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ANALYSIS OF TUBULIN BETA-4A CHAIN ROLE IN CEREBRAL ATROPHY: AN IN SILICO STUDY

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ABSTRACT: Cerebral Atrophy commonly occurs in most of the neurodegenerative diseases. When there is attenuation in the size of the brain cells which may be caused due to progressive loss of cytoplasmic proteins in the brain tissue. The primary effect of atrophy is the loss of neurons and connection between them. Atrophy can be general, which means that it has affected brain tissues in the entire brain; or it can be focal, affecting a particular area of the brain causing diminution in the functions controlled by that area of the brain. Naturally active compounds from various plants have been utilized to treat different diseases. In the present study, tubulin isoform tubulin beta class IVA protein sequence from Homo sapiens was taken from uniprot. The homology model was developed by using Modeller 9.21 version. The structure of the peptide *GTP-Tubulin in complex with a DARPIN from Ovis aries* (PDB id: 4DRX) used as a template. Molecular docking studies were performed using Autodock4.2. Twenty natural compounds were docked against modelled protein. Every one of the compounds showed great binding energy. 1,7-bis-(4-hydroxyphenyl)-1,4,6-heptatrien-3-one showed with lesser energy of -11.66 Kcal/mol.

KEYWORDS: Homology modelling, Docking, TUBB4A, Natural compounds.

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1.INTRODUCTION

Neurodegenerative Disorders are pathologically caused by accumulation of abnormal proteins in the brain. The most common proteins are β-amyloid [1], the microtubule-associated protein tau [2], the synaptic vesicle protein α-synuclein [3], and the proteasomal protein ubiquitin [4]. The protein TDP-43 has also recently been reported in association with ubiquitin inclusions however its specificity needs to be confirmed [5]. Cerebral Atrophy commonly occurs in most of the

neurodegenerative diseases. Tissues atrophy when there is a decrease in the size of the brain cells which may be caused due to progressive loss of cytoplasmic proteins in the brain tissue [6]. The primary effect of atrophy is the loss of neurons and connection between them. Atrophy can be in general, which means that it has affected brain tissues in the entire brain; or it can be focal, affecting a particular area of the brain causing diminution in the functions controlled by that area of the brain [7]. Leukodystrophies are conditions that include variations of the nerve sensory system's white issue, which comprises of nerve fibers secured by a fatty substance called myelin [8]. Myelin protects nerve filaments and advances the fast transmission of nerve impulses. In particular, TUBB4A-related leukodystrophy involves hypomyelination, which means that the nervous system has a reduced ability to form myelin. In some affected individuals, myelin may also break down, which is known as demyelination [9]. A recent study of heterozygous mutations of TUBB4A (encoding the Tubulin Beta Class IVA isoform: Tubb4a) in individuals with hypomyelination point out that other CNS cell types besides oligodendrocytes may play a role in leukodystrophies. More than 30 heterozygous pathogenic mutations have been recorded in TUBB4A that can result in a broad phenotypic spectrum including primary dystonia (DYT4-OMIM#128101) [10,11] spastic diplegia [12–14] infantile encephalopathy [15,16] isolated hypomyelination, [17] and hypomyelination with atrophy of basal ganglia and cerebellum (H-ABC-OMIM #612438)) [5,10,11,13]. At the most severe end of TUBB4A related leukodystrophy is the condition which is called hypomyelination with atrophy of the basal ganglia and cerebellum (H-ABC). Most affected individuals have delayed development of the motor skills or in other cases, the motor skills develop but are lost in early childhood. In addition, individuals with H-ABC also have other movement abnormalities, such as dystonia, choreoathetosis, muscle rigidity, and ataxia. These individuals also often suffer from dysarthria, dysphonia, and dysphagia. Some also develop seizures. In addition the tissues in some parts of the brain atrophies, most commonly in the region called putamen. Atrophy also occurs in the regions of cerebellum and cerebrum which causes neurological deficiencies [9]. In the present study, in silico studies were performed due to the absence of crystal structure for GTP-Tubulin in complex with a DARPIN protein. The homology model of the protein was established using Modeller 9.21 [18] and confirmed by using Procheck [19]. Protein-Ligand binding energies and molecular interactions of tubulin isoform tubulin beta class IVA (Tubb4a) were studied by performing docking studies using autodock4.2 [20].

2. MATERIALS AND METHODS

Sequence alignment and structure prediction

The amino acid sequence of Tubulin beta-4A (chain) (Having Uniprot accession number: P04350) from the species Homo sapiens was retrieved from the UniProtKB database [21]. Template selection was done after performing a BLAST (Basic Local Alignment Search Tool) search. The

Chain A, Structure of the Peptide GTP-Tubulin in complex with a DARPIN from Ovis aries (PDB ID: 4DRX_A) [22] was selected on the basis **E-value, identity, positives**. The three dimensional structure was generated using Modeller 9.21. The respective templates were retrieved from protein database like PDB [23]. 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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CLUSTAL O(1.2.4) multiple sequence alignment


sp|P04350|TBB4A_HUMAN      MREIVHLQAGQCGNQIGAKFWEVISDEHGIDPTGTYHGDSDLQ--LERINVYYNEATGGN 58
pdb|4DRX|A                 MRECISIHVGQAGVQIGNACWELYCLEHGIQPDGQMPSDKTIGGGDDSFNTFFSETGAGK 60
                           *** : ::.**.* ***   **: . ****:* *   .*. :    : :*.::.*: .*:

sp|P04350|TBB4A_HUMAN      YVPRAVLVDLEPGTMDSVRSGPFGQIFRPDNFVFGQSGAGNNWAKGHYTEGAELVDAVLD 118
pdb|4DRX|A                 HVPRAVFVDLEPTVIDEVRTGTYRQLFHPEQLITGKEDAANNYARGHYTIGKEIIDLVLD 120
                           :*****:***** .:*.**:* : *:*:*:::: *:..*.**:*:**** * *::* ***

sp|P04350|TBB4A_HUMAN      VVRKEAESCDCLQGFQLTHSLGGGTGSGMGTLLISKIREEFPDRIMNTFSVVPSPKVSDT 178
pdb|4DRX|A                 RIRKLADQCTGLQGFLVFHSFGGGTGSGFTSLLMERLSVDYGKKSKLEFSIYPAPQVSTA 180
                            :** *:.*  **** : **:*******: :**:.::  :: .:    **: *:*:** :

sp|P04350|TBB4A_HUMAN      VVEPYNATLSVHQLVENTDETYCIDNEALYDICFRTLKLTTPTYGDLNHLVSATMSGVTT 238
pdb|4DRX|A                 VVEPYNSILTTHTTLEHSDCAFMVDNEAIYDICRRNLDIERPTYTNLNRLISQIVSSITA 240
                           ******: *:.*  :*::* :: :****:**** *.*.:  *** :**:*:*  :*.:*:

sp|P04350|TBB4A_HUMAN      CLRFPGQLNADLRKLAVNMVPFPRLHFFMPGFAPLTSRGSQQYRALTVPELTQQMFDAKN 298
pdb|4DRX|A                 SLRFDGALNVDLTEFQTNLVPYPRIHFPLATYAPVISAEKAYHEQLSVAEITNACFEPAN 300
                           .*** * **.** :: .*:**:**:** :  :**: *  .  :. *:* *:*:  *:  *

sp|P04350|TBB4A_HUMAN      MMAACDPRHGRYLTVAAVFRGRMSMKEVDEQMLSVQSKNSSYFVEWIPNNVKTAVCDIPP 358
pdb|4DRX|A                 QMVKCDPRHGKYMACCLLYRGDVVPKDVNAAIATIKTKRSIQFVDWCPTGFKVGINYQPP 360
                            *. ******:*:: . ::** :  *:*:  : ::::*.*  **:* *...*..:   **

sp|P04350|TBB4A_HUMAN      RGL--------KMAATFIGNSTAIQELFKRISEQFTAMFRRKAFLHWYTGEGMDEMEFTE 410
pdb|4DRX|A                 TVVPGGDLAKVQRAVCMLSNTTAIAEAWARLDHKFDLMYAKRAFVHWYVGEGMEEGEFSE 420
                             :        : *. ::.*:*** * : *:..:*  *: ::**:***.****:* **:*

sp|P04350|TBB4A_HUMAN      AESNMNDLVSEYQQYQDATAEEGEFEEEAEEEVAaa 446
pdb|4DRX|A                 AREDMAALEKDYEEVGV------------------- 437
                           *..:*  * .:*::
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Figure 1: Sequence alingnment of Tubulin beta-4A (chain) protein and template 4DRX

MODELLER 9.21 was then used to generate reasonable models; an automated approach to homology modelling by satisfaction of spatial restraints. ClustalX and ClustalW [24] platforms were used to get the sequence alignment with protein and template sequences (Figure 1). Modeller program: Modeller 9.21 was used to construct the homology model of the selected protein [25]. The alignment input files were manually modified in MODELLER 9.21 to match the query and template sequence and 20 models were generated. Modeller Objective Function is studied out of which the best model is selected on the basis of the lowest value. PROCHECK software was used to evaluate the stereo chemical quality of the given model which can be used for further [26]. Ramachandran plot was generated by PROCHECK which explains 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 (4DRX_A) over the generated model to access the accuracy and reliability of the generated model by using SPDBV [27].

Docking methodology

Identification of active site pockets: The active site prediction was carried out using Tripo's Sybyl 6.7 [28]. Three active site pockets were found. The amino acids in pocket one were Ser138, Gln15, Ile16, Val169, Pro171, Ser172, Val175, Ile202, Asn204, Leu207, Tyr222, Leu225, Asn226, Val229, Thr232, Met233, Gly235, Val236, Cys239, Leu246, Asn247, Leu253, Pro268, Met300, Val316, Ala352, Thr366, Asp67 and Cys12.

A total of twenty natural compounds were chosen from NCBI. Sybyl 6.7 was used to sketch the molecules and they were minimized by adding Gasteiger-Huckel charges and saved in mol2 format. AutoDock4.2 software was used to perform molecular docking studies on all the natural compounds separately using Lamarckian Genetic Algorithm (LGA) and empirical free energy function was also implemented[29].The modelled of Tubulin beta-4A (chain) protein was loaded and hydrogens were added before saving it in PDBQT format. The ligands were then loaded and conformations were set and it was 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 Coordinates were selected on the basis of the amino acids present in the active site predicted in sybyl 6.7 biopolymer module. Default parameters were used to run the AutoDock.

3. RESULTS AND DISCUSSION

Homology modelling and model evaluation

The current study reports that the template protein (PDB ID: 4DRX _A) having high degree of homology with P04350 protein, was used as a template and it had a good atomic resolution of its crystal structure. The target sequence of Tubulin beta-4A (chain) (uniprot accession number: P04350_Human) bearing 444 amino acid residues was collected from the uniprot protein sequence database having Accession No. P04350. The target protein was run against the pdb database in protein BLAST and the template 4DRX_A was identified and selected. The template 4DRX_A was selected on the basis of its identity percentage which was 41%. Modeller9.20 was used for structure modelling. By using protein structure and PROCHECK, the generated structure was substantiated. The generated mode showed 92.8% of amino acid residues in core region with 361 amino acids, 7.2% of amino acid residues in additionally allowed region having 28 amino acids, with no amino acids present in generously allowed region and disallowed region. The template PDB shows 89.0% of amino acids in the core region, 10.7% of amino acid residues in the additionally allowed region, 0.3% of amino acids in the generously allowed region and no amino acid residues in the disallowed region. Figure.2 shows the cartoon model of secondary structure of the modelled protein and figure.4 shows the image of the Ramachandran plot. RMSD was calculated for template and generated model by using SPDBV [30]. PDB ID of both template and query were loaded and superimposed using the alpha carbon and RMSD was calculated. It showed

RMSD of 1.61Å, which indicates that the generated model shows similarity to the template (Figure 3)

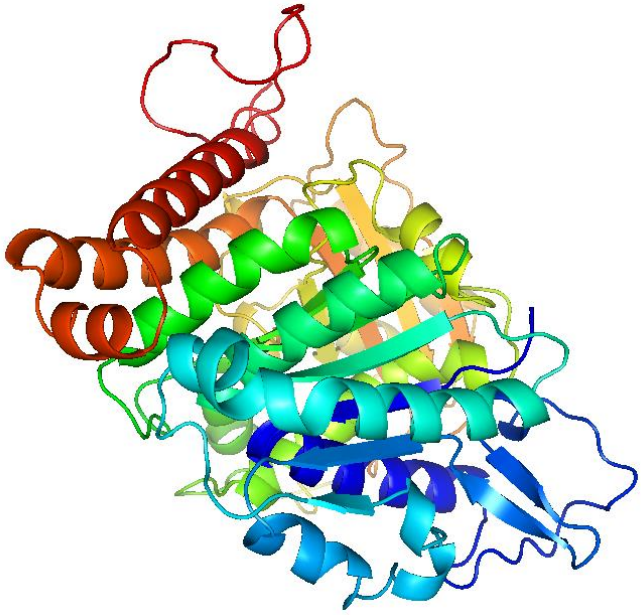


Figure 2 : The cartoon model of Tubulin beta-4A (chain) modelled protein

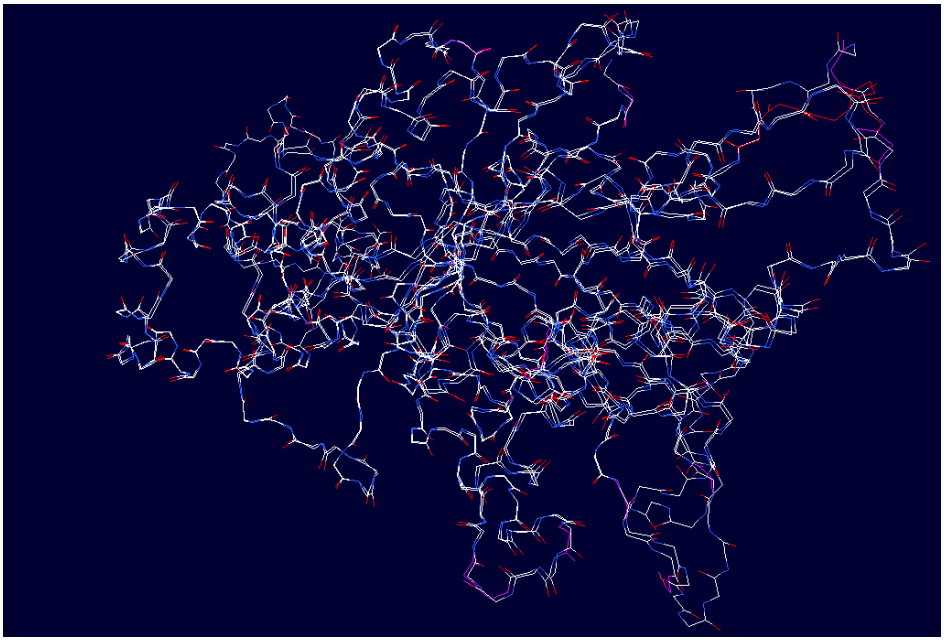


Figure 3: superimposed model of modelled Tubulin beta-4A (chain) protein and template protein

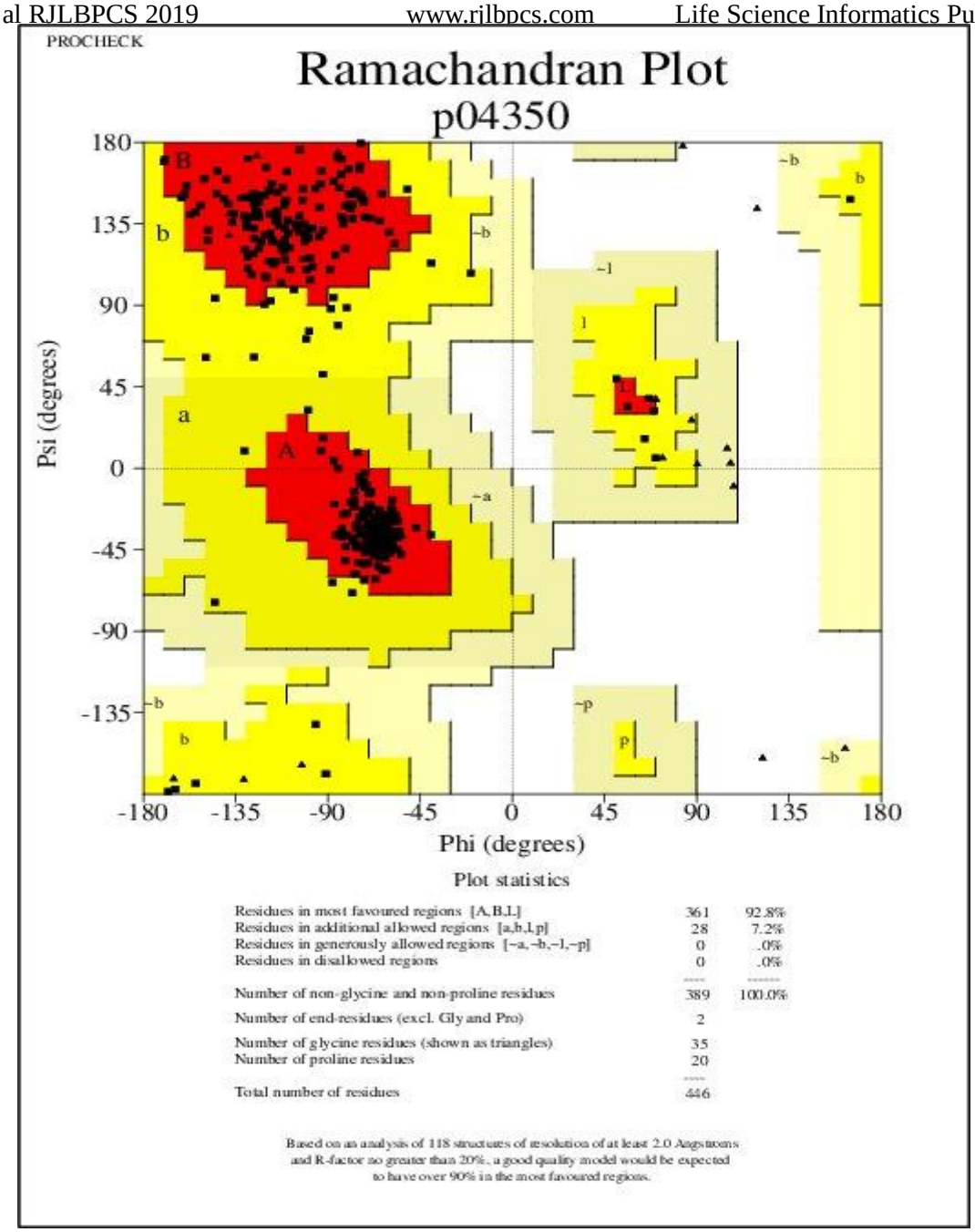


Figure 4: Ramachandran plot of the modelled Tubulin beta-4A (chain) protein exhibited 92.8% amino acid residues in most favored region.

Molecular docking results

The most extensively used method for the calculation of protein-ligand interactions is Molecular docking. It is an efficient method to predict the potential ligand interactions. The present study uses secondary metabolites (ligands) of native plants which have been identified as potent Tubulin beta-4A(chain) inhibitors. The best binding conformation is assigned by the binding free energy assessment through AutoDock4.2 which uses genetic algorithm. Standard drugs were used as controls which were used to compare the activity of docked ligand molecules. In total, twenty natural compounds were docked against modelled Tubulin beta-4A (chain). However, the compounds 1,7-bis-(4-hydroxyphenyl)-1,4,6-heptatrien-3-one and Piperine showed better

interactions and lower free energy values, indicating more thermodynamically favored interactions. Both the compounds exhibited binding energy of less than -11.0 Kcal/mol. Specifically, 1,7-bis-(4-hydroxyphenyl)-1,4,6-heptatrien-3-one exhibited the highest binding energy of value -11.66 K.cal/mol while interacting with Asn247 and Piperine exhibited binding energy of -11.34 K.cal/mol with interacting Ile202 and Ile368. When compared to the standard drugs i.e., (Tolcapone, Diacomit, Xagol, Rytary) 1,7-bis-(4-hydroxyphenyl)-1,4,6-heptatrien-3-one exhibited highest binding energy. Piperine exhibited binding energy of -11.34 Kcal/mol while interacting with Ile202 and Ile368. The selected compounds showed good binding energy with modelled protein. Two compounds exhibited binding energy less than -10.00 Kcal/mol, five compounds exhibited binding energy of less than -8.00 KCal/mol. Table 1 and figure 5 shows interactions and binding energies of the query protein with their corresponding natural compounds. Table 2 and figure 6 shows interactions and binding energies of the query protein with standard drugs that are taken as a control measure.

Docking Results Table

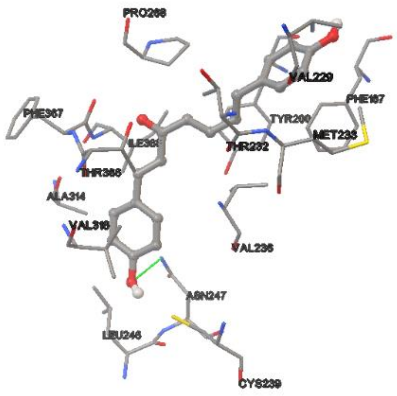
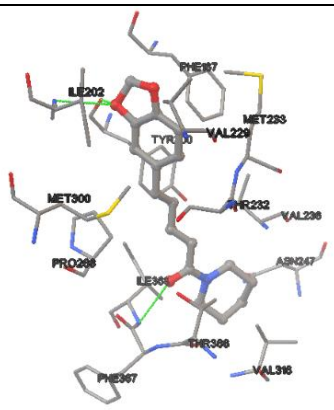
Table 1: Interactions and binding energies of the query protein with their corresponding natural compounds

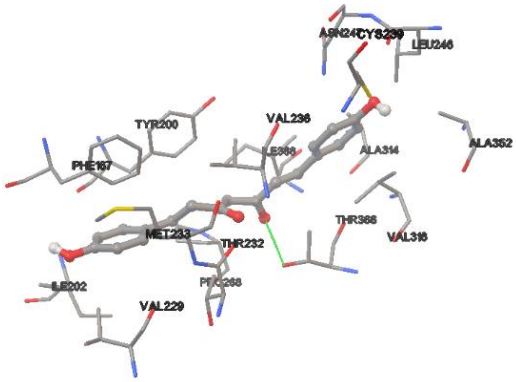
S.No	Compound Name	Interacting Amino Acids	Binding Energy	Dissociation Constant
1	1,7-bis-(4-hydroxyphenyl)-1,4,6-heptatrien-3-one	Asn247	-11.66	2.85nM
2	Piperine	Ile202, Ile368	-11.34	4.87nM
3	1,7-bis(4-hydroxyphenyl)-1-heptene-3,5-dione	Thr366	-10.87	10.76nM
4	Curcumin	Cys239	-10.18	34.64nM
5	Bisdemethoxycurcumin	Ile368, Val236	-10.81	1.97nM
6	2,5-bis(4-hydroxy-3-methoxy benzylidene) cyclopentanone	Thr366	-9.04	235.3nM
7	Resveratrol	Val229, Cys201,Thr366	-8.51	578.91nM
8	Alpha Atlantone	Thr366	-8.31	815.01nM
9	Demethoxycurcumin	Cys239	-8.18	1.01 µM
10	Shagol	Asn247	-8.12	1.11 µM
11	Termilignan	Tyr200	-7.87	1.69 µM

12	Isopentyl Ferulate	Asn247	-7.38	3.88 µM
13	Apigenin	Val316, Pro358	-7.27	99.1 µM
14	Luteolin	Val316, Arg359	-6.68	12.76 µM
15	Caffeic Acid	Val229	-6.61	14.26 µM
16	Ferrullic Acid	Thr36	-6.61	14.38 µM
17	Coumaric Acid	Thr366	-6.44	19.06 µM
18	Thannilignan	Phe367	-6.58	15.1 µM
19	Scopoletin	Val316	-6.39	20.86 µM
20	Genistrin	Met233	-6.35	22.01uM

Table 2: Interactions and binding energies of the query protein with their corresponding Standard Drugs

S.No	Compound Name	Interacting Amino Acids	Binding Energy	Dissociation Constant
1	Tolcapone	Asn247, Thr366	-7.23	5.02 µM
2	Diacomit	Thr366	-7.16	5.65 µM
3	Xagol	Asn247, Gly235	-8.22	940.1nM
4	Rytary	Val236, Asn247	-2.87	7.91 µM

 1)	 2)



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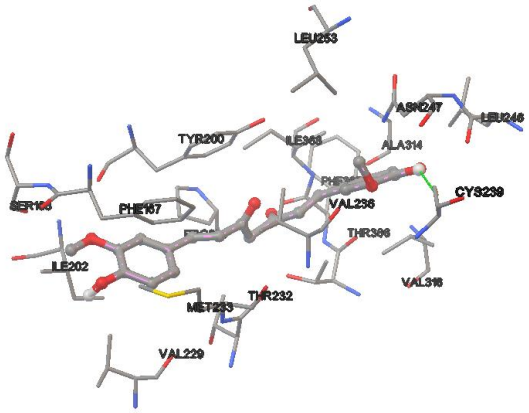

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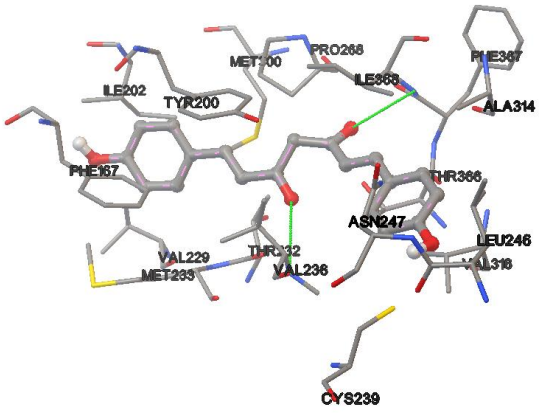

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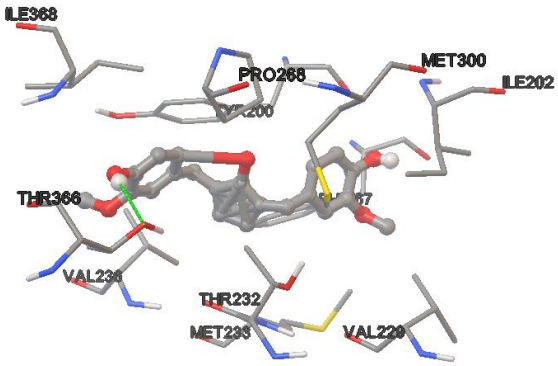

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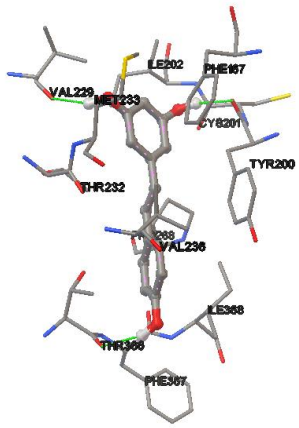

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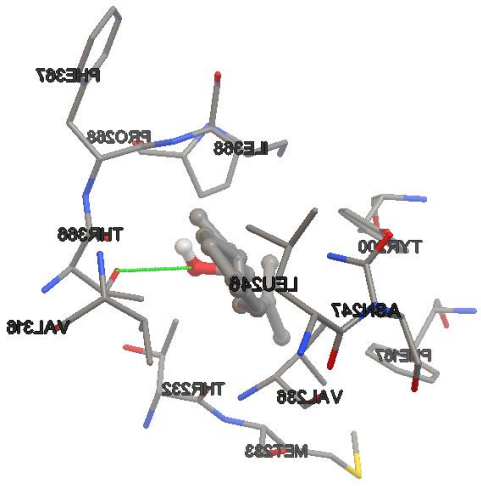

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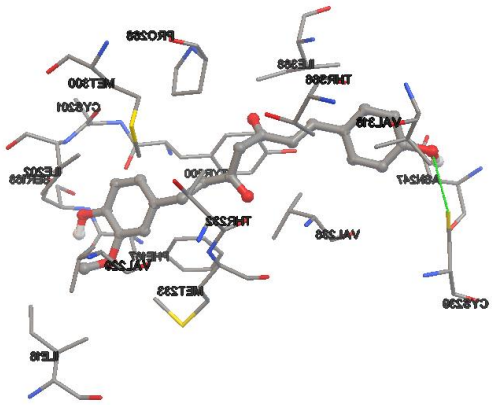

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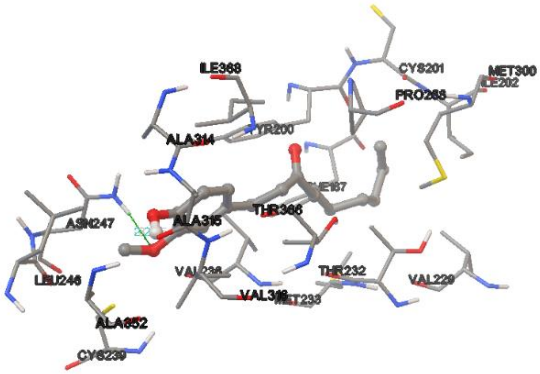

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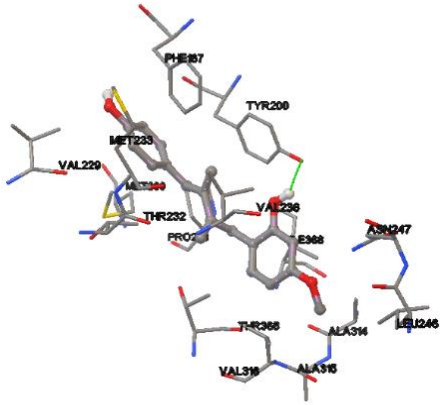

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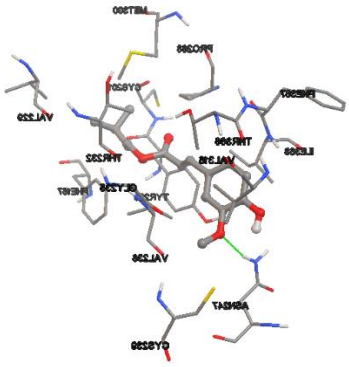

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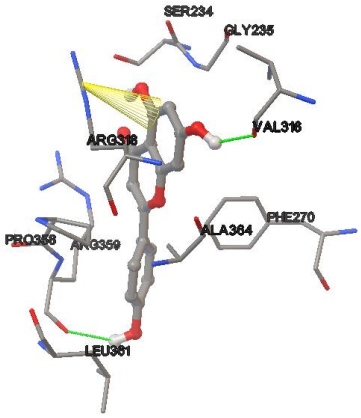

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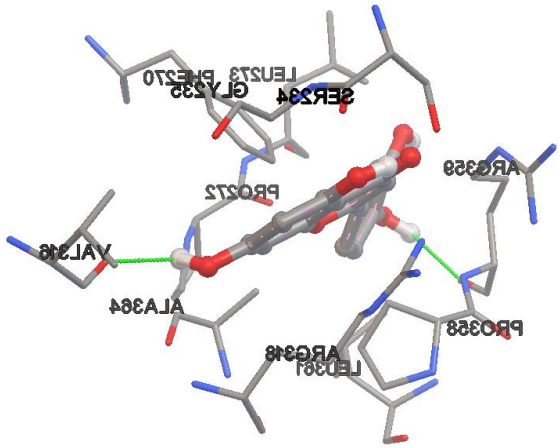

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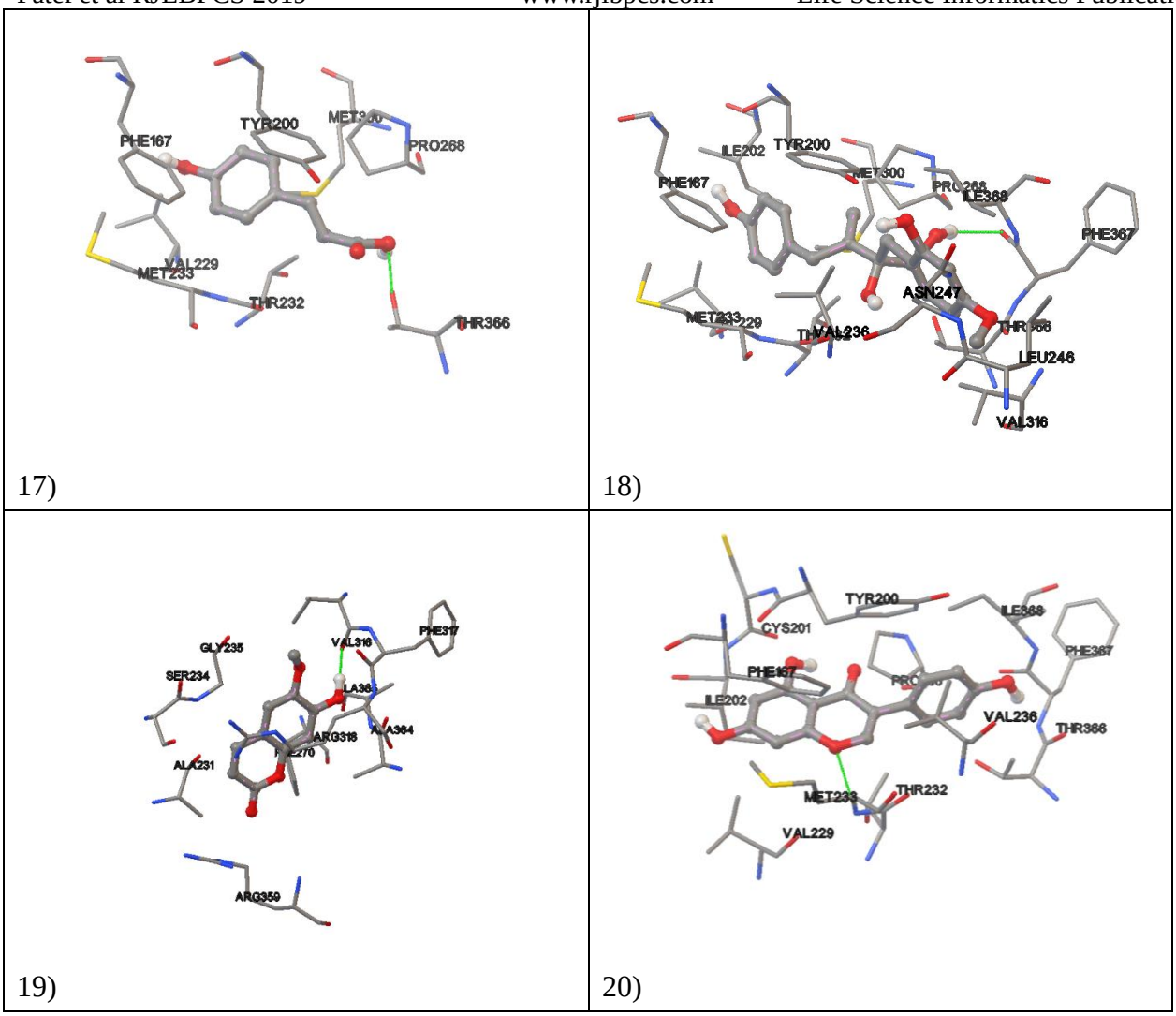

Figure 5: Interactions and binding energies of the query protein with their corresponding natural compounds

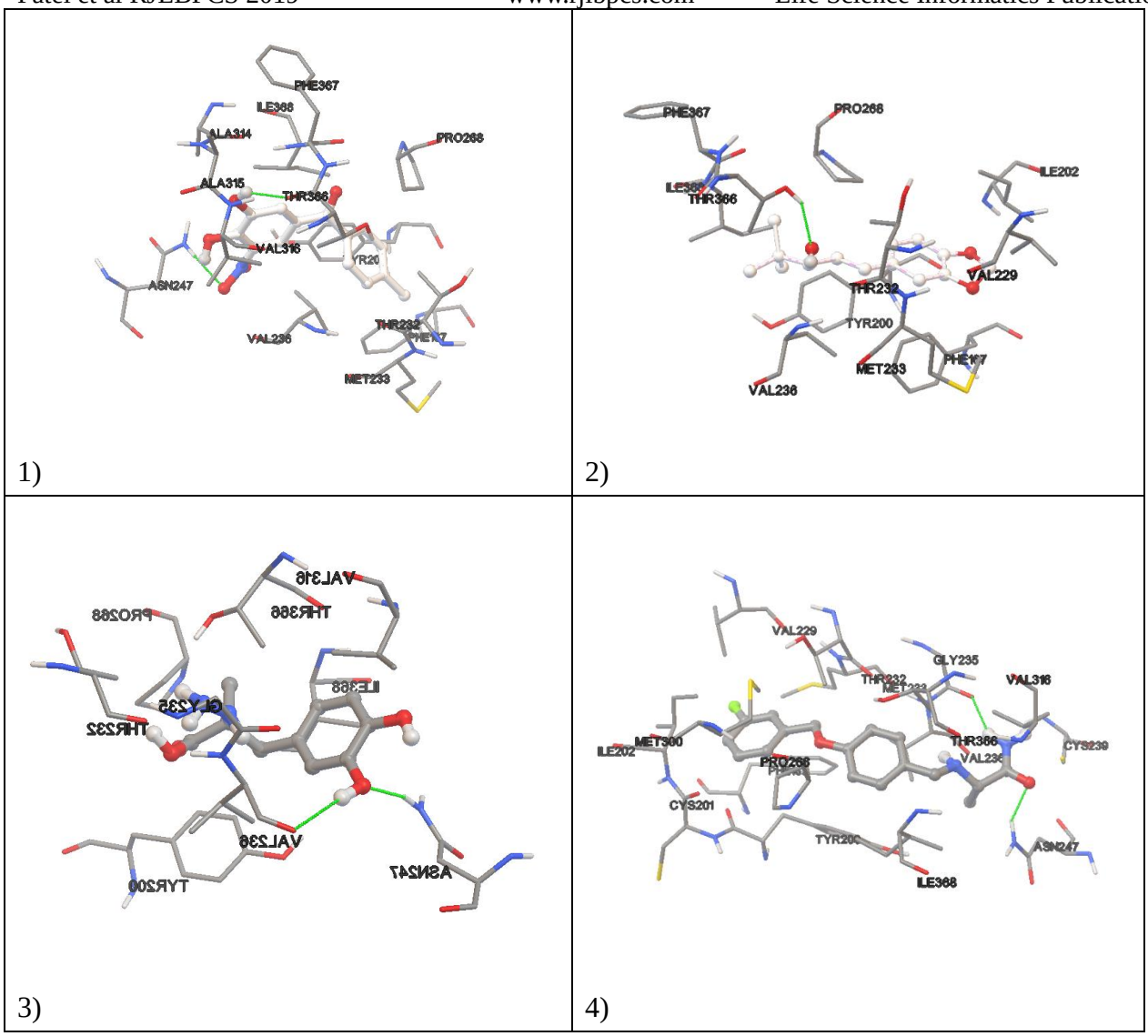

Figure 6: Interactions and binding energies of the query protein with their corresponding standard drugs

4. CONCLUSION

The selected query sequence that is obtained from uniprot does not contain the crystal structure (3D structure) in the PDB database. The crystal structure was built by performing homology modeling using Modeller 9.21. The modelled protein was affirmed using PROCHECK. The generated model showed 92.8% of amino acid residues in the most favored region. The generated model was then docked with twenty natural compounds and also docked with already existing drugs which were taken as controls. The natural compounds were noted to show better binding energies than already existing drugs. 1,7-bis-(4-hydroxyphenyl)-1,4,6-heptatrien-3-one exhibited highest binging energy of -11.66 Kcal/mol with interacting Asn247. The study proves that naturally existing compounds are more effective than already existing drugs for Cerebral Atrophy.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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