Research Article

In-Silico Discovery of Novel Inhibitors Against PrP Protein (*E196K, V203I and E211Q*): Creutzfeldt Jakob Disease

Swetha Pavani Rao*, Shama Mujawar, Sameer Chaudhary, Inigo Benze Thobias
RASA Life Science Informatics, 301, Dhanashree Apartments, Opposite Chittaranjan Vatika, Model colony, Shivaji Nagar, Pune, MH, India.
***Corresponding author's E-mail:** dr.swethapavani@gmail.com

Accepted on: 08-04-2015; **Finalized on:** 30-04-2015.

ABSTRACT

Creutzfeldt - Jakob disease (CJD) is a disorder of an abnormal protein (PrP), characterized by microscopic vacuoles in brain, loss of neurons and astrocytosis. The disease forms aggregates of the abnormal protein in neuronal cells. This alters the cell functions which may include synaptic plasticity, a role in neuronal development, neuronal myelin sheath maintenance, and iron uptake and iron homeostasis. Our research aim to identify novel drugs inhibits the abnormal PrP by using computer based drug design. We here designed three dimensional structures by homology modelling of mutant PrP using modeller, validated using Ramachandran plot. Various computer based premises has been developed including ligand based pharmacophore, active site prediction, virtual screening. Based on binding energy two drugs were selected and subjected to toxicity prediction and ZINC3830922 emergent as a potent inhibitor for mutant PrP.

Keywords: Creutzfeldt - Jakob disease, CJD, Prion Protein, PrP, Drug design, Homology Modeling.

INTRODUCTION

Prions are protein molecules that are typically much smaller than viruses and are detectable through electron microscope only as clusters. They cause wide array of degenerative neurological disorders that include Bovine Spongiform Encephalopathy (mad cow) and Creutzfeldt-Jakob disease. Familial CJD (transmissible spongiform encephalopathies) is a rare fatal neurodegenerative disorder of central nervous system. It occurs in humans as well as animal species sporadically at an annual rate of one in a million.[1] It is characterized by microscopic vacuoles in brain, loss of neurons and astrocytosis.[2]

The causative agent for familial CJD is a normal host Prion protein (PrP/PRNP). Genetic mutations in the gene which codes for PrP were shown to be associated with the human disease which indicates that the modified form of the PrP causes the disorder.[3] This protein is a membrane glycosylphosphatidylinositol anchored glycoprotein that tends to aggregate into rod-like structures. The encoded protein contains a highly unstable region of five tandem octa-peptide repeats. The gene encoding PrP is found on chromosome 20p13, approximately 20 kbp upstream of a gene which encodes a biochemically and structurally similar protein to the one encoded by this gene.(http://www.ncbi.nlm.nih.gov/gene/5621).

Mutations in the PrP gene are the source for familial CJD. Inherited prion diseases are caused by germ-line mutations in the PRNP gene. PrP modification appears to lie in its shape. An abnormally folded form of PrP makes it very stable and resistant to proteolysis. The accumulation of protease resistant protein in the brain for prolong interval appears to be the basis of neural degeneration causing the disease.[4] Hence, drugs effective on the PrP should be discovered so as to inhibit the protein aggregation. Some of the drugs like Astemizole, Amantadine, Acyclovir, Curcumin, Doxycycline, Flupirtine, Quinacrine, pentosan sulphate, vidarabine have been found to alleviate the symptoms associated with fCJD.

Humans lack immune response against prion protein as it is an abnormal protein with point mutations and also protease resistant. It is not recognized by our immune system as a foreign particle.[5] Hence, prion aggregation has to be controlled either by drugs or by different means which may include miRNA, gene silencing, antibodies etc.[5-8]

Our present study is aim to design new lead molecule by using bioinformatics tools.

Three dimensional structure of the mutant protein modelled using modeller. Ligand based pharmacophore design and Virtual screening was used to find the potent inhibitor. Drug like properties and toxicity was evaluated to validate the lead drug.[9-11]

MATERIALS AND METHODS

Target Identification

Bovine Spongiform Encephalopathy (BSE) is a most common disease found in cow herds of UK which transmitted to humans.[12-13] The origin of transmission is unclear. The transmission of BSE to humans as vCJD[12] gave rise to a vast public concern, with a death toll of 119 people so far.[14]

Currently, there is no medicine available to combat the disease. To inhibit the causative agent, below is some of the in-silico experiments performed to find a lead which may act against the agent. The causative agent of the Creutzfeldt Jakob Disease (CJD) is found to be a PRION

Protein (PrP) in humans. Prion having mutations at E196K, V203I, and E211Q has been taken for discovering drug which causes Creutzfeldt-Jakob disease. [1]

Target Modeling

FASTA sequence of protein has been retrieved from UniprotKB database (Accession No. P04156). Retrieved FASTA sequence has been mutated at E196K, V203I, E211Q using RASMOL and its primary structure was analyzed using Protparam tool. Protparam is a tool to estimate the physical and chemical properties of the protein sequence entered. The physical and chemical property is displayed in (Table-1). The parameters include molecular wt., theoretical pI, amino acid composition and extinction coefficient. Secondary structure prediction was done with GOR, SOPMA and CFSSP. SOPMA gives accurate prediction as CFSSP does not give information about the coils in protein. The results are shown in (Table-2). Detection of the tertiary structure of the protein was performed using Modeller, PS2, Phyre2, SwissModel, Geno3D and RaptorX. These models have been evaluated using PDBSUM and RAMPAGE. As shown in (Table-3) and (Figure-1).[15-21]

Table 1: Physical and chemical parameters of PrP protein sequence was analyzed by Protparam tool of Expasy's[28] server

No. of Amino acids	253
Molecular Weight	27673.2
Theoretical pI	9.43
No. of Positively charged molecules	22
No. of Negatively charged molecules	13
Estimated Half – Life	30 hours
Instability Index	42.85
Hydropathicity	-0.568

Hot Spot (Pocket Detection)

A site where Ligand binds to the protein actively is known as Active site/Pocket. Active site is crucial to dock a drug at a specific site of the protein. These pockets are determined using servers COACH, CASTp, TM Site. Binding sites from the different servers were compared and the best three are selected for docking. Out of three the best selected pocket has the conformations in x, y, z axis are -22.297, 15.527 and 5.113 respectively where interaction between protein and drug was executed.[22-25]

Pharmacophore Model Generation

In 1909, Ehrlich defined pharmacophore as 'a molecular framework that carries the essential features responsible for a drug's biological activity'.[26] According to IUPAC, Pharmacophore is "an ensemble of steric and electronic features that is necessary to ensure the optimal supramolecular interactions with a specific biological target and to trigger (or block) its biological response".[27] Drugs used for pharmacophore design are: Amantidine, Amphotericin, Curcumin, Heparitin, Pentosan Sulphate, Quinacrine, Quinapyramine, Tetracycline and Thioflavine. These drugs have been identified to work against the symptoms of the disease but do not directly inhibit the protein. Pharmagist is a well-known server for the detection of pharmacophores. Using the pharmacophore features of these drugs, a new pharmacophore was developed. This pharmacophore is used as a principal for the detection of ligands having the same features.[28]

Ligands Database Screening

ZincPharmer is a freely available database contain drug like molecule, and an online interface for searching the purchasable compounds of the ZINC database.[29] The generated pharmacophore was entered in ZincPharmer to screen the molecules using ZINC drug database filters. The compounds that match with the submitted pharmacophore serve as a lead for a drug discovery. The database searches 176 million conformers out of which 1107 drugs have been identified with desired pharmacophore features. The drugs thus obtained from the ZincPharmer will be used to dock and check affinity with PrP protein.

Receptor - Ligands Interaction (Docking)

AutoDock is the docking tool designed by Molecular graphics laboratory, reliable software to detect protein ligand interactions. Genetic algorithm method was used to predict the protein ligand docking. Vina is an open-source program for virtual screening. All the screened Ligands (1107 conformers) were allowed to interact with the protein at a specific active site with x, y, z axis -22.297, 15.527 and 5.113 respectively. Multiple docking of the protein was done using AutoDock Vina by Cygwin software. Nine conformations were used for docking.[30]

Lead Identification

A total of 1107 Docked drugs were analyzed using MGL Tools where binding energy and repeated conformations are noted, out of which 27 best drugs were found to be interacting with the protein at its specified binding site. These drugs are having bond energy in the range of 8.1 - 6.1 kJ which is considered to be good while ideal binding energy is nearly 10 kJ.

Loop Docking

Drugs which are following Lipinski Rule were re-docked to verify the stability of their conformations. Loop Docking of each drugs were performed 10 times increasing the conformations to 60. Re-docking was executed on MGL tools by Cygwin software and the best leads were identified. Drug likeness and toxicity is visualized in Table-6.

RESULTS AND DISCUSSION

Homology Modeling and Validation

Protein sequence accessed from the UniProtKB database (Access ID P04156) and mutate the sequence in E196K, V203I, and E211Q using RASMOL. Further parameters

were analyzed by using Protparam and displayed in (Table-1).

In addition to that; Alpha helix, extended strand, Beta turns and Random coils were analyzed using SOPMA, CFSSP and GOR as shown in Table-2.

We validate the quality of the protein using Ramachandran plot. We predict the quality using RAMPAGE and PDBSUM.

Based on RMAPAGE prediction 98% are in the favored region and 2% falls in the allowed region whereas PDBSUM predict 94.2% in favored and 5.8% in favored region.

The predicted model shows 100% in favored and allowed region indicates our modelled protein is in higher quality. The estimated values are displayed in Table-3 and the modelled protein visualized in Figure 1.

Protein Sequence retrieved from UniProtKB database having accession ID-P04156

MANLGCWMLVLFVATWSDLGLCKKRPKPGGWNTGGSRYPG QGSPGGNRYPPQGGGG

WGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQ GGGTHSQWNKPSKPKTNMK

HMAGAAAAGAVVGGLGGYMLGSAMSRPIIHFGSDYEDRYYRE NMHRYPNQVYYRPM

DEYSNQNNFVHDCVNITIKQHTVTTTTKG**K**NFTETD**I**KMMERV V**Q**QMCITQYERESQA YYQRGSSMVLFSSPPVILLISFLIFLIVG

Table 2: Showing Secondary structure prediction using GOR, SOPMA and CFSSP

Method	Alpha Helix %	Extended Strand %	Beta Turns %	Random Coils %
SOPMA	28.85	17.79	5.53	47.83
CFSSP	36.8	-	15.8	-
GOR	18.97	23.32	0.0	57.71

Table 3: Estimation of the 3D models of the protein using RAMPAGE and PDBSUM showing favored, allowed and disallowed regions.

	Favored Regions		Allowed Regions		Disallowed Regions	
Servers	RAMPAGE %	PDBSUM %	RAMPAGE %	PDBSUM %	RAMPAGE %	PDBSUM %
Geno3D	84.6	81.6	14.3	17.2	1.1	0.0
Modeller	98	94.2	2	5.8	0.0	0.0
Phyre2	92.9	87.7	5.7	11.5	1.4	0.8
PS2	99.1	96.9	0.0	2.1	0.9	1.0
RaptorX	94.9	89.1	10.9	4	0.6	0.0
SwissModel-1	94.2	89.3	5.0	10.7	0.7	0.0
SwissModel-2	100	99	0.0	1.0	0.0	0.0
SwissModel-3	100	99	0.0	1.0	0.0	0.0

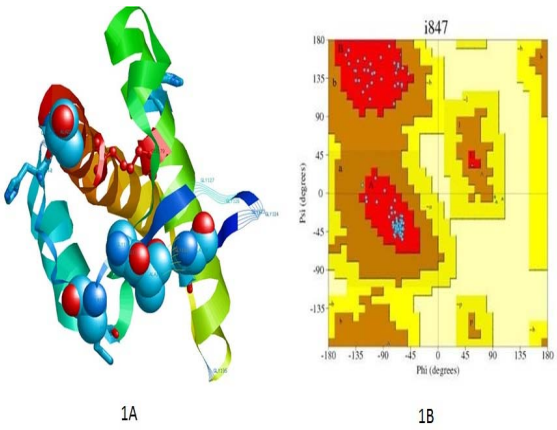

Figure 1: (1A) Shows the visualization of the 3D – model of protein (1B) is the Ramachandran plot of the modelled protein.

Pharmacophore Model Generation

In our research we use Pharmagist a freely available web server. We aligned drugs like Amantidine, Amphotericin, Curcumin, Heparitin, Pentosan Sulphate, Quinacrine, Quinapyramine, Tetracycline and Thioflavine with Amphotericin as the pivot molecule. Best aligned pharmacophore file was further proceeded for zinc pharmer and following virtual screening.

Ligand Screening

Virtual screening was performed to gain the most promising inhibitor to bind the mutant PrP. We got a collection of 27 drugs which inhibit mutant protein (Table-4). On subsequent Re-Docking two potent drugs obtained, shows optimal interactions with mutant protein and the result visualize in Table-5. Further toxicity of the drug was estimated and the results were presented in Table-6.

Table 4: Top 27 leads with best binding energies were identified out of 1107 molecules.

S. No.	Molecule - ZINC ID	Afinity (kcal/mol)	Interaction
1	ZINC00020253	-6.3	TYR162 : OH
2	ZINC00601265	-6.1	THR190 : OG1
3	ZINC00897225	-6.4	TYR162 : OH, THR190 : OG1
4	ZINC00601305	-6.6	THR190 : OG1, TYR162 : OH1
5	ZINC00968279	-7.2	THR190 : OG1
6	ZINC03616640	-6.1	THR190 : OG1
7	ZINC03830383	-7.6	LYS194 : NZ
8	ZINC03830385	-7.9	THR190 : OG1
9	ZINC03830431	-6.3	THR190 : OG1 , TYR162 : OH
10	ZINC03830432	-7	THR190 : OG1
11	ZINC03830922	-7.3	THR190 : OG1
12	ZINC03830924	-7.8	THR190 : OG1
13	ZINC03831159	-6.4	HIS187 : ND, TYR162 : OH
14	ZINC03831242	-6.3	THR190 : OG1
15	ZINC03874498	-5.9	TYR162 : OH
16	ZINC03920266	-6.9	THR190 : OG1
17	ZINC04097304	-6	TYR162 : OH
18	ZINC08552018	-6.3	HIS187 : ND1
19	ZINC11592618	-6.4	THR190 : OG1
20	ZINC11592622	-6.5	THR190 : OG1
21	ZINC11592929	-6.4	HIS187 : ND1, TYR162 : OH
22	ZINC11678081	-7.7	THR190 : OG1 , TYR162 : OH
23	ZINC11678088	-7.9	THR190 : OG1
24	ZINC11678097	-8.1	THR190 : OG1
25	ZINC11678102	-6.9	TYR162 : OH , THR190 : OG1
26	ZINC14879972	-6.5	THR190 : OG1
27	ZINC52955754	-7.4	THR190 : OG1

Table 5: Potent drug obtained from loop docking (Re-Docking)

Drugs	Docking	Binding affinity	Interacting residue
ZINC03830922	Dock1	-7.4	THR190: OG1
	Dock2	-7.4	THR190: OG1
	Dock3	-7.4	THR190: OG1
	Dock4	-7.4	THR190: OG1
	Dock5	-7.4	THR190: OG1
	Dock6	-7.4	THR190: OG1
	Dock7	-7.4	THR190: OG1
	Dock8	-7.4	THR190: OG1
	Dock9	-7.4	THR190: OG1
	Dock10	-7.4	THR190: OG1
Zinc03831242	Dock1	-6.3	THR190:OG1
	Dock2	-5.8	THR190:OG1
	Dock3	-6.5	TYR162:OH
	Dock4	-5.5	THR190:OG1
	Dock5	-6.3	THR190:OG1
	Dock6	-6.3	THR190:OG1
	Dock7	-6.3	THR190:OG1
	Dock8	-6.4	THR190:OG1
	Dock9	-5.6	THR190:OG1
	Dock10	-5.6	THR190:OG1

Table 6: Toxpredit result for ligands "ZINC03831242" and "ZINC3830922"

ADMET Property	ZINC03830922	ZINC03831242
Bioavailability	YES	YES
Carcinogenic	Non Carcinogen	Non Carcinogen
Irritant	No irritation	No irritation
Drug score	0.37	0.75
Molecular Wt	498.508	400.436
Log P	0.89	2.03
Lipinski	Yes	Yes
Hydrogen bond donor	7	1
Hydrogen bond acceptor	10	8
Chemical name	Idarubicin	Micropenin

CONCLUSION

Creutzfeldt - Jakob disease, rare neurodegenerative cause's brain disorder. There are no drugs to control or cure CJD. Advance therapeutic findings and novel drugs are needed to control CJD. Our current work focused on ligand based virtual screening on mutant modelled protein. Our research outlines that ZINC03830922 popularly known as Idarubicin, an antitumor antibody class of drug shows inhibitory signs by binding with THR190:OG.

Significantly our other drug ZINC03831242 popularly known as Micropenin inhibit the mutant prion protein THR190:OG. We conclude that ZINC03830922 shows good binding interactions and there will be no signs of carcinogenic and displays a very good bioavailability and there is no signs of irritation. Furthermore clinical studies are needed to validate the drug against CJD.

REFERENCES

1. McKintosh E, Tabrizi SJ, Collinge J, Prion diseases, Journal of NeuroVirology, 9, 2003, 183–193.
2. Klingeborn M, "The Prion Protein in Normal Cells and Disease: Studies on the Cellular Processing of Bovine PrPC and Molecular Characterization of the Nor98 Prion [dissertation], Swedish University of agricultural science, Uppsala, (2006), 1-58.
3. Peoc'h K., Manivet P., Beaudry P., Attane F., Besson G., Didier H., Delasnerie-Laupretre N., Laplanche J.-L, Identification of three novel mutations (E196K, V203I, E211Q) in the prion protein gene (PRNP) in inherited prion diseases with Creutzfeldt-Jakob disease phenotype, Hum Mutat, 15, 2000, 482.
4. Prusiner SB, Scott MR, DeArmond SJ, Cohen FE, "Prion Protein Biology", cell, 93, 1998, 337-348.
5. Manuelidis L, Virulence, Infectious Particles, Stress, and induced prion amyloids: a unifying perspective, Virulence, 4, 2013, 373-383.
6. Linden R, Martins VR, Prado MAM, Cammarota M, Izquierdo I, Brentani RR, "Physiology of the Prion Protein", Physiol Rev, 88, 2008, 673-728.
7. Harris DA, "Cellular Biology of Prion Diseases, Clinical Microbiology" Reviews, 12, 1999, 429-444.
8. Dealler S, Rainov NG, Pentosan polysulfate as a prophylactic and therapeutic agent against prion disease, IDrugs, 6, 2003, 470-478.
9. Shoichet BK. Virtual screening of chemical libraries, Nature, 432, 2004, 862–865.
10. Kuck D, Singh N, Lyko F, Medina-Franco JL, Novel and selective DNA methyltransferase inhibitors: Docking-based virtual screening and experimental evaluation, Bioorg. Med. Chem, 18, 2010, 822–829.
11. Taylor RD, Jewsbury PJ, Essex JW. A review of protein-small molecule docking methods. J. Comput. Aided Mol. Des, 16, 2002, 151–166.
12. Hill AF, Desbruslais M, Joiner S, Sidle KCL, Gowland I, Collinge J, Doey LJ, Lantos P, The same prion strain causes vCJDand BSE, Nature, 389, 1997, 448-450.
13. Collinge J, Sidle KCL, Meads J, Ironside J, Hill AF, Molecular analysis of prion strain variation and the aetiology of 'new variant' CJD, Nature, 383, 1996, 685-690.
14. Robotin M, Evaluation of the Australian CJD surveillance system, Communicable disease intelligence, 26, 2002.
15. Cavasotto CN, Phatak SS, Homology modelling in drug discovery: current trends and applications, Drug Discov. Today, 14, 2009, 676–683.
16. Krieger E, Nabuurs SB, Vriend G, Homology modelling Methods, Biochem. Anal, 44, 2003, 509–523.
17. Pierri CL, Parisi G, Porcelli V, Computational approaches for protein function prediction: a combined strategy from multiple sequence alignment to molecular docking-based

virtual screening. Biochim. Biophys. Acta, 1804, 2010, 1695–1712.

18. Larkin MA, Blackshields G, Brown NP, Chenna R, Mc Gettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, Thompson JD, Gibson TJ, Higgins DG, Clustal W and Clustal X version 2.0, Bioinformatics, 23, 2007, 2947-48.
19. Eswar N, Marti-Renom MA, Webb B, Madhusudhan MS, Eramian D, Shen M, Pieper U, Sali.A, Comparative Protein Structure Modelling With MODELLER, Current Protocols in Bioinformatics, 15, 2006.
20. de Beer T A P, Berka K, Thornton J M, Laskowski R A, PDBsum additions, Nucleic Acids Res, 42, 2014, D292-D296.
21. Gasteiger E., Hoogland C., Gattiker A., Duvaud S., Wilkins M.R., Appel R.D., Bairoch A, Protein Identification and Analysis Tools on the ExPASy Server, The Proteomics Protocols Handbook, 2005, 571-607.
22. Kore P, Mutha M, Antre R, Oswal R, Kshirsagar S, Computer-Aided Drug Design: An Innovative Tool for Modelling, Open Journal of Medicinal Chemistry, 2, 2012, 139-48.
23. Aparoy P, Reddy KK, Reddanna P, Structure and Ligand Based Drug Design Strategies in the Development of Novel 5-LOX Inhibitors, Curr Med Chem, 19, 2012, 3763-3778.
24. Kapetanovic IM, Computer-aided drug discovery and development (CADDD): *In silico*-chemico-biological approach, Chem-Biol Interact, 171, 2008, 165–176.
25. Shaikh SA, Jain T, Sandhu G, Latha N, Jayaram B, From drug target to leads-sketching a physicochemical pathway for lead molecule design *in silico*, Curr. Pharm. Design, 13, 2007, 3454-70.
26. Ehrlich P, Ueber den jetzigen Stand der Chemotherapie, Ber. Dtsch. Chem.Ges, 42, 2006, 17–47.
27. Wermuth CG, Ganellin CR, Lindberg P, Mitscher LA, Glossary of terms used in medicinal chemistry (IUPAC Recommendations 1998), Pure and Applied Chemistry, 70, 1998, 1129-43.
28. Schneidman-Duhovny D, Dror O, Inbar Y, Nussinov R, Wolfson HJ, PharmaGist: a webserver for ligand-based pharmacophore detection, Nucleic Acids Research, 2008.
29. Koes DR, Camacho CJ, ZINCPharmer: pharmacophore search of the ZINC database, Nucleic Acids Research, 40, 2012, W409-W-414.
30. Trott O, Olson AJ, AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading, J Comput Chem, 31, 2011, 455-461.

Source of Support: Nil, **Conflict of Interest:** None.