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MOLECULAR INTERACTION STUDIES OF CRYSTAL STRUCTRUES OF QUINOLINE DERIVATIVES AGAINST BACE1

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ABSTRACT: Alzheimer's disease (AD) is a severe neurodegenerative disorder and the most common type of dementia in the elderly. Developing small compound based drugs targeting the β-secretase (BACE1) enzyme is one of the most promising strategies in treatment of the Alzheimer's disease. As this enzyme shows the activity based on the acid-base reaction at a very narrow pH range, the protonation state of aspartic acids with the residue number 32 and 228 (Asp32 and Asp228), which forms the active site dyad, along with the protonation state of the ligand (substrate or inhibitor) play very critical role in interactions between the ligand and enzyme. Thus, understanding the nature of the protonation state of both enzyme's active site dyad and ligand is crucial for drug design in Alzheimer's disease field. Here we have studied the through molecular docking studies three different protonation states of the Asp32 and Asp228 residues (Ash32, Ash228 and Ash32 – Ash228) in the presence of a three crystal structure compounds (163, 187 and 216) and cocrystallized inhibitor. BACE1 enzyme all protonation states were performed using Schrodinger Glide Induced fit docking protocol. From this docking results revels all three compounds having good binding affinity with important catalytic sites of target of BACE1. Finally, these all three crystal compounds and cocrystal inhibitor were employed in ADME property calculations, all compounds shows the good Blood Brain barriers (BBB) and lesser molecular weight and its obey the Lipinski rules. This finding of protonation states is most important to designing new inhibitors to useful for therapeutic efficacy for the prospective treatment of AD.

KEYWORDS: Alzheimer's disease, BACE1, Quinolone, Induce Fit Docking, ADME

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1.INTRODUCTION

Alzheimer's disease is an irreversible, brain disorder that progressively destroys memory, reducing thinking skills and as a result, makes incapable to carry out even simplest tasks. Generally, Alzheimer's symptoms could be witnessed in mid-60s of a human being. Speaking or writing, loss of reasoning skills, and delusions were also reported as symptoms[1]. At present, therapies to AD aiming management of symptoms and yet no disease altering treatment exists. β-Secretase, also known as beta-site amyloid precursor protein cleaving enzyme 1 (BACE1) which is an aspartyl protease found in the early cascade of biological events which causes disease progression. Since it is an enzyme, searching for suitable inhibitor could make drug designing for AD as a direct strategy[2]. Apart from, neuritic plaques on the brain and neurofibrillary tangles cause AD pathology. Insoluble bundles of fibres make neurofibrillary tangles are generally composed of phosphorylated tau protein and located in the perinuclear cytoplasm. Appearance of tangles in the brain of AD patient could be due to neuronal responses to the formation of plaque[3]. Plaques are spherical lesions which consists of extracellular aggregates of amyloid-β protein (Aβ)[4]. Realizing the significance of Aβ, the biosynthesis pathway for its production is studied in detail. Two important proteases, β-Secretase 1 (BACE1) and γ-secretase are found to have role in cleaving amyloid precursor protein (APP) into Aβ[5]. Even with the understanding that BACE1 involve in post-translational processing and intracellular trafficking, BACE1 inhibition was considered as AD therapy[6]. Primarily, BACE1 cleaves APP at the N-terminus of the Aβ peptide domain and γ-secretase involve in the cleavage (proteolysis) of transmembrane domain of APP thus leading to the secretion of Aβ peptide. BACE1, as the initial enzyme exists widely in the AD brain and also the fact that Aβ is not detectable in neurons of BACE1 knockout rats, initiated the strategy to consider BACE1 as a novel target for AD therapy[7],[8],[9]. Also, BACE1 is found to regulate Adult hippocampal neurogenesis (AHN) which is a lifelong process that is important for learning and memory.[10] The research efforts to identify BACE1 inhibitors had been initiated long back about 15 years ago. At that time, BACE1 was confirmed as membrane-bound aspartic proteinase with 501 amino acids.[11] It also confirmed that formation of Aβ as a rate-limiting step and so BACE1 as rate limiting enzyme.[12] Structure-based designing attempts were rendered to have selective memapsin 2 (BACE1) inhibitors and pyrazole substituted ligands were proposed.[13] Also hydroxyethylamine isosteres are also proposed as inhibitors based on cell line studies.[14] Dihydroquinazoline-derived compounds also were proved to have BACE inhibition.[14] With the consideration that BACE1 reduces cerebral levels of Aβ, it has been proposed as the target to treat prevention of AD.[15],[6] Also folic acid was found to decease BACE1 mRNA and protein expression along with BACE 1 inhibiting capacity.[16] Drug repurposing is also been suggested as an alternative solution to identify efficient BACE-1 inhibitors so as happened in the discovery of sildenafil, bupropion, thalidomide, and many other

drugs for various ailments.[17] Recently, from this lab, Pharmacophore based inhibitor designing was reported[18] Peptidomimetic molecules which are initially analyzed are potent BACE1 inhibitors, mainly because the large open active site for catalyzing polypeptide substrates. However, these peptidomimetic inhibitors do not possess optimum drug-like properties such as oral bioavailability, long serum half-life, or blood–brain barrier penetration. It has proven challenge to have an inhibitor big enough to occupy the large active site with high affinity yet small enough to satisfy pharmacokinetics characteristics and achieve adequate blood-brain penetration. Targeting the β secretase BACE1 for Alzheimer's disease.[6] By the realization that among the two ASP active site residues (Asp Dyad), possibility of various protonated/unprotonated combination as well as ligand induced protonation, BACE 1 inhibitor designing has taken different approaches.[19],[20],[21] Since many of the proposed inhibitors are pseudo peptides and synthetic compounds, natural oligoscharides were also analyzed to design BACE 1inhibitors.[22] Although BACE1 inhibitor drug development has proven challenging, several promising BACE1 inhibitors have recently entered human clinical trials.[6] Since Alzheimer's is one of themes of the lab, structure based drug designing protocols were recently carried out against PKC, BACE1 and MaoB enzymes. The crystal structures presented in the previous chapter were subjected to Swiss Target predication server for target prediction and the results revealed the possibility of BACE1. With that encouragement, molecular docking studies were carried out with human BACE1 enzyme. Three different dyad combinations (Asp/Asp, AsH/Asp, Asp/Ash, AsH/AsH) were employed for the docking analysis.

2. MATERIALS AND METHODS

2.1 Biological Target Prediction

Crystal structures of all three compounds were employed to finding the biological target predication using online Swiss Target Prediction server.[23] Molecular insight into the mode of action of bioactive small molecules is key to understanding observed phenotypes, predicting potential side effects or cross-reactivity and optimizing existing compound. Accurately predict the targets of bioactive molecules based on a combination of 2D and 3D similarity measures with known ligands. Predictions can be carried out in five different organisms, and mapping predictions by homology within and between different species is enabled for close paralogs and orthologs.

2.2 Protein Preparation

For molecular docking study, modifications were carried out in human BACE1 (PDB ID: 2FDP). Missing hydrogen atoms were added and correct bond orders were assigned, and then formal charges and orientation of various groups were fixed. Following this, optimization of the amino acid orientation of hydroxyl groups, amide groups were carried out. All amino acid flips were assigned and H-bonds were optimized. BACE1 catalytic aspartic dyad (Asp32 and Asp228) are modified in charged to neutral four different protonation states ASP32 & ASP228 (Deprotonated) ASH32 & ASP228

(Mono protonated), ASP32 & ASH228 (Mono protonated), ASH32 & ASH228 (Die protonated). No hydrogen atoms were minimized until the average root mean square deviation reached default value of 0.3 Å. Sitemap 2.3 was used to explore the binding site in the docking studies.[24]

2.3 Ligand Preparation

Crystal structure of three small molecule (163, 187 and 216) compounds was built using builder panel in Maestro. The compounds were taken for ligand preparation by Ligprep 2.3 module[25] (Schrödinger, USA) which performs addition of hydrogen, 2D to 3D conversion, realistic bond lengths and bond angles, low energy structure with correct chiralities, ionization states, tautomers, stereo chemistries and ring conformations.

2.4 Induced Fit Docking

Induced fit docking (IFD) is one of the main complicating factors in docking studies which predicts accurate ligand-binding modes and concomitant structural movements in the receptor using Glide and Prime modules. In IFD, when a ligand binds to the receptor, it undergoes a side chain or backbone conformational changes or both in many proteins. These conformational changes allow the receptor for better binding according to the shape and binding mode of the ligand.[26] Here, the prepared protein was loaded in the workspace and the sitemap predicted active site was specified for IFD. The grid was calculated about 20 Å to cover all the active site residues defined by the site map. The van der Waal's radii of the non-polar receptor and ligand atoms were scaled by a default factor of 0.50. IFD calculations were carried out for crystal structure of three small molecule compounds (163, 187 and 216) against human BACE1. Following this, 20 conformational poses were calculated where the best conformational pose was selected based on the docking score, glide energy, hydrogen bonding and hydrophobic bonding interactions.

2.5 Adme Properties

The synthesized all three crystal structures compounds of drug likeness was determined by using the “Lipinski rule of five”. ADME properties were ADME and Toxicity studies were considered by taking the parameters as mentioned below. We have analyzed various physiochemical descriptors and pharmaceutically significant properties of three crystal compounds (Quin1, Quin2 and Quin3) using QikProp v3.0[26] tool of Schrodinger software.

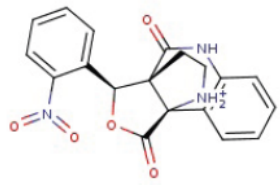
3. RESULTS AND DISCUSSION

From the biological target predication results all three crystal compounds (Quin1, Quin2 and Quin3) shows Beta secretase 1 aspartic protease enzyme (Figure: 1), so further all three molecules were carried out molecular docking studies.

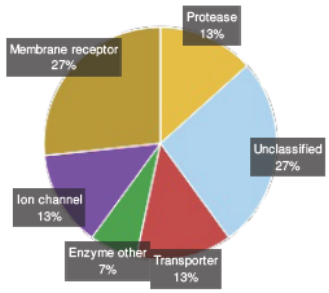
SwissTargetPrediction report:

Reference:
Gfeller D., Michielin O. & Zoete V. Shaping the interaction landscape of bioactive molecules, *Bioinformatics* (2013) 29:3073-3079.

Query Molecule



Frequency of Target Class

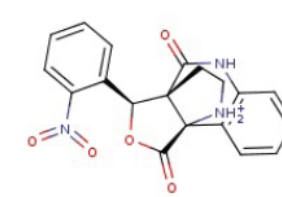


Target	Uniprot ID	Gene code	ChEMBL ID	Probability	# sim. cmpds (3D / 2D)	Target Class
Beta-secretase 1	P56817	BACE1	CHEMBL4822		5 / 9	Aspartic Protease
Beta-secretase 2 *(by homology)*	Q9Y5Z0	BACE2	CHEMBL2525		5 / 9	Aspartic Protease
Muscleblind-like protein 1	Q9NR56	MBNL1	CHEMBL1293317		155 / 4	Unclassified
Muscleblind-like protein 2 *(by homology)*	Q5VZF2	MBNL2			155 / 4	Unclassified

SwissTargetPrediction report:

Reference:
Gfeller D., Michielin O. & Zoete V. Shaping the interaction landscape of bioactive molecules, *Bioinformatics* (2013) 29:3073-3079.

Query Molecule



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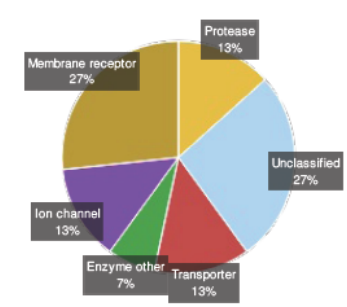
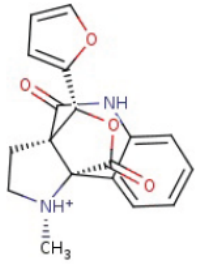


Target	Uniprot ID	Gene code	ChEMBL ID	Probability	# sim. cmpds (3D / 2D)	Target Class
Beta-secretase 1	P56817	BACE1	CHEMBL4822		5 / 9	Aspartic Protease
Beta-secretase 2 *(by homology)*	Q9Y5Z0	BACE2	CHEMBL2525		5 / 9	Aspartic Protease
Muscleblind-like protein 1	Q9NR56	MBNL1	CHEMBL1293317		155 / 4	Unclassified
Muscleblind-like protein 2 *(by homology)*	Q5VZF2	MBNL2			155 / 4	Unclassified

SwissTargetPrediction report:

Reference:
Gfeller D., Michielin O. & Zoete V. Shaping the interaction landscape of bioactive molecules, *Bioinformatics* (2013) 29:3073-3079.

Query Molecule



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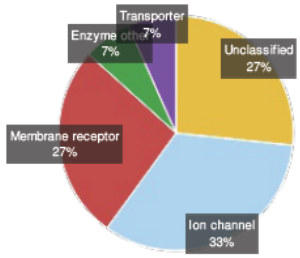


Target	Uniprot ID	Gene code	ChEMBL ID	Probability	# sim. cmpds (3D / 2D)	Target Class
Microtubule-associated protein tau	P10636	MAPT	CHEMBL1293224		125 / 2	Unclassified
Sodium channel protein type 9 subunit alpha	Q15858	SCN9A	CHEMBL4296		6 / 1	Ion channel
Sodium channel protein type 1 subunit alpha *(by homology)*	P35498	SCN1A	CHEMBL1845		6 / 1	Ion channel
Sodium channel protein type 2 subunit alpha *(by homology)*	Q99250	SCN2A	CHEMBL4187		6 / 1	Ion channel

Figure 1: showing Target prediction efforts for compounds Quni1, Quin2 and Quin3.

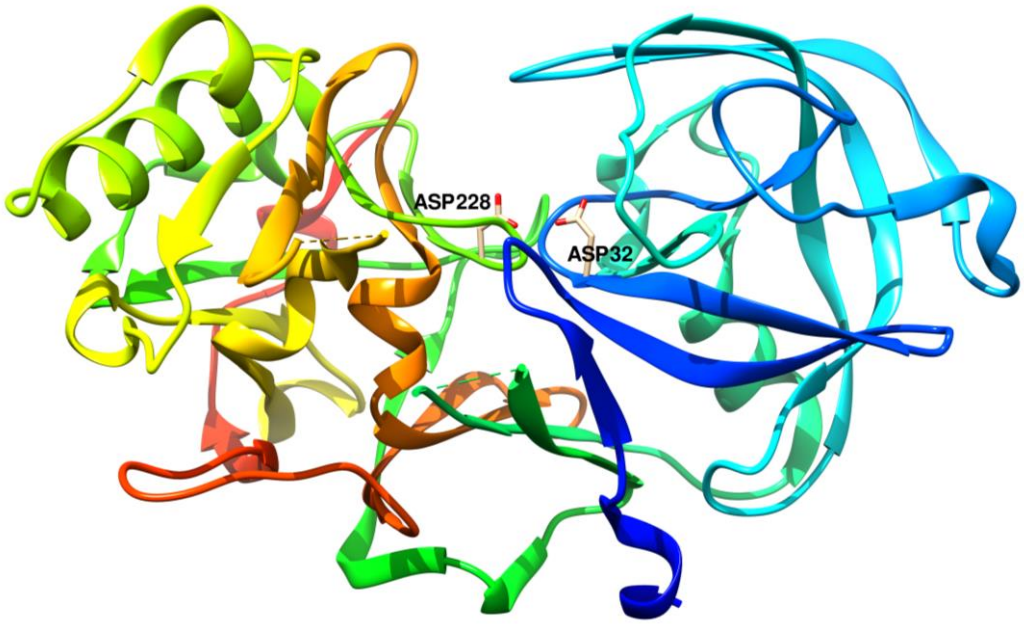
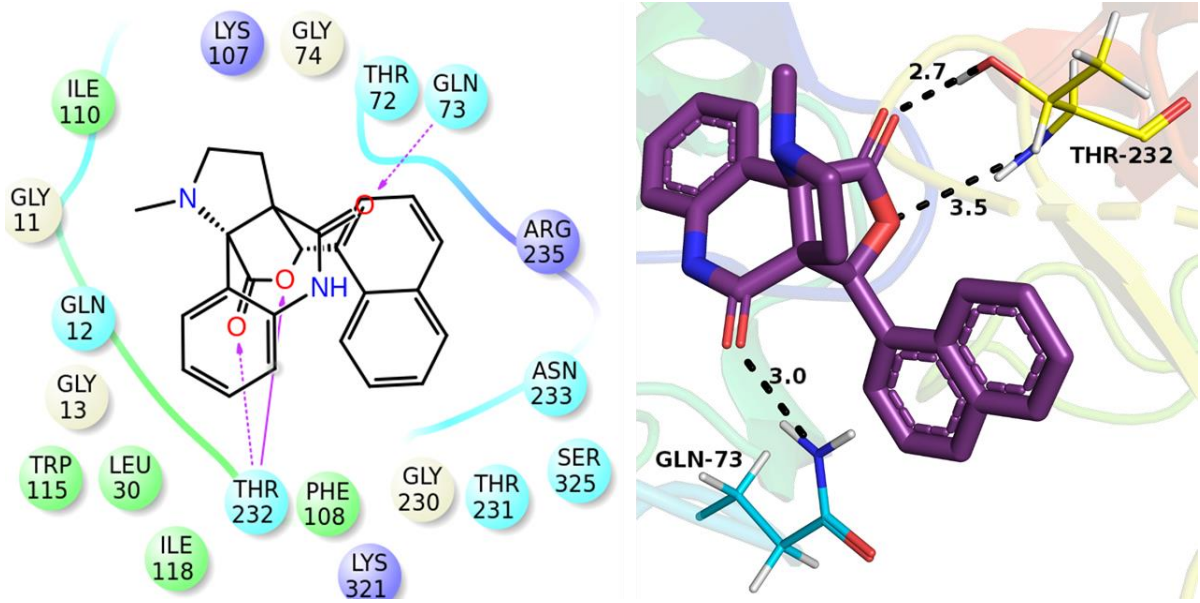


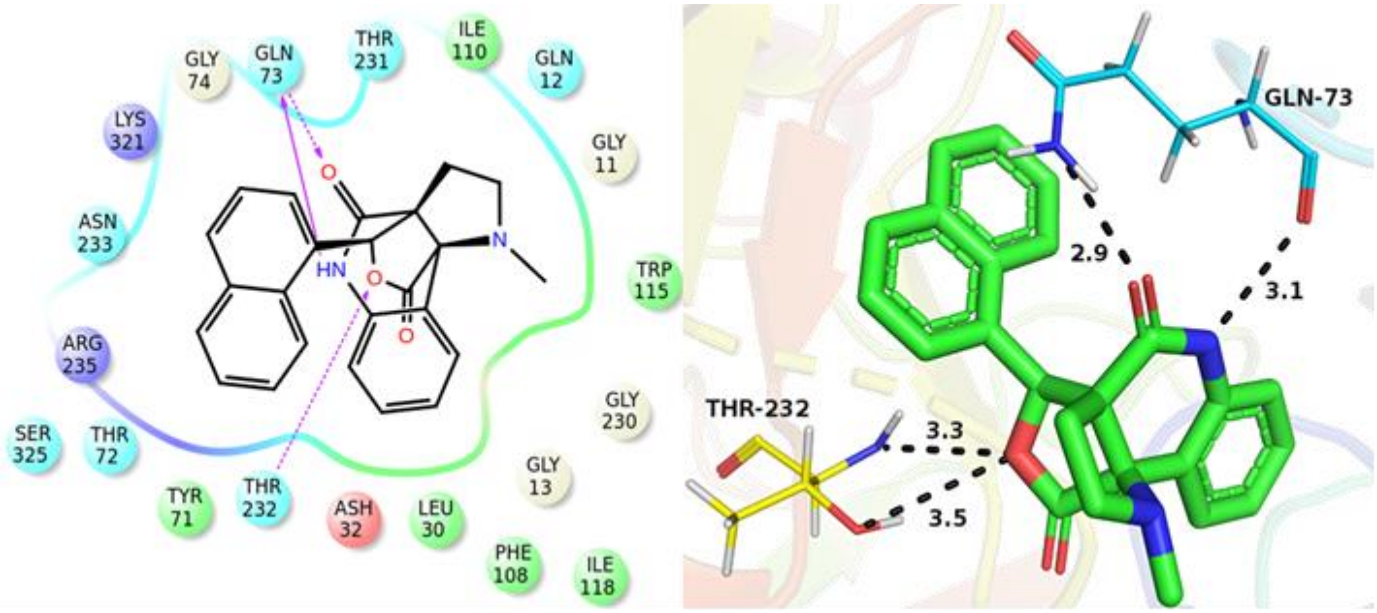
Figure 2: Cartoon representation of human BACE1 enzyme PDBID 2FDP (apo form).

***** Aspartic dyad has labeled in active sites**

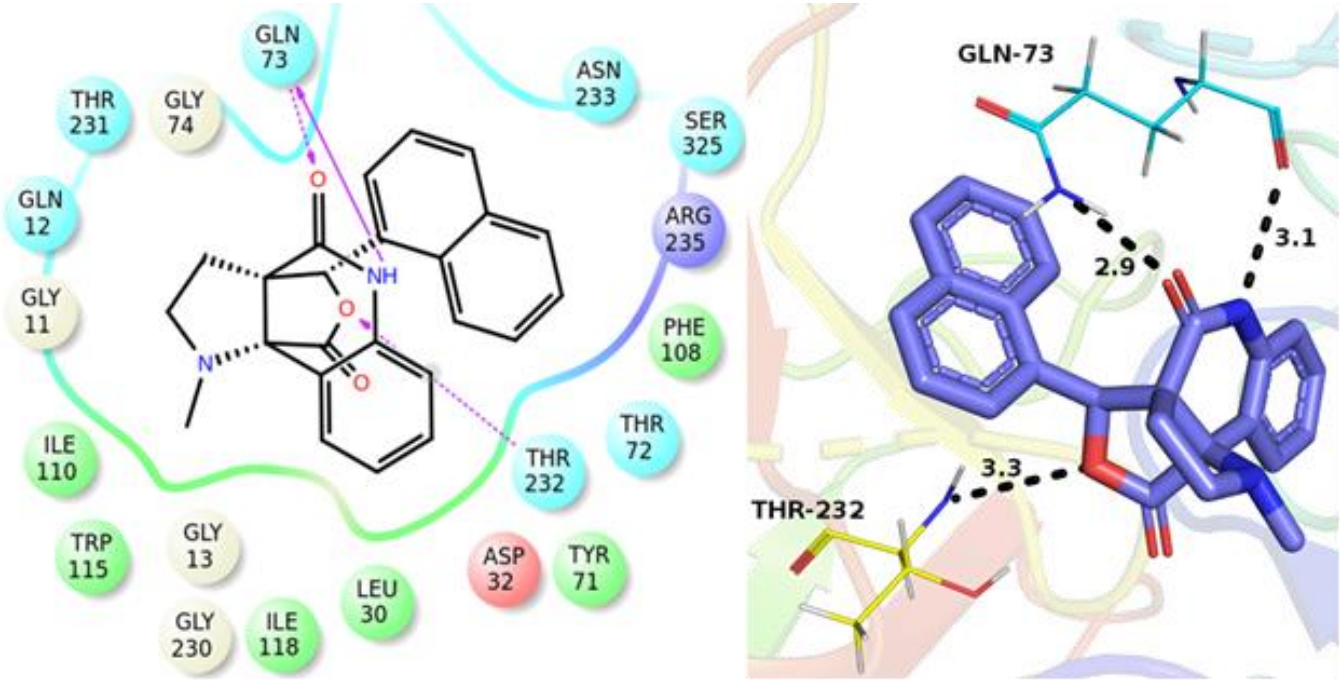
Human BACE1 structure was utilized to understand the binding characteristics of our compounds. From RCSB, the structure with PDBID 2FDP was retrieved and after protein preparation (Figure: 2), it was subjected to docking protocols. Induced fit docking procedures were carried out to understand the dynamic behavior of binding by allowing both the ligand and protein to undergo conformational changes. As it was mentioned in the introduction, different protonation states for the catalytic residues were considered for docking calculations. Table 1 are summarizes the findings. Among four different protonated combinations of catalytic residues compound 163 shows high binding energy (-56.31 kj/mol) with dual protonated states (ASH32 & ASH228) compared to other three states. At this best binding pose, the compound 163 exhibit hydrogen bond interaction with important beta harpin loop residue Gln73 which makes tight binding at the active site. Best ranked poses of enzyme-compound 163 interactions reveal Glide Scores of -6.39, -5.35, -5.1 and -8.19 respectively with ASP32 & ASP228, ASH32 & ASP228, ASP32 & ASH228 and ASH32 & ASH228 combinations (Figure:3). Compound 187 exhibits docking with BACE1 and the resulted Glide Scores are -7.3, -5.33, -6.76 and -7.27 respectively with ASP32 & ASP228, ASH32 & ASP228, ASP32 & ASH228 and ASH32 & ASH228 combinations. The better binding of compound 187 is found with ASP/ASP combination as evidenced from binding Glide energy of -55.67 (Figure: 4).

(a)

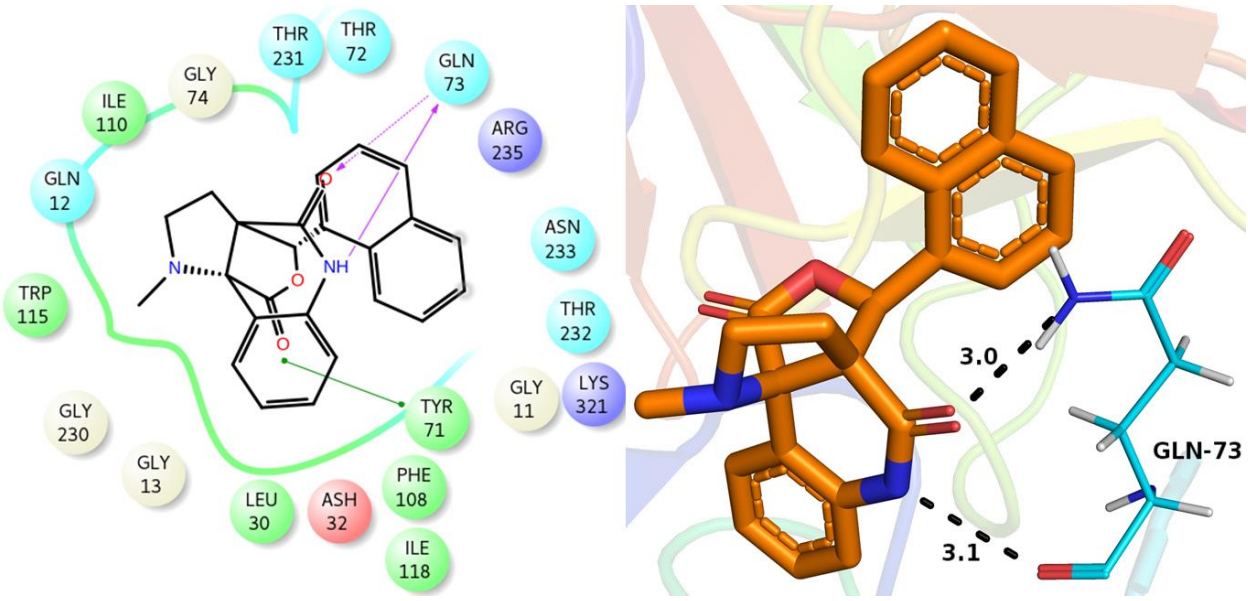




(b)

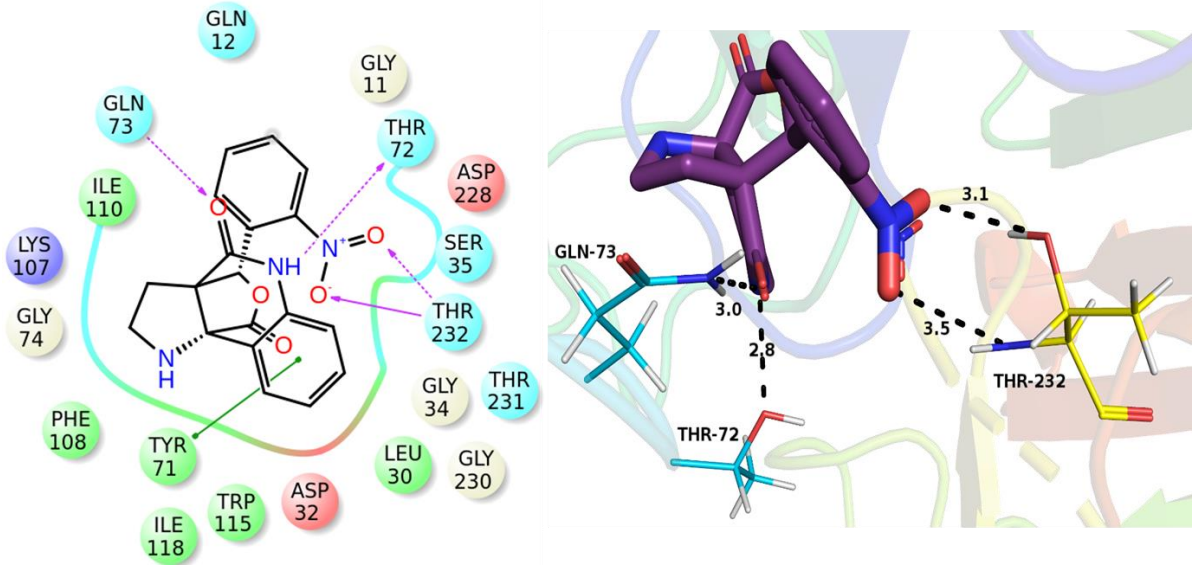


(c)

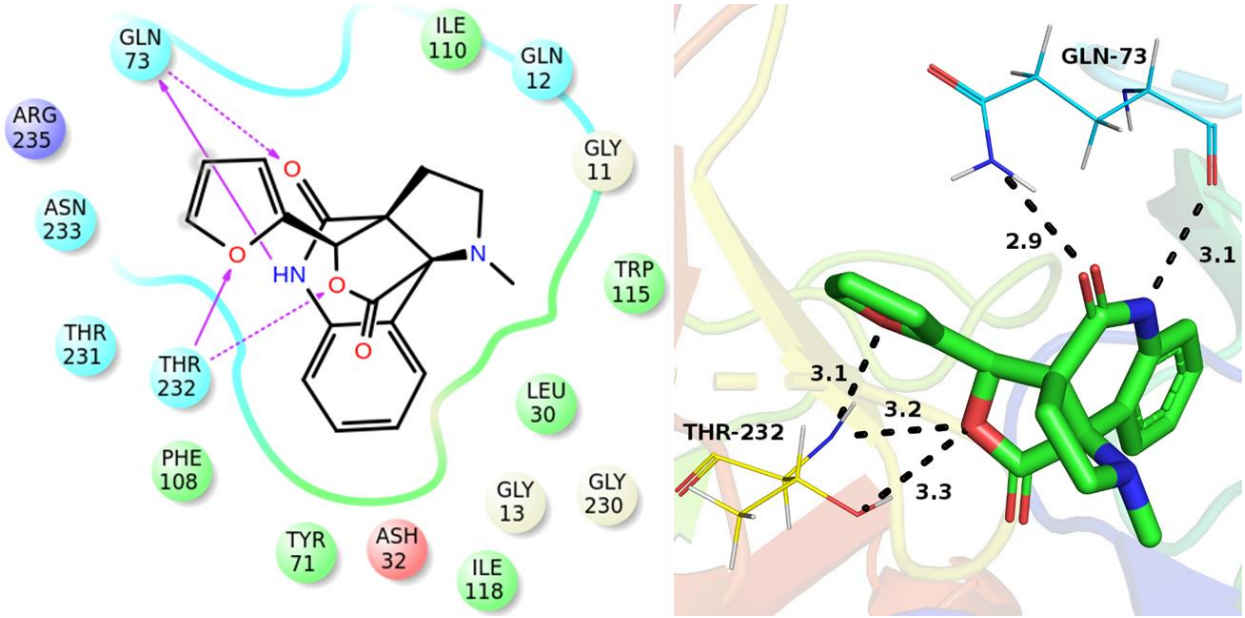


(d)

Figure 3: Interaction diagram of compound 163 (Schrodinger) revealing the ligand's interaction with protein for ASP32-ASP228 (a), for ASH32-ASP228 (b), ASP32-ASH228 (c) and for ASH32-ASH228 (d).



(a)



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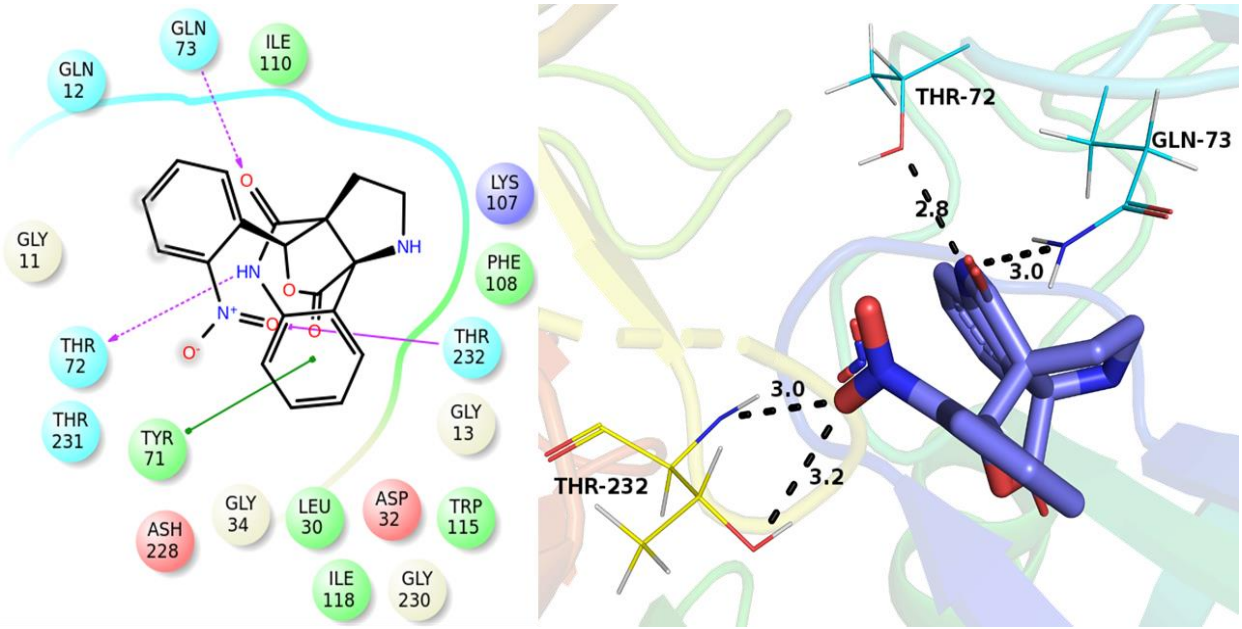

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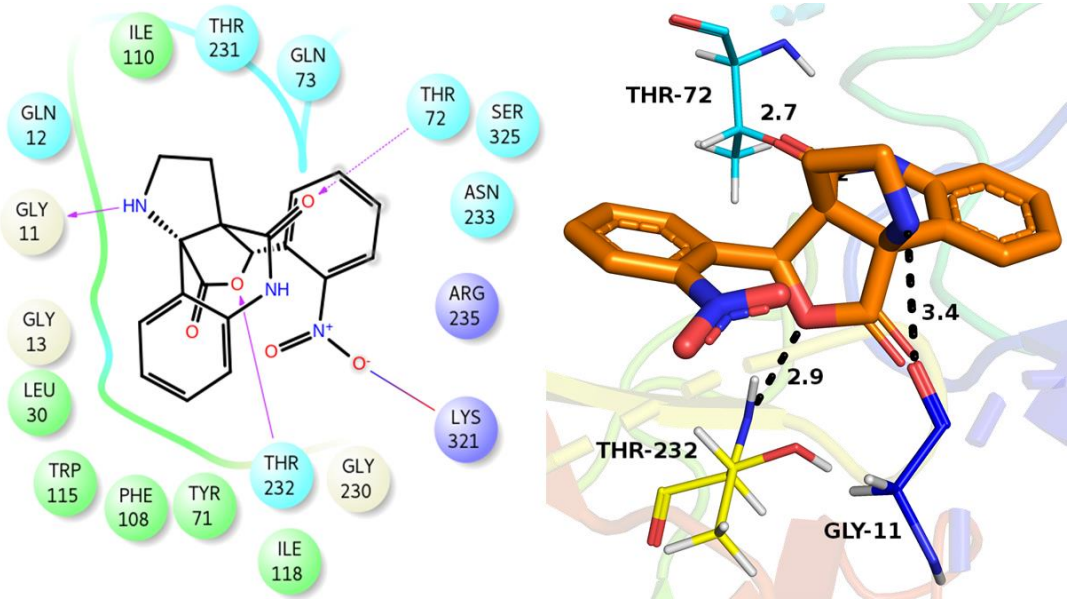
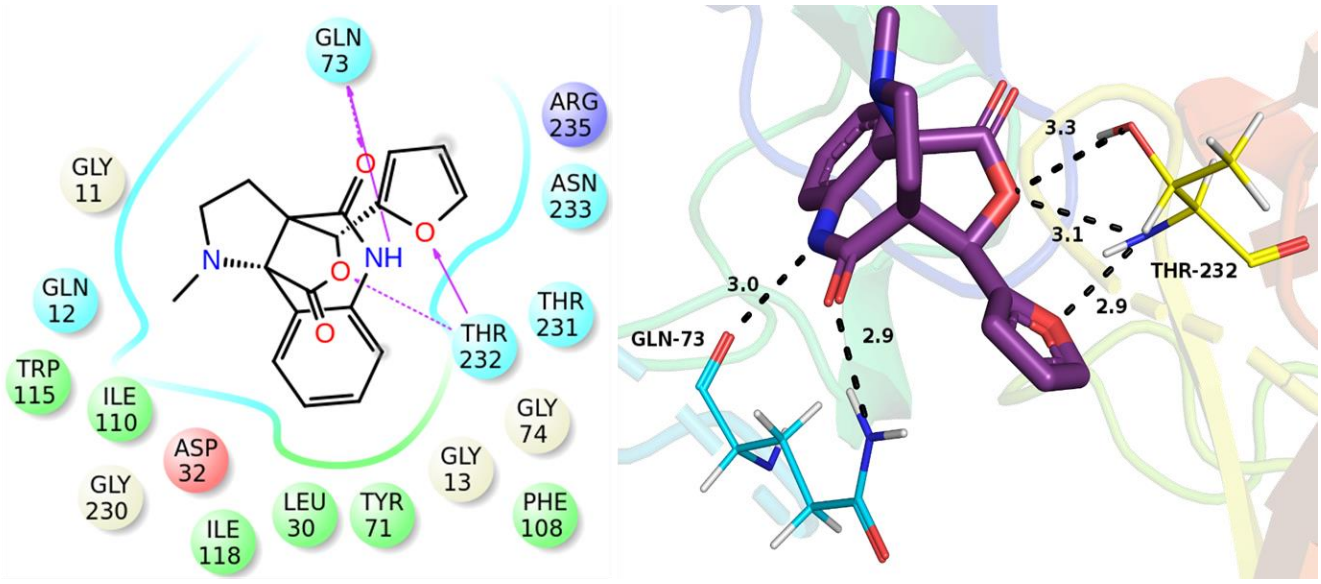


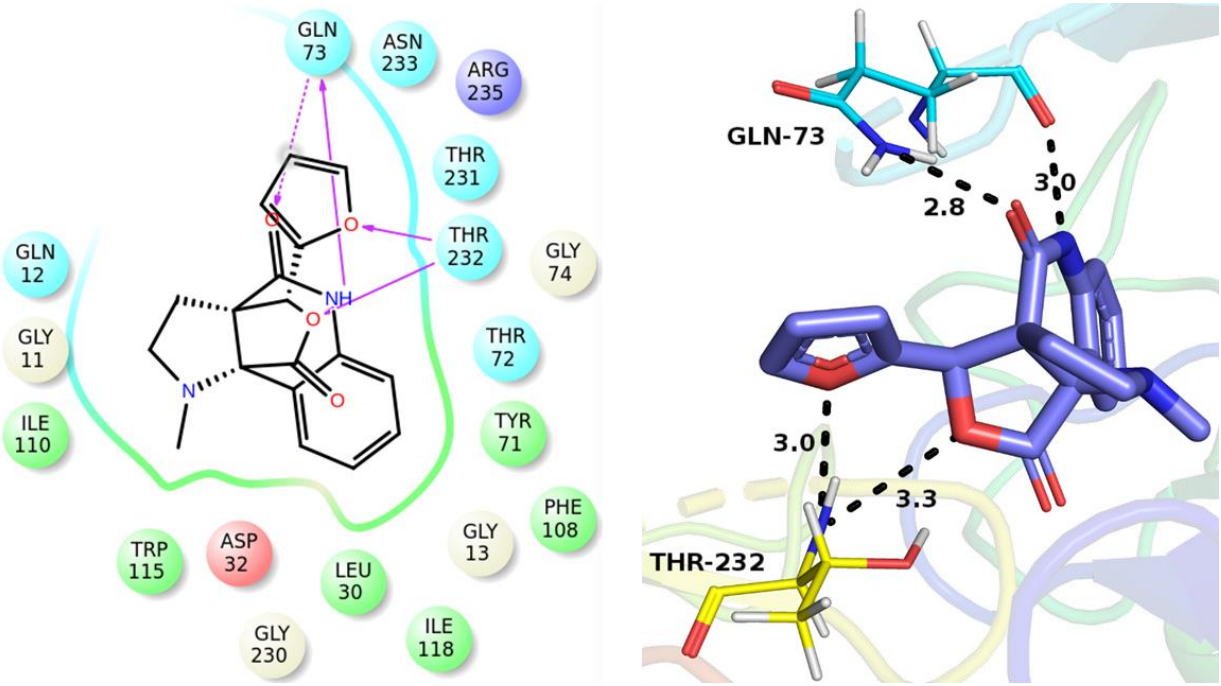
(d)

Figure 4: Interaction diagram of compound 187 (Schrodinger) revealing the ligand's interaction with protein for ASP32-ASP228 (a), for ASH32-ASP228 (b), ASP32-ASH228 (c) and for ASH32-ASH228 (d).

Similarly compound 216 also found to show fitting at the active site of with BACE1 and the Glide Scores are -5.7, -6.02, -6.63 and -6.71 respectively with ASP32 & ASP228, ASH32 & ASP228, ASP32 & ASH228 and ASH32 & ASH228 combinations. And relatively high binding energy was -54.19 as the result of binding with ASH/ASH combination (Figure: 5).



(a)



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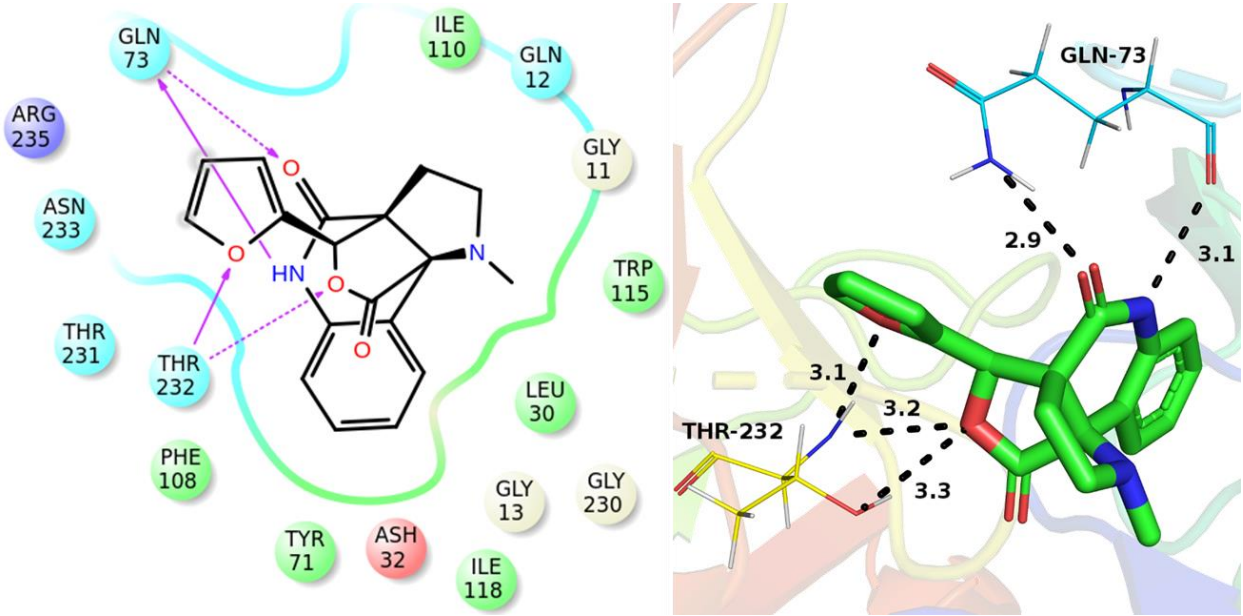

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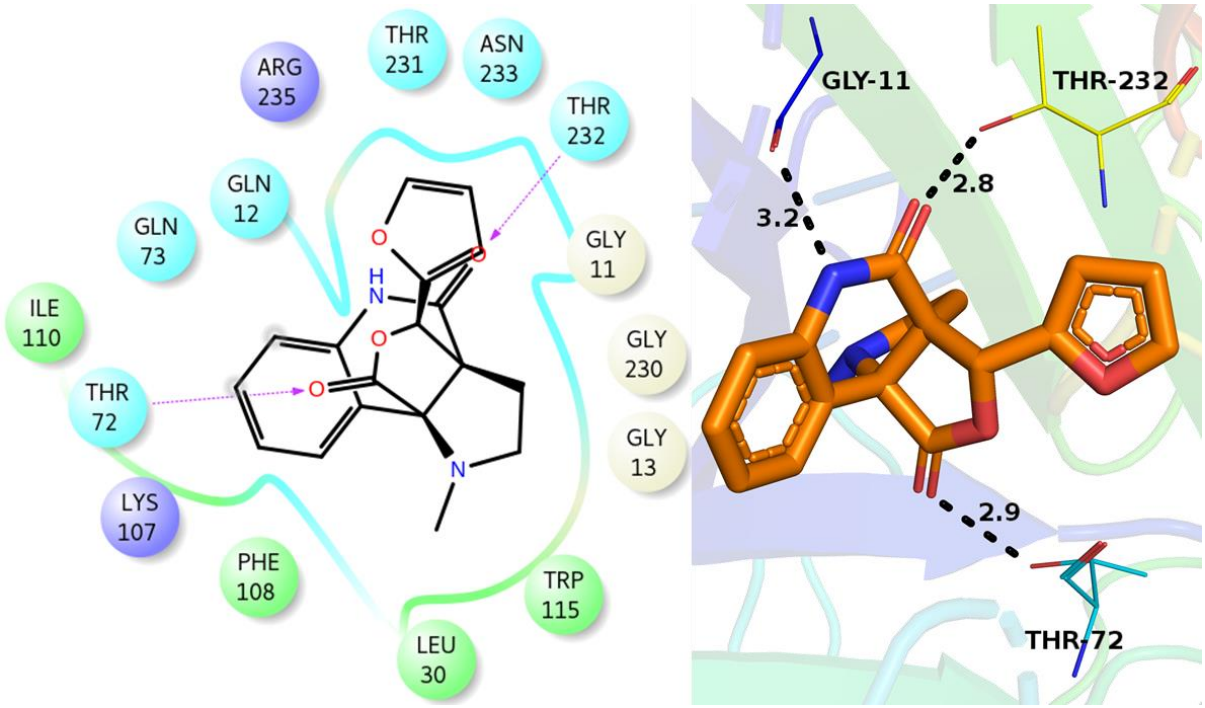
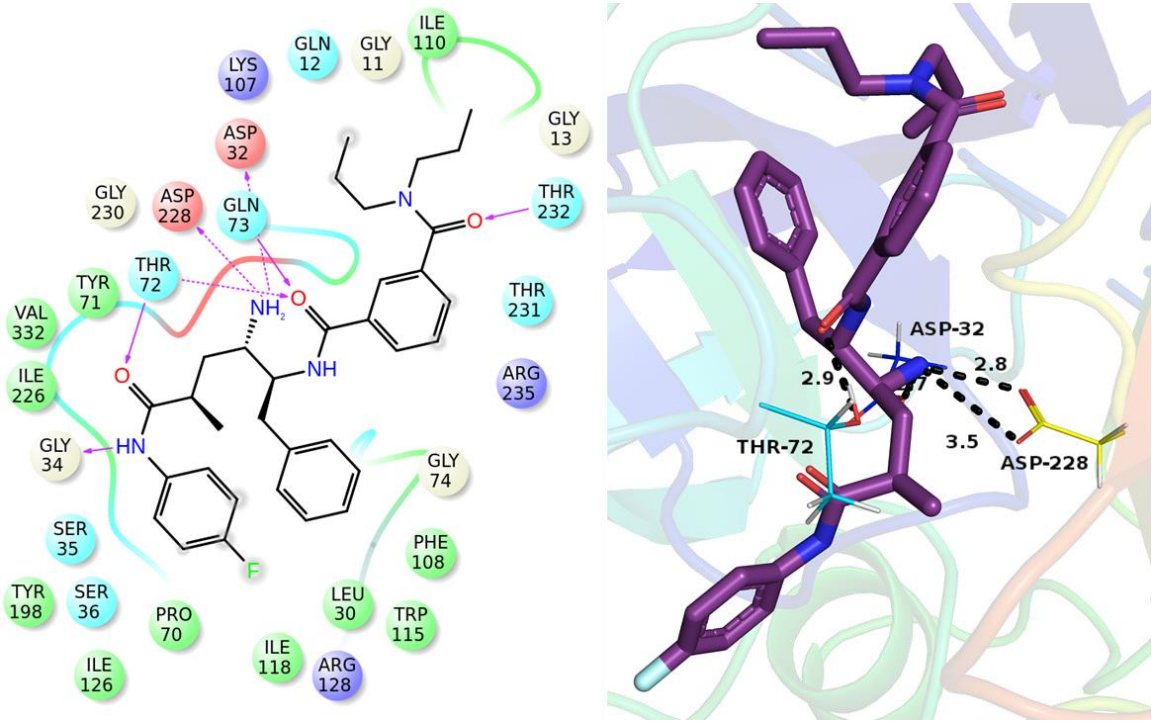


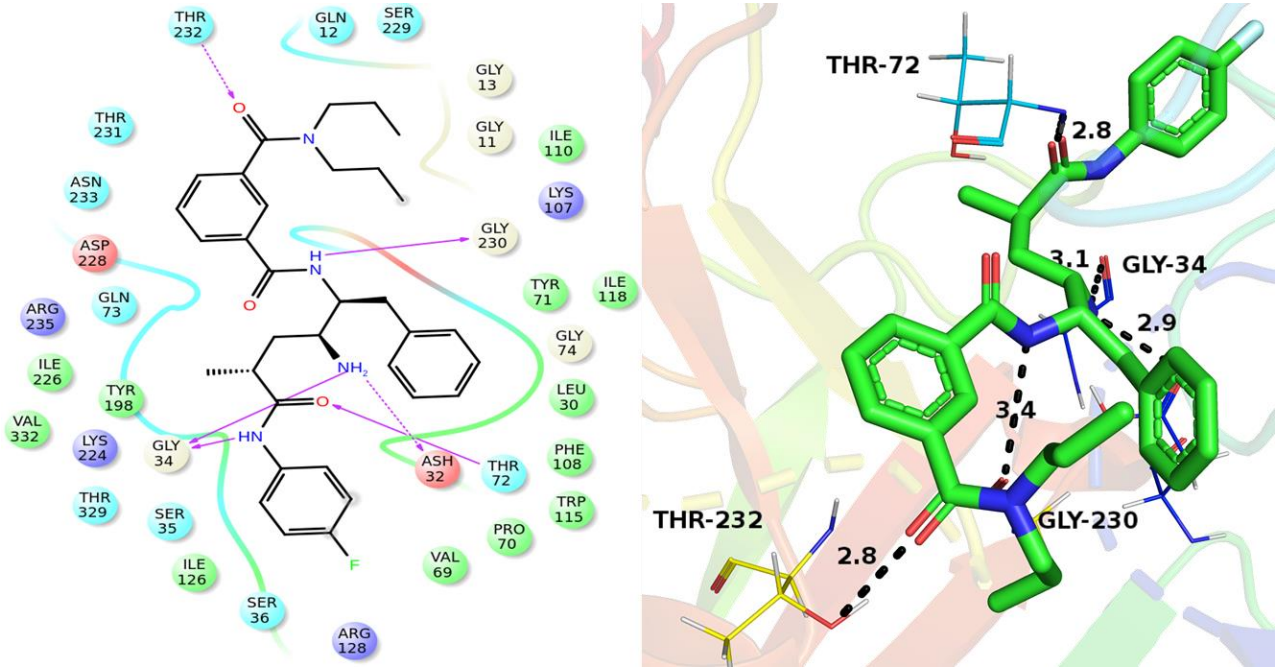
(d)

Figure 5: Interaction diagram of compound 216 (Schrodinger) revealing the ligand's interaction with protein for ASP32-ASP228 (a), for ASH32-ASP228 (b), ASP32-ASH228 (c) and for ASH32-ASH228 (d).

The ligand which is found complexed with BACE1 (PDBID: 2FDP) show better binding than all the other three compounds. Corresponding Glide score (enzyme-cocrystal) is double when compared to enzyme-compound163/compound187/compound216 complexes. Also the cocrystal significantly reduces the energy of enzyme-cocrystal complex. Mainly due to its big size to cover almost entire active site, individual interaction of many atoms with protein residues could reduce the total Glide energy which is ranged between -78.48 and -89.49 k/joule. It should be noted that the cocrystal ligand is designed based on the traditional approach of modifying peptide substrate analogue. The co crystal ligand contains four nitrogen atoms with basic characteristics and two carbonyl oxygen atoms along with aryl groups 3-dimensionally distributed (Figure : 6).



(a)



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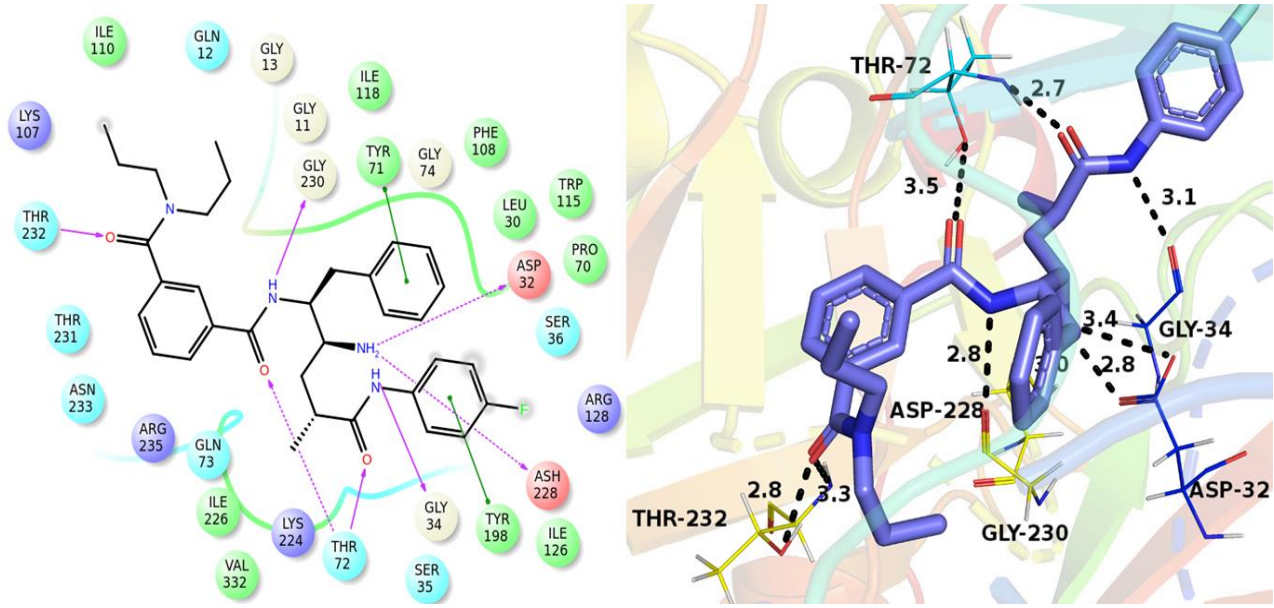

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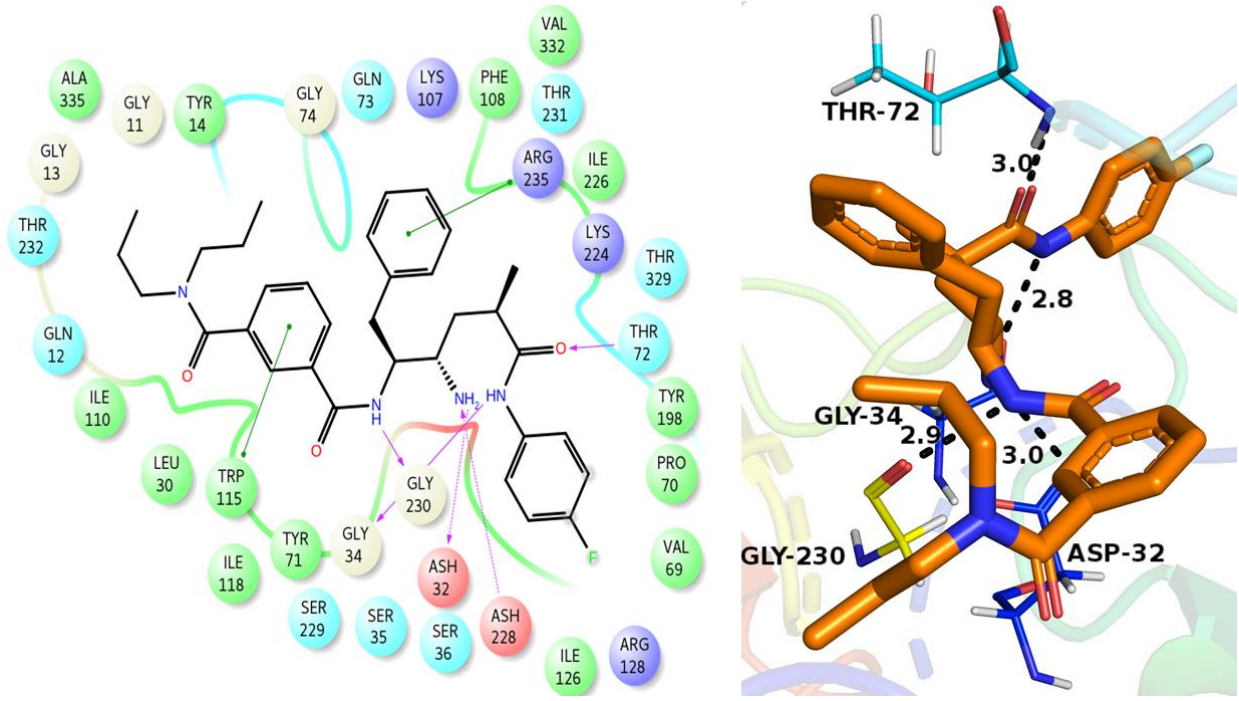

(d)

Figure 6: Interaction diagram of Cocrystal ligand (2FDP) (Schrodinger) revealing the ligand's interaction with different protonation's ASP32-ASP228 (a), for ASH32-ASP228 (b), ASP32-ASH228 (c) and for ASH32-ASH228 (d).

Table 1: Interactions of compounds and cocrystal at the active site of human BACE1 with four different protonation states of dyad (Asp32 and Asp228)

Compounds	**Docking score (Kcal/mole)**	**Glide energy (Kcal/mole)**	**Hydrogen bond interaction**
Dual Unprotanated (ASP32 & ASP228**)**			
Quin1	-6.39	-42.90	**Gln73**, Thr232.
Quin2	-7.3	-55.67	Thr72,**Gln73**, Thr232.
Quin3	-5.70	-44.81	**Gln73**, Thr232.
Cocrystal (Amino-ethylene inhibitor)	-15.68	-89.49	Asp32, Asp228, Thr72, **Thr72**.
Mono Protonated (ASH32 & ASP228**)**			
Quin1	-5.35	-53.28	**Gln73**, Thr232.
Quin2	-5.33	-48.24	Thr72, Thr232.
Quin3	-6.02	-46.33	**Gln73**, Thr232.
Cocrystal (Amino-ethylene inhibitor)	-15.46	-85.70	Thr72, Gly230, Gly34, Thr232.
(ASP32 & **ASH228) Mono Protonated**			
Quin1	-5.1	-53.64	Gln73, Thr232.
Quin2	-6.76	-52.54	Gln73, Thr72, Thr232.
Quin3	-6.63	-46.87	Gln73, Thr232.
Cocrystal (Amino-ethylene inhibitor)	-14.16	-78.48	Asp32, Ash22, Thr72, Gly34, Gly230, Thr232.
Dual Protonated (ASH32 & ASH228)			
Quin1	-8.19	-56.31	Gln73.
Quin2	-7.27	-46.43	Thr72, Thr232, Gly11.
Quin3	-6.71	-54.19	Thr72, Thr232.
Cocrystal (Amino-ethylene inhibitor)	-15.53	-84.92	Ash32, Ash228, Gly230, Gly34, Thr72.

INTERACTION WITH ACTIVE SITE

BACE1 has a wide active site along with the catalytic residues Asp32 and Asp2228 (Dyad). This cavity consists of three binding subsites. S1 subsite having Trp115, Phe108, Ile118, and Leu30 amino acids residues form site 1; Asp228,Thr232, Thr231, Val326, Ile226, Lys224, Thr72, Asn233 and Gly234 form site 2 and site 3 consists of Tyr71, Pro70, Val69, Arg128, Asn77, Ile126, Tyr198, Ser35, and Asp32. It can be realized that a bulky ligand or a ligand with three to four flexible groups may be needed to cover the entire site. Also a flap (loop) consists of Tyr71---Gly74 act as a lid to restrict the approach of substrate of ligand. From the results, it is noteworthy to mention that all the ligand i.e compounds 163,187 and 216 show interaction with Gln73 and Thr232 residues. The quinolinone carbonyl oxygen makes hydrogen bonding with Gln73 in all the compounds. Furanone moiety involve in interaction with Thr232 in 163 and 187 where as such interaction is rendered by nitro oxygen in case of compound 187. The residue Thr 72 is also an important amino acid present in the flap tip which covers the active site and the cocrystal is found to maintain its interaction with this residue in all protonation combinations. Compound 187, through quinolone nitrogen fulfill this interaction. This flap interaction which could be important to keep lid closed always is maintained by 163 and 216 via their binding to Gln73. None of the three compounds interact with catalytic dyad residues Asp32 and Asp228 which is very unusual whereas the cocrystal firmly binds with these residues in almost all the protonation combinations through a primary amine (Table 2a-c).

Table 2: (a) Interaction of Quin1 at the active site

(ASP32 & ASP228)	-6.39	-42.90
(ASH32 & ASP228)	-5.35	-53.28
(ASP32 & ASH228)	-5.1	-53.64
(ASH32 & ASH228)	-8.19	-56.31

Table 2: (b) Interaction of Quin2 at the active site

(ASP32 & ASP228)	-7.3	-55.67
(ASH32 & ASP228)	-5.33	-48.24
(ASP32 & ASH228)	-6.76	-52.54
(ASH32 & ASH228)	-7.27	-46.43

Table 2: (c) Interaction of Quin3 at the active site

(ASP32 & ASP228)	-5.70	-44.81
(ASH32 & ASP228)	-6.02	-46.33
(ASP32 & ASH228)	-6.63	-46.87
(ASH32 & ASH228)	-6.71	-54.19

DRUG LIKELINESS (ADME PROPERTIES)

In a computational approach, the interaction of the ligand with a protein target, particularly at the active/binding studies are analyzed. Even then, the compound's physico chemical properties also have to be analyzed to understand the possibility to elevate them as drugs for practical use. Apart from the structural features, the practical use is determined by its size, solubility, pH tolerance with reference to biological relevance, getting excreted if any unused quantity and with low or nil toxicity. From ADME analysis (Table 3), all the three compounds show better CNS (-2 inactive and +2 active as per standard) than that of cocrystal. Blood-Brain barrier crossing efficiency as per QPlogBB (desired values: -3 to 1.2) of all the compounds are encouraging. Interestingly, bioavailability in terms oral absorption is found remarkably high for the compounds when compared to cocrystal.

Table 3: ADME Properties of the three ligands and the Cocrystal: Though number of hydrogen donors and acceptors for making interactions are low, other parameters are highly desirable for all compounds.

Compound	CNS	Molecular weight	Donor HB	Accpt HB	QPlogBB	Human Oral Absorption	Percent Human Oral Absorption
Quin1	1	384.434	1	6.5	0.198	3	91.654
Quin2	1	365.345	2	7.5	-0.578	3	64.895
Quin3	1	324.335	1	7	0.262	3	82.248
Cocrystal	-2	560.71	4	9	-1.388	1	75.205

4. CONCLUSION

The compounds with furanone, quinolone and pyridine moieties were analyzed for their possible binding with AD target BACE1. The active site is composed of two aspartic residues which may undergo protonation or ligand induced protonation. Hence molecular docking was carried out through induced fit docking procedures and found all the compounds have reasonable binding at the active site particularly the lid region which may prevent enzyme starved for the poly peptide

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CONFLICT OF INTEREST

No conflict of interest related to the study.

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