Inhibitory Activity of Betulin (Lup-20(29)-Ene-3β, 28-Diol) On Pknb Serine/Threonine Kinase from *Mycobacterium Tuberculosis*: A Molecular Modeling Study

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Abstract

Ser/Thr Kinase is one of the four *M. tuberculosis* kinases that are conserved in the downsized genome of *Mycobacterium leprae* and are therefore presumed to play an important role in the processes that regulate the complex life cycle of mycobacteria. It is known that there are two main superfamilies of protein kinases, one including STPKs1 and PTKs and that of His kinases. For a long time, the former were only found in eukaryotes, and the latter were only found in prokaryotes. In this paradigm, proteins from each superfamily were supposed to play analogous roles in the essentially different organization of signal transduction in both phyla. In this study we report the binding mode of Ser/Thr kinase with derivatives of Betulin ((lup-20(29)-ene-3β,28-diol) on the basis of structural similarity, substructure, isomers & conformers. Molecular docking approach using Lamarckian Genetic Algorithm was carried out to find out the potent inhibitors for Ser/Thr Kinase on the basis of calculated ligand-protein pairwise interaction energies. Study was carried out on 3000 molecules which were virtually screened from different databases on the basis of the structural similarity of Betulin. The grid maps representing the protein were calculated using auto grid and grid size was set to 60*60*60 points with grid spacing of 0.375 Å. Docking was carried out with standard docking protocol on the basis of a population size of 150 randomly placed individuals; a maximum number of 2.5 *107 energy evaluations, a mutation rate of 0.02, a crossover rate of 0.80 and an elitism value of 1. Fifteen independent docking runs were carried out for each ligand and results were clustered according to the 1.0 Å rmsd criteria.

The docking result of the study of 3000 molecules demonstrated that the binding energies were in the range of -12.72 kcal/mol to -1.71 kcal/mol, with the minimum binding energy of –12.72 kcal/mol. 6 molecules showing hydrogen bonds with the active site residue VAL 95. Further in-vitro and in-vivo study is required on these molecules as the binding mode provided hints for the future design of new derivatives with higher potency and specificity.

KEYWORDS: In- Silico Drug Discovery, Protein Kinase PKnB, *Mycobacterium Tuberculosis*, Betulin, Molecular Docking.

INTRODUCTION

The knack of *Mycobacterium tuberculosis*, pathogen liable for Tuberculosis (TB), to adapt to the changing environmental conditions requires an efficient way of sensing and transducing extracellular signals [1]. Multidrug resistance strains of *Mycobacterium tuberculosis* are creating havoc worldwide, more particularly in immune-compromised individuals. Thus, exploring newer drugs for MDR tuberculosis is a challenge for scientists. One of the mechanisms used in *Mycobacterium* to assure a tight regulation of cell growth and division involves the reversible phosphorylation on serine/threonine residues, a well-established process for eukaryotic signaling networks [2]. *M. tuberculosis* PKnB (EC - 2.7.11.1) is a trans-membrane Ser/Thr protein kinase (STPK) highly conserved in Gram-positive bacteria and apparently essential for mycobacterial viability [3]. Earlier it was shown that PKnB is regulated by auto-phosphorylation an dephosphorylation by the Ser/Thr protein phosphatase [4] and [5] and recent work showed that PKnB is predominantly expressed during exponential growth, where as its over expression causes morphological changes linked to defects in cell wall synthesis and cell division [6]. Aberrant kinase activity is implicated in numerous human diseases and, not surprisingly, protein kinases represent today one of the most important groups of drug targets [7] and [8]. In the present study we try to elucidate computationally the inhibitory activity of Betulin, its derivatives and isomers on PKnB protein using Autodock4.2 software.

MATERIALS AND METHODS

Protein structure retrieval:

The 3D structure of cytosolic domain of the PKnB Ser/Thr Kinase receptor protein from *Mycobacterium tuberculosis* was retrieved from RCSB Protein Data Bank (PDB). The protein model with PDB ID: 2FUM [1] was chosen for active site predictions and further docking studies.

Protein Active Site Predictions:

The residues forming the active site of the protein were predicted using CASTp Calculations [9]. The active site having the catalytic amino acid Asp138 was chosen for further docking studies of Betulin and its derivatives. Analysis of the catalytic amino acids was done through Uniprot (Primary Accession Number: P0A5S4) [10].

In silico screening

3000 compounds from different chemical databases were screened, including the PubChem database [11]. They were docked into the active site of the *M. tuberculosis* PKnB protein structure model, 2FUM using the program AutoDock4.2 [12].

Substrate selection

3000 structures most 2D-similar to Betulin (Fig 1) were chosen based on screening from the PubChem. The chosen ligands have conformational stability and structural diversity in relation to the bound ligands to the crystal structure. Ligands were identified as per the pharmacokinetic parameter and solubility. The molecules were searched by similarity

search compound collection, by similarity to a structure (may be specified via a SMILES string, or drawn with JME Molecular Editor). The active site in the protein interacts with the functional groups of the ligands and gives rise to the catalytic activity to test ligands that helps in determining the binding pattern of the ligands to the active site of PKnB (PDB ID: 2FUM).

Docking setup

Docking was performed using Autodock4.2, which combines energy evaluation through grids of affinity potential employing various search algorithms to find the suitable binding position for a ligand on a given protein [12]. While docking, polar hydrogen's were added to ligands using the hydrogen's module in Autodock tool and thereafter, Kollman united atom partial charges were assigned. Docking of ligands onto PKnB protein from *Mycobacterium tuberculosis* was carried out using LGA with standard docking protocol on the basis a population size of 150 randomly placed individuals; a maximum number of $2.5*10^7$ energy evaluations, a mutation rate of 0.02, a crossover rate of 0.80 and an elitism value of 1. Ten independent docking runs were carried out for each ligand and results were clustered according to the 1.0 Å rmsd criteria. The grid maps representing the proteins were calculated using auto grid and grid size was set to 60*60*60 points with grid spacing of 0.375 Å. The coordinate of the docked protein along with the ligand was visualized using UCSF chimera [13] within 5 Å region.

RESULTS AND DISCUSSION

The PKnB protein (2FUM) from *Mycobacterium tuberculosis* was selected for our docking study. A total of 3000 three dimensional structures of Betulin and its derivatives were selected from PubChem databases and were screened against the functional site of 2FUM through AutoDock4.2 software.

The active site analysis of the protein was performed using CASTp Calculations. Amongst all the binding sites obtained, site 1 was highly conserved and the most favorable site for docking. The residues in site 1 were found to be Leu17, Gly18, Phe19, Gly20, Val25, Ala38, Lys40, Glu59, Ala63, Leu66, Ile71, Val72, Ala73, Val74, Met92, Glu93, Tyr94, Val95, Gly97, Val98, Thr99, Asp102, Asp138, Lys140, Ala142, Asn143, Met145, Met155, Asp156 and Phe157.

The docking result of the study of 3000 molecules in the active site of PKnB protein model: 2FUM; demonstrated that the binding energies were ranging from -12.72 kcal/mol to -1.71 kcal/mol, with the minimum binding energy of -12.72 kcal/mol (Fig 2).

The number of hydrogen bonds and the amino acid residues involved in bonds between the target protein and the molecules, in the docked protein model was illustrated by Chimera. The protein-ligand complex showed Hydrogen bond with the active site residue. The final docked conformations obtained for the different ligands were evaluated based on the minimum binding energy and the number of hydrogen bonds formed. (Fig 3- 6)

The two molecules Ilekudinol C and Roxburghiadiol A showed best results and were tested for Drug Likeness Score and ADMET studies. The tests showed promising results as shown in Fig 7.

Designing drugs against a disease based on structural interactions with the ability to work at high resolution with both proteins and drug compounds marks the importance of Structure Based Drug Designing in drug design. Further there is need to generate in vitro and in-vivo activity of the generated data to synthesize and test so to design drug with better specificity and metabolism. The work is significant in emphasizing the potent inhibitory effects of Betulin (lup-20(29)-ene-3β, 28-diol) and its derivatives on PKnB activity and further their application in anti-malarial drug design.

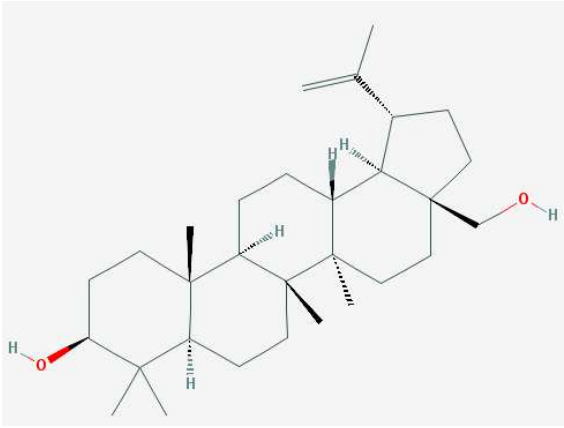


Fig 1: Betulin- Also known as: Betulinol, Trochol, Betuline, Betulol, Lup-20(29)-ene-3beta,28-diol, 473-98-3, NSC 4644, EINECS 207-475-5, Lup-20(30)-ene-3beta,28-diol

RMSD TABLE

Rank	Sub-Rank	Run	Binding Energy	Cluster RMSD	Reference RMSD	Grep Pattern
1	1	9	-12.31	0.00	48.53	RANKING
1	2	4	-12.21	0.35	48.64	RANKING
1	3	10	-12.14	0.40	48.77	RANKING
1	4	1	-11.80	0.76	49.05	RANKING
2	1	7	-12.07	0.00	48.34	RANKING
2	2	8	-11.99	0.17	48.42	RANKING
2	3	3	-11.84	0.56	48.63	RANKING
2	4	2	-11.65	0.80	48.14	RANKING
2	5	5	-11.38	1.48	48.66	RANKING
3	1	6	-11.90	0.00	48.08	RANKING

Fig 2: RMSD Table for the best binding energy.

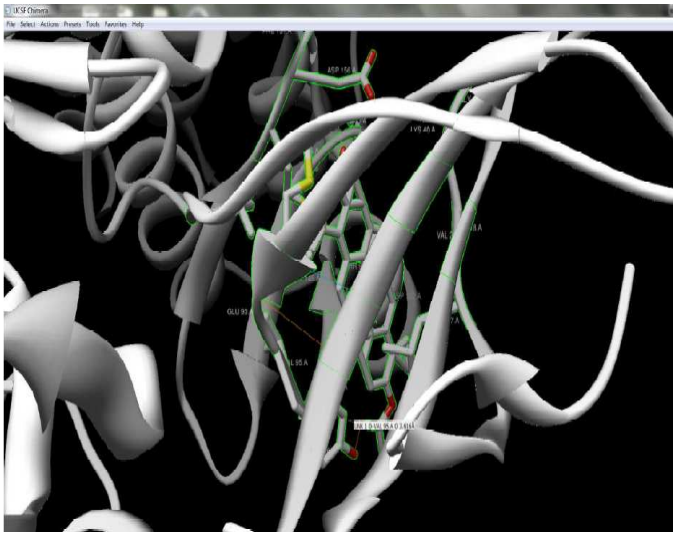


Fig 3: Ligand showing H Bond interaction with VAL 95

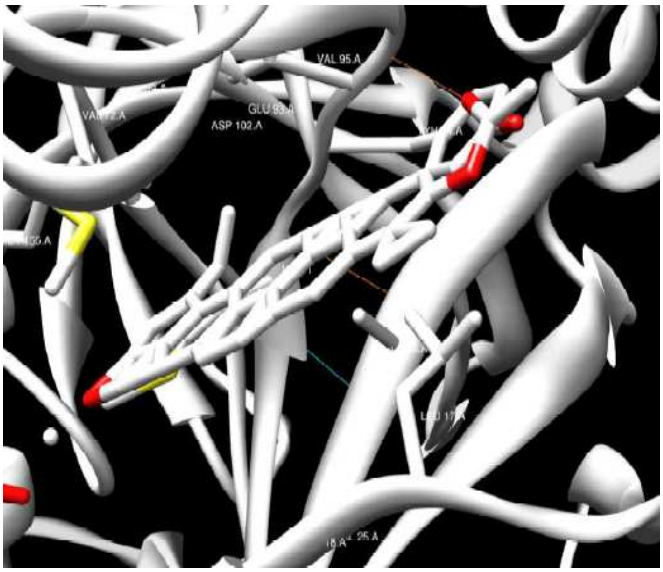


Fig 4: Ligand bound to active site residue

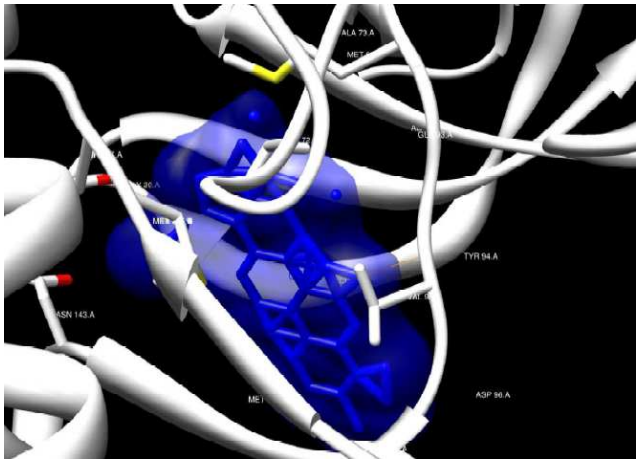


Fig 5: Ligand binding area

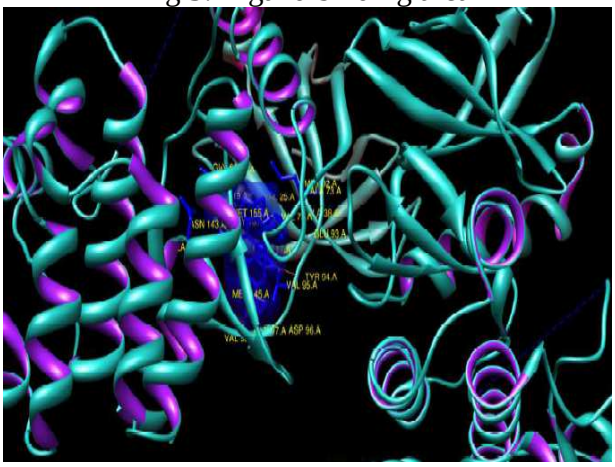
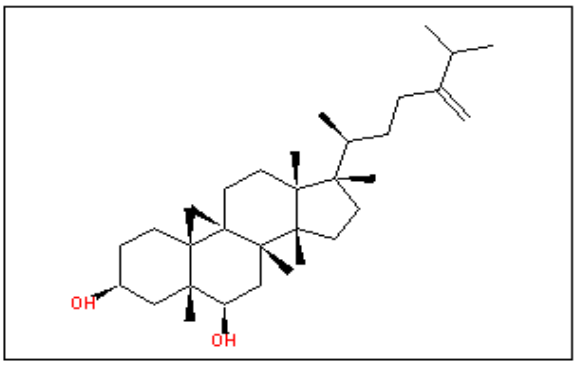


Fig 6: Ligand bound

Molecular Properties and Drug-likeness.



Molecular formula: C32 H54 O2
Molecular weight: 470.41
Number of HBA: 2
Number of HBD: 2
MolLogP : 1.29
MolLogS : -8.80 (in Log(moles/L)) 0.00 (in mg/L)
MolPSA : 33.05 A^2
MolVol : 639.88 A^3
Number of stereo centers: 10

Drug-likeness model score: 0.57

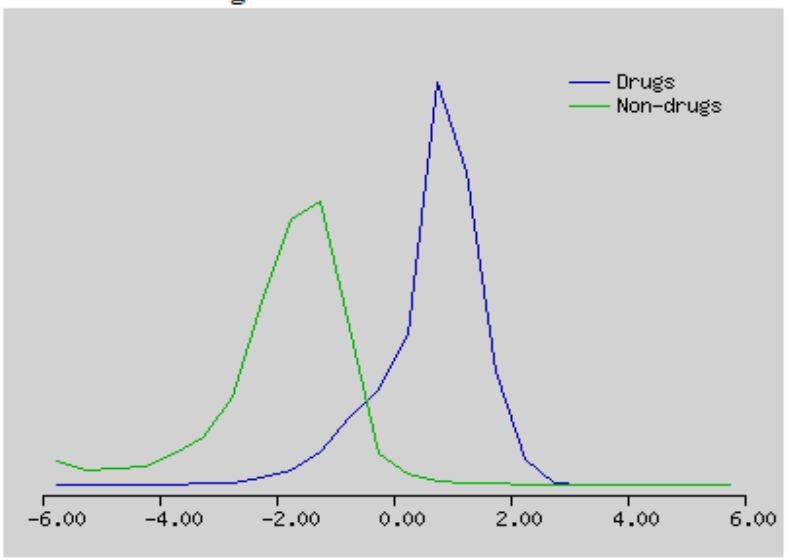
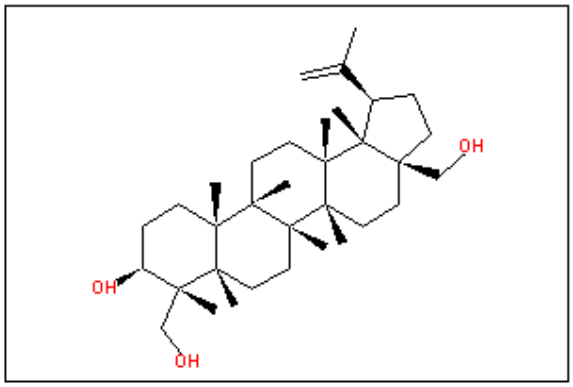


Fig 6: Drug Likeness Model for Roxburghiadiol A

Molecular Properties and Drug-likeness.



Molecular formula: C34 H58 O3
Molecular weight: 514.44 (> 500)
Number of HBA: 3
Number of HBD: 3
MolLogP : -0.43
MolLogS : -8.38 (in Log(moles/L)) 0.00 (in mg/L)
MolPSA : 50.92 A^2
MolVol : 695.73 A^3
Number of stereo centers: 11

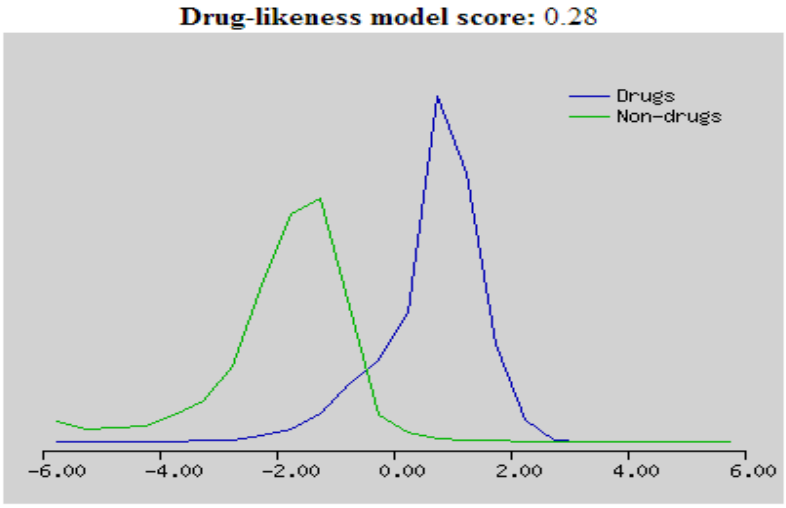


Fig 7: Drug Likeness Model for Ilekudinol C

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Table 1: Binding Energy: Betulin Compounds

S. No.	Minimum Binding Energy
1	-12.72
2	-12.46
3	-12.42
4	-12.31
5	-12.27
6	-12.11
7	-12.07
8	-11.98
9	-11.97
10	-11.93
11	-11.81
12	-11.73
13	-11.7
14	-11.69
15	-11.68
16	-11.67
17	-11.66
18	-11.55
19	-11.54
20	-11.49