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Original Article

Synthesis and Evaluation of 2, 5-substituted Pyrazolone for Neuroprotective Potential in SH-SY5Y Human Neuroblastoma Cells

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ABSTRACT

Aim: To carry out Synthesis and Evaluation of novel 2, 5-substituted Pyrazolone for Neuroprotective Potential in SH-SY5Y Human Neuroblastoma Cells. **Materials and Methods:** Alzheimer's Disease (AD) has become a serious public health concern as a result of people living longer than ever before. It is critically necessary to discover a way to halt and postpone the illness. The present study aims to determine if new synthetic analogue pyrazole derivatives may protect against toxicity caused by Aβ25-35 and its underlying mechanisms in neuroblastoma cells like SH-SY5Y. PyRx 0.9 software was used for molecular docking studies. The cells of SH-SY5Y were preincubated for 30 min with varying doses of the generated compounds (C1-C10) to induce neurotoxicity. They were then grown in Aβ25-35 (25 mol/L) for 48 hr. Cell viability was determined by MTT assay. **Results:** Compounds C5 and C8 exhibited a better binding score -8.4k/cal compared to other analogues. Synthesized compounds C5 and C8 inhibited Aβ25-35-induced apoptosis in SH-SY5Y cells and protected neural cells from damage. **Conclusion:** The MTT assay confirmed that compounds C5 and C8 significantly reduced Aβ25-35-induced toxicity among human neuroblastoma SH-SY5Y cells, demonstrating its neuroprotective properties.

Keywords: Cell viability, MTT assay, Neurodegenerative diseases, Neuroprotective agent, Pyrazole derivatives, SH-SY5Y cells.

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INTRODUCTION

Alzheimer's Disease, also known as dementia (AD) is a neurodegenerative disorder that mostly affects the elderly and results in progressive and irreversible memory loss due to the death of neurons in the brain.[1] The appearance of senile plaques and the associated neuronal loss is a hallmark of AD on the histological level. The β-amyloid peptide (Aβ) is the primary component of amyloid plaques and plays a crucial role in the development of Alzheimer's disease.[2-5] Evidence has shown that the apoptotic pathway's activation may be responsible for the neurotoxic effects of Aβ.[6-9] Because neuronal death is the main factor contributing to neuronal loss in AD patients, neuroprotection has been proposed as a viable therapeutic strategy for slowing the rate at which associated brain cells in AD patients undergo apoptosis.[10] Since iproniazide showed in vitro effectiveness in treating CNS depression, researchers have focused on creating heterocyclic hydrazines and hydrazides and their potential as therapeutic agents.[11,12] Inhibitory action against MAO was shown in tests of 1, 3, 5-triphenyl-2-pyrazolines containing a cyclic hydrazine moiety.[13-16] These results have motivated us to synthesize ever pyrazole derivatives and test them for theirdiverse amine oxidase inhibitory capabilities, including those against Bovine Serum Amineoxidase (BSAO), Monoamine Oxidase (MAO), and Semicarbazide Sensitive Amine Oxidase (SSAO). Several of the synthesized compounds had inhibitory activity against MAO, BSAO, and SSAO that was on par with or even better than that of the original reference compounds.[17-19] Licensed for the treatment of cognitive impairment in Alzheimer's disease, several of the pyrazoles first described as particular MAO-B inhibitors have been shown to specifically and non-competitively decrease the antioxidant enzyme AChE actions in human erythrocyte and plasma.[20]

Discovering drugs with various activities is a contemporary method to treating AD and other diseases including HIV/AIDS, cancer, and cardiovascular difficulties. The pathophysiology of a disease depends on a several factors. In this context, substances like pyrazoles, which have a variety of biochemical and pharmacological effects, may hold promise for the treatment and suppression of the disease's progression. We sought to

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synthesize a new set of 2-Akyl/Aryl-5-[(E)-2-phenylethenyl]-2,4-dihydro-3H-pyrazol-3-one derivatives to investigate them as innovative possible treatments for AD.

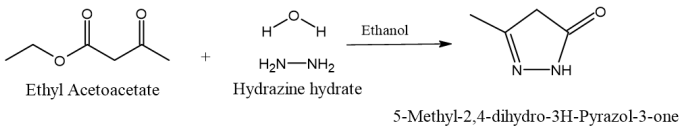
MATERIALS AND METHODS

Compounds C1, C2, C3, C4, C5, C6, C7, C8, C9 and C10 of Ten new pyrazolones (Table 1) were created as reversible and selective MAO-A and MAO-B inhibitors. Commercial supplies of chemicals and solvents were purchased from Merck, India, for use in experimental operations.

Chemistry

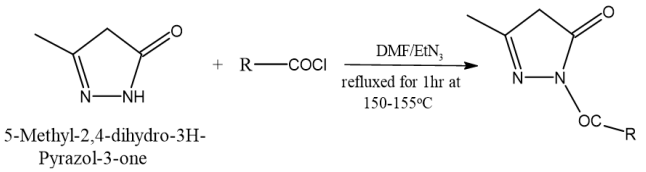
Step 1. Synthesis of 5-methyl-2,4-dihydro-3H-pyrazol-3-one (A)

In 40 mL of ethanol, add hydrazine hydrate (0.5 mmol) drop-wise with a steady string and keep the temperature about 60°C. The reaction mixture was stirred for 1 hr at 60°C, and the crystalline product was separated. More crystals of the product were produced after chilling the mixture. The product is filtered and then recrystallized in cold ethanol.



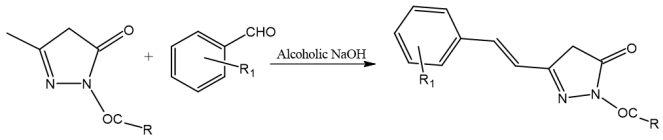
Step 2. Synthesis of 2-Akyl/Aryl-5-methyl-2,4-dihydro-3H-pyrazol-3-one (B)

The compound A (0.01 mmol) is dissolved in 40 mL of dimethyl formamide, and this solution is added to the various substituted acid chlorides (0.012 mmol) in triethylamine (0.012 mmol), and it was refluxed for 1 hr at 150°C - 155°C until the disappearance of starting materials, which was checked by TLC using ethyl acetate and h-hexane (4:6) as mobile phase. After cooling the reaction mixture, the precipitate of 2-benzoyl-5-methyl-2,4-dihydro-3H-pyrazol-3-one (B) was separated. The chemical B was refined by recrystallising with ethanol.[21]



Step 3. Synthesis of (E)-5-(2-substituted)-2-(substituted-2-carbonyl)-2,4-dihydro-3H-pyrazol-3-one (C)

The chalcone derivatives of (E)-5-(2 substituted)-2-(substituted-2-carbonyl)-2,4-dihydro-3H-pyrazol-3-one is made by condensing compound B (0.1 mmole) with different substituted aromatic aldehydes (0.1 mmole) in the presence of 10% alcoholic NaOH (10 mL) and stirring at 25°C. The reaction mixture was left at room temperature for 24 hr before adding HCl to neutralize any unreacted NaOH, and the precipitate was separated and rinsed with cold water.[22]



In silico molecular docking

Studies Devices and materials

To better understand the target ligand-receptor connection and the binding orientation of the target lead molecule with its protein receptor, docking is often used in modern drug design. Relationships between the intended parts may also be discovered with its help. Bioinformatics tools were used to conduct the research *in silico*. Additionally, we make use of offline programs like Marvin Sketch, the PubChem database, and the Protein Data Bank (PDB) (www.rcsb.org/pdb). The molecular docking investigations were done using PyRx 0.9.

Preparation of protein

With the help of the offline software Protein Data Bank (PDB ID: 4EY6), we were able to capture the enzyme acetylcholinesterase at a resolution of 1.90Å. We first isolated the protein (4EY6) from its crystal water before adding the missing hydrogen ions, protonation, ionization, and energy minimization. Using the Swiss-Protein Data Bank Viewer (SPDBV) force field, we were able to keep the energy levels constant. Protein quality may be checked using the Ramachandran plot.

Preparation of Ligands

Using the Marvin sketch tool, the molecules are organized into two- and three-dimensional arrangements. The molecule was then 3D-optimized in Marvin Sketch before being exported to pdb format.

Identification of active sites

Google can be used to access offline tools such as Protein-Ligand Interaction Profile to determine whether the protein's active

amino acids are present. This led us to the active amino acid of the protein.

Cell culture

Sigma Aldrich's human SH-SY5Y cells from neuroblastoma were cultured at 37°C in a humidified 5% CO_2 atmosphere using DMEM-d_6 supplemented with 10% foetal bovine serum. The studies used a confluence of 80%.

Determination of cell viability

An established MTT reduction test was used to measure cell viability.[23] Using three replicates, the experiment was carried out. Cells were seeded in 96-well plates at a density of 2.0 x 104 cells per well in 100 L of fresh medium containing 10% FBS. Once everything had settled down for a day, the cells underwent a 2-hr pretreatment with five different test chemical concentrations (Solubilized in DMEM with 10% FBS at concentrations of 25, 50, 100, 250, and 500 µg/mL). After 2 hr, 10 mM Aβ25-35 was added to the mixture, and the jar was incubated at 37°C with 5% carbon dioxide for another 24 hr. The control condition for the statistical analysis was DMEM + 10% FBS. After the treatment period, the culture mediums were removed and 500 µg/mL of MTT was added to each well. The plates were then left in an incubator for 4 hr. After the MTT solution was drained, the dark blue crystals were dissolved by adding 100 µL of DMSO-d_6 to each well. The plates were briefly shaken before being scanned at 540 nm using a Thermo Plate scanner (Thermo Plate, China). After compiling the data, we determined the percentage difference from the control group.[24-26]

Test stock solution and working solution

All five of the created compounds were used to make test compound stock solutions, which were sterilized by filtering and kept at 4°C after being dissolved in a mixture of cell medium and alcohol (70/30v/v). Before use, the stock solution was diluted in a cell medium to a working concentration of (4.0 x 103 µg/mL).

Aβ25–35 stock solution and working solution

The concentration of A-25-35 in the distilled water is 1 µM. Each well was treated with an overall concentration of 10 µM using a working solution of 100 µM and cell media supplemented with 10% FBS. The following formula was used to compute the percentage Cell Inhibition (CI):

$$\% \text{ Cell Inhibition} = \frac{\text{ControlOD} - \text{SampleOD}}{\text{ControlOD}} \times 100$$

$$\% \text{ Cell Viability} = 100 - \text{Cell inhibition}$$

The concentration of test samples required to suppress cell growth by 50% was calculated by creating dose-response curves for each cell line.

RESULTS

Experimental analysis

(E)-2-acetyl-5-(2-chlorostyryl)-2,4-dihydro-3H-pyrazol-3-one(C1)

Compound C1 was synthesized according to the general procedure. Yield75%. Mp.140°–142°C. FT-IR (ν,cm−1): 2917.69, 1628.69, 1510.85, 1255.90, 1212.88, 805.70; m/z: Calculated for C1 for 262.05 observed 262.05(100.0%), 264.05(32.0%), 263.05(14.1%), 265.05(4.5%). ^{1}HNMR (500MHz, DMSO-d_6) δ7.37–7.21(m,3H), 7.19–7.07(m,3H), 3.62(s,2H), 2.39(s,3H). ^{13}CNMR (126MHz, DMSO-d_6) δ170.31, 157.06, 155.82, 154.34, 134.70, 134.50, 129.99, 129.36, 129.30, 128.79, 128.02, 127.88, 127.26, 127.02, 126.63, 124.45, 123.71.

(E)-2-acetyl-5-(2-methoxystyryl)-2,4-dihydro-3H-pyrazol-3-one(C2)

Compound C2 was synthesized according to the general procedure. Yield 80%. Mp162°–165°C. FT-IR (ν,cm−1): 2917.27, 1627.96, 1525.19, 1384.92, 1214.88, 11190.60; m/z: Calculated for C2 for 258.10 observed 258.10(100.0%), 259.10(15.1%), 260.11(1.1%). ^{1}HNMR (500MHz, DMSO-d_6) δ7.27(s,2H), 7.16(d,J=0.7Hz,4H), 6.93–6.90(m,1H), 6.90(s,1H), 6.90–6.56(m,5H), 3.84–3.80(m,6H), 3.61–3.57(m,4H), 2.41–2.37(m,6H). ^{13}CNMR (126MHz, DMSO-d_6) δ170.31, 157.06, 155.82, 154.34, 134.70, 134.50, 129.99, 129.36, 129.30, 128.79, 128.02, 127.88, 127.26, 127.02, 126.63, 124.45, 123.71, 119.40, 22.94, 21.72.

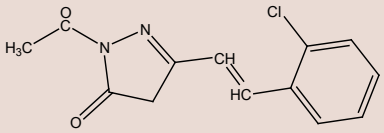
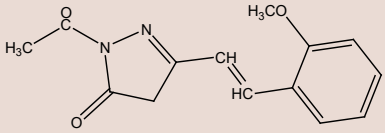
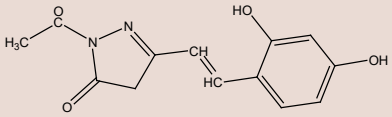
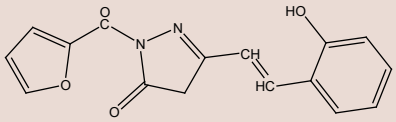
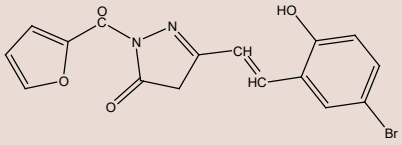
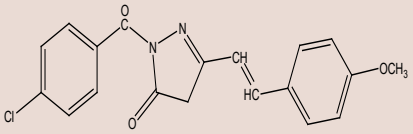
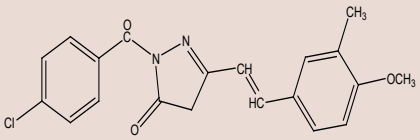
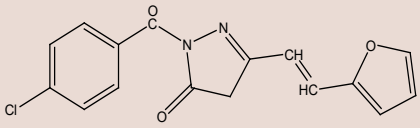
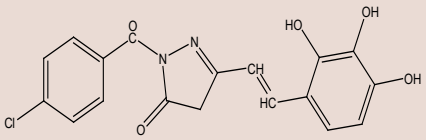
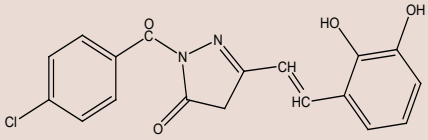
(E)-2-acetyl-5-(2,4-dihydroxystyryl)-2,4-dihydro-3H-pyrazol-3-one(C3)

Compound C3 was synthesized according to the general procedure. Yield 80%. Mp151°–153°C. FT-IR (ν,cm−1): 3108.16, 2917.33, 1628.73, 1571.60, 1362.72, 1500.15, 1173.22. m/z: Calculated for C3 for 260.08 observed 260.08(100.0%), 261.08(14.1%). ^{1}HNMR (500MHz, DMSO-d_6) δ6.99(d,*J*=30.8Hz,2H), 6.65(s,1H), 6.32(s,1H), 6.27(s,1H), 4.31(s,1H), 4.07(s,1H), 3.61–3.57(m,2H), 2.41–2.37(m,3H). ^{13}CNMR (126MHz, DMSO-d_6) δ170.31, 157.06, 155.82, 154.34, 134.70, 134.50, 129.99, 129.36, 129.30, 128.79, 128.02, 127.88, 127.26, 127.02, 126.63, 124.45, 123.71, 119.40, 119.01, 108.40, 107.37, 67.61, 64.87, 22.94, 21.72.

(E)-2-(furan-2-carbonyl)-5-(2-hydroxystyryl)-2,4-dihydro-3H-pyrazol-3-one(C4)

Compound C4 was synthesized according to the general procedure. Yield 85%. Mp145°–148°C. FT-IR (ν,cm−1): 3267.94, 1698.38, 1527.01, 1364.77, 1224.4, 1040.36. m/z: Calculated for C4 for 296.08 observed 373.99(100.0%), 375.99(97.3%), 376.99(16.8%), 374.99 (16.2%), 377.99(1.2%), 374.99(1.1%), 376.00(1.1%). ^{1}HNMR (500MHz, DMSO-d_6) δ9.60(s,1H), 7.51(dd,*J*=7.4,1.5Hz,1H), 7.13(dd,*J*=7.4,1.5Hz,1H),

Table 1: List of Designed Compounds.

Sl. No.	Compounds code	Structure
1	C1	
2	C2	
3	C3	
4	C4	
5	C5	
6	C6	
7	C7	
8	C8	
9	C9	
10	C10	

7.06–6.96(m,3H), 6.85–6.70(m,3H), 6.43(t,*J*=7.5Hz,1H), 4.27(s,1H), 3.60(s,2H). ^{13}CNMR (126MHz, DMSO-d_6) δ170.30, 155.81, 154.37, 134.50, 130.00, 129.31, 128.03, 127.26, 127.03, 124.45, 119.01, 108.40, 22.31.

(E)-5-(5-bromo-2-hydroxystyryl)-2-(furan-2-carbonyl)-2,4-dihydro-3H-pyrazol-3-one(C5)

Compound C5 was synthesized according to the general

procedure. Yield 75%. Mp167°–169°C. FT-IR (ν,cm−1): 3053.71, 1698.38, 1597.59, 1487.37, 1215.60, 1173.79, 838.72. m/z: Calculated for C5 for 373.99 observed 373.99(100.0%), 375.99(97.3%), 376.99(16.8%), 374.99(16.2%), 377.99(1.2%), 374.99(1.1%), 376.00(1.1%). ^{1}HNMR (500MHz, DMSO-d_6) δ9.60(s,1H), 7.51(dd,*J*=7.4,1.5Hz,1H), 7.13(dd,*J*=7.4,1.5Hz,1H), 7.06–6.96(m,3H), 6.85– 6.70(m,3H), 6.43(t,*J*=7.5Hz,1H), 4.27(s,1H), 3.60(s,2H). ^{13}CNMR (126MHz, DMSO-d_6) δ170.30, 155.81, 154.37, 134.50, 130.00, 129.31, 128.03, 127.26, 127.03, 124.45, 119.01, 22.31.

Table 2: **Docking Score of the Designed Compounds.**

Sl. No.	Designed compounds	Docking score
1.	C1	-8
2.	C2	-8
3.	C3	-8.1
4.	C4	-8
5.	C5	-8.4
6.	C6	-8.1
7.	C7	-8.1
8.	C8	-8.4
9.	C9	-8
10.	C10	-8.1

(E)-2-(4-chlorobenzoyl)-5-(4-methoxystyryl)-2,4-dihydro-3H-pyrazol-3-one(C6)

Compound C6 was synthesized according to the general procedure. Yield 92%; MP: 187° – 188°C; FT-IR (ν,cm−1): 2918.69; 1632.81; 1539.87; 1001.14; 758.51. m/z: Calculated for C6 for 354.08 observed 354.08 (100.0%), 356.07 (32.0%), 355.08 (20.5%), 357.08 (6.6%), 356.08 (2.0%); ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.79 – 7.65 (m, 2H), 7.53 – 7.38 (m, 2H), 7.36 – 7.22 (m, 2H), 7.19 (s, 1H), 6.93 – 6.79 (m, 2H), 6.75 (s, 1H), 3.83 – 3.79 (m, 3H), 3.60 – 3.56 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 179.24, 155.97, 134.70, 134.50, 129.99, 129.36, 129.30, 128.79, 128.02, 127.88, 127.26, 127.02, 126.63, 124.45, 123.71, 119.40, 119.01, 64.87, 18.59.

(E)-2-(4-chlorobenzoyl)-5-(4-methoxy-3-methylstyryl)-2,4-dihydro-3H-pyrazol-3-one(C7)

Compound C7 was synthesized according to the general procedure. Yield 86%. MP: 291° – 293°C; FT-IR (ν,cm−1): 2918.07; 1597.62; 1544.14; 866.06; 750.38. m/z: Calculated for C7 for 368.09 observed 368.09 (100.0%), 370.09 (32.0%), 369.10 (21.6%), 371.09 (6.9%), 370.10 (2.2%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.80 – 7.65 (m, 2H), 7.53 – 7.39 (m, 2H), 7.22 (d, *J* = 9.3 Hz, 2H), 7.16 (s, 1H), 6.78 (d, *J* = 28.5 Hz, 2H), 3.83 – 3.79 (m, 3H), 3.60 – 3.56 (m, 2H), 2.35 – 2.31 (m, 3H).^{13}C NMR (101 MHz, DMSO-d_6) δ 175.15, 166.21, 163.94, 161.39, 132.62, 131.80,

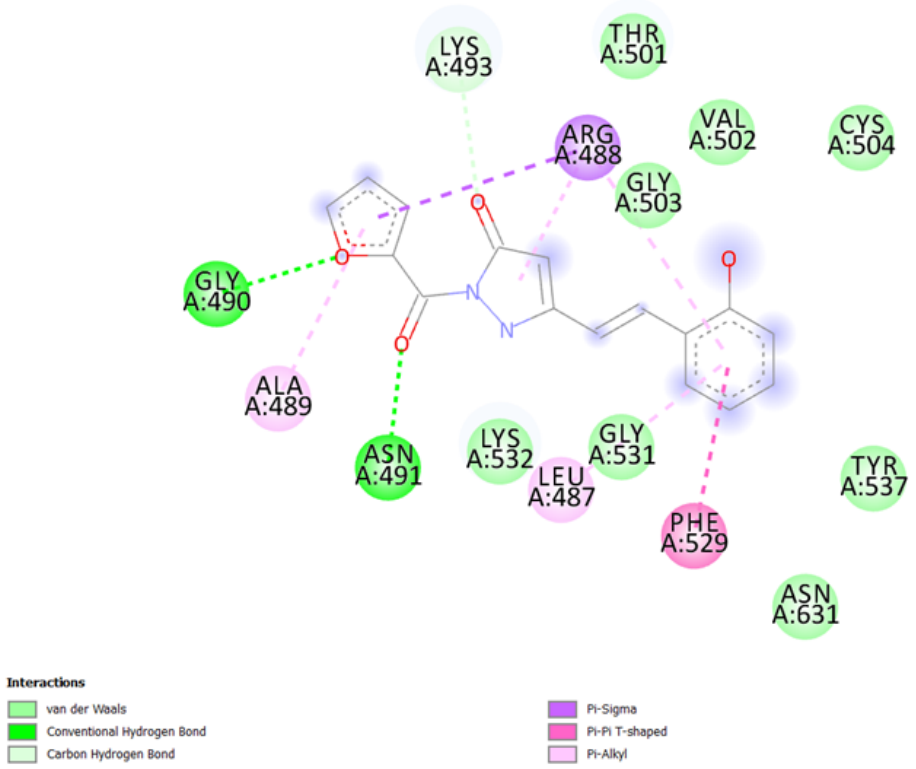


Figure 1: Docking interaction of compound C5 with 4EY6 protein.

Table 3: Effect of compound pyrazolone derivatives on cell viability in cultured shsy5y cells.

Compound code	Tested concentrations µg/mL	OD at 540nm (Mean± SD)	%Viability	IC_{50}
C1	500	0.357 ± 0.0015	61.23	
	250	0.421 ± 0.0015	72.21	
	100	0.512 ± 0.0025	87.82	34.72
	50	0.799 ± 0.0057	62.96	
	25	0.892 ± 0.003	47.00	
	Control	0.583 ± 0.0021		
C2	500	0.210 ± 0.0025	36.02	
	250	0.462 ± 0.0014	79.25	
	100	0.696 ± 0.002	80.62	26.10
	50	0.852 ± 0.0025	53.86	
	25	0.960 ± 0.0051	35.34	
	Control	0.583 ± 0.0021		
C3	500	0.264 ± 0.0021	45.28	
	250	0.481 ± 0.0015	82.50	
	100	0.625 ± 0.02	92.79	45.20
	50	0.793 ± 0.0025	63.98	
	25	0.934 ± 0.0031	39.80	
	Control	0.583 ± 0.0021		
C4	500	0.238 ± 0.0031	40.82	
	250	0.454 ± 0.0018	77.87	
	100	0.552 ± 0.0025	94.68	39.69
	50	0.615 ± 0.0026	60.21	
	25	0.914± 0.001	43.23	
	Control	0.583 ± 0.0021		
C5	500	0.281 ± 0.0021	48.20	
	250	0.462 ± 0.0015	79.25	
	100	0.562 ± 0.0021	96.39	106.29
	50	0.761 ± 0.0015	69.46	
	25	0.958 ± 0.0025	35.67	
	Control	0.583 ± 0.0021		
C6	500	0.234 ± 0.0025	40.13	
	250	0.427 ± 0.001	73.24	
	100	0.548 ± 0.0021	93.99	56.05
	50	0.698 ± 0.002	80.28	
	25	0.915 ± 0.0021	43.06	
	Control	0.583 ± 0.0021		

Compound code	Tested concentrations µg/mL	OD at 540nm (Mean± SD)	%Viability	IC_{50}
C7	500	0.234 ± 0.002	40.14	
	250	0.520 ± 0.0015	89.20	
	100	0.624 ± 0.0021	92.96	82.26
	50	0.767 ± 0.0021	59.34	
	25	0.953 ± 0.003	36.53	
	Control	0.583 ± 0.0021		
C8	500	0.224 ± 0.0015	38.43	
	250	0.457 ± 0.001	78.39	
	100	0.592 ± 0.0021	98.46	145.62
	50	0.737 ± 0.0015	73.58	
	25	0.991 ± 0.0025	30.01	
	Control	0.583 ± 0.0021		
C9	500	0.129 ± 0.0021	77.87	
	250	0.372 ± 0.0057	63.81	
	100	0.702 ± 0.0015	79.58	92.63
	50	0.781 ± 0.0015	66.03	
	25	0.844 ± 0.002	55.23	
	Control	0.583 ± 0.0021		
C10	500	0.323 ± 0.0015	55.41	
	250	0.422 ± 0.0025	72.39	
	100	0.658 ± 0.002	87.13	87.77
	50	0.739 ± 0.0015	73.24	
	25	0.862 ± 0.0021	52.14	
	Control	0.583 ± 0.0021		

131.22, 130.90, 128.64, 127.81, 127.51, 126.30, 56.30, 54.35, 44.56, 18.73.

(E)-2-(4-chlorobenzoyl)-5-(2-(furan-2-yl) vinyl)-2,4-dihydro-3H-pyrazol-3-one(C8)

Compound C8 was synthesized according to the general procedure. Yield 84%.MP: 181° – 183°C; FT-IR (ν,cm−1): 3056.53; 1628.72; 1598.94; 958.46; 748.23. m/z: Calculated for C8 for 314.05 observed 314.05 (100.0%), 316.04 (32.0%), 315.05 (17.3%), 317.05 (5.5%), 316.05 (1.4%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.72 (d, *J* = 7.5 Hz, 2H), 7.58 (dd, *J* = 7.5, 1.6 Hz, 1H), 7.45 (d, *J* = 7.5 Hz, 2H), 6.61 – 6.51 (m, 2H), 6.39 (dd, *J* = 19.1, 11.3 Hz, 2H), 3.57 (s, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 172.98, 170.30, 134.50, 130.00, 129.31, 128.03, 127.26, 127.03, 124.45, 119.01, 20.90, 19.79.

(E)-2-(4-chlorobenzoyl)-5-(2,3,4-trihydroxystyryl)-2,4-dihydro-3H-pyrazol-3-on e(C9)

Compound C9 was synthesized according to the general procedure. Yield 87%. MP: 195° – 196°C; FT-IR (ν,cm−1): 2917.59; 1628.82; 1598.56; 930.41; 748.34. m/z: Calculated for C9 for 372.05 observed 372.05 (100.0%), 374.05 (32.0%), 373.05 (19.5%), 375.05 (6.2%), 374.06 (1.8%), 374.06 (1.0%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.73 (d, *J* = 7.5 Hz, 2H), 7.45 (d, *J* = 7.5 Hz, 2H), 6.97 (d, *J* = 15.0 Hz, 1H), 6.57 (d, *J* = 7.5 Hz, 1H), 6.29 (d, *J* = 15.2 Hz, 1H), 6.16 (d, *J* = 7.3 Hz, 1H), 4.33 (s, 1H), 3.58 (s, 2H), 3.21 (s, 1H), 2.53 (s, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 176.12, 168.50, 156.63, 148.92, 148.39, 132.92, 130.39, 129.49, 127.79, 127.56, 125.64, 124.66, 123.54, 122.11, 121.60, 109.19, 70.35, 58.71, 21.20.

(E)-2-(4-chlorobenzoyl)-5-(2,3-dihydroxystyryl)-2,4-dihydro-3H-pyrazol-3-on e(C10)

Compound C10 was synthesized according to the general procedure. Yield 89%. MP: 191° – 193°C; FT-IR (ν,cm−1): 3056.53; 1628.72; 1598.64; 913.43.; 748.23. m/z: Calculated for C10 for 373.99 observed 373.99(100.0%), 356.06 (100.0%), 358.05 (32.0%), 357.06 (19.5%), 359.06 (6.2%), 358.06 (1.8%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.74 (d, *J* = 7.5 Hz, 2H), 7.46 (d, *J* = 7.5 Hz, 2H), 7.05 (d, *J* = 15.0 Hz, 1H), 6.73 (dd, *J* = 7.4, 1.5 Hz,

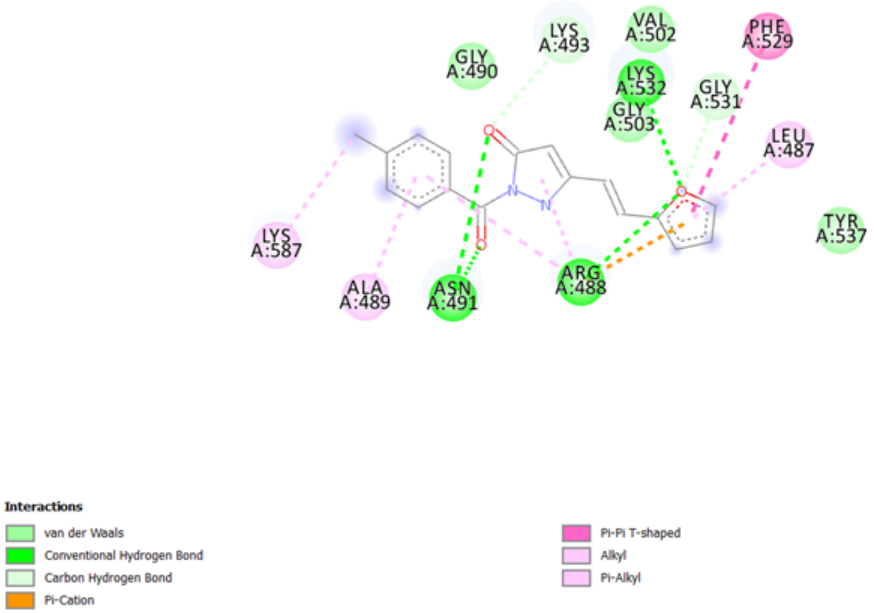


Figure 2: Docking interaction of compound C8 with 4EY6 protein.

1H), 6.66 (t, *J* = 7.4 Hz, 1H), 6.63 – 5.77 (m, 2H), 6.43 (d, *J* = 15.0 Hz, 1H), 6.43 (d, *J* = 15.0 Hz, 1H), 4.72 (s, 1H), 3.60 (s, 2H), 3.50 (s, 1H).[13]C NMR (126 MHz, DMSO-d_6) δ 176.12, 168.50, 156.63, 148.92, 148.39, 132.92, 130.39, 129.49, 127.79, 127.56, 125.64, 124.66, 123.54, 122.11, 121.60, 70.35, 58.71, 21.20.

Docking results

Docking using PyRx 0.9 was performed on the ten designed molecules. Despite having various docking scores (Table 2), the findings demonstrated that all derivatives have a nearly identical binding mechanism. The computational study found that compounds C5 and C8 binding energy was 8.4 Kcal/mol higher than its counter parts. At the enzyme active site, the C5 Furan ring and the phenyl ring provide all of the hydrogen bonds between Arginine (ARG) 488 and Glutamine (GLY) 490 (Figure 1).

Results of biological evaluation

Effect of synthesized compounds on cytotoxicity induced by Aβ25–35 in SHSY5Y cells

Aβμ25-35 (10-50 mol/L) treatment to SH-SY5Y cells for 48 hr led to a dose-dependently substantial reduction in cell viability in the medium. The following research investigated the protective effects of synthetic pyrazole derivatives against Aβ25-35neurotoxicityinSH-SY5Ycells using the 25 μmol/L Aβ25-35. To choose a synthetic drug concentration that won't cause cytotoxicity when studying how synthetic compounds affect Aβ25-35 neurotoxicities, we looked at how different synthetic chemical concentrations such as 25, 50, 100, 250, and 500 μg/mL affected the viability of neuroblastoma cell SH-SY5Y. Pretreatment of SH-SY5Y cells with 25-500g/mL synthetic compounds for 30 min dramatically reduced the cytotoxicity caused by Aβ25-35 and increased cell survival (Table 3).

DISCUSSION

AD is a complex disease marked by neuroinflammation, severe oxidative damage, synapse loss, and neuronal cell death. In this sense, designing multi-target tactics to prevent or slow its progression is a difficult task. AD has been linked to a decrease in AChE levels in the brain, which causes aberrant cholinergic neurotransmission and affects various brain functions, including attention and memory impairment.[27] Alzheimer's Disease (AD) affects around 35 million people worldwide, making it the most common form of dementia. There are no effective treatments or preventive actions. According to studies, substantial breakthroughs in the molecular pathways have occurred since the lethal progressive neurodegenerative sickness was first identified in 1907. AD pathogenesis is characterized by the accumulation of Aβ-containing plaques in the brain over time. Research suggests that Ab has a role in the disease's development.[28] Neuronal apoptosis was observed in human AD brains.[29,30] In the present study, a compact library of such diazoles was synthesized using a multistep procedure. Hydrazinolysis of ethyl acetoacetate was followed by heating with hydrazine hydrate to produce the intermediate 5-methyl-2, 4-dihydro-3H-pyrazolone. The intermediate was subsequently refluxed with acid chloride to get 2-alkyl/aryl-5-methyl-2,4-dihydro-3H-pyrazolone. In the penultimate phase, it interacted with aldehyde to produce a pyrazolone derivative via alcoholic alkali. The synthesized

moieties were identified using IR, ^{1}HNMR,[13] CNMR, and Mass Spectroscopy and tested for anti-alzheimer activity. Docking studies were used to investigate the compounds (C1–C10). The five designed compounds underwent docking with PyRx 0.9. Despite having different docking scores (Table 2), the results showed that all derivatives have substantially identical binding mechanisms. The computational investigation discovered that compounds C5 and C8's binding energy was 8.4 Kcal/mol higher than its counterparts. At the enzyme active site, the C5 Furan ring and the phenyl ring form all of the hydrogen bonds between Arginine (ARG) 488 and Glutamine (GLY) 490 (Figure 1).

For instance, excessive exposure of neurons to the amino acid glutamate causes neuronal damage and apoptosis.[31] The neuroblastoma SH-SY5Y cell culture has been widely employed as an in vitro model for AD research.[32] including the examination of the Aβ1-42 inhibitory action of several chemical substances.[33] This work investigated the neuroprotective effects of pyrazolone derivatives pre-treatment in Aβ and l-glutamate-induced SH-SY5Y cells. Treatment of SH-SY5Y cells with Aβ25-35 (10-50 mol/L) for 48 hr significantly reduced cell viability in the medium in a dose-dependent manner. The study tested synthetic pyrazolone derivatives for their ability to protect SH-SY5Y cells from Aβ25-35 neurotoxicity at a concentration of 25 μmol/L. To avoid cytotoxicity when evaluating the effects of synthetic substances on Aβ25-35 neurotoxicity, we tested the viability of neuroblastoma cell SH-SY5Y at various concentrations (25, 50, 100, 250, and 500 μg/mL). Pretreatment of SH-SY5Y cells with 25-500g/mL synthetic chemicals for 30 min significantly reduced cytotoxicity from Aβ25-35 and boosted cell survival (Table 3).

Among those, (E)-2-(4-chlorobenzoyl)-5-(2-(furan-2-yl)vinyl)-2,4-dihydro-3H-pyrazol-3-one(C5)and(E)-2-(4-chlorobenzoyl)-5-(2-(furan-2-yl)vinyl)-2,4-dihydro-3H-pyrazol-3-one(C8) (Figure 2) were discovered to be the most notable for their *in vitro* cytotoxicity activity against Human SH-SY5Y neuroblastoma cell lines, while the rest of the synthesized derivatives were determined to be moderate. Many researches show that Aβ induces apoptosis in various cell types *in vitro*.[34-36] Aβ25-35 is commonly used in *in vitro* models of

Alzheimer's disease because of its neurotoxic effects, which are similar to those of Aβ1-40/42. These effects include learning and

memory deficits, neuronal death, cholinergic nerve dysfunction, and oxidative damage.[37-39]

CONCLUSION

The purpose of this work was to develop, synthesize, and test the anti-Alzheimer activity of substituted pyrazole derivatives (Table 1). A set of five new substituted pyrazolone compounds was created. Based on the binding score of 8.4 Kcal/mol, the computational investigation concluded that compounds C5 and C8 exhibited better binding energy than other analogues. The synthesized compounds C5 and C8 considerably prevented Aβ25-35 neuron toxicity and lowered the proportion of SH-SY5Y cells that are apoptotic.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

AD: Alzheimer's disease; **Aβ:** Amyloid beta-protein; **ND:** Neurodegeneration; **MAO:** Mono Amino Oxidase; **SSAO:** Semicarbazide-Sensitive Amino Oxidase; **AChE:** Acetylcholine; **PDB:** Protein Data Bank; **SPDBV:** Swiss-Protein Data Bank Viewer; **DMEM:** Dulbecco's Modified Eagle Medium; **FBS:** Fetal Bovine Serum; **DMSO:** Dimethyl Sulfoxide; **ARG:** Arginine; **GLY:** Glutamine; **C:** Compound; **BuChE:** Butylcholine.

SUMMARY

In the current work, the Pyrazolone derivatives are synthesized. The synthesis involved hydrazinolysis of ethyl acetoacetate followed by heating with hydrazine hydrate to yield the intermediate 5-methyl-2,4-dihydro-3H-pyrazolone. This intermediate was then refluxed with acid chloride to form 2-benzoyl-5-methyl-2, 4-dihydro-3H-pyrazolone. In the final step, it reacted with aldehyde to form pyrazolone derivative by alcoholic alkali. The prepared moieties were identified by IR, ^{1}HNMR, ^{13}CNMR & Mass spectroscopy. The synthesized compounds have been developed and tested using molecular docking through PyRx 0.9 and then they are evaluated for Anti-alzheimer's activities. Every synthetic chemical underwent screening to determine its ability to cause *in vitro* cytotoxicity activity against Human SH-SY5Y neuroblastoma cell lines. Cell viability was determined by MTT assay. Compounds C5 and C8 exhibited a better binding score -8.4k/cal respectively compared to other analogues. Synthesized compounds C5 and C8 inhibited Aβ25-35-induced apoptosis in SH-SY5Y cells and protected neural cells from damage. The MTT assay confirmed that compounds C5 and C8 significantly reduced Aβ25-35-induced toxicity among human neuroblastoma SH-SY5Y cells, demonstrating its neuroprotective properties.

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