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Research Article

INSILICO DOCKING STUDIES TO IDENTIFY POTENT INHIBITORS OF ALPHA-SYNUCLEIN AGGREGATION IN PARKINSON DISEASE

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ABSTRACT

Background: Parkinson's disease (PD) is the second most common neurodegenerative disorder after Alzheimer's disease. Etiology of PD is progressive loss of dopaminergic neurons in Substantia nigra pars compacta (SNpc). One of the pathological hallmarks of PD is the presence of intracellular proteinaceous substances termed 'Lewy bodies' composed of aggregated alpha-synuclein which is responsible for its toxic effect on SNpc. Hence any therapeutic target which blocks α-synuclein aggregation will provide a new channel to cure PD.
Objective: The aim of the present study is to identify potent inhibitors (ligands) which binds to active site of α-synuclein and prevents self-association.
Methods: In this study, insilico molecular docking was done against α-synuclein using five plant derived compounds namely (a) stimovul (b) 7,8dihydroxycoumarin, (c) etorphine (d) propoxyphene and (e) pentazdine. These compounds were analyzed for their Lipinski and ADMET properties using Accelrys Discovery studio 3.5. Molecular docking was performed between ligand and protein using Lead IT.
Results: Results revealed that the best fit ligands against active site of α-synuclein were identified as Stimovul with a docking score of -4.5122 and the interacting amino acids were found to be SER 87 and VAL 95 followed by other compounds.
Conclusion: These compounds which have the ability to bind to α-Synuclein *insilico* can be further developed using *invitro* and *in vivo* studies as a potent anti-parkinson drug.

Keywords: Parkinson disease, Substantia nigra, Molecular docking, Lipinski, ADMET.

INTRODUCTION

The second most common neurodegenerative disorder, Parkinson's disease [PD] is a debilitating motor related disease and is presently incurable [1]. PD disorder affects more than 0.1 % of the total population older than 40 years of age[2].The developing neuroprotective therapies are not effective due to limited understanding of the key molecular event that stimulate neurodegeneration. The finding of PD genes has led to the hypothesis that misfolding of protein & dsyfuntion of ubiquitin proteasome pathway, mitochondrial dysfunction and oxidative stress are causes for PD pathogenesis[3].The key pathological characteristic of PD are loss of nigral neurons [loss of pigmentation in this area] at cellular level. PD is characterized by the aggregation of Lewy body plaques in neurons of the substantia nigra. Clinical behavior symptoms of PD are linked to uncontrolled motor deficits such as akinesia [absence of movement, or temporary paralysis] bradykinesia [abnormal slowness of movement], abnormalities in gait, resting tremor, rigidity, and memory loss can also develop as later symptom [1]. New diagnostic techniques and promising neuroprotective pharmacological agents are becoming a reality enabling the next stage in PD therapy. Clinical symptoms arise when 50 % of substantia neurons are already lost and at the end stage of the disease the loss of dopamine neurons, can exceed 95% [4]. Alpha synuclein is a member of a small family of protein that are expressed preferentially within the substantia nigra [5]. It is mainly found in neuronal presynaptic terminals, which is close to synaptic vesicles [6]. α-synuclein was first isolated from TORPEDO CALIFORNIA CHOLINGERIC Synaptic vesicles and later as the non-amyloid component of plaques from Alzheimer diseased brain [7]. Its main function is still unknown but it is found to be the major component of Lewy bodies [LB] which are neuronal cytoplasmic inclusions of aggregated protein that are characteristic hallmarks of idiopathic and familial forms of PD [8]. It is thought that cause of idiopathic PD may be an interaction of environment and genetic factors [9]. Mutations in the gene encoding wild-type (WT) alpha synuclein like alanine^{30}-proline (A30P) and alanine^{53}-threonine (A53T) are cause autosomal-dominant forms of PD [10]. Amino acid residue 64-100 of alpha synuclein is identified as the binding region responsible for self association. Small molecules or ligands that can block, slow down or reverse α-synuclein aggregation especially at it early stage can provide an attractive and effective therapeutic approach for targeting the underlying PD disease progression [11]. Mitochondrial function and oxidative stress responses related genes have been identified including Parkin, ubiquitin-carboxy-terminal-hydrolase-L1, PINK1, DJ-1 and LRRK2 (Dardarin) responsible for PD. However the first gene associated with PD was SNCA which codes for a protein called α- synuclein of unknown function [7].

Oxidative stress is thought to be an important factor in PD due to the destructive effect of free radicals and enhanced fibrillation of α-synuclein which is toxic to neuronal cells [12]. Antioxidants represent a large class of potential therapeutic agents for neurodegenerative diseases. These compounds are intended to prevent oxidation of other molecules reducing overall free radical levels and cellular oxidative stress [7].Various research report have indicated that a range of pure compounds derived from herbal materials, herbal extracts/ fractions & herbal formulations are effective on *invitro*/ *invivo* PD models [13]. With the help of Computer aided drug design (CADD), potent compound that has the ability to bind to the active site of alpha synuclein can be identified effectively. Nowadays, molecular docking studies are very frequently used in modern drug design molecules [14]. Docking was performed between compounds and active binding region (64-100) of α-synuclein using Accelrys discovery studio 3.5 and Lead IT [15]. 5 compounds have been selected from the literature based on their anti-oxidant activity. Based on docking score, the results obtained will compare the potency of compound with other natural compounds which will provide insights in understanding the activities of compounds as inhibitors and potent compound can be brought to light for further trials.

MATERIALS & METHODS

All the works were performed using HP Workstation Z220 with Next-generation 22nm processors, including the Intel® Xeon® processor E3-1200v2 family with 16 GB RAM, 1 TB Hard disk,

NVIDIA Quadro 2000, Windows 7 Ultimate 64 bit Software Used: Discovery Studio Client 3.5, Biosolve IT, CLC Genomic Workbench 5.1.

Ligand

The ligand used for this study has been selected based on various literatures and given in Table 1. 3D structure of the compound has been downloaded from Pubchem and www.chemicalize.org. Marvin Sketch software developed by Chemaxon was used to draw and optimize the 3D structure of compounds which were not available in the databases. 3D structure optimized of the ligands has been employed further for docking study.**Table:1**

Drug likeliness evaluation

The Compounds drug likeness property was examined with the help of Lipinski drug filter using Accelrys Discovery Studio 3.5. This filter predicts the Lipinski rule of 5 for the compounds based on its 2D structure and provides an important information whether that chemical compound contain properties of pharmacological or biological activity that would make it a likely orally active drug for human consumption [16].

ADME-Toxicity investigation

ADME-Toxicity studies were performed through Accelrys Discovery Studio 3.5 .The Absorption, Distribution, Metabolism, Excretion (ADME) and Toxicology studies were studied which provides insights into the pharmacokinetic property of the compounds. ADMET properties includes Aqueous solubility, Blood brain barrier level, CYP 2D6, Hepatoxicity and Plasma Protein Binding level.

Molecular Simulation studies

Protein Minimization

Protein Minimization: Protein Minimization was performed by applying CHARMm forcefield using Accelrys Discovery Studio 3.5. The protocol prepares the proteins by inserting missing atoms in incomplete residues, modeling missing loop regions based on SEQRES information, deleting alternate conformations, removing waters, standardizing atom names, protonating titratable residues using predicted pKs. The potential energy, Van der Waals energy, Electrostatic energy and RMS gradient was checked for the protein before and after minimization.

Ligand Minimization

Ligand minimization was performed using CHARMm and MMF forcefield using Accelrys Discovery Studio 3.5. This optimization is done through chemical modification of the hit structure, with modifications chosen by employing Structure – Activity analysis (SAR) as well as structure – based design if structural information about the target is available. Bond energy, CHARMm energy, Dihedral energy, Electrostatic energy, Initial potential energy and Initial RMS gradient were calculated.

Target Protein

The structure of the target protein was retrieved from Protein Data Bank [PDBID- 1XQ8].The protein structure of favored regions were evaluated through Ramachandran plot analysis via PROCHECK [17] from EBI server (www.ebi.ac.uk/thornton-srv/software/PROCHECK/) to investigate the quality of the target protein structure.

Active site Prediction

Based on the study by Omar *et al.,* 2004, [12] 64-100 amino acid residues has been identified as the active site responsible for α-synuclein aggregation. Hence the ligand binding to this region would interact with the same region in the full-length α-syn molecule, and block its self-association into oligomers and resultant mature amyloid fibril formation.

Molecular Docking

The possible docking modes between the ligands and the target protein (1XQ8) were studied using LeadIT. The docking algorithm in the LeadIT suite is the FlexX docking approach. Before docking, first the molecules were prepared and bonds, bond orders, explicit hydrogens, charges, flexible torsions were assigned if they were missing to both the protein and ligands [18]. It flexibly places ligands into the active site with an incremental buildup algorithm. It starts with selecting a base fragment, which is placed into the active site based on superposing interaction points of the fragment and the active site. The base fragment is then incrementally built up to the complete compound by modeling the ligand flexibility with a torsion library for the added components. The procedure is described in detail elsewhere. Using the FlexX algorithm, up to 200 poses are generated for each compound and the best pose wise and the compound with the best docking score was selected as a potent inhibitor for alpha-synuclein aggregation.

RESULTS

In recent years, use of natural antioxidant obtained from food and other biomaterial has been increasing due to their safety, and nutritional and therapeutic value. Mostly natural antioxidant comes from plants in the form of phenolic compounds (flavanoids, phenolic acids, alcohols, stibenes, tocopherols). Plants are rich source of bioactive chemicals which are beneficial without any side-effects there by an increased interest to identify natural compounds with antioxidant activity. Biomedical research on the health benefits of these compounds are of great interest. Natural compounds with antioxidant activity can act as an antiparkinson drug by inhibiting the fibril formation of α- synuclein protein and thereby hindering Lewy body formation, we simulate the theoretical binding of the ligands (chemicals) to the active site of α- synuclein protein and its ability to inhibit fibril formation. Ramachandran plot revealed that the protein structure is suitable for docking studies **(Figure 1)**.

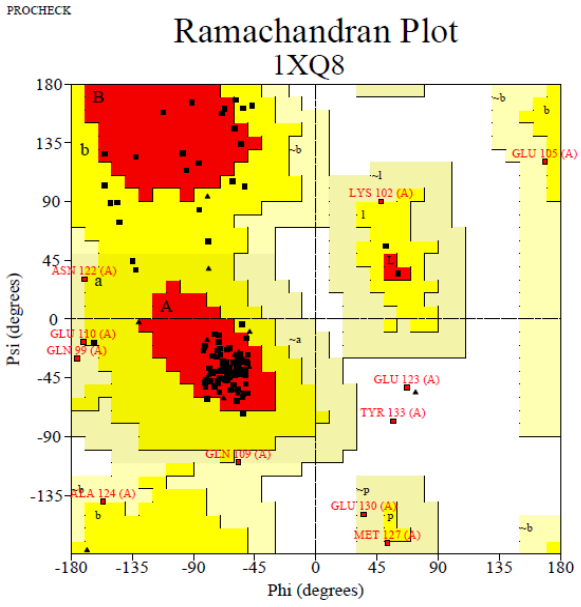


Fig 1: Ramachandran plot for 1XQ8

Molecular simulation studies

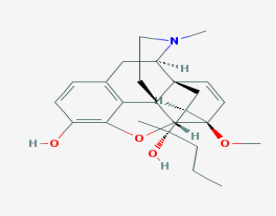
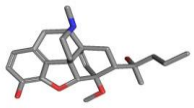
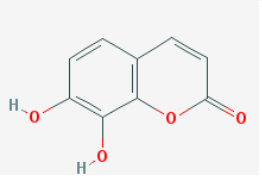
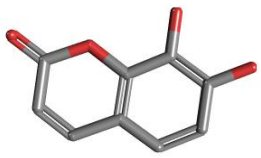
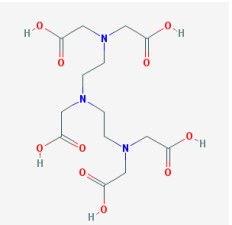
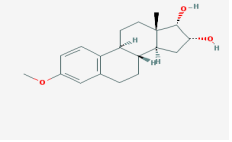
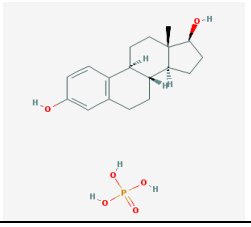
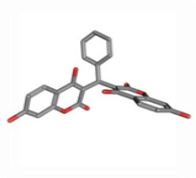
CHARMm is a highly flexible molecular mechanics and dynamics program. It derives from the program CHARMM (Chemistry at HARvard Molecular Mechanics). CHARMm performs well over a broad range of calculations and simulations, including calculation of geometries, interaction and conformation energies, local minima, barriers to rotation, time-dependent dynamic behavior, and free energy [19]. Energy minimization is performed on structures prior

to dynamics to relax the conformation and remove steric overlap that produces bad contacts. The results obtained before and after minimization of protein and ligand.

Drug likeness evaluation

The Lipinski rule of five for the compounds was predicted via Lipinski drug filter **(Table 1)**.

Table 1: Structure of Ligands with their Molecular properties

S.NO	Compound Name	Properties	2D Image	3D Image
1	Etorphine	Compound Id:443407 Molecular weight:411.53382(g/mol) Molecular formula:C25H33NO4 XLog P3-AA:3.1 Hydrogen bond donor:2 Hydrogen bond acceptor:5 Lipinski's rule:yes		
2	7,8 dihydroxycoumarin	Compound Id: 8144195 Molecular Weight: 178.14154 [g/mol] Molecular Formula: $C_9H_6O_4$ XLogP3-AA: 1.2 H-Bond Donor: 2 H-Bond Acceptor: 4 Lipinski's rule: yes		
3	Pentazdine	Compound Id:3053 Molecular weight:393.34652(g/mol) Molecularformula:C14H23N3O10 XLogP3-AA: 5 Hydrogen bond donor:5 Hydrogen bond acceptor:13 Lipinski's rule:no		3D Structure unavailable
4	Stimovul	Compound Id:244809 Molecular weight:302.40794(g/mol) Molecular formula:C19H26O3 XLogP3-AA:2.8 Hydrogen bond donor:2 Hydrogen bond acceptor:3 Lipinski's rule: yes		
5	Estradurin	Compound Id:68576 Molecularweight:370.377142(g/mol) Molecular formula:C18H27O6P Hydrogen donor:5 Hydrogen acceptor:6 Lipinski's rule :yes		

The cut off values include:

- Molecular mass less than 500Da
- Less than 5 hydrogen bond donors
- Less than 10 hydrogen bond acceptors
- High lipophilicity (expressed as Log *P* less than 5)

The results show that the compounds obey Lipinski rule of five and they can be strongly recommended as a drug.

ADME investigation

The ADME (Absorption, Distribution, Excretion, and Metabolism) properties of the compounds are predicted using pre ADMET server to know the pharmacokinetics property of the drug. These results show that the **Lead** compounds (1-5) possess good pharmacokinetic properties and it satisfies all the parameters to be taken over as a good drug **(Table 2)**.

Table 2: Comparison of the ADME values of Ligands

Descriptor	Stimovul	7_8-dihydroxycoumarin	Estradurin	Etorphine	Pentazoline
AQ SOl LEV	2	4	2	2	3
BBB LEV	1	3	1	2	0
CYP 2D6	-2.74915	-7.62557	-4.44542	-2.00959	2.40545
HEPATOX	-7.27411	-2.47233	-10.4084	-3.54958	-15.3293
PPB LEV	10.6359	-6.08494	2.64501	-15.6501	-27.0942

Note: SOLUBILITY: 0-2 highly soluble, BBB:1-high penetration, 2- medium penetration and 3- Low penetration, CYP2D6: -ve - non-inhibitors & +ve – inhibition. HEPATOX: <1: Non-toxic, PPB: Greater the value greater the binding capacity.

Molecular docking simulation

Molecular docking studies were performed using LeadIT. The results of interaction between α- synuclein protein with the compounds (a) stimovul (b) 7,8dihydroxycoumarin, (c) etorphine (d)propoxyphene and (e) pentazdine were shown in **Figure 2.**

Table 3: Ligand-Protein interaction with docking scores

S.NO	COMPOUND NAME	LEAD-IT SCORE	H-BOND	LEAD-IT (DOCKING)			
				AMINO ACID	AMINO ACID ATOM	LIGAND ATOM	H-BOND LENGTH (Å)
1	Stimovul	-4.5122	3	SER87	HG_	O1	1.74603
				VAL95	HN_	O3	1.87547
				SER87	OG_	H41	2.08543
2	7_8_ dihydroxycoumarin	-4.3881	3	VAL95	HN_	O1	1.83773
				VAL95	HN_	O4	2.04063
				GLY93	O_	H18	1.9792
3	Estradurin	-3.7081	2	GLY93	O_	O2	2.81667
				GLY93	O_	H44	1.96871
4	Etorphine	-3.0914	3	GLY86	O_	O4	2.75013
				GLY86	O_	H60	1.879
				GLY93	O_	H64	1.89926
5	Pentazoline	-2.9219	1	ALA90	O_	H49	1.8844

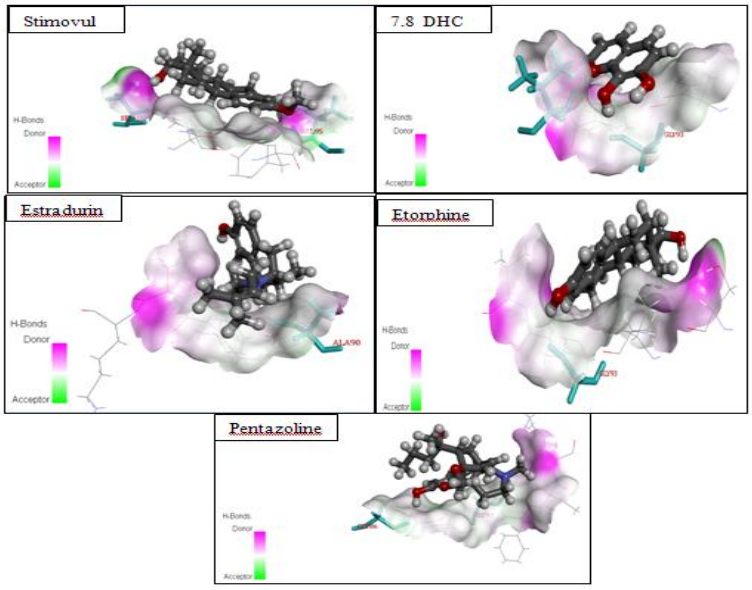


Fig 2: Representation of binding site of Ligands on 1XQ8 using Discovery Studio 3.5. Ligands are shown in Ball & Stick pattern. Interacting residues are shown in Stick pattern.

DISCUSSION

Molecular docking approaches are routinely used in modern drug design to help understand drug receptor interaction. It has been shown in the literatures that these computation techniques can strongly support and help the design of novel, more potent inhibitors by revealing the mechanism of drug receptor interaction [20] .Computer aided drug design (CADD) helps in identifying small molecules by orienting and scoring them in the active binding site a protein. By using Lipinski's rule of five which was derived empirically from the world drug index is used to filter the drug for its capability of drug for human use. In the present study, all the compound satisfied Lipinski rules are expected to be active in humans after oral administration. The molecular properties which influence ADMET are recognized a long side therapeutic potency as key determinants of whether a molecule can be successfully developed as a drug [21] ·The novel ligands biology activity was helpful to predict by scoring function. Recent study showed that identified amino acid residues 64-100 are responsible for self aggregation. The present docking study is carried out for five compounds against target protein within the active site 64-100 amino acids that favors protein fibril formation and thereby Lewy body inclusions. According to Cheng & Merz, 2003 [22] aqueous solubility helps to predict the solubility of the compound in water at 25^0C and the solubility level ranges from 0-6. Previous reports conclude that lower solubility is favorable for good and complete oral absorption. In connection with this context, the compounds are observed to have low solubility and hence they should have a good and complete oral absorption for effective dosage. Egan & Lauri, 2003 concluded that BBP level shows the penetrating efficacy of compound towards the brain. If the level falls between 0&1: high penetrating efficacy, level 2: medium penetrating efficacy, level 3: low penetrating efficacy & 4: zero penetrating efficacy. Susnom & Dixon, 2003 [23] reported that Cytochrome 4502D6 (CYP 450) predicts CYP2D6 emzyme inhibition. It has two levels, 0 for non-inhibitors and 1 for inhibitors. The compounds stimovul, 7,8 dihydroxycoumarin, etrophine, pentazdine, Estadurin satisfied Lipinski's rule and ADMET properties [24].

Using Lead IT program we selected the best docked based on two criteria:

- Ligand binding position
- Fitness Function scores comparison.

The parameter used for identifying the best ligand binding position was the root-mean square distance (RMSD) value [25, 26]. Higher binding capacity of the molecule to the protein Lead score Stimovul (-4.5122) interact with (Ser87, Val97, Ser-87) 7,8-dihydroxycoumarin (-4.3881) interact with val95, gly93, Estadurin(-3.7081) with Gly93, Gly93, Etrophine (-3.0914) interact with Gly86, Gly86, Gly93. Pentazdine (-2.9219) with Ala90. Stimovul, 7,8 dihydroxycoumarin, estradurin and etrophine were having 3 hydrogen bonds and pentazdine have one hydrogen bond. The interaction of these compounds with target protein was found to be strong in docking models. Based on the docking score stimovul have high score ranking compare to other compounds. These results revealed that stimovul has the ability to bind towards the active site of α-synuclein and prevents the self-association of protein to form a

toxic aggregate. Hence stimovul can be further developed as a potential drug for Parkinson disease.

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Conflict of Interest:

No conflict of interest.

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