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Insilico Investigation of Potential Inhibitors Targetting Protein Associated with Vitiligo Disorder

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Abstract
Vitiligo is a depigmentation disorder. There were various hypotheses proposed over the years. Based on the previous studies, VIT1 protein has been selected for this study to control the depigmentation by controlling apoptosis process of melanocytes. In this present study computational docking study performed for the following ligands Berberine, Chrysin, Pinocembrine, Piperine against VIT1 protein. Docking study results were analyzed based on the scores and protein-ligand interactions. Docking scores reflects their binding affinity, and hydrogen and hydrophobic interactions reflects protein-ligand stability. Binding affinity as well as protein-ligand stability associated with drug efficacy.

Keywords: Vitiligo, depigmentation, VIT1 protein, Docking

1. Introduction
Vitiligo is a rare and chronic autoimmune disease in which the body attacks its own melanocytes in the epidermis, causing depigmentation in irregular patches of skin and hair. Vitiligo affects 0.5-2% of the world population [1]. Currently there is no cure for vitiligo. Those with the disease often use topical creams and concealers to diminish the appearance of depigmented patches. Those with severe cases have sometimes resorted to skin grafts and photo therapies [2, 3]. However with advent of research that seeks to understand the genetic basis of vitiligo and its connection to other autoimmune disease at their genetic root.
VIT1 gene encodes VIT1 protein and it is located on 2p16 chromosome (Accession number: AAF76888) [4]. It has a single domain putative zinc finger region in N-recognin. N-recognin is an ubiquitin protein ligase, and a part of N-end rule pathway. N-end rule pathway is a main initiator of cellular apoptosis by destruction of specific proteins. The presence of this ubiquitin ligase in VIT1 gene may influence the apoptosis process and leads to the melanocyte death without significant inflammation [5]. By inhibiting VIT1 protein can prevent further melanocyte loss. Some of the herbal constituents like Berberine, Chrysin, Pinocembrine, Piperine selected to study the inhibitory activity against VIT1 protein using computational docking study.
VIT1 protein-Vitiligo associated protein1 has 141 amino acids has no X-ray crystal structure. So Homology modeling used to predict three dimensional structure for further docking analysis.

2 Materials and Methods
2.1 Modeling of VIT1 protein
In molecular modeling following steps are involved (i) Target identification (ii) Template selection (iii) Sequence alignment and model prediction (iv) Validation of the predicted model.

2.1.1 Target Identification
>gi|8571450|gb|AAF76888.1|AF264714_1vitiligo-associated protein VIT-1 [Homo sapiens]
MKDNKIMNNQDAIEKAVSRGQCLYKISSYTSYPMHDFYRCHTCNTTDRNAICVNCI
KKCHQGHDVEFIRH
DRYVAHLLDILPNYFPPHFSNIWVSFCFRFFCDCGAGTLSNPCTLAGEPTHDTDTLYD
SAPPIESNTLQHN. Target structure identified based on the literature survey.

2.1.2 Template Identification
Template has been identified using PSIPRED server which is normally predicts secondary structure for the query sequence. PSIPRED server can also used to predict template sequence for the given VIT1 protein [6].

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Table 1: Query proteins and its templates with sequence identity

S. No	Target	Template	Sequence identity
1	VIT1	3NY1(Ubiquitin ligase-Human)	25.9% (LALIGN)

2.1.3 Sequence alignment and build model

VIT1 alignment was carried out using Clustal-W web based server with the PIR output format. Aligned sequences were used as an input for the modeling of query sequences. Then modeling of the query proteins was carried out using modeler 9v7. From the modeler results least scoring functions were chosen for further validation process [7, 8, 9]

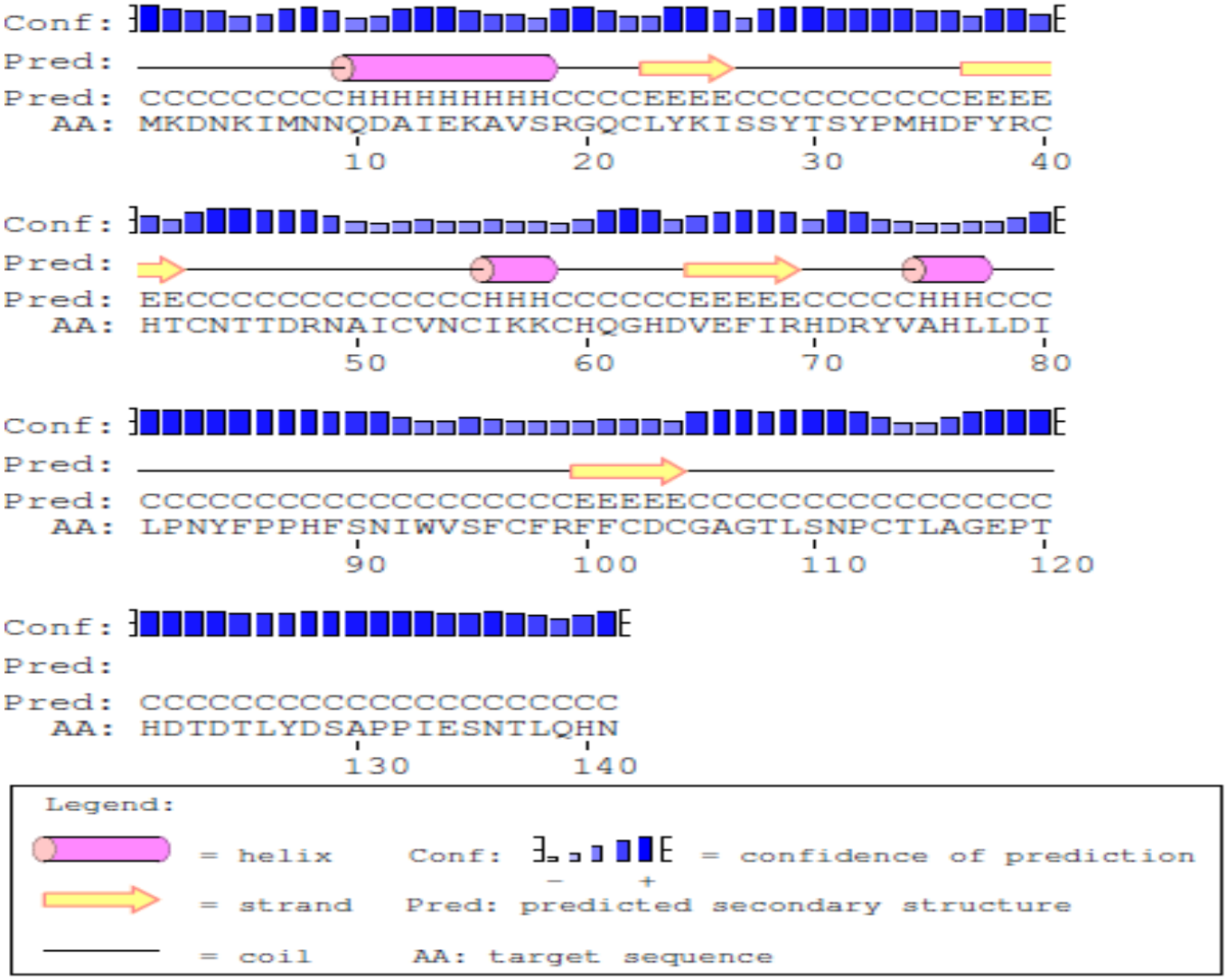


Fig 1: Predicted Secondary structure for VIT1 protein, (PSIPRED Server)

2.1.4 Validation of the Predicted models

The modeled target protein structure then subjected for validation process to ensure the backbone conformation using PROCHECK web based server with the help of RAMACHANDRAN Plot statistics and Reliability of the modeled structures calculated RMSD value by superimposing it to template using UCSF Chimera software.

2.2 Docking studies

2.2.1 ADME Toxicity predictions

ADME prediction based on Lipinski's rule of five they are as follows:

(i) Molecular mass less than 500 Dalton
(ii) High lipophilicity (Express in logP less than 5)
(iii) Less than 5 hydrogen bond donors
(iv) Less than 10 hydrogen bond acceptors
(v) More refractivity should be in between 40-130.

Nearly 40% of drug candidates fail in clinical trials due to poor ADME (Absorption, Distribution, Metabolism and Excretion) properties.

2.2.2 Molecular docking studies

Autodock is widely using molecular docking software. It is freely available for research community. Here Auto dock employed for VIT1 protein docking.

(a) Protein preparation

The selected macromolecule (Protein) preparation process consisting the addition of polar hydrogen, addition of Kollaman charges, and then save the protein file as PDBqt file format.

(b) Ligands Preparation

Ligands are imported in to the Auto dock workspace and set the number of torsions. The ligand needs torsion information to be added depending on which torsions are to be treated as rotatable during the docking. Then the output ligand file should get saved in PDBqt file.

Table2: List of Ligands selected to perform docking studies against VIT1 protein

S. No.	Proteins	Ligands	References
1	VIT1 Protein	1)Berberine 2)Chrysin 3)Pinocembrine 4)Piperine	Primary data (Folklore medicine)

3. Results and Discussion

3.1 Modeled structure prediction and model validation

In this present study recently identified VIT1 gene encoding VIT1 (Vitiligo associated protein 1) protein is also responsible for melanocyte loss in vitiligo patients. So here VIT1 protein took it as target protein and the sequence is retrieved from NCBI protein database. Then similar template structure 3NY1 selected based on the sequence similarity using PSIPRED web based server. Based on the template (3NY1) selection target protein (VIT1) modeled using MODELLER software (Figure 2). Predicted model RMSD value while super imposing on the template structure is 0.862Aº (Figure 4), and the RAMACHANDRAN PLOT statistics shows 96% of the amino acids are in the favored and additional allowed region(Figure 3) (Table 3). The results were in allowed region. So based on the RMSD and RAMACHANDRAN PLOT STATISTICS values theoretically predicted modeled region shows good quality.

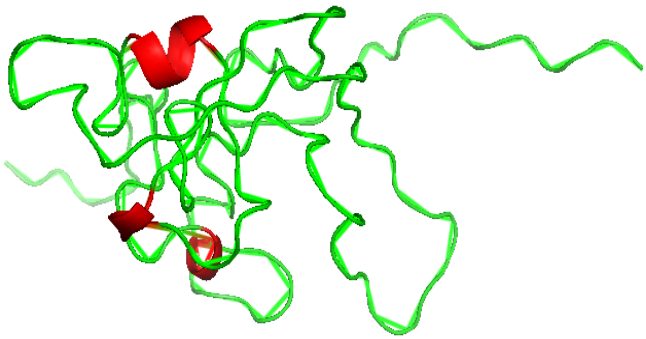


Fig 2: Predicted three-dimensional structure of VIT1 protein model. structures are shown in secondary structure mode using Pymol; αhelices, loops,and βsheets shown Red, Green and Yellow respectively.

Table 3: Ramachandran plot statistics for Modeled protein structures calculated using PROCHECK

Parameter	Value for VIT1
Most favored region (%)	73.8
Additional allowed region (%)	22.2
Generously allowed region (%)	0.8
Disallowed region (%)	3.2
G.factor	-0.39
Main chain bond length	-0.35
Main chain bond angle	-0.98

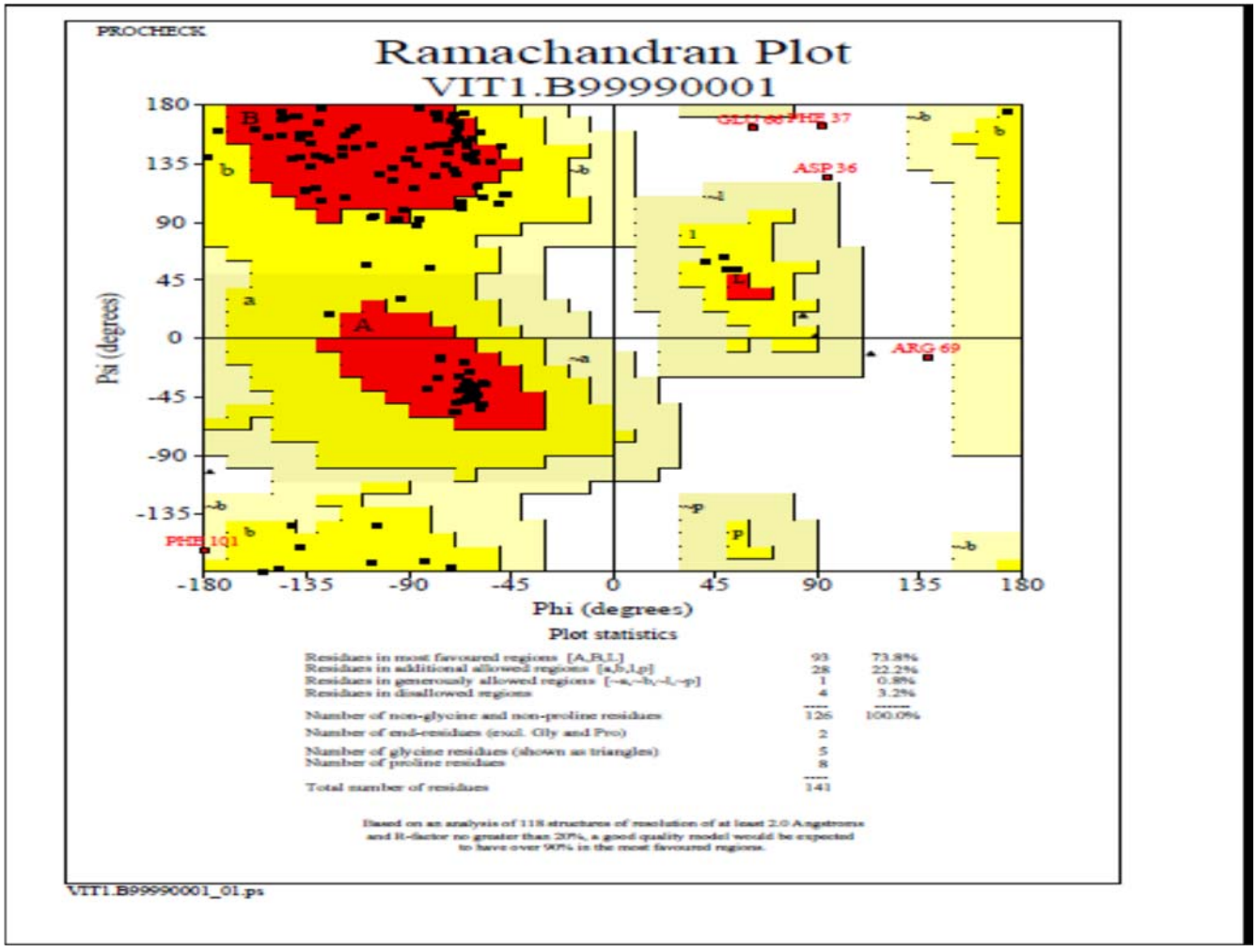


Fig 3: Ramachandran plot for the 3D modeled VIT1 protein. The most favoured regions are colored red; additional allowed, generously allowed and disallowed regions as shown as yellow, light yellow and white fields respectively

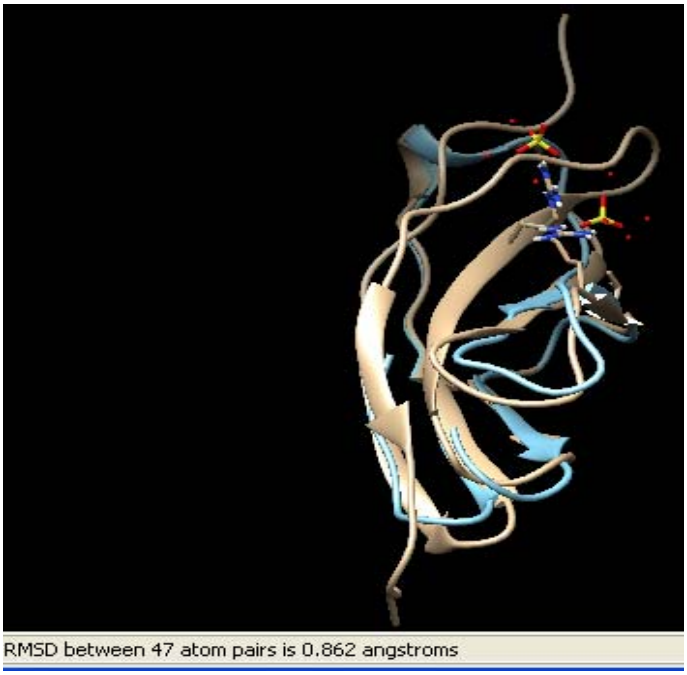


Fig 4: superimposition of modeled VIT1 (target) on 3NY1 (template).

3.2 Molecular Docking studies

(i) Analysis of hydrogen bond

Based on the Auto dock results, Binding site residues and binding energy were analyzed (Table 4). Here this protein is responsible for N-end rule pathway, in VIT1 protein 34-64 amino acids in its length responsible for this pathway. Pinocembrine and Chrysin are the ligand molecules forming hydrogen bond with following amino acid residues ASN49, THR45, GLY117(Figure 5). From these **ASN49** amino acid is present in **the zinc finger region** which is responsible for **N-end rule pathway.** So based on this **Pinocembrine and chrysin** may has the property **to inhibit the N-end rule pathway** [10]. It helps to **prevent** further **melanocyte loss** in Vitiligo patients.

Table 4: Interaction energies and Molecular hydrogen bond interactions of VIT1 protein against ligands

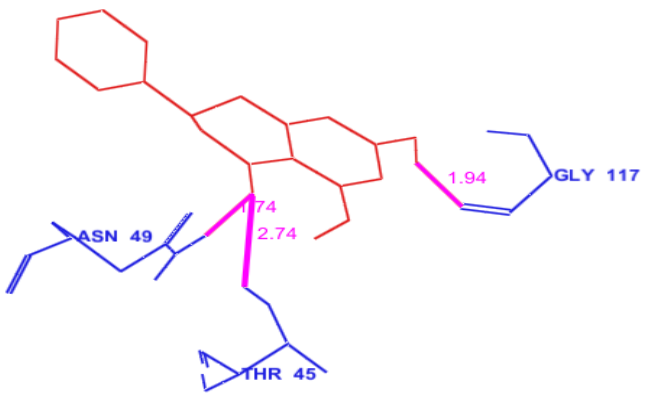
S. No	Ligand name	Ligand atom: VIT1Protein	Interacting Residues	Bond Length Å	Interaction Energy (kcal/mol)
1	Pinocembrine	H:OG1 O:HD21 O:HN H:O	THR45 **ASN49** GLY117 GLY117	2.74 1.74 1.94	-8.0
2	Chrysin	H:O O:HD21	GLY117 **ASN49**	1.88 1.87	-7.77
3	Piperine	O:HN	VAL17	1.95	-8.81
4	Berberine	O:HZ3 O:HN	LYS15 VAL17	2.05 1.75	-8.19

(i) Analysis of hydrophobic interactions

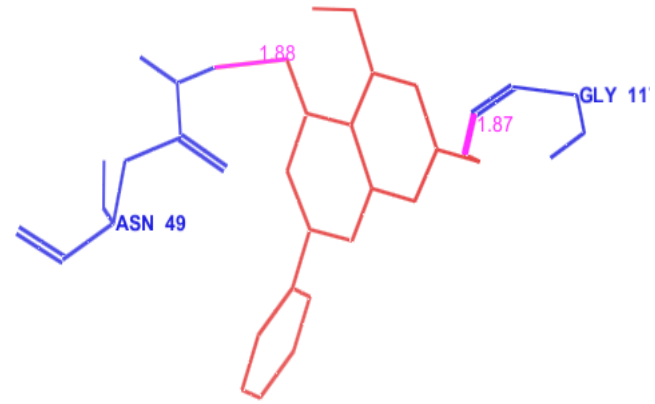
PHE96, PHE98, PHE100, PHE101, ALA106, ALA116, PRO119 are the common hydrophobic interacting residues found in top scoring docked poses of Pinocembrine and chrysin.

4. Conclusion

Molecular docking studies (Table 2) screened ligands were selected to perform docking studies against VIT1 protein to inhibit the protein to control further melanocyte loss. Berberine, Chrysin, Piperine, Pinocembrine ligands were tested for toxicity predictions and results were all in acceptable range. Selected screened ligands allowed to dock against VIT1 protein (Vitiligo associated protein 1) and said to be bound well in the active site of the VIT1 protein based on the more negative scores. Based on the docking studies results scores reflects their binding affinity towards target protein, their hydrogen and hydrophobic interactions reflects their protein-ligand stability. Binding affinity as well as protein ligand stability plays important role in the therapeutic activity of the ligand. This preclinical evaluation helps to screen compounds for further *invivo and invitro* analysis.

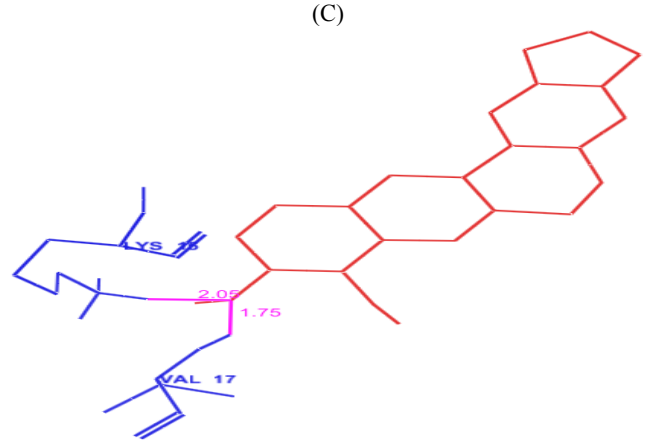


(A)



(B)

(C)



(D)

Figure 5: Interacting residues of VIT1 protein with top scoring ligand poses of (a) Pinocembrine (b) Chrysin. (C) Piperine (D) Berberine

5. References

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