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Research Article

IDENTIFICATION OF NOVEL SELECTIVE ANTAGONISTS FOR BESTROPHIN-1 PROTEIN BY HOMOLOGY MODELING AND MOLECULAR DOCKING

PRIYA RAJENDRA RAO

Research Associate, School of Bio Science and Technology, Plant Biotechnology Division, VIT University, Vellore-632014, Tamil Nadu.
Email: priya.bioinforamtics@gmail.com

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ABSTRACT

Bestrophin-1 (BEST1), also known as VMD2 belongs to the bestrophin gene family. It codes for a protein that may act as a channel to control the movement of negatively charged chloride ions into or out of cells in the retina or may regulate voltage-gated L-type calcium-ion channels. Thus the mutation in this gene leads to progressive vision loss known as Best Vitelliform macular dystrophy. It is inherited in an autosomal dominant manner. Hence, Bestrophin-1 is an important constituent of this disease which is chosen for structure prediction using homology modeling, with the aid of software called insight II. The 3D structure is predicted and the final model is refined by energy minimization. The quality of the refined model is assessed using PROCHECK. The interaction between the predicted structure of Bestrophin-1 and its potential inhibitors namely Verteporfin, Fluorescein, L- Cystine and Fluocinolone Acetonide is analyzed by *in silico* method with the help of Autodock. The results indicate that certain residues like ARG 82, ARG 84 and HIS 197 are highly conserved across the active site stretches of bestrophin-1 and the inhibitor Verteporfin gives best interaction in this active site than other compounds. This study provides an insight into the structure of bestrophin-1 and also gives an idea about potential sites responsible for inhibitory action that could further be substantiated by experimental investigations.

Keywords: Bestrophin-1, Vitelliform macular dystrophy, *Pseudomonas syringae*, Insight II, Autodock 4.0.

INTRODUCTION

Vitelliform macular dystrophy is a genetic eye disorder that can cause progressive vision loss. This disorder affects the retina, specifically cells in a small area near the center of the retina by causing a fatty yellow pigment (lipofuscin) which build up in cells under the macula[1,2,3]. The macula is the yellow oval spot at the center of the retina (back of the eye) that contains blood vessels and nerve fibers. The macula is responsible for sharp central vision. The abnormal accumulation of this lipofuscin can damage cells that are critical for clear central vision, which is needed for detailed tasks such as reading, driving, and recognizing faces. As a result, people with this disorder often lose their central vision and may experience blurry or distorted vision. Vitelliform macular dystrophy does not affect side (peripheral) vision or the ability to see at night. There are two forms of vitelliform macular dystrophy with similar features. The early-onset form known as Best Vitelliform macular dystrophy usually appears in childhood; however, the onset of symptoms and the severity of vision loss vary widely. The adult-onset form begins later Adult vitelliform macular dystrophy usually in middle age, and tends to cause relatively mild vision loss. The two forms of vitelliform macular dystrophy each have characteristic changes in the macula that can be detected during an eye examination.

Best disease, the early-onset form of vitelliform macular dystrophy, it inherited in an autosomal dominant pattern of inheritance, which means one copy of the altered gene in each cell is sufficient to cause the disorder. It characterized by large deposits of lipofuscin-like material in the sub retinal space, which creates characteristic macular lesions resembling the yolk of an egg ('vitelliform'). Later, the affected area becomes deeply and irregularly pigmented and a process called 'scrambling the egg' occurs. The disorder is progressive and loss of vision may occur. In most cases, an affected person has one parent with the condition. Vitelliform macular dystrophy is a rare disorder; its incidence is unknown.

Bestrophin 1(VMD2), also known as hBest1, is a human gene. The BEST1 gene is located on the long (q) arm of chromosome 11 at position 13[4,5,6]. This gene encodes a member of the bestrophin gene family. Mutations in the VMD2/Best1 gene cause Best vitelliform macular dystrophy. The VMD2/Best1 gene provides instructions for making this bestrophin protein. Bestrophin is characterized with highly conserved N-terminus with four to six trans membrane domains[14,15]. Although its exact function is unknown, this protein likely act as a channel that controls the movement of negatively charged chlorine atoms (chloride ions) into or out of cells in the retina or may regulate voltage-gated L-type calcium-ion channels[8,9,10,11]. Mutations in the VMD2/Best1 gene probably lead to the production of an abnormally shaped channel that cannot regulate the flow of chloride. How these malfunctioning channels are related to the buildup of lipofuscin in the macula and progressive vision loss is uncertain. Mutations in the peripherin/RDS gene have been found in a small proportion of cases suggesting that AVMD is a genetically heterogeneous phenotype[17].

Bestrophin are generally believed to form calcium-activated chloride-ion channels in epithelial cells but they have also been shown to be highly permeable to bicarbonate ion transport in retinal tissue. The Cl– currents in *Drosophila* S2 cells, which are mediated by bestrophin, are dually regulated by Ca2+ and cell volume[12]. Mutations in this gene are also responsible for juvenile-onset vitelliform macular dystrophy.

Bestrophin is found in a thin layer of cells at the back of the eye called the retinal pigment epithelium (RPE). This cell layer supports and nourishes the retina, which is the light-sensitive tissue that lines the back of the eye. The retinal pigment epithelium is involved in the growth and development of the eye, maintenance of the retina, and the normal function of specialized cells called photoreceptors that detect light and color. when bestrophin-1 expressed in RPE-derived cells, bestrophin-1 altered the characteristics of L-type Ca2+ channel activity, suggesting that bestrophin-1 plays a role in regulation of Ca2+ entry via L-type Ca2+ channels[13].

Bestrophin functions as a channel across cell membranes in the retinal pigment epithelium. Charged chlorine atoms (chloride ions) are transported through these channels in response to cellular signals. Some studies suggest that bestrophin may also help regulate the entry of charged calcium atoms (calcium ions) into cells of the retinal pigment epithelium. Other potential functions of bestrophin are under study.

METHODS AND MATERIALS

Target sequence retrieval

The amino acid sequence of vitelliform macular dystrophy 2 (Best disease, bestrophin), was obtained from the NCBI database (accession no.: EAW73983, entry name: bestrophin-1 and alternative name Vitelliform macular dystrophy 2, TU15B). It was ascertained that the three dimensional structure of bestrophin-1 from VMD gene is not available in PDB database, hence an attempt has been made in the present study to determine the structure of bestrophin-1.

Template identification

Using the Gapped-BLAST[20] through NCBI[21], the homologous structure of Crystal Structure of Tabtoxin Resistance was identified, which was used as template for the homology modeling of the bestropin-1. The sequence alignment was done using the online version of ClustalW[22]. The results yielded by NCBI BLAST against the PDB database revealed that Tabtoxin Resistance from *pseudomonas syringae* (PDB ID: 1j4j) with a resolution of 1.55 Å as a suitable template. The template and the target have 42% of residues identical with an *E*-value of 9.0.

Primary and secondary structure prediction:

Primary structure prediction for bestrophin-1 target protein were performed using the protparam web Server to confirm the sequences details, which show 604 amino acid in length with molecular weight of 69096.2 and theoretical pI of 6.37.It is also used to identify the composition of each amino acid residues and its atomic composition. The total number of negatively charged residues (Asp + Glu): 63 and total number of positively charged residues (Arg + Lys): 56 are also evaluated. Secondary structure predictions were performed using the predator web-server and the Multivariate Linear Regression Combination (MLRC) in order to confirm that the bestrophin-1 protein is not having proper secondary structure; hence an attempt has been made to model the structure.

Model generation

Homology modeling is a type of computational techniques in protein 3-dimensional structure prediction that seek to generate a model of a protein's tertiary structure based on its amino acid sequence. Homology modeling relies on a pair wise sequence alignment between the target amino acid sequence and the template sequence whose structure has been experimentally determined. The three dimensional structure of bestrophin-1 has been predicted using insight II[18]. The insight II is the command based program in which the homology module is used to load the reference molecule and target molecule to perform an alignment between the target and template sequence. Then the alignment module is used to perform pairwise alignment. A rough 3D model was then obtained using the build module based on the generated alignment.

Refinements

The rough model generated was subjected to energy minimization using insight II module to eliminate bad contacts between protein atoms. In insight II the Discover 3 module is used to calculate the energy of the modeled protein. The energy minimization is done by using various force fields such as CFF, CVFF, AMBER, CHARMm and ESFF which are specified through the force field/ select command

Structure validation and Loop refinement

The 3D rough model of bestrophin-1 constructed were evaluated using PROCHECK suite of programs[19] that provides a clear check on the stereochemistry of a protein structure. The output of PROCHECK comprises a number of plots in different types of file format and a comprehensive residue-by-residue listing. The best bestrophin-1 model was selected based on the PROCHECK analysis, which includes checks on dihedral angles, covalent geometry, chirality, planarity, non-bonded interactions, disulphide bonds, stereo chemical parameters, main-chain hydrogen bonds, parameter comparisons, and residue-by-residue analysis. The Ramachandran plot for the rough models obtained from comparative prediction was validated in PROCHEK analysis. The Ramachandran plot used to visualize dihedral angles φ against ψ of amino acid residues in protein structure. The possible conformations of φ and ψ angles for a polypeptide are clearly predicted using the plot. In Ramachandran plot, the white region indicates disallowed region for all amino acids except glycine, which does not have side chain. The red regions are allowed regions namely the beta-sheet and alpha-helical conformations where there are no steric clashes. The yellow areas are called partially allowed regions of left handed helix where in the atoms are allowed.

Active site Identification

The ligand binding site is a small region or pocket, where ligand molecules can bind to activate the target protein and produce the desirable effect. Thus, identification of the binding site residues in the target protein structure is of high importance in computer aided drug designing. But identification of accurate binding site residues is difficult because the proteins are capable of undergoing conformational changes[24]; but still there are few computational tools like Ligsitecsc[25], Q site finder[26] and CASTp[27] that can capably predict the binding site residues. Q site finder identifies the favorable ligand binding sites by using the Van der Waal's probes and interaction energy. The possible ligand binding sites of final modeled bestrophin-1was searched using Q-Site Finder. From the Q-'Site Finder possible 10 binding sites were obtained for bestrophin-1.The predicted sites are interesting and its shows the amino acid residues position 17- 22, 78-98 and 197-208 are predicted to conserved with the active site. Thus in this study ARG 82, ARG 84 and HIS 197 are chosen as the more favorable sites to dock the substrate.

Docking the inhibitors with the active site of bestrophi-1

The modeled structure of bestrophin -1 protein was subjected to docking studies, the following four compounds namely Verteporfin, Fluorescein, L- Cystine and Fluocinolone Acetonide as show in fig were found to effectively inhibit bestrophin-1 protein which causes the diseases called vitelliform macular dystrophy. The ligand, are taken from Drug bank database. The structure was imported to Auto Dock software version 4.0 and subsequently docking grid was generated. Polar hydrogen were added to the receptor, Kollaman charges were assigned and solvation parameters were added with the "Addsol" option in AutoDock. Docking was carried out using the default parameters, the Lamarkian genetic algorithm by adding Gasteiger charges (computed using ANTECHAMBER) and running 10,000 steps of energy minimization, the generalized amber force field was used. Throughout the docking simulation, the target protein is kept rigid in the grid (50 X 50 X 50A ˚ in the x, y and z dimensions). The ligand being docked is usually flexible, and therefore explores an arbitrary number of torsional degrees of freedom in addition to the six spatial degrees of freedom spanned by the translational and rotational parameters. Docking simulations were carried out with an initial population of 50 individuals, and a maximum number of 25,000 energy evaluations were used as the docking parameters for obtaining the final docked structures. The best ligand-receptor structure from the docked structures was chosen based on lowest energy and minimal solvent accessibility of the ligand.

RESULTS AND DISCUSSION

Homology modeling of Bestrophin-1

The absence of the three dimensional structure for bestrophin-1 in PDB prompted us to construct the 3D model. On the basis of sequence similarity with the template protein (PDB ID: 1J4J) 3D structure and functional aspects has been predicted. This method involves the selected template, aligning the target protein sequence, loop building and structure validation.

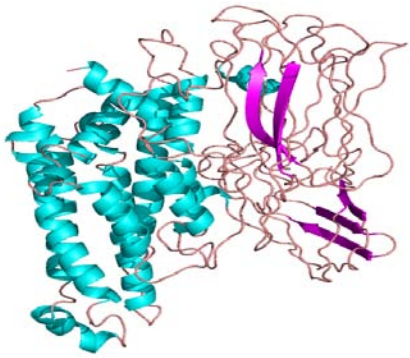


Fig. 1: The final 3D structure of Bestrophin-1 obtained after energy minimization. The α-helix is represented in cyan color, β-sheet by purple arrows and loops by warm pink lines.

The fasta sequence of the target protein bestrophin-1 was obtained from the NCBI database. Known template protein structure selection was carried out by NCBI blast tool. Homology modeling was carried by insight II software. The non-conserved residues were refined structurally with help of loop build method. The three dimensional structure provides valuable insight into molecular function and also enables the analyses of its interactions with suitable inhibitors. Among the ten conformations generated, the one with the least value was considered to be thermodynamically stable and chosen for further refinement and validation. Figure 1.

Validation of the predicted structure

The stereochemistry of the constructed model of Bestrophin-1 was subjected to energy minimization and the stereochemical quality of the predicted structure was assessed. The Ramachandran plot for the model showed 97.5% of the residues in the core region, 1.5% residues in the allowed regions and 1.0%, residue in the disallowed region (Fig 2). This residue (Lys177) was found to occur in the loop region of secondary structure. Hence, it was subjected to loop refinement and further energy minimization. In an analysis of the final model, 97.5% of the residues were found to occupy the core region. The residue in the disallowed region had been shifted to the generously allowed region, thereby optimizing and stabilizing the overall conformation of the predicted structure.

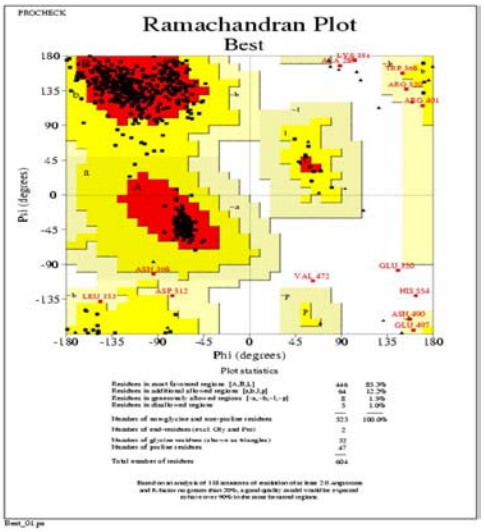


Fig. 2: Ramachandran plot of Bestrophin-1 protein built using insight II software. The plot calculations on the 3D model of Bestrophin-1 were computed with the PROCHECK server

Superimposition of the template (1j4j) with predicted structure of Bestrophin-1

The weighted root mean square deviation of C^{α} trace between the template and the final refined model was 0.4 Å with a significant *Z*-score of 7.6. Figure 3

Fig. 3: It shows the Superimposition of target and template. Green color represent target and cyan color represent template

Active site identification of Bestrophin-1

The active site amino acid of modelled protein was found with the help of Q-site finder. The active site amino acids strings are THR476, VAL477, GLU478, PHE479, ASN480, LEU481, MET48, ALA516, LEU517, PRO535, THR536, PRO537, ALA538, SER539, LEU540, ALA541, PHE546, HIS548, LEU560, LYS581, ASP596, and LEU598. These strings were loaded as an input in autodock4.0 which deletes other amino acid residues and select only the active site amino acids of the target protein. From the description of Q-site finder it suggests that there is at least one successful prediction in the top three predicted site. In this study the active site amino acids of target protein are present in the second predicted site volume of 424 Cubic Angstroms and Protein Volume of 57449 Cubic Angstroms. Figure 4

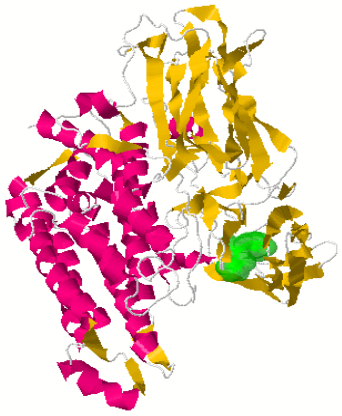


Fig. 4: Active site residues taken for docking analysis. Pink and yellow color indicates the proteins and green color indicates the active site

Docking of Bestrophin-1 with potential inhibitors

Docking of Bestrophin-1 was performed with four inhibitors namely Verteporfin, Fluorescein, L- Cystine and Fluocinolone Acetonide. Docking was carried out only in the active site of the modeled protein so as to facilitate maximum possible fits. Energy minimization for the modeled structure of bestrophin-1 was carried out in insight II. The inhibitors which are taken from Drug bank data base are energy minimized by using chimera software Fig 5. The ligand being docked is usually flexible and the receptor molecule is kept rigid throughout the docking program. Then, the protein Bestrophin-1 and the ligand were saved in PDBQT file format, for input to the Autodock version 4.0. A grid- based approach is used to reduce the overall run time of the docking simulation and to approximate the energy calculations used by the energy function. The grid formed for the protein Bestrophin-1 as a cube of 58Å on each side, centred on the protein (X_Y_Z coordinates: 0.097_ -39.461_28.667) and the search space was a box with xyz dimensions 82Å×82Å×32Å respectively. A grid is formed for all type of atom in the ligand such as carbon, oxygen, nitrogen and hydrogen and more over the grid showing the electrostatic potential with a point charge of +1 as the probe. This develops the chance of finding the lowest energy binding conformation.

The root-mean-square-displacement (RMSD) is used to calculate the initial root conformation. The four final docked conformations obtained for the different inhibitors were evaluated based on the number of hydrogen bonds formed and bond distance between atomic co-ordinates of the active site and inhibitor Fig 6 and Table 3. The binding energy value of each compound is differs due to its atom present in ligand. The binding energy values of the inhibitors are given in Table 1 and the physiochemical properties of each drug candidate from drug bank is given in Table 2. Among the four drug molecules verteporfin show more number of interaction. L- Cystine does not have any interaction. From this we hypothesized that the drug verteporfin having more affinity with the modeled Bestrophin-1 protein than other drug molecule.

Table 1: Autodock binding energy values of the four inhibitors.

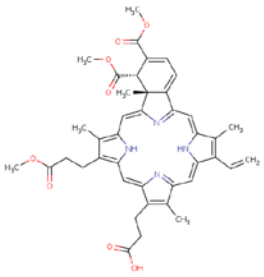
S. No.	Chemical molecule	Binding energy Kcal/mol
1.	Verteporfin	-5.08 Kcal/mol
2	Fluorescein	-5.01 Kcal/mol
3	L-Cystine	No interaction
4	Fluocinolone Acetonide	-4.02 Kcal/mol

Table 2: Physiochemical Cahracteristics of Compounds

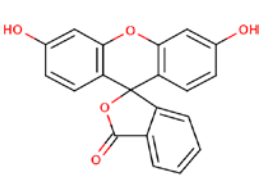
S. No.	Property	Verteporfin	Fluorescein	L-Cystine	Fluocinolone Acetonide
1.	logP	5.02	2.64	-3.16	2.47
2.	logS	-4.72	-4.11	-1.16	-3.92
3.	pKa	15.62	9.32	2.26	13.903
4.	H-bond donor count	7	3	6	6
5.	hydrogen donor count	3	2	4	2
6.	polar surface area	173.56	75.99	126.63	93.06
7.	rotatable bond count	12	0	7	2
8.	Refractivity	199.08	91.22	54.87	111.4
9.	polarizability	81.28	33.13	22.66	44.96

Table 3: Hydrogen bonds along with their distances between modeled protein Bestrophin-1 and Verteporfin

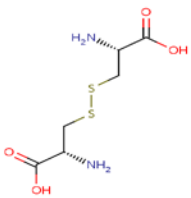
Residue	Atom	Compound	Distance (A)
Lys508	N	O29	2.97
Thr506	O	O28	2.74
Asn502	O	O28	3.14
Ser539	O	N21	3.25
Ser539	N	O4	2.96
Ala538	N	O4	2.78
Lys581	N	O44	2.88
Lys581	N	O45	2.72
Pro533	N	O51	3.37
Glu478	OE1	N13	2.95
Glu478	OE2	N13	2.95
Fluocinolone Acetonide			
Asp436	N	O11	3.35
Leu15	O	H26	3.31
Ala372	O	H35	2.85
Asp436	N	O11	3.35
Leu15	O	H26	3.31
Ala372	O	H35	2.85
Asp436	N	O11	3.35
Leu15	O	H26	3.31
Ala372	O	H35	2.85
fluoroescein			
Asn480	O	O2	2.52
Asn480	O	N1	2.72
L-cystine	**No interaction**		



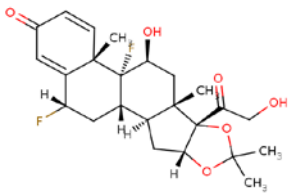
a) Verteporfin



b) Fluorescein

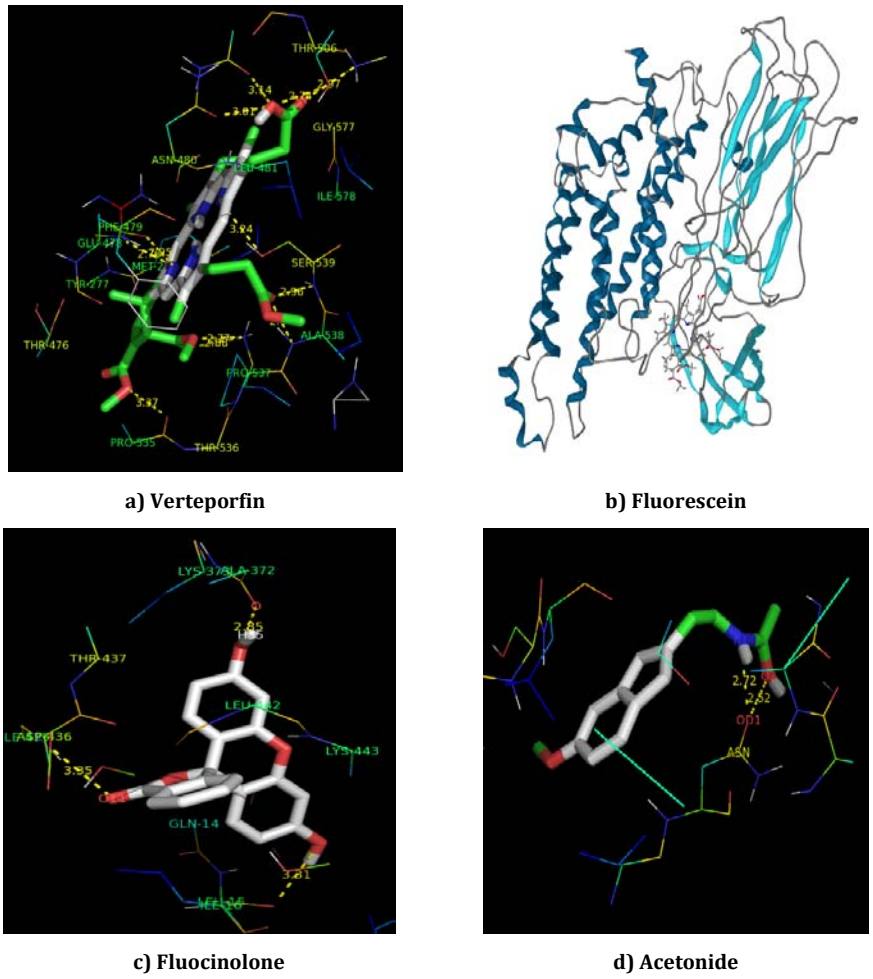


c) L-Cystine



d) Fluocinolone Acetonide

Fig. 5: It shows the structure of inhibitors such as Verteporfin, Fluorescein, L- Cystine and Fluocinolone Acetonide.



a) Verteporfin **b) Fluorescein**

c) Fluocinolone **d) Acetonide**

Fig. 6: (a) Bestrophin-1 in complex with Verteprophin and showing best binding affinity. (b) Bestrophin-1 in complex with Fluorescein. (c) L- Cystine is not bind with Bestrophin-1. (d) Bestrophin-1 in complex with Fluocinolone Acetonide.

CONCLUSION

In this work homology modeling and structure based drug designing studies were carried out to explore the structure elucidation of bestorphin-1 protein and the binding affinity of bestrophin-1 inhibitors. 3D Structure of bestorphin-1 was elucidated and catalytic binding sites were analyzed. The binding models of the all four inhibitors show clearly that, how the compounds interact with bestrophin-1. The binding energies of these drugs were predicted by docking algorithm. Among the chosen inhibitors for this study, Verteporfin has best conformational binding affinity with the modeled protein bestrophin-1. Thus Verteporfin have the potential as lead which certainly aid in designing new drug for Vitelliform macular dystrophy in short span of time. This data enabled the structure guided development of small molecule antagonists of bestropin-1. This drug can further be modified and used for second generation drug development .As whole results throw light for future development of more potent and drug like inhibitor for the treatment of disease Vitelliform macular dystrophy.

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