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A SEARCH FOR NATURAL ANALOGS OF PSORALEN INVOLVED IN THE TREATMENT OF VITILIGO: A COMPUTATIONAL APPROACH

SUJATHA, P L[1], KUMARASAMY, P[2], PREETHA S P[3] & BALACHANDRAN C[4]

[1]College of Food and Dairy Technology, Koduvalli, Chennai, Tamil Nadu, India

[2]Department of Bioinformatics, Madras Veterinary College, Chennai, Tamil Nadu, India

[3]Department of Veterinary Pharmacology, Madras Veterinary College, Chennai, Tamil Nadu, India

[4]The Dean, Madras Veterinary College, Chennai, Tamil Nadu, India

ABSTRACT

Vitiligo is an acquired skin disease which includes patterned loss of melanin pigment due to destruction of melanocytes. Vitiligo associated protein-1, also called as F-box only protein 11 is responsible for this disease which is encoded by a gene VIT-1 located at the chromosome 2p16 position. The so far known drug compound to inhibit this protein is psoralen. Similar structural natural compounds were selected to study the inhibition of the protein like Ginko-Biloba (maidenhair tree leaves), Turmeric (Curcumin), khella (Ammivisnaga). Analogue search is done for each natural compound and virtual screening was performed for all analogs (300) from which best 5 lead compounds were selected using Argus Lab. Further with resultant lead compounds docking is performed with the target protein using Discovery Studio 4.0. Best analogue from each natural compound was selected by their binding energy values. Since the compounds are only modified variants of natural template molecules, they may be presumed to have more activity but less toxicity compared to their counterparts in modern medicine. The product(s) is thus envisaged to be better variant(s) of existing natural molecule(s) designed using bioinformatics tools and semi-synthetically prepared under laboratory conditions for further testing and validation as potential drug candidates.

KEYWORDS: Vitiligo, Psoralen, Virtual Screening, Analogs, Interaction Studies

INTRODUCTION

Vitiligo is one of the most common skin disorders, affecting about 1% of the world population. Vitiligo (vit-ill-EYE-go) is a disorder in which white patches of skin appear on different parts of the body. This happens because the cells called melanocytes which that make pigment (color) in the skin are destroyed. Vitiligo can also affect the mucous membranes (such as the tissue inside the mouth and nose) and the eye. It is associated with autoimmune and inflammatory diseases, commonly thyroid over expression and under expression. Vitiligo affects both genders and all races, but is more noticeable in people with darker skin. The famous pop singer late Michael Jackson had this disorder. Half the people who have vitiligo develop it before age 20; most develop it before their 40th birthday. Vitiligo seems to be somewhat more common in people with certain autoimmune diseases. These autoimmune diseases include hyperthyroidism (an overactive thyroid gland), adrenocortical insufficiency (the adrenal gland does not produce enough of the hormone called corticosteroid), alopecia areata (patches of baldness), and pernicious anemia (a low level of red blood cells caused by the failure of the body to absorb vitamin B_{12}). Vitiligo may also be hereditary; that is, it can run in families. Children whose

parents have the disorder are more likely to develop vitiligo.

Figure 1: Vitiligo

(PhotoCourtesy: http://img3.rnkr-static.com/user_node_img/79/1574447/870/michael-jackson-recording-artists-and-groups-photo-u38.jpg)

Many people with vitiligo can feel depressed and start lacking confidence because of their white spots. These people are tensed and want to get back their lost skin pigmentation. For such people a number of therapies are available. The most proven techniques out of these are UV therapy and herbal treatments. Herbal treatments are mainly based on Psoralens. Psoralen occurs naturally and sensitizes human skin to the tanning effect of sun light by enhancing pigmentation.

MATERIALS AND METHODS

Biological databases such as PDB, PDBSUM, GENBANK, Drug Bank, PIR were used for collecting sequences and structures. Tools such as Corina, Blast, Modeller, ChemSpider, Vega, Argus Lab and Discovery Studio were used for the research work.. The amino acid sequence of target protein VIT-1(Vitiligo Associated Protein) was retrieved from NCBI which consists of 141aa. 1JA9 was used as template for modelling. The model obtained through modeller was validated through ramachandran plot. It has been proved from studies that some of the natural compounds play an effective role in treating vitiligo disease. Among those compounds; three major effective compounds were taken for this Study. They are as follows

- khellin(ammivisnaga)
- curcumin(turmeric)
- ginkgobiloba (maidenhair tree leaf extract)

Analogs for the tree individual natural ligands were identified based on the similarity in compounds and their conformers. For each ligand, 100 analog were chosen, hence a total of 300 of analogs were screened to find the most efficient analog for the VIT-1 protein receptor. The various analogs selected are as follows.

Table 1: Analogs

ANALOGS FOR KHELLIN		ANALOGS FOR GINKO BILOBA		ANALOGS FOR CURCUMIN	
CID_3828	CID_762657	CID_441293	CID_10049419	CID_146723	CID_5431653
CID_10167	CID_762926	CID_441294	CID_10071931	CID_238783	CID_5468330
CID_108104	CID_799827	CID_441295	CID_10344455	CID_382903	CID_5468459
CID_621572	CID_904636	CID_441296	CID_10431574	CID_396718	CID_5469424
CID_631791	CID_928054	CID_6324617	CID_11145311	CID_442783	CID_5469426
CID_6976557	CID_1323630	CID_6325203	CID_11154476	CID_969516	CID_5470596
CID_6976559	CID_5319436	CID_6325204	CID_11385169	CID_3525493	CID_5470600
CID_8016427	CID_5320355	CID_6325205	CID_11502738	CID_3544536	CID_5470601
CID_8016506	CID_5320357	CID_6325206	CID_11826309	CID_4436278	CID_5470829
CID_9973037	CID_6451552	CID_6325207	CID_11876970	CID_5247675	CID_5703486
CID_10022767	CID_6936044	CID_6419993	CID_11876971	CID_5281767	CID_5855330
CID_10563005	CID_10804438	CID_6708664	CID_11988297	CID_5316043	CID_5856764
CID_10662089	CID_10861100	CID_6917929	CID_15944764	CID_5317593	CID_5898023
CID_10733108	CID_10970882	CID_9867869	CID_15944765	CID_5324476	CID_5924427
CID_11515902	CID_11090240	CID_9909368	CID_15965401	CID_5354238	CID_6440133
CID_11522089	CID_11427240	CID_15972426	CID_15966490	CID_5372323	CID_6441913
CID_11537346	CID_11494340	CID_15972884	CID_15971012	CID_9796428	CID_6448631
CID_11550827	CID_11508881	CID_15973422	CID_15971266	CID_9800110	CID_6474893
CID_11552041	CID_11560799	CID_15973638	CID_21631697	CID_9978040	CID_6477182
CID_11552115	CID_11566472	CID_15976427	CID_21631699	CID_10267390	CID_6477468
CID_11559445	CID_11579779	CID_15976688	CID_21725806	CID_10424759	CID_6477469
CID_11616230	CID_11602815	CID_15976709	CID_21725807	CID_10469828	CID_6477637
CID_11623610	CID_11638263	CID_15976789	CID_21733097	CID_10469829	CID_6604598
CID_11625928	CID_11660367	CID_15977923	CID_21733099	CID_10500096	CID_6610332
CID_11630455	CID_11683195	CID_16211418	CID_21733100	CID_10543821	CID_6977116
CID_11631720	CID_11697442	CID_16219435	CID_22836325	CID_10573616	CID_6989249
CID_11780237	CID_11709791	CID_16406429	CID_22836632	CID_10595440	CID_7946294
CID_11848270	CID_11710039	CID_16759509	CID_23229639	CID_10666836	CID_8854691
CID_11848272	CID_11716667	CID_17751010	CID_23230326	CID_10760444	CID_9548762l
CID_11848273	CID_11717270	CID_17759915	CID_23230335	CID_10763284	CID_10892754
CID_11848335	CID_12305449	CID_20704512	CID_23232093	CID_10764042	CID_10904292
CID_11848959	CID_12714981	CID_23304392	CID_23232291	CID_10812252	CID_11257493
CID_11848961	CID_12838742	CID_23307235	CID_23267563	CID_10861871	CID_11382654
CID_11848962	CID_12919412	CID_23307248	CID_23304389	CID_14837578	CID_11474949
CID_11849012	CID_12919414	CID_23307634	CID_23304391	CID_14839244	CID_11495229
CID_11849018	CID_12919431	CID_23640525	CID_24883995	CID_14839249	CID_11550934
CID_11849078	CID_12963177	CID_23747848	CID_24883996	CID_15552284	CID_11617052
CID_12221187	CID_12988082	CID_23817960	CID_24893495	CID_15941759	CID_11617870
CID_13300793	CID_13142723	CID_24721483	CID_24893496	CID_15986781	CID_11618620
CID_13300794	CID_13300788	CID_24721502	CID_25078430	CID_15986862	CID_11717379
CID_13830798	CID_13300789	CID_24728714	CID_25081385	CID_15986863	CID_14145089
CID_14183076	CID_13300790	CID_24741540	CID_25199910	CID_15987054	CID_14214498
CID_14214851	CID_16637748	CID_24883855	CID_25200019	CID_16087293	CID_14407221
CID_14721979	CID_21679807	CID_24883856	CID_25200020	CID_16087300	CID_14695154
CID_15482560	CID_21785683	CID_24883857	CID_25200021	CID_16087306	CID_17874170
CID_15568808	CID_23258519	CID_24883993	CID_25200031	CID_16718107	CID_19888953
CID_24995141	CID_23258521	CID_24883994	CID_25200072	CID_16739270	CID_20728512
CID_24995448	CID_23876358	CID_42616587	CID_25200283	CID_16760039	CID_20835150
CID_24995451	CID_24832508	CID_42616677	CID_25200290	CID_2084238	CID_21160552
CID_24995452	CID_26435482			CID_20842398	CID_21632890

A database file was constructed for 300 analogs using Vega ZZ software. Screening was done using Argus Lab. From the virtual screening results, top five analogs from each compound were selected according to their ranking and are short listed for docking. Docking is performed using Discovery studio tool. Resulting interactions can be viewed using

DISCOVERY STUDIO 2.5 molecular viewer,

Analog 1, Analog 2, analog 3 which exhibited superior binding energy and inter molecular interactions were compared to find the most effective ligand. Hence, from my research and analysis, I conclude that analog of curcumin is found to be very efficient and thus it could be used as a drug for vitiligo disease after testing its toxicity and side effects

RESULTS AND DISCUSSIONS

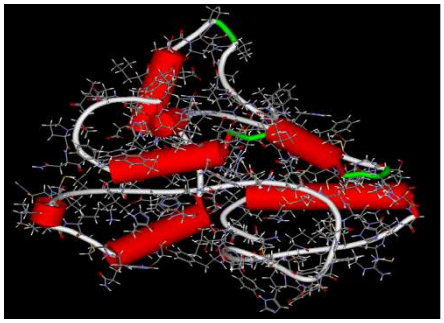

Figure3: Modelled Structure of VIT-1 Protein

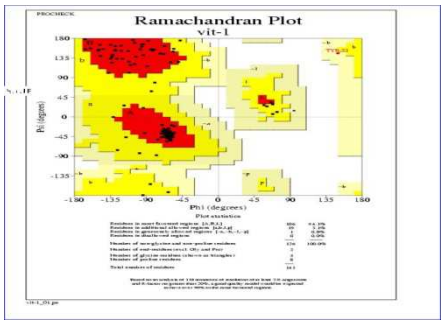

Figure 4: Ramachandran Plot for VIT-1 Protein

RAMACHANDRAN PLOT

PLOT STATISTICS

Number of residues in favoured region: 106-94.1%

Number of residues in additional allowed region: 19-5.1%

Number of residues in generously allowed region: 1-0.8%

Number of residues in disallowed region: 0-0%

VIRTUAL SCREENING

A database file was constructed for 300 analogs using Vega ZZ software. Screening was done using Argus Lab.

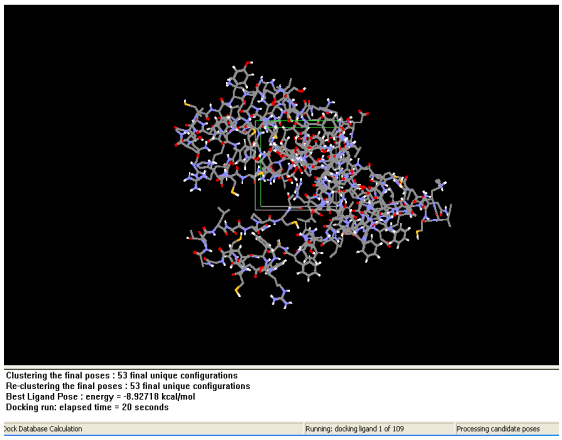

Figure 5: Virtual Screening of Compounds in Argus Lab

SELECTION OF POTENTIAL LIGANDS

From the virtual screening result, top five analogs from each compound were selected according to their ranking and are short listed for docking. Those best analogs have been screened and the top 5 from each natural ligand are listed below

Table 2: Top Five Potential Ligands

	BEST ANALOGS OF NATURAL COMPOUNDS		
S. NO	KHELLIN	GINKO BILOBA	CURCUMIN
1.	CID11848272	CID441294	CID6440133
2.	CID11848335	CID15976789	CID14214498
3.	CID11848961	CID25200290	CID6448631
4.	CID11849078	CID24721483	CID3525493
5.	CID11849018	CID24883993	CID19888953

MOLECULAR DOCKING

The molecular interaction between protein VIT1 and the ligands were obtained using AUTO DOCK 4.0. The molecular docking was performed using Genetic Algorithm - Least Square (GA-LS) algorithm optimized with Auto Dock tool. From the several poses of docking, the complex formed with least energy and with the top rank chosen as the stable conformation.

DOCKING RESULTS

Table 3: Energy Table for the Best Analogs Obtained Using AUTODOCK

ANALOGS OF	COMPOUND ID	COMPOUND NAME	CHEMICAL FORMULA	BINDING ENERGY (Kcal/mol)
KHELLIN	CID11849018	1-(4-methoxy-7-phenethyloxy-benzofuran-5-yl)ethanone	$C_{19}H_{18}O_4$	-6.99
CURCUMIN	CID3525493	1-(3,4-dihydroxyphenyl)-7-(3-hydroxy-4-methoxy-phenyl)hepta-1,6-diene-3,5-dione	$C_{20}H_{18}O_6$	-8.99
GINKO BILOBA	CID441294	ginkgolide B	$C_{20}H_{24}O_{10}$	-5.12

INTERACTION BETWEEN VIT-1 AND BEST THREE ANALOGS

ANALOG 1

Name: 1-(4-methoxy-7-phenethyloxy-benzofuran-5-yl) ethanone

Analog of: khellin Comp id: CID11849018

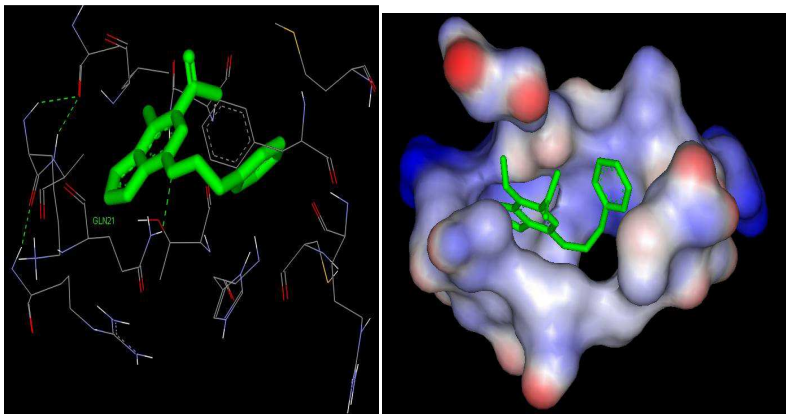

Figure 6: Interaction between VIT-1 and Analog 1

BINDING ENERGY BETWEEN VIT-1 AND ANALOG 1

Binding Energy : -6.99

kI : 7.58uM

Intermolecular Energy : -8.27

Internal Energy : -0.95

Torsional Energy : 1.79

Unbound Extended Energy: -0.44

Cluster RMS : 0.0

Ref RMS : 81.82

ANALOG 2

Name : 1-(3, 4-dihydroxyphenyl)-7-(3-hydroxy-4-methoxy- Phenyl) hepta-1,6-diene 3,5-dione

Analog of : CURCUMIN

Comp id : CID3525493

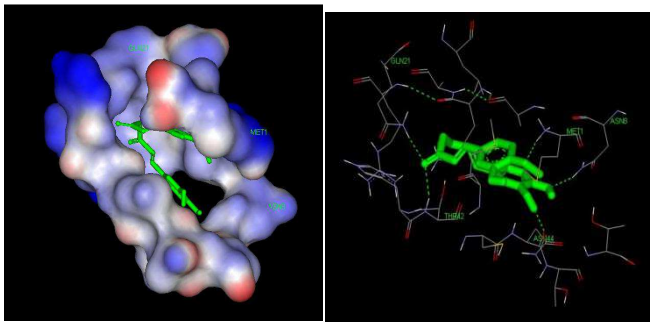

Figure 7: Interactions between VIT-1 and Analog 2

BINDING ENERGY BETWEEN VIT-1 AND ANALOG 2

Binding Energy	:-8.99
kI	:257.42nM
Intermolecular Energy	:-10.77
Internal Energy	:-1.74
Torsional Energy	: 2.98
Unbound Extended Energy	:-0.54
Cluster RMS	: 0.0
Ref RMS	: 84.31

ANALOG 3

Name	: ginkgolide B
Analog of	: Ginko biloba
Compound Id	: CID441294

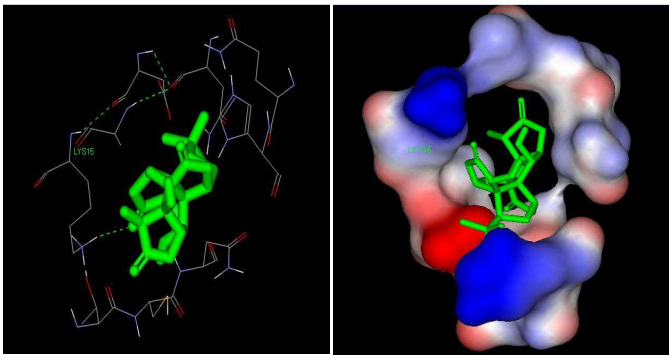

Figure 8: Interaction between VIT-1 and Analog 3

BINDING ENERGY BETWEEN VIT-1 AND ANALOG 2

Binding Energy	: -5.12

kI :176.63uM

Intermolecular Energy :-5.88

Internal Energy :-0.4

Torsional Energy : 1.19

Unbound Extended Energy: 0.03

Cluster RMS : 0.0

Ref RMS: 77.0

INTERACTION DETAILS OF ANALOGS AND COMMERCIAL DRUGS

The Protein-Ligand interaction plays a significant role in structural based drug designing. In the present work we have taken the receptor Vitiligo-associated protein (VIT-1) and identified the drugs that were used against Vitiligo.

When the receptor was docked with the commercial drugs the energy value obtained were L-phenylalanine (-4.83), Methoxsalen (-6.1), Monobenzone (-5.92). When the natural analogs were docked against the same receptor the energy value obtained was Curcumin Analog (-8.99), Ginkobiloba Analog (-5.12), Khellin(-6.99) from this we can conclude that Curcumin, an analogue of natural active constituents is better than the commercial drugs available in the market.

Table 4: Drug Interactions

COMMERCIAL DRUGS	BINDING ENERGY (kcal/mo)	INTERACTING RESIDUES	NATURAL ANALOGS	BINDING ENERGY	INTERACTING RESIDUES
L-phenyl alanine	-4.83	THR41, ARG39	**Curcumin**	**-8.99**	**MET1, ASN8, GLN21, THR42, ASN44**
Methoxsalen	-6.1	HIS41, THR42	Ginkobiloba	-5.12	LYS15
Monobenzone	-5.92	ARG39	Khellin	-6.99	LEU23, ARG49

Analog 1, 2 and 3 which exhibited superior binding energy and the inter molecular interactions were compared to find the most efficient ligand. Hence, curcumin (analog 2) is found to be very efficient because of its binding energy with the target protein VIT-1.

ADMET/TOX ANALYSIS FOR CURCUMIN ANALOG

ABSORPTION (4 Items)

	Human Intestinal Absorption (HIA, %)	**90.521780**
	in vitro Caco-2 cell permeability (nm/sec)	19.0353
	In Vitro MDCK Cell Permeability (Nm/Sec)	**25.8989**
	in vitro skin permeability (logKp, cm/hour)	-2.92731

DISTRIBUTION (2 Items)

	In Vitro Plasma Protein Binding (%)	**88.836084**
	in vivo blood-brain barrier penetration (C.brain/C.blood)	0.533855

TOXICITY

Ames Test (7 Items)

	Ames TA100 (+S9)	**Negative**
	Ames TA100 (-S9)	negative
	Ames TA1535 (+S9)	**Negative**
	Ames TA1535 (-S9)	negative
	Ames TA98 (+S9)	**Negative**
	Ames TA98 (-S9)	negative
	Ames test	non-mutagen

Carcinogenicity (2 Items)

	Carcinogenicity (Mouse)	**Negative**
	Carcinogenicity (Rat)	positive

Analog 1, Analog 2, analog 3 which exhibited superior binding energy and inter molecular interactions were compared to find the most effective ligand. Hence, from my research and analysis, I conclude that analog of curcumin is found to be very efficient and thus it could be used as a drug for vitiligo disease after testing its toxicity and side effects.

CONCLUSIONS

In order to treat Vitiligo disease using natural compounds as ligands, the target protein VIT-1 was modeled using MODELLER9v7. It was validated through RAMPAGE server and the ramachandran plot was obtained to be 95% of residues in the favoured region. The binding sites of VIT-1 were spotted using Q-site finder. The analogs of the natural compounds were virtually screened using ARGUS LAB, to find the potential analogs from each natural compounds based on the best docking score. As a result of screening, 5 analogs for each compound were docked to find analyse their interaction with the target protein.

Analog 1, Analog 2, analog 3 which exhibited superior binding energy and inter molecular interactions were compared to find the most effective ligand. Hence, from our research and analysis, we conclude that analog of curcumin is found to be very efficient and thus it could be used as a drug for vitiligo disease after testing its toxicity and side effects.

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