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In silico analysis of the efficacy of some natural compounds as antituberculosis agents

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Tuberculosis (TB) remains a disease of global importance with approximately two million deaths annually worldwide. Effective treatment of TB has been hampered by the emergence of drug resistant strains of *Mycobacterium tuberculosis*. Natural products have a proven global history of treating TB diseases and ailments. Available vast reservoir of chemically diverse natural products that provide new templates for drug design can be scrutinized and the most efficient may be chosen by molecular docking studies with the TB proteins. In the present study, an attempt has been made to find out a potential natural product to inhibit *M. tuberculosis* – protein kinase B (PknB) protein by molecular docking method. Docking has been performed for around 40 natural products against *M. tuberculosis* – PknB protein target to determine their potentiality against TB diseases. The anti-TB ability has been analysed in terms of binding energy. The results indicate that 80% of the natural products (B.E ≥ -7 kcal/mol) under study, exhibit good anti-TB activity. It is known that most of the natural product under study is found to possess greater binding activity than that of the conventional anti-TB drugs.

Keywords: Anti-TB agents, Molecular docking, Protein kinase B (PKnB) Protein

Tuberculosis (TB) is an infectious disease and is caused by the bacterium *Mycobacterium tuberculosis.* TB generally affects the lungs, but can also affect other parts of the body. In recent years, researchers have revealed that the *M. tuberculosis,* which is the causative agent of TB, is getting resistant towards conventional drugs used for the treatment. The developments of drug resistant in *M. tuberculosis* have frightened the global health community. Multidrug-resistant tuberculosis (MDR-TB) is a condition where the *M. tuberculosis* strain is resistant to the two most frequently used first-line oral drugs specifically Isoniazid and Rifampicin. Development of new drugs in the field of TB continues to be more challenging because of the emergence of drug resistance organisms. However, synthetic drugs such as Nitroimidazoles (PA-824 & PA-1343), Nitroimidazooxazoles (OPC-67683), Diarylquinoline TMC-207(R207910) Oxazolidinones (Linezolid, DA - 7157 and RBX8700), Ethylene diamine SQ - 109, Pyrrole derivatives (BM212 & LL3858), Phenazines (B4128 & B4169), 2'–monosubstituted Isoniazid derivatives etc., are currently in clinical phase studies[1]. Most of the TB drugs have side effects and are also costly. Hence, research on anti TB drug discovery to find new therapy than the existing one, to develop novel and potentially anti TB drug that would significantly reduce the treatment time and to combat MDR and XDR-TB becomes more challenging. In parallel, there is an increasing inclination towards the use of an alternative source of medicine, especially based on the medicinal plants. It is noteworthy that one of the ancient medical practice of India called as Ayurveda (Ayur = Life, Veda = Science), defines more than 250 medicinal plants for treating TB disease[2,3]. Medicinal plants that possess anti-mycobacterial activity against MDR-TB have also been reported across the globe[4-7]. And also, the medicinal plants that are found to be antimycobacterially active were ethno medically used for the treatment of tuberculosis or related symptoms such as cough and other respiratory diseases[8,9]. But, it is tedious to determine the most potential antimycobacterial medicinal plant by analysing and comparing the efficacy of vastly available medicinal plant samples by experimental evaluation. But nowadays, bioinformatics have opened a new horizon in the field of drug discovery. Molecular docking is used to predict the binding orientation and binding affinity of a particular chemical component with the target protein. Thus, interfacing docking methodologies with the knowledge of natural products may pave way to investigate the potential anti-TB drugs. Therefore, in the present work, we sought to screen some of the natural products for their anti-TB property by docking them with *M. tuberculosis* – protein kinase B (PknB) protein target.

Actually, genome of *M. tuberculosis* codes for about 4000 proteins. *M. tuberculosis* pathogen requires an efficient way of sensing and transducting extracellular signals to adapt to the changing

environmental conditions[10]. For cell signals, the regulation of cell growth and cell division involving the reversible phosphorylation on Serine/Theronine residues are critical in the bacterium[11]. Hence, kinases are attractive drug targets due to the range of crucial cellular processes in which they are involved. *M. tuberculosis* – (PknB) is an essential receptor like protein kinase involved in cell growth control. It is a transmembrane Ser/Thr protein kinase and can be considered as a suitable drug target for tuberculosis[12]. PknB is predicted to consist of 626 amino acids with a transmembrane segment dividing the protein into an N-terminal intracellular domain and a C-terminal extracellular domain. The N-terminal of PknB includes a kinase domain and juxtamembrane linker of 52 residues[13]. And also, infection studies with a mouse model reveal that depletion of PknB results in clearance of pathogens from the host tissues, indicating definitively that PknB is essential for survival of the pathogen within the host[14]. Although docking studies of natural products with *M. tb* proteins are enormous, there are limited or no studies related to docking with *M. tuberculosis* – PknB protein. Thus, PknB is chosen as the drug target in the present study. It was hoped that the present study, being the first to identify a good natural inhibitor for PknB target and a potential lead natural product against the TB. The study will highlight the efficacy of the chosen natural products as potent anti-TB agents from their calculated binding energies with the target protein and by comparison of that with the binding energies of conventional drugs recommended for the treatment of various forms of tuberculosis.

Computational details

Ligand and Protein Preparation

Natural products chosen for this work were selected from literature sources. For each compound, a molecular structure file was generated by using Chemsketch[15]. The crystal structure of the protein (PDB ID: 2fum) was retrieved from the Protein Data Bank. All the bound substances (ligand and co-factors) and solvent molecules were removed from the protein molecule. Docking experiment was performed for the natural products against PknB protein using Auto Dock 4.0[16,17]. The Lamarkian Genetic Algorithm was used during the docking process to explore the best conformational space for the ligand. The other parameters were set as default. The ligand binding domain of target protein PknB was predicted using the Site Finder module of Molecular Operating Environment. UCSF Chimera software[18] is used to visualize the best docked conformations and hydrogen bonding interactions.

Results and Discussion

Natural products as Ligands

A set of 40 natural products that are found to obey Lipinski's rule have been chosen for the docking analysis (Table 1) and their corresponding structures are presented in Supplementary data, Table S1.

Drug-like properties

In silico profiling of all the natural products under study to predict their drug-like properties, as summerised in Lipinski's "Rule of Five" (for orally administered drugs, which have molecular weight (M. W.) ≤ 500, Clog P ≤ 5, H-bond donors (HBD) ≤ 5

Table 1 — Drug-like properties of the natural products chosen for the study and calculated binding energies of natural products to target protein PknB of *M. tuberculosis*—(Contd.)

S. No.	Natural Products	Botanical & (Family) name	M.W.	No of H–bond donor	No. of H–bond acceptor	No. of rotatable bonds	M.R. cm^3	B.E. (kcal/mol)
1	Allin	*Allium sativum* (Lilliaceae)	177.22	3	4	5	43.49 ± 0.3	–4.42
2	Gallic acid	*Allium sativum* (Lilliaceae)	170.12	4	5	1	38.82 ± 0.3	–4.85
2	α –Pinene	*Rosmarinus officinalis* (Lamiaceae)	136.23	0	0	0	43.96 ± 0.3	–5.20
4	Coumarin	*Dipteryx odorata* (Fabaceae)	146.14	0	2	0	39.76 ± 0.3	–5.57
5	Farnesol	*Vachellia farnesiana* (Fabaceae)	222.37	1	1	7	72.77 ± 0.3	–5.60
6	1,4- Naphthoquinone	*Juglans nigra* (Juglandaceae)	158.15	0	2	0	42.90 ± 0.3	–5.86
7	Chelerythrine	*Chelidonium majus* (Papaveraceae)	348.37	0	5	2	-	–6.15

(*Contd.*)

Table 1 — Drug-like properties of the natural products chosen for the study and calculated binding energies of natural products to target protein PknB of *M. tuberculosis*—(Contd.)

S. No.	Natural Products	Botanical & (Family) name	M.W.	No of H–bond donor	No. of H–bond acceptor	No. of rotatable bonds	M.R. cm^3	B.E. (kcal/mol)
8	Methazolamide	*Punica granatum* (Lythraceae)	236.27	2	7	2	53.06 ± 0.5	–6.26
9	Andrographolide	*Andrographis paniculata* (Acanthaceae)	350.45	3	5	3	94.13 ± 0.4	–6.48
10	Abruquinone B	*Abrus precatorius* (Fabaceae)	390.38	0	8	6	97.26 ± 0.4	–6.73
11	Acetazolamide	*Punica granatum* (Lythraceae)	222.25	3	7	2	45.95 ± 0.4	–6.81
12	Plumbagin	*Plumbago zeylanica* (Plumbaginaceae)	188.18	1	3	0	49.49 ± 0.3	–6.90
13	Xanthone	*Canscora decussata* (Gentianaceae)	196.2	0	2	0	55.53 ± 0.3	–7.04
14	Sparsiflorine	*Croton sparsiflorus* (Euphorbiaceae)	283.32	3	4	1	79.61 ± 0.3	–7.47
15	Aristolactum	*Piper nigrum* (Piperaceae)	341.27	1	8	3	88.25 ± 0.3	–7.58
16	Quindoline	*Crytolepis sanguinolenta* (Apocyanaceae)	218.25	1	2	0	72. 30 ± 0.3	–7.59
17	Graveolinine	*Ruta graveolens* (Rutaceae)	279.29	0	4	2	79.61 ± 0.3	–7.66
18	Cryptolepine	*Cryptolepis sanguinolenta* (Apocyanaceae)	232.28	0	2	0	72.30 ± 0.5	–7.90
19	Plumericin	*Plumeria bicolor* (Apocynaceae)	290.27	0	6	2	69.72 ± 0.4	–7.95
20	Pachypodol	*Agastache rugosa* (Lamiaceae)	344.31	2	7	4	86.77 ± 0.4	–8.04
21	Crotsparine	*Croton sparsiflorus* (Euphorbiaceae)	283.32	2	4	1	78.38 ± 0.4	–8.11
22	Kaempferol	*Acalypha indica* (Euphorbiaceae)	286.24	4	6	1	71.43 ± 0.3	–8.13
23	Obtusifoliol	*Acacia obtusifolia* (Aristolochiaceae)	426.72	1	1	5	133.00 ± 0.4	–8.13
24	Diosyprin	*Diospyros montana* (Ebenaceae)	374.34	2	6	1	97.40 ± 0.3	–8.17
25	Piperine	*Piper nigrum* (Piperaceae)	285.34	0	4	3	82.14 ± 0.3	–8.26
26	Pinostrobin	*Cajanus cajan* (Fabaceae)	270.28	1	4	2	-	–8.26
27	Luteolin-7-o-glucoside	*Gentianopsis paludosa* (Gentianaceae)	286.24	4	6	1	71.73 ± 0.3	–8.34
28	Homoeriodictyol	*Eridictyon californium* (Boranginaceae)	302.28	3	6	2	76.93 ± 0.3	–8.35
29	Tangeretin	*Citrus reticulate* (Rutaceae)	372.37	0	7	6	97.59 ± 0.3	–8.49
30	Berberine	*Berberis aristata* (Beriberidaceae)	336.36	0	5	2	-	–8.55
31	Rhamnetin	*Syzygium aromaticum* (Myrtaceae)	316.26	4	7	2	78.11 ± 0.3	–8.62
32	Eriodictyol	*Citrus limon* (Rutaceae)	288.25	4	6	1	72.13 ± 0.3	–8.78
33	Fisetin	*Cotinus coggygria* (Anacardiaceae)	288.24	4	6	1	71.43 ± 0.3	–8.81

(Contd.)

Table 1 — Drug-like properties of the natural products chosen for the study and calculated binding energies of natural products to target protein PknB of *M. tuberculosis*—(Contd.)

34	Emodin	*Ventilago maderaspatana* (Rhamnaceae)	270.24	3	5	0	69.13 ± 0.3	–8.98
35	Quercetine	*Allium cepa* (Liliaceae)	302.24	5	7	1	73.31 ± 0.3	–9.04
36	Aromadendrin	*Pinus sibirica* (Pinaceae)	288.25	4	6	1	71.84 ± 0.3	–9.07
37	Aloe emodin	*Aloe barbadensis miller* (Asphodelaceae)	270.24	3	5	0	69.13 ± 0.3	–9.13
38	Isorhamnetin	*Brassica nigra* (Brassicaceae)	316.26	4	7	2	78.11 ± 0.3	–9.18
39	Sobachalcone	*Dorstenia barteri* (Moraceae)	324.37	3	4	5	96.10 ± 0.3	–9.62
40	Stilpulin	*Dalbergia stipulacea* (Fabaceae)	392.49	3	4	7	119.45 ± 0.3	–10.41

and acceptors (HBA) ≤ 10), was performed, and the details are presented in Table 1[19]. The entire natural product chosen for the current study had a molecular weight less than 500.

Docking calculations

Docking studies are widely used methods in lead discovery because it is advantageous in the elimination of undesired molecules from compound libraries and the reduction of cost and time in drug discovery projects. In the current research work, docking method is used to predict the binding efficacy of the chosen natural compounds with PknB protein.

The chosen 40 natural products were docked with the anti-TB target protein (PknB), to predict their binding energies and possible binding modes using Autodock tools[16,17]. The three dimensional structure of the receptor protein – PknB is represented in the Fig. 1. The best docked conformations were selected, visualized, and analyzed using Autodock tools[16,17] and Chimera softwares[18]. The interaction of the ligands with the active site residues of the target PknB was analysed in terms of the binding energy, number of hydrogen bonds established by the ligand with residues of the active site. Autodock uses the following empirical formula to calculate the Free energy of binding:

Binding energy (ΔG) = Intermolecular energy + (vanderWaal's hydrogen bond desolvation energy + Electrostatic energy) + Total internal energy + Torsional energy – Unbound energy of the system

The dock score of Autodock is reported in kcal/mol. The calculated binding energies of all the natural products under study are presented in the Table 1. The conventional anti-TB drugs such as Isoniazid, Pyrazinamide and Ethambutol are also docked with the same target protein to deliberate the anti-TB ability of the natural products chosen for the study and are presented in the Table 2.

The best docking score (free energy with a more negative value) indicates the highest ligand–protein affinity. A closer look of the docked binding energies of all the natural products under study in Table 1 revealed that 80% of the chosen natural compounds have good binding affinities (BE ≥ –7 kcal/mol) with the selected target protein. From the Table 1 and 2, it is known that most of the natural products under study were found to possess greater binding ability than that of the anti-TB drugs considered for the comparison purpose. The results indicate that the natural products are better to treat the TB disease when compared to the synthetic drug having severe side effects.

Among the natural products under study, the top lead molecules with best binding affinity are Stipulin (B.E. ~ –10.41 kcal/mol), Sobachalcone, (B.E. ~ –9.62 kcal/mol), Isohamnetin, (B.E. ~ –9.18 kcal/mol), Aromadendrine (BE ~ –9.07 kcal/mol) and Quercetin, (B.E ~ –9.04 kcal/mol). These top lead molecules are analyzed further, in terms of the parameters such as number of hydrogen bonds established by the ligand with the residues of the active site and conformations oriented by the ligand within the active site (Table 3). It is known that desolvation energy is a prime parameter that decides a molecular interaction with its pharmacodynamic target and it is found to be good for all the top lead molecules (Table 3). In the biological environment, all the drug binding pockets of a target protein remain solvated and hence a ligand cannot as such occupy the active site unless it dislodges the water molecules.

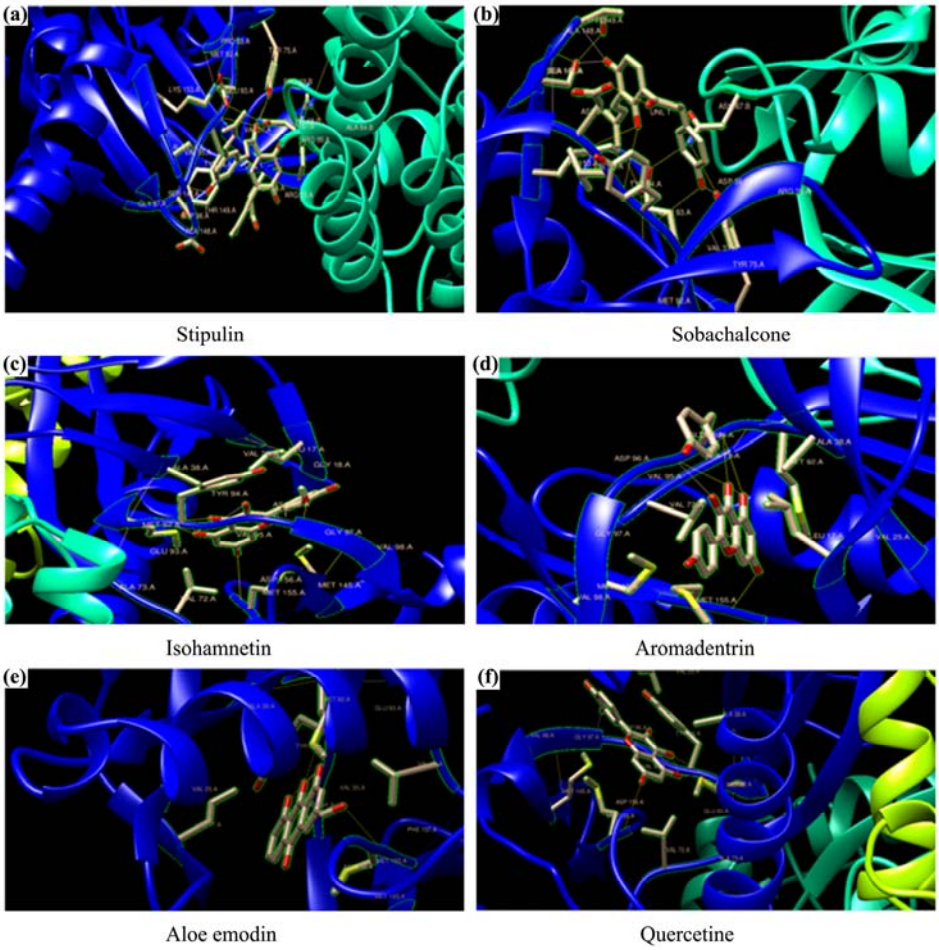

Fig. 1 — The ligand with its most likely binding conformation is docked into the binding cavity of the receptor and the intermolecular interactions (H-bonds) are identified (a–f).

The top lead molecules with its most likely binding conformation are docked into the binding cavity of the receptor and the intermolecular interactions (H–bonds) are identified. The identified hydrogen bonding interactions between the ligand and the protein was illustrated in the Fig. 1 (a–f). For all the top lead molecules, the bond conformation was stabilized by strong interactions with polar and non-polar side chains of the amino acids in the binding pockets owing to the presence of hydrogen bond donors in them. Stipulin is anchored by four hydrogen bonds with amino acid residues such as Tyr94, Tyr75, Asp96 and Asp36 of the active site of PknB protein. Sobachalcone interacted with Tyr94, Tyr75, Tyr94, Asp96, Thr149 and Ser147, by forming seven hydrogen bonds and Aromadendrin established five H–bonds with Glu93, Val95 and Asp156, of the active site of PknB protein. Isohamnetin forms five H–bonds with the amino acid residues such as Asp 156, Val 95, Glu 93, Gly 97 and Aloe emodin interacted by forming four hydrogen bonds with Val 95, Asp 156, Glu 93, of the active site of PknB protein. Quercetine established six H – bonds with the amino acid residues such as Val 95, Asp 156, Gly 96, Glu 93 of the active site of PknB protein. In addition to the usual medicinal uses of all the lead compounds, the results of our docking study support the fact that they also possess a greater binding affinity towards the PknB protein and hence have greater potential to combat the TB disease. But, further *in vitro* investigation is necessary to confirm their actual

Table 2 — Predicted binding energies of known anti-TB drugs to target protein PknB of *M. tuberculosis*

S. No.	Conventional anti-TB drugs	Binding Energies* (kcal/mol)	Binding Energies (kcal/mol)
1	Isoniazid	–4.0	–4.71
2	Pyrazinamide	–3.9	–4.01
3	Ethambutol	–3.7	–2.14

*The binding energies of the drugs calculated using Autodock Vina[20]

Table 3 — Ligand binding data

Parameter	Predicted values					
	Stipulin	Sobachalcone	Isohamnetin	Aloe Emodin	Aromadendrine	Quercetin
Binding energy (kcal/mol)	–10.41	–9.62	–9.18	–9.13	–9.07	–9.04
Inhibitory constant (nM)	23.41	88.61	86.28	203.46	224.79	238.16
Intermolecular energy (kcal/mol)	12.47	–10.61	–9.99	–9.43	–9.07	–9.64
Total internal energy (kcal/mol)	–1.84	–1.79	–0.34	–0.09	0.00	–0.21
Torsional energy (kcal/mol)	2.39	1.79	0.60	0.30	0.00	–0.30
Electrostatic energy (kcal/mol)	–0.22	–0.06	0.03	–0.07	0.03	0.05
Unbound system energy (kcal/mol)	–1.51	–0.99	–0.55	–0.09	0.03	–0.52
van der Waals Hydrogen bond desolvation energy (kcal/mol)	–12.25	–10.55	–10.02	–9.35	–9.10	–9.69
Number of H–bonds established with amino acid residues of target protein - PknB	4	7	5	4	5	6
Interacting amino acid residues of target protein - PknB & their	Tyr94, Tyr75, Asp96, Asp36	Tyr94, Tyr75, Tyr94, Asp96, Asp96, Thr149, Ser147	Val95, Val95, Glu93, Asp156, Gly97	Val95, Asp156, Glu93, Glu93	Glu93, Glu93, Val95, Val95, Asp156	Glu93, Glu93, Val95, Val95, Asp156, Gly96
Bond length (Å)	2.971, 2.771, 2.813, 2.950	3.030, 2.717, 2.934, 2.943, 2.934, 3.015, 2.969	3.015, 3.128, 2.586, 3.074, 3.142	2.658, 2.861, 2.664, 3.471	2.524, 2.627, 3.049, 2.918, 3.115	2.573, 2.586, 2.725, 3.142, 3.072, 3.092

therapeutic efficacy and drug ability towards the disease.

Conclusions

Literature reports revealed a large set of natural products having anti TB properties, but their isolation, structural characterization and experimental evaluation of their medicinal properties are normally time-consuming, difficult and expensive. To meet the demanding challenge of simplifying the process, molecular docking studies help to chart, navigate, and analyze systematically the chemical space of huge natural products for drug discovery. The results of this *in silico* study indicate that 80% of the natural products (B.E. ≥ -7 kcal/mol) under study, exhibit greater binding activity than that of the conventional anti-TB drugs. The current work suggests that 80% of the natural products under study have the unique capability to counter the deadly tuberculosis pathogen by binding strongly to the PknB protein of it. Though the natural products under study are structurally diverse, they may be considered as the useful templates for the discovery of new pharmaceuticals for the treatment of tuberculosis, but further *in vitro* investigation is needed to confirm their authentic healing prospective towards the disease.

Supplementary data

Supplementary data associated with this article are available in the electronic form at: http://nopr.niscair.res.in/jinfo/ijca/IJCA_59A(02)207-213_SupplData.pdf.

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