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DESIGN & MOLECULAR DOCKING STUDIES OF COUMARIN SUBSTITUTED 1, 3, 4-OXADIAZOLES AS GLYCOGEN SYNTHASE KINASE-3 INHIBITORS.

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ABSTRACT

Glycogen synthase kinase 3 (GSK-3) is a serine/threonine protein kinase that mediates the addition of phosphate molecules onto serine and threonine amino acid residues. First discovered in 1980 as a regulatory kinase for its namesake, Glycogen synthase, GSK-3 has since been identified as a kinase for over forty different proteins in a variety of different pathways. GSK-3 has recently been the subject of much research because it has been implicated in a number of diseases, including Type II diabetes (Diabetes mellitus type 2), Alzheimer's Disease, inflammation, cancer, and bipolar disorder. A plethora of GSK-3 inhibitors has been described and most of the effects were observed in vitro and cellular studies. Present study is aimed at design of GSK-3 Inhibitors, their molecular docking studies using online molecular docking software, i.e. www. Dockingserver.com. Based upon previous studies on 1, 3, 4-oxadiazoles as GSK-3 inhibitors, 1, 3, 4-oxadiazole molecule skeleton was taken as the core skeleton & 4 different modifications were made. The compounds were docked with GSK (PDB ID: 3f88 and PDB ID: 4E7W).The results have shown appreciable molecular docking interactions with the GSK-3 protein amino acid residues. The Est. inhibition constant, Ki values for the ligands were observed in µM values. . It is observed that Ligand I has shown Est. free energy of binding -10.17 which is said to be better than the other 3 ligands & reference ligands.

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INTRODUCTION

Glycogen synthase kinase 3 (GSK-3) is a serine/threonine protein kinase that mediates the addition of phosphate molecules onto serine and threonine amino acid residues. First discovered in 1980 as a regulatory kinase for its namesake, Glycogen synthase,[1]GSK-3 has since been identified as a kinase for over forty different proteins in a variety of different pathways.[2] In mammals GSK-3 is encoded by two known genes, GSK-3 alpha (GSK3A) and GSK-3 beta (GSK3-B). GSK-3 has recently been the subject of much research because it has been implicated in a number of diseases, including Type II diabetes (Diabetes mellitus type 2), Alzheimer's Disease, inflammation, cancer, and bipolar disorder.

Alzheimer's disease (AD) is a neurodegenerative disorder and characterized by the presence of abnormal filamentous protein inclusions in nerve cells of the brain.[3] The neuropathological hallmarks of AD were first reported by Alois Alzheimer and dateback to 1907.[4,5] These inclusions are formed by extracellular amyloid deposits and intracellular microtubule-associated protein tau.[6] Early onset forms of familial Alzheimer's disease (FAD) have been linked to mutations in the amyloid precursor protein (APP),presenilin-1 (PS-1) and presenilin-2 (PS-2). These mutations adversely affect APP processing and result in the increased production of the 40-42 amino acid long b-amyloid (Ab) peptides,which are the major component of amyloid deposits. Several risk factors have been associated with sporadic Alzheimer's disease (SAD). The most prevalent is aging and the presence of specific ApoE isoforms, which have been implicated in Ab clearance. The activation of b-secretase may be involved in Ab generation, which in combination with a deficiency in Ab clearance will result in the accumulation of Ab aggregates.[3,7] Partially phosphorylated tauin the normal adult brain features sequences that support association with tubulin, which entails the stabilization of microtubules. The pathological hyperphosphorylation of tau causes destabilization of microtubules, which in turn interferes with tubulin binding. The misfolding of hyperphosphorylated tau leads to the formation of insoluble neurofibrillary tangles (NFTs) and intraneuronal aggregates of paired helical filaments (PHFs).[8,9] GSK-3 was shown to phosphorylate tau both in vitro and in vivo on multiple sites. Several studies demonstrate that inhibition of GSK-3 induces decreased Ab production and a reduction in tau hyperphosphorylation.[10,11] GSK-3 was identified in the late 1970s and is a constitutively active, ubiquitously expressed serine/threonine kinase, which participates in a number of physiological processes.[3,12] Two related isoforms of GSK-3 exist in mammals,GSK-3A and B, which share 98% homology in their catalytic domains and have similar biochemical properties.[13] The isoforms differ significantly outside of their catalytic domains at their N-terminal regions. Furthermore, an alternative splice variant of GSK-3B: GSK-3B2, has been reported for rodents and humans.[14,15]The crystal structure of GSK-3B was determined in 2001.[16,17] GSK-3 is highly enriched in the brain and several publications indicate that the GSK-3b isoform is a key kinase required for abnormal hyperphosphorylation of tau.[18-20] Lithium chloride was the first GSK-3 inhibitor to be discovered. However there are several other biological targets for lithium cations, which impose limits on the therapeutic window. Considering the homology of GSK-3A and B within the ATP-binding pocket it appears difficult to identify an inhibitor that differentiates the two isoforms. All GSK-3 inhibitors developed until now are able to inhibit the two isoforms with almost similar potency.[21-24]. Current research is aimed at searching for the novel entity which can specifically inhibit these 2 isoforms.

MATERIALS & METHODS

Design of proposed GSK3 inhibitor

Proposed compounds will be designed by keeping in mind the 2, 5-disubstituted mercapto-1, 3, 4-oxadiazole as the basis for GSK3 inhibition with easily changeable functional groups in the 2ndand 5th position of the Oxadiazole ring system.[25]

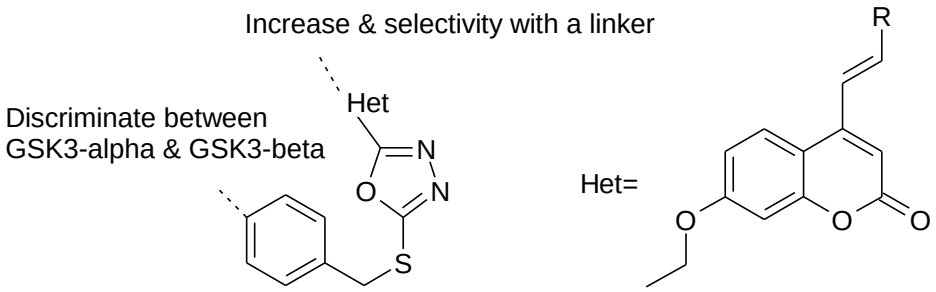


Fig.3. Design of Ligands.

1,3,4-oxadiazole

Oxadiazoles are important five-membered heterocyclic compounds. The synthesis of heterocyclic compounds has always drawn attention of organic chemists over the years mainly because of their important biological properties. The role of Nitrogen and Oxygen Containing hetero compounds which are endowed with unique structure and potent antibacterial activity need to be over emphasized. Moreover Oxadiazoles have played a crucial role in the development of theory in heterocyclic chemistry and also are extensively useful in organic chemistry. In the recent past, it has been observed and reported, that considerable antibacterial and antifungal activity has been exhibited by 1, 3, 4-oxadiazole derivatives, suitably substituted at 2^{nd} and 5^{th} positions.[26] 1,3,4-Oxadiazole with different heterocyclic compounds known to have a wide range of biological activities such as anti-inflammatory[27], analgesic[28], anti-ulcerogenic[29] , anti-microbacterial[30] , anthelmintic[31] , muscle relaxant[32] , anti-viral[33], anti-tubercular[34], anti-cancer[35], anti-convulsant[36] and anti-fungal[37] activities.

Coumarin:

The active compounds isolated from some of the plants such as Scoparone from Artemesia Capillaris,[50] 3-hydroxyumbelliferone derivatives from Bahia ambrosioides,[38] Glycyrin from Glycyrrhiza Uralensis[39] and Marmesin and Umbelliferone ether isolated from Aegle marmelos[40] used traditionally for the management of diabetes and related metabolic disorders contain 7-hydoxycoumarin motif. An alkaloid, Aegeline (N-cinnamoyl-4-methoxyphenylethanolamine) isolated from the same plant Aegle marmelos reported to exhibit both anti-hyperglycemic and anti-dyslipidemic activities.[41]

Molecular docking studies:

The designed compounds were subjected for molecular docking studies to check the interaction of the active ligands against target specific protein (GSK3) using online software called dockingserver.[43-53]

Ligand Preparation:

Docking calculations were carried out using DockingServer (*Bikadi, Hazai, 2009*). The MMFF94 force field (*Halgren, 1998*) was used for energy minimization of ligand molecule I-IV using DockingServer. Gasteiger partial charges were added to the ligand atoms. Non-polar hydrogen atoms were merged, and rotatable bonds were defined.

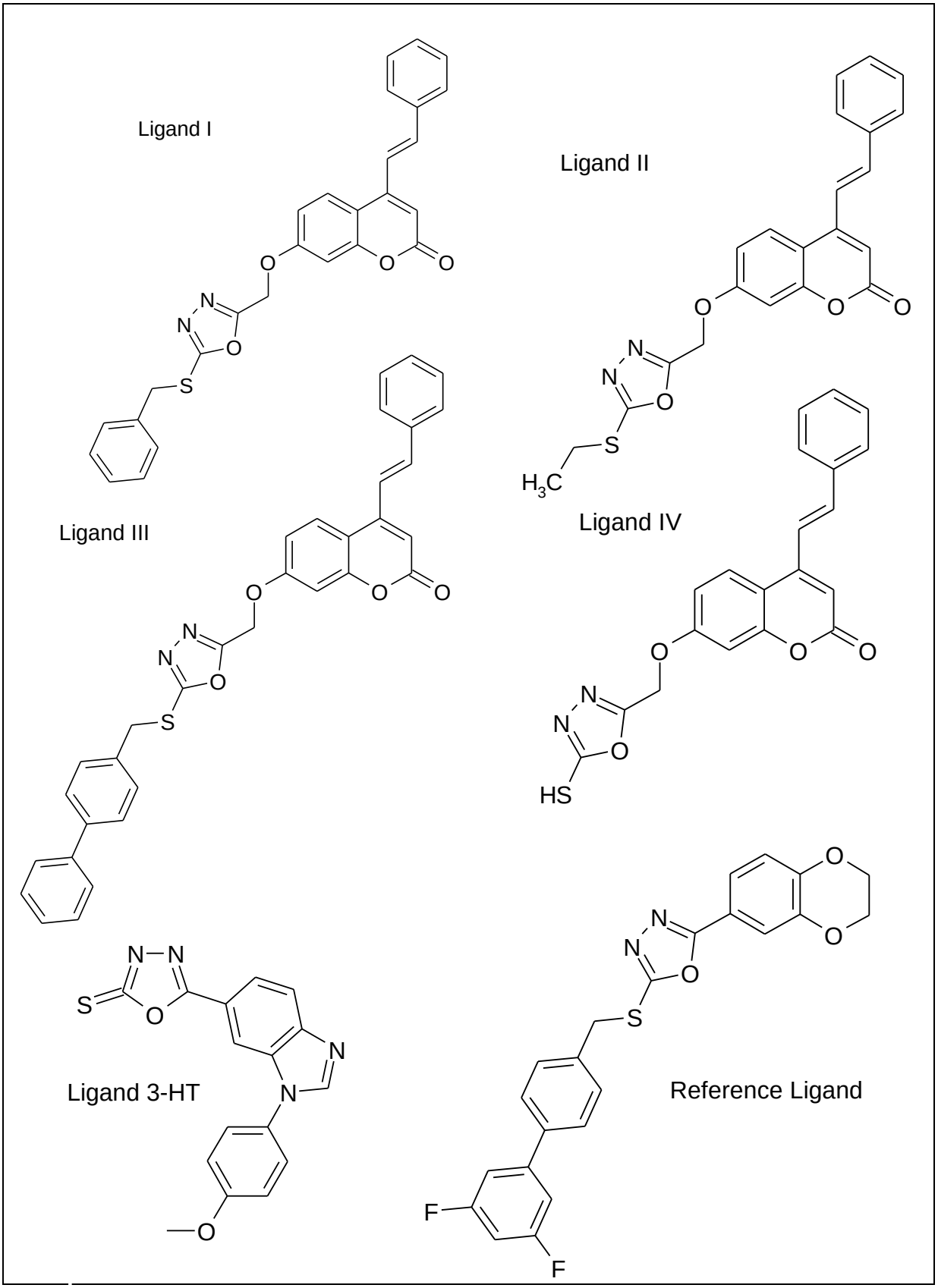


Fig.4: Proposed Ligands.

Protein Preparation:

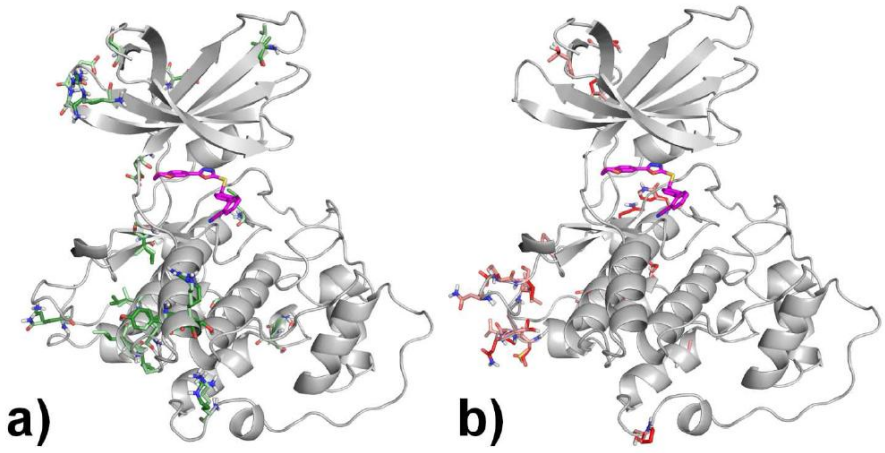


Fig.1: Superposition of 3D structure of GSK-3β (PDB code 3F88) and the homology based 3D structure of GSK-3α.

Both proteins are shown as white cartoons; conservative mutations are displayed as forest and light green stick, for GSK-3β and GSK-3α respectively. (a), while non-conservative mutations are represented as red and pink sticks, for GSK-3β and GSK-3α respectively. (b). It is evident that the majority of the mutations are located at the loop at the C-terminus fragment of the proteins (D345-T363 in GSK-3β and R407-A427 in GSK-3α). [24].

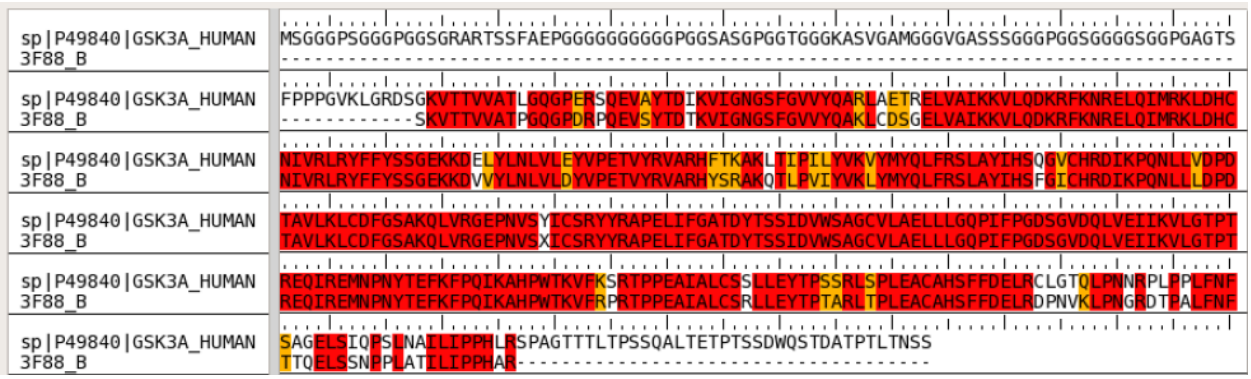


Fig. 2. Alignment between GSK-3α (Swiss-Prot code: P49840) and GSK-3β sequences (PDB code: 3F88). Identical residues are colored in red, conservative residues in orange, non-conservative residues are not painted. Gaps are represented as dashes.[24]

Docking calculations were carried out on GSK3-A modelled protein (Fig.5) & GSK3-B protein (Fig.6 PDB ID-3F88). Essential hydrogen atoms, Kollman united atom type charges and solvation parameters were added with the aid of AutoDock tools (*Morris, Goodsell et al., 1998*). Affinity (grid) maps of 20×20×20 Å grid points and 0.375 Å spacing were generated using the Autogrid program (*Morris, Goodsell et al., 1998*). AutoDock parameter set- and distance-dependent dielectric functions were used in the calculation of the van der Waals and the electrostatic terms, respectively.

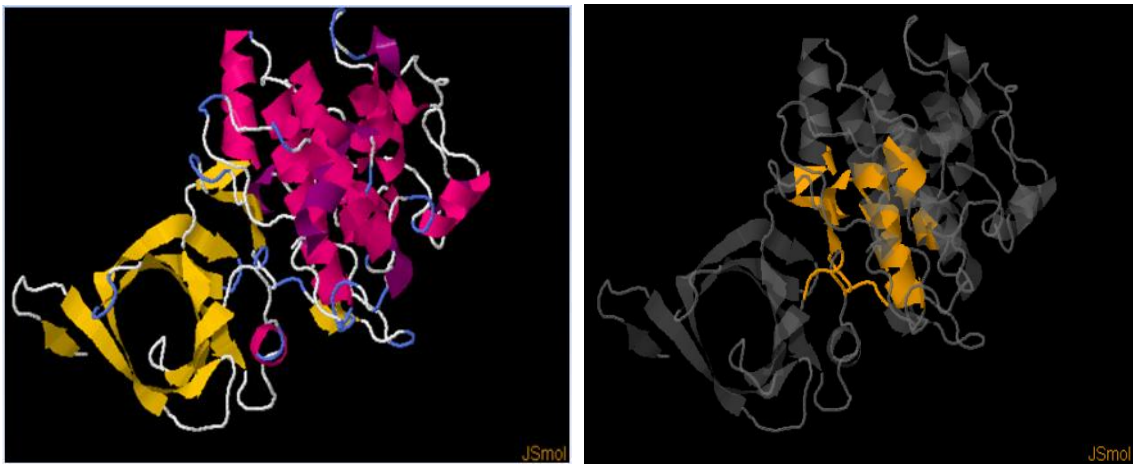


Fig.5: Modelled structure of GSK3-A_HUMAN P49840 (Glycogen Synthase Kinase3-Alpha).

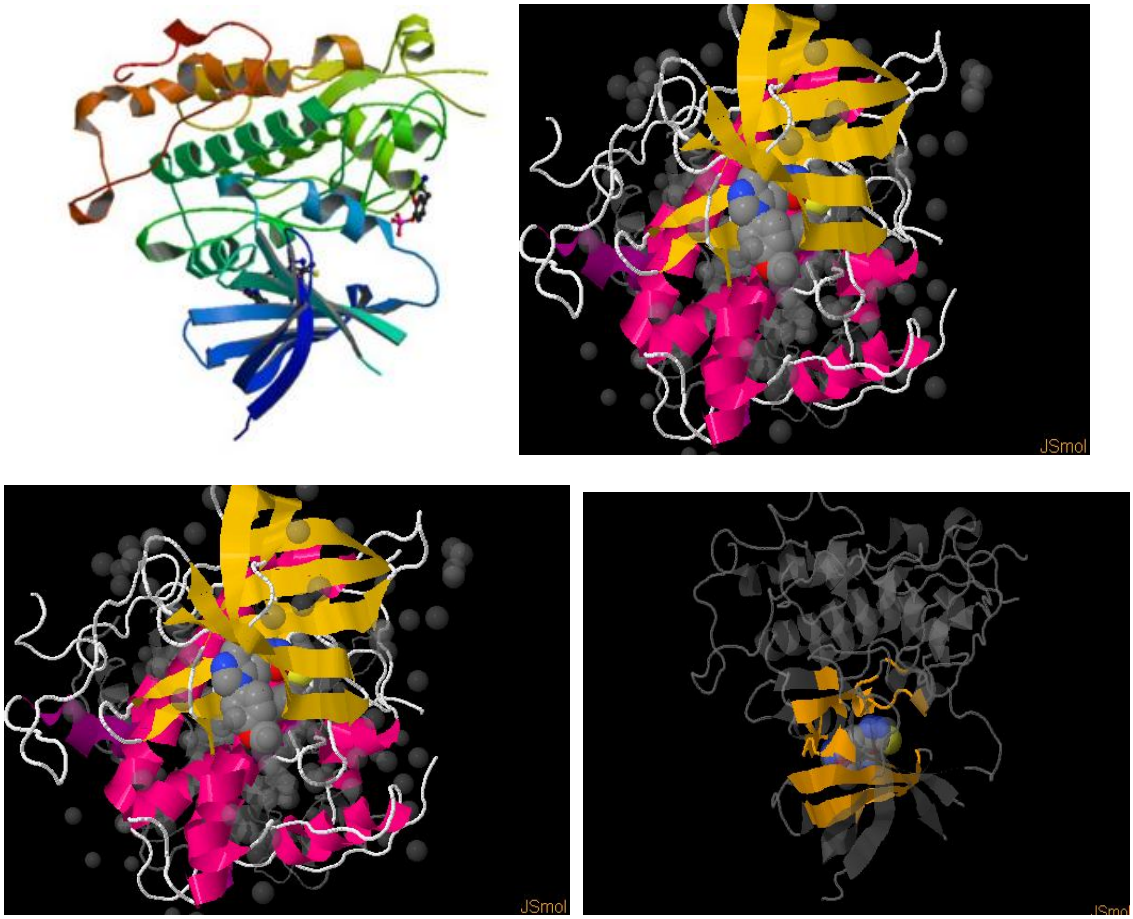


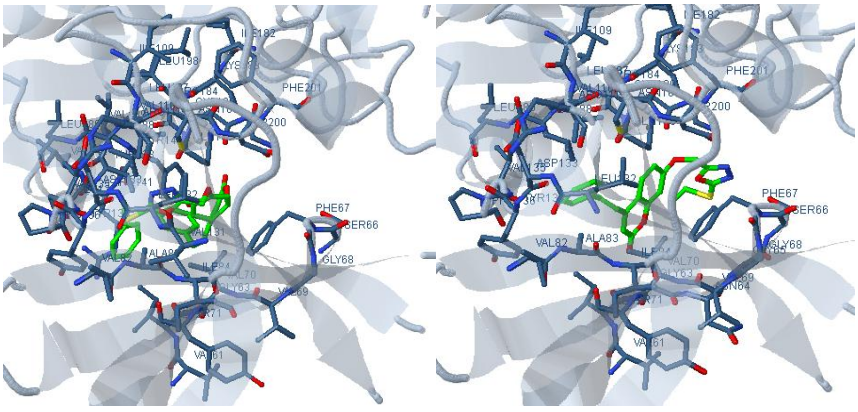
Fig.6: Glycogen Synthase kinase3-β inhibitor complex.

Computational Methods

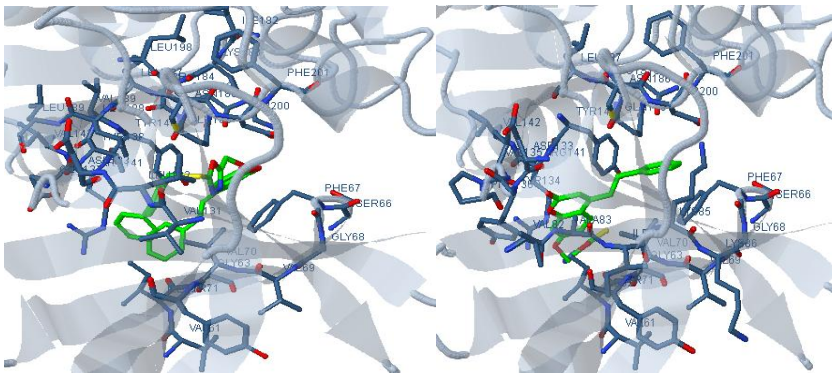
Docking simulations were performed using the Lamarckian genetic algorithm (LGA) and the Solis & Wets local search method (*Solis and Wets, 1981*). Initial position, orientation, and torsions of the ligand molecules were set randomly. Each docking experiment was derived from 10 different runs that were set to terminate after a maximum of 250000 energy evaluations. The population size was set to 150. During the search, a translational step of 0.2 Å, and quaternion and torsion steps of 5 were applied.

RESULTS & DISCUSSION

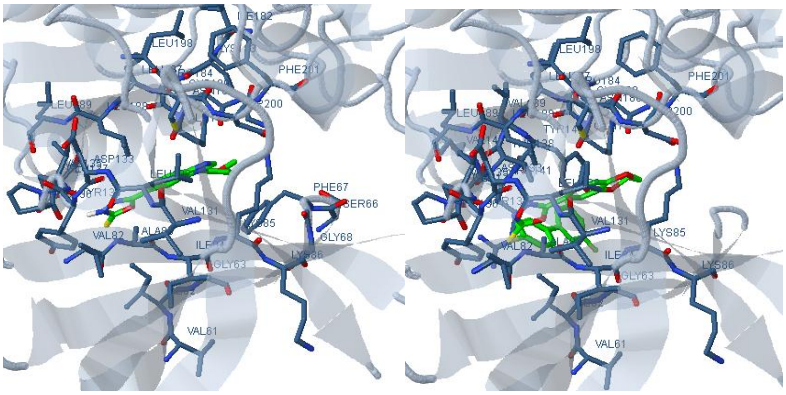
The homology modelling for GSK3-A protein was performed using SwissProt code: P49840 & the protein structure along with the binding site (in yellow) is depicted in Figure 5. The In-silico molecular docking studies were carried out for four different Ligands containing Coumarin substituted 1,3,4-oxadiazole as the core moiety with 3F88 protein. To investigate the potency of the ligands I-IV, we proceeded to examine the interaction of ligands with 3F88. The molecular docking was performed by simulation of ligands into the binding site of GSK-3β protein. The 2D plot and their 3-D geometry were exhibited in Figure 7 and Figure 8 respectively. The docking results reveal that all the ligands inside 3F88 protein are bounded well. The Free Energy of Binding, Inhibition Constant, Vander Waal + Hydrogen bond + dissolve Energy, Electrostatic Energy and Total Intermolecular Energy are given in Table 1.The ligand molecules are bound to protein 3F88 by four binding modes such as Hydrogen bonds, Van der Waals, electrostatic and hydrophobic Interactions. The ligands I-IV have hydrogen bond interaction with receptor. The calculated free energy of binding for ligands is comparable with the 3-HT & Reference ligand . The free energy of binding for ligands I-IV in the inhibitor binding site (IBS) were -10.19, -7.36, -7.51 and -7.46 kcal/mol respectively in the best pose & is depicted in Table.1. The molecular docking of ligand I to GSK3-A model is shown in Figure 9. Ligand I has bound to the pocket cavity impressively. The hydogen bonding interactions along with the other hydrophobic interactions are depicted in 2-D plot Figure 9.



I to 3f88 – TRANSFERASE **II to 3f88 - TRANSFERASE**

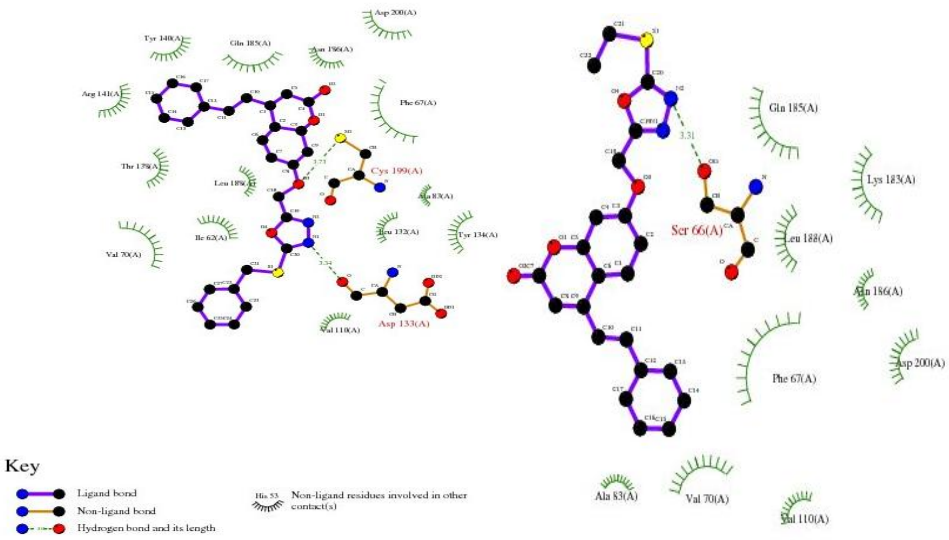


III to 3f88 – TRANSFERASE **IV to 3f88 - TRANSFERASE**

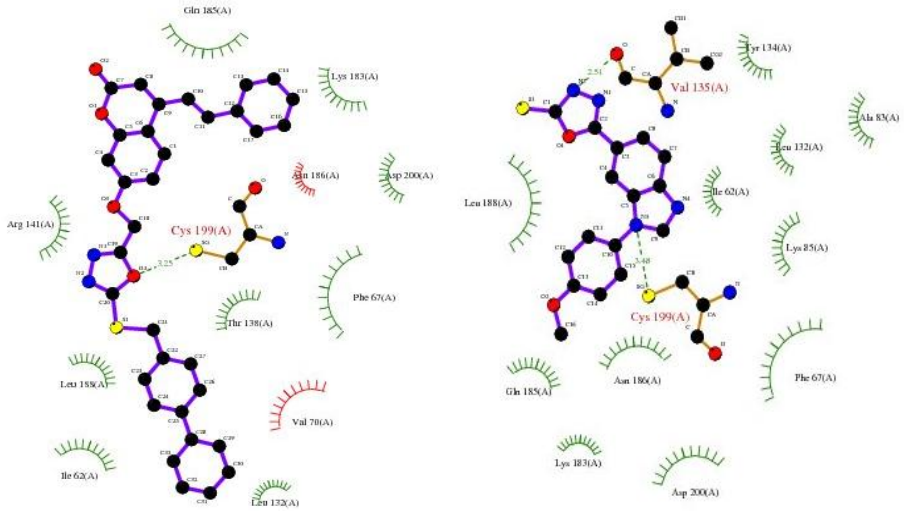


5-[1-(4-methoxyphenyl)-1H-benzimidazol-6-yl] -1,3,4-oxadiazole-2(3H)-thione to 3F88 – TRANSFERASE (Ligand 3HT) **Std to 3F88 – TRANSFERASE**

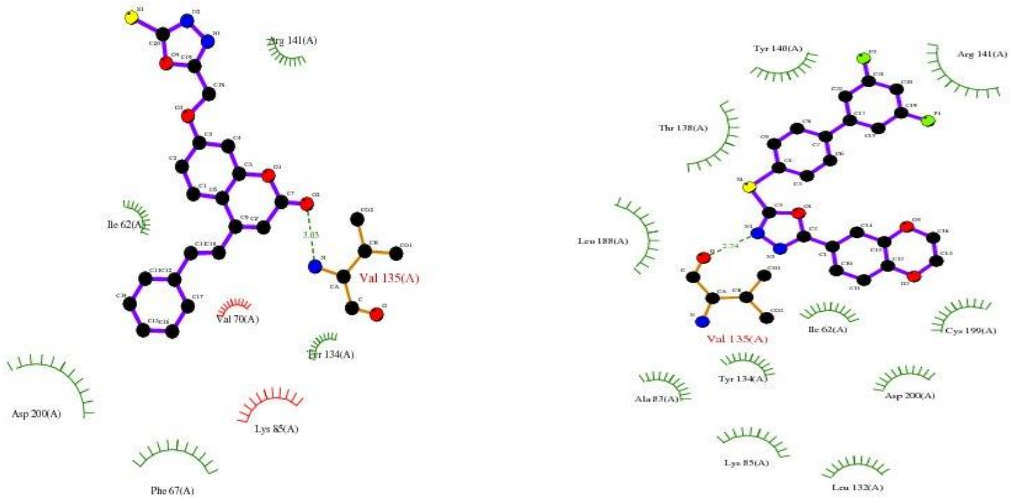
Fig.7:Docking Pose (3D-Geometry) of the Ligands I-IV to the binding site of 3F88 protein.



I to 3F88 - TRANSFERASE **II to 3F88 – TRANSFERASE**



5-[1-(4-methoxyphenyl)-1H-benzimidazol-6-yl]
-1,3,4-oxadiazole-2(3H)-thione to 3f88 - TRANSFERASE
III to 3F88 - TRANSFERASE

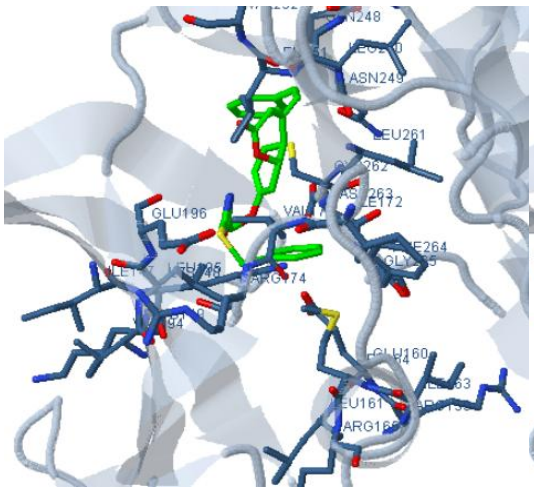


III to 3F88 - TRANSFERASE **Std to 3F88 - TRANSFERASE**

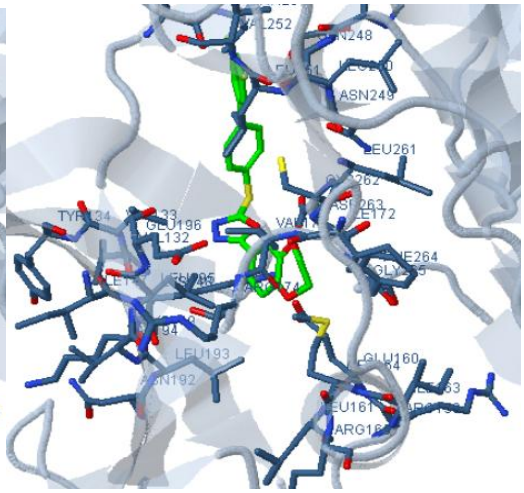
Fig.8: 2D-plot of ligands I-IV to 3F88 protein

Table 1. Molecular docking studies of Ligand I-IV along with the Standard GSK-3 inhibitors.

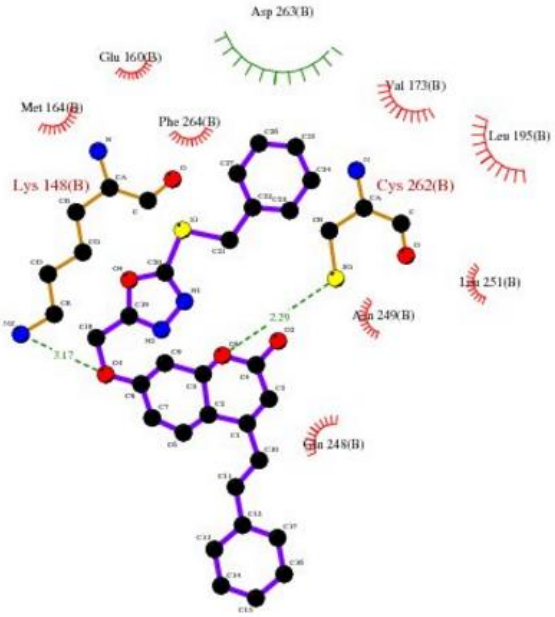
Ligand Code	Est. Free Energy of binding (kcal/mol)	Est.inhibition Constant, Ki	vdW + Hbond + desolv. Energy (kcal/mol)	Electrostatic Energy (kcal/mol)	Total Intermolec. Energy (kcal/mol)	Interact. Surface
I	-10.19	34.10 nM	-11.10	+0.06	-11.84	975.74
II	-7.36	4.04 µM	-9.01	-0.15	-9.17	914.28
III	-7.51	3.14 µM	-9.68	-0.11	-9.79	1090.86
IV	-7.83	1.82 µM	-7.98	-1.32	-9.30	905.44
3-HT	-8.13	1.10 µM	-9.12	-0.05	-9.17	762.11
Reference Ligand	-9.42	125.19 nM	-10.34	-0.19	-10.53	874.65



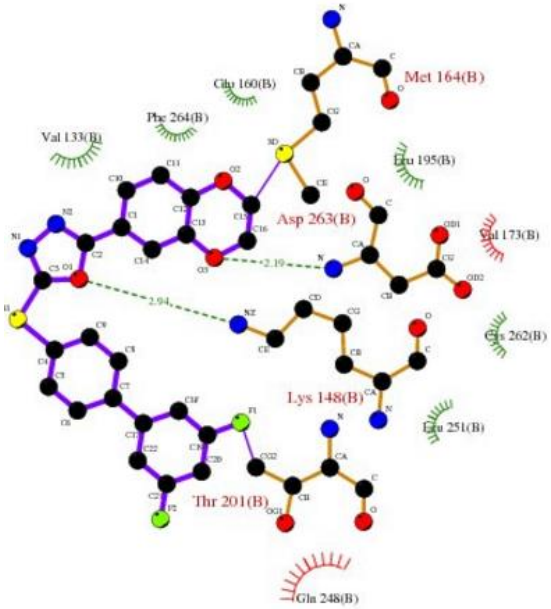
I to GSK3-A model_01.pdb



Std to GSK3-A model_01.pdb



I to GSK3-A model_01.pdb



Std to GSK3-A model_01.pdb

Fig.9: Geometry & 2D-plot of ligands I-IV to GSK3-A modelled protein

CONCLUSION

A series of four different coumarin substituted 1,3,4-oxadiazole ligands were examined for the in silico molecular docking studies with GSK3-β (3F88) protein and modeled protein structure of GSK3-A. Results reveal that all the compounds inside PDB:3F88 protein are bound well. The ligands have shown inhibition to 3F88, but it is not limited to 1,3,4-oxadiazoles substituted with bi-phenyls. It is observed that Ligand I has shown Est. free energy of binding -10.17 which is said to be better than the other 3 ligands & reference ligands. The Est. inhibition constant, Ki is 34.10nM & is comparable to the Reference ligand, i.e. 125.19nM. The Est. inhibition constant, Ki values for rest of the ligands were observed in µM values. It is also observed that these compounds have hydrogen, polar and other interactions which are important for the folding of proteins. It could be concluded that the binding mode of our target ligands & reference ligand are resembling. i.e. in the binding site of 3F88 receptor and modeled protein structure of GSK3-A & so, they might act through inhibition of tubulin .The present can be further recommended for future research.

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Conflicts of Interest:

The authors hereby declare that there is no conflicts of interest for this publication.

Abbreviations:

GSK-3 -Glycogen Sythase Kinase-3
AD -Alzheimer's Disease
FAD -Familial Alzheimer's Disease
APP -Amyloid precursor protein
Presenilin-1 (PS-1)
Presenilin-2 (PS-2)
Ab -b-amyloid
SAD -Sporadic Alzheimer's disease
NFTs -Neurofibrillary tangles
PHFs -Paired Helical Filaments
Est.inhibition Constant, Ki
Kcal/mol- kilocalorie/mol

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