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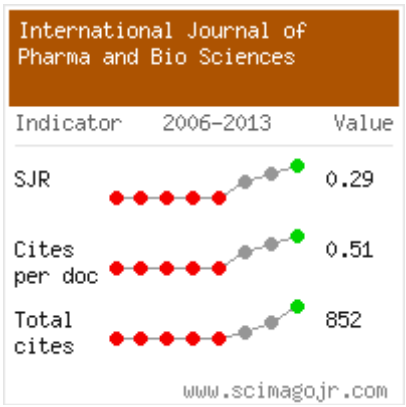

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IN-SILICO SCRUTINY AND MOLECULAR DOCKING ANALYSIS FOR BETA SECRETASE-1 AND PRESENILIN-1

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ABSTRACT

We have performed comparative scrutiny between the canonical and non-canonical isoforms of beta secretase-1 and presenilin-1 by various bioinformatics tools for understanding the disparities existent among them. Conjointly, the PDB structure of these neural proteins were docked with two therapeutic drugs (bexarotene and hesperidin) and a toxin (streptozotocin). Results**:** *In-silico* scrutiny revealed consanguinity among beta secretase-1 isoforms. On the contrary, amongst presenilin-1 isoforms significant disparities were observed. Molecular docking predicted ubieties of bexarotene, hesperidin and streptozotocin binding sites on beta secretase-1 and presenilin-1. Conclusion**:** The study propounds pernicious effect of streptozotocin on essential neural proteins and therapeutic potential of bexarotene and hesperidin in Alzheimer's disease and other neurodegenerative disorders. Additionally, the study raises a strong need for evaluation of functionalities of non-canonical isoforms of beta secretase-1 and presenilin-1, in order to uncover their roles in the pathogenesis of Alzheimer's disease and other neurodegenerative disorders.

KEYWORDS: Alzheimer's disease; Beta secretase-1; Presenilin-1; Bexarotene; Hesperidin; Streptozotocin

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INTRODUCTION

Indispensable neural proteins, beta secretase-1 and presenilin-1 possess promising candidature for therapeutic targeting in the Alzheimer's disease[1]. Numerous studies have affirmed that the autosomal dominant mutations in genes for either amyloid precursor protein or the presenilins ends in the genesis of familial Alzheimer's disease (FAD)[2]. Amyloid precursor proteins are attacked by beta secretase-1 (encoded by BACE1 gene) giving birth to C terminal fragments (CTFs) of amyloid precursor proteins. In the aftermath, γ-secretase complex comes into play and subsequently attacks the C terminal fragment of amyloid precursor protein, for the formation of amyloid beta (40 or 42 amino acids in length) and amyloid beta precursor protein intracellular domain peptide[3,4]; the amassing of long amyloid beta is a prevalent course underlying all forms of Alzheimer's disease[5]. Presenilin-1, a multi transmembrane protein, is imperative for the functioning of γ-secretase-complex[6,7]. The high molecular γ-secretase-complex comprises of presenilin 1 and 2 (encoded by PSEN1 and PSEN2 genes respectively) with nicastrin, APH-1 and PEN-2[6,7,8].

In consonance with the information indexed in the UniProt, both beta secretase-1 and presenilin-1 may exist in multiple isoforms, formed by alternate splicing. *Homo sapiens* possess six isoforms of beta secretase-1, to wit, beta secretase-1 isoform A (BACE1 A), beta secretase-1 isoform B (BACE1 B), beta secretase-1 isoform C (BACE1 C), beta secretase-1 isoform D (BACE1 D), beta secretase-1 isoform 5 (BACE1_5) and beta secretase-1isoform 6 (BACE1_6). Amongst these, the longest sequence of beta secretase-1 represented by isoform A is the canonical sequence. Likewise, seven isoforms have been reported for presenilin-1, to wit, isoform 1 (PSEN1_1), isoform 2 (PSEN1_2), isoform 3 (PSEN1_3), isoform 4 (PSEN1_4), isoform 5 (PSEN1_5), isoform 6 (PSEN1_6) and isoform 7 (PSEN1_7). The longest sequence of presenilin-1 isoform 1 is considered as the canonical sequence for this protein. The beta secretase-1 isoform A and presenilin-1 isoform 1 have been thoroughly investigated by researchers across the globe. Additionally, roles of these isoforms are very well understood in the pathogenesis of Alzheimer's disease and other neurodegenerative disorders[1,2]. Hence, these isoforms are getting maximum attention while other isoforms of beta secretase-1 (that is, beta secretase-1 isoforms B, C, D, 5 and 6) and presenilin-1 (that is, presenilin-1 isoforms 2, 3, 4, 5, 6 and 7) are still experiencing an attention exiguity. Although, not much information is available about these non-canonical isoforms but like their respective canonical isoforms, they may play important roles in the pathogenesis of Alzheimer's disease.

As presented and noticed in various meetings held bi-annually for the critical assessment of techniques for protein structure prediction (CASP), a reliable three-dimensional model of a protein can be generated from its amino acids sequence by virtue of homology modeling[9]. Proteins are modeled for homology, so as to find the conformation space by least possible distortion of the structures that have been already solved by experiments. Homology modeling deals with the calculation of a force field[10]. A well-known fact that the structural conformation of a protein is highly conserved than the sequence of amino acids, forms the basis of this method; the small or medium changes in sequence, if strike, bring only little variations in the three-dimensional structure[11]. Proteins like beta secretase and presenilins can be modeled structurally and ample information regarding various conformational characteristics can be retrieved, viz identification of proper binding sites etc.

Streptozotocin, a glucosamine-nitrosourea compound, is used for inducing type-1 diabetes in the animal models because of its toxicity towards pancreatic β-cells[12,13]. According to the latest reports hesperidin[13], a flavanone, possesses antioxidant and neuro-defensive consequences on the brain tissue in rodent models of streptozotocin-induced diabetes[12]. Bexarotene, an agonist of retinoid X receptor (RXR), is employed for T cell lymphoma treatment[13]. Recently, it was reported to possess neuro-defensive attributes in rodent models of Alzheimer's disease[14]. Bexarotene and hesperidin being the possible therapeutic

agents for Alzheimer's disease demand a precise docking analysis to detect their interactions with the binding sites possessed by beta secretases and presenilins. The objectives of the given study comprised of: (i) *in-silico* scrutiny of the isoforms of *Homo sapiens* beta secretase-1 and presenilin-1. The isoforms of both proteins were respectively, compared at the primary, secondary and tertiary structural levels. Conjointly, the structural consanguinity among the isoforms was respectively, evaluated for both proteins. (ii) Finally, the neuro-pernicious potential of streptozotocin and the therapeutic potential of bexarotene and hesperidin for Alzheimer's disease was assessed by molecular docking. Molecular docking was performed using these ligands and the PDB coordinates of beta secretase-1 and CTF subunit of presenilin-1.

MATERIALS AND METHODS

In-silico scrutiny of beta secretase-1 and presenilin-1

Sequence retrieval and alignment

Amino acid sequence of beta secretase-1 isoforms (A, B, C, D, 5 and 6) and presenilin-1 isoforms (1, 2, 3, 4, 5, 6 and 7) was annexed from the Universal Protein Resource (UniProt); the UniProt ids of the procured isoforms have been mentioned in the table 1. Isoform A and isoform 1 are the canonical sequences of beta secretase-1 and presenilin-1 respectively. Initially, complete pre-processed sequences were downloaded in the fasta format; further, signal and pro-peptide sequences were omitted manually. The resulting sequences representing mature proteins were compared by multiple sequence alignment using Clustal Omega[15] (EMBL-EBI).

Physico-chemical characterization and secondary structure prediction

Physico-chemical characterization of retrieved sequences was computed using ProtParam tool[16] (ExPASy); the computed parameters constituted of amino acids number, theoretical isoelectric point (pI), molecular weight, aliphatic index, instability index and grand average hydropathy (GRAVY)[17]. Subsequently, secondary structures comprising alpha helix, extended strand, beta turn and random coil were evaluated using SOPMA[18].

Three-dimensional structure prediction and validation

Phyre 2 server was used to generate three-dimensional models using the sequence of mature proteins. All structures were designed by intensive modeling mode of Phyre2 server[19]. The three-dimensional structures were designed for the mature beta secretase-1 and presenilin-1 isoforms. The generated three-dimensional models were visualized by Pymol[20] software. Resultant structures were validated on the basis of Z score values obtained using ProSA[21,22].

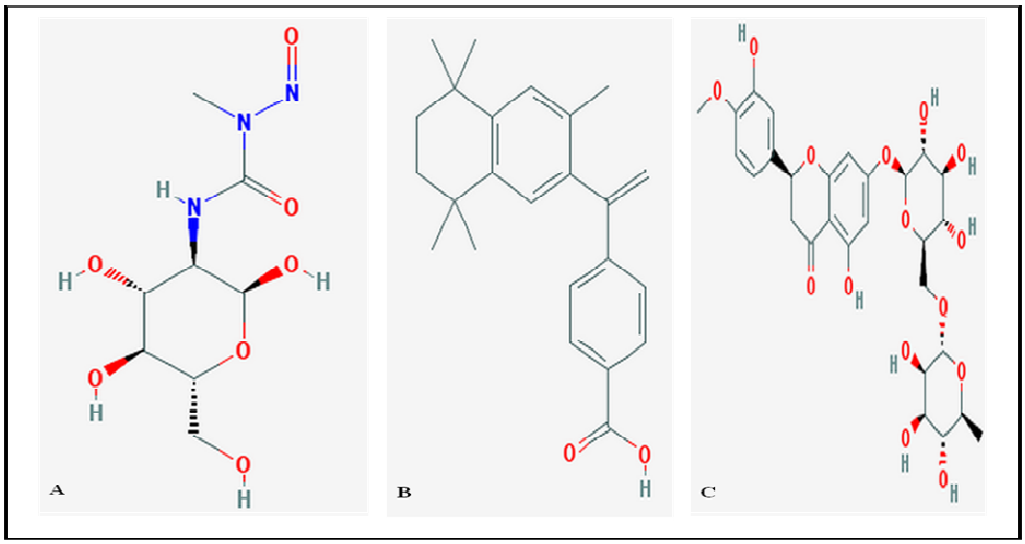
Analogy between three-dimensional structures

Comparative structural propinquity among the isoforms was determined on the basis of RMSD parameter by MultiSeq (VMD 1.9.1) software[23]. The three-dimensional structure of canonical form was superimposed with their corresponding non-canonical isoforms employing Pymol software[20].

Molecular docking

The three-dimensional structure of both imperative neural proteins was docked with a toxin and two therapeutic drugs. The atomic coordinates given in the Protein Data Bank were used for docking. The PDB file containing atomic coordinates of crystal structure limning beta secretase-1 isoform A having PDB ID *4j0p* was acquired from Protein Data Bank; likewise, atomic coordinates of the CTF subunit of presenilin-1 having PDB ID *2kr6* was selected for docking. The toxin used in the study was streptozotocin (PubChem CID *29327*) while bexarotene (PubChem CID *82146*) and hesperidin (PubChem CID *10621*) served as the therapeutic drugs. The three dimensional structure (Figure 1) of ligands was obtained from PubChem (NCBI) in SDF format and was further converted to docking compatible MOL2 format, using the Open Babel software version 2.3.2[24]. The PDB coordinates of proteins and mol2 coordinates of ligands were submitted to iGEMDOCK software[25]. Pymol[20] was used to visualize the best dock ligand poses with three dimensional structure of the protein.

Figure 1
Structure of the ligands. Toxin: streptozotocin (A); therapeutic drugs: bexarotene (B), hesperidin (C).



RESULTS

In-silico scrutiny of beta secretase-1 and presenilin-1

Beta secretase-1

In accordance with the complete amino acid sequence of beta secretase-1, the isoform A possessed highest number (501 amino acids) of amino acids, followed by isoforms B, C, D, 5 and 6 in the order of decreasing polypeptide length (Table 1). The beta secretase-1 isoform 6 was found to bear the maximum percent sequence loss (24.95%) in comparison with the canonical sequence; even the isoforms 5 (19.96%) and D (13.77%) suffered from huge percent sequence loss while the isoforms 2 and 1 separately endured a deficit of only 8.78% and 4.99%. From the complete amino acid sequence the signal and pro-peptide sequences were removed, yielding the amino acid sequence for mature proteins. The signal sequence for isoform A, B, C and D was MAQALPWLLLWMGAGVLPAHG (information provided in the UniProt) while the pro-peptide sequence for these isoforms was TQHGIRLPLRSGLGGAPLGLRLPR (information provided in the UniProt). In case of beta secretase-1 isoform 5 and 6 these sequences were absent; on the contrary, a short stretch of seventeen amino acids (MVPFIYLQAHFTLCSGW) was found to be present prior to a highly conserved region (SSTYRDLRKGVYVPYTQGKWEGELGTDL, Figure 2) common to all the isoforms of mature beta secretase-1. This short region of seventeen amino acids may be functional as signal sequence and hence was omitted out yielding mature sequences for the isoforms 5 and 6 of beta secretase-1. Clustal Omega based multiple sequence alignment generated using mature sequence of beta secretase-1 isoforms, depicted that a region near C-terminal was highly conserved among the isoforms (Figure 2). This conserved sequence constituted of 278 amino acids, starting as LCGAGFPLNQ and culminating as DFADDISLLK. This sequence forms the C-terminal cytoplasmic region, a transmembrane region and more than half of the extracellular catalytic domain (a region towards the C-terminus of extracellular catalytic domain). On the contrary, N-terminal sequence of extracellular catalytic domain displayed high variability. This variable region may be influencing the catalytic activity and disparities in the physiological role(s) (still uncovered) of these isoforms. In addition to high variability, the N-terminal sequence of beta secretase-1 possessed a short stretch of twenty eight amino acids which was present in all isoforms. This conserved stretch of amino acids was: SSTYRDLRKGVYVPYTQGKWEGELGTDL.

There are two catalytic residue essential for beta secretase-1activity, to wit, ASP-48 and ASP-244 residues of the mature sequence of the canonical isoform (according to the information provided in UniProt). In the complete sequence of the canonical isoform these residues occupy 93rd and 289th positions respectively. The deletion of any of this aspartic acid renders the beta secretase-1 isoform A inactive[26]. The isoforms 5 and 6 were lacking the ASP-48 catalytic residue while the ASP-244 residue was present in all isoforms of beta secretase-1. Hence, the isoforms 5 and 6 may be catalytically inactive, yet *in vivo* studies are essential for establishing the reason for their expression and their role in Alzheimer's disease and neurodegenerative disorders.

Table 1
List of proteins. [*: Asterisk represents canonical sequence of the opted essential neural proteins.]

Proteins	UniProt IDs	No. of residues in the sequence procured from UniProt	Percent sequence loss
Human beta secretase-1 Isoform A*	P56817-1	501	0
Human beta secretase-1Isoform B	P56817-2	476	4.99
Human beta secretase-1 Isoform C	P56817-3	457	8.78
Human beta secretase-1 Isoform D	P56817-4	432	13.77
Human beta secretase-1 Isoform 5	P56817-5	401	19.96
Human beta secretase-1 Isoform 6	P56817-6	376	24.95
Human presenilin-1 Isoform 1 *	P49768-1	467	0
Human presenilin-1 Isoform 2	P49768-2	463	0.86
Human presenilin-1 Isoform 3	P49768-3	374	19.91
Human presenilin-1 Isoform 4	P49768-4	184	60.6
Human presenilin-1 Isoform 5	P49768-5	378	19.06
Human presenilin-1 Isoform 6	P49768-6	409	12.42
Human presenilin-1 Isoform 7	P49768-7	434	7.07

Figure 2

The multiple sequence alignment of mature beta secretase-1 isoforms, generated by Clustal omega. The orange, red and light blue colour lines represents the amino acids forming catalytic extracellular domain, transmembrane region and cytoplasmic regions of beta secretase-1 isoforms (regions were defined according to the information provided in UniProt). The red dots represents catalytic residues.

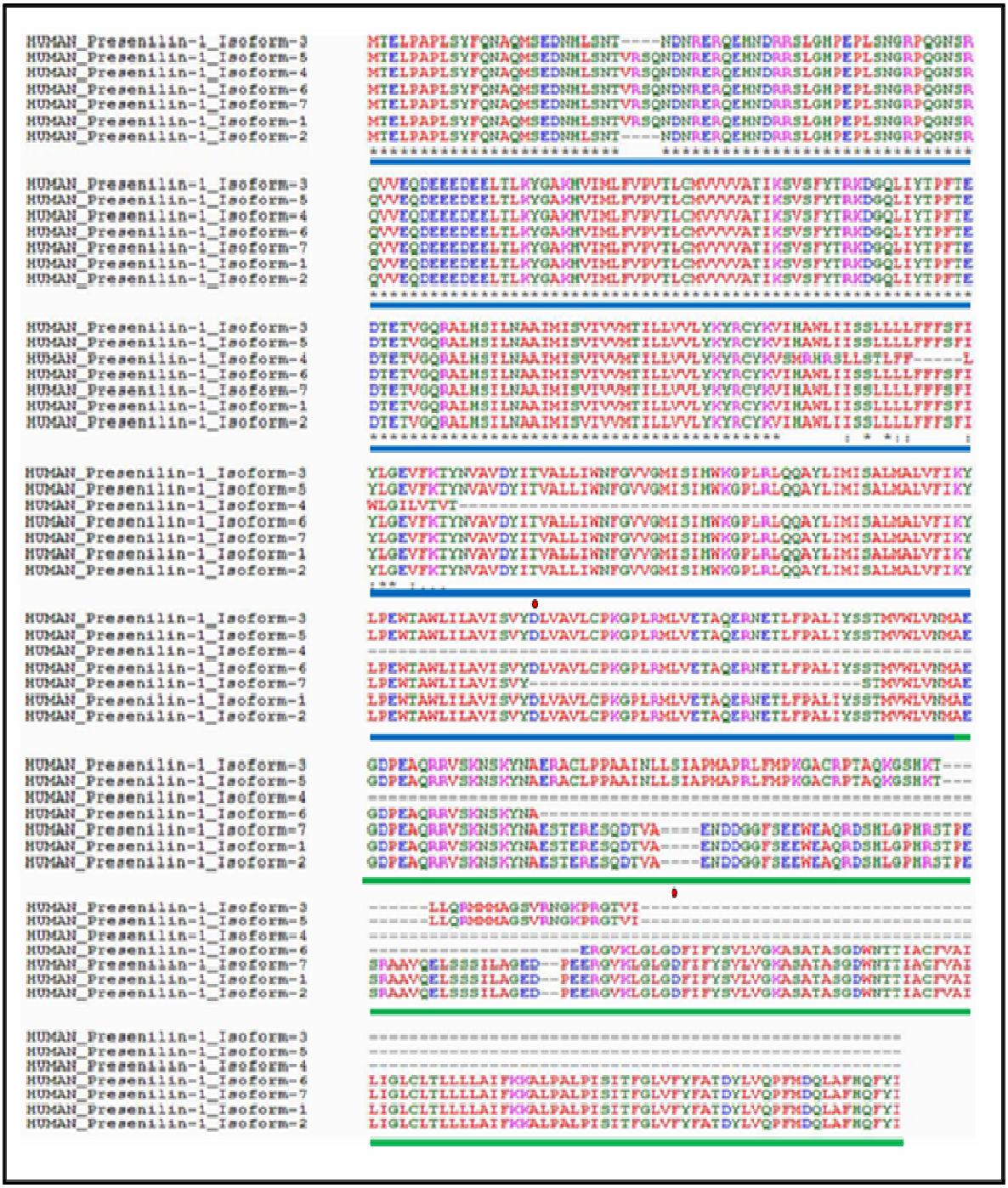
Presenilin-1

In accordance with the complete amino acid sequence of presenilin-1, the isoform 1 possessed highest number (467 amino acids) of amino acids, followed by isoforms 2, 7, 6, 5, 3 and 4 in the order of decreasing polypeptide length (Table 1). The presenilin-1 isoform 4, was found to bear the maximum percent sequence loss (60.6%) in comparison with the canonical sequence; even the isoforms 3 (19.19%), 5 (19.06%) and 6 (12.42%) suffered from huge percent sequence loss while the isoforms 7 endured the deficit of 7.07%. The presenilin-1 isoform 2 possessed minimum percent sequence loss of 0.86%. Clustal Omega based multiple sequence alignment generated using the sequence of presenilin-1 isoforms, depicted a conserved stretch of 137 amino acids towards N-terminal; the region started as NDNRERQEHN and culminated as LVVLYKYRCYKV. The functional presenilin-1 protein is present in heterodimer form comprising of NTF (N-terminal fragment) and CTF (C-terminal fragment), formed by endoproteolytic cleavage of complete presenilin-1. The conserved region was forming approximately half of the NTF. A region towards C-terminal was varying enormously among the seven isoforms (Figure 3). This region constituted half of the NTF and the entire CTF. Thus the isoforms of presenilin-1 were varying in both NTF and CTF. There are two catalytic residues essential for presenilin-1activity, to wit, ASP-257 and ASP-385 (according to the information provided in UniProt). After

endoproteolytic cleavage of complete presenilin-1, the ASP-257 residue is present in NTF while the ASP-385 residue gets located in the CTF. The isoform 4, lacked both catalytic residues also it lacked in few residues of NTF and entire CTF. Also, the isoforms 3 and 5 lacked the ASP-385 residue and their CTF was comparatively shorter. Conjointly, isoform 7 lacked the ASP-257 catalytic residue. Such sequence differences may cause functional differences influencing their physiological roles.

Figure 3

The multiple sequence alignment of mature presenilin-1 isoforms, generated by Clustal omega. The blue and green colour lines represents the amino acids forming NTF and CTF domains of presenilin-1 (regions were defined according to the information provided in UniProt). The red dots represents catalytic residues.



Physico-chemical characterization and secondary structure prediction

Beta secretase-1

Primary structure of beta secretase-1 functional proteins revealed an insight about the physico-chemical parameters of the isoforms. Amongst the six isoforms, number of amino acids and molecular weight was found to reduce progressively from the isoform A to the isoform 6 (Figure 4). The computed values of isoelectric point (pI) for the isoforms were less than 7 indicating proteins to be acidic in nature, consistent with the fact that beta secretase-1 activity is found only in acidic sub-cellular compartments. The instability index[17] (Figure 4) varied widely; the values for isoform A, B, C, D and 5 were exceeding the threshold value of 40. Only the isoform 6 was found to have instability index of 38.87, lower than the threshold value. The instability index provides an estimate of the stability of a protein under *in vitro* conditions. The instability index of the isoform 6 was lower than 40 indicating stability under *in vitro* conditions, indicating that the isoform 6 protein possesses lower degradation rate and may persist in the neural tissue for longer time in comparison to the other isoforms and in turn may contribute significantly in the pathogenesis of Alzheimer's disease. Additionally, a short stretch of twenty five amino acids "PDDSLEPFFDSLVKQTHVPNLFSLQ" may be responsible for increased stability and persistence of the isoform 6 in neural tissues. The aliphatic index[17] indicates about the thermo-stability of a protein. This parameter was evaluated by comparing the aliphatic index values obtained for isoforms with the aliphatic index of a thermostable protein and a heat labile protein. The aliphatic index of thermostable carboxypeptidase of *Sulfolobus acidocaldarius* (UniProt ID *Q4J8B0*) is 89.51 and Heat-labile enterotoxin B chain of *Escherichia coli* (UniProt ID *P32890*) is 85.73. Hence, the isoforms 5 and 6 are thermo-stable while the isoforms A, B, C and D are heat labile. GRAVY[17] for isoform C was highly negative (-0.124) followed by isoform D (-0.112) while the isoforms A and B possessed higher GRAVY values of -0.093 and -0.081 respectively. GRAVY for isoforms 5 and 6 was positive in the magnitude with values of 0.022 and 0.045 respectively. GRAVY is a measure of protein solubility; a positive and negative value respectively implies hydrophobic and hydrophilic nature of a protein. The analysis of GRAVY shows that beta secretase-1 isoforms 5 and 6 are hydrophobic whereas other isoforms are hydrophilic. These results show that absence of seventy two amino acids at N-terminal in isoforms 5 and 6 may be responsible for this change.

The comparative analysis of secondary structures in beta secretase-1 isoforms was achieved by SOPMA. SOPMA analysis was based on the output width 70 with four conformational states (alpha helix, extended strands, beta turns and random coils) at similarity threshold of 8 and window width 17. The SOPMA program employed for analysis, predicted the secondary structure with high accuracy by performing calculations according to the DSSP algorithm[27]. All isoforms were varying in their secondary structural components and amongst the four secondary structures majority of the amino acids were involved in forming random coils (Table 2).

Figure 4
Histograms showing the physico-chemical parameters of beta secretase-1 isoforms, predicted by Protparam.

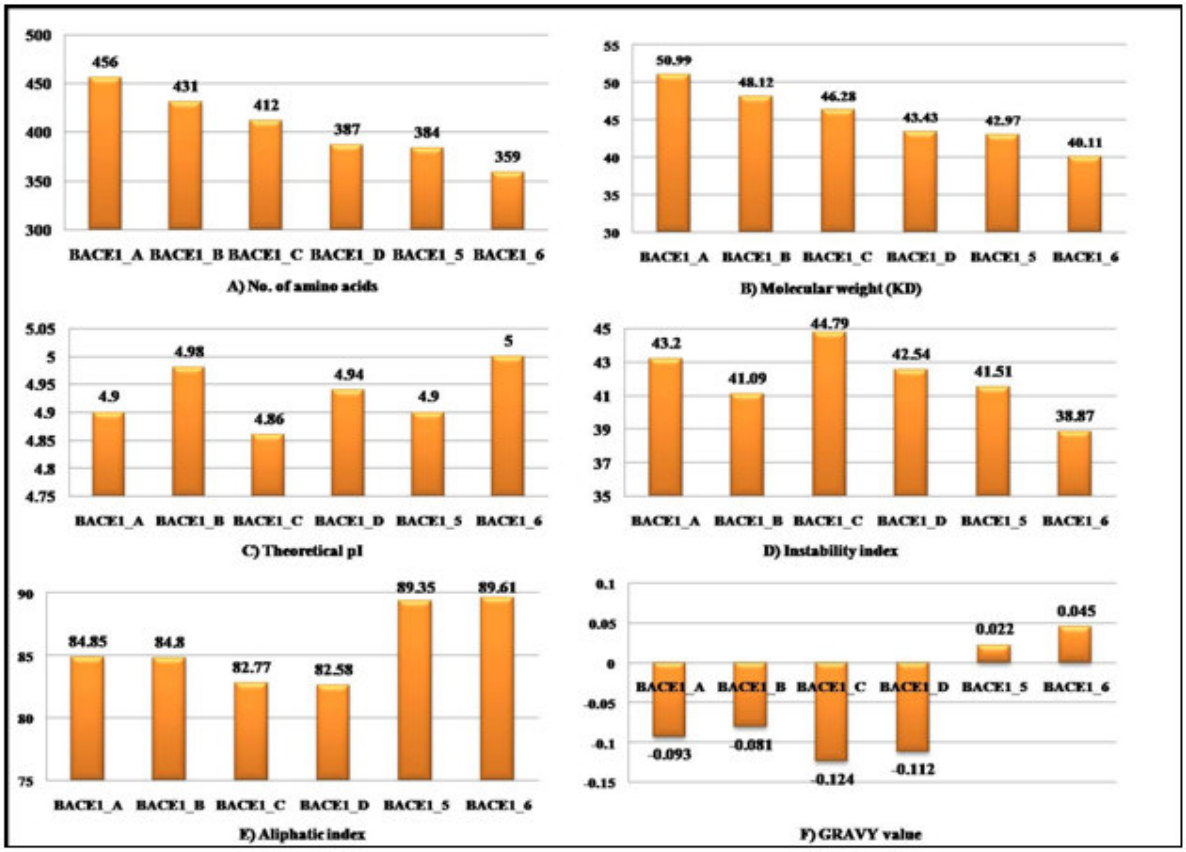


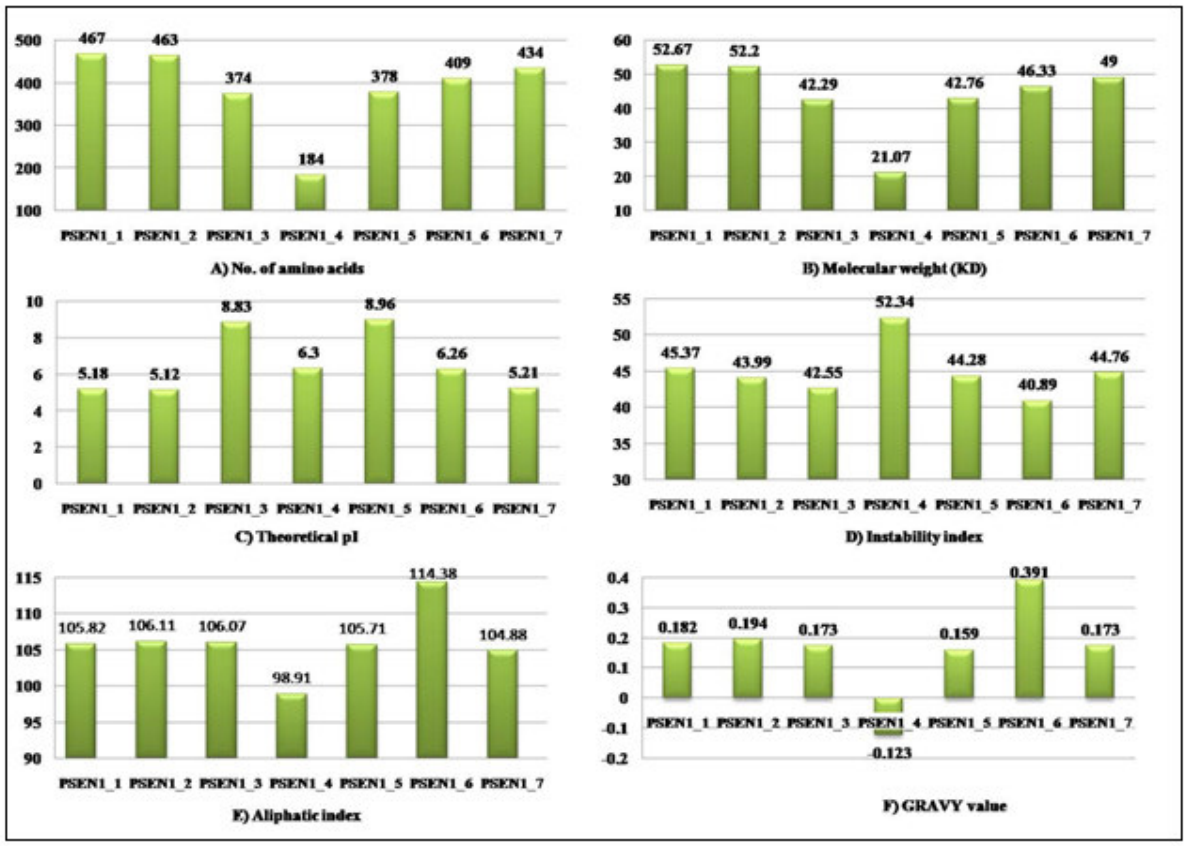
Table 2
Secondary structure prediction by SOPMA. [*: Asterisk represents canonical sequences of the opted essential neural proteins.].

Proteins	Alpha helices	Extended strand	Beta turn	Random coil
Human beta secretase-1 Isoform A*	26.32%	23.68%	8.77%	41.23%
Human beta secretase-1 Isoform B	27.61%	22.74%	7.66%	42.00%
Human beta secretase-1 Isoform C	27.43%	23.54%	9.22%	39.81%
Human beta secretase-1 Isoform D	20.67%	26.36%	5.94%	47.03%
Human beta secretase-1 Isoform 5	28.39%	25.00%	8.59%	38.02%
Human beta secretase-1 Isoform 6	27.30%	25.07%	8.08%	39.55%
Human presenilin-1 Isoform 1 *	35.55%	15.63%	3.00%	45.82%
Human presenilin-1 Isoform 2	36.50%	15.12%	2.38%	46.00%
Human presenilin-1 Isoform 3	35.29%	14.17%	2.94%	47.59%
Human presenilin-1 Isoform 4	39.67%	15.22%	4.89%	40.22%
Human presenilin-1 Isoform 5	35.71%	12.96%	4.50%	46.83%
Human presenilin-1 Isoform 6	44.74%	15.40%	2.93%	36.92%
Human presenilin-1 Isoform 7	40.32%	13.82%	2.53%	43.32%

Presenilin-1

The histogram of amino acids number and molecular weight of full length presenilin-1 isoforms showed a V- shaped pattern (Figure 5). The values were higher towards the ends, but steeped down at the isoform 4 having only 184 amino acids making it the smallest isoform of presenilin-1. The theoretical pI of isoforms 3 and 5 was >7 pH indicating that these isoforms may be located in the basic compartment of the cell while for others the pI point was <7 pH. The instability index[17] for isoforms was more than 40 indicating that the proteins are highly short lived. In comparison to isoform 1, the isoforms 2, 3, 5, and 6 had a lower instability index, they may have relatively longer half lives. In the case of isoform 4, stability was lowest and may make its isolation a difficult task. The aliphatic index[17] values were significantly high, indicating that all isoforms are highly thermo-tolerant. The isoform 6 possessed highest aliphatic index. GRAVY[17] values for all the proteins except isoform 4, were highly positive; indicating them to be more hydrophobic in nature. Only isoform 4 showed negative GRAVY, an indicative about its hydrophilicity. The GRAVY of isoform 6 was very large. In accordance with this information it seems that due to high hydrophobicity, full length presenilin-1 isoforms 1, 2, 3, 5, 6, 7 may coagulate *in vivo* forming protein aggregates that are difficult to be removed rapidly. The comparative secondary structure analysis of presenilin-1 isoforms have been shown in Table2.

Figure 5
Histograms showing the physico-chemical parameters of presenilin-1 isoforms predicted by Protparam.



Homology modeling and analogy between the structures

Beta secretase-1

The three-dimensional structure of proteins were modeled according to the templates selected by Phyre 2 engine on the basis of heuristics to escalate confidence, percentage resemblance and alignment scope between the query and templates. In the isoform A, 85% sequence was modeled at >90% confidence using three templates, to wit *c3ckpB, d2qp8a1* and *c2p83C*; the remaining 15% sequence was modeled by *ab-initio* modeling method. Similarly, for other isoforms the sequence modeled at >90% confidence was as followed: 84% (isoforms B and C), 83% (isoform D) and 99% (isoforms 5 and 6). Four templates were used for isoforms B, D and 6: *c2kncA*, *c3ckpB, d2qp8a1* and *c2p83C;* five templates were used for the isoform C: *c2jp3A, c2jo1A, c3ckpB, d2qp8a1* and *c2p83C;* and six templates were used for the homology modeling of isoform 5: *c2k21A, c2m0qA, c2kncA, c3ckpB, d2qp8a1* and *c2p83C*. Validation of three-dimensional structures of beta secretase-1 isoforms by ProSA validation tool, gave negative Z score values indicating the presence of very less erroneous structures in the designed 3D model. The Z-score values obtained for beta secretase-1 isoforms were as followed: -6.62 (isoform A), -6.01 (isoform B), -5.43 (isoform C), -5.41 (isoform D), -5.41 (isoform 5) and -5.05 (isoform 6). The Z-score for the three-dimensional sructure of beta secretase-1 isoforms were located in the Z-scores plot of all experimentally determined protein chains present in the Protein Data Bank, approving or verifying the designed protein structures. It has been reported that Z score is dependent on the length of the protein and a negative Z-score imply a reliable protein structure[28]. The Z-score represents the overall quality and measures the deviation of the total energy of protein structure. The comparative analysis of beta secretase-1 isoforms, based on the three dimensional structure was achieved by the STAMP structural alignment based on RMSD values. The RMSD[29] value determines the average distance between the atoms of overlapped three dimensional structures. The RMSD value between beta secretase-1 isoforms A and 6 was maximum (2.8793 Å) followed by isoform D (1.1807 Å), isoform C (1.1710 Å), isoform B (1.1289 Å) and isoform 5 (0.9364 Å). Figure 6 depicts the RMSD based tree generated to show structural relation among the beta secretase-1 isoform. The isoform 6 was most disparate while the isoform A was most similar to the isoform 5 followed by the isoform B. The isoform D shared maximum homology with the isoform C. The three-dimensional structure of beta secretase-1 isoform A was superimposed with corresponding non-canonical isoforms, to wit, beta secretase-1 isoforms B, C, D, 5 and 6 (Figure 7). The super positioning between isoforms was performed by employing Pymol software.

Figure 6
The RMSD based tree generated by VMD, showing structural relation among the beta secretase-1 isoforms.

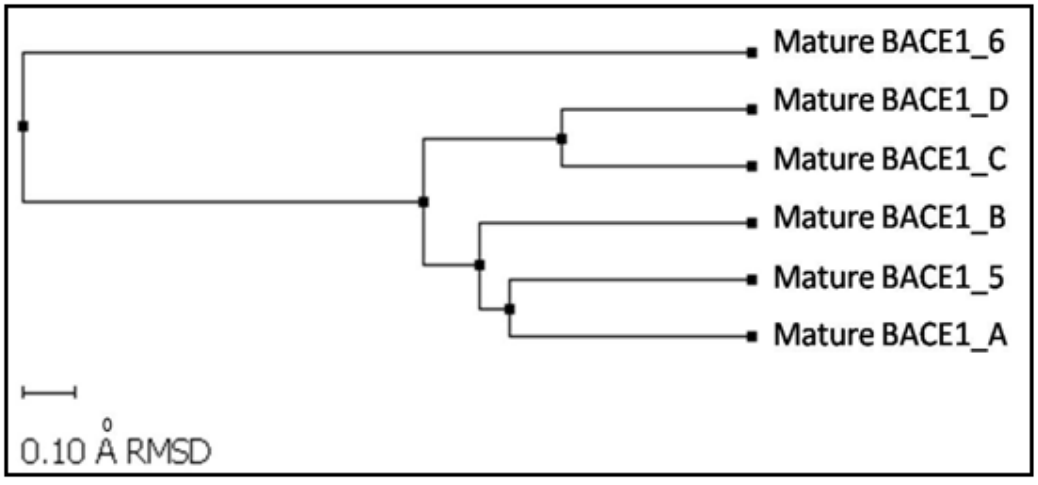
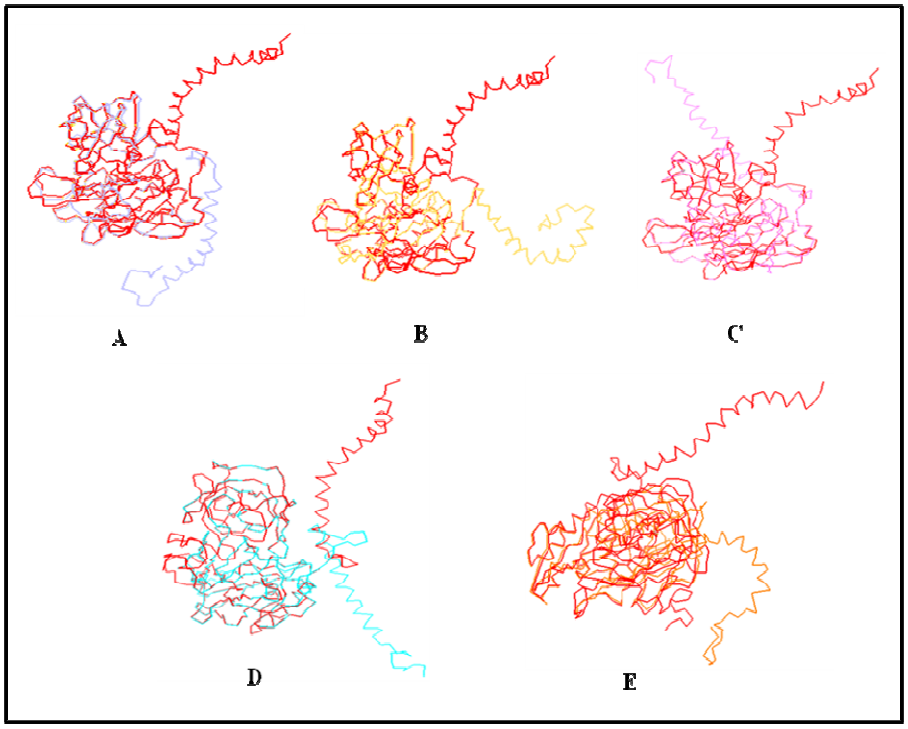


Figure 7

The three-dimensional structure of canonical form of beta secretase-1 superimposed with non-canonical isoforms. The canonical sequence (isoform A) is red colored while non canonical forms have been colored as light blue (isoform B), yellow (isoform C), pink (isoform D), cyan (isoform 5) and orange (isoform 6).

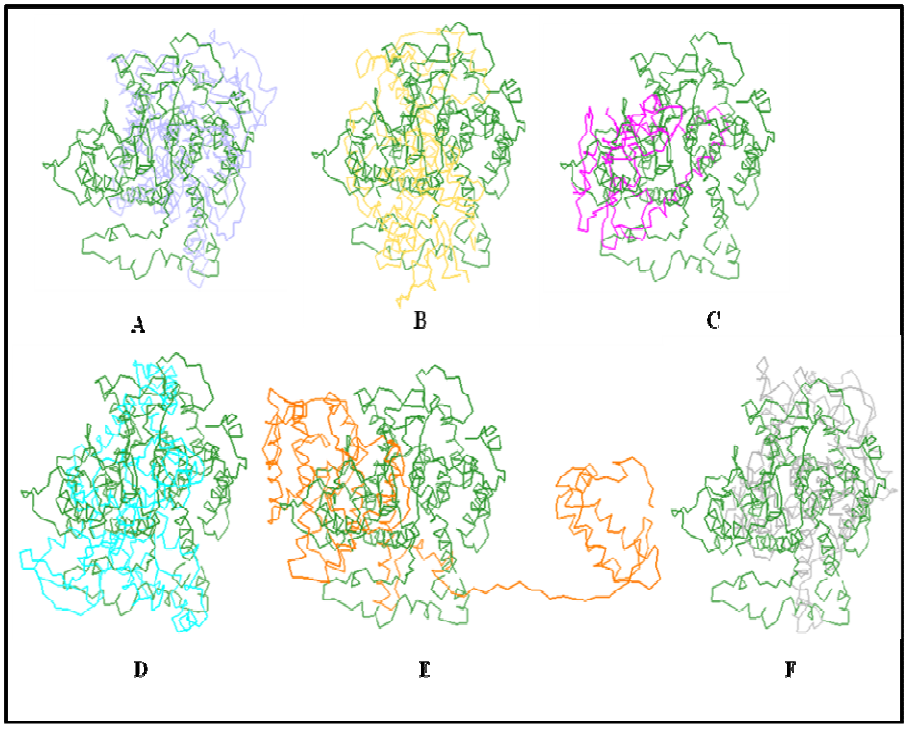


Presenilin-1

In the isoform 1, 69% sequence was modeled at >90% confidence, the remaining 31% sequence was modeled by *ab-initio* modeling method. Similarly, for other isoforms the sequence modeled at >90% confidence was as followed: 77% (isoform 2), 54% (isoform 3), 53% (isoform 5), 68% (isoform 6) and 70% (isoform 7). Two templates were used for isoforms 1, 2, 3, 5, 6, 7: *c4hydA* and *c2kr6A.* The three-dimensional model designed for isoform 4 was having less than 70% overall confidence and the template used was *c4hydA.* The ProSA generated Z-score for the three-dimensional model of presenilin-1 isoforms were negative and positioned in the Z-scores plot of all experimentally determined protein chains present in the Protein Data Bank, validating the designed protein structures. The Z-score values were as followed: -3.42 (isoform 1), -1.18 (isoform 2), -3.45 (isoform 3), -0.74 (isoform 4), -2.96 (isoform 5), -2.36 (isoform 6) and -2.99 (isoform 7). The VMD software was not able to generate the STAMP structural alignment between the presenilin-1 isoforms due to lack of sufficient structural similarity between the seven isoforms; thereby depicting high structural disparity among the isoforms of this neural protein, which may be affecting their functionalities. The three-dimensional structure of presenilin-1 isoform 1 was superimposed with corresponding non-canonical isoforms, to wit, presenilin-1 isoforms 2, 3, 4, 5, 6 and 7 (Figure 8). The superimposed positions clearly reflect high structural dissimilarities between presenilin-1 isoforms and display their potential functional disparities in the neural pathways.

Figure 8

The three-dimensional structure of canonical form of presenilin-1 superimposed with non-canonical isoforms. The canonical sequence (isoform 1) is green colored while non canonical forms have been colored as light blue (isoform 2), yellow (isoform 3), pink (isoform 4), cyan (isoform 5), orange (isoform 6) and grey (isoform 7).



Molecular docking

The atomic coordinates of beta secretase-1 and CTF domain of presenilin-1 were docked with bexarotene, hesperidin and streptozotocin using iGEMDOCK and the best dock poses were identified on the basis of binding energy. According to iGEMDOCK, bexarotene was the best binding ligand of the three in case of beta secretase-1 because of its best fitness (or total energy) of -141.9 Kcal/mol (Table 3). In the case of presenilin-1 also, bexarotene was the best binding ligand and its fitness (or total energy) was -135.13 Kcal/mol. Using iGEMDOCK Post-Screening Analysis Tools, all the docked poses were clustered based on the default "identity consensus residues" parameters where pharmacological energies of Electrostatics, H-bonding and Van der waal's were set at -2.5, -2.5 and -4.0 respectively. Their Z-scores were set at 1.645, 1.645 and 1.645 respectively. The amino acids constituents of streptozotocin binding cleft on beta secretase-1 were TYR-132, THR-133, GLN-134, ASP-289, THR-292, THR-293, and ARG-296; while bexarotene binding cleft had GLY-95, TYR-132, THR-133, GLN-134, LYS-168, PHE-169, ILE-171, ASP-289, GLY-291 and THR-292. On the contrary, hesperidin binding cleft possessed TYR-132, THR-133, GLN-134, LYS-168, PHE-169, ILE-171, ASP-289, GLY-291 and THR-292. The three ligands shared TYR-132, THR-133, GLN-134, ASP-289, and THR-292; ratifying affinity of streptozotocin and the two drugs for same binding cleft located on beta secretase-1 (Figure 9).

In the case of CTF domain of presenilin-1, the design of streptozotocin binding cleft embodied GLU-339, TRP-340, GLU-341, ALA-342, GLN-343, ARG-344, ASP-345, SER-346, LEU-348 and GLY-349; while hesperidin binding cleft comprised of GLU-339, TRP-340, GLU-341, ALA-342, GLN-343, ARG-344, ASP-345, SER-346, LEU-348, GLY-349, PRO-350, HIS-351 and THR-354. On the contrary, bexarotene binding cleft had TRP-340, GLU-341, ALA-342, GLN-343, ARG-344, ASP-345, SER-346, LEU-348, GLY-349, PRO-350 and HIS-351. The three ligands mutually interacted with amino acids

TRP-340, GLU-341, ALA-342, GLN-343, ARG-344, ASP-345, SER-346, LEU-348 and GLY-349; circumstantiating druthers of streptozotocin and the two drugs for tantamount cleft situated on presenilin-1 (Figure 10). Moreover, the value of total binding energy displayed by the toxin was higher (i.e. less negative) on comparing with both drugs; hence these drugs may be anticipated as competitive inhibitors of the acknowledged neurotoxin, streptozotocin.

Table 3

iGEMDOCK predicted stable interactions between ligands and target proteins. T represents total energy; H and V denote (Hydrogen bonding, Van der waal) type of interactions; M and S indicate the main chain and side chain of the interacting residues, respectively.

Ligands	Binding Energy (Kcal/mol)	Composition of residues constituting the binding site
I. Target protein : Beta secretase-1		
Bexarotene	-141.9 (T) -129.35 (V) -12.52 (H)	H-S-THR-133, H-S-GLN-134, H-S-ASP-289, V-M-GLY-95, V-M-TYR-132, V-S-TYR-132, V-M-THR-133, V-M-GLN-134, V-S-GLN-134, V-M-LYS-168, V-S-LYS-168, V-S-PHE-169, V-M-ILE-171, V-S-ILE-171, V-M-GLY-291, V-M-THR-292
Hesperidin	-139 (T) -125.31(V) -13.66 (H)	H-S-GLN-134, H-S-LYS-168, H-S-PHE-169, H-S-ASP-289, V-S-TYR-132, V-M-THR-133, V-M-GLN-134, V-S-GLN-134, V-S-LYS-168, V-M-PHE-169, V-S-PHE-169, V-M-ILE-171, V-S-ILE-171, V-S-ASP-289, V-M-GLY-291, V-M-THR-292
Streptozotocin	-110 (T) -61.3 (V) -48.7 (H)	H-M-THR-133, H-S-THR-133, H-M-GLN-134, H-S-GLN-134, H-S-ASP-289, H-S-THR-292, H-M-THR-293, H-S-ARG-296, V-M-TYR-132, V-M-THR-133, V-S-GLN-134, V-M-THR-292, V-S-ARG-296
II. Target Protein: Presenilin-1		
Ligands	Binding Energy (Kcal/mol)	Composition of residues constituting the binding site
Bexarotene	-135.13 (T) -105.46 (V) -29.66 (H)	H-M-GLU-341, H-M-ALA-342, H-M-GLN-343, H-M-ARG-344, H-M-ASP-345, H-M-SER-346, H-S-SER-346, V-M-TRP-340, V-S-TRP-340, V-M-GLU-341, V-S-GLU-341, V-M-ALA-342, V-M-ASP-345,V-S-ASP-345,V-M-LEU-348, V-M-GLY-349,V-M-PRO-350,V-M-HIS-351
Hesperidin	-133.21 (T) -109.39 (V) -23.82 (H)	H-M-GLU-341, H-M-ALA-342, H-M-GLN-343, H-M-ARG-344, H-M-SER-346, H-S-SER-346,V-M-GLU-339, V-M-TRP-340, V-S-TRP-340, V-M-GLU-341, V-S-GLU-341, V-M-ALA-342, V-S-ASP-345, V-S-ASP-345, V-M-SER-346, V-M-LEU-348, V-M-GLY-349, V-M-PRO-350, V-M-HIS-351, V-S-THR-354
Streptozotocin	-111.63 (T) -65.38 (V) -46.24 (H)	H-M-GLU-339, H-M-GLU-341, H-S-GLU-341, H-M-GLN-343, H-M-ARG-344, H-M-ASP-345, H-S-ASP-345, H-M-SER-346, H-S-SER-346, H-M-GLY-349, V-M-TRP-340, V-S-TRP-340, V-M-GLU-341, V-S-GLU-341, V-M-ALA-342, V-M-AS-345, V-M-LEU-348, V-M-GLY-349

Figure 9
Interaction profiles of streptozotocin (A), bexarotene (B) and hesperidin (C) with beta secretase-1. [Ligands have been highlighted in the yellow coloured circles]

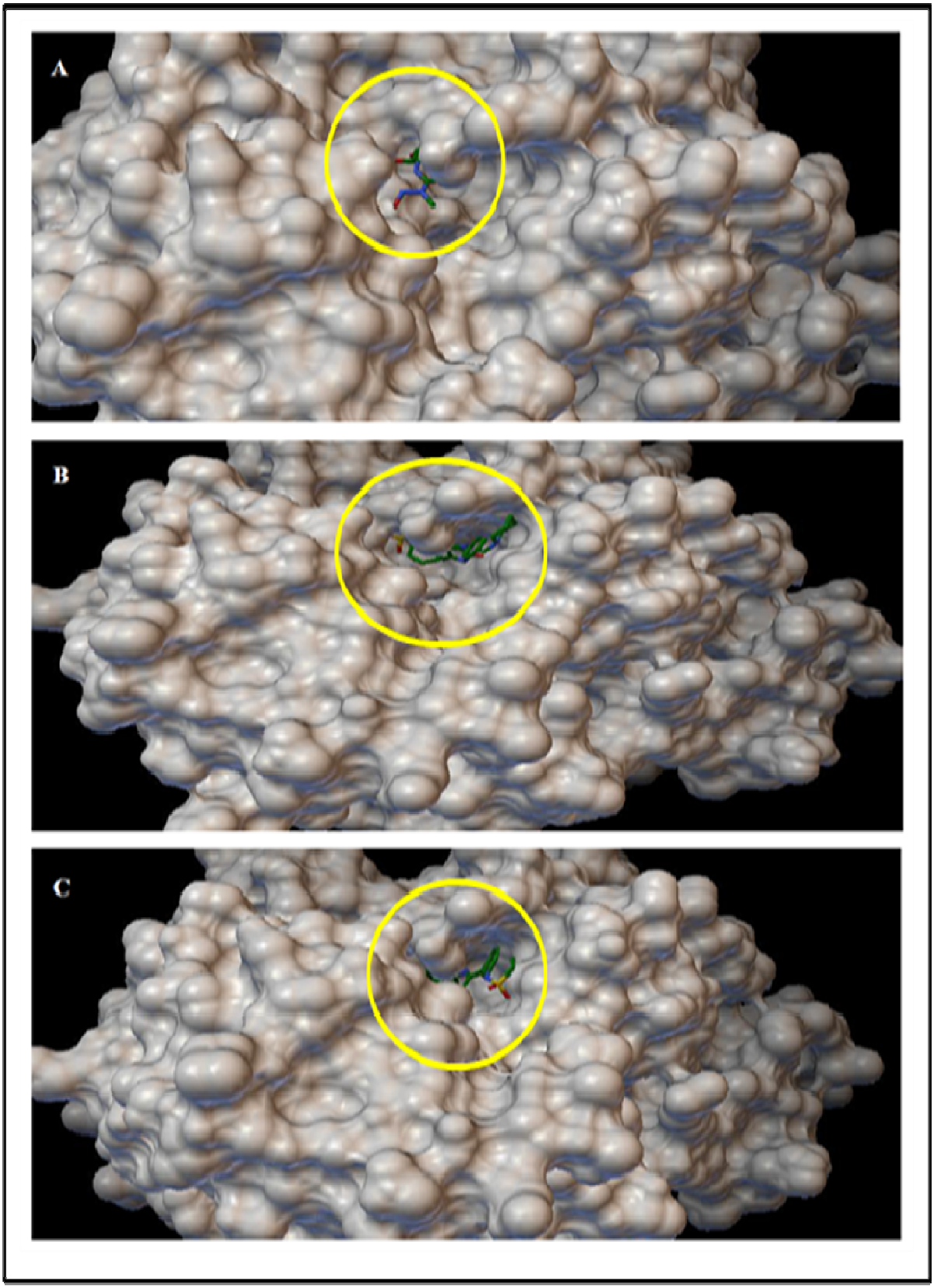
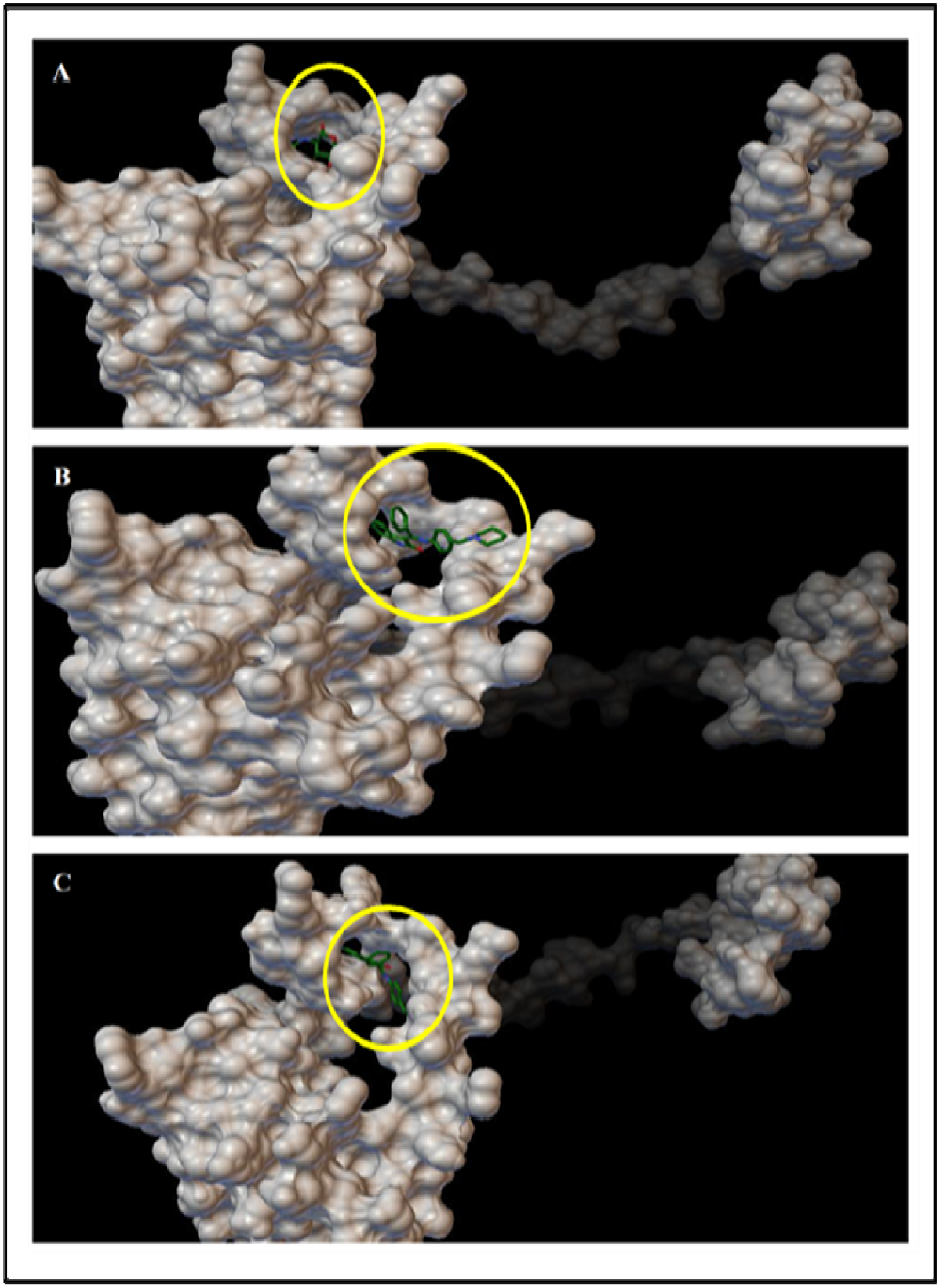


Figure 10
Interaction profiles of streptozotocin (A), bexarotene (B) and hesperidin (C) with presenilin-1. [Ligands have been highlighted in the yellow coloured circles]



DISCUSSION

Alzheimer's disease is a major cause of dementia in elderly human populations across the continents which ultimately repercussions in bereavement of memory and dilapidates regular lifestyle of diseased individuals[30]. Beta secretases-1 and presenilin-1 are essentially known to be involved in the pathogenesis of Alzheimer's disease and therefore are important candidates to serve as therapeutic targets for treating this perilous disease[31]. Beta secretase-1 has six isoforms whereas presenilin-1 has seven isoforms, which are formed by alternative splicing. Canonical isoforms of these essential neural proteins have achieved more attention while only a little information is available related to the non canonical isoforms. UniProt has sequence of all the isoforms; the amino acid sequence missing in the non-canonical isoforms are also given. But knowledge regarding the functionalities of non-canonical isoforms is still experiencing ignorance. We found that in beta secretase-1, C terminal region constituting cytoplasmic, transmembrane and more than half of the catalytic extracellular domain (a region towards the C-terminus of extracellular catalytic domain) is highly conserved among the isoforms. The N terminal region of extracellular domain is highly variable and in turn determines the physiological activity of the isoforms. The isoforms 5 and 6 lack catalytic residue ASP-48 but due to highly positive GRAVY, their hydrophobicity may cause them to aggregate[17] and form proteinous coagulations causing neurodegenerative diseases. Conjointly, the significantly higher *in vivo* life of isoform 6 may result in persistent aggregates in the neural tissues. Also, the six isoforms of beta secretase-1 varied structurally, reflecting potential undiscovered functional differences. The full length presenilin-1 is functionally inactive and activates after endoproteolytic cleavage resulting in separation of the entire sequence into the N-Terminal Fragment (NTF) and C-Terminal Fragment (CTF) domains [32]. The full length presenilin-1 is involved in the pathogenesis of Alzheimer's disease[33]. Hence, we have studied the full length sequence of presenilin-1. The full length presenilin-1 isoforms were highly hydrophobic in nature and may aggregate leading to neurodegenerative changes. Also, the structural disparities were very intense as the structural relationship among the presenilin-1 isoforms could not be resolved due lack of sufficient similarities. Among presenilin-1 isoforms, half of NTF was conserved, but the other half of NTF and entire CTF was highly variable. These results are only suggestive for functioning of the isoforms of the two essential neural proteins and we strongly encourage researchers to explore the same. The resultant knowledge may enlighten the novel pathways involved in the maintenance of healthy neural physiology or leading to pathogenesis of Alzheimer's disease and other neurodegenerative disorders. Molecular docking output acquired from iGEMDOCK, displayed that among the two drugs, bexarotene manifested highly stable interaction with beta secreatse-1 and presenilin-1. Apart from this, hesperidin and bexarotene exhibited respectable binding energy patterns respectively for beta secretase-1 and presenilin-1 implying the capability of the two drugs for binding to both neural proteins. All ligands had mutual binding clefts and possessed varying values of total binding energies for beta secretase-1 and presenilin-1. For both proteins the value of total binding energy displayed by the toxin was higher (or less negative) contrasting to the drugs; this presages an essence of competition between the toxin and the drugs for binding clefts existent on these necessitous brain proteins.

CONCLUSION

From the results, it can be interpreted, that both the therapeutic ligands (bexarotene and hesperidin) have respectable binding energy patterns for the proteins beta secretase-1 and presenilin-1, showing possible therapeutic properties of the ligands (bexarotene and hesperidin) in Alzheimer's disease plausibly by reversing the amyloid plaque formations. Many neurodegenerative diseases are also caused by oxidative stress, which affects the function of various proteins. Both hesperidin and bexarotene may have therapeutic applicability in such diseases as well. Withal,

homologs of bexarotene and hesperidin may be generated using the Qualitative Structure Activity Relationship (QSAR). Streptozotocin (STZ) was also found to interact with beta secretase-1 and presenilin-1 suggesting that the toxic ligand interacts with these proteins, thereby affecting their properties in a negative manner resulting in Alzheimer's like symptoms. Hence, the study displayed the pernicious effect of streptozotocin on essential neural proteins and therapeutic role of bexarotene and hesperidin drugs, in treating Alzheimer's diseases and other neural disorders. Additionally, the *in-silico* scrutiny of the isoforms of beta secretase-1 and presenilin-1 raises a strong need for thorough exploration of these essential neural proteins for better understanding of their roles in the normal physiology and neurodegenerative disorders.

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ABBREVIATIONS

AD: Alzheimer's diseases; BACE: Beta secretases; PSEN: Presenilin; STZ: Streptozotocin; APH-1: Anterior pharynx-defective 1; PEN-2: Presenilin enhancer 2

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