Virtual screening and pharmacophore studies for ftase inhibitors using Indian plant anticancer compounds database

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Abstract:
Farnesyl transferase (FTase) is an enzyme responsible for post-translational modification in proteins having a carboxy-terminal CaaX motif in human. It catalyzes the attachment of a lipid group in proteins of RAS superfamily, which is essential in signal transduction. FTase has been recognized as an important target for anti cancer therapeutics. In this work, we performed virtual screening against FTase with entire 125 compounds from Indian Plant Anticancer Database using AutoDock 3.0.5 software. All compounds were docked within binding pocket containing Lys164, Tyr300, His248 and Tyr361 residues in crystal structure of FTase. These complexes were ranked according to their docking score, using methodology that was shown to achieve maximum accuracy. Finally we got three potent compounds with the best Autodock docking Score (Vinorelbine: -21.28 Kcal/mol, Vincristine: -21.74 Kcal/mol and Vinblastine: -22.14 Kcal/mol) and their energy scores were better than the FTase bound co-crystallized ligand (L-739: –7.9 kcal/mol). These three compounds belong to Vinca alkaloids were analyzed through Python Molecular Viewer for their interaction studies. It predicted similar orientation and binding modes for these compounds with L-739 in FTase.Thus from the complex scoring and binding ability it is concluded that these Vinca alkaloids could be promising inhibitors for FTase. A 2-D pharmacophore was generated for these alkaloids using LigandScout to confirm it. A shared feature pharmacophore was also constructed that shows four common features (one hydogen bond Donar, Two hydrogen bond Acceptor and one ionizable area) help compounds to interact with this enzyme.

Keywords: Virtual Screening, Indian Plant Anticancer Compounds Database, Signal Transduction, Autodock, LigandScou.

Background:
Many intracellular proteins are post-translationally modified by the attachment of lipid through a process called farnesylation (a type of prenylation) **[1].** This modification process has been identified in numerous protein located in eukaryotic organisms, including RAS proteins, which plays an important role in the signal transduction pathway that leads to constant activation of the protein, ultimately resulting in uncontroll cell proliferation **[2].** The high prevalence of mutated ras gene, are found in 30% of all human cancer **[3].** Since the farnesylation of oncogenic RAS protein is required for cellular transformation; a promising way of interfering with RAS function seemed to be the inhibition of Farnesyl- Transferase (Ftase) which catalyze the formation of thioether linkages between the C1 atom of farnesyl (15-carbon by Ftase) and –SH of the cystine residue at or near the C-terminus of RAS protein **[4].** This enzyme recognizes a common CAAX amino acid sequence located at C-terminus of substrate protein. In CAAX motif, C is the cystine residue to which farnesyl group is attached; A, A are aliphatic amino acids and X is the carboxyl terminal residue. Crystal structure of human Ftase was resolved at 2.30 Å resolution and is a heterodimer consisting of 44865.4 Dalton alpha subunit & 48822.9 Dalton beta subunit **[5, 6].** Several classes of compound having selective Farnesyl transferase inhibitory activity have been tested in clinical trials for example: L778123 **[7]**, tipifarnib **[8],** lonafarnib **[9],** FTT-277 **[10]** & L744832 **[11]**. The promising results in preclinical models were not confirmed in the clinic. Unexpectedly, tumors containing non-mutated RAS were also sensitive to the Farnesyl transferase inhibitors (FTIs). Thus there is still a need for novel, selective and potent Ftase inhibitors **[3]**.Traditional synthesis of a series of new compounds through high-throughput screening can be carried out at high cost and also are time consuming; whereas on the other hand, screening small molecule databasesfor novel compounds represents an alternative process. Docking various ligands to the protein of interest followed by scoring to reveal the strength of interaction and to determine the affinity of binding has become increasingly important in the context of drug discovery.Screening large databases of compounds can provide a feasible, alternative technique against high-throughput screening, but depends on the fast and accuracy of the docking algorithm **[12].**

In this paper we made an afford to develop a selective & potent Ftase inhibitors by screening a set of compounds from Indian Plant Anticancer Database (InPACdb) **[13]** against FTase protein, with bound ligand L-739, 750 extracted from Protein Databank, **[14]** by utilizing exhaustive docking software AutoDock 3.0.5 **[15]**. On the basis of Docking result a pharmacophore map were constructed for those compounds, which are having high score.

Methodology:
Receptor X-ray structure:
The 3D coordinates of the crystal structure of Human Protein Farnesyl Transferase Complexed with Farnesyl diphosphate and the peptidomimetic inhibitor L-739, 750 (PDB code: 1JCQ) [14] was selected as the receptor model in flexible Docking program. Before Docking all heteroatoms (Farnesyl Diphosphate, acetic acid, sucrose, Zinc ion, 739) & water molecules are removed from Protein file 1JCQ. After removing the water molecule H-atom were added to protein for correct ionization and tautomeric states of aminoacid residues such as Asp,Ser, Glu, Arg and His.
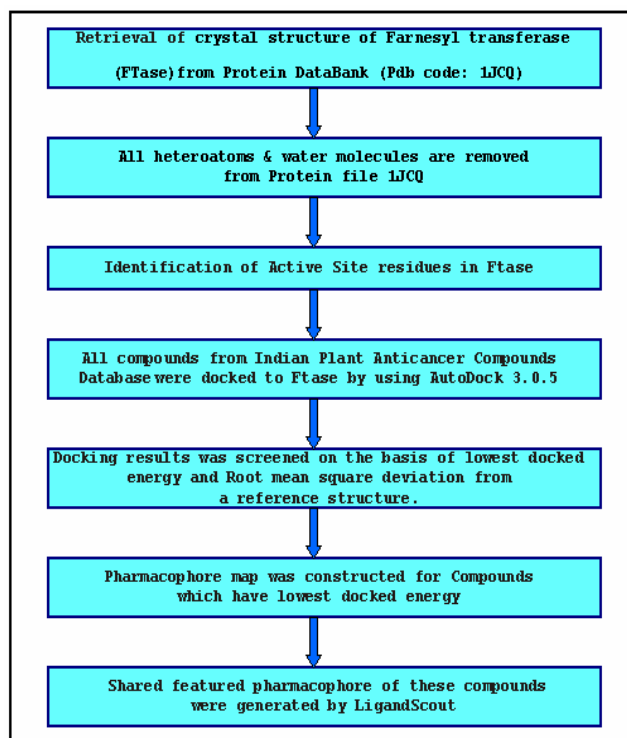
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Database

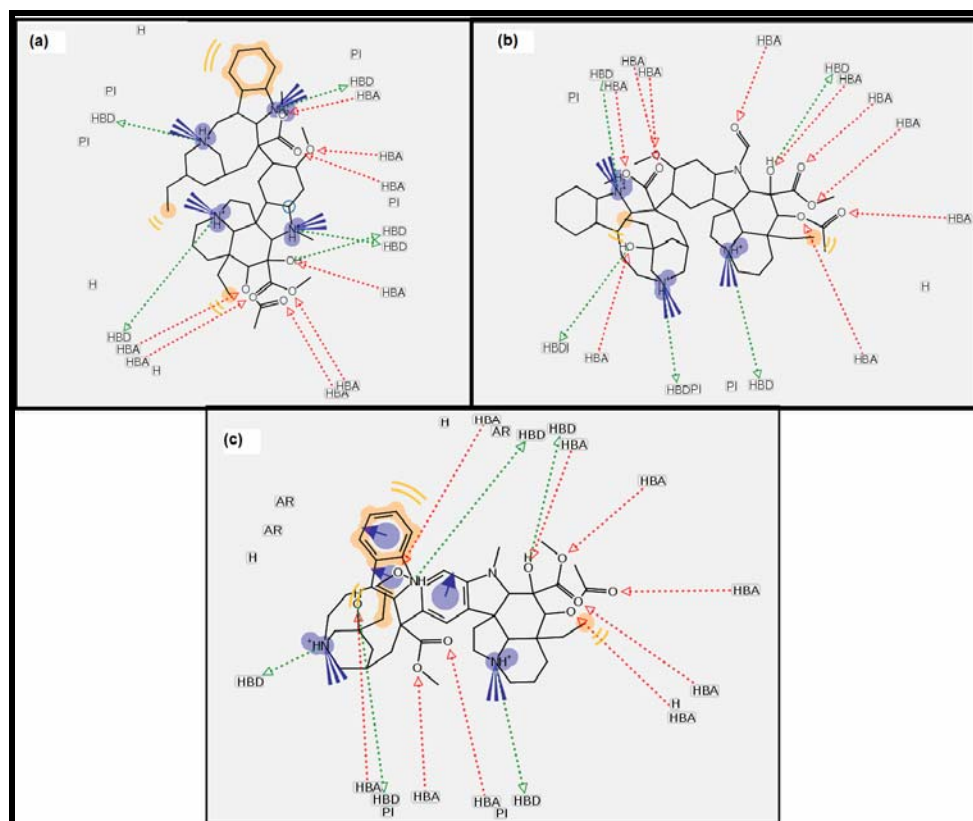**Figure 1:** A detailed flowchart summarizing the methodology implemented is shown.

Figure 2: 2-D Pharmacophore model of alkaloid compound (a) Vinorelbine, (b) Vincristine, (c) Vinblastine generated by LigandScout.
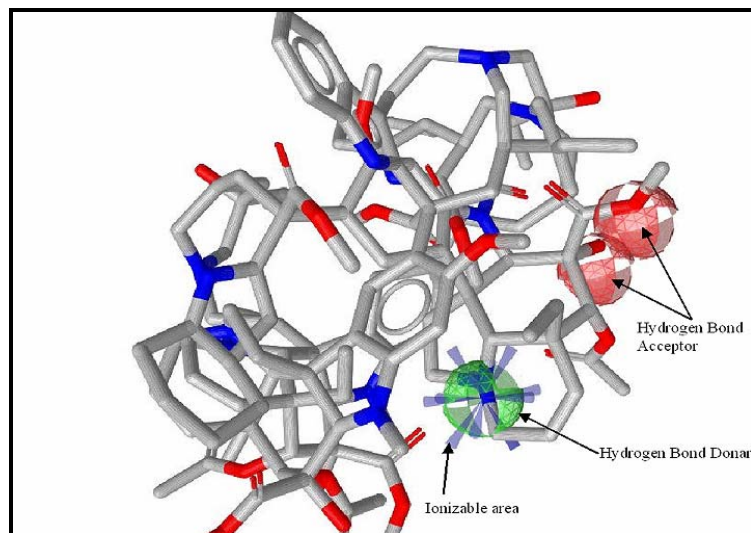
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Figure 3: Shared Feature Pharmacophore of Vinorelbine, Vincristine and Vinblastine showing four common features (one hydrogen bond Donar: green sphere, two hydrogen bonds Acceptor: Red sphere and one ionizable area: Blue asterisk).

Active Site Analysis:
The active site was analyzed by selecting neighbors within 3Å around Ligand 739,which is already bound to the crystal structure of protein Farnesyl Transferase (PDB code: 1JCQ). The active site contains Lys164, Tyr300, His248 and Tyr361 residues.

Ligand preparation:
Indian Plant Anticancer Compounds Database (InPACdb) [13] is a free online database of compounds that possess anticancer properties. These compounds are derived from various plant species that have an Indian origin. It contains 125 compounds in ready-to- dock 3D formats, available at the URL http://www.inpacdb.org/. Molecules in InPACdb are annotated by molecular property that include molecular weight, molecular formula, number of rotatable bonds, calculated XLogP, number of hydrogen-bond donors, hydrogen-bond acceptors, refractivity, Topological Polar Surface Area & Van der Waals surface area (3D). InPACdb provides several search criteria such as IUPAC name, Plant name, Cancer type & Refractivity.

Virtual Screening:
Docking of the entire 125 anticancer compounds of InPACdb against Farnesyl Transferase protein structure was done using molecular docking program AutoDock v3.05 [15]. Gasteiger charges are added to the ligand &maximum 6 numbers of active torsions are given to the lead compounds using AutoDock Tool [16]. Kollman charges and the solvation term were then added to the protein structure using the same. The grid for docking calculations was centered on Lys164, Tyr300, His248, and Tyr361 for 1JCQ in the docking studies. An $52 \times 52 \times 52$ 3D affinity grid with 0.381 spacing was calculated, respectively, for each of the following atom types C, A (aromatic C), N, O, S, H and e by Autogrid 3.0.The Lamarckian genetic algorithm implemented in Autodock was used. Docking parameters were as follows: 10 docking trials, population size of 150, maximum number of energy evaluation ranges of 25,0000, maximum number of generations is 27,000, mutation rate of 0.02, cross-over rate of 0.8, Other docking parameters were set to the software's default values. After docking, the ligands were ranked according to their docked energy as implemented in the AutoDock program.

Pharmacophore Modeling:
Three-alkaloid compounds were taken on the basis of higher scoring function for pharmacophore modeling, which is one of the most powerful techniques to classify & identify key features from a group of molecules. And a 3D-pharmacophore is a set of interactions (chemical features or

functionalities) aligned in three-dimensional space.This pharmacophore model will furnish a new insight to design novel molecules that can enhance or inhibit the function of the target and will be useful in drug discovery strategies [17]. LigandScout [18] was used to develop pharmacophore model of these three lkaloids derived from *Vinca rosea*. Different types of chemical features for each alkaloid molecule were examined using feature directory from LigandScout software. By consolidating all the available features, five common features were used to generate pharmacophore: Hydrogen bond donar

All these three compounds with lowest docked energy were categorize under vinca alkaloids [20], a class of alkaloids from the genus of apocynaceous woody herbs including Periwinkle. They are some of the most useful antineoplastic agent. However several compounds having selective Farnesyl transferase inhibitory activity (L778123 [7], tipifarnib [8], lonafarnib [9], FTT-277 [10] & L744832 [11]) is in clinical trial but these compounds are not very promising in preclinical models. Pharmacophore map was constructed for compounds (Vinorelbine, Vincristine and Vinblastine) using Ligand Scout [18] which are shown in **figure 2** (a, b, c). After that shared featured pharmacophore of these compounds was generated by aligning pharmacophores and molecules using reference points as shown in **Figure 3.**

Conclusion:
Flexible docking of ligand from chemical database to receptor is an emerging approach and is extensively used to reduce cost and time in drug discovery. The approach utilized in this study is successful in finding three potent Ftase inhibitors from Indian Plant Anticancer Database. Individually these three compounds are from group of vinca alkaloid & show lowest docked energy and hydrogen bonding stabilizes the interaction. Hydrogen bonding plays an important role for the structure and function of biological molecules, especially for inhibition in a complex. Thus the result demonstrates that Vinorelbine, Vincristine and Vinblastine are potential inhibitor for Farnesyl Transferase, which is a promising way of interfering with RAS function. Four shared feature pharmacophore (one hydrogen bond Donor, Two hydrogen bond Acceptor & one ionizable area) are generated using LigandScout to discover the essential features of ligand, which are invaluable to examine the potential lead of Farnesyl Transferase. Further the work can be evaluated experimentally to study the receptor-ligand interactions, which would help in designing of compounds based on virtual screening.

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Hypothesis

Supplementary material:

Table 1: Best Docked Anticancer Compound & its docked energy with Reference Root Mean Square Deviation

S.No.	Alkaloid Compound Name	IUPAC name	Molecular formula and Mol. Mass	Docked Energy (Kcal/mol)	Ref Root Mean Square Deviation
1.	Vinorelbine	4-(acetyloxy)-6,7-didehydro-15- ((2*R*, 6R, 8S)-4-ethyl-1, 3,6,7,8,9-hexahydro- 8-(methoxycarbonyl)- 2,6-methano- 2*H*-azecino(4,3- *b*) indol-8-yl)-3-hydroxy- 16- methoxy-1-methyl-, methyl ester, (2beta, 3beta, 4beta, 5alpha, 12R, 19al pha)- aspidospermidine-3- carboxylic acid	$C_{45}H_{54}N_4O_8$ 778.932 g/mol	-21.28	148.3
2.	Vincristine	methyl (1*R*,9*R*,10*S*,11*R*,12*R*,19*R*)-11-(acetyloxy)-12-ethyl-4-[(13*S*,15*S*,17*S*)-17-ethyl-17-hydroxy-13-(methoxycarbonyl)-1,11-diazatetracyclo[13.3.1.0^{4,12}.0^{5,10}]nonadeca-4(12),5,7,9-tetraen-13-yl]-8-formyl-10-hydroxy-5-methoxy-8, 16-diazapentacyclo[10.6.1.0^{1,9}.0^{2,7}.0^{16,19}]nonadeca-2,4,6,13-tetraene-10-carboxylate	$C_{46}H_{56}N_4O_{10}$ 824.958 g/mol	-21.74	128.56
3.	Vinblastine	dimethyl (2β,3β,4β,5α,12β,19α)- 15-[(5*S*,9*S*)-5-ethyl-5-hydroxy-9-(methoxycarbonyl)- 1,4,5,6,7,8,9,10-octahydro-2*H*-3,7-methanoazacycloundecino[5,4- *b*]indol-9-yl]-3-hydroxy-16- methoxy-1-methyl-6,7- didehydroaspidospermidine-3,4- dicarboxylate	$C_{46}H_{58}N_4O_9$ 810.974 g/mol	-22.14	128.56