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Synthesis, characterisation, *in silico & in vitro* anti-cholinesterase activity of some novel 2 amino 5-substituted oxadiazoles

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Abstract

An individual with Alzheimer's disease (AD) experiences cognitive decline, memory loss, and behavioral abnormalities. The complex condition known as AD is brought on by acetylcholinesterase (AChE), which breaks down acetylcholine. The current investigation aimed to evaluate synthetic AChE inhibitors that may be utilized to treat AD. To do this, synthetic two amino five substituted oxadiazole derivative compounds (1a-5e) were assessed and shown to be potential AChE inhibitors, with IC50 values ranging from 73 ± 0.67 to 98 ± 0.7 µmol/min/mg of tissue. *In silico* docking investigations were performed using Schrodinger, revealing that most of the compounds are held together by π–π and hydrogen bonding and interact with the anionic subsite of AChE. Chem Bio Draw Ultra 12.0 (www.cambridgesoft.com) was used to create the 2D structures of each drug. 2. The RSC PDB (www.rscb.org) provided the crystallographic three-dimensional structure of AChE target receptors, with PDB ID: 4EY5 &604 w for AChE. The most potent compound is 5e; consequently, these compounds can potentially be used as therapeutic agents to treat AD and its related conditions because of their AChE inhibitory capacity cytotoxicity safe profile.

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INTRODUCTION

Numerous biological components necessary for life are found in heterocyclic molecules. For instance, nucleic acid comprises lengthy chains of hetero units with various materials. With the molecular formula $C_2H_2N_2O$, oxadiazole is a heterocyclic aromatic chemical compound belonging to the azole family [1]. There are four isomers of oxadiazole, namely, 1,2,3 oxadiazole, 1,2,4 oxadiazole,1,2,5 oxadiazole, and 1,3,4 oxadiazole. When two methane groups are

swapped out for two nitrogen atoms of the pyridine type, the resultant compound is oxadiazole [2]. The most widely utilized synthetic pathway for synthesizing 1,3,4-oxadiazole is the reaction of acid hydrazides with acid chlorides. In synthesizing oxadiazole, which yields related esters and hydrazides, substituted aromatic acids are typically employed as a starting material. Fischer esterification produces ethyl ester, which combines with hydrazine hydrate in the presence of ethanol to make a hydrazide derivative [3]. The chemistry of oxadiazole has undergone significant development and continues to do so. The oxadiazole moiety is present in several therapeutically utilized medications and different heterocyclic rings. Due to this, an endeavor was started to create a new class of 1,3,4-oxadiazoles by condensation reaction with an aldehyde moiety, and the pharmacological activity of the new compounds was assessed. *In silico* and *in vitro* studies were performed. Elmann's technique screened the compounds for ACHE inhibitory action [4].

MATERIALS:

In open capillary tubes, the experimental melting points were measured. Infrared spectra were taken utilizing the KBR disc method on an FT-IR spectrometer.

METHODS:

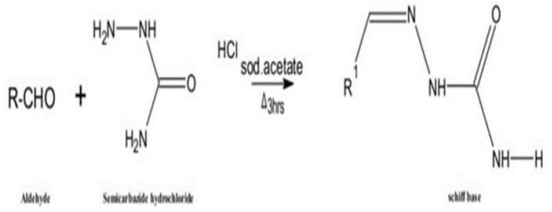
Twenty milliliters of ethanol were used to dissolve a mixture of semi-carbazide hydrochloride (0.05 mole) and aldehyde (0.05 mole). A buffer solution, such as sodium acetate, was added to the mixture to keep its pH stable. The mixture was refluxed at 100°C for three hours [5]. The residue left after the solvent was distilled out was employed for further processing.

To the residue, as mentioned above (0.01 mole), sodium carbonate (0.01 mole), Iodine (0.01 mole), and potassium iodide (0.01 mole) were refluxed for 2 hrs. After the reaction mixture was concentrated and allowed to cool, the solid result was filtered and cleaned with water, and then methanol was used to re-crystallize it [6].

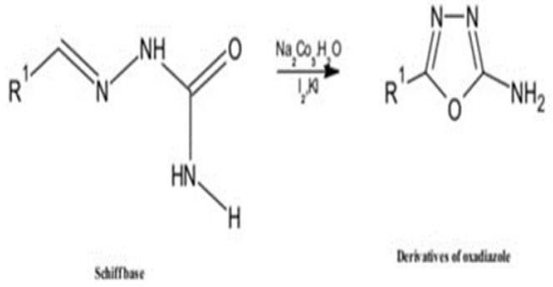
Pharmacological Evaluation:

Silicon approaches: We created pharmacophore models for this study using AChE inhibitors accumulated from the Protein database. In addition to elucidating the quantitative structure-activity link for known AChE inhibitors, the pharmacophore characteristics were utilized to find effective AChE inhibitors [7]. The most effective quantitative model was used to create 3D search queries that screened the Protein databases to discover novel AChE inhibitors that might block both the peripheral and catalytic anionic sites. After being found, the hit compounds underwent molecular docking filtering to enhance the quality of the recovered hits [8]. To see and create novel AChE inhibitors with improved selectivity, the virtual screening approach can be used with molecular docking, consensus scoring, and pharmacophore modeling. All of the compounds' 2D structures were created using Chemsketch (www.acdlabs.com), and Gaussian 09,1 with the small basic set small hf/3-21g* was used to develop the energy-minimized 3D structures [9]. Target receptors and the crystallographic three-dimensional structure of AChE were recovered from the RSC PDB (www.rscb.org); AChE's PDB ID is UNK 900. Docking was done using these energy-minimized molecules and AChE receptors [10].

Step I



Step II



***In-Vitro* Approaches**:

AChE enzyme was calculated from the whole brain using the Ellamn technique, and the reaction assay

Table 1 Five derivatives of 2 amino 5-substituted oxadiazoles derivatives have been synthesized

Code	Molecular formula	Molecular weight	Solubility	%yield	RF Values	m.p(c)
1a	$C_{10}H_9N_3O_3$	219.2 g/mol	H2O, Ethanol, acetone.	72.61%	0.61	46-48ºc
2b	$C_9H_7N_4O_4$	235.178	H2O, isopropanol, ethanol, toluene, acetone ethyl acetate.	85.083%	0.54	58-61ºc
3c	$C_{11}H_9N_3O_2$	215.212	H2O, ether chloroform	76.822%	0.53	47-49ºc
4d	$C_{10}H_9N_3O_3$	219.2	H2O, methanol acetone.	77.671%	0.65	44-50ºc
5e	$C_8H_6N_3OCl$	195.61	H2O, methanol acetone	81.2%	0.68	48-51^{0}c

Spectral data of 5-(4-chlorophenyl)-1,3,4-oxadiazol-2-amine (5e):
IR (KBr, cm-1):3501.81 (-OH of oxime), 2953.87, 2805.10(Aromatic –CH Str)
1H NMR (DMSO-*d6*, δ ppm): 7.35 (s, 2H, -NH2), 7.59-7.82 (m, 4H, aromatic -C-H)
13C NMR (DMSO *d6*, δ ppm): 123.17, 126.68, 128.46, 129.29, 134.83 (Ar-C), 156.50 (C-NH2), 163.94 (C of Oxadiazole)

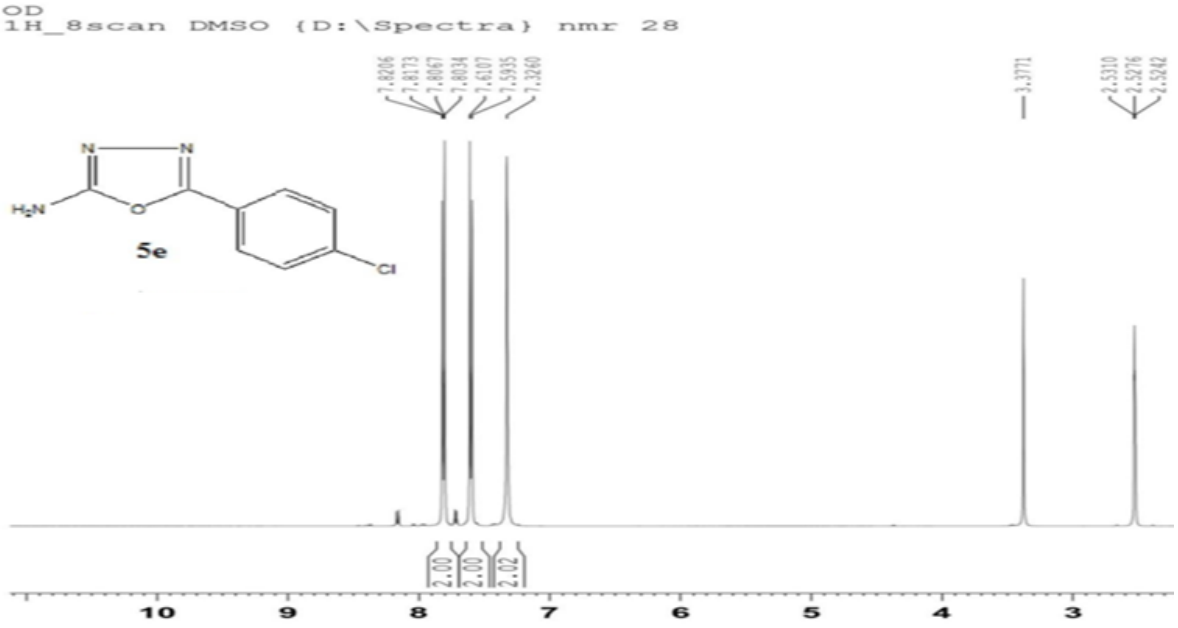


Figure 1 Spectral data of 5-(4-chlorophenyl)-1,3,4-oxadiazol-2-amine (5e)

mixture included 3 ml of phosphate buffer (PH 8.0), 0.1 ml of an aqueous solution of test or standard (donepezil), with concentrations ranging from 0 µg/ml to 200 µg/ml.

In phosphate buffer pH 7.0 with 120 mM sodium bicarbonate, the result was the development of a yellow hue as a result of the reaction of thiocholine from acetylthiocholine iodide in the presence of Dithionitrobanzoate (DTNB) (0.3 mM) [11].

The reaction mixture was vortexed after a 30-minute pre-incubation period at 37°C. Acetyl thiocholine iodide (ATCI) in 0.1 m was added to start the reaction. Next, for a quarter of an hour at room temperature, the absorbance (AA) at 412 nm was measured [12].

RESULTS AND DISCUSSION:

Five derivatives of 2 amino 5-substituted oxadiazoles derivatives have been synthesized. In the 72–85% range, all compounds with the titled names produced products.

The compounds' melting points were between 45 and 610 degrees Celsius. All compounds exhibited a single migration spot from the origin on TLC plates, proving their purity.

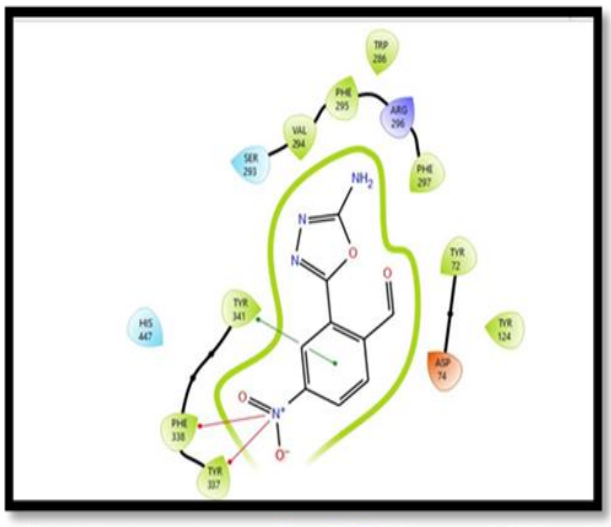
Table 2 Binding energies of synthetic3-(5-amino-1,3,4-oxadiazol-2-yl) derivatives and standard inhibitors against AChE receptor

S. No	Compound code	Binding Energies (Kcal/mol)
1.	1a	-7.64
2.	2b	-6.24
3.	3c	-6.28
4.	4d	-7.59
5.	5e	-10.61
6.	Donepezil	-14.61

Using Schrodinger software, the primary goal of this portion of the investigation is to conduct a docking analysis of synthetic 2 amino 5 substituted oxadiazole derivatives on AChE receptors. to establish a likely association between the binding affinities of the 1a–5e with receptor and the in vitro Alzheimer's activity outcomes. Chem Bio Draw Ultra 12.0 (www.cambridgesoft.com) was used to create the 2D structures of each drug. The RSC PDB (www.rscb.org) provided the crystallographic three-dimensional structure of AChE target receptors, with PDB ID: 4EY5&604 w for AChE. Using the UCSF Chimera 1.10.1 protein preparation wizard program, the receptors were cleaned and made suitable for docking by removing any water and ligand molecules that were already present. AChE receptors and such energy-minimized molecules were used for docking. The majority of the synthesized compounds exhibit strong binding affinities. Of all the synthetic compounds, compound 5e exhibits a good affinity for binding.

Table 3 In vitro estimation of Acetylcholinesterase inhibition activity

Treated groups	Dose(mg/ kg)	AChElevels(µmol/min /mg of tissue)
Control	-	66±2.8
Donepezil	5 10	79.5±0.7*** 74±4.9***
1a	5 10	89±64** 83.7±5.3***
2b	5 10	73±8.9*** 77±3.9****
3c	5 10	78.6±0.7*** 74.5±3.5****
4d	5 10	88±8.5*** 84±9.7****
5e	5 10	98±5.7* 90±6**



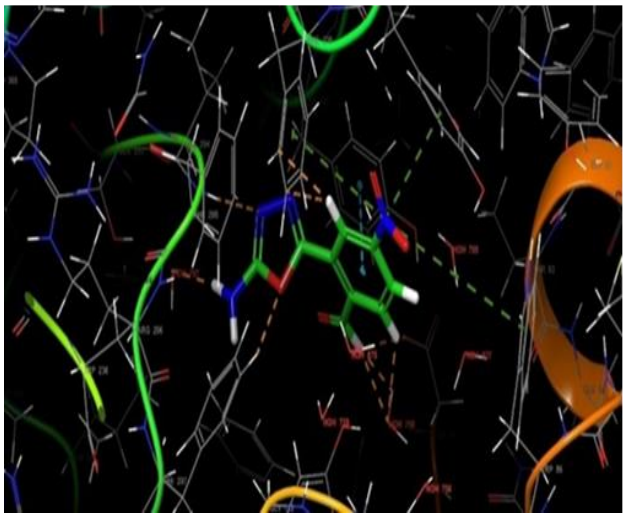


Figure 2 The docking data of the various 5e

Values are expressed as Mean ±SEM (n=6) ****P<0.0001, ***P<0.001, **P<0.01, *P<0.05 (Dunnetts Multiple Comparison tests) using one-way ANOVA

Ellman's approach was used to test five compounds for their ability to inhibit ACHE in vitro. A graph has been generated by noting the percentage inhibitions of the standards and samples. Determined the value of IC 50.

Compared to normal donepezil, almost all synthesized compounds have demonstrated inhibitory action, with compound 5e demonstrating potent inhibition.

CONCLUSION:

The silicon and in vitro ACHE inhibitory actions of five substituted oxadiazoles were synthesized, described, and assessed. Compound 5e, the most promising oxadiazole, can potentially be a novel

ACHE inhibition template. Given the noteworthy actions of the compounds under investigation, it is anticipated that additional optimization of these found chemical leads would likely result in the synthesis of molecules with even higher activity levels. After appropriate structural alterations, future research is suggested to determine their in vivo efficacy and receptor interaction.

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Author Contribution

All authors made substantial contributions to the conception, design, acquisition, analysis, or interpretation of data for the work. They were involved in drafting the manuscript or revising it critically for important intellectual content. All authors gave final approval of the version to be published and agreed to be accountable for all aspects of the work, ensuring its accuracy and integrity.

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