

Interactome analysis and design of inhibitors against selected protein targets of Ser/Thr protein kinase (STPK) signaling pathways in *Mycobacterium tuberculosis* H37Rv

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ABSTRACT. Tuberculosis continues to be a major cause of mortality worldwide despite significant advances in chemotherapy and development of the BCG vaccine. Although curable, the tuberculosis treatment period (6-9 months) presents many concerns, including patient noncompliance and the development of drug toxicity and drug resistance. This study aimed to understand the protein-protein interactions of key proteins involved in the *Mycobacterium tuberculosis* STPK signal transduction pathway (such as PknB, PknE, and PstP); in addition, we attempted to identify promising leads for the inhibition of protein-protein interactions. Interactome analyses revealed the interactions of these protein targets with several other proteins, including PknG and PbpA. Drug-like candidates were screened based on Lipinski's rule of five and the absorption digestion metabolism

excretion toxicity. Molecular docking of the target proteins with the selected ligands identified cryptolepine HCl to be a common molecule interacting with all protein targets (with a good docking score). The generation of a pharmacophore model for cryptolepine HCl revealed three pharmacophoric regions: aromatic hydrocarbon, hydrogen bond acceptor, and hydrogen bond donor, which play important roles in its interaction with the protein targets. Therefore, cryptolepine HCl appears to be a promising drug candidate for further optimization and validation against *M. tuberculosis*.

Key words: Ser/Thr protein kinase; Pharmacophore modeling; Docking; Interactome; Signal transduction

INTRODUCTION

Mycobacterium tuberculosis is the causative agent of tuberculosis (TB), which is the leading cause of death worldwide. Recent estimates have suggested that a third of the population worldwide asymptotically harbors a dormant or latent form of *M. tuberculosis*, with a lifelong risk of disease reactivation (Koul et al., 2011). Despite tuberculosis being curable, two developments regarding the recent TB resurgence are of great concern: co-infection with HIV-AIDS (Tufariello et al., 2003) and the emergence of multi-drug resistant strains of *M. tuberculosis* (Zvi et al., 2008). The latter problem can be attributed to the lengthy treatment period and patient noncompliance. Most of the currently available TB drugs are ineffective against persistent bacteria (Zhang, 2005); in addition, a comprehensive vaccine for TB also remains elusive (Gupta et al., 2012). Such problems warrant the need for new anti-TB drugs that could help to eliminate multidrug-resistant strains of *M. tuberculosis*.

Protein phosphorylation is the principal means by which environmental signals are converted to appropriate cellular responses in both prokaryotes and eukaryotes. The two-component systems (TCS), consisting of histidine sensor kinase/response regulator partner proteins play a key role in this process in bacteria, with phosphorylation occurring at the histidine/aspartic acid residues. In contrast, the Ser/Thr protein kinases (STPKs) and phosphoprotein phosphatases constitute the mainstay of signal transduction pathways in eukaryotes. Mycobacteria are characterized by the presence of both classes of signal transduction systems, that is, the TCS and eukaryotic-like STPK proteins (Tyagi and Sharma, 2004). The *M. tuberculosis* genome includes 11 STPK genes (*pknA* to *pknL*) (Cole et al., 1998), and their domain organization indicates that only two enzymes (PknG and PknK) are soluble proteins. All other mycobacterial STPKs are predicted to be transmembrane (receptor-like) proteins (Av-Gay and Everett, 2000), with their N-terminal kinase domain connected through a single transmembrane helix to one or more C-terminal domains, which presumably serve as signal sensors. The *M. tuberculosis* genome also includes three genes (*pstP*, *ptpA*, and *ptpB*) encoding eukaryotic-like protein phosphatases in addition to STPKs. PstP is a metalloenzyme that belongs to the PPM family of Ser/Thr protein phosphatases, which is known to dephosphorylate various mycobacterial STPKs and their substrates (Boitel et al., 2003; Chopra et al., 2003; Durán et al., 2005).

The *M. tuberculosis* serine/threonine protein kinases are promising drug targets because of their significant role in serine/threonine phosphorylation in *M. tuberculosis*. PknA and PknB are essential kinases regulating *M. tuberculosis* (Sassetti et al., 2003; Kang et al.,

2005; Fernandez et al., 2006), while PknG plays an important role in bacterial survival within the macrophage either via its role in glutamate metabolism (Cowley et al., 2004) or via the modulation of host cell trafficking pathways (Walburger et al., 2004).

Protein-protein interactions are important mechanisms that drive many physiological processes in the cell; these have also been implicated in the pathogenesis of diseases such as Alzheimer's disease, cervical cancer, bacterial infection, and prion diseases (Cohen and Prusiner, 1998; Selkoe, 1998; Loregian et al., 2002). Because of the wide diversity of protein-protein interactions, all possible interacting partners of a given protein must be thoroughly understood and identified. The establishment of protein interaction networks using a bioinformatics approach plays a crucial role in the drug discovery process, especially when protein-protein interactions are implicated in the development of diseases. Rational drug design has largely benefited from the introduction of the concept of 'pharmacophore', introduced in 1909 by Ehrlich (1909); since then, approaches utilizing pharmacophores have been widely used in virtual screening and drug design. A pharmacophore is a molecular framework that carries the essential features responsible for the biological activity of the drug (Ehrlich, 1909). A pharmacophore model can be ligand-based (extracting common chemical features from a set of bioactive ligands) or structure-based (studying the protein-ligand interactions).

The aim of this study is to understand the protein-protein interactions of key proteins such as PknB, PknE, and PstP, involved in the *M. tuberculosis* STPK signal transduction pathway and to identify the novel leads that can inhibit the protein-protein interactions. The target proteins were chosen based on their involvement in the phosphorylation and de-phosphorylation mechanisms required for signal transduction; therefore, targeting these proteins could help inhibit protein-protein interaction and inhibit the growth of *M. tuberculosis* within the cells.

MATERIAL AND METHODS

Retrieval of target protein sequences

The primary sequences of the target proteins protein kinase B (PknB), protein kinase E (PknE), and serine/threonine phosphatase (PstP) were retrieved from the UniProt database (www.uniprot.org).

Prediction of functional protein partners

Functional protein partners for the three protein targets PknB, PknE, and PstP were predicted using STRING 9.1 (Szklarczyk et al., 2011). STRING is a freely accessible database consisting of known and predicted protein-protein interactions, which quantitatively integrates the interaction data from several sources for a large number of organisms.

Determination of the essentiality of the selected target proteins and their functional partners

The basic local alignment search tool (BLAST) was used to determine the essentiality of the target proteins and their functional partners in the database of essential genes (DEG) (Zhang et al., 2004). Essential genes are indispensable for the survival of an organism and are therefore considered to form the foundation of life. DEG hosts the records of currently avail-

able essential genes among a wide range of organisms.

Determination of the homology for PknB, PknE, and PstP in humans using BLAST

The target proteins PknB, PknE, and PstP and their functional partners PknG and PbpA were subjected to a National Center for Biotechnology Information BLAST search against *Homo sapiens*, in order to identify the possible homology of these target proteins with humans.

Retrieval of the three-dimensional structures of the proteins and structural analysis

The three-dimensional structures of the target proteins were downloaded from protein data bank (PDB) (www.rcsb.org). The Procheck tool (<http://nihserver.mbi.ucla.edu/SAVES/>) was used to analyze the stereochemistry of the protein structures of the target proteins, via the analysis of residue-by-residue geometry.

Preparation of ligands

The three-dimensional structures of antimicrobial compounds were retrieved from the PubChem database (<https://pubchem.ncbi.nlm.nih.gov/>) in the 3D SDF format; those compounds whose structures were not available in the PubChem database were modeled using the ChemSketch tool.

Screening of ligands

The ligands were screened with the Molinspiration tool (<http://www.molinspiration.com/>) by applying the rule of five; the absorption digestion metabolism excretion (ADME) toxicity analysis of the filtered compounds was performed using the Osiris tool. Molinspiration (<http://www.organic-chemistry.org/prog/peo/>) predicts the bioactivity of the compounds based on the following criteria: a) mlogP (octanol-water partition co-efficient) ≤ 5 , b) topology polar surface area (TPSA) ≤ 140 , c) number of atoms (nATOMS) = 20-70, d) molecular weight ≤ 500 , e) electron acceptor (nON) ≤ 10 , f) electron donor (nOHN) ≤ 5 , g) number of violations (nviolation) = 0, h) number of rotatable bonds (nRotB) ≤ 10 . Osiris predicts the drug likeness and toxicity (including mutagenicity, irritancy, and reproductive effect), based on the following criterion: a) lipophilicity (cLogP) ≤ 5 , b) solubility > -4 , c) drug likeness ≥ 0 , d) drug score ≥ 0.5 ; green color indicates “no risk”, orange color indicates “medium risk”, and red color indicates “high risk”.

Prediction of active site residues

The binding site residues were predicted for two protein targets PbpA and PstP using CastP, while the binding sites for the other proteins (PknG, PknE, and PknB) were defined from the ligand already bound to the crystal structures.

Molecular docking

Molecular docking was performed using the GOLD software (v.5.2). All water mol-

ecules and ligands were extracted from the protein structures. The binding sites were defined for each protein. The binding sites of PknB, PknG, and PknE were defined at approximately 6Å from the bound inhibitor and predicted for PbpA and PstP using CastP. Twenty genetic algorithm (GA) runs were performed for each compound. The selection pressure for each GA run was set to 1.1; one hundred thousand GA operations were performed on a set of five islands, with a population size of 100 individuals. The operator weights for crossovers, mutations, and migrations were set to the default values. The protein-ligand interactions were further studied using the LigPlot tool (<http://www.ebi.ac.uk/pdbsum/>). The LIGPLOT program automatically generates schematic 2-D representations of protein-ligand complexes from a standard protein data bank file input.

Pharmacophore modeling

A pharmacophore model defines the minimum necessary structural characteristics that a molecule must possess in order to bind to the target. The best twelve docked ligands were used to build a pharmacophore model using the V-life MDS software v3. The ligands were loaded, and ligand 10 was selected as the reference ligand, as it was observed to superimpose well with all 11 ligands.

RESULTS AND DISCUSSION

The interactome analyses predicted the interaction of the three protein targets (PknB, PknE, and PstP) with several other proteins (Figure 1), with high confidence scores (Table 1). The common predicted functional partner proteins were PknG and PbpA. The selected protein targets and their predicted functional partners were predicted to be important for the survival of the organism, based on the percentage of identity of these proteins against those of the prokaryotic organisms of the DEG database (Table 2). The selected protein targets showed no significant homology with their human counterparts (Table 3), indicating that the inhibitors designed for the targets would show less cross-reactivity *in vivo*, possibly resulting in lesser side-effects. Binding site residues were predicted for two protein targets PbpA and PstP (Figure 2). The binding sites for the other targets were retrieved from the ligands bound to the crystal structures. The three dimensional structures of the target proteins were considered to be stereochemically good as a majority of the residues existed within the most favored regions (Figure 3, Table 4), which signified a lower number of steric clashes between the backbone and the side chains in the protein structures. Twenty-five of the 253 antimicrobial compounds (142 natural and 111 synthetic compounds) were selected for molecular docking studies based on the drug-likeness and ADME properties (Tables 5-7).

Molecular docking of the target proteins with the 25 ligands suggested that the ligand 7 to be the common ligand interacting with all protein targets (PknB, PknE, PstP, PknG, and PbpA) with good docking scores (45.1423, 40.7455, 49.6259, 54.6775, and 63.8415, respectively; Table 8). Ligand 7 mostly formed hydrophobic interactions with PknB, and hydrogen bonds and hydrophobic interactions with the remaining protein targets (Figure 4). A pharmacophoric analysis of Ligand 7 (Figures 5-6) revealed that the nitrogen (N) atom of the pyrrole ring acted as pharmacophoric atom, where the ligand interacted with the target proteins. In addition, three pharmacophoric regions, aromatic hydrocarbon (AroC), hydrogen bond acceptor (HAc), and hydrogen bond donor (HDr), were also discovered in this ligand.

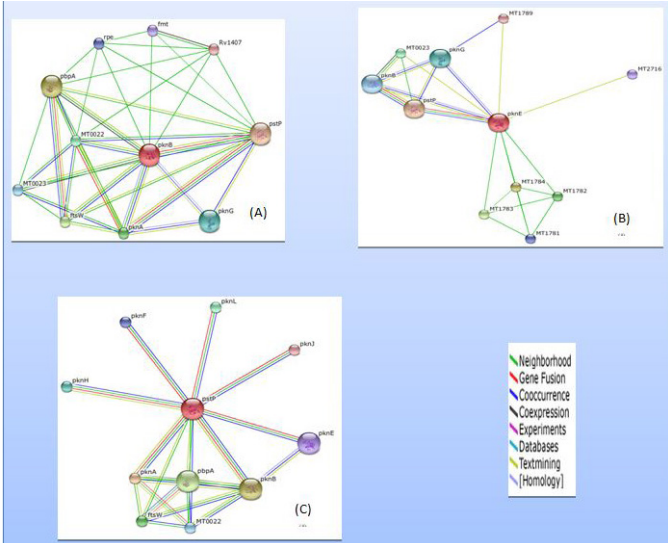


Figure 1. Protein network view for the target proteins-(A) PknB (B) PknE (C) PstP. The central red spheres indicate target proteins interconnected by other spheres which represent predicted functional partners. On the bottom right panel shows color codes for the active String prediction methods.

Table 1. Predicted functional partners of target proteins (PknB, PknE, and PstP) and their string score.			
S. No.	Protein target	Interacting partners	String score
1.	PknB	PstP	0.998
		PbpA	0.996
		Ftsw	0.982
		MT0023	0.978
		Rv1827	0.972
		MT0022	0.946
		GlmU	0.908
		PknA	0.908
		MT0537	0.900
		EmbR	0.864
2.	PknE	PstP	0.972
		MT0537	0.926
		MT1789	0.843
		MT0023	0.843
		Rv1365c	0.840
		EmbR	0.839
		PknG	0.759
		PbpA	0.724
		PknK	0.614
		Ag84	0.607
3.	PstP	PknB	0.998
		PknA	0.997
		PbpA	0.997
		Ftsw	0.983
		PknL	0.972
		PknE	0.972
		PknF	0.972
		PknH	0.970
		PknG	0.940
		Mt0022	0.936

The highlighted rows indicate the common interacting partners PstP and PbpA.

Table 2. Blast results from Database of Essential Genes (DEG).

Proteins	DEG ID	Organism	Identity
PknG	DEG10100048	<i>Mycobacterium tuberculosis</i> H37Rv	96%
PknB	DEG10100003	<i>Mycobacterium tuberculosis</i> H37Rv	92%
PknE	DEG10100003	<i>Mycobacterium tuberculosis</i> H37Rv	37%
PstP	DEG10070095	<i>Streptococcus pneumoniae</i> TIGR4	33%
PbpA	DEG10050014	<i>Haemophilus influenzae</i> Rd KW20	25%

Table 3. Blast results (*Mycobacterium tuberculosis* protein against Human taxid: 9606).

Proteins	BLAST results						
	Accession	Description	Max. score	Total score	Query coverage	E value	Max. identity
PknB	EAX08908	NIMA (never in mitosis gene a)-related kinase 3, isoform CRA_d	117	117	40%	7E-28	32%
PknE	ADX95745	SadB kinase short isoform	118	118	38%	3E-28	31%
PstP	Q86UP3	Full=Zinc finger homeobox protein 4	35.8	35.8	11%	0.55	29%
PknG	3BQR_A	Chain A, Crystal Structure Of Human Death Associated Protein Kinase3 (Dapk3)	82	82	27%	4E-16	30%

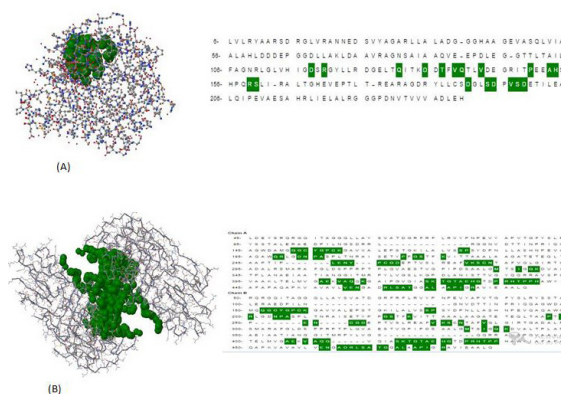
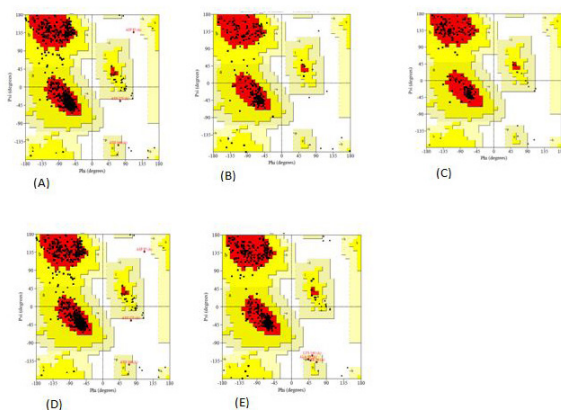
**Figure 2.** CASTp result showing active site residues of the protein PstP (A) and PbpA (B) in green.**Figure 3.** Ramachandran plot for target proteins PknB (A), PknE (B), PstP (C), PknG (D), and PbpA (E).

Table 4. Summary of Ramachandran plot statistics.

Proteins	PDB entry	Residues in most favored regions (%)	Residues in additional allowed regions (%)	Residues in generously allowed regions (%)	Residues in disallowed regions (%)	No. of non-glycine & non-proline residues (%)
PknB	1o6y	92.0	7.6	0.0	0.4	100
PknE	2h34	86.7	13.3	0.0	0.0	100
PstP	1txo	96.0	4.0	0.0	0.0	100
PknG	2pzi	91.9	7.6	0.2	0.4	100
PbpA	3lo7	91.2	7.9	0.9	0.0	100

Table 5. Physico-chemical properties of selected compound with Molinspiration.

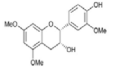
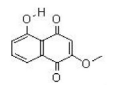
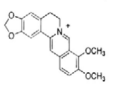
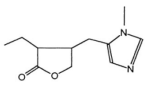
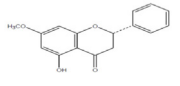
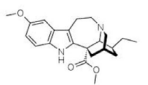
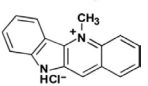
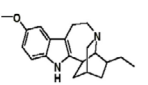
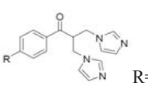
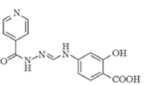
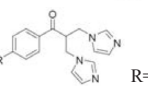
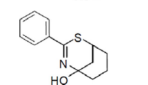
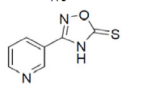
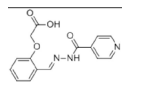
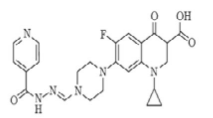
Compounds	CID	miLogP	TPSA	natoms	MW	nON	nONNH	nviolations	nrotb	vol
1	-	2.28	77.392	24	332.352	6	2	0	4	296.725
2	-	1.385	63.604	15	204.181	4	1	0	1	172.149
3	2353	0.196	40.821	25	336.367	5	0	0	2	296.302
4	5910	-0.236	44.131	15	208.261	4	0	0	3	199.563
5	73201	3.132	55.767	20	270.284	4	1	0	2	239.772
6	73255	4.203	54.568	27	368.477	5	1	0	4	347.713
7	156093	3.857	17.826	18	232.286	2	0	0	0	213.791
8	363272	4.27	28.263	23	310.441	3	1	0	2	303.508
9	-	2.989	52.723	27	356.429	5	0	0	7	330.106
10	-	1.06	123.908	22	300.274	8	4	0	5	253.363
11	-	1.643	52.723	22	294.358	5	0	0	6	275.258
12	-	2.796	32.592	16	233.336	2	1	0	1	212.305
13	-	0.526	54.715	12	179.204	4	1	0	1	141.554
14	5391612	1.064	100.887	22	299.286	7	2	0	6	258.73
15	-	0.838	119.303	34	466.473	10	1	0	5	397.394
16	-	4.05	63.083	21	282.343	4	1	0	4	267.744
17	-	1.178	29.853	15	201.273	3	1	0	5	199.471
18	-	-0.98	109.236	20	270.252	8	2	0	4	229.015
19	4472	0.377	83.115	22	298.346	6	3	0	7	276.875
20	-	1.598	77.373	16	261.332	7	1	0	2	201.513
21	-	-1.042	108.371	21	284.279	8	3	0	5	245.574
22	-	2.819	29.098	15	217.293	2	1	0	3	194.352
23	4908	2.104	60.176	19	259.353	4	3	0	6	256.91
24	5340	0.834	85.084	16	255.324	5	3	0	3	197.13
25	65720	-0.564	101.392	22	302.29	8	2	0	4	256.881

miLogP, octanol-water partition coefficient; TPSA, topology polar surface area; nATOMS, number of atoms; MW, molecular weight; nON, electron acceptor; nONNH, electron donor; nviolations, number of violations; nrotb, number of rotatable bonds; vol, volume.

Table 6. Toxicity risk of selected compounds as predicted by Osiris. No risk of mutagenic, tumorigenic, irritant and reproductive effectiveness were found in all the cases analyzed.

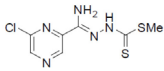
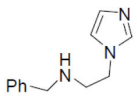
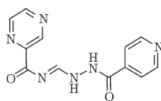
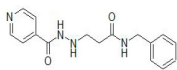
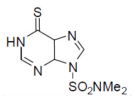
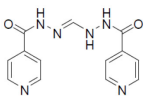
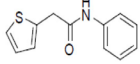
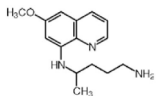
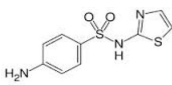
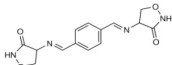
Compounds	CID	cLogP	Solubility	Molecular weight	Drug likeness	Drug-Score
1		2.46	-2.71	332	1.7	0.8
2		1.57	-2.6	204	0.95	0.8
3	2353	2.05	-2.88	339	2.95	0.84
4	5910	0.4	-1.2	208	1.17	0.85
5	73201	3	-3.25	270	2.05	0.8
6	73255	2.97	-3.86	368	1.8	0.7
7	156093	2.64	-3.71	232	3.75	0.83
8	363272	4.18	-3.97	310	3.69	0.69
9		2.06	-3.05	356	2.45	0.81
10		1.09	-2.83	300	1.5	0.82
11		0.69	-1.3	294	2.6	0.91
12		3.43	-3.95	233	0.91	0.67
13		1.96	-2.11	179	1.93	0.88
14	5391612	1.2	-2.72	299	6.87	0.9
15		-0.16	-3.16	466	3.88	0.74
16		0.91	-2.41	261	0.95	0.8
17		0.61	-0.91	201	1.94	0.91
18		-1.16	-1.46	270	3.24	0.94
19	4472	0.47	-2.42	298	4.3	0.91
20		-2.07	-1.65	261	2.19	0.91
21		-0.12	-2.5	284	4.78	0.92
22		2.71	-3.1	217	0.28	0.69
23	4908	2.56	-3.1	273	3.34	0.85
24	5340	1.13	-2.76	255	4.51	0.91
25	65720	-1.12	-2.93	302	2.5	0.87

Table 7. Final twenty five ligands both Synthetic(S) and Natural(N) compounds selected for molecular docking.

S. No.	Compounds	Type	Source	Chemical structures	Reference
1	(2R,3R)-2-(4-hydroxy-3-methoxyphenyl)-5,7-dimethoxy-3,4-dihydro-2H-chromen-3-ol Flavanol	N	-		Chen et al. (2005)
2	3-methoxyjuglone	N	<i>Engelharda roxburghiana</i>		Lin et al. (2005)
3	Berberine	N	<i>Hydrastis canadensis</i>		Gentry et al. (1998)
4	Pilocarpine	N	<i>Pilocarpus racemosa</i>		Rastogi et al. (1998)
5	Pinostrobin	N	<i>Teloxys graveolens</i>		Camacho-Corona et al. (2009)
6	Voacangine	N	<i>Tabernaemontana citrifolia</i>		Rastogi et al. (1998)
7	CryptolepineHCl	N	ATCC6841		Gibbons et al. (2003)
8	Ibogaine	N	<i>Tabernaemontana citrifolia</i>		Rastogi et al. (1998)
9	1-(biphenyl-4-yl)-3-(1H-imidazol-1-yl)-2-(1H-imidazol-1-ylmethyl)propan-1-one	S			Chauhan et al. (2010)
10	2-hydroxy-4-((E)-[2-(pyridin-4-ylcarbonyl)hydrazinylidene]methyl}amino)benzoic acid	S			Rogoza et al. (2010)
11	3-(1H-imidazol-1-yl)-2-(1H-imidazol-1-ylmethyl)-1-(4-methylphenyl)propan-1-one	S			Chauhan et al. (2010)
12	3-phenyl-2-thia-4-azabicyclo[3.3.1]non-3-en-5-ol	S			Chauhan et al. (2010)
13	3-(pyridin-3-yl)-1,2,4-oxadiazole-5(4H)-thione	S			Chauhan et al. (2010)
14	Aconiazide	S			Lougheed et al. (2009)
15	(1-cyclopropyl-6-fluoro-1,2,3,4-tetrahydro-4-oxo-7-(4-((E)-(pyrazine-2-carboxoylimino)methyl)piperazin-1-yl)quinoline-3-carboxylic acid)	S			Rogoza et al. (2010)

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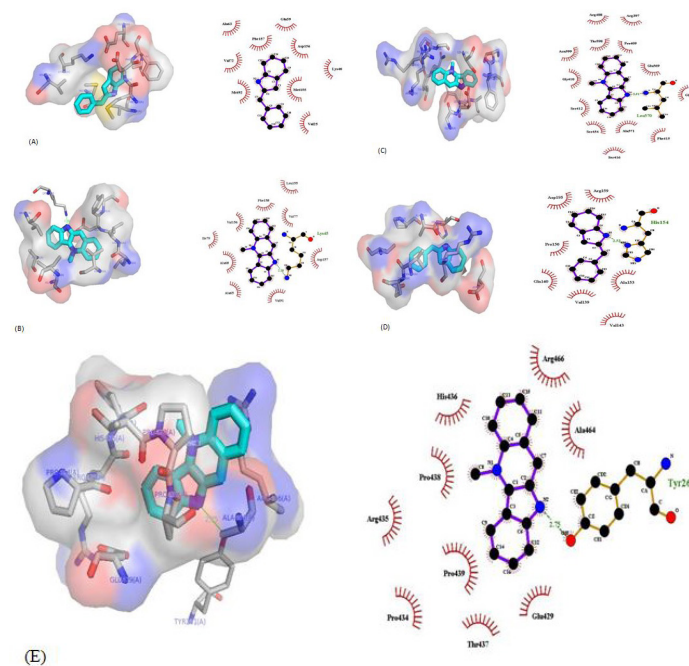
Table 7. Continued.

S. No.	Compounds	Type	Source	Chemical structures	Reference
16	methyl (2Z)-2-[amino (6-chloropyrazin-2-yl) methylidene] hydrazinecarbodithioate	S			Chauhan et al. (2010)
17	N-benzyl-2-(1H-imidazol-1-yl)ethanamine	S			Chauhan et al. (2010)
18	N-[(E)-[2-(pyridin-4-ylcarbonyl)hydrazinyl] methylidene]pyrazine-2-carboxamide	S			Rogoza et al. (2010)
19	Nialamide	S			Lougheed et al. (2009)
20	N,N-dimethyl-6-thioxo-1,4,5,6-tetrahydro-9H-purine-9-sulfonamide	S			Chauhan et al. (2010)
21	N',N''-(E)-methylidenedipyridine-4-carbohydrazide	S			Chauhan et al. (2010)
22	N-phenyl-2-(thiophen-2-yl)acetamide	S			Chauhan et al. (2010)
23	Primaquine	S			Lougheed et al. (2009)
24	Sulfathiazole	S			Lougheed et al. (2009)
25	Terizidone	S			Ginsberg (2008)

Cryptolepine HCl (Ligand 7) is an important lead obtained via virtual screening, molecular docking, and pharmacophore analysis. Cryptolepine HCl is an indoloquinoline alkaloid obtained from the West African medicinal plant *Cryptolepis sanguinolenta* (Gibbons et al., 2003). The pyrrole ring of this compound can be a scaffold for new derivatives, as deduced from the pharmacophore analysis. Therefore, it could be a promising drug candidate; cryptolepine HCl must be further optimized to increase its specificity against the target proteins and must be further validated against *M. tuberculosis* by wet laboratory analysis.

Table 8. Molecular docking result of selected ligands against target proteins.

Ligands	PknB		PknE		PstP		PknG		PbpA	
	Gold Score	No. of H bonds	Gold Score	No. of H bonds	Gold Score	No. of H bonds	Gold Score	No. of H bonds	Gold Score	No. of H bonds
1	34.0163	3	33.6761	2	42.433	3	49.1906	3	47.1717	0
2	24.6372	3	27.2217	3	32.2145	4	35.837	5	42.8637	3
3	38.774	0	37.9058	0	47.8694	0	52.586	1	50.7661	0
4	30.1701	2	24.9583	2	35.5759	6	36.1021	2	40.7669	2
5	32.001	1	31.1421	4	41.0338	1	47.1787	0	48.3621	2
6	30.3857	0	31.1225	1	41.5973	2	42.1372	0	41.6364	1
7	45.1423	0	40.7455	1	49.6259	1	54.6775	1	63.8415	1
8	31.4683	1	37.1498	0	45.3188	2	45.7499	0	44.3443	0
9	36.0543	2	36.2102	0	55.653	2	55.815	1	55.6936	1
10	33.9736	2	38.7658	1	43.5762	4	48.9865	1	51.8879	4
11	33.538	2	35.0406	1	47.9865	0	47.3505	0	50.5594	2
12	28.7978	3	29.7057	0	38.8607	1	41.8713	1	43.0599	1
13	29.1463	3	28.5153	3	31.6979	1	35.7697	1	39.9714	2
14	37.9305	6	34.4247	1	44.7016	5	51.5384	2	52.0856	0
15	39.7701	2	35.3683	2	47.8084	1	53.6807	0	57.3783	0
16	29.641	1	27.3238	1	38.0435	2	43.1819	1	45.3366	1
17	33.3467	1	31.4097	0	40.0934	1	42.1258	0	48.5045	1
18	31.1304	2	33.2719	2	42.1134	3	47.1551	0	48.1866	2
19	36.8033	3	39.4367	1	48.6808	2	53.96	0	52.9506	1
20	26.2689	3	27.1396	1	32.8871	4	37.9968	0	41.0372	1
21	32.1609	3	35.226	0	44.7341	3	49.1733	1	50.0253	2
22	32.4412	1	33.1535	0	41.9012	2	42.3789	0	49.717	0
23	32.0395	0	35.3142	1	42.7173	2	49.5323	1	46.918	1
24	30.2808	0	29.5629	2	41.3743	2	42.2825	2	51.4701	0
25	38.408	6	30.8581	1	40.8181	4	46.4917	2	49.6914	2

**Figure 4.** Ligand 7 in the binding site pocket of target proteins (A-E) where the ligand is shown in cyan and 2-D representation of the interaction of Ligand7 with target proteins PknB (A), PknE (B), PstP (C), PknG (D), and PbpA (E).

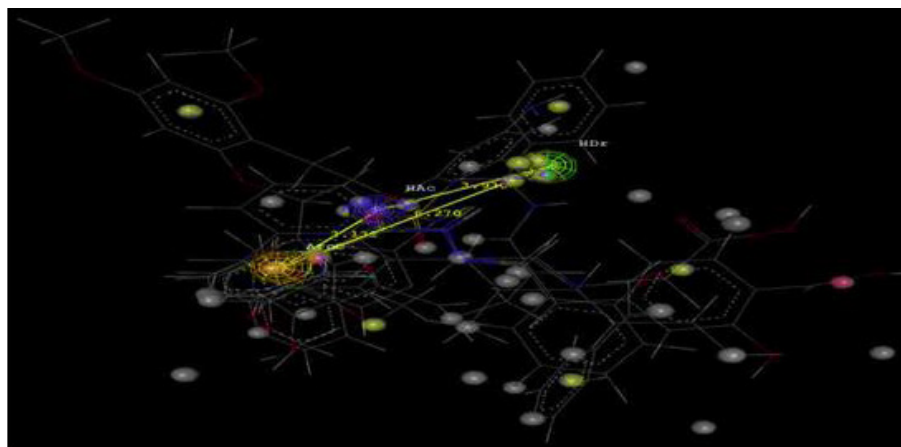


Figure 5. Superimposition of the best docked 12 ligands using ligand 10 as a reference.

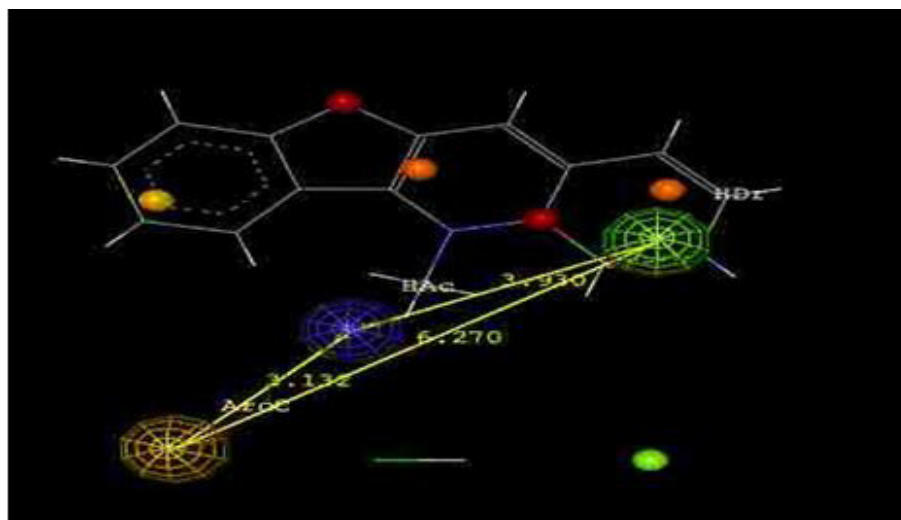


Figure 6. Pharmacophore model generated for ligand 7 (Cryptolepine HCl): stick model representation of the ligand 7. Pharmacophoric regions are indicated by green (Hydrogen bond donor), blue (Hydrogen bond acceptor) and yellow spheres (Aromatic carbon) connected by lines representing distance constraints in the pharmacophore model. Small spheres in orange, yellow, and green indicate additional pharmacophore regions.

Conflicts of interest

The authors declare no conflict of interest.

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