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Targeting Gamma Secretase with Natural Compounds from Zinc Database-An Insilico Approach

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Abstract: Alzheimer disease is a chronic degenerative neurological disorder which contributes to dementia condition in about 60% to 70% of Alzheimer patients. The causative agent for Alzheimer disease is the deposition of amyloid beta (Aβ) peptide which results in neural toxicity, deterioration of memory, cognitive and behavioural changes. According to statistics about 2.11 million of Indian population and 5.3 million of US population is facing the problem related to Alzheimer disease. Gamma secretase is the enzyme involved for unusual production of Aβ42 and has four subunits in it namely APH-1, Presenilin, Pen-2 and Nicastrin. The options available till date have not been too effective for Alzheimer patients. This work mainly focuses in screening of potential zinc modulators which would aid better living for the Alzheimer patients. The structure prediction, binding site prediction, docking and analysing the ADMET properties for the zinc compounds were done using various effective and efficient bioinformatics online tools available at authorised sites. The modulators screened in this work served for overcoming the disadvantages of the existing options and providing a new view to focus in terms of treating the Alzheimer disease.

Introduction

Bioinformatics field have emerged worldwide playing its role in designing of new drug compounds. The drugs are designed by bioinformatics tools with higher specificity and efficiency. Alzheimer disease is a chronic degenerative neurological disorder[12] in which neurons progressive loss leads to dementia[1]. The pathogenic features of Alzheimer disease are amyloid plagues accumulation, neurofibrillary tangles and neural death in a selective manner inside the brain[12] and involve deterioration of memory, cognitive and behavioural changes in Alzheimer patients[3]. The disease progresses in three stages namely preclinical AD, mild cognitive impairment (MCI) and dementia[3]. The enzyme involved in Alzheimer disease is gamma secretase[11]. The cleavage made by gamma secretase which is an intramembrane aspartyl protease[7] at specific position of APP leads to production of Alzheimer disease Aβ42 peptides[5]. The subunits of gamma secretase are nicastrin, presenilin (PS), anterior defective pharynx-1 (APH-1) and presenilin enhancer 2 (PEN-2)[10] and its molecular weight togheter accounts to about 170kDa[4].

The drugs that are available till date for treating Alzheimer disease are first generation gamma secretase modulators flurizan[2], gamma secretase modulator derived from natural products extracted from Actaea racemosa[8], Phenylimidazole-type GSM[10], Sulindac sulfide[5], Icariin[12], donezepil, rivastigmine and galanthamine (acetylcholinesterase (AChE) inhibitors), and memantine (N-methyl-D-aspartate (NMDA) receptor antagonists)[3].

In this study we have first obtained the structures of all the four subunits of presenilin using Swiss and I-Tasser online tools and compared their results to carry forward the best result to the next step. The essential justification for the structure was given using Ramachandran plot. The binding site for the active site presenilin was found using Raptor-X tool. Based on presenilin binding site compounds were screened using RASPD online server available at IIT, delhi website. The efficiency of the obtained 76 compounds was evaluated using BSP-SLIM tool to obtain the docking score for all the 76 compounds and shortlist the top 10 compounds among them. The top ten compounds again underwent a docking process via molecular docking server and Auto Dock Vina software to obtain the energy values and interaction sites between presenilin and screened top 10 compounds. The ADMET property for all the top 10 compounds was analysed and resulted in potential five modulators that would contribute in modulating the preseniln active site and would help the Alzheimer patient a better living way.

Materials and Methods

Structural modelling and validation

The amino acid sequence for the all the four subunits of gamma secreatase enzyme namely Nicastrin, APH-1, PEN-2 and Presenilin was obtained from the NCBI site in FASTA format. The sequence was given as input for SWISS AND I-Tasser web servers to obtain the structure for all the four subunits. The obtained results from the two tools were compared for finalising which result has to be taken for the next step. The structural validation was done using Ramachadran plot.

Binding site prediction

It was proved that presenilin is the active site responsible for the Aβ42 production leading to Alzheimer disease. Presenilin's binding site was identified using Raptor-X tool. For this the amino acid sequence in FASTA format was given as input and the online tool provided with the entire possible binding site present over the presenilin molecule along with its pocket multiplicity and solvent acess.

Screening of compounds and Docking

The obtained binding site of presenilin was utilised to screen the potential zinc compounds which could act as modulators via RASPD online tool available at IIT, Delhi website. This tool provided with entire possible no. of zinc compounds that could act as modulators of presenilin.

The compounds obtained from RASPD tool was docked initially with the help of BSP-SLIM tool to obtain the docking score for all the compounds and screen from them top 10 efficient compounds. The top 10 compounds were then docked in molecular docking server and Auto Dock Vina software to obtain the energy values and interaction sites between the presenilin and screened top 10 compounds.

Visualization of the compounds

PYMOL software was used to visualise the bonds between the zinc compounds and the presenilin molecule. This software in turn helped to cross check the interaction site obtained in molecular docking server.

ADMET

ADMET functionality test was performed for the top 10 compounds to estimate results of ametset, bioavailability, health effects and physiochemical properties. On analysing the results for the 10 compounds five compounds only commenced as potential modulators for presnilin's active site.

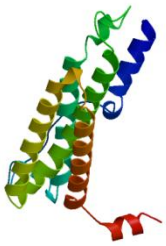
Results

Structural modelling and validation

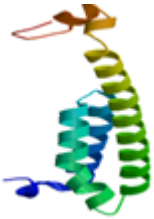
Swiss Port

Structure predicted of four subunits is:

Presenilin APH-1



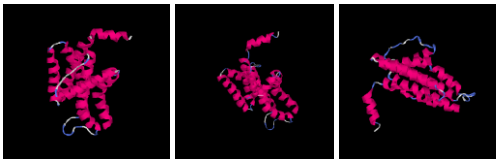
Nicastrin PEN-2



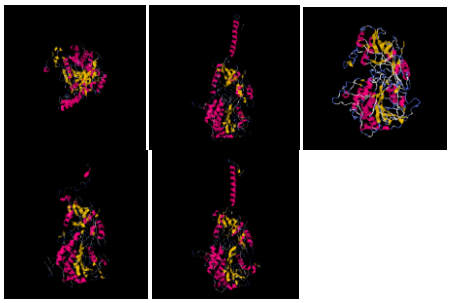
I-Tasser

Structure predicted of the four subunits are:

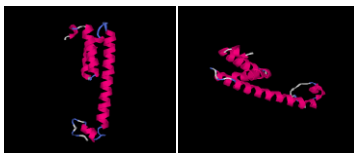
APH-1



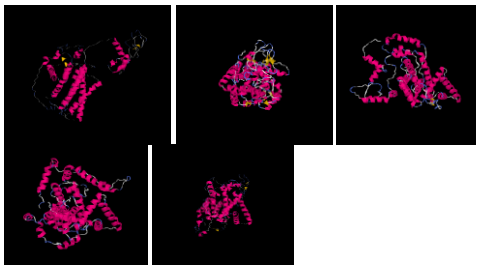
Nicastrin



PEN-2



Presinilin



Name of the subunit	No. of coils	No. of strands	No. of helix
APH-1	40	14	135
Nicastrin	385	94	231
PEN-2	389	224	91
Presenilin	67	17	238

Name of the subunit	Z score	TM score	C score
APH-1	3.39	0.957	-0.65
Nicastrin	11.26	0.825	-2.93
PEN-2	5.96	0.962	-1.34
Presenilin	9.11	0.843	-1.03

Ramachandran Plot

This is the justification for considering model 1 of structure obtained from I-TASSER software.

Name of the subunit	QMEAN value	GMQE	Solvation value	Torsion
Presenilin	-6.06	0.50	-3.29	-4.40
APH-1	-5.99	0.97	-1.75	-5.07
Nicastrin	-3.34	0.96	-0.22	-3.39
PEN-2	-2.42	0.99	0.63	-2.69

APH-1:

Model no.	Favoured region	Allowed region	Outlier region
1	171(92.9%)	9(4.9%)	4(2.2%)
2	172(93.5%)	10(5.4%)	2(1.1%)
3	33(91.7%)	2(5.6%)	1(2.8%)

Nicastrin:

Model no.	Favoured region	Allowed region	Outlier region
1	595(89.7%)	50(7.5%)	18(2.7%)
2	508(81.7%)	65(10.5%)	49(7.9%)
3	519(82%)	65(10.3%)	49(7.7%)

PEN-2:

Model no.	Favoured region	Allowed region	Outlier region
1	96(98%)	1(1%)	1(1%)
2	24(96%)	0(0%)	1(4%)
3	22(100%)	0(0%)	0(0%)

PS-1:

Model no.	Favoured region	Allowed region	Outlier region
1	370(95.4%)	9(2.3%)	9(2.3%)
2	121(69.5%)	44(25.3%)	9(5.2%)
3	339(86%)	32(8.1%)	23(5.8%)

Binding site prediction

The binding site for presenilin found via RAPTOR-X tool.

Domain1

Pocket	Multiplicity	Ligand	Binding residues
1	1	CL	G382 L383 G384 F386
2	1	IOD	G378 V379 S390
3	1	GOL	T407 F411 I414

Domain 2

Pocket	Multiplicity	Ligand	Binding residues
1	11	MG	F205 V208 Y225 M228
2	4	DC	Q223 L226 I227 I229 S230
3	4	DC	S170 I213 H214 W215K216 L219 Q222 Q223 L226
4	3	CL	F179 Y195

Domain 3

Pocket	Multiplicity	Ligand	Binding residues
1	39	C	Y106 K109 D110 G111 Q112 I114
2	36	C	V82 L85 F86
3	36	G	K80 H81 M84
4	26	G	L91 V94 V95 A98
5	25	G	T74
6	25	G	L73 Y77
7	18	C	K101
8	16	G	V95 A98 S102
9	14	G	T74 L75
10	9	PLM	H81 M84 L85 P88 V89 C92 V95

Screening and docking of compounds

Sreening of compounds

Using RASPD tool about 76 zinc compounds were screened based on the binding site of presenilin.

Docking of the compounds

BSP-SLIM software is utilised to obtain the docking score for the screened 76 zinc compounds.

The docking server provided the docking energy values, interaction tables and protein interaction site for the top 10 compounds.

Compound id	Docking Score
ZINC14534804	9.032
ZINC08437367	7.746
ZINC19113735	7.688
ZINC04045011 ZINC03001639	7.479 7.065
ZINC00999063	7.044
ZINC00718613	7.015

ZINC12845408	6.967
ZINC14742886	6.938
Curcumin	7.053

Compounds	**H bond+ Desov energy**	**Total inter Molecular energy**	**Interactions surface**
Zinc 12845408	-6.44	-6.45	512.18
Zinc 08437367	-5.46	-5.48	543.866
Zinc 14534804	-5.62	-5.38	626.767
Zinc19113735	-6.89	-6.71	688.033
Zinc20256707	-4.80	-4.67	571.938
Zinc14742886	-7.78	-7.76	586.759
Zinc 00718613	-6.75	-6.74	763.332
Zinc 03001639	-6.10	-6.11	662.174
Zinc 04045011	-6.08	-6.06	550.958
Zinc 05354562	-9.54	-9.06	796.146

1) **ZINC12845408**
Protein interaction sites are
26: LEU
27: PRO
28: PHE
29: LEU
32: VA
2) **ZINC08437367**
Protein interaction sites are
27: PRO
28: PHE
64: PHE
67: TRP
68: VAL
71: LEU
72: THR
3) **ZINC14534804**
Protein interaction sites are
18: TYR
26: LEU
28: PHE
29: LEU
32: VAL
36: TRP
4) **ZINC19113735**
Protein interaction sites are
17: LYS

18: TYR
20: LEU
24: ALA
28: PHE
29: LEU
32: VAL

5) **ZINC20256707**
Protein interaction sites are
18: TYR
26: LEU
28: PHE
29: LEU
32: VAL

6) **ZINC14742886**
Protein interaction sites are
26: LEU
27: PRO
28: PHE
29: LEU
31: LEU
35: PHE
64: PHE
68: VAL
71: LEU

7) **ZINC00718613**
Protein interaction sites are
14: LEU
17: LYS
18: TYR
26: LEU
28: PHE
29: LEU

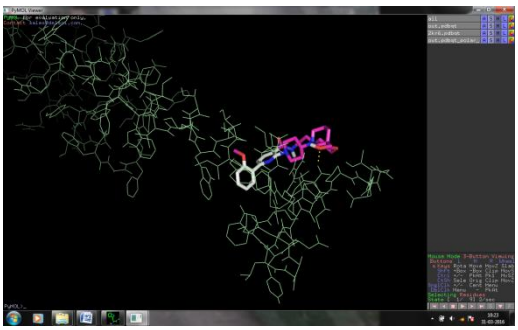
8) **ZINC03001639**
Protein interaction sites are
14: LEU
17: LYS
18: TYR
26: LEU
28: PHE
29: LEU

9) **ZINC04045011**
Protein interaction sites are
17: LYS
18: TYR
26: LEU
27: PRO
28: PHE
29: LEU
32: VAL

10) **ZINC05354562**
Protein interaction sites are
27: PRO
28: PHE
31: LEU
64: PHE
68: VAL
71: LEU

Visualisation of the compounds

Pymol software is used to visualise the molecular interaction between the screened 10 compounds and the presenilin molecule.



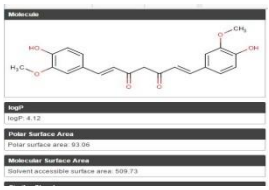
ADMET

ADMET analyse the amestest, bioavailability, health effects and physiochemical properties for the 10 compounds.

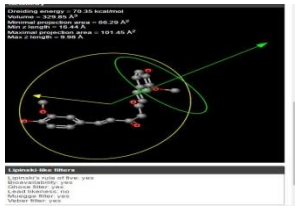
a) ACD Labs

Compund	Blood	kidney	Liver	Lungs
ZINC12845408	0.47	0.52	0.58	0.74
ZINC08437367	0.4	0.84	0.32	0.99
ZINC14534804	0.68	0.42	0.69	0.56
ZINC19113735	0.9	0.82	0.81	0.82
ZINC20256707	0.9	0.82	0.81	0.82
ZINC00999063	0.8	0.85	0.64	0.66
ZINC01479923	0.83	0.48	0.8	0.4
ZINC00848762	0.32	0.58	0.74	0.94
ZINC04045011	0.72	0.72	0.53	0.49
ZINC05354562	0.99	0.86	0.8	0.89
CURCUMIN	0.7	0.42	0.69	0.56

b) Chemicalize



Properties of ZINC12845408



Geometry and lipinski's rule in chemicalize

of ZINC12845408

Results and Discussion

Among the 79 compounds screened from the zinc databases compounds screened based on their docking score 10 compounds have been identified and docked with the protein molecule to find some more properties which resulted in reliable potential 5 modulators for Gamma Secretase.

Compond	**Free binding energy (kcal/ml)**	**Lipkinsi's Rule**
ZINC14534804	-7.7	YES
ZINC08437367	-6.8	YES
ZINC19113735	-6.8	YES
ZINC04045011	-6.3	YES
Curcumin	-6.10	YES

Conclusion and Future Work

Alzheimer is a neurodegenerative disease which further results in dementia. The problems faced by the patients of Alzheimer disease are memory loss, aggression, repetition, sleeping issues, confusion and sun downing. To aid the living of this patients the idea of modulating the active site of gamma secreatase enzyme was put forward. Firstly the structure of the four subunits of gamma secreatase was predicted via Swiss and I-Tasser online web tools. On further referencing presenilin is the active site could be concluded by Raptor-X online server. Based on the binding site present in presenilin 87 zinc compounds were screened which could act as modulators using RASPD online tool. To find the most effective modulators first screening was performed via BSP-SLIM to obtain the docking score for all the 87 compounds and short list the top 10 modulators then the screening was done by docking server and autodocksoftwares to find the effectiveness of the top 10 modulators. Finally the ADMET properties were analysed and final 5 effective, efficient and best modulators were put forward for modulating presenlin.

The five potential modulators explored by verifying the ADMET properties could be further utilised to develop drug in constitution with the potential modulators and could be implemented in clinical trial phase to know the effect of the drug in human body, the extent to which it provide relief for the Alzheimer patients and help the Alzheimer patient to live a better life. This developed drug could help to modulate the gamma secreatase enzymes presenilin subunit without affecting the notch signalling pathway and other regular and normal functions occurring inside the body.

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