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Identification of Potential Inhibitors Against *Mycobacteriaum tuberculosis* Protein Targets: An *In-Silico* Approach

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Abstract

Mycobacterium tuberculosis (*Mtb*) is gradually gaining resistance to anti-tuberculosis drugs, which have imposed novel drug targets on *Mtb* for further discovery. Seven proteins involved in the cellular processes of *Mtb* with their respective PDB IDs, namely rpoC (5UH5), PstS3 (4LVQ), ICDH-1 (4HCX), cpn10 (1P3H), PstS1 (1PC3), PknD (1RWI) and devR/dosR (3C3W), were obtained from the RCSB Protein Data Bank (PDB) and thoroughly analyzed. Literature curated 80 compounds were virtually screened using various bioinformatics tools such as Molinspiration, Osiris Property Explorer, ProTox-II. Molecular docking interactions of *Mtb*-Serine/threonine-protein kinase PknD with Alliin ligand shows better binding energy (-6.07 kcal/mol) and the specific key residues such as SER27, GLY28, VAL29, GLN70, GLY71, LEU72, GLU111, GLY112, ASP153, GLY154, GLY196, ILE197, and ALA238 play a vital role in ligand interaction using hydrogen bonds, van der Waals and hydrophobic interaction. Therefore, the finding shows alliin is the ideal phyto-compound that could be used for the optimized synonymous compound design for safe and efficient tuberculosis treatment specific to the PknD protein target.

Keywords: Drug targets, *Mycobacterium tuberculosis*, Lipinski's rule, Molecular docking, Serine/threonine-protein kinase PknD

Introduction

Tuberculosis (TB) is one of the most common infectious diseases known to exist. It is known as one of the key causes of mortality, as almost two million people die from this disease each year. The World Health Organization (WHO) reports about 10 million people to get the disease every year, out of which 3 million of them "missed" their health problems (https://www.cdc.gov/) [1]. If we equate TB to numerous other diseases caused by a single microorganism, tuberculosis is the world's second-most killer. In 2019, around 1.4 million people died because of TB, where 10 million got infected [2].

Mycobacterium tuberculosis, is the key causative agent for TB. It is a thin, aerobic, gram-positive, non-motile bacillus. This pathogen's high lipid content primarily accounts for a significant number of its specific clinical features [3]. They were found to contain a high content of lipid and mycolic acid in their cell walls [4]. *Mtb* can resist fragile disinfectants and can live for quite a long time in a dry state. The *Mycobacterium tuberculosis* complex (MTBC) refers to a somewhat similar genetic group of species (*M. tuberculosis*, *M. canettii*, *M. africanum, M. microti*, *M. bovis*, *M. caprae*, and *M. pinnipedii*) [5]. Severe virulence factors including but not limited to bacterial genes/proteins have evolved in MTBC members as a response to the host immune reaction. From these, *Mtb* is the most

well-known infectious disease that causes disease in more than 33% human population in the world. Based on their role and molecular features or cellular localization, the virulence determinants of *Mtb* have been classified into many categories. Most of them are involved in lipid and fatty acid metabolism, cell envelope development, apoptosis, regulation of transcription, phagosome arrest, reactions to oxidative and nitrosative stress, inhibition of antimicrobial effectors of macrophages etc. [6]. Seven pathogenic proteins (rpoC (DNA-directed RNA polymerase subunit beta'), PstS3 (Phosphate-binding protein PstS3), ICDH-1 (Isocitrate dehydrogenase [NADP]), cpn10 (10 kDa chaperonin), PstS1 (Phosphate-binding protein PstS1), PknD (Serine/threonine-protein kinase PknD), and devR/dosR (DNA-binding transcriptional activator)) have been identified as a key players in the cellular process of *Mtb*. Each of these pathogenic proteins are involved in various functional roles including but not limited to catalysis, which induces phagosome maturation arrest, ATPase inhibition, membrane transportation, phosphorylation, and signal transduction. A potentially active compound for TB inhibition was discovered by virtual screening and molecular docking studies. We have established a TB target that is aligned with our lead compound. This present study on *Allium sativum* derived active compounds that contribute to anti-TB drug targets recognition and thus describes the mechanism of action.

Materials and Methods

Target preparation: Seven *Mycobacterium tuberculosis* protein PDB structures involved in the *Mtb* cell process with the following PDB IDs: rpoC (5UH5), PstS3 (4LVQ), ICDH-1 (4HCX), cpn10 (1P3H), PstS1 (1PC3), PknD (1RWI) and devR/dosR (3C3W) have been obtained from the RCSB (Research Collaboratory for Structural Bioinformatics) PDB (Protein Data Bank) website (https://www.rcsb.org/). For further studies, the cleaned and energy-minimized protein structures were used [7].

Ligand preparation: A set of eighty different phytocompounds, that are effective against *Mtb* proteins were selected from the literature. The selected phytocompounds' two-dimensional structure was retrieved from Pubchem database (https://pubchem. ncbi.nlm.nih.gov/). Physicochemical properties for the phytocompounds were calculated using the molinspiration web server (http://www. molinspiration.com). Properties like partition coefficient, polar surface area, molecular weight, amount of hydrogen bond donor & acceptor and toxicity properties [8,9] were assessed ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) properties of phytochemicals can be predicted by using the Osiris Property Explorer (http://www.organic-chemistry.org/prog/peo/) and the ProTox-II server [10]. Tumorigenic, mutagenic, irritant, reproductive effects and drug-related properties as well as the extent of oral toxicity of phytocompounds are the properties that were further assessed. Using the Open Babel tool, SDF-format (Structure Data File) of the chosen phytocompounds were converted into PDB-formats [11]Chemical Markup Language. These compounds were further optimized using CORINA Classic.

Active site selection: The target region for the inhibition of tuberculosis is the protein's active site residues. CASTP (Computed Atlas of Surface Topography of proteins) server is used to predict the protein's active site [12]. The protein file without processed heteroatoms was uploaded and the most appropriate pocket/region was selected for docking.

Molecular docking: Using AutoDock software, the energy-minimized structure of Alliin and Galacturonic acid ligands were docked with chosen protein targets. The protein active site residues were enclosed within a grid box with a grid spacing of 0.375 Å for docking, maintaining the protein rigid and the ligand as a flexible molecule. To look for the best conformers, the Lamarckian Genetic Algorithm (LGA) was chosen. A maximum of 10 conformers were considered for each ligand during the docking [13]. Docking

runs were performed from the command prompt after specifying the binding site and protein-ligand preparation. For the entire binding site, the interaction energy between the ligand and the receptor was measured and expressed as energy (kcal/mol). Using PYMOL, the interactions of protein-ligand complex representations were analyzed.

Eighty chemical compounds belonging to different families of medicinal plants were chosen for this study based on their common use for the treatment of tuberculosis. These inhibitors have been identified in previous studies based on tuberculosis protein targets (Table 1).

Results and Discussion

Table 1: Phytocompounds identified in the medicinal plant from previous studies

S. No	Compounds	Plant	Plant family	Part	Ref
1	1'-acetoxychavicol acetate	*Alpinia galanga*	Zingiberaceae	rhizome	[14]
2	2-Demethylcolchicine	*Gloriosa superba*	Colchicaceae	-	[15]
3	2-hydroxy-4-methoxy-benzaldehyde	*Periplocasepium*	Apocynaceae	Root bark	[16]
4	3,3'-Biplumbagin	*Ceratostigma plumbaginoides*	Plumbaginaceae	Leaf	[17]
5	3,7,11,15–Tetramethyl–2–hexadecen –1–ol (Terpene alcohol)	*Solanum xanthocarpum*	Solanaceae	Leaves	[18]
6	3-Desmethylcolchicine	*Gloriosa superba*	Colchicaceae	-	[15,19]
7	Abruquinone B	*Abrus precatorius*	leguminosae	Aerial	[20]
8	Ajoene	*Allium sativum*	Amaryllidaceae	Bulbs	[21,22]
9	Allicin	*Allium sativum*	Amaryllidaceae	Bulbs	[21,22]
10	Alliin	*Allium sativum*	Amaryllidaceae	Bulbs	[21,22]
11	Alliodorin	*Cordia alliodora*	Boraginaceae	Leaf, root	[23]
12	Allyl sulfide	*Allium sativum*	Amaryllidaceae	Bulbs	[21]
13	alpha mangostin	*Garcinia mangostana*	Clusiaceae	Fruit	[24]
14	alpha sapinine	*Sapium indicum*	Euphorbiaceae	Fruit	[25]
15	Ambroxol	*Adhatodavasica*	Acanthaceae	Leaves, Roots	[26,27]
16	Andrographolide	*Andrographis paniculata*	Acanthaceae	Leaves	[28]
17	Ascorbic acid	*Ampelocissus latifolia*	Vitaceae	Aerial	[29]

18	Bakuchiol	*Psoralea corylifolia*	Fabaceae	Seeds, roots, and leaves	[30]
19	Bauhinoxepin J	*Bauhinia purpurea*	Leguminosae	Roots	[31]
20	Benzoic acid	*Pinus ponderosa*	Pinaceae	-	[32]
21	beta sitosterol	*Adhatodavasica*	Acanthaceae	Leaves	[26]
22	Bidebiline E	*Polyalthiacerasoides*	Annonaceae	Roots	[33]
23	Bisbenzylisoquinoline	*Stephania epigaea*	Menisperma-ceae	Tubers	[34]
24	Bromhexine	*Adhatodavasica*	Acanthaceae	Leaves, Roots	[27]
25	Butein	*Rhus verniciflua*	Anacardiaceae	Bark	[35]
26	Butin	*Butea monosperma*	Fabaceae	Flower	[36]
27	Choline	*Abrus precatorius*	Fabaceae	Leaves, roots, and seeds	[37]
28	Chrysophanic acid	*Rheum rhabarbarum*	Polygonaceae	Roots and rhizomes	[38]
29	Colchicine	*Gloriosa superba*	Colchicaceae	seeds	[39]
30	Cordiachrome B	*Cordia fragrantissima*	Boraginaceae	Timber	[40]
31	Cordiachrome C	*Cordia fragrantis-sima*	Boraginaceae	Timber	[40]
32	Cordiachromene	*Cordia globifera*	Boraginaceae	Roots	[23]
33	Cycloalliin	*Allium Cepa*	Amaryllidaceae	Bulbs	[41]
34	Cycloartenol	*Morindacitrifolia*	Rubiaceae	Leaves	[42]
35	Diallyl Disulfide	*Allium sativum*	Amaryllidaceae	Bulbs	[21,22]
36	Diallyl Trisulfide	*Allium sativum*	Amaryllidaceae	Bulbs	[21,22]
37	Diospyrin	*Diospyros glandulo-sa Lace*	Ebenaceae	-	[43]
38	Diterpenoids	*Andrographis panic-ulata*	Acanthaceae	Whole plant, leaf and stem	[44]
39	Elaeagin	*Cordia globifera*	Boraginaceae	Roots	[45]
40	Eugenin	*Pisonia aculeata*	Nyctaginaceae	Leaves	[46]
41	Ferruginol	*Salvia sclarea*	Lamiaceae	-	[47]
42	Galacturonic acid	*Cordia myxa*	Boraginaceae	Fruit	[23]
43	Gingerol	*Zingiber officinale*	Zingiberaceae	Rhizome	[43]
44	Glabridin	*Glycyrrhiza glabra*	Papilionaceae/ Fabaceae	Roots	[48]
45	Globiferin	*Cordia globifera*	Boraginaceae	Root	[45]
46	Grayanin	*Prunus grayana*	Rosaceae	bark	[49]

47	Greveichromenol	*Harrisoniaperforata*	Simarouba-ceae	leaves, wood and root-bark	[50]
48	Ibogaine	*Voacangaafricana*	Apocynaceae	Leaves, root	[51]
49	Isoambreinolide	*Vitex trifolia*	Verbenaceae	Leaves	[52]
50	Isoliquiritigenin	*Glycyrrhiza uralensis*	Fabaceae	roots	[53]
51	kaurane	*Croton zambezicus*	Euphorbiaceae	leaves	[54]
52	Lansine	*Micromelumhirsutum*	Rutaceae	Stem Bark	[55]
53	Licochalcone A	*Glycyrrhiza glabra*	Fabaceae	root	[56]
54	liquiritigenin	*Glycyrrhiza uralensis*	Fabaceae	Leaves	[57]
55	Maackiain	*Derris indica*	Leguminosae	Root & stem	[58]
56	Maritinone	*Diospyros anisandra*	Ebenaceae	-	[59]
57	Methylallyl trisulfide	*Allium sativum*	Amaryllidaceae	Bulbs	[60]
58	Micromeline	*Micromelumhirsutum*	Rutaceae	Stem Bark	[55]
59	n–Hexadecanoic acid (Palmitic acid)	*Costusspeciosus*	Costaceae	stem-flower	[61]
60	Obtusifoliol	*Struthanthusmarginatus*	Loranthaceae	Aerial	[62]
61	Oleanolic acid	*Duroia macrophylla*	Rubiaceae	leaves	[63]
62	Perforamone B	*Harrisoniaperforata*	Simarouba-ceae	leaves, wood and root-bark	[50]
63	Perforamone D	*Harrisoniaperforata*	Simarouba-ceae	leaves, wood and root-bark	[50]
64	Phytol (Diterpene)	*Croton zambezicus*	Euphorbiaceae	leaves	[54]
65	Piperine	*Piper nigrum, Piper longum*	Piperaceae	pepper seeds	[64]
66	Plumbagin	*Plumbago indica*	Plumbagina-ceae	Root	[65]
67	Plumericin	*Plumeria bicolor*	Apocynaceae	Bark	[66]
68	Potamogetonin	*Potamogetonmalaianus*	Potamogetona-ceae	Whole plant	[67]

69	Rumexneposide A	*Rumex nepalensis*	Polygonaceae	root	[68]
70	Salicylic acid	*Cocos nucifera*	Arecaceae	-	[69]
71	S-allylcysteine	*Allium sativum*	Amaryllidaceae	Bulbs	[21]
72	Sandracopimaric acid	*Juniperus excelsa*	Cupressaceae	Fruit	[70]
73	Sclareol	*Salvia sclarea*	Lamiaceae	aerial	[71]
74	Scorodonin	*Marasmiusscorodonius*	Omphalotaceae	-	[72]
75	Sitosterol	*Etlingera elatior*	Zingiberaceae	Rhizomes	[43]
76	Squalene	*Andrographis serpyllifolia*	Acanthaceae	leaves	[73]
77	Stigmasterol	*Croton zambezicus*	Euphorbiaceae	leaves	[54]
78	Thiosulfinate	*Allium sativum*	Amaryllidaceae	bulbs	[74]
79	Ursolic acid	*Duroia macrophylla*	Rubiaceae	leaves	[63]
80	Wutaiensal	*Zanthoxylum wutaiense*	Rutaceae	root wood	[75]

The physicochemical properties of these compounds were evaluated (Molecular weight (g/mol) less than 500 daltons, Num. Acceptors of H-bonds < 10, Num. Donors of H-bonds <5, molar refractivity (40 to 130), TPSA (<140Å2) and Log P (<3)), prediction of toxicity (mutagenicity, carcinogenicity, irritant and other disorderly function). Out of 80, 24 compounds obeyed the drug-like properties and further oral toxicity was examined. The potential compound were observed to be alliin and galacturonic acid from *Allium sativum* and *Cordia myxa* respectively (Table 2).

Table 2: Toxicity prediction of phytocompounds

S. No	Compounds	Predicted toxicity class	Predicted LD50 (mg/kg)
1	3,3'-Biplumbagin	2	6
2	Abruquinone B	4	500
3	Ajoene	4	6
4	Allicin	4	874
5	Alliin	6	8000
6	Ambroxol	5	2380
7	Andrographolide	5	5000
8	Ascorbic acid	5	3367
9	Butin	4	2000
10	Cycloalliin	5	4000
11	Diallyl Disulfide	3	260
12	Diallyl Trisulfide	3	100
13	Diospyrin	2	16
14	Eugenin	3	100
15	Galacturonic acid	6	10000
16	Lansine	4	1250
17	liquiritigenin	4	2000
18	Maritinone	2	16
19	Methylallyl trisulfide	3	260
20	Perforamone B	2	44
21	Perforamone D	4	522
22	Plumericin	4	2000
23	S-allylcysteine	5	4000
24	Thiosulfinate	4	570

By geometry optimization of the protein structure, the potential energy was subsequently minimized. Based on the interactions and binding energies between *Mtb* proteins and the phytocompounds, the product of the docking

analyzed. From the study, we noticed that alliin showed substantial affinity to PknD with -6.07 kcal/mol binding energy, followed by -6.02 kcal/mol Cpn10, and -5.68 kcal/mol ICDH-1. The PknD:alliin complex was recognized to have a stronger binding affinity with the least binding energy of all complexes (Table 3).

Table 3. Interacting of *Mtb* proteins with alliin and galacturonic acid complex energy values

S. No	Protein	Binding energy with Alliin (Kcal/mol)	Binding energy with galacturonic acid (Kcal/mol)
1	PstS1	-4.16	-3.46
2	PknD	-6.07	-5.42
3	DevR / DosR	-5.57	-5.01
4	ICDH-1	-5.68	-4.3
5	Cpn10	-6.02	-3.97
6	PstS3	-4.47	-4.54
7	rpoC	-4.55	-4.8

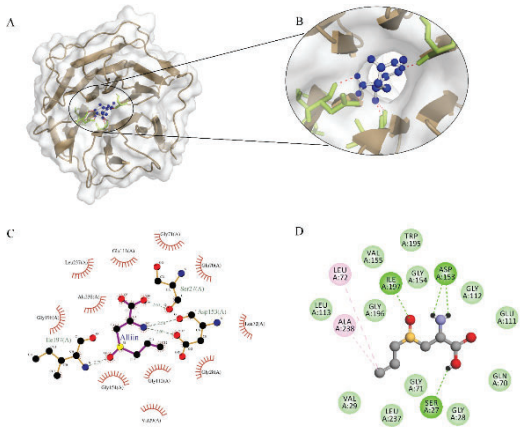

Figure 1: Serine/threonine-protein kinase PknD in complex with a selective inhibitor alliin compound. Two-dimensional structure of PknD-alliin as indicated. (A) PknD-alliin complex structure in 3D view in PYMOL (B) closed view of PknD-alliin complex (C) LigPlot view (D) Discovery studio view.

Best binding affinity, lowest binding energy of -6.07 Kcal/mol and H-bond formation with residues SER27, ASP153 and ILE197 were identi-

Table 4a: Different interaction residues for major *Mtb* proteins with alliin complex measured through Discovery studio visualizer

Docked complexes with interaction residues (Alliin)			
Complexes	Interactions		
Target	Hydrogen bond	Hydrophobic	Van der Waals
Cpn10	GLU18, GLU20, GLU84	-	ALA19, GLU83, GLY82
PstS1	ASN297, ARG299	-	GLY75, GLY96, ASN298, GLN300
PknD	SER27, ASP153, ILE197	LEU72, ALA238	GLY28, VAL29, GLN70, GLY71, GLU111, GLY112, LEU113, GLY154, VAL155, TRP195, GLY196, LEU237
devR / dosR	ARG56, GLU195	VAL55, LEU161, VAL185, LEU189	ASN61, SER186, GLN199
ICDH-1	ASN187, TYR222, MET225	-	MET185, TYR186, PHE188, LYS189, ILE192, PHE226
PstS3	ASP104, ASN196, THR296, ALA313, TYR315	PRO106, VAL293	VAL105, ARG199, LEU294, ASP295, ASP297, TYR300, LEU312
rpoC	ARG6, GLU27	PRO25	GLN5, PRO7, THR8, LEU26, PHE189

Table 4b: Different interaction residues for major *Mtb* proteins with galacturonic acid measured through Discovery studio visualizer

Docked complexes with interaction residues (Galacturonic acid)			
Complexes	Interactions		
Target	Hydrogen bond	Van der Waals	Unflavorable
Cpn10	ALA19, THR21, ILE29, PRO30, LYS36	GLU20, ASP31, LYS72	-
PstS1	GLN300, LYS301	ARG299, ASP302, SER351	-
PknD	VAL29, LEU72, VAL155, ILE197, VAL239	GLY28, ALA30, ALA73, LEU113, ALA114, GLY154, ALA156, GLY196, ALA198, ALA238, ALA240	ILE197
devR/dosR	ARG56, ASN167, LYS182	VAL55, VAL185, SER186, LEU189, GLU195	-
ICDH-1	ASN187, LYS189	PHE188, GLU190, MET225	-
PstS3	ASP104, ASN196, ARG199, LEU294	VAL105, PRO106, ASP295, THR296, ASP297, LEU312, ALA313, TYR315	-
rpoC	VAL216, ARG223	LEU215, PHE219, GLY220	-

fied in the PknD-alliin complex (Figure 1). The findings of the docking studies suggest that amino acid residues GLY28, VAL29, GLN70, GLY71, GLU111, GLY112, LEU113, GLY154, VAL155, TRP195, GLY196, LEU237 (Van der Waals); LEU72, ALA238 (Hydrophobic) play a significant role in drug interaction (Table 4a and 4b).

Conclusion

Notably, the discovery of inhibitors from natural sources has been the subject of several studies. This study concludes that Alliin from *Allium sativum* is an efficient lead compound that will be useful for the development of novel, non-toxic and highly effective tuberculosis treatment drug. To validate this result, Alliin should be subjected to further *in vitro* and *in vivo* experimental studies. This research found that SER27, ASP153 and ILE197 of PknD play a significant role in the hydrogen bonding with Alliin.

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Conflicts of interest

We declare no conflict of interests.

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