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Research Article

Inactivation of Phosphoserine/Threonine Phosphatase PstP in H37Rv *Mycobacterium tuberculosis* by *In silico* Drug Design Approach

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I N F O

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A B S T R A C T

Introduction: Mycobacterium tuberculosis (Mtb) is the aetiological agent of the infectious disease tuberculosis (TB). According to the World Health Organization's most recent study, kinases play a crucial role in phosphorylation by transferring phosphate moieties to target proteins, while phosphatases reverse this process by dephosphorylating substrates or regulating kinase activity, restoring proteins to their unphosphorylated state.

Method: Several software, programs and databases were used to predict and calculate the interaction between target protein and ligand molecules.

Results: Protein phosphatases are essential components in cellular signalling pathways mediated by phosphorylation. Despite the presence of eleven serine/threonine protein kinases in Mtb, only one of them, namely phosphoserine/threonine phosphatase (PstP), has been identified. PstP stands out as a promising drug target due to its singular role as the exclusive phosphatase in Mtb. Serine/threonine protein phosphatase (PDB ID: 2cm1) was docked using AutoDock Vina software with ligands collected from other studies conducted on *Mycobacterium tuberculosis*. Virtual screening was carried out using ZincPharmer to find the homologous molecules to load the pharmacophore.

Conclusion: Mycobacterium tuberculosis H37Rv has multiple targets for new drug design procedures. This is due to the variation in the proteins. PstP protein has five pockets and was given a good docking score with eight safe inhibitors for further studies, and eight tested molecules with PubChem_IDs 50956528, 7388777, 46780845, 3635532, 46954451, 1494562, 540267, 79107978, which have good features and can bind to PstP protein.

Keywords: *In silico* Simulation, Phosphorylation Enzymes, CADD

Introduction

Mycobacterium tuberculosis (Mtb) is the aetiological agent of tuberculosis (TB). According to a recent study by the World Health Organization, 1.45 million deaths and 10 million new cases of TB were estimated to have occurred in 2018 due to Mtb infection.[1] The current regimen of DOTS (directly observed treatment short course), which is highly intricate and time-consuming, includes medications that block either DNA replication, transcription, or cell wall biosynthesis. Several factors, such as inadequate patient compliance or treatment failure, have led to the emergence of various drug-resistant TB strains, encompassing multidrug-resistant, extensively drug-resistant, and fully drug-resistant variants.[2,3] The challenges in TB drug development include identifying drug targets that exhibit weakness *in vitro*, discovering scaffolds with novel mechanisms of action to potentially shorten chemotherapy, and developing drugs that can effectively target drug-resistant and latent bacteria while remaining compatible with current TB and anti-retroviral therapy. Peptidoglycan, forming the innermost layer of the bacterial cell wall, consists of glycan chains interconnected by small peptides. Kinases play a crucial role in phosphorylation by transferring phosphate moieties to target proteins, while phosphatases reverse this process by dephosphorylating substrates or regulating kinase activity, restoring proteins to their unphosphorylated state. In addition to the well-established two-component systems that target His/Asp residues in bacteria, phosphorylation also commonly targets Ser, Thr, and Tyr residues. Mtb possesses 11 Ser/Thr protein kinases (STPKs PknA-L, excluding C), one tyrosine kinase (PtkA), one Ser/Thr phosphatase (phosphoserine/threonine phosphatase - PstP), and two tyrosine phosphatases (PtpA and PtpB). Numerous mycobacterial proteins have been identified as substrates regulated through phosphorylation by STPKs, with some of these substrates undergoing dephosphorylation by PstP. PstP belongs to the PP2C phosphatase (PPM family). Its activity is strictly dependent on Mn^{2+} ions. The intricate interplay between kinases and phosphatases in Mtb underscores the significance of phosphorylation in regulating various cellular processes.[4] An intriguing aspect of Mtb Ser/Thr signalling molecules is the co-localisation of both essential STPKs, PknB (Rv0014c) and PknA (Rv0015c), along with the sole Ser/Thr phosphatase PstP (Rv0018c) within the same genomic cluster, a conservation observed in various mycobacterial species. Previous transcriptional analyses have revealed similar expression profiles for PknA, PknB, and PstP, suggesting a need for stringent regulation due to the functional counteraction between these enzyme classes. This study unveils a novel finding regarding the regulation of PstP activity through phosphorylation, marking the first report on post-translational modification influencing a bacterial Ser/Thr phosphatase. The investigation demonstrates that PstP undergoes differential phosphorylation by both PknA and PknB, both *in vitro* and within the surrogate host.

Furthermore, recent studies have identified zinc ions (Zn^{2+}) and inorganic phosphate (Pi) as inhibitors of PstP activity. Protein phosphorylation plays a pivotal role in modulating the transduction of extracellular signals into cellular responses. The demonstration of protein phosphorylation in prokaryotes was initially established simultaneously in *Salmonella typhimurium* and *Escherichia coli*.[5] The evolving functions of STPKs indicate their capacity not only to regulate numerous intracellular metabolic processes but also to influence host signalling cascades. Exploiting such networks selectively provides an avenue for designing novel pharmaceutical strategies to combat the disease. In accordance with theories of molecular evolution, a gene with a crucial function would exhibit high conservation in its sequence across species. Therefore, in the quest to develop new anti-TB drugs, assessing the evolutionary conservation of the target becomes imperative.[6,7] The objective of TB drug discovery and development is to attain drugs and drug regimens for TB that surpass the current options in terms of efficacy, rapidity of action, safety, tolerability, user-friendliness for all patient demographics, and accessibility.[8] Over the past decade, the processes involved in discovering and manufacturing new drugs have undergone continuous evolution, leading to direct improvements in the quality of life for the global population.[9] One approach to mitigate limitations in drug discovery and manufacturing, reducing both costs and time impacts, involves the utilisation of computer-aided drug design (CADD), also referred to as molecular modelling. In this method, the design and analysis stages of drugs are conducted through a cyclic-assisted process entirely reliant on in silico simulations, which enable the evaluation of crucial factors in drug discovery, such as toxicity, activity, biological activity, and bioavailability, even before progressing to *in vitro* and *in vivo* clinical trials.

An initial step in the CADD approach is the screening of virtual compound libraries, known as virtual screening (VS), which is a method for classifying molecules based on biological or chemical properties present in extensive datasets. According to the International Union of Pure and Applied Chemistry (IUPAC), VS involves computational methods that categorise molecules in a database based on their potential to exhibit biological properties against a specified molecular target.[10] The primary objective of a VS procedure is to identify a collection of structurally diverse hit

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compounds, which can subsequently undergo refinement during optimisation stages. Notably, the outcomes of a VS approach, particularly when employing receptor-based methods, can provide insights into the molecular foundations of the activity of bioactive compounds.[11]

Method

In silico studies were performed using the following programs, software and databases.

Target Determination

Target determination involved the utilisation of the following programs and databases:

- NCBI database (http://www.ncbi.nlm.nih.gov/)
- UniProt database (http://www.uniprot.org/)
- KEGG database (http://www.genome.jp/kegg/)
- Tuberculist database (http://tuberculist.epfl.ch/)
- PDB database (https://www.rcsb.org/)

Ligands Search and Pharmacophore Building

- Zinc database (http://zinc.docking.org/)
- PubChem database (https://pubchem.ncbi.nlm.nih.gov/)
- PyMOl software (https://pymol.org/#download)
- CB dock (http://cadd.labshare.cn/cb-dock2/)
- Auto Dock Vina (https://vina.scripps.edu/)
- ZincPharmer (http://zincpharmer.csb.pitt.edu/)
- T.E.S.T. Toxicity Estimation Software Tool (http://www.epa.gov/nrmrl/std/cppb/qsar/index.html#TEST)

Results & Discussion

Target Validation

Essential genes, defined as crucial for replication and viability, make an organism susceptible to death upon the deletion, interruption, or blocking of the protein expressed by such genes. This characteristic renders essential genes attractive targets for drug development. By identifying a protein vital to Mtb and developing inhibitors for that specific protein, new antimicrobials can be discovered.[12] Protein phosphatases play crucial roles in cellular signalling mediated by phosphorylation. In Mtb, despite the presence of 11 Ser/Thr protein kinases, only one Ser/Thr phosphatase, PstP, has been recognised. PstP stands out as a promising drug target due to its status as the sole phosphatase in Mtb.[4] It performs a crucial function in the regulation of cell division and growth through reversible phosphorylation signalling. Consequently, it plays a pivotal role in controlling cellular metabolism and signalling pathways, potentially influencing the growth and development of the cell, leading to the establishment and maintenance of infection.[4,13] Serine/threonine protein phosphatase (EC:3.1.3.16) consists of 514 amino acids resulting from 1545 nt. 2cm1 is a crystal structure of the catalytic domain of PstP; the PDB file was downloaded and removed the connected molecules and used in the primary docking with selected molecules which are used as drugs in the treatment of TB in addition to cinnamic acid as a natural product and has a killing activity for Mtb[12,14] as shown in Table 1.

Molecular Docking

More than four thousand genes are the total content of the *Mycobacterium tuberculosis* H37Rv genome, the majority of which are of unknown functions. Efforts have been directed towards computationally modelling and docking one of its proteins. Additionally, there exists a wide array of software tools designed to simulate the molecular interactions between a target protein and small molecules.[15] Serine/threonine protein phosphatase (PDB 2cm1) was docked using the AutoDock Vina software with ligands collected from other studies conducted on Mtb as mentioned in Table 1. The binding pose and affinity between a ligand and enzyme are very important parts of information for CADD.[15] The results of docking are shown in Figure 1 and Table 2.

Table 1.Chemical Properties of Ligands in this Study[16,17]

S.NO	Ligands	Chemical Formula	Compound CID	MW (g/mol)
1	Cinnamic acid	$C_9H_8O_2$	444539	148.16
2	Ethambutol	$C_{10}H_{24}N_2O_2$	14052	204.31
3	Isoniazid	$C_6H_7N_3O$	3767	137.14
4	Pvrazinamide	$C_5H_5N_3O$	1046	123.11
5	Rifabutin	$C_{46}H_{62}N_4O_{11}$	135415564	847.00
6	Rifampin	$C_{43}H_{58}N_4O_{12}$	135398735	822.90
7	Rifapentine	$C_{47}H_{64}N_4O_{12}$	135403821	877.00

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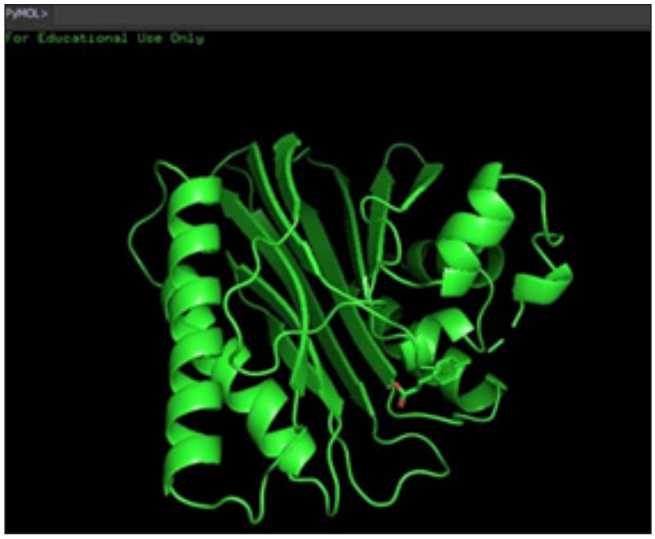

Figure 1.Docking Result between PstP Protein and Cinnamic Acid Illustrated by PyMOL Software (https://pymol.org/#download)

Table 2.Best Results of AutoDock's Affinity (kcal/mol)[17]

S.NO	Molecules_PubChem ID	Docking Scores
1	Cinnamic_444539	-5.0
2	Ethambutol_14052	-4.5
3	Isoniazid_3767	-5.4
4	Pyrazinamide_1046	-4.7
5	Rifampin_135398735	-7.2

Rifampin_135398735 was eliminated from pharmacophore building and virtual screening due to high molecular weight (822.9 g/mol); cinnamic_444539 and isoniazid_3767 were used in the pharmacophore building by employing LigandScout software which is a comprehensive platform for precise virtual screening utilising three-dimensional chemical feature pharmacophore models.

Virtual Screening

The process of assessing a compound library using a computational model to rank and screen for molecules with desired characteristics is referred to as Virtual Screening (VS). In this study, ligand-based VS was employed, and a pharmacophore model derived from a collection of known ligands was utilised in the VS process to identify specific inhibitors against the PstP protein. VS was carried out using ZincPharmer (http://zincpharmer.csb.pitt.edu/pharmer.html)[16] by loading the features of Pharmacophore. (29,101) thousand hits molecules were obtained from Zinc database after filtering through Lipinski's rules of five, all molecules were tested for their carcinogenicity, mutation elucidation ability and toxicity with T.E.S.T. software and excluded the irritant ones, in addition to filtration depending on Lipinski's rules of five, the filtered molecules were listed in Table 2 with molecular weight and SMILES string of each one which collected from PubChem which is a public repository for information on chemical structures and their biological activities "(https://pubchem.ncbi.nlm.nih.gov/)".[17] These molecules were docked using CB dock program. It is a protein-ligand docking technique that automatically detects binding sites, computes their centre and size, adjusts the docking box dimensions based on the query ligands, and subsequently conducts molecular docking using AutoDock Vina.[18] Figures 3–10 indicate the pocket ID of the target protein and Vina score after docking with selected molecules. A cavity found on the surface or within the interior of a protein, exhibiting favourable characteristics for binding a ligand, is commonly known as a binding pocket. The physicochemical characteristics of a binding pocket are determined by the set of amino acid residues surrounding it. This, in conjunction with its shape and position within a protein, establishes its functionality.[19]

Models depending on 3D ligands are employed to analyse the structure-activity relationship. Molecular docking, on the other hand, delves into the conformational space and utilises a scoring function to categorise the poses of ligands within the active site of a protein. It relies on the geometric complementarity between the ligand and the receptor. Consequently, the positions of atoms play a crucial role in identifying a protein region characterised by favourable ligand-receptor interactions.[20,21]

Table 3.Selected Molecules after Pharmacophore Virtual Screening[17]

PubChem CID	SMILES String	Molecular Formula	Molecular Weight
79107978	c1cc(c(c(c1)F)S(=O)(=O)[N]c2ccc(cc2)Cc3[n]nnn3)F	$C_{16}H_{29}N_3O$	349.322
540267	CC(=O)O[C@@H]1[C@@H](N(c2c(non2)N1[N+](=O)[O-])[N+](=O)[O-])OC(=O)C	$C_8H_8N_6O_9$	332.180
50956528	c1ccc(cc1)c2c(n(cn2)Cc3[n-]nnn3)c4ccc(o4)c5ccn[nH]5	$C_{18}H_{14}N_8O$	358.400
46780845	c1ccc(c(c1)c2ccccc2c3[n-]nnn3)c4[n-]nnn4	$C_{14}H_{10}N_8$	290.280

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3635532	c1ccc(cc1)S(=O)(=O)[N-]/N=C\c2ccc(s2)Br	$C_{11}H_9BrN_2O_2S_2$	345.200
46954451	COc1cccc(c1OC)CN(Cc2c(n[nH]n2)c3ccccc3)C[C@H]4CCCO4	$C_{23}H_{28}N_4O_3$	408.500
1494562	c1ccc(cc1)COc2ccccc2/C=C/[N+](=O)[O-]	$C_{15}H_{13}NO_3$	255.270
7388777	Cc1cccc(c1)[C@H]2[C@H](C([C@@H]([C@@H](N2)c3cccc(c3)C)[N+](=O)[O-])(C)C)[N+](=O)[O-]	$C_{21}H_{26}N_3O_4^+$	384.400

SMILES: Simplified Molecular Input Line Entry System

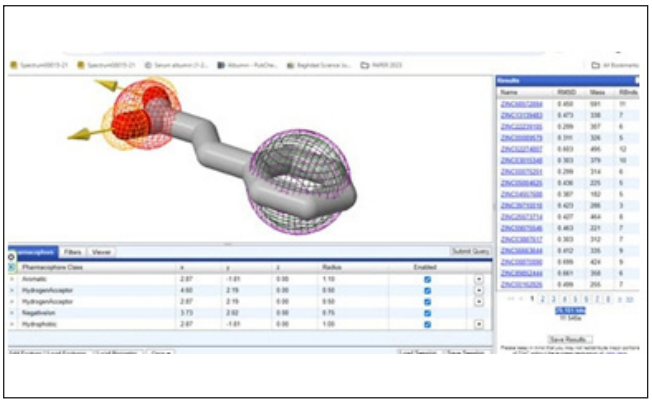

Figure 2. Pharmacophore Features in ZincPharmer[16]

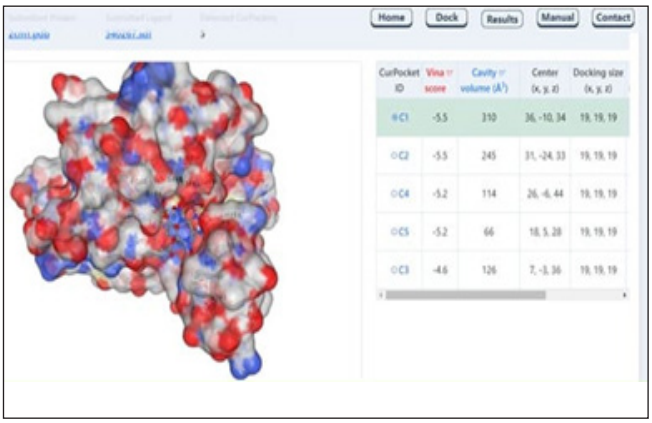

Figure 3.Vina Score of Docking Result for 540267 with Target Protein (Pocket 1)[18]

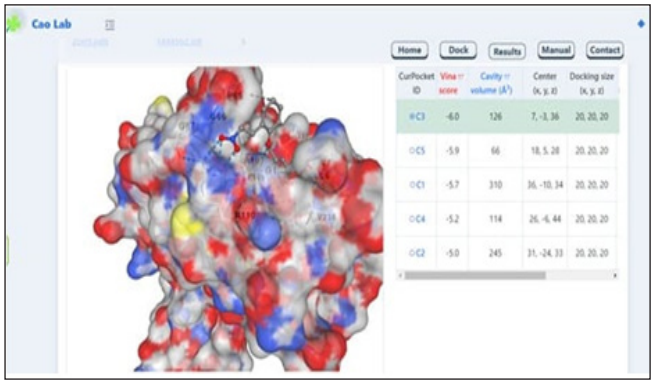

Figure 4.Vina Dock Score of Docking Result for 1494562 with Target Protein (Pocket 3)[18]

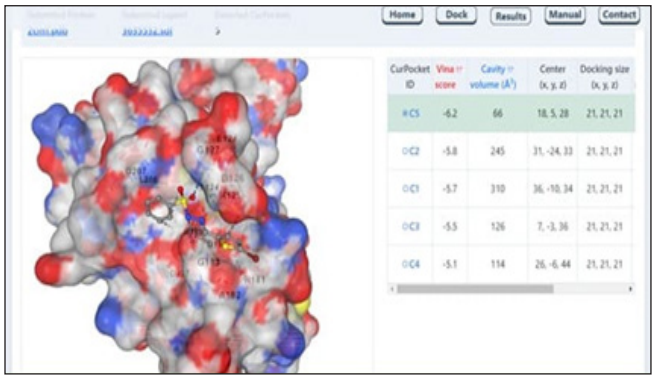

Figure 5.Vina Dock Score of Docking Result for 3635532 with Target Protein (Pocket 5)[18]

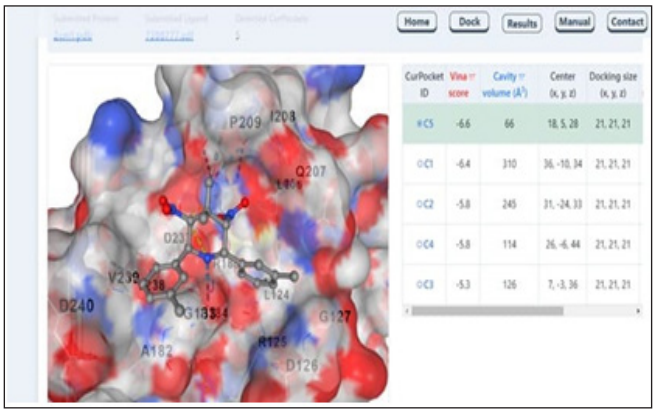

Figure 6.Vina Dock Score of Docking Result for 7388777 with Target Protein (Pocket 5)[18]

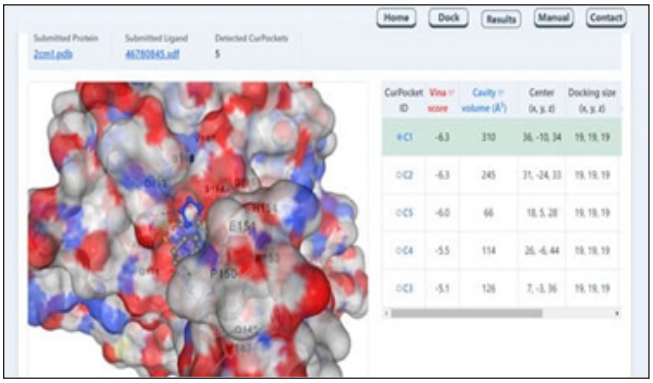

Figure 7.Vina Dock Score of Docking Result for 46780845 with Target Protein (Pocket 1)[18]

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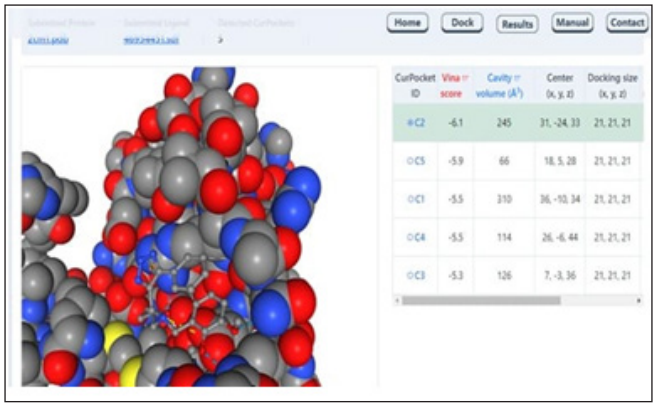

Figure 8.Vina Dock Score of Docking Result for 46954451 with Target Protein (Pocket 2)[18]

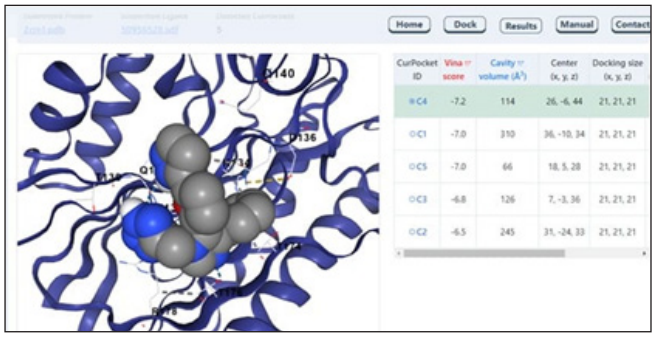

Figure 9.Vina Dock Score of Docking Result for 50956528 with Target Protein (Pocket 4)[18]

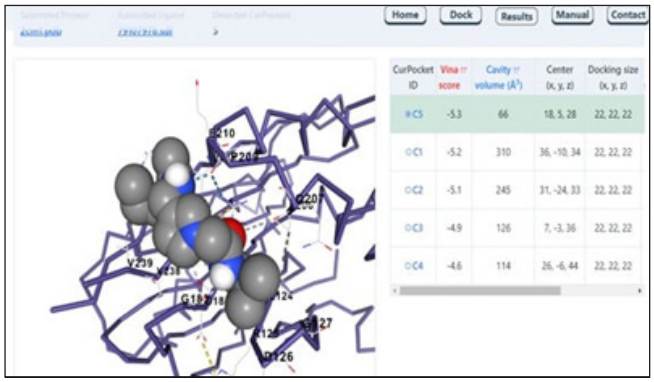

Figure 10.Vina Dock Score of Docking Result for 79107978 with Target Protein (Pocket 5) [18]

Table 4.Final Docking Scores for Selected Molecules as Inhibitors for PstP Protein[18]

PubChem CID	CurPocket ID	Vina Score
50956528	C4	-7.2
7388777	C5	-6.6
46780845	C1	-6.3
3635532	C5	-6.2
46954451	C2	-6.1
1494562	C3	-6.0
540267	C1	-5.5
79107978	C5	-5.3

Conclusion

Mycobacterium tuberculosis has multiple targets for new drug design procedures. This is due to the variation in the proteins. PstP protein has five pockets and was given a good docking score with eight safe inhibitors for further studies, and eight tested molecules with PubChem IDs 50956528, 7388777, 46780845, 3635532, 46954451, 1494562, 540267, 79107978, which have good features and can bind to PstP protein. Our data demonstrated that PstP is a promising a good target for new lead compounds against drug-resistant tuberculosis.

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Conflict of Interest: None

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