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Research Article

Synthesis and Virtual Screening of Some Novel Quinazolinone Derivatives as Potent Cholinesterase Inhibitors against Alzheimer's Disease

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Abstract

In the past few decades, the cholinergic theory of Alzheimer's disease has been promoted as a crucial tool for the creation of new drugs. In this study, a series of novel quinazolinone scaffold were synthesized, docked and predicted for their ADMET studies for cholinesterase inhibitors against Alzheimer's disease. Docking study were performed, using Autodock 4.2 for the synthesized compounds **4a-c** and were observed to be well accommodated in the active site of AChE compared to standard Donepezil. Compounds **4d-f** were most suggested novel quinazolinone derivative that the inhibitor exhibited two hydrogen bonding interactions with AChE. *In-silico* drug-likeness and pharmacokinetic properties was predicted using Swiss ADME, pkCSM software. All synthesized compounds **4a-f** having better pharmacokinetic profile for potential to act as a cholinesterase inhibitors against Alzheimer's disease.

Keywords: Alzheimer's disease, Quinazolinone, Pharmacokinetic profile, Cholinesterase, Acetylcholinesterase (AChE).

1. INTRODUCTION

Dementia is the loss of cognitive functioning - thinking, remembering, and reasoning - to such an extent that it interferes with a person's daily life and activities. The most common type of dementia is Alzheimer's disease (AD), which is defined as a slowly progressive neurodegenerative disease. AD is characterised by death of cholinergic neurons, oxidative stress, neuritic plaques and neurofibrillary tangles as a result of amyloid-beta peptide (Aβ) accumulation in the most affected area of the brain.[1,2] Alois Alzheimer discovered the Alzheimer disease while analysing the brain of his patient who suffered from memory loss and behavioural changes before dying. AD is termed medical disorder by Emil Kraepelin for the first time.[3,4]According to WHO predictions, AD will be more common than AIDS, cancer, and cardiovascular disorders in the coming century.[5] By 2050, it's estimated that more than 46 million individuals would have AD, and that number will have increased threefold.[6] Alzheimer's disease is a neurological illness that develops over time and is caused by the loss of neurons. It usually begins in the hippocampus's entorhinal cortex. Both early and late-onset Alzheimer's disease have been linked to a hereditary component. Numerous risk factors have been connected to Alzheimer's disease. The major risk factor for Alzheimer's disease is growing older. Traumatic head injury, depression, cardiovascular and cerebrovascular disease, parental age, smoking, family history of dementia, elevated homocysteine levels, and the presence of the APOE e4 allele have all been linked to an increased risk of Alzheimer's disease.[7,8,9]

Acetylcholine are hydrolytically metabolized by cholinesterase enzyme which are two type acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE). Human brain ACh levels are markedly reduced in AD, various approaches to enhance Ach level have been tried. AchE is a key component of cholinergic brain synapses and neuromuscular junctions. AChE inhibitor is a successful strategy to treat AD. Four inhibitors of AChE currently are approved by the FDA for treatment of Alzheimer's disease: tacrine, donepezil, rivastigmine and galantamine (Figure 1). And memantine is only drug that can be antagonist N-methyl- aspartate (NMDA) receptor.[10,11]

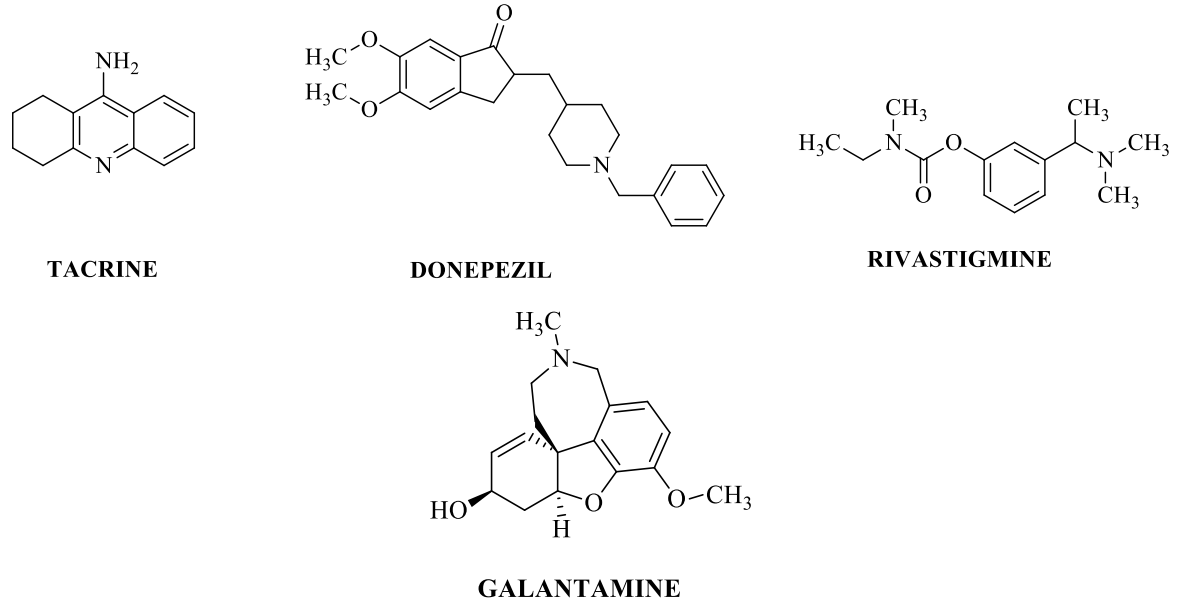


Figure 1: AChE inhibitors drugs used for the treatment of AD

Quinoline/quinazolines are one of the versatile nucleus that will show many type of interactions and effective for their CNS activities. Based on the study done by Maurizio Anzini *et al.* amongst the six quinoline-piperonal hybrids, structure **(X)** was evaluated as potential drugs against AD. Theoretical analysis of the pharmacokinetic and toxicological properties of the compounds suggest that they present good oral bio-availability and are also capable of penetrating the blood–brain barrier, qualifying as leads for new drugs against AD. Evaluation of their inhibitory capacity against AChE and BuChE through Ellman's test showed that three compounds present promising results with one of them being capable of inhibiting both enzymes.[12] These compounds have quinoline moiety and hydrazine-carboximidamide in their structure which is helpful foe the enzyme interections.

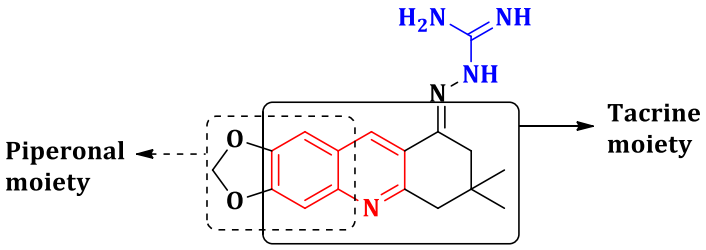


Figure 2: Structure of Quinoline-Piperonal Hybrids **(X)**

The acridine derivative, tacrine **(I)** was one of the earliest ChEI developed to treat AD. Some natural compounds from plant and animal sources are studied for AD, among them oroidin **(II)** from Agelas oroides (Turkish marine sponges) shows a moderate level of AChE inhibition and possesses cyclic guanidine in the structure.[13] Based on above literature, the structure of tacrine (tricyclic ring system) **(I)** and the guanidine base ring system **(II)** it is proposed to synthesize quinazolinone derivatives **(III)** as shown in Figure-2, which was then docked on active site of AchE and evaluated for their ADME properties.

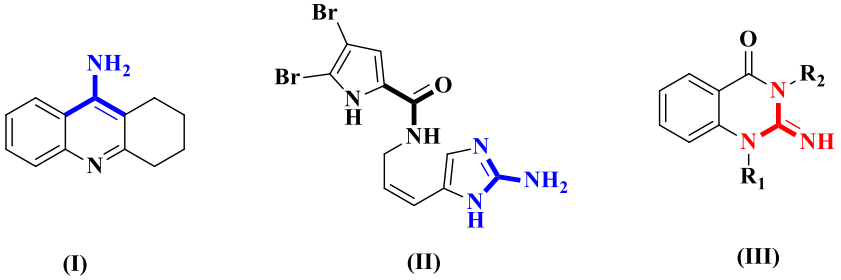
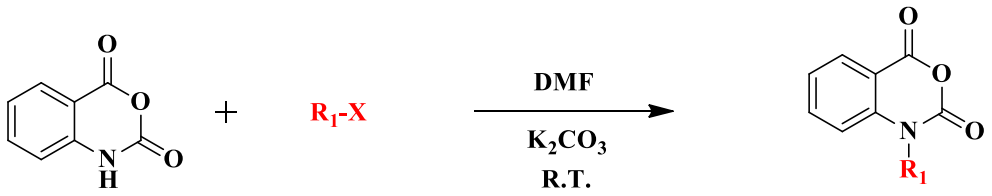


Figure 3: Proposed synthesize quinazolinone derivatives **(III)**

2. RESULT AND DISCUSSION

2.1. Chemistry

Isatoic anhydride **1**, in aliphatic nucleophilic substitution reaction, reacts with aryl/alkyl halide in the presence of potassium carbonate (K_2CO_3) to form intermediate **2a-f** as depicted in Scheme 1. Compounds **2a-f** react with different amines for nucleophilic attack on carbonyl carbon of N_1 substituted isatoic anhydride to form compounds **3a-f** which undergoes the cyclization reaction with cynogen bromide to form final compounds **4a-f** in competitive yields. All the final compounds were purified by column chromatography using appropriate solvents.

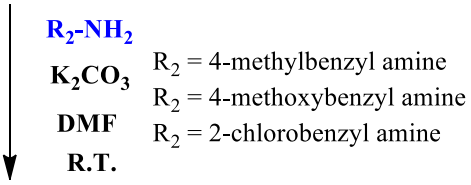


1

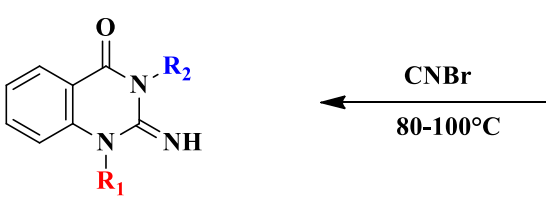
R_1 = 2,4-dichlorobenzyl chloride
R_1 = 4-chlorobenyl chloride
R_1 = 4-methylbenzyl chloride
R_1 = 2-methylbenzyl chloride
R_1 = 4-chlorobenzyl chloride
R_1 = 4-chlorobenzyl chloride

2a-f

2a: R_1 = 2,4-dichlorobenzyl chloride
2b: R_1 = 4-chlorobenyl chloride
2c: R_1 = 4-methylbenzyl chloride
2d: R_1 = 2-methylbenzyl chloride
2e: R_1 = 4-chlorobenzyl chloride
2f R_1 = 4-chlorobenzyl chloride



R_2 = 4-methylbenzyl amine
R_2 = 4-methoxybenzyl amine
R_2 = 2-chlorobenzyl amine



3a-f

3a: R_1 = 2,4-dichlorobenzyl chloride, R_2 = 4-methylbenzylamine
3b: R_1 = 4-chlorobenyl chloride, R_2 = 4-methoxybenzyl amine
3c: R_1 = 4-methylbenzyl chloride, R_2 = 4-methylbenzyl amine
3d: R_1 = 2-methylbenzyl chloride, R_2 = 4-methoxybenzyl amine
3e: R_1 = 4-chlorobenzyl chloride, R_2 = 2-chlorobenzyl amine
3f: R_1 = 4-chlorobenzyl chloride, R_2 = 4-methylbenzyl amine

4a-f

4a: R_1 = 2,4-dichlorobenzyl chloride, R_2 = 4-methylbenzylamine
4b: R_1 = 4-chlorobenyl chloride, R_2 = 4-methoxybenzyl amine
4c: R_1 = 4-methylbenzyl chloride, R_2 = 4-methylbenzyl amine
4d: R_1 = 2-methylbenzyl chloride, R_2 = 4-methoxybenzyl amine
4e: R_1 = 4-chlorobenzyl chloride, R_2 = 2-chlorobenzyl amine
4f: R_1 = 4-chlorobenzyl chloride, R_2 = 4-methylbenzyl amine

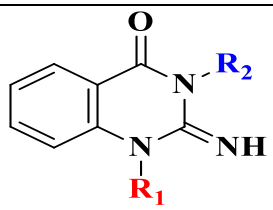
Scheme 1: Synthesis of final compounds **4a-f**

FTIR spectra of the intermediate **2a-f** showed N-H stretching vibration peak of isatoic anhydride disappeared in N_1 substituted isatoic anhydride and two stretching vibrations peak for carbonyl group of anhydride were intact in the corresponding substituted isatoic anhydride. An IR spectrum showed peaks at the range 1780-1760 cm^{-1} and 1725-1715 cm^{-1} for carbonyl stretching of isatoic anhydride and they appeared at lower wave number than the usual anhydride carbonyl stretching due to the presence of nitrogen in the ring. All intermediate **2a-f** showed a peak in the region of 1300-900 cm-1 due to C-O stretching. FTIR spectra of compounds **3a-f** show N-H stretching peak near 3300 cm^{-1}. The carbonyl stretching was observed near 1625 cm^{-1}. Two N-H bending peaks near 1540 and 1515 cm^{-1} along with bands 820, 750 and 700 due to para-disubstituted, ortho-disubstituted and mono-substituted aromatic rings were observed. FTIR spectrum of all final compounds **4a-f** indicated N-H stretching peak in a region of 3350-3270 cm^{-1}. Aromatic C-H peak is in region of 3150-3050 cm^{-1}. Carbonyl stretching of amide gives peak around 1625 cm^{-1}; carbonyl gives at lower wavenumber due to presence of adjacent nitrogen. Resonance effect is observed when the unpaired electrons on nitrogen atom conjugate with the carbonyl group, resulting in increased single bond

character and a lowering of the C=O absorption frequency. A peak around 1600 cm-1 is due to C=N stretching.

^{1}H NMR spectra of all final compounds **4a-f** showed C=NH proton at around δ value 8.2 and aromatic protons in the range of 6.2-7.5 and all alkanyl proton in the range of 4.43-4.62 ppm. For the purpose of confirming the predicted structures of all the final compounds **4a-f**, the ESI mass spectra of all the compounds displayed peaks at relevant M+ and M+2 m/z and complimented the FTIR & ^{1}H NMR spectra.

Table 1: Physical data of novel quinazolinone derivatives **4a-f**



Com.	R_1	R_2	M. wt	M.P (°C)	Rf*	% Yield
4a	2,4-dichlorobenzyl chloride	4-methylbenzyl amine	424	152-154	0.4	70
4b	4-chlorobenzyl chloride	4-Methoxybenzyl amine	405	180-184	0.6	75
4c	4-Methylbenzyl chloride	4-Methylbenzyl amine	369	131-133	0.7	82
4d	2-Methylbenzyl amine	4-Methoxybenzyl amine	385	141-145	0.8	60
4e	4-chlorobenzyl chloride	4-Methoxybenzyl amine	410	179-183	0.6	83
4f	4-chlorobenzyl chloride	4-Methoxybenzyl amine	389	158-160	0.7	87

* Mobile phase: n-hexane: ethyl acetate

2.2. DOCKING STUDIES

2.2.1. Preparation of Receptor

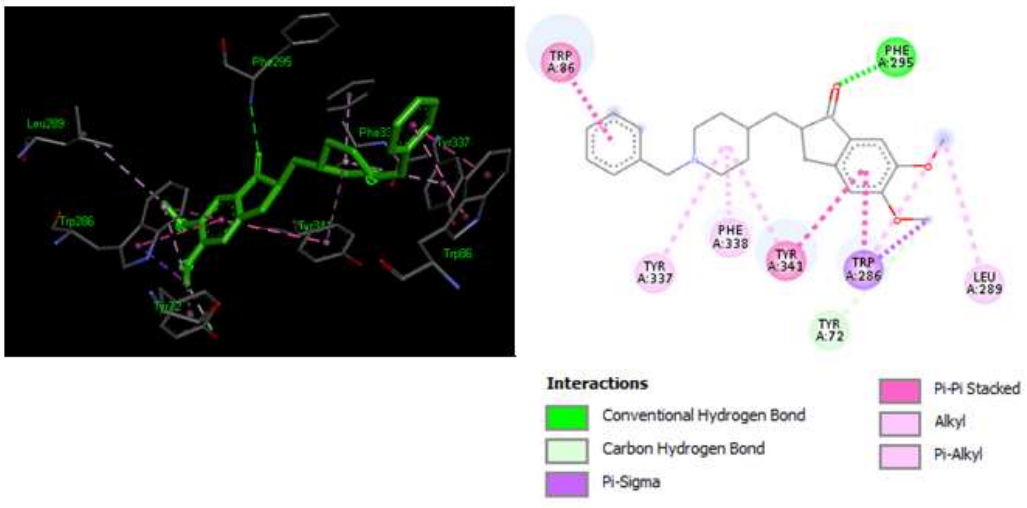
The X-ray crystal structure of recombinant human Acetylcholinesterase (PDB ID: 4EY7, resolution = 2.35 Ă) recovered from Protein Data Bank (https://www.rcsb.org). The protein structure was created by removing water molecules and co-crystallized ligand, adding missing hydrogen atoms, and adding rotatable bonds using the Discovery Studio Visualizer (version 3.1) and AutoDock Tools (ADT; version 1.5.4). For further analysis, the file was saved in the pdbqt file format.

2.2.2. Preparation of ligands

The chemical structures of novel quinazolinone derivatives were constructed using ChemDraw12.0 and they saved in PDB format. The acyclic dihedral angles were given free to rotate and flexible torsions were assigned in order to optimized the structures using "Prepare Ligands" in AutoDock 4.2. After that, the file was stored in pdbqt file format for further analysis.

2.2.3. Moleular docking method

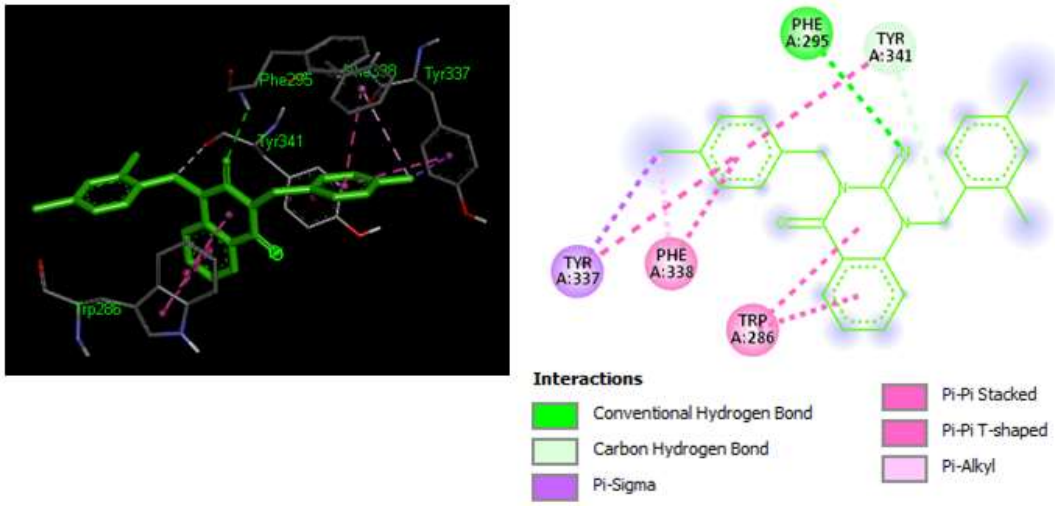
The docking performed using AutoDock 4.2. software in which grid box were generated without water molecule. The appropriate ligand binding site in the protein structure served as the centred point for the grid maps. After, docking were carried out generated output file that contain best computational binding pose. This output file were open into Discovery Studio Visulizer (version 3.1) with appropriate receptor (PDB ID: 4EY7) and created 3D and 2D ligand-receptor interaction mode.



(A) **(B)**

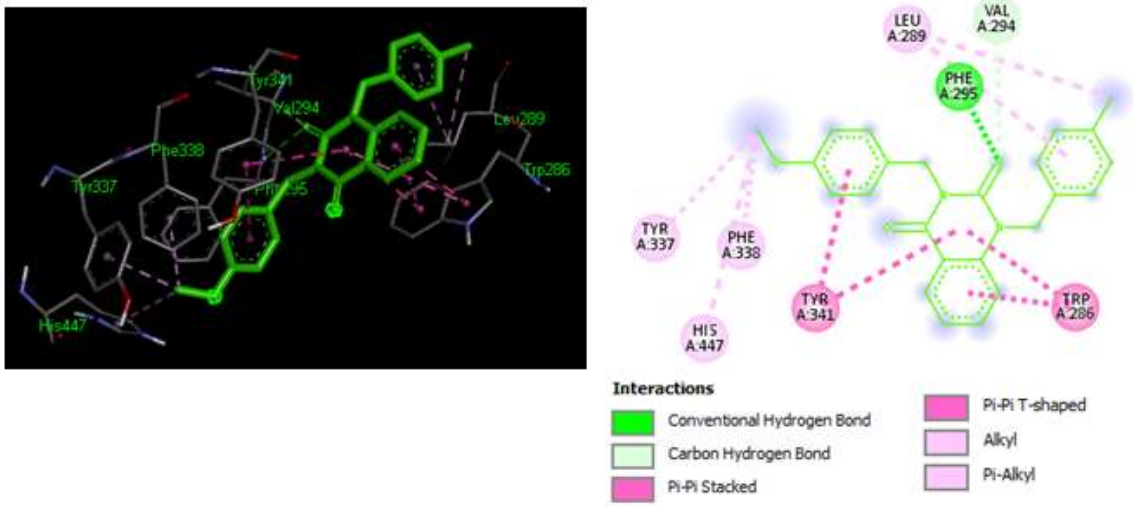
Figure 4: Molecular docking of compound **Donepezil** (A) 3D interaction (B) 2D interaction

Docking studies of Donepezil indicated 1H-inden-1-one formed conventional hydrogen bond with PHE A:295, π-sigma bond with TRP A:286. While benzene ring showed π-π stacked interaction with TRP A:86. Also, piperidine ring formed π-π stacked interaction with TYR A:341 and PHE A:338. Ligand interaction for Donepezil is shown in Figure 3.



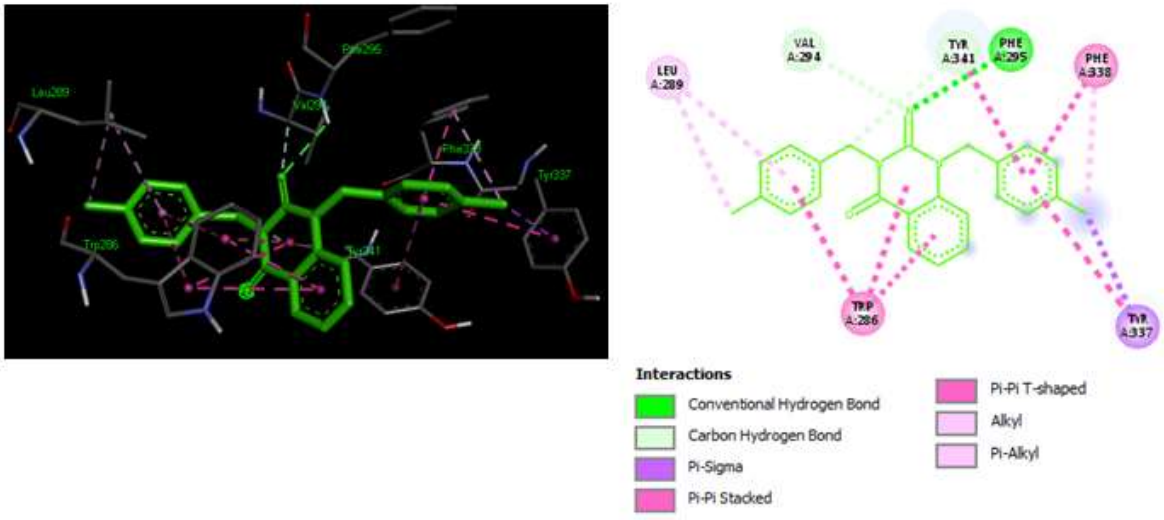
(C) **(D)**

Figure 5: Molecular docking of compound **4a** (C) 3D interaction (D) 2D interaction



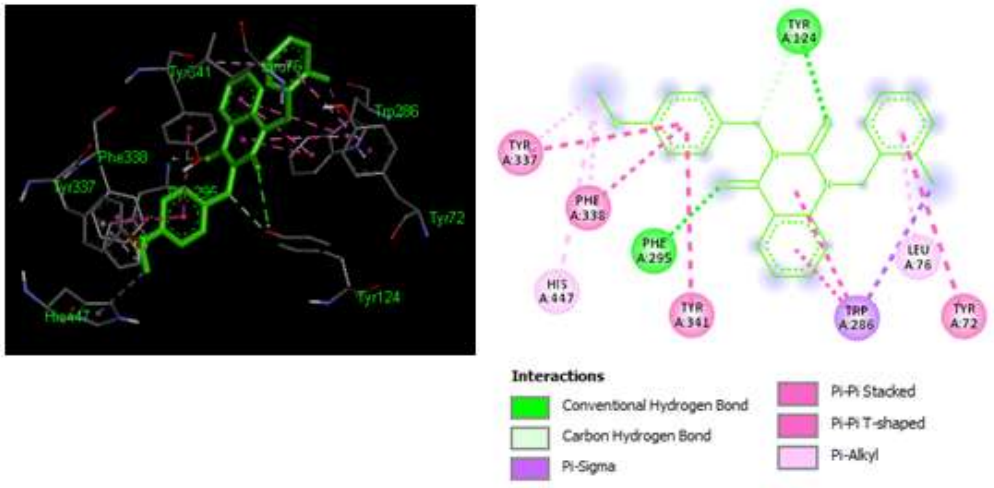
(E) **(F)**

Figure 6: Molecular docking of compound **4b** (E) 3D interaction (F) 2D interaction



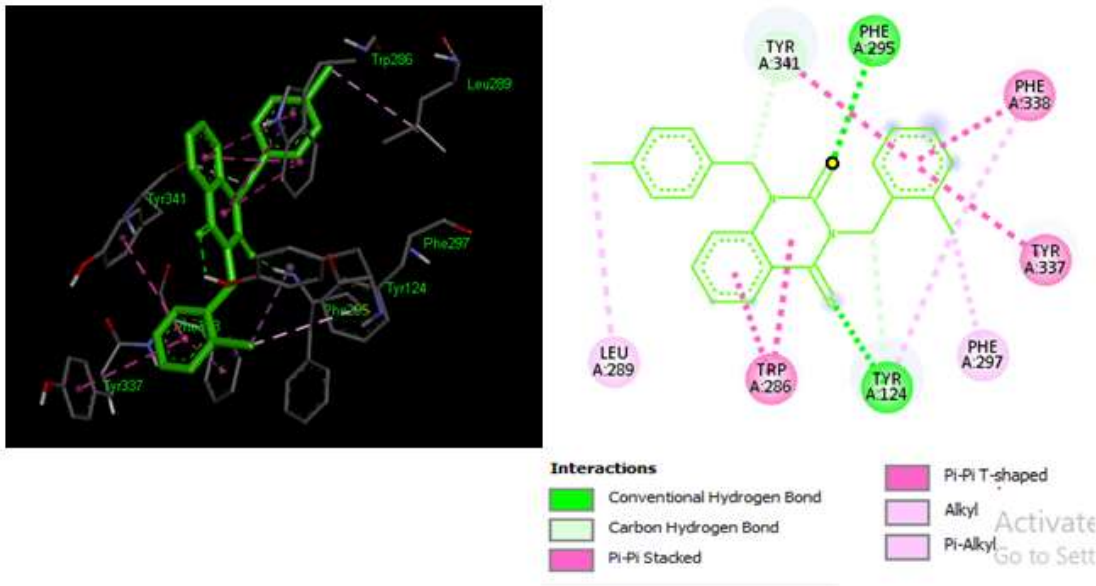
(G) **(H)**

Figure 7: Molecular docking of compound **4c** (F) 3D interaction (H) 2D interaction



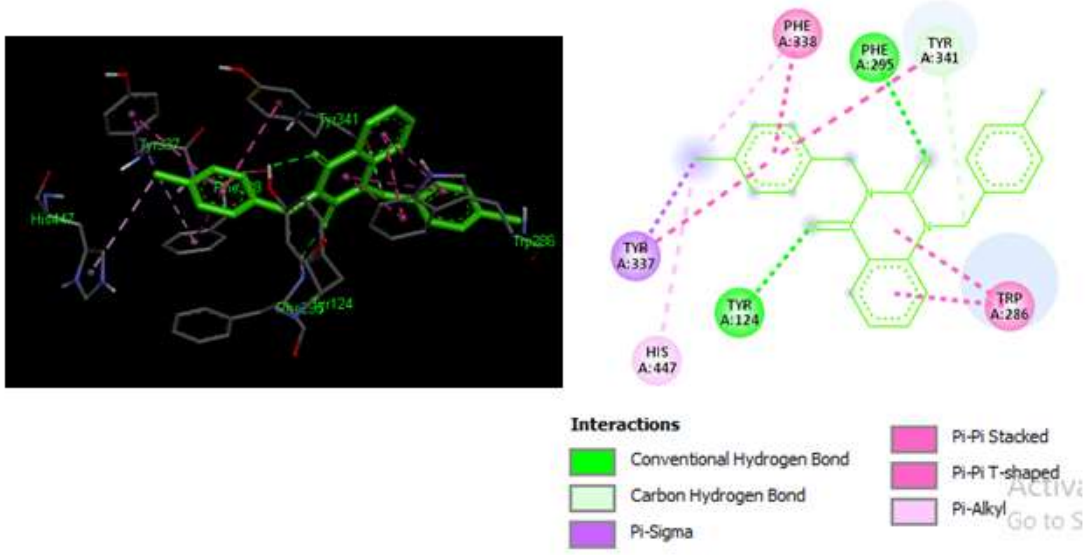
(I) **(J)**

Figure 8: Molecular docking of compound **4d** (I) 3D interaction (J) 2D interaction



(K) **(L)**

Figure 9: Molecular docking of compound **4e** (K) 3D interaction (L) 2D interaction



(M) **(N)**

Figure 10: Molecular docking of compound **4f** (M) 3D interaction (N) 2D interaction

According to analysis of the optimum conformational binding pose of all the synthesized quinazolinone derivatives. The compounds **4a-c** showed at least one hydrogen bond interaction with PHE A:295 within the binding pocket of AChE same as standard donepezil and The molecular docking results of most active compound among the compound **4d-f** revealed that the

inhibitor exhibited two hydrogen bonding interactions, with TYR A:124 and PHE A:295.

2.2.4. Prediction of *In-silico* drug-likeness properties

The theoretical pharmacokinetic properties of the ligands were evaluated with the help of in-silico prediction studies using pkCSM online software to predict the drug-likeness of the ligands. Pharmaceutically important features such molecular weight, H-bond donors and acceptors, octanol-water partition coefficient (LogP), lipinski rule violations, and rotatable bonds were determined by the software.

Table 2: Prediction of *in-silico* drug-likeness properties novel quinazoline derivatives[a]

Com.	MW	Molecular formula	HBD	HBA	Rotatable bonds	LogP	Lipinski rule violations
4a	424.32	$C_{13}H_{19}Cl_2N_3O$	1	2	4	3.43	0
4b	405.88	$C_{23}H_{20}ClN_3O_2$	1	3	5	3.75	0
4c	369.46	$C_{24}H_{23}N_3O$	1	2	4	3.58	0
4d	385.46	$C_{24}H_{23}N_3O_2$	1	3	5	3.30	0
4e	410.30	$C_{22}H_{17}Cl_2N_3O$	1	2	4	3.55	0
4f	389.88	$C_{23}H_{20}ClN_3O$	1	2	5	3.57	0

[a] Abbreviations: MW = Molecular weight, LogP = partition coefficient, HBD = hydrogen bond donor, HBA = hydrogen bond acceptor.

All of the final compounds **4a–f** were found to have expected lipophilicity (reported as LogP) values that were significantly higher than the standard cut-off value of 5 considered for drug design. The adequate molecular weight (MW 500) required for a successful penetration across biological membranes was shown by quinazolinone derivatives in this study. For all the compounds, the number of hydrogen bond acceptors (HBA ≤10) and donors (HBD ≤5) followed Lipinski's rule of five.

2.2.5. Prediction of pharmacokinetic properties

The pharmacokinetic properties were carried out by using SwissADME software to predicted the absorption study (water solubility, intestinal absoption, $CaCO_2$ and skin permeability), distribution study [volume of distribution (VD), CNS and BBB permeability], metabolism study (CYP2C9 inhibitor, CYP3A4 inhibitor, CYP2D6 substrate, CYP1A2 inhibitor and CYP2C19 inhibitor) and Excretion study [Total Clearance and Renal OCT2 (organic cation transporter 2) substrate].

Table 3: Prediction of ADME properties

Absorption					
Compound No.	**Water solubility**	**$CaCO_2$ permeability**	**Intestinal Absorption (human)**	**Skin permeability**	**p-glycoprotein substrate**
4a	-5.135	0.995	94.755	-2.748	Yes
4b	-4.962	1.008	96.147	-2.77	Yes
4c	-4.904	1.001	97.27	-2.755	Yes
4d	-4.884	1.018	97.97	-2.728	Yes
4e	-4.956	1.021	93.788	-2.729	Yes
4f	-4.972	0.999	95.812	-2.752	Yes
Distribution					
Compound No.	**VD (human)**	**Fraction unbound (human)**	**BBB permeability**	**CNS permeability**	
4a	-0.082	0.126	0.295	-1.528	
4b	-0.103	0.169	0.172	-1.828	
4c	-0.07	0.166	0.326	-1.582	
4d	-0.074	0.16	0.191	-1.86	
4e	-0.063	0.116	0.162	-1.832	
4f	-0.072	0.159	0.313	-1.542	
Metabolism					
Compound No.	**CYP2C9 Inhibitor**	**CYP3A4 Inhibitor**	**CYP2D6 substrate**	**CYP1A2 inhibitor**	**CYP2C19 Inhibitor**
4a	Yes	Yes	No	Yes	yes

4b	Yes	Yes	No	Yes	Yes
4c	Yes	Yes	No	Yes	Yes
4d	Yes	Yes	No	Yes	Yes
4e	Yes	Yes	No	Yes	Yes
4f	Yes	Yes	No		

Excretion		
Compound No.	**Total Clearance**	**Renal OCT2 substrate**
4a	0.13	No
4b	0.11	No
4c	0.269	No
4d	0.368	No
4e	0.021	No
4f	0.024	No

All quinazolinone derivatives showed high water solubility ranging from -2.674 to -5.513 log mol/L, good $CaCO_2$ permeability, good intestinal absorbance and skin permeability, Further, all quinazolinone compounds have low VD, poor BBB permeability and moderate CNS permeability. All compounds inhibition toward the metabolizing enzyme CYP2C19, CYP3A4 inhibitor. Total clearance of all compounds was found to be in range of -0.049 to 0.437ml/min/kg and all compounds are not renal OCT2 substrate thus, these all compounds **4a-f** will not have any reaction and no effect on renal clearance.

3. CONCLUSIONS

AChE inhibitors provided sufficient platform to design a novel quinazolinone derivatives to act as a potent anti- Alzheimer's agents. All the designed novel quinazolinone derivatives **4a-f** have been successfully synthesized. Synthesized compounds were characterized by IR, MASS and NMR spectroscopic methods.

In-silico docking studies were carried out to see the binding interactions of the synthesized compounds along with standard Donepezil. The synthesized compounds found to be accommodated in the active of AChE and orienting towards the active site similar to standard drug. In this study, compounds **4d-f** were most recommended quinazoline derivatives to bind with AChE active site. Physicochemical properties are also predicted by using SwissADME and pkCSM software. All synthesized novel quinazolinone derivatives having good BBB and CNS permeation, low volume of distribution and no negative effect of renal clearance.

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