IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)
e-ISSN: 2278-3008, p-ISSN:2319-7676. Volume 7, Issue 6 (Sep. – Oct. 2013), PP 39-44
www.iosrjournals.org

Insilico Designing and Development of Drug Inhibitor to PSEN 1 Protein in Alzheimer's disease

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Abstract: *There are at least four well-confirmed genes responsible for Alzheimer's disease (AD), the most common form of dementia. In addition, many reports indicate an association between the disease and genetic variations in different gene candidates. Approximately 5-10% of patients develop an early age-at-onset AD (before 65 years). The disease in up to 50% of such cases is explained by mutations in one of three genes: APP, presenilin 1 (PS1), and presenilin 2 (PS2) . Pathological mutations in these genes PS1 on Chromosome 14q24.3 and PS2 Genes on Chromosome 1q31-q42 are responsible for an autosomal dominant trait and cause A-beta accumulation in the brain.[1] However, the pathological consequence of some mutations detected in small AD families is uncertain and needs further investigation. Recently, we proposed to use a systematic algorithm to classify mutations in known AD genes as possibly, probably, or definitely pathogenic. Leads were identified based on several physiochemical properties and we created our library with those new molecules that were generated based on Lipinski's rule of five. We carried out high throughput screening and molecular docking on 65 molecules from scaffolds selected. Screening of molecules was based on the criterions such as, TOPKAT and ADMET properties. Among the ligands used, only three compounds were identified to have interaction within the targeted domain. To be more specific about our drug candidate we suggest noname 3 to be the most potent molecule in terms of physiochemical and docking properties. These results suggest that the identified compounds have the potential to inhibit PSEN-1 binding in Alzheimer.*
Keywords*: PSEN-1, modeling, TOPKAT, ADMET, receptor, ligand, docking, pharmacophore*

I. Introduction:

Alzheimer's disease (**AD**), also known in medical literature as **Alzheimer disease**, is the most common form of dementia. There is no cure for the disease, which worsens as it progresses, and eventually leads to death. It was first described by German psychiatrist and neuropathologist Alois Alzheimer in 1906 and was named after him. Most often, AD is diagnosed in people over 65 years of age although the less-prevalent early-onset Alzheimer's can occur much earlier. In 2006, there were 26.6 million sufferers worldwide.

More recent studies demonstrate that PSEN mutation involves formation of Presenillin-1(PSEN-1) protein which interact with molecule gamma secretase responsible for the formation of amyloid plaque and neurofibrillary tangles. Research indicates that the disease is associated with plaques and tangles in the brain. Current treatments only help with the symptoms of the disease. There are no available treatments that stop or reverse the progression of the disease.

In the current study we analyse the protein sequence of surface subunit of human PSEN gene PSEN type-1 deposited at PDB (2Kr6) and KEGG. Taking to the next stage we carried out further studies on PSEN binding domain(aa440) of surface subunit in PSEN-1 protein. *In-silico* methods have been applied to pharmacology hypothesis development and testing. These *in-silico* methods include databases, quantitative structure-activity relationships, similarity searching, pharmacophores, and other molecular modelling, machine learning, data mining, network analysis tools and data analysis tools that use a computer.

During our study, we designed 65 ligands having potential as inhibitor to PSEN-1 binding and inhibit the protein to interact with gamma secretase. This inhibitory action of the ligand could minimize the action of gamma secretase. All the ligands were screened for Lipinski's Rule of 5[46], toxicity and later on docking was done. These ligands and receptor was also energetically minimized during those process. Majority of our work was performed using Accelrys Discovery studio 2.5.

II. Materials and Methods:

Disease selection	Receptor Identification	Structure Modelling	Structure Validation	Scaffold Identification	Lead library design	screening	Molecular docking	Pharmacophore Analysis

***3.1. Target identification and validation*:**

Surface subunit PSEN-1 sequence was retrieved from PDB (2Kr6) and KEGG and analysed for domains using Blastp against Protein Data Bank. Meanwhile sequences of PSEN-1 deposited in GenBank at various periods were subjected to sequence alignment to determine the quantity and quality of mutations occurred.The structure was validated for Ramachandran plot using Procheck and Discovery studio.

***3.2. Scaffold Selection*:**

Searching for drugs having potent inhibitory action on protein with similar function, we analysed drugs from KEGG database and Drug Bank.Inhibitor for gamma secretase against PSEN gene and surface protein PSEN-1 were used for lead designing based on various literatures substantiating their biological functionality.

***3.3. Lead library Designing*:**

Lead library was designed based on Lipinski's rule of five. The functional group of all leads were kept unchanged on the course of our designing. Lead design was performed with ChemSketch Freeware. No inclusion of heavy atoms or carcinogenic atoms to the molecule was done.

3.4. Virtual screening:

ADMET and TOPKAT protocols were used in Discovery studio to screen ligand molecules. Six models such as NTP Carcinogenicity Call (Male Mouse) (v3.2), FDA Carcinogenicity Female Mouse Single vs. Mult (v3.1), Developmental Toxicity Potential (DTP) (v3.1), Rat Oral LD50 (v3.1), Skin Irritation (v6.1) and Aerobic Biodegradability (v6.1) were deployed for TOPKAT analysis(Table.2).

Molecules screened with TOPKAT were later subjected to ADMET protocol. ADMET -after oral administration.

3.5. Receptor and Ligand Preparation:

The validated model was then determined for largest binding site using CASTp server and Accelrys Discovery Studio 2.5. The sphere was defined for the binding site; charge typing was carried out by CHARMm force field (Momany-Rone parital charges methods). Minimization was carried out in Accelrys Discovery Studio 2.5 using 2200 cycles of conjugate gradient; a constant potential energy of -22322.84878 kcal/mol was obtained.

The screened compounds were typed similarly using CHARMm for partial charges set up and minimized by Conjugate Gradient until a constant potential energy -4106.66943 kcal was obtained.

3.6.Receptor-Ligand Docking:

The minimised receptor and ligand was docked with LibDock, a relatively fast algorithm thatconducts 'HotSpots' matching of ligand conformation.

The interactions for the pose with low LibDock Score are studied and intermolecular hydrogen bonds and intermolecular bumps were further examined(Table.3).

3.7.Pharmacophore Analysis:

The ligands were analysed for pharmacophore using the common purpose pharmacophore in Pharmacophore protocol available in Discovery studio.Pharmacophore analysis include aromatic group, donor molecule, positive and negative ionizing group ,hydrophobic group and hydrophilic group(Fig.5).

III. Results:

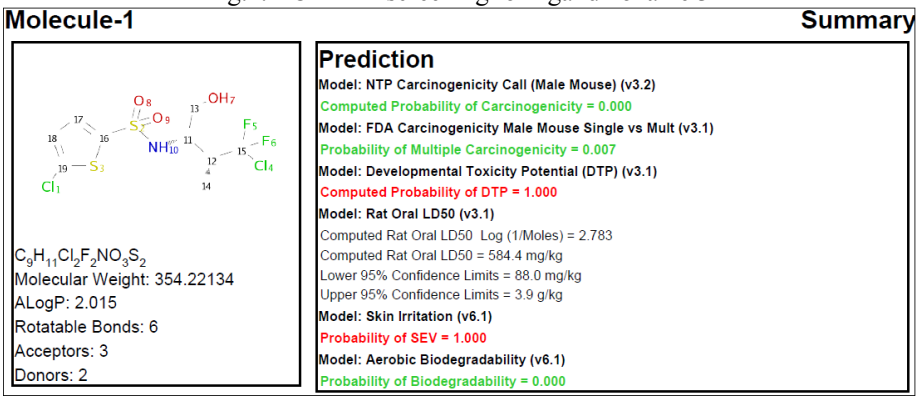
Table.1: Molecular properties of molecules used for docking.

Mol. Name	ALogP	Molecular Weight	Num_H Acceptors	Num_H Donors	Num_ Rotatable Bonds	Num_ Rings	Num Aromatic Rings	Molecular_ Fractional Polar Surface Area
Noname 3	2.015	354.22134	3	2	6	2	2	0.142

Inference:65 molecules were designed and those molecules which obey the Rule of five were selected for further screening and later docking studies

Inference: three ligand molecules were identified to show positive results. Molecules screened for TOPKAT screening were given flexible criterion on Skin irritation, DTP and Rat oral LD50.

Fig.1. TOPKAT screening for ligand noname 3



Inference: The ligand noname 3 had good TOPKAT score and are well suited for ADMET screening. Tolerance to skin irritation and DTP were given to these molecule to a certain limit.

Table.2. ADMET screening for molecules used for docking studies.

NAME	ADMETT_BB	ADMET_BB_LEVEL	ADMET_Absorption_Level	ADMET_Solubility	ADMET_Solubility_Level	ADMET_Hepatoxicity	ADMET_Hepatoxicity_Probability	ADMET_CYP2D6	ADMET_CYP2D6_Probability	ADMET_PPB_Level	ADMET_AlogP98	ADMET_Unknown_AlogP98	ADMET_PSA_2D
NONAME1	-0.467	2	0	-3.885	3	0	0.456	0	0.247	0	2.48	0	68.227
NONAME3	-0.467	2	0	-3.885	3	0	0.456	0	0.247	0	2.48	0	68.27
NONAME4	-1.051	3	0	-3.63	3	1	0.675	0	0.217	0	1.934	0	94.458
NONAME50	0.38	1	0	-4.08	2	1	0.615	0	0.316	2	3.681	0	38.116
CL3	1.12	0	0	-5.017	2	1	0.629	1	0.712	2	4.58	0	8.93
CL7	0.326	1	0	-4.056	2	1	0.523	1	0.623	2	3.567	0	39.35
CL14	0.226	1	0	-4.532	2	1	0.635	0	0.465	0	3.795	1	50.132
CL15	0.431	1	0	-5.277	2	1	0.715	1	0.673	1	4.459	1	50.132

Inference: ADMET screening of ligand molecules demonstrated their Blood- Brain Penetration and Hepatotoxicity. Flexible criterions based on probability(<0.6) were given to certain molecule having potential for further analysis.

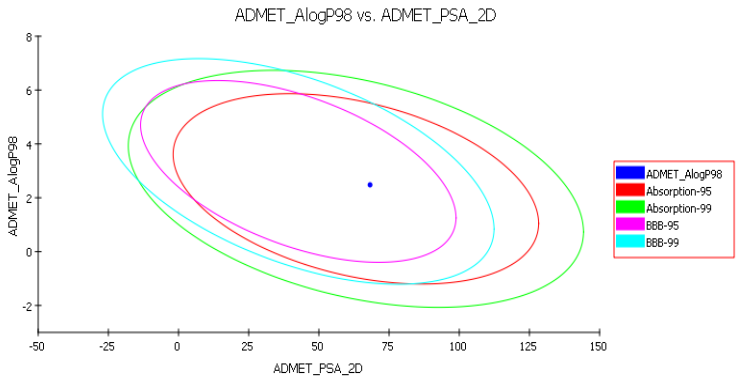


Fig.3. ADMET description plot for noname 3 ligand molecule.

Inference: ADMET description of ligand nonmae 3 makes it a good candidate for Docking and Pharmacophore studies.

Table.3. Docking studies on ligand molecules having interactions within the Receptor-Ligand complex.

Name	absolute energy	conf number	mol_number	relative energy	pose number	libdock score	hot spots
noname 3							
Molecule-1	31.9961	113	1	9.39411	1	69.0827	3.06,68.89,-6.76,A,8,1 1.66,66.29,-5.36,A,10,3 0.26,63.69,-6.96,A,7,9
Molecule-1	36.9004	177	1	14.2983	2	66.0143	-7.94,66.29,7.84,A,53,3 -10.14,65.49,8.84,A,65,20 -10.14,66.89,9.04,A,68,21
Molecule-1	33.1622	140	1	10.5602	3	65.441	-7.94,66.29,7.84,A,53,3 -10.14,65.49,8.84,A,65,20 -10.14,66.89,9.04,A,68,21
Molecule-1	29.7288	77	1	7.12676	4	65.3645	-7.94,66.29,7.84,A,53,3 -10.14,65.49,8.84,A,65,20 -10.14,66.89,9.04,A,68,21
Molecule-1	31.4233	104	1	8.8213	5	64.9248	2.86,67.09,9.04,A,69,1 6.46,66.09,0.24,A,29,6 4.06,67.49,0.64,A,31,8
Molecule-1	24.8538	7	1	2.25176	6	64.5077	-7.94,66.29,7.84,A,53,3 -10.14,65.49,8.84,A,65,20 -10.14,66.89,9.04,A,68,21

Infernence :-Total 96 poses were obtained for the ligand molecule no name 3

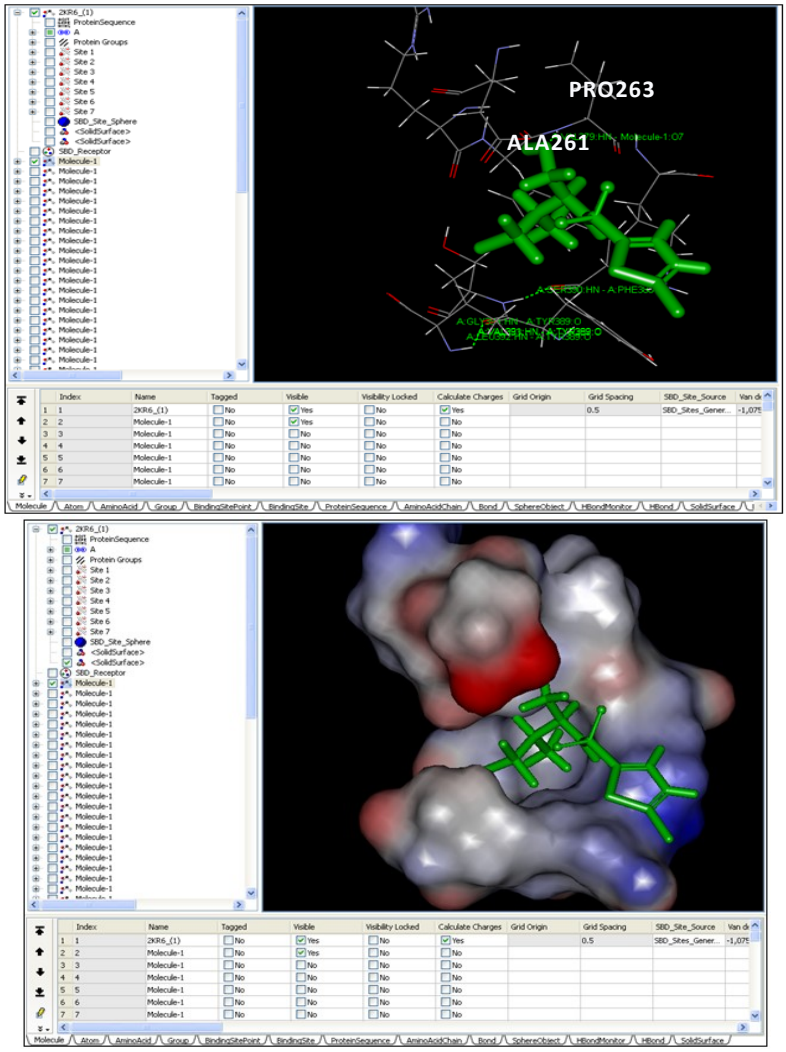


Fig.4: Intermolecular interactions ligand noname 3 "5-chloro-*N*-[(2*S*,3*R*)-4-chloro-4,4- difluoro-1-hydroxy-3-methylbutan-2-yl]thiophene-2-sulfonamide" with the receptor.

Inference: Docking studies on selected molecules express a variety of interaction within the Receptor- Ligand complex. Molecules noname 3,showed promising interactions within PSEN-1 binding domain(aa440). Screening of molecules on the basis of all parameter we used for selection of good ligand candidate, we identified molecule noname 3 as a good drug candidate to inhibit PSEN-1.

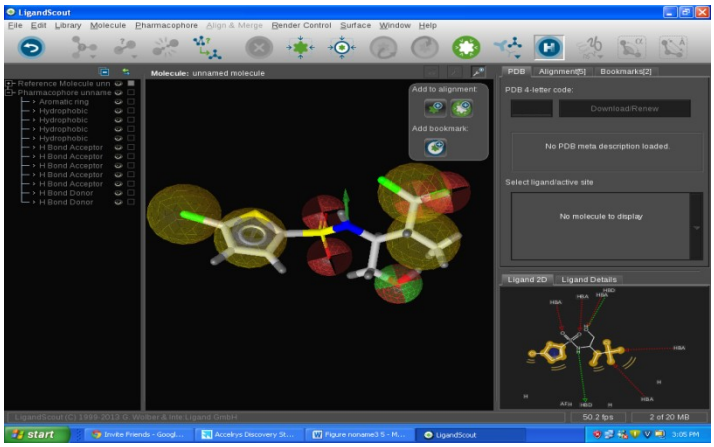


Fig.5: Pharmacophore analysis of noname3 in LigandScoutt.

IV. Discussion:

The ADMET results showed that noname 3 has a positive result as depicted in the Table.3. The probability values for TOPKAT screening with noname 3 were between 0.0 to 0.30, and are likely to produce a negative response in an experimental assay.The computed rat oral bioavailability was found to be less than 70% and the predicted LD50 values in rat was found to be 1.6 g/kg when administered orally for these molecule. The ADMET Absorption_PSA_2D level was between 5.8-6.2(99%). The probability of ADMET hepatotoxicity was (0.0),. On the basis of several drug parameters the ligand noname 3 can be suggested as a good ligand with least toxicity.

A few inhibitors predicted to inhibit the PSEN-1 are not effective in all forms. The three potential inhibitors of the PSEN-1 were screened by docking and several pharmacological parameters were evaluated. It is clear that the nonmae 3(a novel gamma secretase domain inhibitor) satisfied almost all properties like drug toxicity value, drug score, lower logP values and Lipinski's rule of five. Thus noname 3 can be treated as a potential inhibitor of PSEN-1, and can be considered as a good drug candidate for and suggested for further clinical trials. The drug constructed has been passed with several tests such as ADMET, TOPKAT etc. and can be helpful in curing the Alzheimer disease. It can be used as the potential drug for the disease which can be made available commercially after passing through different phase of clinical tests and FDA approval.

V. Conclusion:

The potentiality of our studies are immense in terms of good target and the ligand molecules. The protein of our interest shows the quality and importance of our studies. In our studies all the molecules that we designed qualified the criterions of Lipinski's rule of five like the number of hydrogen donors and the number of hydrogen acceptors, molecular weight, logP values. But surprisingly only a few molecules passed TOPKAT and ADMET. After docking the molecule noname 3 had the best LibDock score (69.0827) among all of the molecules .

From all our ligands we identify noname 3 and noname 1 as potent inhibitors to PSEN-1 binding domain(aa440). To be more specific about the molecule at the best we recommend ligand noname 3 having most potentiality among all our ligands designed. Further studies are valuable on the basis on our report and the drug can be taken for *in vitro* studies.

Acknowledgement

Javed Ahmad would like to thank Dr.Manas Ranjan Barik,chief scientific officer at DNA Labs India for providing all the necessary facilities and guidense to do the research work. We also acknowledge Dept. of Biotechnology, Bhagwant University, Ajmer, for their constant encouragement. I express my deep sense of gratitude to the DNA LABS INDIA, Hyderabad, for guidance and support of research work. At last I am thankful to my parents as without them I am unable to reach this point.

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