DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION OF SUBSTITUTED OXADIAZOLEBENZAMIDE DERIVATIVES AS NEW SCAFFOLDS AGAINST ALZHEIMER'S DISEASE

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Abstract

Novel anti-butyrylcholinesterase inhibitors containing, oxadiazole derivatives, were designed and these molecules were subjected to molecular docking study. The docking study revealed that the designed compounds possess significant to moderate interaction with the targeted enzyme butyrylcholinesterase. Among them compound 3 (10.8k/cal) and 52 (10.4 k/cal) showed similar docking compared to **donepezil (12.76 K/cal).** Remaining compound shows good to moderate activity compared to standard drug. The target compounds were evaluated for their inhibitory activity against the BuChE. Among these new derivatives, 3 (71.02±4.3nM) was found to inhibit cholinesterase catalyticdomain most significantly, and then compound 52 (72.37±4.3nM) possessed significant cholinesterase. Compound 3 and 51 have more inhibitory activity than the remaining tested compounds.This may be due to the presence of more electronegative atoms (OCH_3) in thesubstitution. The remaining tested compoundshave moderate to good inhibitory activity against the BuChE. In addition, ADMET prediction results indicated that these compounds might be less toxic and display more interesting pharmacokinetic properties.

Keyword: Anti-butyrylcholinesterase, oxadiazole, Molecular docking study, Donepezil, ADMET.

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Introduction

Alzheimer's disease (AD) is an advanced and irremediable disorder ofthe brain, which is marked by abnormal behaviours, cognitive impairmentand memory loss. Although its precise mechanism is not well understood,enzymes performing critical roles in biochemical pathways pertainingthe cholinergic system and AD progression are well documented [1, 2, 3].

These enzymes are the cholinesterases belonging to the carboxylicester hydrolase family. Two distinct cholinesterases, viz, acetylcholinesterase(AChE; EC 3.1.1.7) and butyrylcholinesterase (BChE; EC 3.1.1.8)are found in vertebrates [1].

Even though these enzymes share about 50% sequence similarity, their tissue distribution, kinetic behaviour andinhibitor sensitivity show a considerable variation [4, 5].

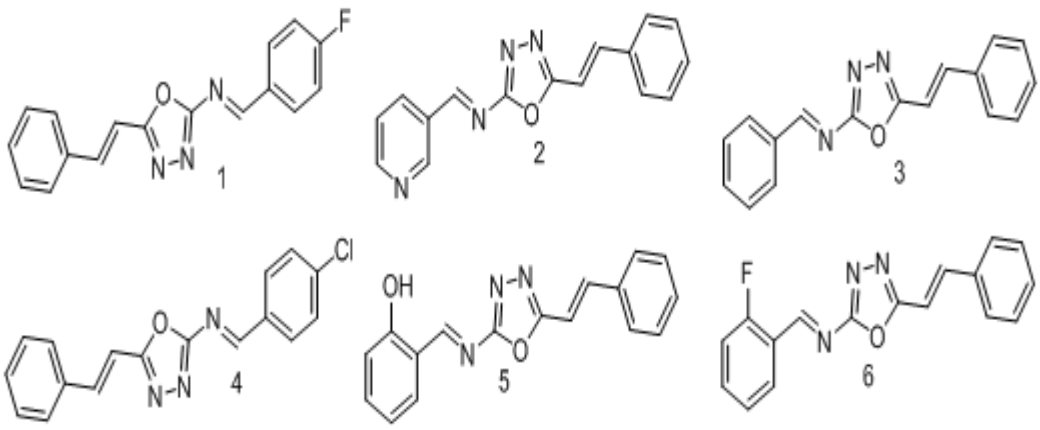
Cholinesterasesregulate cholinergic neurotransmission by aiding the swift removal ofacetylcholine (ACh), and the supposed cholinergic theorem propoundsthat reduced cholinergic neurotransmission facilitates the deepening ofcognitive dysfunction in AD [6]. Other hallmarks of AD pathogenesisinvolve: (i) intracellular neurofibrillary tangles formation (ii) amyloid-β(Aβ) peptides accumulation in neuritic plaques and (iii) loss of cholinergicneurons [7].

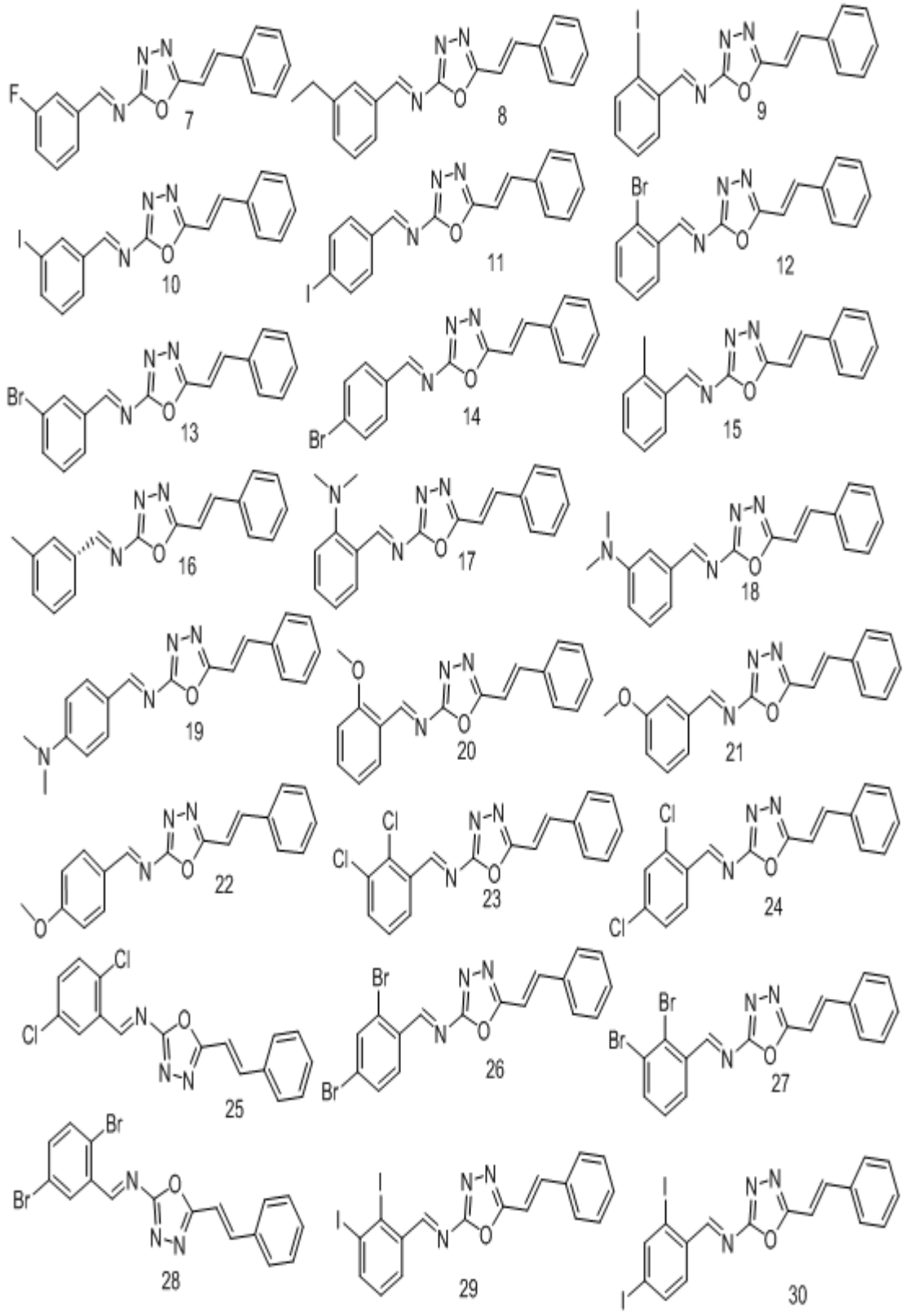
Drugs designed to slow down the disease progression are available; however, these medicines have central and peripheral side effects which are the main drawback of current therapy. Thus, the demand for safe and neweracetylcholine esterase inhibition has become important.Donepezil, a selective AChE inhibitor with a low anti-BuChE effect, is currently administered as an anti-AD medication due to possessing fewer side effects and longer half-life. Moreover, it has been considered as an appealing lead compound for designing new agents. However, this medicine could only relieve the symptoms of the disease, and it is incapable of being effective on moderate to severe AD [8]. Accordingly, the starting point of our investigation was donepezil in order to design a structure with the improved inhibitory effects on AChE that might be promising in the latter phase of the disease [9].

Oxadiazoles are frequently occurring motifs in drug like molecules, and they are often used with the intention of being bioisosteric replacements for ester and amide functionalities.Some of the recent review proclaimed that 1,3,4-oxadiazoles and its derivatives were reported to acquire antimicrobial [9], tuberculostatic [10], anti-inflammatory [11], antifungal [12], antibacterial [13] anticancer [14], analgesic [15] activities, anti-convulsant [16], anti-hepatitis B, antiparasitic [17].

Many drugs containing oxadiazole are in late clinical trials, including zibotentan[18] as an anticancer agent, ataluren for the treatment of cystic fibrosis and raltegravir[19] an antiretroviral drug for the treatment of HIV infection. Some compounds having 1,3,4-oxadiazole unit are currently used as medicines: Fenadiazole is a hypnotic drug [20], MK-0633 p-toluenesulfonate is a 5-lypoxynase inhibitor [21], Nesapidil is an antihypertensive agent [22] and ABT-751-oxadiazole [23] and Furamizole are antibiotics [24]. Several methods have been reported in the literature for the synthesis of 1,3,4-oxadiazoles.

Therefore, we designed the novel donepezil-like compounds by maintaining the amide moiety and replacing the indanone segment with a 1,3,4-oxadiazole ring that was connected to an aromatic ring with different substituents **(Figure 1)**. In the current study, synthesis, biological evaluation, docking study, and ADME prediction were reported.

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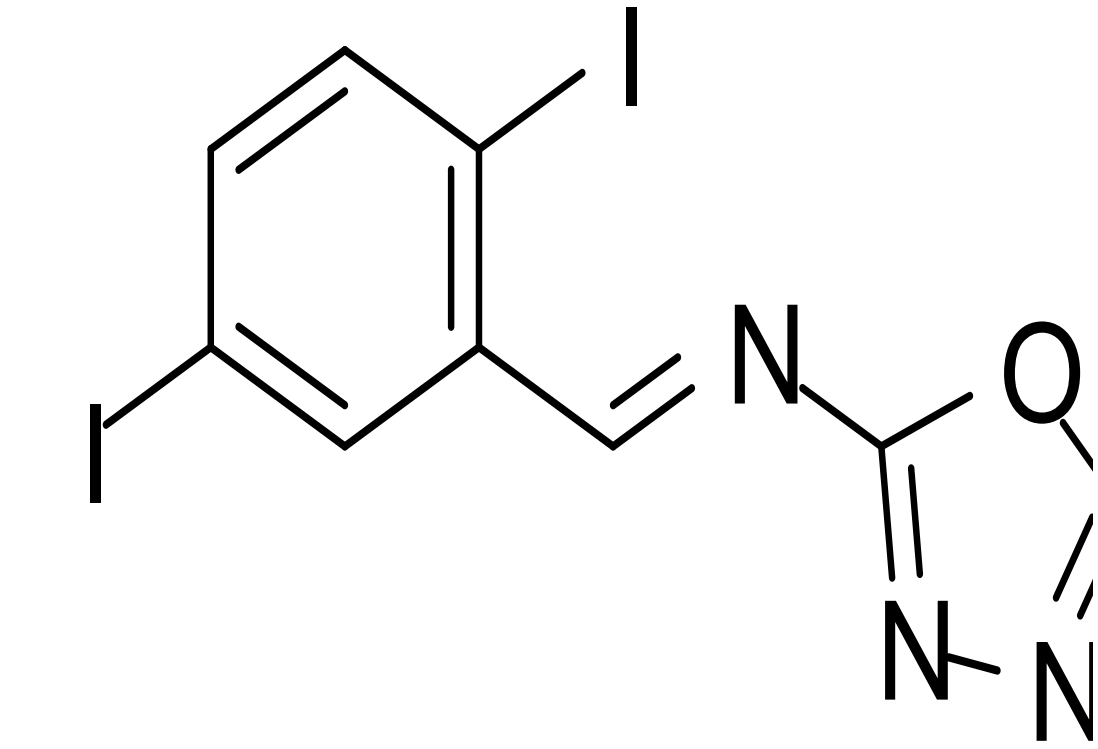
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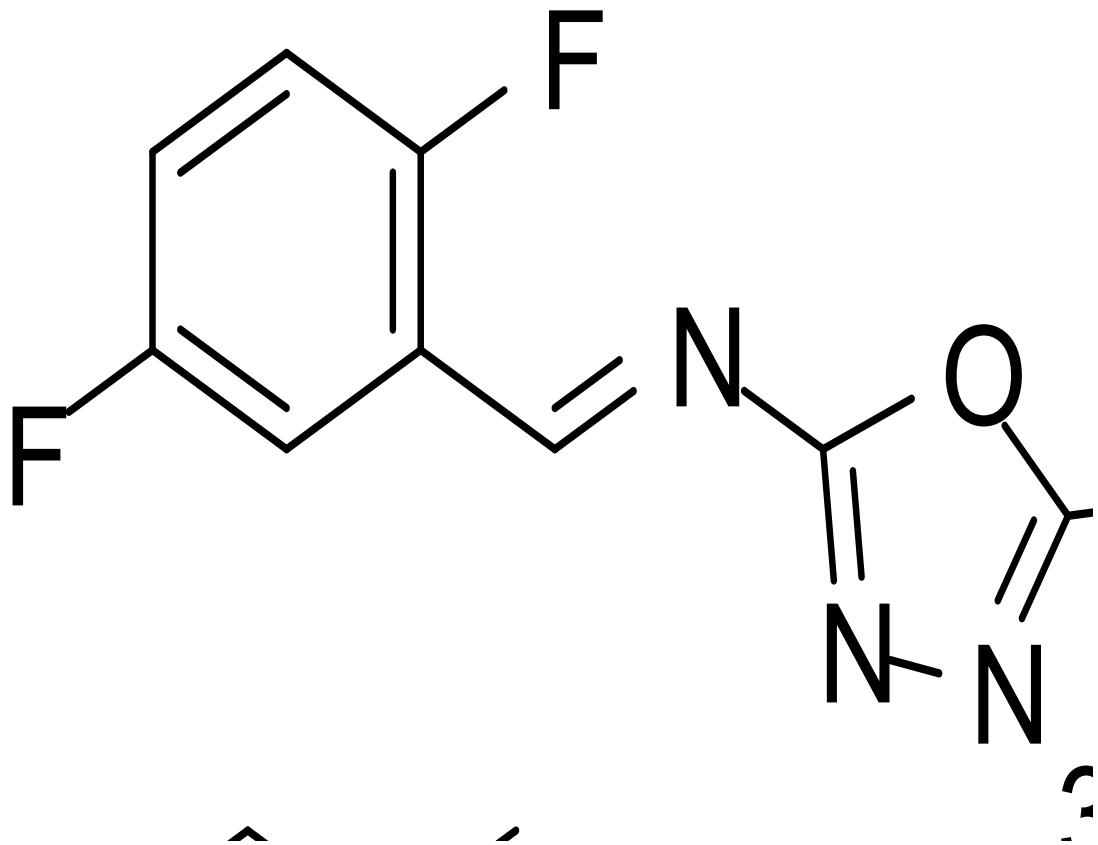
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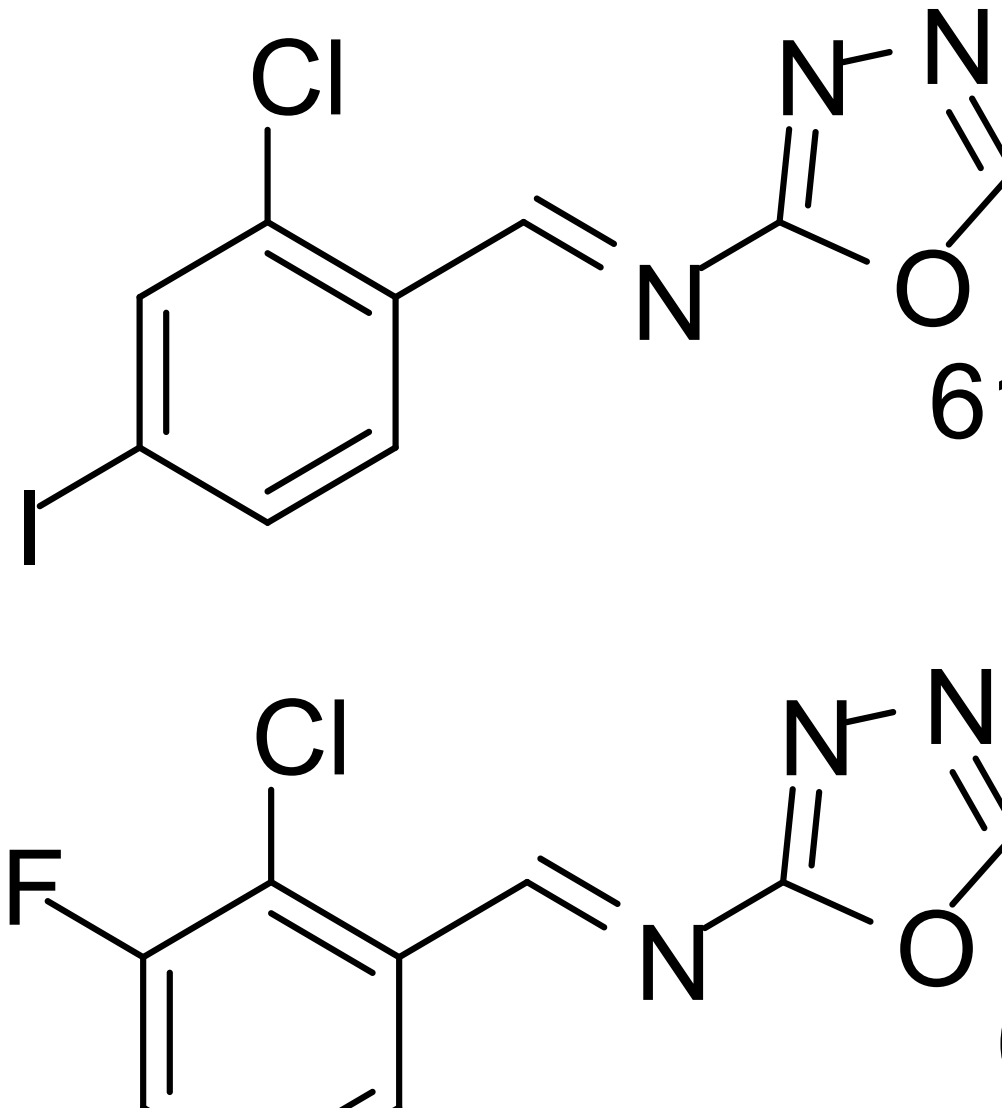

Figure 1. 1,3,4-oxadiazole ring that was connected to an aromatic ring with different substituents

Materials and methods

Experimental section

In-silico molecular docking studiesDevices and materials

In the molecular scenario in the modern drug design, the docking is commonly used to understand the interaction between the target ligand-receptor and the target lead molecule's binding orientation with its protein receptor and is quite frequently used to detect the associations between the target components. The research work was done *in-silico* by utilizing bioinformatics tools. Also, we utilize some of the offline programming's like protein data bank (PDB) www.rcsb.org/pdb, PubChem database, Marvin sketch. The molecular docking studies were carried out through PyRx 0.9 [25].

Preparation of protein

By utilizing the offline program protein data bank, we take the BuChE(PDB: 2XQF) with a resolution of 2.10A^{o} was obtained. From the protein we removed the crystal water, followed by the addition of missing hydrogens, protonation, ionization, energy minimization. The SPDBV (swiss protein data bank viewer) force field was applied for energy minimization. Prepared protein is validated by utilizing the Ramachandran plot [26].

Identification of active sites

Identification of active amino acid present in the protein is detected by using Protein-ligand interaction profile (PLIP) https://plip-tool.biotec.tu-dresden.de/plipweb/plip/index offline tool in google. From this, we found the active amino acid present in the protein [27].

Preparation of Ligands

By utilizing the Marvin sketch tool, the molecules are designed in two and three-dimensional structures. After designed molecule, the structure was optimized in 3D optimization in Marvin sketch and saved as a pdb format [28, 29].

In silico ADMET prediction

A computational study was conducted to predict the pharmacokinetic properties (ADMET) of designed compounds using swiss ADME prediction. Here we calculated molecular volume (MV), molecular weight (MW), number of acceptors of hydrogen bonds (n-ON), number of donors of hydrogen bonds (n-OHNH), total CNS activity, % of oral absorption for humans, polar surface area (PSA), 1-Octyl alcohol-water distribution constant (log P o/w), and BBB penetration. The described properties help to understand the ADME properties of any drug/synthesized molecule. The drug likeness, rule-of-five and rule-of-three violations were also ascertained. For a molecule to be administered via oral route, it should possess distribution constant 5, molecular mass 500, number of donors of H-bonds 5 and number of acceptors of H-bonds 10 and only one violation of the above criteria is acceptable. [30]

BChE Inhibition Assays

Recombinant human BChE (50 mU/mL, 6.54 nM for colorimetric readout; 10 mU/mL, 1.31 nM for fluorescence readout) in 50 mM phosphate-buffered saline (PBS) at pH 7.4 was dispensed (4 μL/well) into black clear-bottom 1536-well plates (Greiner Bio-One North America, Monroe, NC). BChE in the presence of 0.05% Triton was used in a parallel colorimetric assay to rule out compound aggregation. Ethopropazine and physostigmine, known BChE inhibitors, were used as positive controls. Controls and test compounds (at eight different final concentrations from 0.37 nM to 28.75 μM) were transferred into the assay plates (23 nL/well) using a Wako Pintool station (Wako Automation, San Diego, CA). After a 30 min incubation period at room temperature, 4 μL of colorimetric detection cocktail solution (DTNB, butyrylcholine) or 4 μL of fluorometric detection cocktail solution (Thiolite Green, butyrylcholine) was added to each well using a BioRaptr Flying Reagent Dispenser (FRD) (Beckman Coulter, Brea, CA). The final DMSO concentration in the assay well was 0.29%. Assay plates were incubated at room temperature for another 10 min before measuring absorbance readout (405 nm) or measuring fluorescence readout (excitation = 480, emission =540) using an Envision plate reader (PerkinElmer, Shelton, CT) [31].

Results and Discussion

Molecular docking studies

Based on literature studies of oxadiazole derivatives,the 100 compounds were designed for our study and these 100 compounds were subjected to molecular docking studies. Molecular docking was carried out through PyRx 0.9 to predict the interactions model of the protein to its inhibitors. The molecular docking was performed to elucidate the bindingmode competence of BuChE and 100oxadiazoleanalogues. The designed molecules

were docked along withthe native ligand and a reference standard, donepezil. Thedocking energy of our designed compounds ranged from 7 to 10.8kcal/mol indicated good binding affinities to the targetreceptor, and the results are depicted in **Table 1**.Among the docked compounds, derivatives 3 (-10.8 kcal/mol)showed a significant binding energy towards the targeted enzyme. Thecompounds 2 hydrogen bond between amino acids ASP404.The rolesof certain crucial amino acids in the ligand-binding domainof the human Acetylcholinesterase inhibitors were also established.Major non-covalent interactions between the studiedligands and the ligand-binding domain of the Acetylcholinesterase inhibitors were investigated. These amino acidshave been repeatedly implicated during ligand interactionwith the BuChE inhibitors and also play importantrole in the inhibition of the ligand-binding domain of Acetylcholinesterase inhibitors. These non-covalent interactions,van der Waals, columbic interaction, π–π interaction, andhydrogen interaction, are shown in **Figure 4 to 12**.

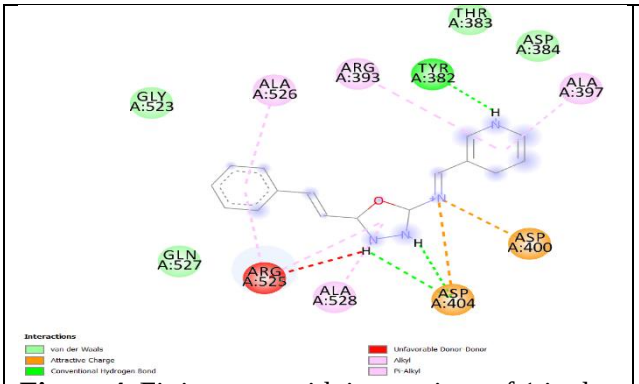

Figure 4. Fitting pose with interactions of 1 in the pocket of 2XQF in 2D view.

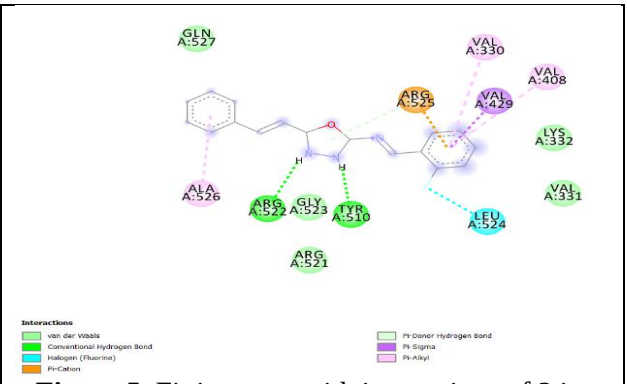

Figure 5. Fitting pose with interactions of 2 in the pocket of 2XQF in 2D view

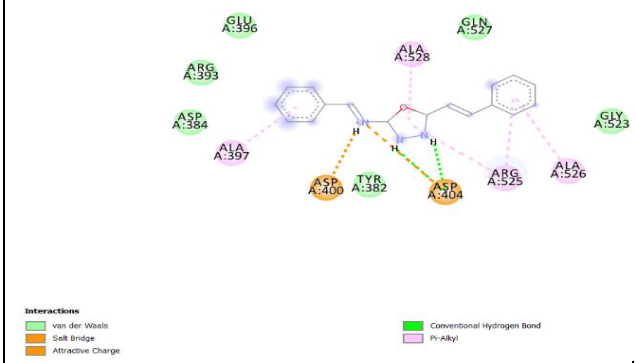

Figure 6. Fitting pose with interactions of 3 in the pocket of 2XQF in 2D view

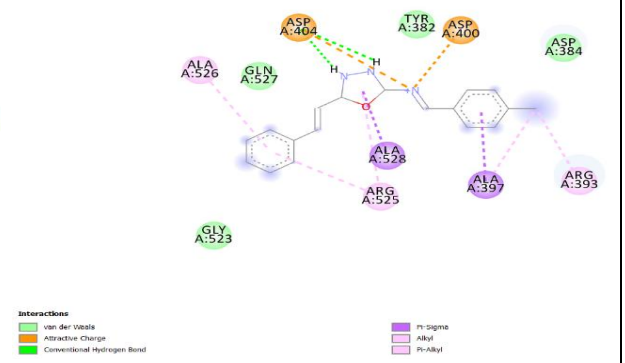

Figure 7. Fitting pose with interactions of 4 in the pocket of 2XQF in 2D view

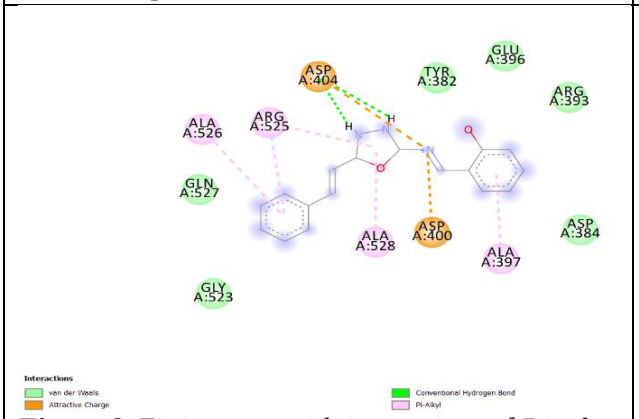

Figure 8. Fitting pose with interactions of 5 in the pocket of 2XQF in 2D view.

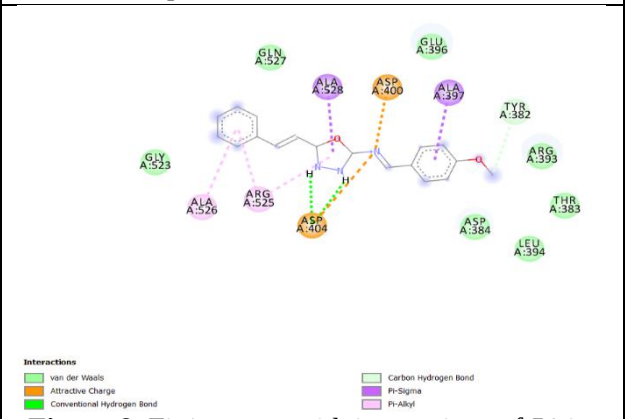

Figure 9. Fitting pose with interactions of 51 in the pocket of 2XQF in 2D view.

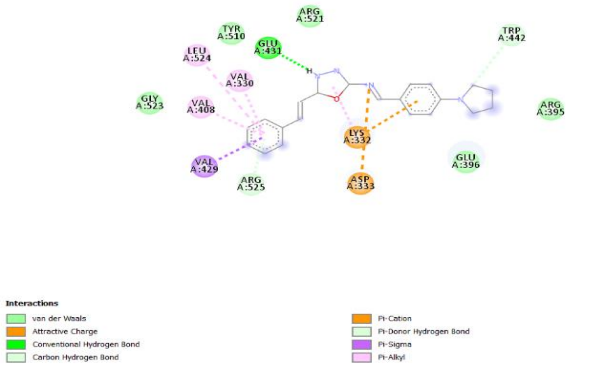

Figure 10. Fitting pose with interactions of 52 in the pocket of 2XQF in 2D view.

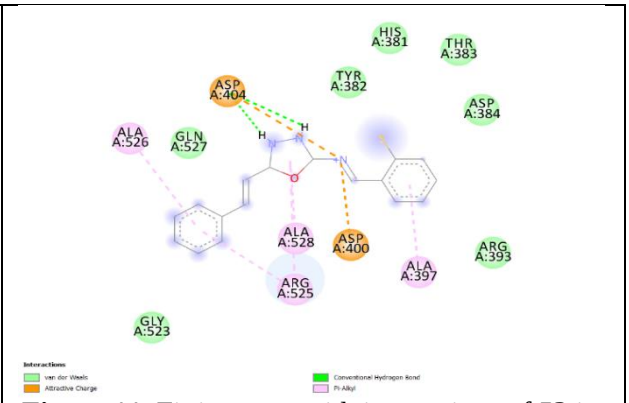

Figure 11. Fitting pose with interactions of 53 in the pocket of 2XQF in 2D view.

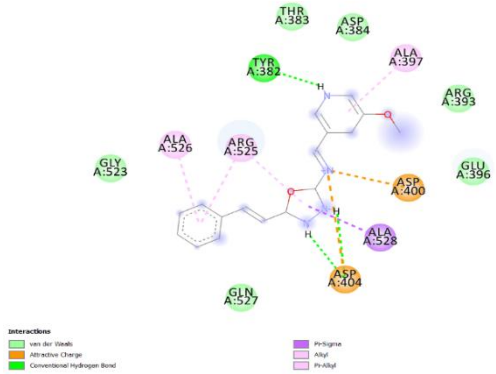

Figure 12. Fitting pose with interactions of 54 in the pocket of 2XQF in 2D view.

Table 1: docking score of designed compounds

Ligand	Binding Affinity	Ligand	Binding Affinity	Ligand	Binding Affinity	Ligand	Binding Affinity
1	**-9.9**	26	-7.5	**51**	**-9.5**	76	-7.1
2	**-9.8**	27	-7.2	**52**	**-10.4**	77	-7.1
3	**-10.8**	28	-7.6	**53**	**-9.2**	78	-7.5
4	**-9.7**	29	-7.2	**54**	**-9.4**	79	-7.3
5	**-10.3**	30	-6.4	**55**	**-9.7**	80	-7.1
6	-6.3	31	-8.4	56	-6.6	81	-7.0
7	-7.4	32	-7.0	57	-8.6	82	-7.1
8	-8.9	33	-7.5	58	-8.8	83	-8.2
9	-7.5	34	-8.2	59	-7.5	84	-8.0
10	-8.4	35	-7.9	60	-8.7	85	-8.1
11	-8.6	36	-7.8	61	-7.8	86	-7.9
12	-8.5	37	-7.5	62	-8.2	87	-7.8
13	-8.2	38	-7.2	63	-8.5	88	-7.6
14	-8.1	39	-7.4	64	-8.5	89	-7.7
15	-8.4	40	-7.3	65	-8.4	90	-7.6
16	-8.5	41	-8.1	66	-8.3	91	-7.3
17	-8.1	42	-8.6	67	-8.7	92	-7.1
18	-8.0	43	-8.2	68	-7.4	93	-7.5
19	-8.6	44	-8.1	69	-7.0	94	-8.1
20	-8.8	45	-8.3	70	-8.2	95	-7.4
21	-8.3	46	-8.3	71	-8.1	96	-7.1
22	-8.1	47	-8.4	72	-8.3	97	-7.7
23	-6.7	48	-8.4	73	-8.2	98	-7.7
24	-6.9	49	-8.6	74	-8.3	99	-7.5
25	-7.4	50	-6.8	75	-8.2	100	-7.8

BuChE inhibition assay

The target compounds were evaluated for their inhibitory activity against the BuChE. Among these new derivatives, 3 (71.02±4.3nM) was found to inhibit cholinesterase catalyticdomain most significantly, and then compound 52 (72.37±4.3nM) possessed significant cholinesterase. Compound 3 and 52 have more inhibitory activity than the remaining tested compounds.This may be due to the presence of more electronegative atoms (OCH_3) in thesubstitution. Of these compounds, compound 2 (85.44±4.4nM) have moderate inhibitory activity against BuChE. The remaining tested compoundshave moderate to good inhibitory activity against the cholinesterase enzyme. The result isshown in **Table 3.**

Table 3. BuChE inhibiting activity of compounds

Compound	IC_{50} (nM)
1	109.92±5.4
2	85.44±4.4
3	71.02±4.3
4	97.90±4.5
5	135.74±6.2
51	93.35±3.4
52	72.37± 4.3
53	98.01±3.6
54	108.88± 5.2
55	189.96±3.4
Donepezil (Standard)	17.96±2.3

Conclusion

The oxadiazole molecules were designed and this investigated molecules displayed a similar manner to protein binding tothe active site of BuChEprotein**(PDB ID:2XQF)** in molecular docking studies. The calculateddocking energies indicated that its interaction with cholinesterase is favourable, but only to a limitedextent. The enzyme inhibitory assay indicated that the enzyme inhibitory activities of 3, 52 and 2 against BuChE enzyme were similar to those of donepezil. In addition, ADMET prediction results indicated that these compounds might possessless toxicity and pharmacokinetic properties. The study thus serves as an attempt to progresstoward the discovery of novel BuChE drugs. Additional derivatives may be prepared and furtherextended in-depth investigations into *in-vivo* activity would be implemented to establish aSAR (Structural activity relationship) for rational study. From the present investigation, it may beconcluded that oxadiazolebenzamidederivatives need to undergofurther investigation to develop as a potential candidate drug for Alzheimer.

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CONFLICT OF INTEREST

The authors declare that there are no Conflicts of Interests

FUNDING SUPPORT

Nil

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