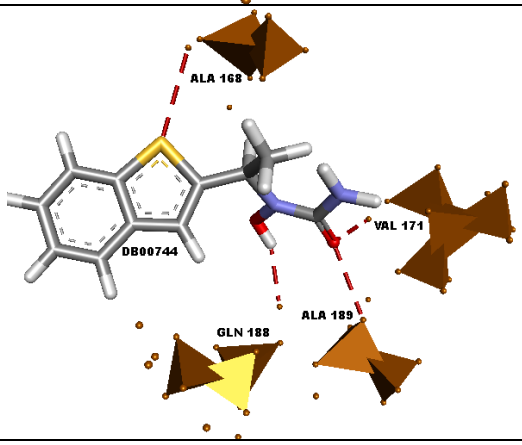
Fig 2: Interacting orientation of the ten selected compounds within the binding pocket.

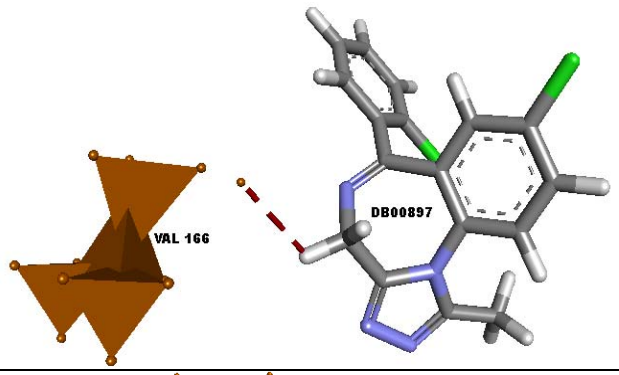
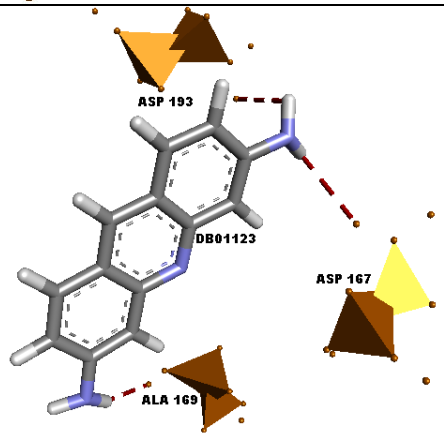
Table 1: Represent top ten compounds with their XP glide score and interacting residues.

S. No.	Compound ID	Name	Smile Notations	Glide score	Interacting Amino acids
1	DB00349	Clobazam	CN1C2=C(C=C(Cl)C=C2)N(C2=CC=CC=C2)C(=O)CC1=O	-11.80	Val 171, Ala168, Ala189
2	DB00348	Nitisinone	[O][N+](=O)C1=C(C=CC(=C1)C(F)(F)F)C(=O)C1C(=O)CCCC1=O	-11.32	Val 171, Pro 170, Gly191
3	DB04552	Niflumic Acid	OC(=O)C1=C(NC2=CC=CC(=C2)C(F)(F)F)N=CC=C1	-11.09	Met 178, Lys 164
4	DB00821	Carprofen	CC(C(O)=O)C1=CC2=C(C=C1)C1=C(N2)C=CC(Cl)=C1	-11.06	Ala 168, Arg 140
5	DB00643	Mebendazole	COC(=O)NC1=NC2=C(N1)C=C(C=C2)C(=O)C1=CC=CC=C1	-11.02	Asp167, Arg140, Gln188, Gly191, Glu61
6	DB08621	Thiamphenicol	[H][C@](CO)(NC(=O)C(Cl)Cl)[C@]([H])(O)C1=CC=C(C=C1)S(C)(=O)=O	-10.97	Val 171, Lys 164, Arg140, Asp193
7	DB00498	Phenindione	O=C1C(C(=O)C2=CC=CC=C12)C1=CC=CC=C1	-10.92	Ala 168, Gln188
8	DB00744	Zileuton	CC(N(O)C(N)=O)C1=CC2=CC=CC=C2S1	-10.69	Ala168, Val171, Gln188, Ala 189
9	DB00897	Triazolam	CC1=NN=C2CN=C(C3=CC=CC=C3Cl)C3=C(C=CC(Cl)=C3)N12	-10.56	Val 166
10	DB01123	Proflavine	NC1=CC2=NC3=C(C=CC(N)=C3)C=C2C=C1	-10.20	Asp 167, Asp 193, Ala169

Table 2: Selected Drug Bank compounds (element color) and Interacting amino acids Polyhedron style (Orange color).

Compound ID	Interaction of Selected Drug Bank compounds
DB00349	
DB00348	
DB04552	

DB00821	
DB00643	
DB08621	
DB00498	
DB00744	

DB00897	
DB01123	

5. References

1. Organization WH, Programme GT. Global Tuberculosis Control: WHO Report: WHO. 2008.
2. Karakousis PC. Mechanisms of action and resistance of antimycobacterial agents. Antimicrobial drug resistance: Springer. 2009, 271-291.
3. Ramaswamy S, Musser JM. Molecular genetic basis of antimicrobial agent resistance in *Mycobacterium tuberculosis*: 1998 update. Tubercle and Lung disease. 1998; 79(1):3-29.
4. Scorpio A, Zhang Y. Mutations in pnc A, a gene encoding pyrazinamidase/nicotinamidase, cause resistance to the antituberculous drug pyrazinamide in tubercle bacillus. Nature medicine. 1996; 2(6):662-667.
5. Scorpio A, Lindholm-Levy P, Heifets L *et al.* Characterization of pncA mutations in pyrazinamide-resistant *Mycobacterium tuberculosis.* Antimicrobial agents and chemotherapy. 1997; 41(3):540-543.
6. Juréen P, Werngren J, Toro JC, Hoffner S. Pyrazinamide resistance and pncA gene mutations in *Mycobacterium tuberculosis.* Antimicrobial agents and chemotherapy. 2008; 52(5):1852-1854.
7. Zhang N, Torrelles JB, McNeil MR *et al.* The Emb proteins of mycobacteria direct arabinosylation of lipoarabinomannan and arabinogalactan via an N-terminal recognition region and a C-terminal synthetic region. Molecular microbiology. 2003; 50(1):69-76.
8. Shen K, Hines AC, Schwarzer D, Pickin KA, Cole PA. Protein kinase structure and function analysis with chemical tools. Biochimica et Biophysica Acta (BBA)-Proteins and Proteomics. 2005; 1754(1):65-78.
9. Chao J, Wong D, Zheng X *et al.* Protein kinase and phosphatase signaling in *Mycobacterium tuberculosis* physiology and pathogenesis. Biochimica et Biophysica ACTA (BBA)-Proteins and Proteomics. 2010; 1804(3):620-627.
10. Thakur M, Chakraborti PK. Ability of PknA, a mycobacterial eukaryotic-type serine/threonine kinase, to transphosphorylate MurD, a ligase involved in the process of peptidoglycan biosynthesis. Biochemical Journal. 2008; 415(1):27-33.
11. Alber T. Signaling mechanisms of the *Mycobacterium tuberculosis* receptor Ser/Thr protein kinases. Current opinion in structural biology. 2009; 19(6):650-657.
12. Wehenkel A, Bellinzoni M, Grana M, *et al.* Mycobacterial Ser/Thr protein kinases and phosphatases: physiological roles and therapeutic potential. Biochimica et Biophysica Acta (BBA)-Proteins and Proteomics. 2008; 1784(1):193-202.
13. Ravala SK, Singh S, Yadav GS, Kumar S, Karthikeyan S, Chakraborti PK. Evidence that phosphorylation of threonine in the GT motif triggers activation of PknA, a eukaryotic-type serine/threonine kinase from *Mycobacterium tuberculosis*. FEBS journal. 2015; 282(8):1419-1431.
14. Chopra P, Singh B, Singh R *et al.* Phosphoprotein phosphatase of *Mycobacterium tuberculosis* dephosphorylates serine–threonine kinases PknA and PknB. Biochemical and biophysical research communications. 2003; 311(1):112-120.
15. Wehenkel A, Fernandez P, Bellinzoni M *et al.* The structure of PknB in complex with mitoxantrone, an ATP-competitive inhibitor, suggests a mode of protein kinase regulation in mycobacteria. FEBS letters. 2006; 580(13):3018-3022.
16. Wishart DS, Knox C, Guo AC *et al.* DrugBank: a comprehensive resource for in silico drug discovery and exploration. Nucleic acids research. 2006; 34(suppl 1):D668-D672.
17. Chatterjee A, Cutler SJ, Doerksen RJ, Khan IA, Williamson JS. Discovery of thienoquinolone derivatives as selective and ATP non-competitive CDK5/p25

inhibitors by structure-based virtual screening. Bioorganic & medicinal chemistry. 2014; 22(22):6409-6421.
18. Unger M, Eichhoff AM, Schumacher L *et al.* Selection of nanobodies that block the enzymatic and cytotoxic activities of the binary Clostridium difficile toxin CDT. Scientific reports. 2014; 5:7850-7850.
19. Halgren T. New Method for Fast and Accurate Binding-site Identification and Analysis. Chemical biology & drug design. 2007; 69(2):146-148.
20. Nayal M, Honig B. On the nature of cavities on protein surfaces: application to the identification of drug-binding sites. Proteins: Structure, Function, and Bioinformatics. 2006; 63(4):892-906.
21. Friesner RA, Banks JL, Murphy RB *et al*. Glide: a new approach for rapid, accurate docking and scoring. 1. Method and assessment of docking accuracy. Journal of medicinal chemistry. 2004; 47(7):1739-1749.