Modelling and Docking Studies on Natural Compounds against Parkinson's Disease

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ABSTRACT

Parkinson's disease has a large clinical effect on patients, families, and parental figures through its dynamic degenerative effects on body movement and muscle control. The motor symptoms of PD are ascribed to the loss of striatal dopaminergic neurons, in spite of the fact that the presence of nonmotor symptoms bolsters neuronal loss in non-dopaminergic regions as well. The term parkinsonism is an indicator utilized to depict the motor features of PD, which incorporate resting tremor, bradykinesia, and muscular rigidity. Three dimensional structure of the protein Protein/nucleic acid deglycase DJ-1 was built using MODELLER 9.20 using the chain A structure of the peptide methionine sulfoxide reductase from Escherichia coli (PDB ID: 4E08_A) as template. The generated model was validated by using Ramachandran plot, which showed a model of good quality having 93.1% of amino acid residues in the most favoured region. Molecular docking studies also showed lower binding energy for all the compounds. Cinnamic acid exhibited the lowest binding energy of value -6.26 K.cal/mol while interacting with Arg98.

Key Words: Homology modelling, Parkinson's disease, Docking, Natural compounds.

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INTRODUCTION

Parkinson's disease is one of the most common neurological disorders that have been observed in humans. This disorder was first described in 1817 by Dr. James Parkinson. The characteristic feature of this particular condition is the loss of motor function leading to loss of control over the limbs [1].The cause for Parkinson's disease has been credited to a mixture of genetic and environmental factors such as family, heredity, age, pesticide exposure, etc. In light of such factors, disease prevalence is expected to shoot up as the population ages, leading to concerns regarding the present health care systems. Parkinson's disease is generally identified by comparing the presented symptoms with those of the Parkinsonism syndrome which include b radykinesia, tremors, gait impairment and involuntary limb movements [2]. As of now, there is no prescribed course of treatment for this disease. The analysis of Parkinson's disease is altogether clinical. It is characterized histologically as lost pigmented neurons in the substantia nigra, alongside trademark eosinophilic cytoplasmic considerations known as Lewy bodies. The pathologic neurochemical process is an inadequacy of striatal synapses, mostly dopamine, which must be diminished to 20% to 30% of its normal quantity before the clinical symptoms can be observed in the patients [3]. A major breakthrough in the understanding of Parkinson's disease came when dopamine deficiency was discovered in the corpus striatum and SN of brains taken from patients. The hypothesis that Parkinson's is a disease of dopamine loss led to the development of therapies aimed at correcting this deficiency. After some initial hiccups, the dopamine precursor levodopa proved to be a powerful treatment for this disease [4].

Out of the six genes unequivocally connected to genetic, monogenic - Parkinson's disease, mutations in Parkin (PARK2), PINK1 (PARK6), DJ-1 (PARK7), and ATP13A2 (PARK9) are responsible for Parkinson's disease that shows an autosomal recessive method of appearance [5]. The Parkinsonism-related protein DJ-1/Park7 is hypothized as a multifunctional oxidative stress reaction protein that is considered to be a member

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of the PfpI/Hsp31/DJ-1 superfamily, whose individuals have a preserved uncovered cysteine engaged with environmental stress resistance [6].

PARK7 encodes for DJ-1, a small protein of 189 amino acids (AA), which is not only completely expressed and localized to the cytoplasm but also found in the nucleus and associated with mitochondria [7]. It has been hypothized that DJ-1 secures neurons against oxidative stress by going about as a redox-dependent chaperone. The protein is ordinarily found in the cytoplasm and oxidative stress elevates migration to mitochondria where it defends against mitochondrial toxins. However, it remains to be seen as to how oxidation of Cys106 prompts antioxidative or potentially cytoprotective effects through conformational changes and how PD related DJ-1 mutations influence these procedures [8].

In the present study, in silico studies were performed due to the absence of crystal structure for Protein/nucleic acid deglycase DJ-1 protein. The homology model of the protein was developed using Modeller9.20 and validated by using Procheck. To study the binding affinity of protein-ligand and molecular interactions of Protein/nucleic acid deglycase DJ-1 docking studies were performed using autodock4.2.

METHODOLOGY

Sequence alignment and structure prediction

The amino acid sequence of Protein/nucleic acid deglycase DJ-1 (Uniprot accession number: Q99LX0) from the species Mus musculus (Mouse) was retrieved from the UniProtKB database [9]. A BLAST (Basic Local Alignment Search Tool) search was performed to select the template. the chain A structure of the peptide methionine sulfoxide reductase from *Escherichia coli* (PDB ID: 4E08_A) [10] was selected. The three dimensional structure was generated using Modeller9.20. The respective templates were retrieved from protein database like PDB [11]. When choosing the template, it is important to consider the sequence identity and resolution of the template. When both parameters are high the resulting model would be sufficiently good to allow structural and functional research.

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sp|Q99LX0|PARK7_MOUSE     --MASKRALVILAKGAEEMETVIPVDVMRRAGIKVTVAGLAGKDPVQCSRDVMICPDTSL 58
pdb|4E08|A                GSHMSKSALVILAPGAEEMEFIIAADVLRRAGIKVTVAGLNGGEAVKCSRDVQILPDTSL 60
                              ** ****** ****** :* .**:************ * : *:***** * *****

sp|Q99LX0|PARK7_MOUSE     EDAKTQGPYDVVVLPGGNLGAQNLSESPMVKEILKEQESRKGLIAAICAGPTALLAHEVG 118
pdb|4E08|A                AQVA-SDKFDVVVLPGGLGGSNAMGESSLVGDLLRSQESGGGLIAAICAAPTVLAKHGVA 119
                           :.  .. :********  *:: :.** :* ::*:.***  ********.**.*  * *.

sp|Q99LX0|PARK7_MOUSE     FGCKVTTHPLAKDKMMNGSHYSYSESRVEKDGLILTSRGPGTSFEFALAIVEALVGKDMA 178
pdb|4E08|A                SGKSLTSYPSMKPQLVNNYSYVD-DKTVVKDGNLITSRGPGTAYEFALKIAEELAGKEKV 178
                           * .:*::*  * :::*.  *   :. * *** ::*******::**** *.* *.**: .

sp|Q99LX0|PARK7_MOUSE     NQVKAPLVLKD- 189
pdb|4E08|A                QEVAKGLLVAYN 190
                          ::*   *::
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Fig. 1: Sequence alingnment of Protein/nucleic acid deglycase DJ-1 and template 4E08

MODELLER 9.20 was then used to generate satisfactory models; an automated approach to homology modelling by satisfaction of spatial restraints. Sequence alignments using the protein and template sequences was then carried out using platforms like ClustalX and ClustalW [12] (Figure 1). Homology models for the chosen protein were then constructed using modeller programs like Modeller 9.20 [13]. After manually modifying the alignment input file in MODELLER 9.20 to match the query and template sequence, 20 models were generated. The best model is determined by the lowest value of the objective function in MODELLER. The stereochemical quality of the given models was then evaluated using software like PROCHECK [14] and the model can be used for further structural or functional study. PROCHECK generated a Ramachandran plot which explains residue by residue listing that facilitates the in-depth calculation of Psi/Phi angles and the backbone conformation of the models. The RMSD (root mean square deviation) was calculated by superimposing (4E08_A) over the generated model to access the accuracy and reliability of the generated model by using SPDBV [15].

Docking methodology

Identification of active site pockets. The active site prediction was carried out using Tripo's Sybyl6.7 [16]. It showed two active site pockets. The amino acids in pocket one were Leu58, Ala61, Gly65, Tyr67, Leu7, Val70, Ile91, Glu94, Gln95 and Arg98. Amino acid

residues present in active site pocket two are Gly65, Tyr67, Leu7, Val70, Ile91, Glu94, Gln95 and Arg98

Twenty different plant secondary metabolites and four already existing drugs were selected for molecular docking with modelled protein. All the molecules were sketched in Sybyl 6.7 and minimized by adding Gasteiger-Huckel charges and saved in .mol2 format. Molecular docking studies were performed on all the natural compounds separately by using AutoDock4.2 [17] program, using the Lamarckian Genetic Algorithm (LGA) and empirical free energy function was implemented. Initially, the modelled mitochondrial peptide methionine sulfoxide reductase protein was loaded and hydrogens were added before saving it in PDBQT format. Later the ligand was loaded and conformations were set and saved in PDBQT format. The grid parameters were selected and calculated using AutoGrid. For all the dockings, a grid-point spacing of 0.375 Å was applied and grid map with 60×60×60 points were used. X, Y, Z (27.126, -18.024, 28.939) coordinates were selected on the basis of the amino acids present in the active site predicted in sybyl6.7 biopolymer module. Default parameters were used to run the Autodock.

RESULTS AND DISCUSSION:

Homology modelling and model evaluation:

The present study reports that the template protein (PDB ID: 4E08_A) having high degree of homology with Q99LX0 protein was used as a template with good atomic resolution of its crystal structure. The target sequence of Protein/nucleic acid deglycase DJ-1 (uniprot accession number: Q99LX0_Mouse) bearing 189 amino acid residues was retrieved from the uniprot protein sequence database with accession no. Q99LX0. Using BLAST, PDB ID 4E08_A was identified and selected as template to predict the model. The structure was modelled using Modeller9.20. The generated structure was validated using the protein structure and by PROCHECK. The generated model showed 93.1% of amino acid residues in core region with 149 amino acids, 5.6% of amino acid residues in additionally allowed region having 9 amino acids, 1.3% of the amino acid residues in the generously allowed region with 2 amino acids and there are no amino acids present in disallowed region. The template PDB shows 94.6% (295 aa) of amino acids in core region, 4.8% (15 aa) of the amino acid residues in additionally allowed region, and there are 2 amino acid residues (0.6%) in disallowed region. Cartoon model of secondary structure of the modelled protein is shown in figure 2 and Ramachandran plot is shown in figure 3. RMSD was calculated for template and generated model by using SPDBV. Both the models were loaded and superimposed using the alpha carbon and RMSD was calculated (Figure 4). It showed RMSD of 0.32Å, which indicates that the generated model shows similarity to the template.

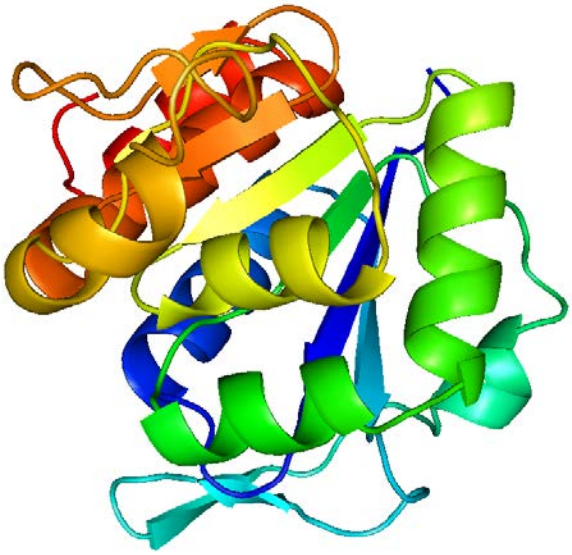

Fig. 2: The cartoon model of Protein/nucleic acid deglycase DJ-1 protein

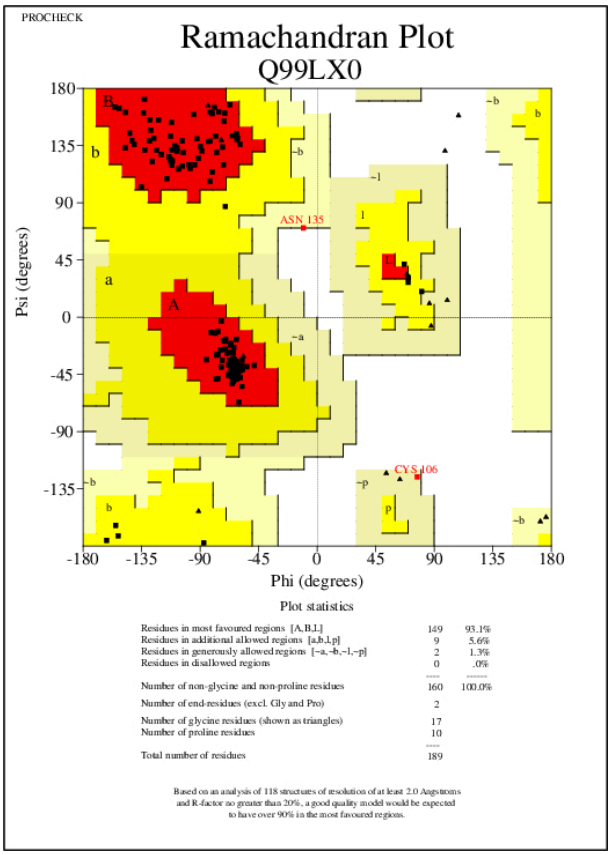

Fig. 3: Ramachandran plot of the modelled Protein/nucleic acid deglycase DJ-1 protein exhibited 93.1% amino acid residues in most favored region.

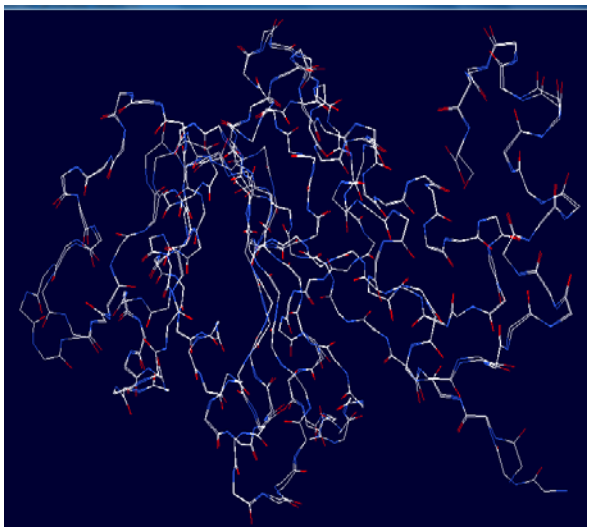

Fig. 4: superimposed model of modelled Protein/nucleic acid deglycase DJ-1 protein and template protein

Molecular docking results

Molecular docking is the most extensively used method for the calculation of protein-ligand interactions. It is an efficient method to predict the potential ligand interactions. In the present study, the native plant secondary metabolites (ligands) have been identified as potent protein/nucleic acid deglycase DJ-1 inhibitors. AutoDock4.2 uses (genetic algorithm) binding free energy assessment to assign the best binding conformation**.** Further, the activity of docked ligand molecules was compared to that of standard drugs which were controls. In total, twenty natural compounds were docked against modelled protein/nucleic acid deglycase DJ-1 protein.

However, the compounds cinnamic acid and eugenin showed better interactions and lower free energy values, indicating more thermodynamically favoured interactions. Both the compounds exhibited binding energy of less than -6.26 Kcal/mol and -6.17 Kcal/mol respectively. Specifically, cinnamic acid exhibited the highest binding energy of value -6.26 Kcal/mol while interacting with Arg98 and eugenin exhibited binding energy of -6.17 Kcal/mol while interacting with Gln94. When compared to the standard drugs (i.e., melevodopa, istradefylline and safinamide) cinnamic acid exhibited highest binding energy. Sulfinamide exhibited binding energy of -4.73 Kcal/mol while interacting with Gly100. All the compounds showed good binding energy with modelled protein. The natural compounds with their corresponding interactions and binding energies are shown in Table 1 and figure 5.

Table 1: Binding energy, dissociation constant and interacting amino acids of twenty natural compounds and four existing drugs.

S.No	Compound name	Interacting amino acids	Binding energy ΔG (Kcal/Mol)	Dissociation Constant (kI) (μM)
1	Caffeic acid	Lys62	-6.10	34.04
2	Cinnamic acid	Arg98	-6.26	25.62
3	Eugenin	Glu94	-6.17	29.89
4	Eugenol	Lys62	-5.13	174.16
5	Ferulic acid	Gly65	-5.17	160.99
6	Gallic acid	Glu94	-5.35	120.34
7	Kaempferol	Ala71, Tyr67	-4.74	332.99
8	Naringenin	Ala2, Arg98, Asp68	-5.17	162.36
9	Quercetin	Lys4, Asp68	-4.20	831.74
10	Rhamnetin	Arg5, Tyr67, Gln64	-4.97	226.45
11	Vanillic acid	Gly65	-5.31	127.18
12	3,6-O dimethoxy-5,7,3',4'-tetrahydroxy-flavone	Arg5, Tyr67, Gln64	-4.60	428.03
13	7,4'-O-dimethoxy-5-hydroxy-flavone	Arg5	-4.99	219.22
14	7,4'-O-dimethoxy flavone	Arg5	-5.23	147.45
15	7-O-methoxy-4',5-hydroxy-flavone	Arg98,Ala171	-5.35	118.91
16	7-O-methylnaringenin	Arg98,Ala171	-5.54	86.98
17	3',4',5-trihydroxy-7-methoxy-6,8-C-dimethyl-flavone	Arg5,Arg98,Ala2,Asp68	-4.59	428.88

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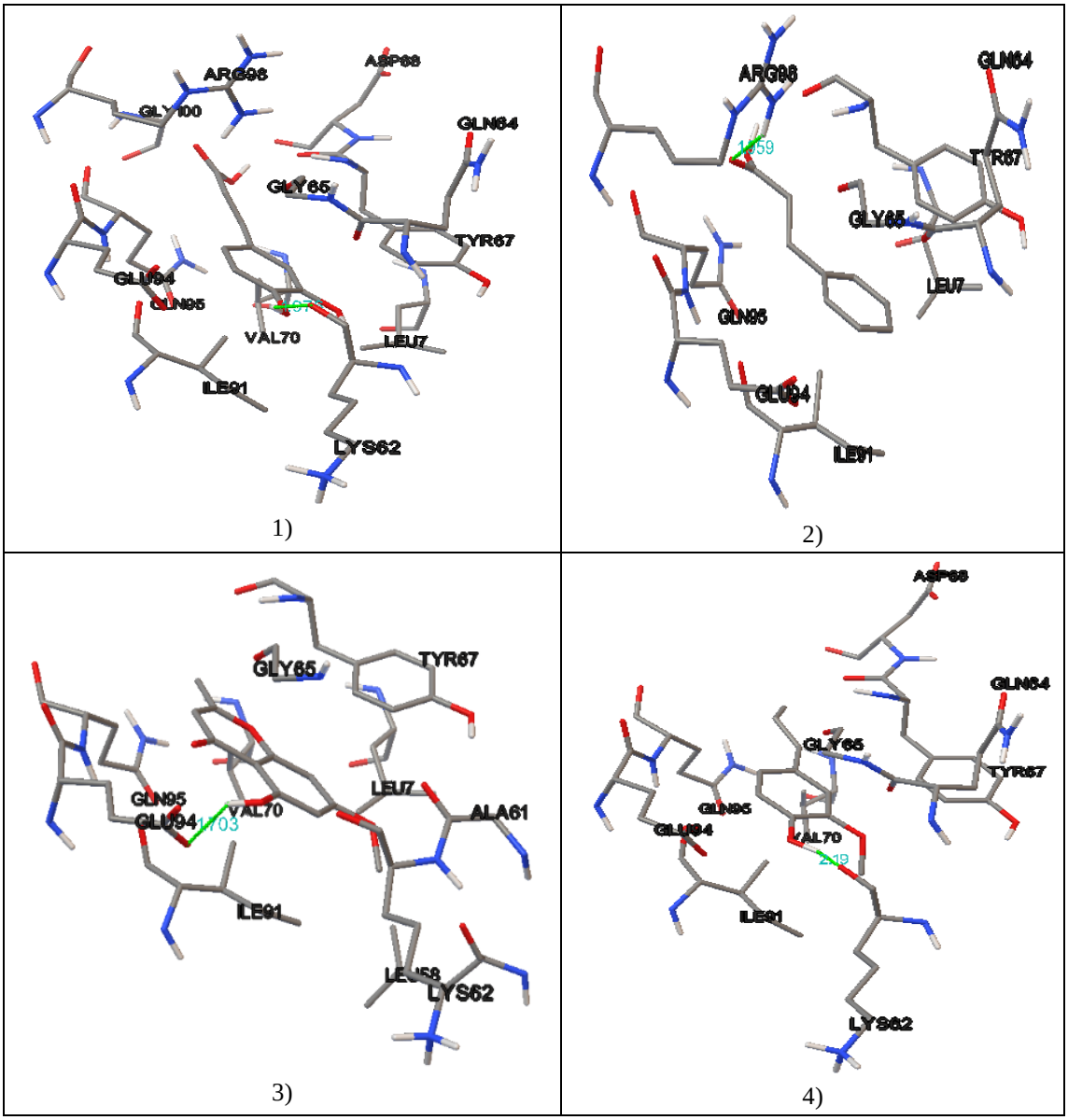
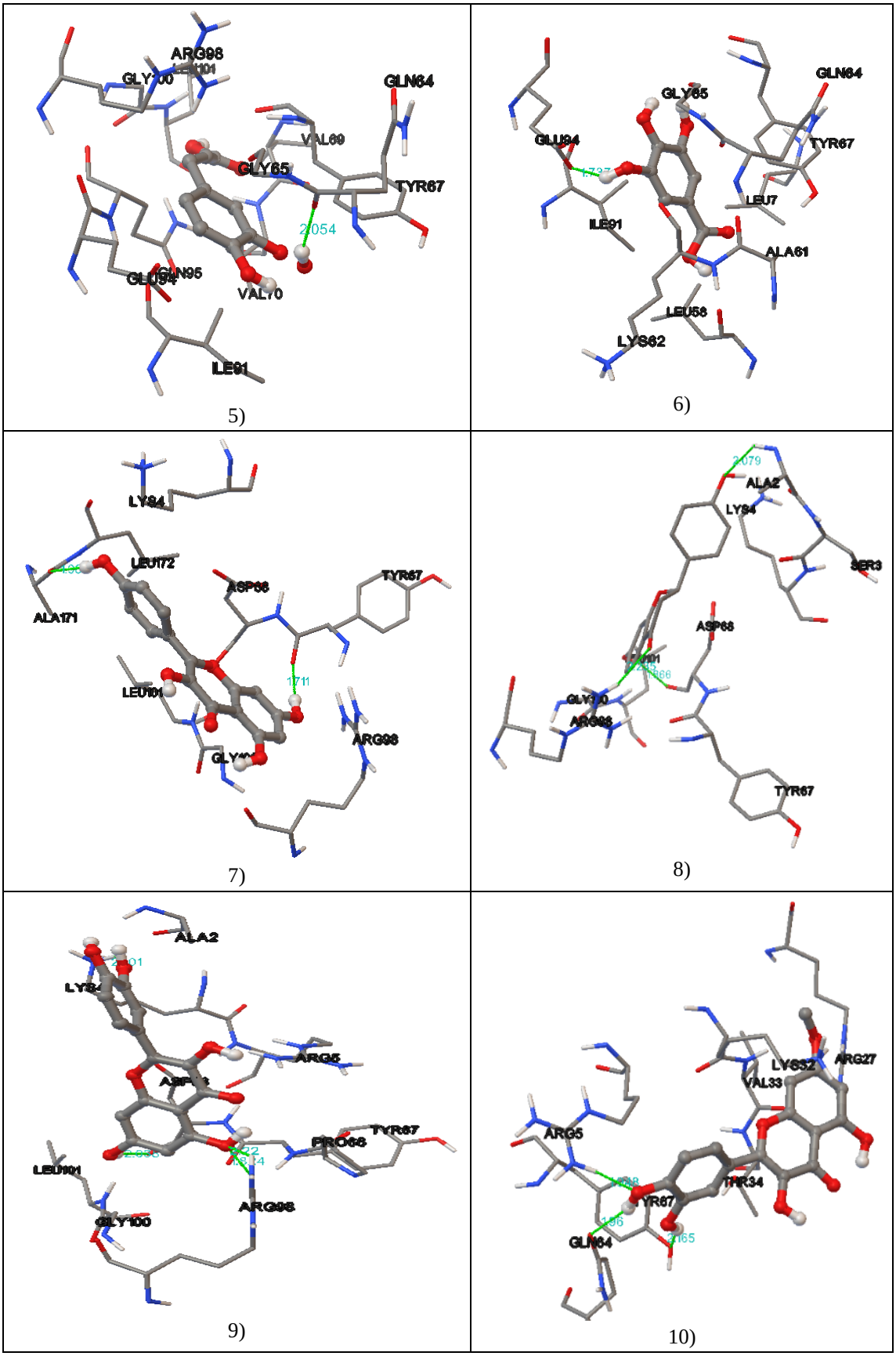
18	3,5,7-hydroxy-3,4'-methoxy 6-8 methyl flavone	Arg98 (2), Ala2, Asp68	-5.57	83.03
19	4',5,7-trihydroxy 6,8-C-dimethylflavone	Ala2, Arg98 (2), Ser3,Asp68	-5.46	99.34
20	8-formyl-3',5,7-trimethoxy-6-C-methylflavanone	Val33	-5.06	193.92
21	Melevodopa	Arg98(2)	-3.63	2.17
22	Istradefylline	Val33, Arg27	-4.41	586.99
23	Safinamide	Gly100	-4.73	338.39
24	2-Butyl-9-methyl-8-(triazol-2-yl)purin-6-amine	Arg98	-4.89	259.90




ARG98
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2054
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VAL70
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ILE91
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LEU58
LYS62
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1711
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ARG98
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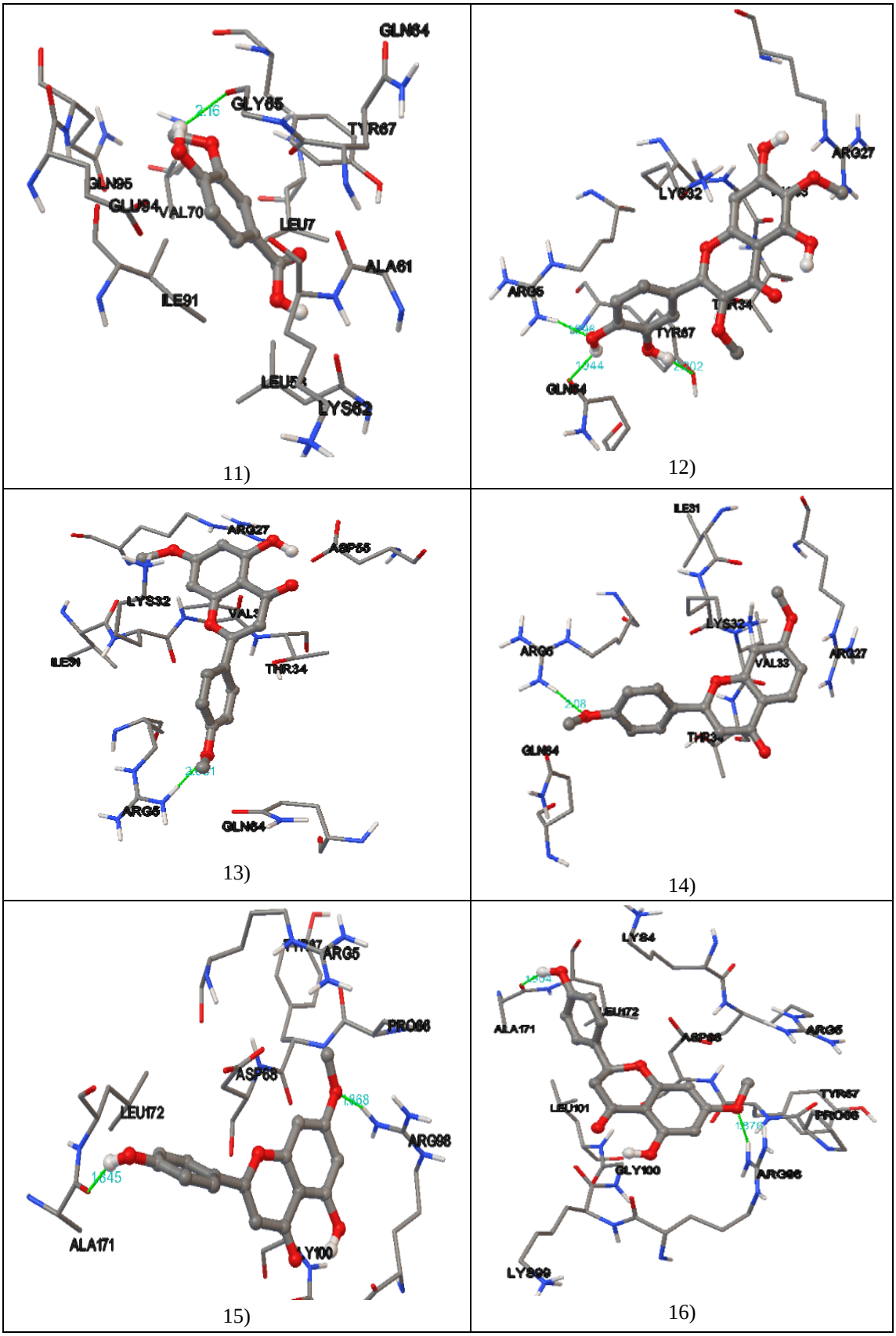

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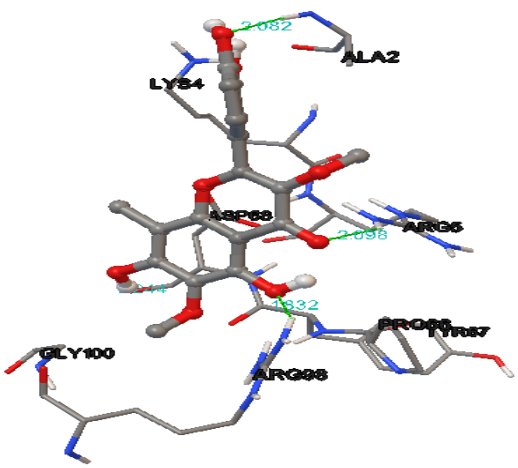

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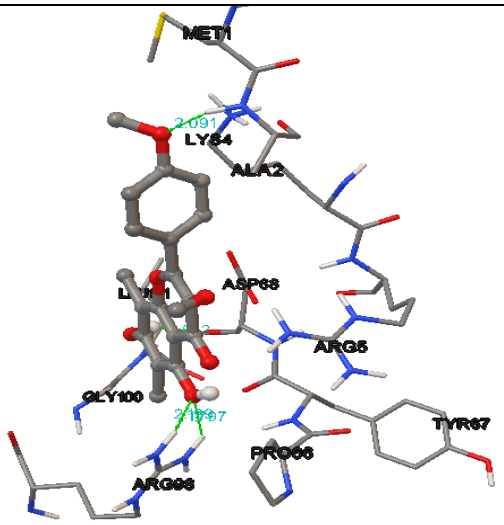

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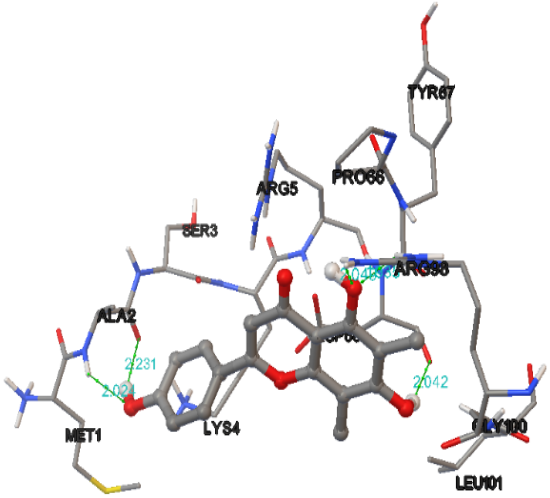

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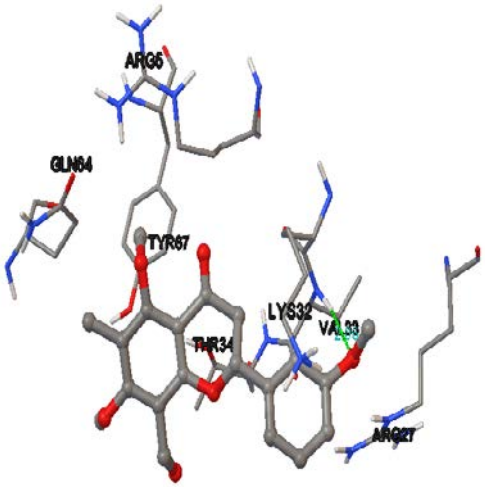

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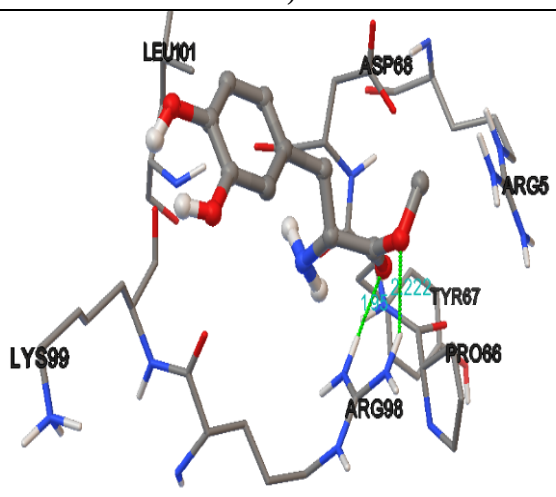

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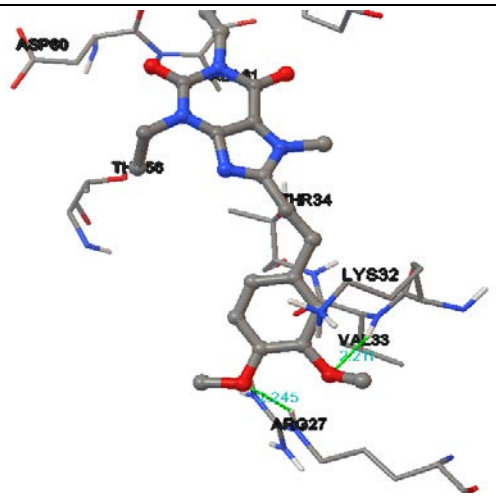

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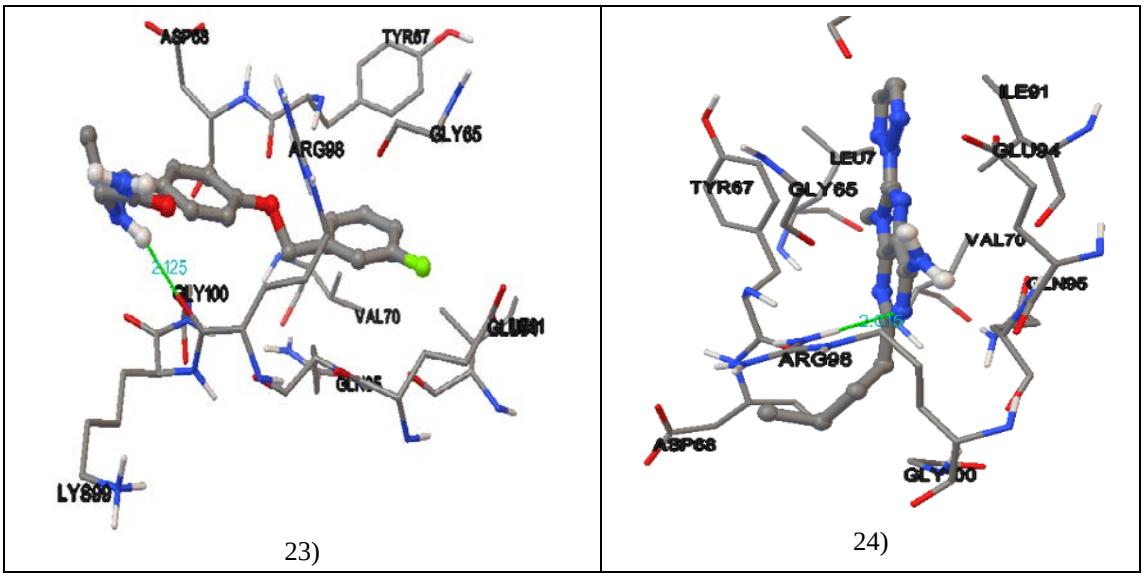

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Fig. 5: Molecular docking interactions of modelled protein with twenty natural compounds and four controls.

CONCLUSION

The sequence obtained from uniprot does not contain the crystal structure (3D structure) in the PDB database. The crystal structure was built by homology modeling using MODELLER 9.20. Modelled protein was validated by using procheck. The generated model showed 93.1% of amino acid residues in the most favored region. The generated model was then docked with twenty natural compounds and also docked already existing drugs as controls. Natural compounds showed better binding energies than already existing drugs. Cinnamic acid exhibited highest binging energy of -6.26 Kcal/mol with interacting Arg98. The study explains natural compounds are more potent than already existing drugs for Parkinson's disease.

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