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DESIGN OF NOVEL INHIBITORS TARGETING THE MUTATED PROTEIN OF ALPHA SYNUCLEIN USING COMPUTATIONAL APPROACH

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ABSTRACT

Researchers have discovered that the indication of Parkinson disease-Clumps of a protein Alpha synuclein, which are also called lewy bodies.Alpha synuclein(SNCA) has been believed to be a useful strategy for the treatment of Parkinson disease (PD) at a molecular level. However, no effective alpha synuclein inhibitors are currently available for this proteins. But In this current inspection, the mutated structure of alpha synuclein was modeled by using the present crystal structure of human alpha synuclein(PDBID-1XQ8). A structure-based pharmacophore model was developed based on the available crystal structure of alpha synuclein(1XQ8) with the two reported antioxidants, i.e., MitoquinoneMesylate (PubchemID:11388331) and Ubiquinone-10 (PubchemID:5281915).The best pharmacophore model consisted of 2 hydrogen bond donor and one hydrogen bond acceptor. The pharmacophore model was then used as a 3D-query in virtual screening to determine potential hits from DrugBank database.Finally, 27 hits were identified as potential inhibitors which were further validated by Molecular docking with the help of VlifeMDS software package.

KEYWORDS: ***Parkinson's disease, Alpha synuclein, Lewy bodies, Antioxidants, Hydrogen bond, Ubiquinone-10,Mitoquinone Mesylate***

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INTRODUCTION

After Alzheimer(AD) disease, if focus to the neurodegenerative disorder Parkinson disease (PD)is coming to the second position in the united state, for this 15 million individual are affecting. By now from the experimental evidence of PD ~1-2% of the population above age 65 and 4-5% above age 85 are suffering from this genetic disorder.[1] There are two different type of pathological sign in the PD; firstly the mid brain reason is affected by the loss of dopaminergic neurons present in the Lewy bodies and Lewy neurites.There are several symptoms included in PD these are shaking, tremors, rigidity, Bradykinesia, postural instability. Other symptoms include loss of sense of smell,sleep, and emotional problems. PD is indicating the amyloid disease that finds in the alpha-synuclein aggregation. The clues include the finding that alpha-synuclein is the major fibrillar protein in Lewy bodiesand the discovery that point mutations in the alpha-synuclein protein (i.e., A30P,E46K,H50Q,A53T) are inherited as an autosomal dominant form.[2-4] The protein alpha-synuclein is found in huge proportion in many parts of human brain muscles, heart and some other tissue are also contain a low amount of this protein. Alpha-synuclein is present in the nerve cell (neuron) at the tip position which is called as presynaptic terminals, for this, the alpha-synuclein interacts with phospholipids and proteins. The chemical messenger known as the neurotransmitter is release at presynaptic terminals that relay the signal between the neuron and is unfavorable for the normal brain function.[5]Synucleins family of proteins are soluble commonly found in vertebrates, primarily found in the neural tissue.[6]The synuclein family includes three common protein: α-synuclein, β-synuclein, and gamma-synuclein.[7] Alpha synuclein is made by the combination of a 140 different amino acid attached the protein to the cytoplasmic or membrane that is basically found in the presynaptic terminals of neuron cells. The architecture of alpha synuclein revealed that the presence of N-terminal region composed of incomplete …KTKEGV…motifs, which is amphipathic in nature where as the C-terminal region of alpha synuclein which is rich in acidic residues has been shown to regulate fibril formation.[8-9]This protein has the hydrophobic portion that is the sequences position 61-95 and this region is defined as the responsible for beta sheet formation and alpha synuclein aggregation.[10-11] It does not determine the exact function of the alpha-synuclein, but the different evidence indicate that the function of that is related to its capacity and that interact directly with the membrane phospholipids, particularly vesicles that is highly curved. One other important function of alpha- synuclein is the vesicletrafficking that occurs due to neurotransmission release .the protein that does not define structure at the aqueous salutation and that normally referred to the unfolded protein with the negative charge. At the pathological condition (such as mutations in the SNCA gene, oxidative stress, and post-translational modifications) the protein Alpha-synuclein adoptoligomer and fibrillar conformations. Mounting evidence suggests that the alpha-synuclein in pathology species include the post-translationally modified,oligomer, mutant, or aggregated forms. There are several mechanisms to the pathological species may bind toxicity that such as disrupting the normal function in neurotransmission release of alpha-synuclein and that may act as a negative regulator of Dopamine release. [12] In this current study, we investigate the alpha synuclein protein at the mutated level to find out the novel molecules.After modeling of the mutated structure, we were determined a structure-based pharmacophore model based on two reported antioxidants, i.e., Mitoquinonemesylate and Ubiquinone-10.The structure based pharmacophore model was generated by using Biopredicta module of VlifeMDS software package. Hydrogen bond donor/acceptor,hydrophobic,aromatic these are the pharmacophore featuresdetermined automatically.The pharmacophore model was then taken as a query in virtual screening to determine the best hits from the Drug bank database.Here we were making only 100 molecules from Drug Bank database according to the capacity of software VlifeMDS.

MATERIALS AND METHODS

Overall Study Design

The human alpha synuclein was first searched in the Universal protein sequence Database (UniProt-P37840).The retrieved protein sequence was manually mutated as per the previously reported mutation. The 3D structure was build using homology modeling.MD is a simulation process that optimized the three-dimensional structure. The optimized 3D structure of alpha synuclein was docked by using an automated server Patch-Dock. Finally, VlifeMDS was used for structure based pharmacophore model generation. In this study, we were found 27 novel ligand molecules which help in the interaction of the query pharmacophore features of the pharmacophore model. The mutated structure was then docked with the 27 ligand molecules to find out the novel molecule may act as a potent activator.

Sequence Retrieval and Analysis

The reviewed sequence of human alpha synuclein was retrieved from universal protein sequence database UniProt (http://www.uniprot.org/) having (UniProt ID-P37840).The given protein we use the ProtParam (http://web.expasy.org/protparam/tool)[13] to compute the physical and chemical parameter. The domain and family was inferred by using SMART[14]

Homology modeling of Alpha synuclein

Homology modeling was considered as one of the most accurate computational methods to determine the most reliable structure to generate a theoretical model from its primary amino acid sequences which are not present through experimentally. The 140 amino acid sequence of protein was subjected manually mutated in position A30P and A53T. Then the mutated sequence was subjected to BLASTp against PDB. Based on maximum sequence identity, query coverage and ain A, human micelle-bound alpha synuclein(1XQ8 A), chain A, the Crystal structure of human fused to maltose binding protein(3Q26 A), chain A crystal structure of human fused to maltose binding protein(3Q27).

Structure Validation

The prosa-web[15] tool is a protein structure analysis server using to validation of the generated model. The function of the protein was predicted using ProFunc(

http://www.ebi.ac.uk/thornton-srv/databases/ProFunc) [16]web server.

Molecular Docking

After getting the theoretical model, we docked the model protein with two antioxidants i.e. MitoQ (Mitoquinone Mesylate) having PubChem-11388331 and CoenzymeQ10(Ubiquinone-10) (Figure 1.) having PubChem-5281915.[17]

Mitoquinone mesylate structure

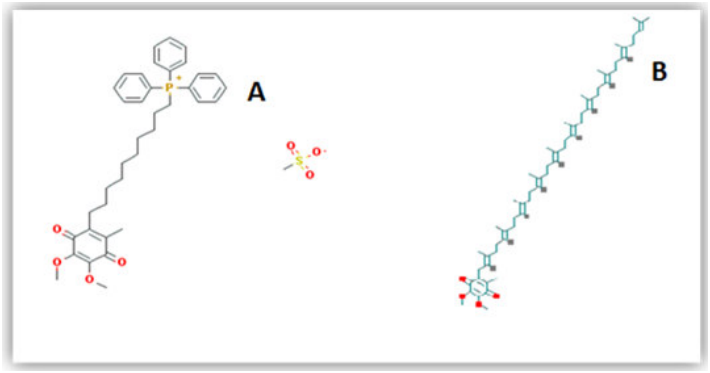

Figure 1
Structure of Mitoquinone mesylate (A) and Ubiquinone-10(B)

pharmacophore modeling

Pharmacophore model was generated based on molecular features that are necessary for molecular recognization of a ligand by a biological macromolecule. Using a test set of the molecule to created a pharmacophore query that considering the aromatic center contain some pharmacophore feature, H-bond donors & acceptors and Hydrophilic centroids of the molecules.To identify the similar pharmacophore model and active compound separate from the database, we use this query againstthe database. The molecules that were not matching with the query pharmacophore features were excluded from the search. Then the test data conformations were aligned for clear visualization.

Structure-Based Approaches

In this current study, the aim is to find the crystal structure of the protein. That gives us to be a chance to find the new scaffolds that could bind in the active site of the protein. The human alpha synuclein crystal structure is used in the structure based virtual screening method for the novel hit search. This screening method is used to estimation the modes of receptor ligand binding with calculate the affinity of the binding site, and the process depend on fast and accurate computational method.

Compound Library Filter

Drug Bank is a freely available database of unique bioinformatics and chemo informatics resource which comprises the detailed drug with comprehensive drug target. It contains more than 8250 drug entries. From which we will take 100 compounds for pharmacophore modeling. The pharmacophore features were generated by using BioPredicta modules ofVlifeMDS software package.

Virtual Screening

After the Prediction of pharmacophore features like H-bond donor,H-bond acceptor,positive charge,negative charge and hydrophobic centroids,we will use the Molsign module from VlifeMDS software package to find out the features in the same cavity of the alpha synuclein protein. We screened against 100 molecules taken from Drug bank database to identify the similar hits. We found 27 hits molecules which are again docked with the pharmacophore model by using Biopredicta modules. In this study, the cavity of the structure was automatically determined (Figure 2, Figure 3) On this basis of docking score, we calculated the best drug binding with the structure of alpha synuclein.

Pharmacophore model of the protein alpha synuclein

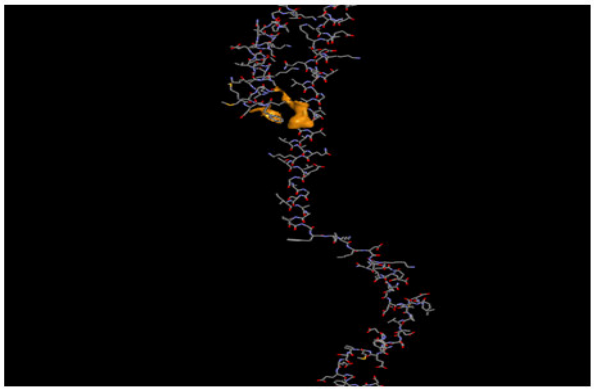

Figure 2
Predetermined cavity of pharmacophore model of the protein alpha synuclein

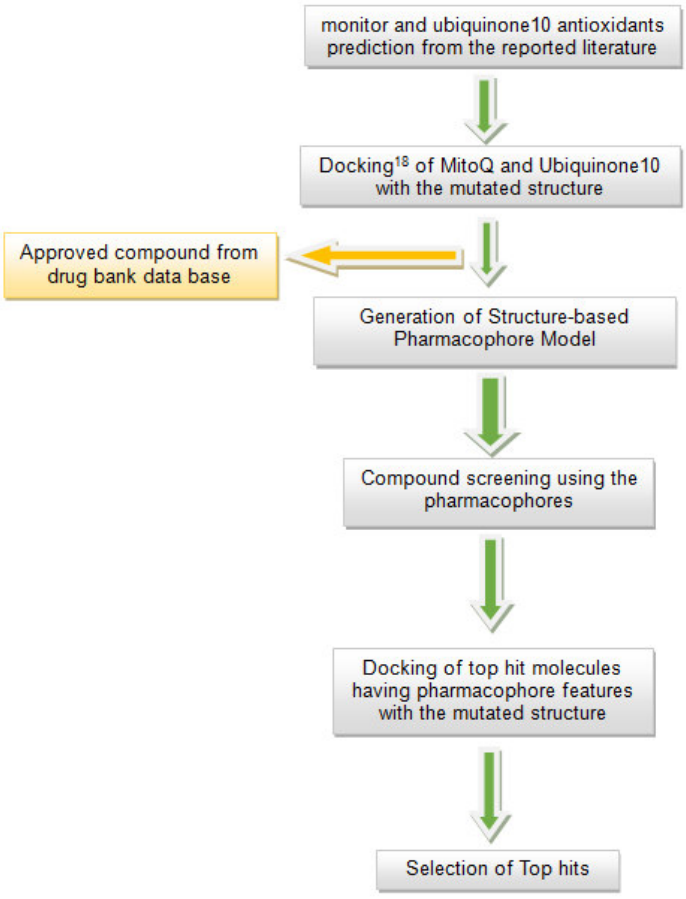

Figure 3
Flow chart represents methodology for structure-based pharmacophore modeling.

RESULTS AND DISCUSSIONS

Sequence analysis

Reviewed alpha synuclein sequence was acquired from the universal protein sequence databaseUniProt and we manually mutate the protein in 30th and 53rd position (Figure 4) to identify how this missense mutation helps in causing Parkinson disease.

Wild type sequence of human alpha synuclein and Mutated Sequence of the protein

```
>sp|P37840|SYUA_HUMAN Alpha-synuclein OS=Homo sapiens GN=SNCA
PE=1 SV=1

MDVFMKGLSKAKEGVVAAAEKTKQGVAEAAGKTKEGVLYVGSKTKEGVVHGVATVAEKTK
EQVTNVGGAVVTGVTAVAQKTVEGAGSIAAATGFVKKDQLGKNEEGAPQEGILEDMPVDP
DNEAYEMPSEEGYQDYEPEA


>sp|P37840|SYUA_HUMAN Alpha-synuclein OS=Homo sapiens GN=SNCA
PE=1 SV=1
                                30                              53
MDVFMKGLSKAKEGVVAAAEKTKQGVAEAPGKTKEGVLYVGSKTKEGVVHGVTTVAEKTK
EQVTNVGGAVVTGVTAVAQKTVEGAGSIAAATGFVKKDQLGKNEEGAPQEGILEDMPVDP
DNEAYEMPSEEGYQDYEPEA
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Figure 4
The above figure showing wild type sequence of human alpha synuclein(first one) and Mutated Sequence of the protein in 30th and 53rd position in the sequence which causes Parkinson disease.

The various physicochemical property analysis of alpha synuclein is provided the acidic nature information of the protein (Isoelectric point=4.67).Aliphatic index (AI) is used to find the relative volume aliphatic side chain with a positive factor for the increase of thermos-stability of the protein.Here the high aliphatic index (69.64) was

reflecting the protein with stable in nature for a wide range of temperature.Instability index is a measure of proteins used to determine whether the protein will be stable in a test tube or not. The alpha synuclein protein was stable in nature as its instability index was reported to be 25.47 which is less than 40.To calculate the sum of hydropath value of all amino acid divided by the number of residue in the sequence is used to GRAVY value of a protein.There is very low index (-0.403) CAD2 was found in GRAVY and that indicating the better interaction with water.

Domain and Family Analysis

The sequence of alpha synuclein from Homo sapiensbelonging to the family synuclein was retrieved, and the analysis of SMART and CD search revealed that it comprised of one domain which is starting from position 1 to 131. So most of the amino acids belonged to domain region of the protein. Synuclein proteins are the small soluble protein expressed primarily in neural tissue in certain tumors.Alpha synuclein was mutated to the several families with autosomal dominant Parkinson disease.

Homology modeling

Protein who have evolutionarily related these are expressed the similar sequences and similar structure are found to be the naturally occurring protein. Homology is a clear relationship between the target protein sequences and the identification of any known structure, for this approach based on the tertiary structure if the sequences are related and two different proteins have the similar tertiary structure if their sequences are related. Alignment between the target sequences and the template structure is more reliable when the high-level sequences identity is found.Employing homology modeling protocol, the structure of alpha synuclein from Homo sapiens (Human) was constructed by using the crystal structure of Human (PDB ID-1XQ8).BLASTp search revealed that 100% identity with 1XQ8. Based on the target-template alignment, Modeler facilitated in the development rough models.(Figure 5.)

3D model structure of alpha synuclein

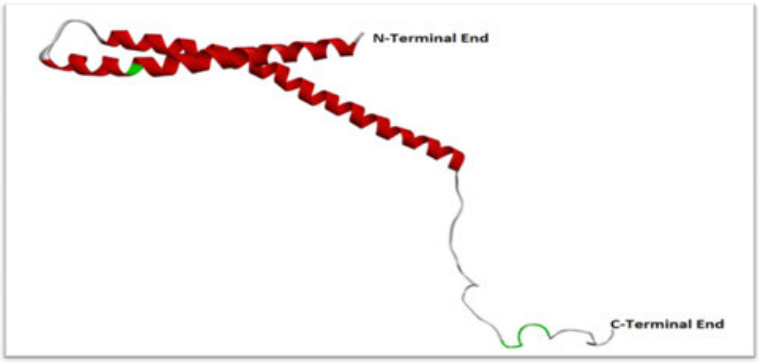

Figure 5
3D model structure of mutated protein alpha synuclein by using Discovery studio visualizer

Model Validation

After a round of complete energy minimization process, this optimized model used various model validation server for identification of each residue stereo-chemical quality evaluation. Analysis of the Ramachandran plot Procheck retrieves that the alpha synuclein model had 87.7% phi and an angle is the core region of its residue. (Figure 6.)ProSA revealed a Z-score of -1.63 for modeled reflecting the overall quality of the alpha synuclein model.

Protein structure analysisRamachandran plot of modeled protein

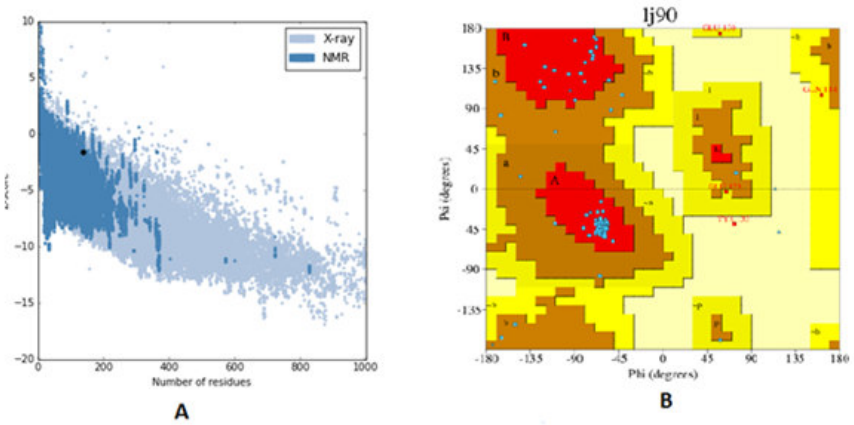

Figure 6
Protein structure analysis (A) and Ramachandran plot of modeled alpha Synuclein(B)

Molecular Docking

Molecular docking is an important tool which use to solve the optimization problem in the field of structural biology and computer aided drug designing. The goal of this protein and antioxidant binding was to identify better the interactions with the two reported antioxidant with the help of structural biology tools.From the docking analysis different binding poses of MitoQ and Ubiquinone10 was screened and ranked by the global energy scores. Here MitoQ was showing -60.95 global energy whereas,Ubiquinone was showing off about -41.02 global energy which is far better than Mitoquinonemesylate(MitoQ).

Solid surface representing

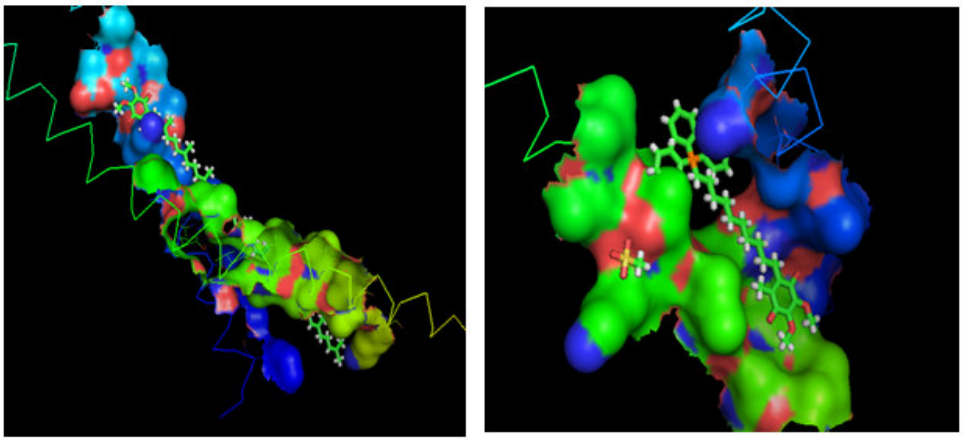

Figure 7

Solid surface representing the interaction of two antioxidants MiUbiquinone 10(Left)

Generation of Structure Based Pharmacophore Modelling

A set of pharmacophore features was generated by using Molsign module of VlifeMDS 4.6. Each of the features contains the three features like two Hydrogen bond donor and one Hydrogen bond acceptor. Before going to the steps, virtual screening the All drugs molecules are aligned for occupying all pharmaco features of the model(Figur 8) The Figure 9. Showing the distances generated by using VlifeMDS package between the three features.

synuclein pharmacophore model composed of Hydrogen bond

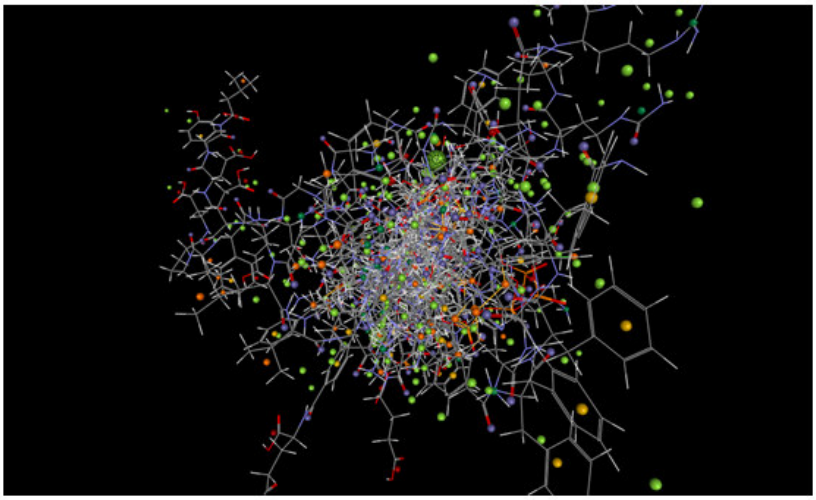

Figure 8

Alpha synuclein pharmacophore model composed of Hydrogen bond donor/acceptor And aromatic features.All the compounds are superimposed as occupying all three regions of the model

In Silico Screening
Distance constraints generated by VlifeMDS software package by using Biopredicta module

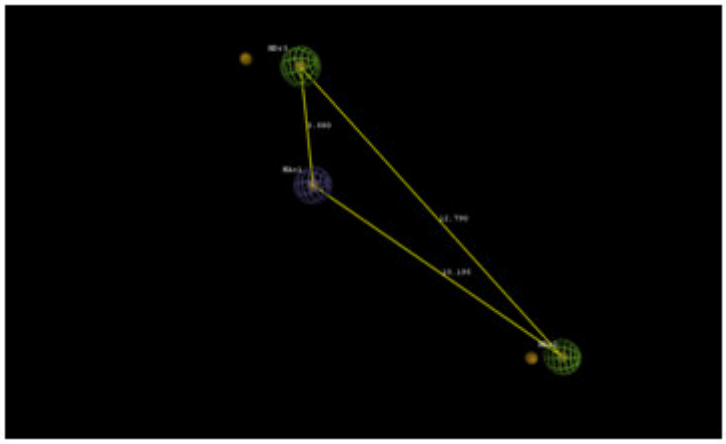

Figure 9
Alpha Synuclein pharmacophore model with its distance constraints generated by VlifeMDS software package by using Biopredicta module. Features are color coded with green: two hydrogen bond donor, violet: one hydrogen bond acceptor.

CONCLUSION

Alpha synuclein is a protein which is the hallmark of Parkinson's disease. Now date the novel hit molecule is discovered for the drug development by using the structure based virtual screening method.In this study, various structural biology tools are used to design novel activators of Parkinson's disease.The resulting pharmacophore model has three chemical features one hydrogen bond acceptor,two hydrogen bond donor.From virtual screening, we get 27 hit molecules with the help of pharmacophore model.Molecular docking of these 27 hit molecules by using Molsign module of VlifeMDS resulted in one novel hit molecule.The pharmacophore model uses a query to search for the novel candidate in another database of alpha synuclein activators at mutated level.Finally, One hit molecules are used as a novel molecule which is having docking score -198.0169.

CONFLICT OF INTEREST

Conflict of interest declared none.

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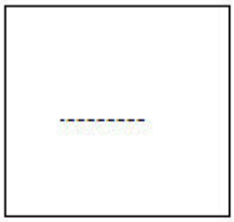

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