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A PROMISING IN-SILICO APPROACH FOR COMPUTER-ASSISTED DRUG DESIGNING FOR PARKINSON'S DISEASE

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Parkinson's disease, In-silico drug discovery, SNCA gene, molecular docking.

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ABSTRACT

Parkinson's is a neurodegenerative disease that is linked to the locus SNCA, as it encodes the protein alpha-synuclein. This protein, alpha-synuclein, is mainly found in the brain, where its role is membrane trafficking and synaptic signaling. A defective or mutated form of these protein the s in SNCA leads to the deposition of the protein alpha-synuclein which is dangerous in the degenerative pathways in this study, an in-silico approach was employed to discover the drug using computational comparative analysis to prevent Parkinson's disease through bioinformatics tools, such as Genescan, CELLO, and ProtParam, attempts have been made to determine protein's role and presence in disease as well as how its structure will appear. Phylogenetic analysis creates an understanding of the evolutionary trends of the SNCA gene in various species, which is important in considering the use of models generated by the mega11 tool to analyze its function. Utilizing Levodopa in ligand-based drug design includes building structural libraries and screening them against the interacting proteins. Analogues are prepared, and their ADMET properties are evaluated to explore Levodopa's potential efficacy. This interdisciplinary approach aims to optimize Levodopa's therapeutic benefits for Parkinson's disease. Molecular docking studies also predict compounds that might interact with alpha-synuclein to provide a control for its deposition. This could be useful in drug development by Swiss Dock and Seam Dock. Out of the 62 analogs constructed, the ligand {3-[(2S)-3-fluoraniumylidene-2, 3-dihydroxypropyl] phenyl} sodium and 3-(3-hydrazine-4-phosphanylphenyl) propanoic acid efficiently bind to the receptor, i.e., target molecule with the energy -6.56896 AND -4.9

1. INTRODUCTION

Parkinson's disease belongs to a group of conditions collectively called movement disorders; every bodily movement is a careful coordination between the nervous system. It is associated with two major pathological alterations, namely: Interestingly, Parkinson's disease is associated with two characteristics: (a) Preferential and selective degeneration of dopaminergic neurons; and (b) the serotonergic fibers of the gut have profound pathology and imprint perfect alpha-synuclein inclusions that set Parkinson's disease apart. The order of these processes

is still unclear, and it is unknown which one occurs first. This progression is evidenced in pathological analyses, where the few lost neurons and the rest, for many years, degenerate and are all pathological for Parkinsonism. When the motor features are apparent, neurodegeneration of dopaminergic neurons in the substantia nigra seen histopathologically, ranges from 30-70 %. In the current management, the main ideas are changing the lost dopamine by dopaminergic therapy and rewiring the circuits. Negative affectivity, depressive symptoms, and impulsive measures are the result of dopamine deficiency in the regions that are located beyond the basal ganglia and by changes within the serotonergic and noradrenergic systems. The changes of the ANS have been described in the pathological conditions not only of the brain and the cranial nerves but also of the spinal cord and the peripheral autonomic nervous system [1].

Parkinson's is a difficult and progressive illness that worsens with time, and the most common manifestations are shaking, stiffness, and slow movement, but some patients will have postural abnormalities in the later stages of PD. This disease was first discovered as a disease by James Parkinson in the year 1817 and further advanced by Jean-Martin Charcot, with much-improved knowledge about this disease today. Parkinsons disease is a type of brain disease that happens. After Alzheimer's disease, it affects about 0.5-1% of people aged 65-69, and 1-3% of those who are 80 years or older. Moreover, PD accounts for the bulk of the forecasted burden in 2030: "over 30% 30%, thanks to the aging population, the disorder is expected to have a higher prevalence, as well as a higher incidence rate, if before 2030 direct and indirect medical costs will emerge". The Petri dish is based on the loss of striatal dopaminergic terminals at the nigrostriatal pathway level, but neurodegeneration continues beyond the nigra dopaminergic cells, involving all neuronal cells in a network. This kind of extension in its pathology is what puts PD on the list of highly unreliable heterogeneous conditions. At this moment, however, there is no reliable diagnostic examination that can be performed [2].

Several studies indicate that neuropathologic evaluations show the clinical diagnosis of parkinsons disease can vary in accuracy ranges from 65-93%, based on the principles used and stages of the disease. This study includes both clinical and neurological data to assess how well people with Parkinson's disease can be diagnosed over time, and how this relates to how long they had the disease, how they respond to medicine, and the symptoms

they show. Parkinsonism is presently classified as a multisystem neurodegenerative disorder affecting the CNS, ENS, ANS, AIS, and GI tract [4]. Parkinson's disease is a common brain condition, part of a group called Lewy body disease. It affects about 160 out of every 100000 people in Western Europe. In the past year, this number has gone up, and now about 4% of people who are 80 years or older have the disease. Due to the general trend of aging, the management of Parkinsonism raises acute and essential issues for neurologists and general physicians. In the past decade, several genes have been identified that could help explain the underlying pathology of the disease in sporadic cases of PD [5].

2. MATERIAL AND METHODS

2.1 DISEASE GENE INFORMATION:

SNCA was the first gene identified for the number of mutations that cause autosomal dominant Parkinsonism. SNCA genetic mutations are associated with early onset Parkinsonism (EOPD, onset at the age of 50) and an initial favorable response to levodopa therapy.

SNCA genetic mutations are linked to early onset parkinsonism (EOPD), which starts before the age of 50, and these patients often respond well to levodopa treatment. The SNCA gene has six parts called exons that make a normal protein called alpha-synuclein, which is about 140 amino acids long. The structure of alpha-synuclein has three important parts : (i)the first part, from amino acid 7-87, has sections of 11 amino acids each repeated seven times, but this are overlaps with (ii)a key area that helps the protein interact with other molecules, covering amino acids 61-95, and (iii)the last part, from amino 96-140, is made up of acidic amino acids. All three types of mutations that change a single amino acid affect the first part of the protein. The primary structure of a-synuclein, apart from certain natively unfolded regions, but once it interacts with the membranes containing phospholipids and small repeat motifs of amino acid sequence (71-82) located at the amino-terminal, a-synuclein takes up a coil conformation containing a helix-rich domain [6].

The SNCA gene was identified as the first connective gene for the autosomal dominant form of Parkinson's disease. The common strategy in identifying the disease-associated genes and their variants was primarily based on identifying the loci through positional cloning of large families employing an artificial chromosome library. This approach resulted in the identification of SNCA [7].

Dopamine metabolite 3, 4-dihydroxyphenylacetaldehyde (DOPAL) binds to α-synuclein and forms a covalent

adduct to the protein, causing its aggregation and reduced degradation in neurons. The aggregation of DOPAL-modified α-synuclein impairs neuronal adaptive capacity, degrades the quality of synapses, and stresses the protein quality control system in neuritis [8].

2.1.IDENTIFICATION OF THE LOCATION OF THE GENE IN THE GENOME SEQUENCE

This gene, involved in the production of alpha-synuclein protein, was the first gene found to have a link with Parkinson's [9].

Once a genome sequence is obtained from NCBI, whether it is the sequence that has been cloned or any chromosome, then a GHMM-based program embedded in GENSCAN (which helps to identify complete gene structure in genomic DNA) can be used to find the present location of the genes. These methods may be broadly categorized into the methods that visually scan the sequence revealed by the technique for the presence of the features characteristic of gene sequences with the help of the naked eye or more often with the help of a computer, and the methods that identify genes using experimental approaches to the DNA sequence [10]. This SNCA gene

2.2.PROTEIN INFORMATION

Alpha-synuclein is a protein (amino acid 140) coded by the SNCA gene, which occurs mainly within the human brain and particularly at the presynaptic junctions, where it is intimately involved with the nervous system.

Alpha-synuclein mainly exists in a natively unfolded form, and it is therefore flexible and can exist in various conformations depending on the conditions. The first-ever identification of the SNCA gene associated with familial Parkinson's disease which had a gene associated with it was in 1997, when the A53T point mutation was found in the Contursi kindred. Other mutations, including E46K and gene duplications/triplications, have now been identified as also being associated with PD more recently. Such genetic modifications tend to increase the amounts of the alpha-synuclein protein, making it aggregated [11]. The molecular mass of the protein sequence is 14460 Da.and size of the protein sequence is 140 amino acids.

2.3.PREDICTION OF SUBCELLULAR LOCALIZATION OF PROTEIN ALPHA-SYNUCLEIN.

Approximately 1000 nuclear-encoded proteins are localized in four locations are radicalized in mitochondria, the matrix, the inner membrane, the intermembrane space, and the outer membrane [12]. Alpha-synuclein is a 140-amino acid protein highly expressed in nervous tissues [13].

Subcellular localization prediction for each homologous sequence, the CELLO tool is used to predict the subcellular localization of the protein found in proteomic data, as given in Figure 1: Prediction of Subcellular Localization of Protein Alpha-Synuclein Using the Cello Tool [14].

2.4.PHYSICOCHEMICAL PROPERTIES

The above studies indicate that groups of protein properties that non-synonymous SNPs affect depend on protein stability, dynamics, and interactions. Mutational analysis of many of the diseases [16].protparam is a tool which used to predict the physical and chemical properties of the alpha synuclein protein. It allows users to calculate different physical and chemical characteristics for a protein sequence that is either from swiss-prot, tr-embl.or entered directly by the user, and their amino acid composition is given in Figure 2: A Amino acid composition1 using protparam tool.Figure 3: Atomic composition2 using protparam tool. [17].-

2.5.COMPARISON OF ORGANISMS TO OTHER ORGANISMS

- **Sequence collection:**

The UniprotKB database was used to gather information about SNCA and related data, including proteins, the organisms they come from, amino acids, annotation scores, and whether the protein exists[18]. A list of all the protein sequences used is provided in the supplementary data files. The selected species include: Homosapiens(human), Pan troglodytes(chimpanzee), Macaca mulatta(monkey), Mus musculus(Mouse), Sus scrofa(Pig), Bos taurus(Cow), Equus caballus(Horse), and all these species' protein sequences compared with each other using Omega Clustal W in mega11 tool are given in Figure 4: Multiple sequence alignment using the Mga11 tool..[19].

- **Sequence analysis:-**

The molecular phylogenetic analysis of the protein was done using the best model in Mega11. The phylogenetic tree for the SNCA family was made using the neighbor-joining method. The paper also includes information from the tree. Only uncorrected p-distance-based neighbor-joining and multiple sequence alignments were used to find where these domains and motifs are located in the paralogous proteins of SNCA in humans, and Orthologs in different species obtained from Uniprot-KB are given in Figure 5.[19]

2.6. IDENTIFICATION OF DRUG TARGET:

The Alpha-synuclein was identified as a drug target from GeneCards. GeneCards: The Human Gene Database is a regularly updated online tool containing information about human genes and genetic diseases.

The Genecard tool helps predict which genes are in humans and what each gene does. The Genecards database lets you search for these predicted genes and includes information from more than 150 different sources, such as HGNC, Ensembl, and NCBI. The list of genes is based on data from NCBI, Ensembl, and gene names approved by the HUGO Gene Nomenclature.

2.7.RETRIEVAL OF 3D STRUCTURE OF DRUG TARGET:

The 3D shape of the drug target, SNCA, was obtained from the PDB database based on the information found in the Genecrds database. The Protein Databank serves the interests of students and researchers in different fields of biology and agriculture, including processes within human health and illness, like the synthesis of protein synuclein and other attributes. For this, PDB ID 1XQ8 or the SNCA gene was used [21].

2.8.IDENTIFICATION OF LIGAND:

The information on the drug molecule Levodopa, which is important for the treatment of Parkinson's disease, was retrieved from the literature, and its structure was downloaded from the ChemSpider database. ChemSpider is used to identify ligand molecules of the compound.

The detectives need to figure out if the ligand is a receptor, if it has a known lead, and whether it's an antibody, a small molecule, or a peptide. They also have to determine if it's an inhibitor or a substrate.They should check its strength in binding to the receptor, how stable it is,how many receptor molecules are on the cell's outer surface, and its movement in the body, including if it attaches to the SNCA protein.A target is considered valid if it has a known role, like being an enzyme or receptor involved in the natural behavior of a molecule that might act as its usual substrate or ligand.

2.9.CONSTRUCTION OF SMALL MOLECULAR LIBRARY OF LIGANDS:

ACD/ChemSketch Freeware is a software tool that allows users to draw chemical structures like organic compounds, organometallics, and polymers. This approach treats drug-likeness as a clinical standard based on Marie E.C. Lipinski's rule of five. This rule basically says that if a drug is meant to be taken by mouth, it should not break more than one of the rules. First, we opened the structure of Levodopa in Chemskech and made changes by adding or removing bonds and atoms, but kept the structure's function the same. Then we did a 3D optimization of the structure and gave it a name. Finally, we saved the structure in

". mol" format. After constructing small molecular library, we select 2 analogs is given in Table 4 .

2.10. DRUG-LIKENESS ASS0ESSMENT

- **Check the ADMET properties:**

In silico evaluation according to the in-silico evaluation approach, the physicochemical and ADME (absorption, distribution, metabolism, and excretion) descriptive parameters, pharmacokinetic characteristics, and drug nature were assessed. Using data from admetSAR software, the compound also met Veber's rule, showed blood-brain barrier likeness, and had both unweighted and weighted quatitative estimates of drug-likeness(QED).This is an important part of drug discovery where the compounds shape and how it fits into the target binding site are studied after study we check the ADMET properties of both selected analogs is given in Table 5 and Table 6 [25][26].

2.11. MOLECULAR DOCKING:

The receptors were examined, and the amino acids that had strong interactions through hydrogen bonds, van der Waals forces, and other methods were identified. The water molecules were taken out, and a clear structure of the receptor was saved as a "PDB" file from the PDB database. Observing at how the protein and ligands interact is an important part of finding good ligands in virtual screening. The Swiss Dock program was used for the first round of molecular docking. The receptors were examined, and the glycine amino acids that had strong interactions through hydrogen .The ligand molecule was retrieved from ChemSpider, and that structure was converted into ".mol2" format (as per SwissDock requirement) using the open babel tool for the first docking analysis, and submitting both receptor and ligand molecule in SwissDock and interpreting the result.

The ligand molecule was converted into PDB format after being retrieved from ChemSpider for second docking analysis, and the target molecule was converted into PDB format (as per Seamdock requirement), and both molecules were used in Seamdock. Swiss Dock and Seam dock were used to dock the ligand with the target, and the results were obtained. Some of the ligand derivatives have an RMSD value mentioned in references [27[[28]. In both cases, the ligand-receptor pairs with the highest docking scores were examined in more detail at the molecular level. The study looked at the formation of van der Waals bonds, regular hydrogen bonds, carbon-hydrogen bonds, and interactions were shown for the best ligand-protein combinations for each receptor[29].

2.12. OPTIMIZATION OF INTERACTION

The chosen docking1 and docking2 results were obtained from SwissDock and Seamdock in structure data files format to reduce their energy, with the compound's net charge being as low as possible, ideally zero. Docking was done using UCSF Chimera Software Version 1.17.3. After the energy was minimized, all selected compounds were converted into "mol2" format, which is necessary for molecular docking analysis [30].To view another protein-ligand docking complex structure, select "Show 2D diagram" from the Receptor-Ligand interactions menu. This visualization displays the interactions in a 2D format and helps see the interactions that took place in the ligand. This type of visualization is often used together with 3D visualization. In this case, both docking and visualization were carried out using SeamDock software [31].

3. RESULT

3.1.INFORMATION OF DISEASE GENE:

Diseased gene information is shown in the following figure, which shows the symbol report for SNCA, their HGNC data for SNCA showing approved symbol, approved name, locus type, HGNC ID, Symbol status, previous symbol, previous names, alias symbols, alias names, and chromosomal locations in Table *1*.

Table 1: HGNC data for SNCA

Attributes	Data
Approved symbol	SNCA
Approved name	Synuclein alpha
Locus type	Gene with protein product
HGNC id	HGNC:11138
Symbol status	Approved
Previous status	PARK1; PARK4
Previous name	Parkinson's disease (Autosomal dominant, Lewy body) 4, Synuclein, alpha (a non-A4 component of Amyloid Precursor)
Alias symbols	NACP; PD1
Alias name	non-A4 component of Amyloid Precursor, Alpha-synuclein, α-synuclein.

Chromosomal location	4q22.1

The HGNC (HUGO Gene Nomenclature Committee) provides standardized nomenclature for human genes, including the SNCA gene, which encodes alpha-synuclein. Alpha-synuclein is mainly expressed in the brain. The Human Gene Sequence presents 1549 base pairs, CDS is 53.475, mRNA is in Linear format, Base Count A is 417, C is 248, G is 342, and T is 488is given in Table 2, And their translation is given in Table 3.

Table 2: ORIGIN of Human Sequence from Genebank

1	gctctcggagtggccattcgacgacagtgtggtgtaaaggaattcattagccatggatgt
61	attcatgaaaggactttcaaaggccaaggagggagttgtggctgctgctgagaaaaccaa
121	acagggtgtggcagaagcagcaggaaagacaaaagagggtgttctctatgtaggctccaa
181	aaccaaggagggagtggtgcatggtgtggcaacagtggctgagaagaccaaagagcaagt
241	gacaaatgttggaggagcagtggtgacgggtgtgacagcagtagcccagaagacagtgga
301	gggagcagggagcattgcagcagccactggctttgtcaaaaaggaccagttgggcaagaa
361	tgaagaaggagccccacaggaaggaattctggaagatatgcctgtggatcctgacaatga
421	ggcttatgaaatgccttctgaggaagggtatcaagactacgaacctgaagcctaagaaat
481	atctttgctcccagtttcttgagatctgctgacagatgttccatcctgtacaagtgctca
541	gttccaatgtgcccagtcatgacatttctcaaagtttttacagtgtatctcgaagtcttc
601	catcagcagtgattgaagtatctgtacctgccccccactcagcatttcggtgcttccctttt
661	cactgaagtgaatacatggtagcagggtctttgtgtgctgtggattttgtggcttcaatc
721	Tacgatgttaaaacaaattaaaaacacctaagtgactaccacttatttctaaatcctcac
781	tatttttttgttgctgttgttcagaagttgttagtgatttgctatcatatattataagat
841	ttttaggtgtcttttaatgatactgtctaagaataatgacgtattgtgaaatttgttaat
901	atatataatacttaaaaatatgtgagcatg aaactatgcacctataaatactaaatatga
961	aattttaccattttgcgatgtgttttattcacttgtgttt gtatataaatggtgagaatt
1021	aaaataaaacgttatctcat tgcaaaaatattttattttt atcccatctcactttaataa
1081	taaaaatcatgcttataagcaacatgaattaagaactgacacaaaggacaaaaatataaa
1141	gttattaatagccatttgaagaaggaggaa ttttagaagaggtagagaaaatggaacatt
1201	aaccctacactcggaattccctgaagcaacactgccagaagtgtgttttggtatgcactg
1261	gttccttaagtggctgtgattaattattgaaagtggggtgttgaagaccccaactactat
1321	tgtagagtggtctatttctcccttcaatcctgtcaatgtttgctttatgtattttgggga
1381	actgttgtttgatgtgtatgtgtttataattgttatacatttttaattgagccttttatt
1441	aacatatatt gttatttttg tctcgaaata atttttttagt taaaatctat tttgtctgat

1501	attggtgtga atgctgtacc tttctgacaa taaataatat tcgaccatg

Table 3: SEQUENCE TRANSLATION:

"MDVFMKGLSKAKEGVVAAAEKTKQGVAEAAGKTKEGVLYVGSKTKEGVVHGVAT VAEKTKEQVTNVGGAVVTGVTAVAQKTVEGAGSIAAATGFVKKDQLGKNEEGAPQE GILEDMPVDPDNEAYEMPSEEGYQDYEPEA"

Alpha-synuclein is part of a protein family known as synucleins, which also includes β-synuclein and γ-synuclein. Synuclein is found in high amounts in the brain, and both alpha and beta-synuclein can block the phospholipase (PLD2) enzyme. SNCA plays a key role in how nerve cells send signals and move membranes. Problems with SNCA have been connected to the start of Parkinson's disease. SNCA. SNCA fragments are present in the brains of people with Alzheimer's disease, mostly in the APA area.

Consequently, extensive research has focused on understanding alpha-synuclein's role, critical for separating PD's original mechanisms and developing potential treatments.

3.2. IDENTIFICATION OF THE LOCATION OF THE GENE IN THE GENOME SEQUENCE:

The Genscan output shows the content of the gene and identifies the complete gene structure and sequence format ".fasta", parameter matrix, predicted genes/exons, suboptimal exon with probability, predicted exon sequence, predicted coding sequence, etc. The GenScan output usually provides a detailed description of predicted genes and their characteristics. The output may start with header information, including details about the input sequence, parameters used for prediction, and version information. The main section of the output usually contains predictions of individual genes found within the input sequence. Each gene prediction typically includes the following information: Gene ID or Name, Genomic Coordinates, Strand Orientation, Exon-Intron Structure, and Coding Sequence (CDS, Protein Translation).

3.3. PREDICTION OF SUBCELLULAR LOCALIZATION OF PROTEIN ALPHA-SYNUCLEIN

The results shown specify the location of the protein alpha-synuclein using the CELLO tool, are shows in Figure 1. Tools and algorithms, in computing and machine learning, can be used to estimate where proteins might be located within cells by

analyzing factors like amino acid makeup and properties like sequence motifs and physicochemical characteristics. These forecasts offer perspectives on the parts of cells where alpha-synuclein could exist and help in understanding its role in biology and its impact on conditions of Parkinson's disease.

CELLO RESULTS

SeqID: MDVFMKGLSKAKEGVVAAAEKTKQGVAEAAGKTKEGVLYVGSKTKEGVVHGVATVAEKTK

Analysis Report:

SVM	LOCALIZATION	RELIABILITY
Amino Acid Comp.	Cytoplasmic	0.430
N-peptide Comp.	Cytoplasmic	0.496
Partitioned seq. Comp.	Chloroplast	0.320
Physico-chemical Comp.	Chloroplast	0.621
Neighboring seq. Comp.	Cytoplasmic	0.336

CELLO Prediction:

Cytoplasmic	1.480 *
Chloroplast	1.471 *
Nuclear	0.931
Mitochondrial	0.595
Extracellular	0.233
PlasmaMembrane	0.078
ER	0.071
Peroxisomal	0.042
Vacuole	0.037
Golgi	0.023
Lysosomal	0.021
Cytoskeletal	0.019

Figure 1: Prediction of Subcellular Localization of Protein Alpha-Synuclein Using the Cello Tool

3.4. PREDICTION OF PHYSICOCHEMICAL PROPERTIES FROM PROTPARAM

Protparam result shows the physicochemical properties of the protein sequence. These calculated values cover the molecular weight, predicted pi value, makeup of amino acids, breakdown of atoms, extinction coefficient, estimated half-life, instability index, aliphatic index, and the overall average of hydrophobicity. Protparam on a protein sequence, the output typically includes a comprehensive description of these properties is given in Figure 2 and Figure 3.

Protparam gives the number of times each amino acid appears and the percentage of each in the protein sequence. And which has 140 amino acids. It also provides the calculated molecular weight of the protein, which is the sum of the atomic weights of all the atoms in the protein molecule. It has 14460.16 molecular weight. The theoretical isoelectric point is the PH level where a protein has no overall electric charge. Protparam calculates this value based on the pKa values of the amino acid residues in the protein; it has a 4.67 pi value. The

protparam tool gives a count of all amino acids in the sequence. Amino acid composition refers to the relative abundance of different amino acids in a protein sequence. It provides insight into the protein's biochemical properties, structural features, and functional characteristics, influencing its interactions and stability.

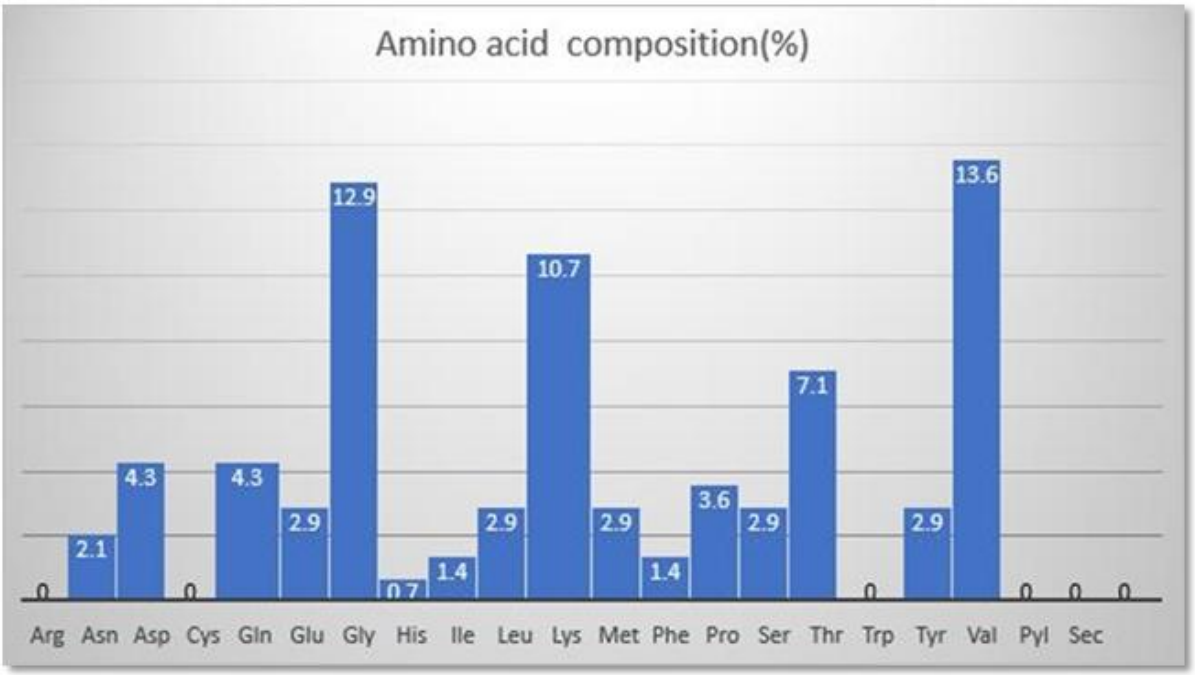


Figure 2: A Amino acid composition1 using protparam tool.

It shows the atomic composition of the protein, including the number of carbons, hydrogen, nitrogen, oxygen, and sulphur atoms.

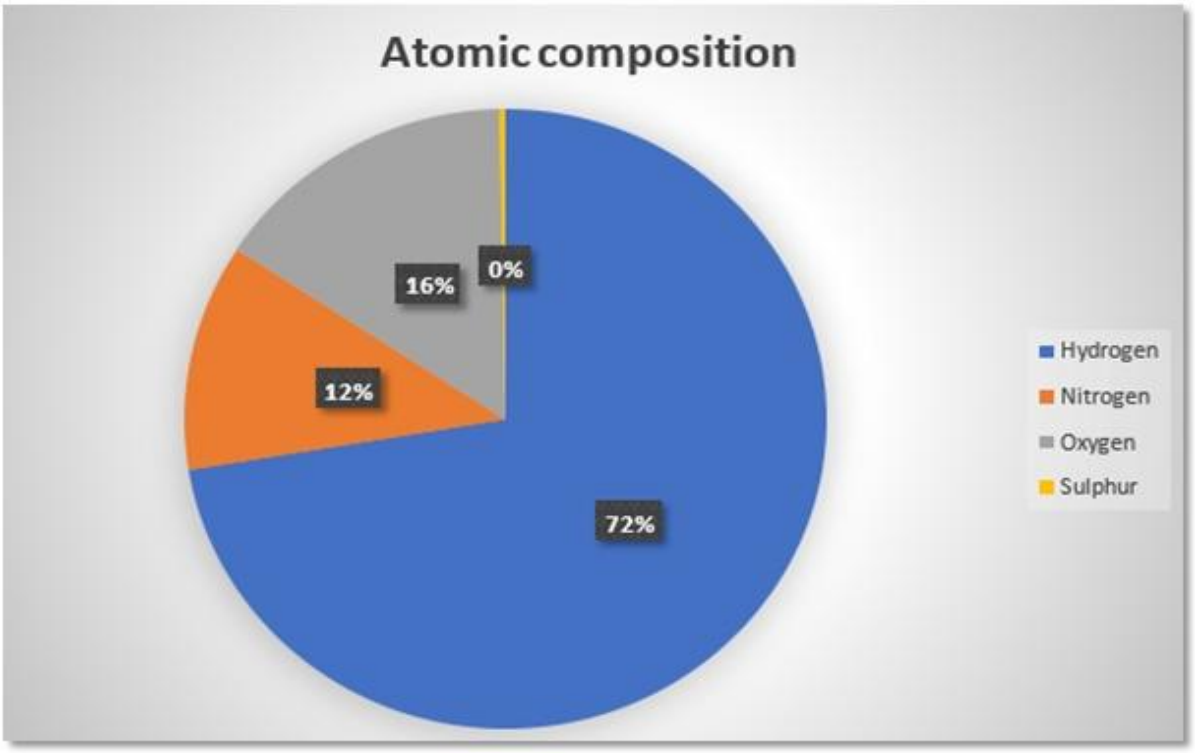


Figure 3: Atomic composition2 using protparam tool.

Protparam calculates the protein's extinction coefficient at 280 nm, which is used to estimate its concentration in solution. The above image provides the absorbance measurement of the protein solution. It also predicts that the instability index higher than 40 is considered unstable. The instability index (II) has been calculated to be 25.47. It classifies the protein as stable. It has an aliphatic index of 69.64. Protparam predicts the fractional composition of different secondary structure elements in the protein, such as alpha helices, beta strands, and coils. It provides the net charge of the protein at a neutral pH, considering the ionization states of amino acid side chains. Protparam may include the pKa values of individual amino acid residues in the sequence, which are used to calculate the isoelectric point and charge of the protein.

3.5. COMPARATIVE ANALYSIS

- **Sequence collection**:

Figure 4 shows the result for the alignment of organisms, which compares every protein sequence with each other by using the Clustal Omega method/tool in the mega11 tool.

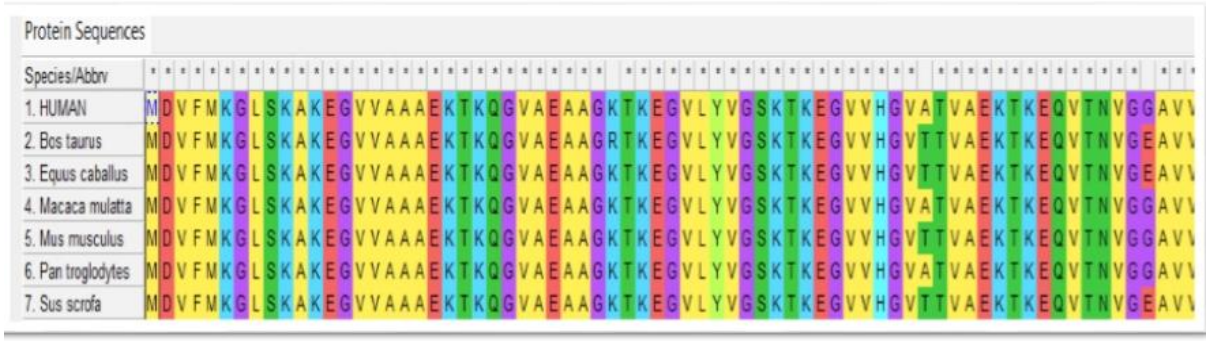


Figure 4: Multiple sequence alignment using the Mga11 tool.

- **Sequence analysis:**

Figure 5 shows study reveals that humans share a connection with chimpanzees, highlighting the relationship between different species like pigs, cows, and horses, with distinct clades such as Bos Taurus and Equus Caballus. The research also indicates that the scale length is measured at 0.01 units.

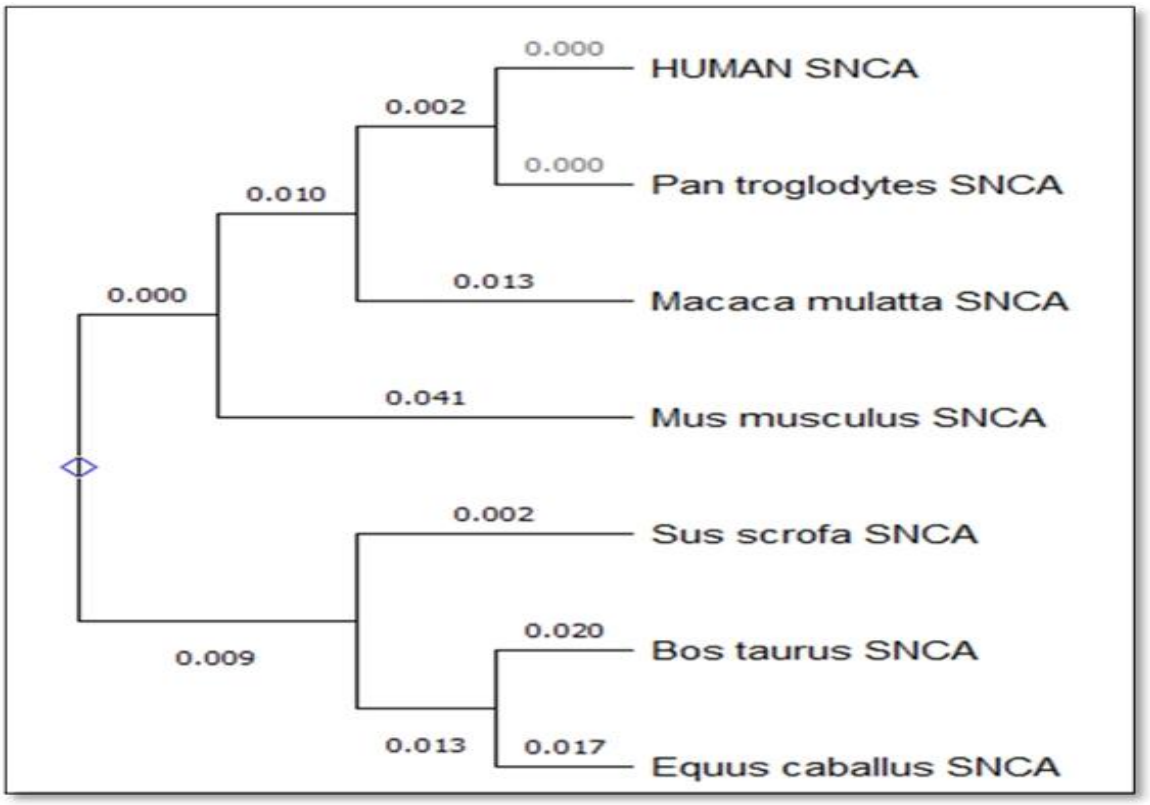


Figure 5:Evolutionary relationship with the common ancestor among 7 groups of organisms in rectangular form using Mega11 software.

The MEGA11 software program lets you explore into the connections between seven groups of living beings by building a family tree known as a phylogenetic tree using genetic data to uncover shared ancestors and points of divergence. The study

uncovers the history of evolution and genetic connections between species while shedding light on how species form in groups of organisms.

3.6.LIGAND PREPARATION AND ANALYSIS OF DRUG LIKELINESS:

Table 4:Prepared analogs.

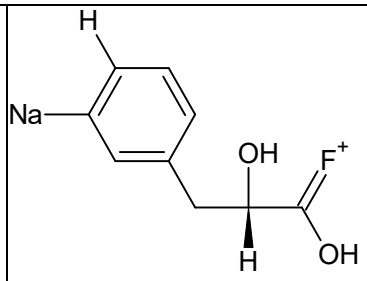
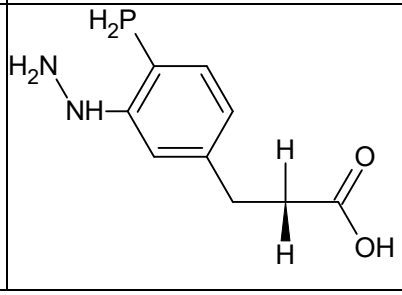
1.		{3-[(2*S*)-3-fluoraniumylidene-2,3- dihydroxy propyl]phenyl}sodium [H]C1=C([Na])C=C(CC([H])(O)C(O)=[F+])C=C1 (4.9)
2.		3-(3-hydrazinyl-4-phosphanylphenyl)propanoic acid [H]C([H])(CC1=CC(NN)=C(P)C=C1)C(O)=O (7.5)

Table 4 represents the compounds and their ADMET properties for perfect binding. These two molecules were selected for interaction, and both molecule shows satisfactory properties for interaction.

Table 5: ADMET properties of {3-[(2S)-3-fluoraniumylidene-2,3-dihhydroxypropyl]phenyl}sodium

Sr. No.	ADMET predicted profile -Regressions	Value	Unit
1.	Water solubility	-1.157	logS
2.	Plasma protein binding	0.726	100%
3.	Acute Oral Toxicity	1.737	log(1/(mol/kg))
4.	Tetrahymena pyriformis	0.311	pIGC50 (ug/L)

Table 6: ADMET properties of 3-(3-hydrazinyl-4-phospanylphenyl)propanoic acid.

Sr. No.	ADMET predicted profile -Regressions	Value	Unit
1.	Water solubility	-1.961	logS
2.	Plasma protein binding	0.731	100%
3.	Acute Oral Toxicity	3.249	log(1/(mol/kg))
4.	Tetrahymena pyriformis	-0.232	pIGC50 (ug/L)

3.7. MOLECULAR DOCKING RESULT USING SWISSDOCK:

Figure 6 and Figure 7 shows results from the molecular docking study were analyzed by looking at how compound 1 binds to α-synuclein protein. This included the number of hydrophobic interactions between the compound1 and α-synuclein protein is given in Figure 8, the number of hydrogen bonds formed is given in Figure 9 and Figure 10 and Figure 11 shows checking the binding affinity. The goal was to find out if there is a specific condition that can explain the difference between molecules that are neuroprotective and those that are neurotoxic. This condition could then be used in computer-based methods to identify potential neuroprotective compounds.

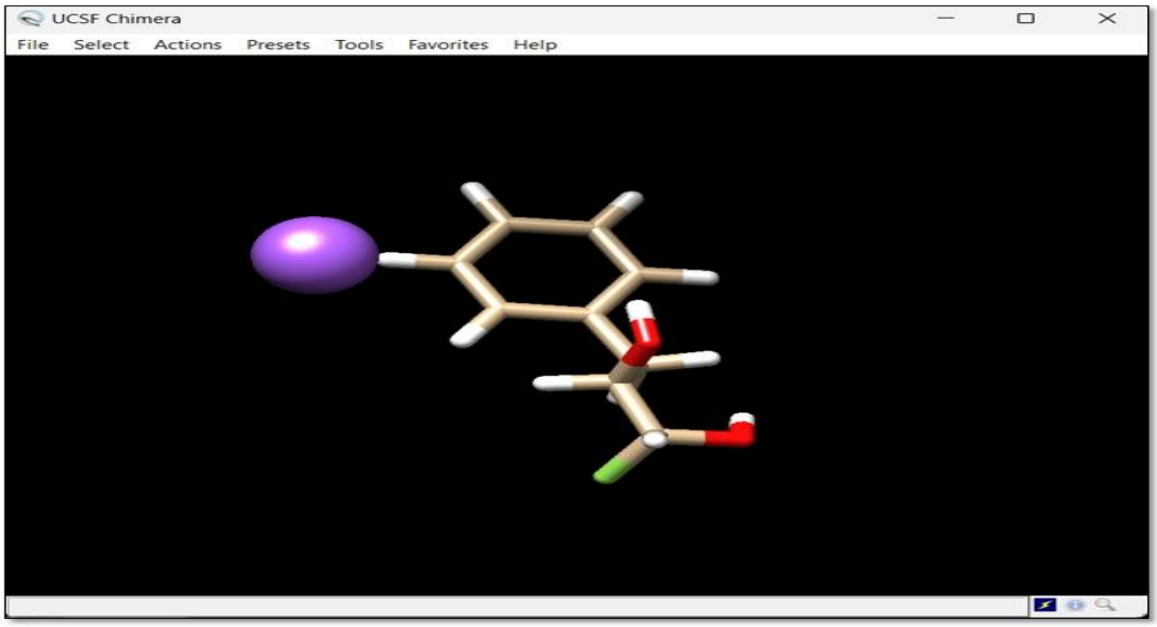


Figure 6: Interaction between target and compound1 (attached with a hydrogen bond)in ribbon form using UCSF Chimera tool.

The RMSD value between the predicted model and the experimental model is -6.56896. The protein backbone is displayed as a ball model. The ligand, water molecules, and residues in the binding site are shown in stick form.

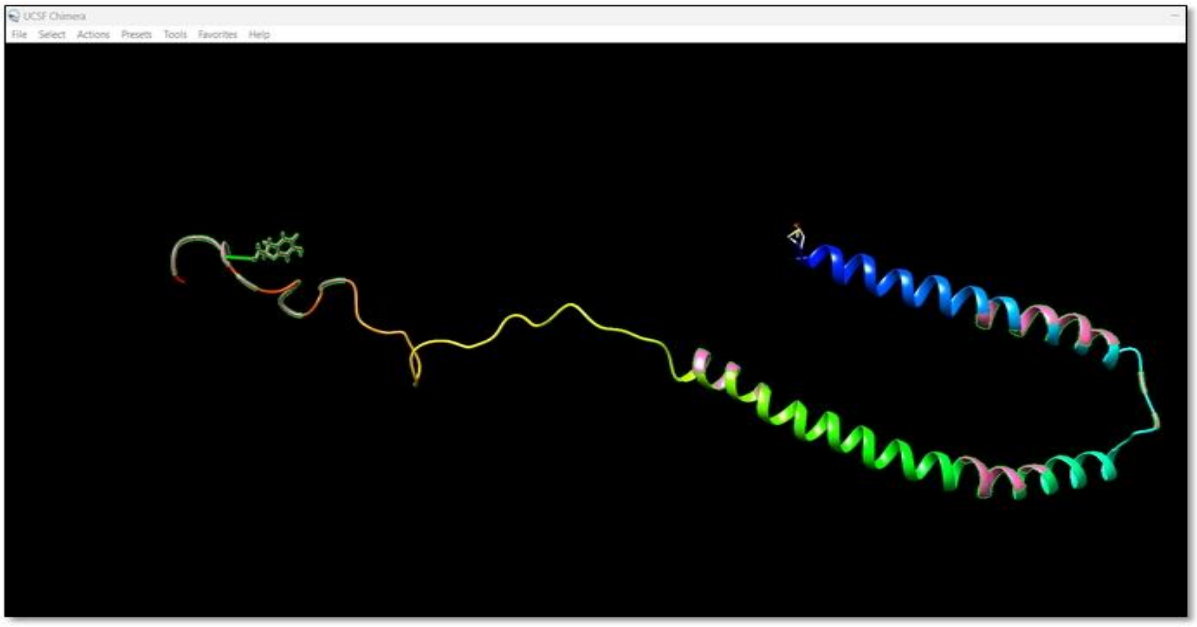


Figure 7:Interaction between target and compound1 without extra atoms (single bond) using UCSF Chimera tool.

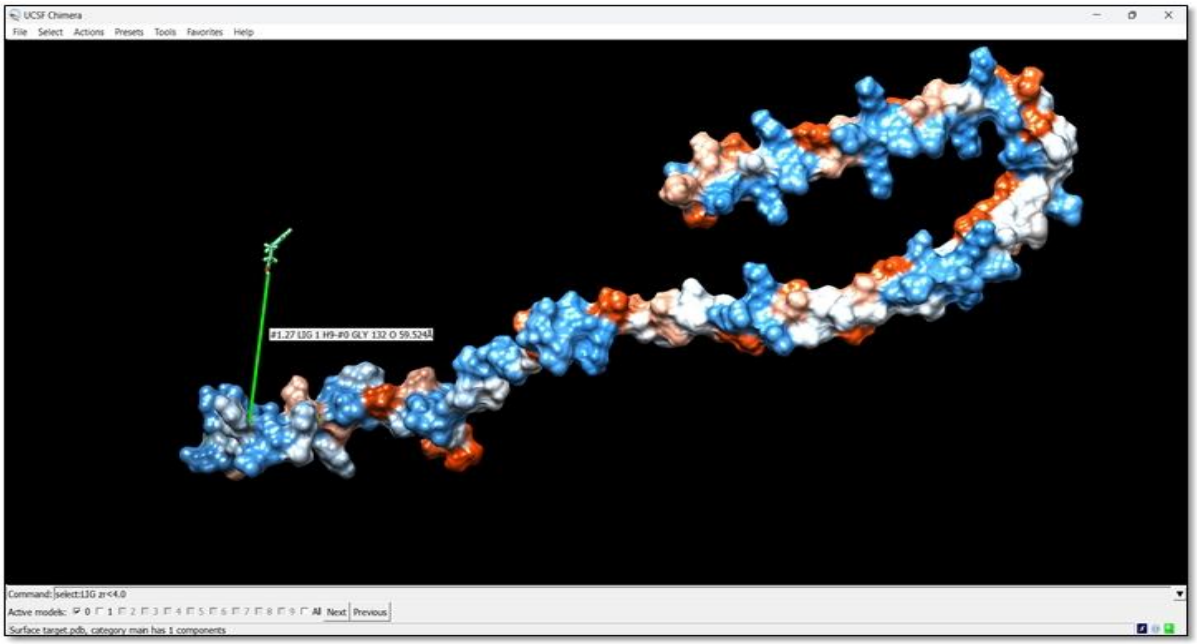


Figure 8: Hydrophobicity of the interaction between target and compound1 without extra atoms (single bond) using UCSF Chimera tool.

The surface shows how hydrophobic the protein is. The area where it binds glycine is shown and marked with green lines. The hydrophobicity of the protein is shown using the Kyte and Doolittle scale. The most polar parts are in medium blue, and the most hydrophobic parts are in tan. The electrostatic charge ranges from negative (red) to positive (blue). Both hydrophobicity and electrostatic charge are shown through the surface colors using the UCSF Chimera tool.

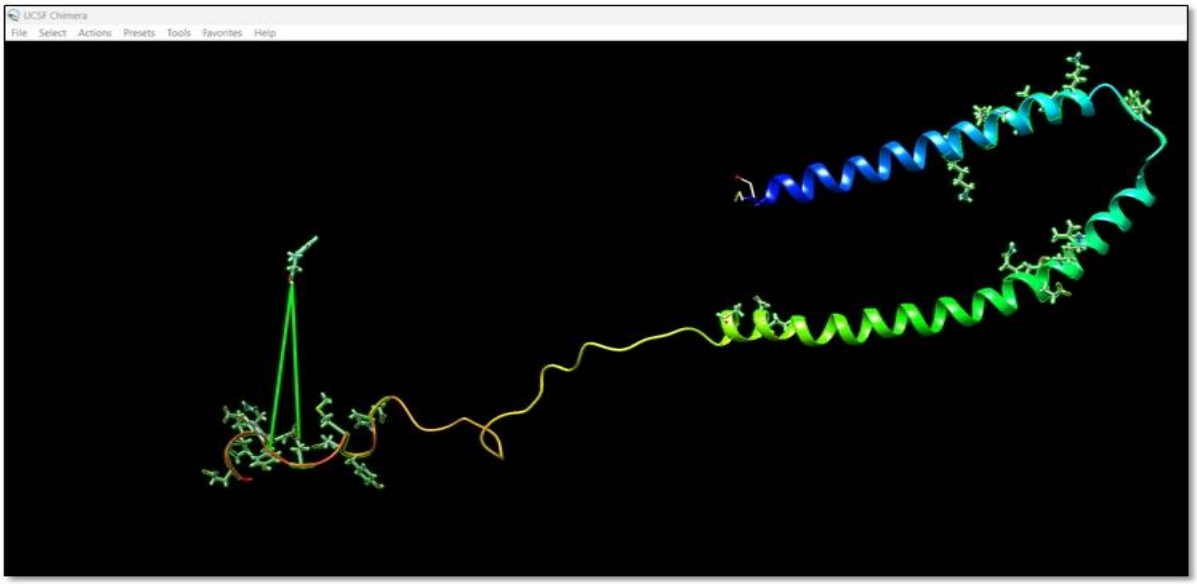


Figure 9:Interaction between target and compound1 with extra atoms (hydrogen bond) using UCSF Chimera tool.

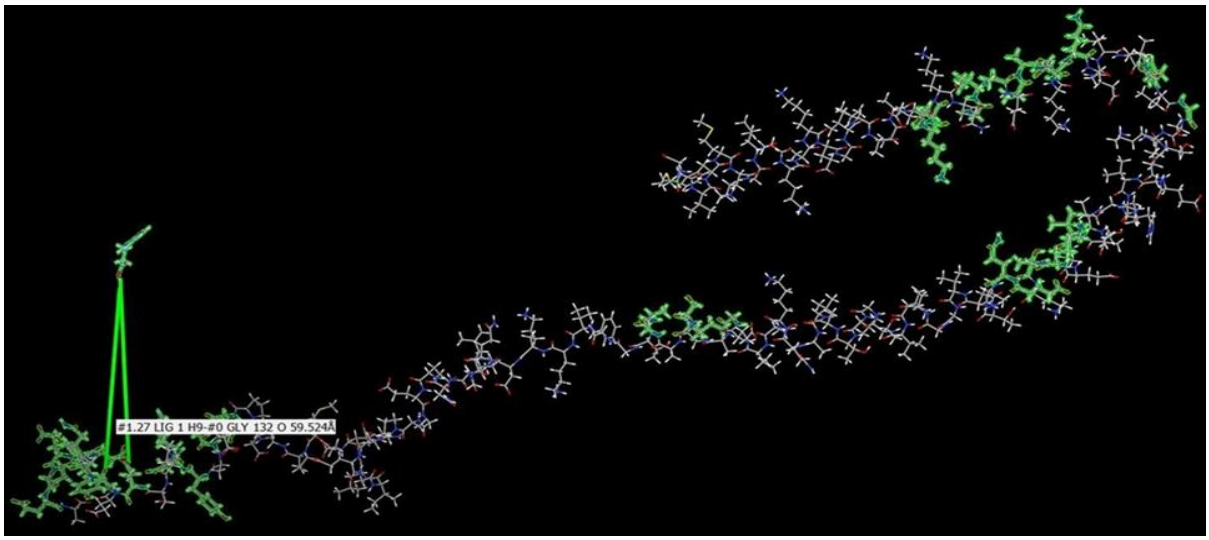


Figure 10: Interaction between target and compound1 with all atoms, which shows binds with glycine, having 59.524A0 using UCSF Chimera tool.

Figure 11 shows E = Interaction energy between target and compound1 having a minimum binding affinity with cluster V3 With Energy Score -6.56896 The interaction of a target protein with Compound 1 has been shown in UCSF Chimera where it estimated that glycine residue undergoes a binding event with a crucial distance of 59.524 Å and this predicted interaction reflects the critical molecular recognition site which is believed to be subjected to hydrogen-bonding or other stabilizing forces. Via visualization of the entire set of atomic interactions, we acquire information about how EXTL2 binds to its ligand and shed light on Compound 1's mechanisms of action and potential for therapeutic relevance. This research achieves the

complex molecular interaction, and this is the only way to extract useful information for drug design, as well as within biological systems, protein-ligand interactions.

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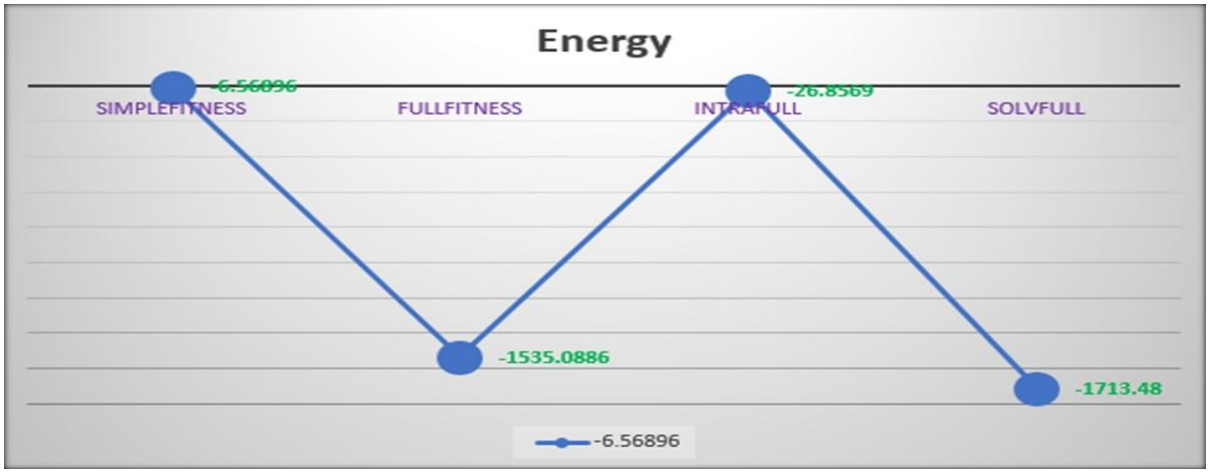

Figure 11: The binding energy (-6.56896) of the drug and the target ung UCSF Chimera tool.

The binding energy was calculated as -6.56896, which evidenced a strong bond between the respective drug and its target molecule. This negative value means an attractive interaction. Thus, a good drug has to bind its target. Therefore, there are stable molecular complexes. The high value of the binding energy indicates that this drug is probably an effective modulator of the target biological function. Understanding this landscape is important for rational drug discovery and design, enabling the selection and/or development of candidate compounds. Moreover, it explains how different molecules talk to each other, which can set the stage for developing more potent and precise therapeutics that bring new hope for treating a wide range of diseases.

3.8 MOLECULAR DOCKING RESULT USING SEAM DOCK

Identifying the hydrogen bond reflects important molecular interactions when using the SEAM Dock tool to study the target molecule with second Compound. Such hydrogen bonds play an important role in the balance of both atoms of the target and Compound2 at their binding sites. This information is of great interest because knowing where this hydrogen bonds are on the interface helps to understand the specificity and strength of interaction mediated by this process (which SEAM Dock should be able to detect and visualize). SEAM Dock illustrates the spatial configuration and geometry of hydrogen bonding, which helps to depict the molecular recognition modeling of how target and Compound2 hold at an atomic

level. Such understanding can provide insights into rational drug design for the optimization of Compound2 to improve its binding affinity and beneficial performance.

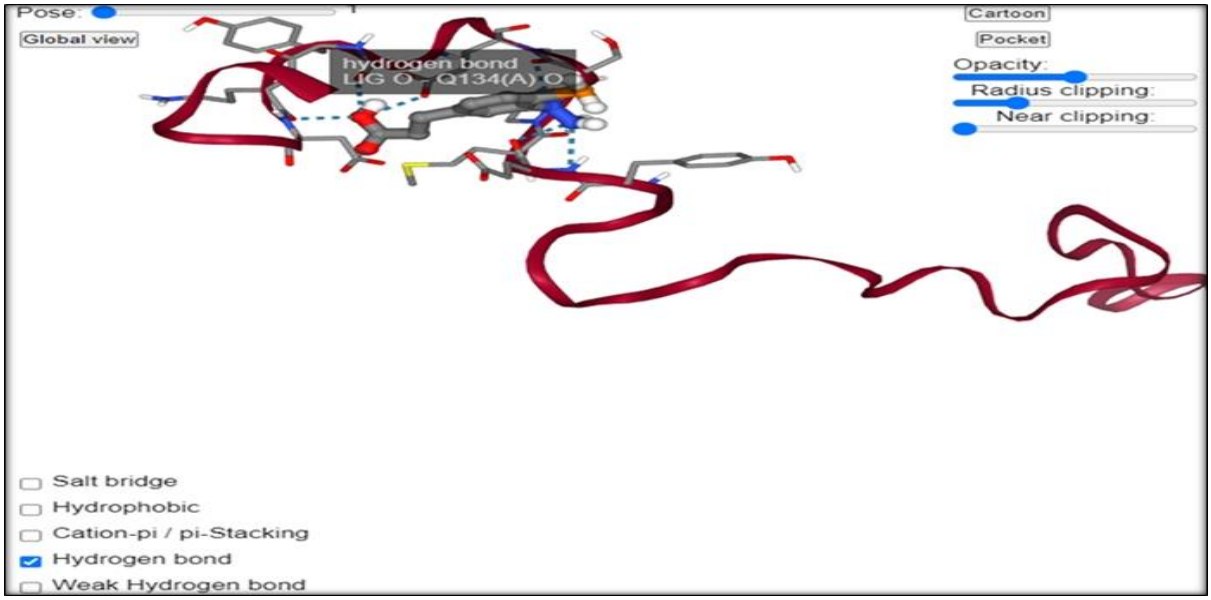

Figure 12: A hydrogen bond between both the target and compound2 using Seamdock tool.

The above Figure 12 shows different types of interactions, such as salt bridges, hydrophobic, cation-π, π stacking, and hydrogen bonds between proteins and ligands. These could be switched on/off from the check box. The bottom of the table shows detailed information for the selected interactions.

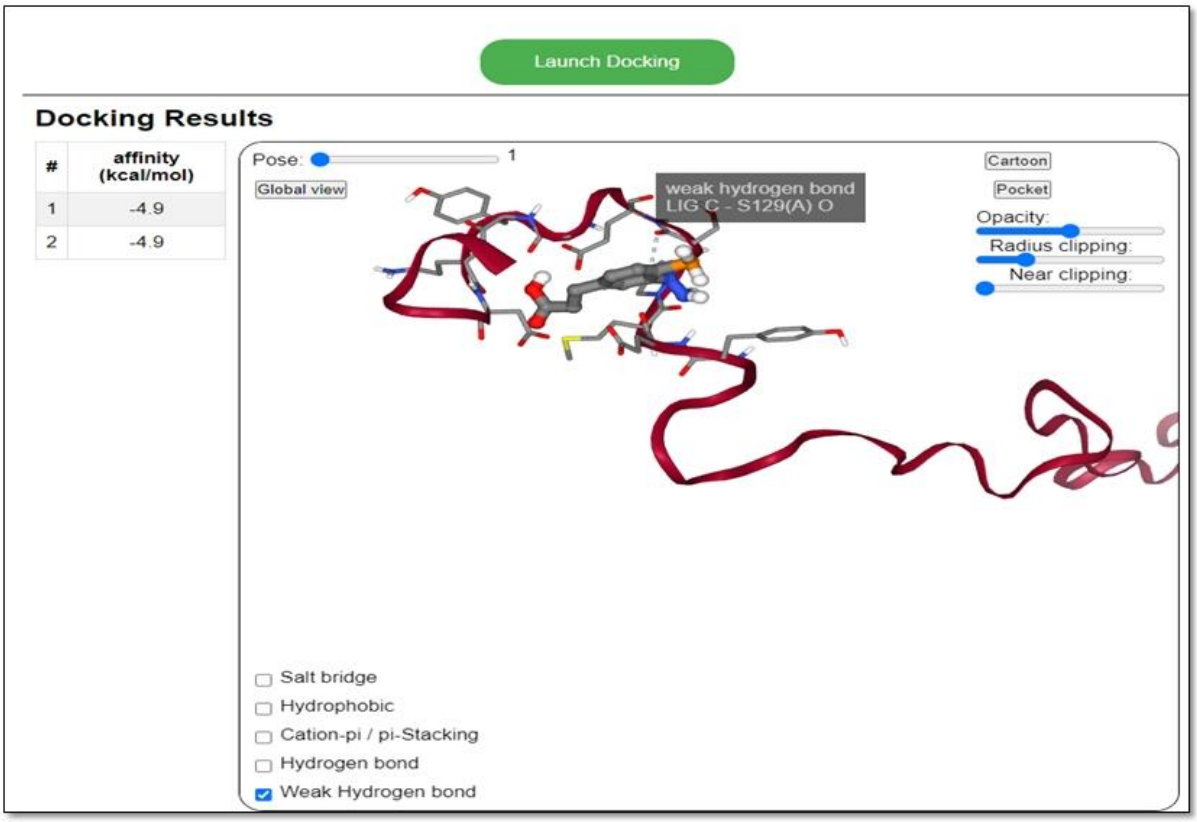

Figure 13: showing a weak hydrogen bond between both the target and compound2 using Seamdock tool.

Once the ligands are placed into the protein, the results are viewed to pick the best ones for further research . The strength of each ligand's binding is determined by the energy from the predicted interaction. Then, the ligands are sorted based on their calculated strength scores and above Figure 13 shows weak hydrogen bond between target and comound2.

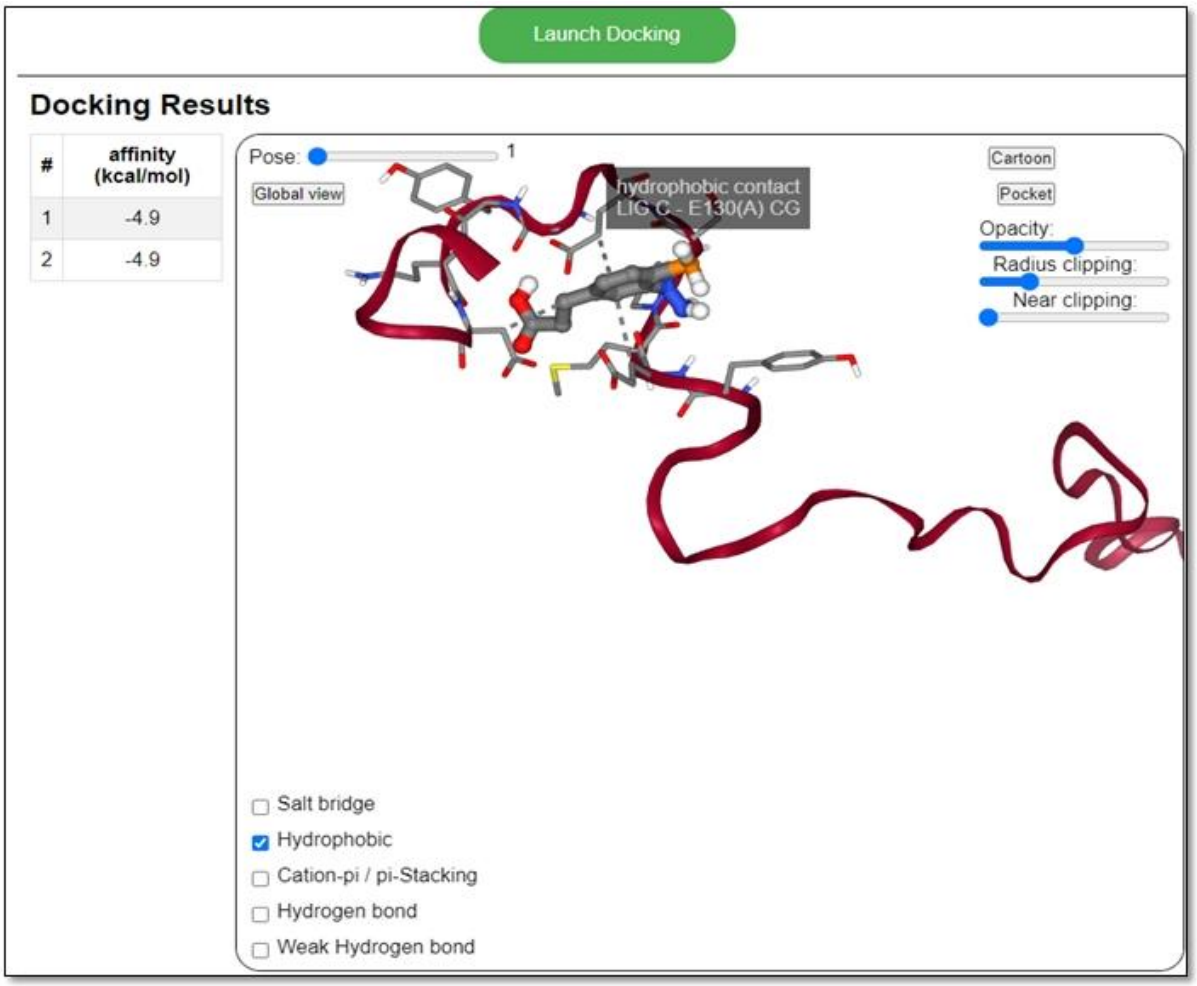

Figure 14: showing Hydrophobic interaction between both target and compound2 using Seamdock tool.

The interaction above the Figure 14 updates every time the docking pose is updated. Highlighting an interaction. One can highlight any of these interactions by either clicking on the corresponding row in the table. The highlighted contact shows up in magenta color. The text in the table for the clicked row will be changed to bold.

Besides, some important interactions, including hydrogen bonding, hydrophobicity, and electrostatic interactions and strong interactions between target and protein is given in Figure 15 and Figure 16, will be studied from the generated docked structures of both ligands with proteins. All these interactions give rich information about the action modes that can be accepted by a ligand and allow one to further optimize those ligand structures.

hydrophobic contact		hydrogen bond		weak hydrogen bond	
Ligand atom	Receptor	Ligand atom	Receptor	Ligand atom	Receptor
C	E126(A) CB	N	E126(A) O	C	S129(A) O
C	E130(A) CG	N	M127(A) O	N	S129(A) CA
C	D135(A) CB	N	P128(A) O		
		N	S129(A) O		
		O	E130(A) OE2		
		O	G132(A) O		
		O	Q134(A) O		
		O	D135(A) OD1		
		N	E126(A) N		

dock_log
dock.pdb

Figure 15: Show interaction information (hydrophobic interaction, hydrogen bond, and weak hydrogen bond) using Seamdock tool.

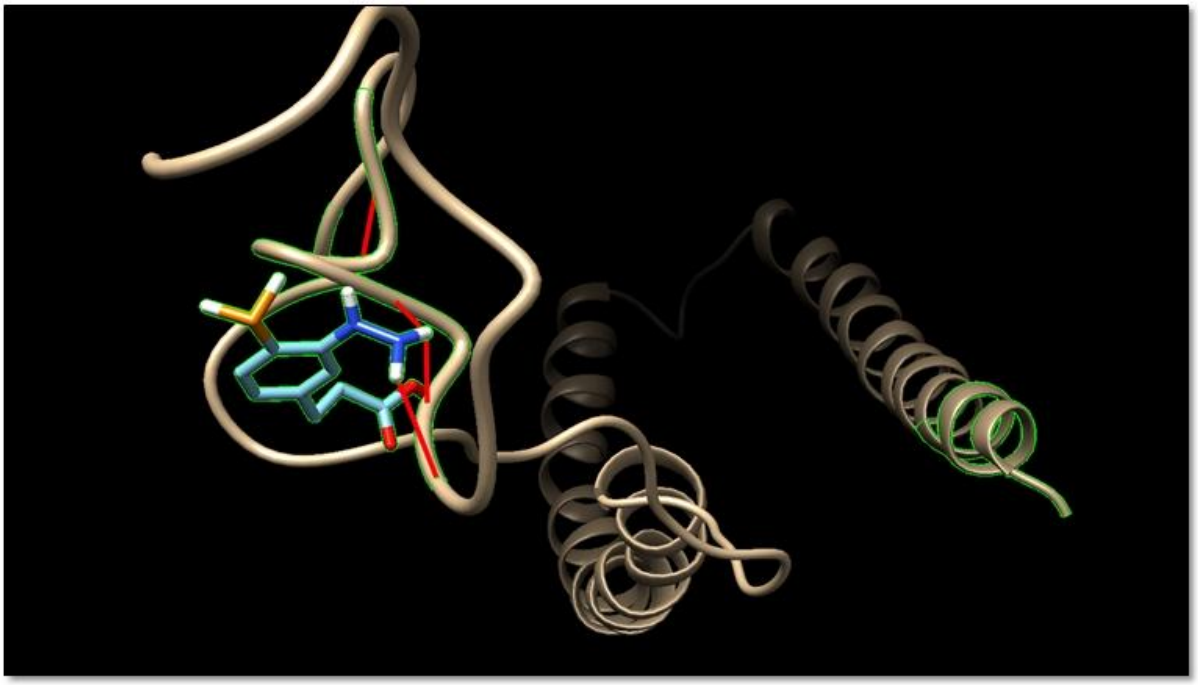

Figure 16: The image shows strong interaction between target and compound2 using Seamdock tool.

4. DISCUSSION

To rapidly address CADD (disease: a growing number of them), it is imperative to carry it out faster. The SNCA gene that encodes the protein alpha-synuclein plays a role in the pathogenesis of neurodegenerative diseases, particularly Parkinson's disease (PD). It should be stressed that alpha-synuclein is primarily expressed in the brain, where it plays roles in synaptic signaling and membrane trafficking. Although the definite physiologic role of the protein is unclear, the disturbance of this protein has been well-known in various neurodegenerative disorders. To such ends, they target alpha-synuclein structure-function interactions in their research to develop treatments. There is a need to keep using it to find effective treatments for patients suffering from Parkinson's disease, and drug development is the end target for the requirements of the patient and their life.

The CELLO tool predicts where, within the compartments of the cell, alpha-synuclein is found. The protein is found presynaptically in most cases, and when there is a defect in normalization, there are disturbances in the trafficking of cells, which is a typical neurodegenerative disease. Localizing the alpha-synuclein in the cell is important in determining its physiologic function and how the function is changed in pathologic conditions.

Another useful tool has been ProtParam, which is directed toward the study of alpha-synuclein's properties, such as molecular weight, pI, and stability. The polypeptide alpha-synuclein has a pI value of about 4.67, which means the protein is acidic and predominantly at a negative charge in physiologic conditions. Moreover, ProtParam advanced the predesigned instability index of α–synuclein and as it turned out, this protein is rather stable under most circumstances. Still, the diseased conditions and environmental factors may contribute to its misfolding and aggregation.

It is important to examine the functional properties of SNCA in different species as they have been acquired throughout evolution. Phylogenetic tools like MEGA11 allow for reconstructing phylogenetic trees that trace SNCA sequences among different species maternally and anthropoid primates. It is this likeness that suggests alpha-synuclein proteins are important to neurons in all species and, therefore, are good model organisms to study in mice and in non-primate animals.

SNCA studies reveal a new direction in developing compounds targeting alpha-synuclein to reduce aggregation. The research aims to cultivate the interactions between small molecules and alpha-

synuclein to explore the possibilities of future therapeutics. In this regard, studies have shown that such compounds possess a reasonably strong binding to alpha-synuclein and are potential candidates for preventing the toxin's oligomerization.

Thus, these compounds appear to be very desirable for use in developing new therapeutics aimed at, among others, controlling the progression of PD and AD. These are the pathologies where abnormal pathological features include the accumulation of misfolded proteins. Targeting alpha-synuclein and preventing or controlling its aggregation is a focus of treatment that can address the underlying pathology of these diseases, rather than treatments that only deal with symptoms. Bioinformatics tools research and molecular docking strategies are advancing, and this brings useful information for understanding alpha-synuclein dysfunction in neurodegenerative diseases and also for developing new therapies.

5. CONCLUSION

Finding compounsds that can protect the brain by sticking to a protein called alpha-synuclein might help create new treatments that stop this protein from forming harmful clumps, which can lead to Parkinson's disease. To make this process faster and cheaper, computer tools are very useful. These tools can help test many chemicals without doing expensive lab experiments. In this study, we looked at how many strong connections, called hydrogen bonds, different chemicals made with alpha-synuclein in three different ways could form.

From the docking study, it was found that Glycine amino acids showed the most prominent site for interaction. It works effectively against Alzheimer’s disease, which is responsible for increasing morbidity and mortality rates in today’s era. SwissDock requires more time for molecule docking and visualization using In-Silico tools UCSF Chimera. The SEAM docking is more efficient and easy implementation to perform the same tasks in a shorter time wich is also called time-consuming, which is beneficial for high-throughput screening of possible beneficial compounds.

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