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Environmental Toxin Induced BACE1 Gene Expression: Selective Inhibition By N-Methyl Piperidinone Analogues Of Curcumin

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Abstract
A series of 1-Methyl-3, 5-bis-(heteroaryl methylene)-piperidin-4-one was synthesised and characterised. Scaffold hoping was performed using in silico tools on N-Methyl piperidinone core structure by substitution at terminal position with six different heterocyclic rings. Combination of ligand-based drug design (LBDD) and structure-based drug design (SBDD) methodology is used for identifying the lead molecule. The hit molecule identified (PM6) was subjected to docking against beta-site amyloid precursor protein cleaving enzyme 1(PDB ID-3IN3). The in vitro MTT assay was conducted for compound code PM6 using neuroblastoma cells. The same experiment was performed against standard drug curcumin. The compound code PM6 exhibited more neuroprotective action on neuroblastoma cells treated with Beta amyloid(10μM) compared to curcumin. Further, the in vitro results are in good correlation with the in silico study conducted.

Key words: *In silico drug design, Monocarbonyl analogues, Curcumin, Neuroprotection.*

INTRODUCTION

Human *β*-Secretase (BACE1) Enzyme

Beta secretase, known as BACE1, is a protease enzyme in humans encoded by the BACE1 gene. The *β*-secretase, *β*-site amyloid precursor protein cleaving enzyme (BACE1; also called Asp2, memapsin 2) is found to be responsible for the formation of *β*-amyloid peptide. This *β*-amyloid peptide is found to be the major constituent in the extracellular aggregations of amyloid protein found in the brain of patients of Alzheimer's disease[1]. This *β*-secretase enzyme acts as a primary target for inhibition of *β*-amyloid peptide and hence slows down the neurodegeneration in Alzheimer's disease. This Amyloid peptide, major component of senile plaques in Alzheimer's disease derived from the cleavage of a larger glycoprotein, namely amyloid precursor protein (APP). This APP plays a crucial role in pathogenesis and is cleaved by *β*-secretase enzyme (BACE1). Drugs inhibit this enzyme in theoretically prevent the accumulation of β-amyloid peptide, which may help to slow down or stop Alzheimer's disease. Studies reported, environmental toxins are one of the major reasons to induce the expression of the BACE1 gene and cause neurodegeneration[2].
Olland AM., deposited three-dimensional structure of human β-Secretase[3] (BACE1) Enzyme with compound 30 in the year 2009 with PDB ID: 3IN3. This belongs to the class of hydrolase enzymes and is derived from Homo sapiens. Molecular docking studies of curcumin and its derivatives were carried out to evaluate their chemotherapeutic property to stop the progression of Alzheimer's disease. This virtual throughput screening,

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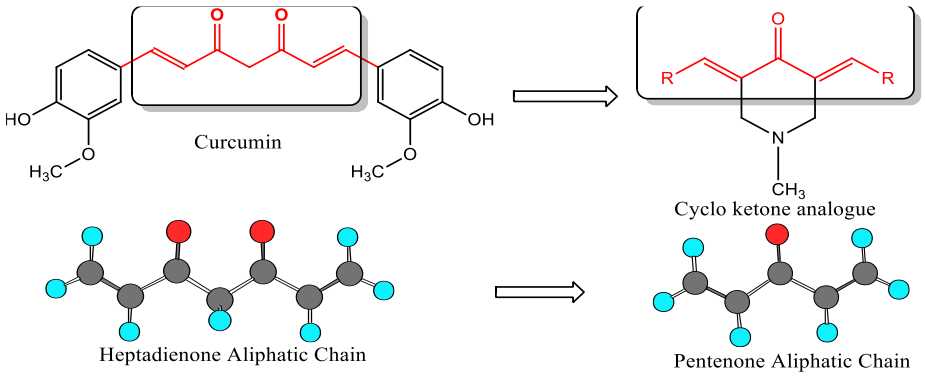
supplemented by studies on cell lines and animal models, was in agreement with confirmation of potential lead compounds. The PDB structure of BACE1 (PDB ID: 3IN3) is provided in Figure 1.

Figure-1. Structure of BACE1with compound 30 of Homo sapiens

Monocarbonyl analogues of Curcumin

Research demonstrates that these cyclo ketone compounds comply with drug likeness regulations. The amount of hydrogen bond providers, acceptors, and total polar surface area, rotatable bonds, and total molecular mass are in good agreement with drug likeness rules. The rise of research in monocarbonyl analogues now replaces the *β*-diketone pharmacophore concept in curcumin research[4]. The swap of heptadienone aliphatic chain with pentenone chain in monocarbonyl analogues is displayed in Figure 2. The studies suggest the monocarbonyl pharmacophore with terminal substitution with aromatic rings is are good scaffold. Additionally, the binding characteristics of these monocarbonyl analogues to various receptors responsible for neurodegeneration have not been explored much. The encouraging outcomes when it docked with COX 2, SARS-CoV-2-spike protein, Human glutathione transferase PI-1, D-alanyl-D-alanine carboxy peptidase, 11β-hydroxysteroid dehydrogenase type-1, Alpha glucosidase enzyme, motivated us to investigate its BACE1 inhibition.

Figure-2. Structural Modification on Heptadienone Scaffold



Experimental Part

1.1. Computer-aided drug design studies

Six analogues of 1-Methyl-3,5-bis-(heteroaryl methylene)-piperidin-4-one prepared from a ligand library of 25 compounds. The ligand library[5] is given in Table 1.

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Table-1. Ligand Library of 1-Methyl-3,5-bis-(heteroaryl methylene)-piperidin-4-one

Code	Smiles
D 1	CN5CC(=Cc2cc1ccccc1[nH]2) C(=O) C(=Cc4cc3ccccc3[nH]4)C5
D 2	CN5CC(=Cc1c[nH]c2ccccc12)C(=O)C(=Cc3c[nH]c4ccccc34)C5
D 3	CN5CC(=Cc1cccc2[nH]ccc12)C(=O)C(=Cc3cccc4[nH]ccc34)C5
D 4	CN5CC(=Cc2ccc1[nH]ccc1c2)C(=O)C(=Cc4ccc3[nH]ccc3c4)C5
D 5	CN5CC(=Cc1cccc2cc[nH]c12)C(=O)C(=Cc3cccc4cc[nH]c34)C5
D 6	CN3CC(=Cc1ccccc1)C(=O)C(=Cc2ccccc2)C3
D 7	CN3CC(=Cc1ccc(O)cc1)C(=O)C(=Cc2ccc(O)cc2)C3
D 8	CN3CC(=CC=Cc1ccccc1)C(=O)C(=CC=Cc2ccccc2)C3
D 9	COc3cc(C=C2CN(C)CC(=Cc1ccc(O)c(OC)c1)C2=O)ccc3O
D 10	CN3CC(=Cc1ccco1)C(=O)C(=Cc2ccco2)C3
D 11	CN3CC(=Cc1cccs1)C(=O)C(=Cc2cccs2)C3
D 12	CN3CC(=CC1CCCCC1)C(=O)C(=CC2CCCCC2)C3
D 13	CN5CC(=Cc2ccc1ccccc1c2)C(=O)C(=Cc4ccc3ccccc3c4)C5
D 14	CN3CC(=Cc1ccc[nH]1)C(=O)C(=Cc2ccc[nH]2)C3
D 15	CN5CC(=Cc2ccc(c1ccccc1)cc2)C(=O)C(=Cc4ccc(c3ccccc3)cc4)C5
D 16	CN3CC(=CN1CCCC1)C(=O)C(=CN2CCCC2)C3
D 17	CN3CC(=CC1CCC(C)=CC1)C(=O)C(=CC2CCC(C)=CC2)C3
D 18	C=C(C)C3CC=C(C=C2CN(C)CC(=CC1=CCC(C(=C)C)CC1)C2=O)CC3
D 19	CN3CC(=CC1CC=CCC1)C(=O)C(=CC2CC=CCC2)C3
D 20	CN3CC(=Cc1nc(C)c[nH]1)C(=O)C(=Cc2nc(C)c[nH]2)C3
D 21	CN3CC(=Cc1cscn1)C(=O)C(=Cc2cscn2)C3
D 22	CN3CC(=Cc1cncnc1)C(=O)C(=Cc2cncnc2)C3
D 23	CN5CC(=Cc2coc1ccccc1c2=O)C(=O)C(=Cc4coc3ccccc3c4=O)C5
D 24	COc3cc(C=CC=C2CN(C)CC(=CC=Cc1ccc(O)c(OC)c1) C2=O) ccc3O
D 25	CN3CC(=Cc1ccc(O)c(O)c1)C(=O)C(=Cc2ccc(O)c(O)c2)C3

The molinspiration search engine[6] was used to assess the ligands' drug-likeness characteristics, and the findings are shown in Table 2. The compounds that were found to have good drug similarity and synthetic feasibility were numbered PM1 to PM6 at the bottom of Table 2.

Table-2. Drug likeness of 1-Methyl-3, 5-bis-(heteroaryl methylene)-piperidin-4-one

Code	Log P	M.W	tPSA	n. HAC	n. HDO
D1	4.5	367.45	51.89	4	2
D2	3.89	367.45	51.89	4	2
D3	3.94	367.45	51.89	4	2
D4	4.35	367.45	51.89	4	2
D5	3.94	367.45	51.89	4	2
D6	3.95	289.37	20.30	4	0
D7	2.99	321.37	60.76	4	2
D8	4.99	341.45	20.30	2	0
D9	2.63	381.42	79.23	6	2
D10	2.10	269.30	46.58	4	0
D11	3.39	301.43	20.30	4	0
D12	4.86	301.47	20.30	0	2
D13	6.31	389.49	20.30	6	2
D14	1.90	267.33	51.89	4	2
D15	4.54	441.57	20.30	2	0
D16	0.47	275.39	26.78	4	2

D17	4.50	325.49	20.30	4	0
D18	5.67	377.57	20.30	2	0
D19	3.89	297.44	20.30	2	0
PM1	3.60	293.41	20.31	2	0
PM2	3.60	293.41	20.31	2	0
PM3	5.18	417.55	20.31	2	0
PM4	5.18	417.55	20.31	2	0
PM5	1.25	291.35	46.09	2	0
PM6	1.12	291.35	97.98	2	0

Swiss ADME target prediction was performed, and Table 3 presents the findings. The compound codes PM1 through PM6 were found to have favourable outcomes with closeness to rules of drug likeness[7]. The Swiss ADME predictions of 1-Methyl-3, 5-bis-(heteroaryl methylene)-piperidin-4-one are outlined in Table 3. The compound codes PM1-PM6 show significant lipophilicity and moderate BBB permeability compared to the other ligands in the library.

Table-3. Swiss ADME prediction of 1-Methyl-3, 5-bis-(heteroaryl methylene)-piperidin-4-one

Code	Log S (ESOL)	BBB permeant	GI absorption	No. of violations
D 1	-4.96	Yes	High	0
D 2	-4.91	Yes	High	0
D 3	-4.91	Yes	High	0
D 4	-4.91	Yes	High	0
D 5	-4.91	Yes	High	0
D 6	-4.19	Yes	High	0
D 7	-3.91	Yes	High	0
D 8	-4.11	Yes	High	0
D 9	-4.64	No	High	0
D 10	-2.91	Yes	High	0
D 11	-3.88	Yes	High	0
D 12	-5.01	Yes	High	0
D 13	-6.48	Yes	High	01
D 14	-2.54	Yes	High	0
D 15	-2.86	Yes	High	01
D 16	-2.15	No	High	0
D 17	-4.06	Yes	High	0
D 18	-5.56	Yes	High	01
D 19	-4.00	Yes	High	0
PM1	-2.89	Yes	High	0
PM2	-3.49	No	High	0
PM3	-2.47	Yes	High	0
PM4	-4.74	Yes	High	0
PM5	-5.25	No	High	0
PM6	-4.04	No	High	0

For PM1-PM6, the findings of the toxicity utilizing the Pro Tox-3.0 program and the predictions made against hepatotoxicity, immunotoxicity, mutagenicity, and cytotoxicity show a strong connection with the prior drug similarity and ADME prediction results[8]. The outcomes are listed in Table 4.

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Table-4. Toxicity class prediction for 1-Methyl-3, 5-bis-(heteroaryl methylene)-piperidin-4-one

Code	LD_{50}(mg/kg)	Toxicity class	Code	LD_{50}(mg/kg)	Toxicity class
D 1	195	03	D 16	750	04
D 2	1012	04	D 17	550	04
D 3	1012	04	D 18	550	04
D 4	1012	04	D 19	550	04
D 5	1012	04	PM1	1190	04
D 6	1012	04	PM2	1016	04
D 7	500	04	PM3	3730	05
D 8	2000	05	PM4	3730	05
D 9	1180	04	PM5	1273	04
D 10	1016	04	PM6	2300	05
D 11	867	04	~	~	~
D 12	550	04	~	~	~
D 13	1012	04	~	~	~
D 14	500	04	~	~	~
D 15	1012	04	~	~	~

PASS bioactivity prediction was carried out for PM1 to PM6. These compounds came out with good anti-bacterial actions. The probability of being active (Pa) and inactive (Pi) for neuroprotective action by BACE1 inhibition is given in Table 5. The compound code PM6 came out with a significant probability for an active lead molecule.

Table-5. PASS online prediction for Pharmacological action and receptor binding

Code	Pa	Pi
PM1	0,308	0,076
PM2	0,720	0,023
PM3	0,726	0,022
PM4	0,713	0,024
PM5	0,195	0,036
PM6	0,684	0,014

Pa= Probability to be active Pi= Probability to be inactive

1.2. Molecular Docking

After being shortlisted and given the names PM1 to PM6, the hit compounds from the early systematic screening approach were put through molecular docking studies. Molecular docking was performed using PyRx and visualized using Discovery Studio Visualizer. Docking was performed to check the binding affinity with BACE1 enzyme. The target protein with PDB ID: 3IN3 was docked with six ligands of PM1-PM6. The binding energy and amino acid interactions, along with hydrogen bond length, are outlined in Table 6. The compound code PM6 showed notable enzyme inhibitions and a good docking score of -8.2k cal/mol.

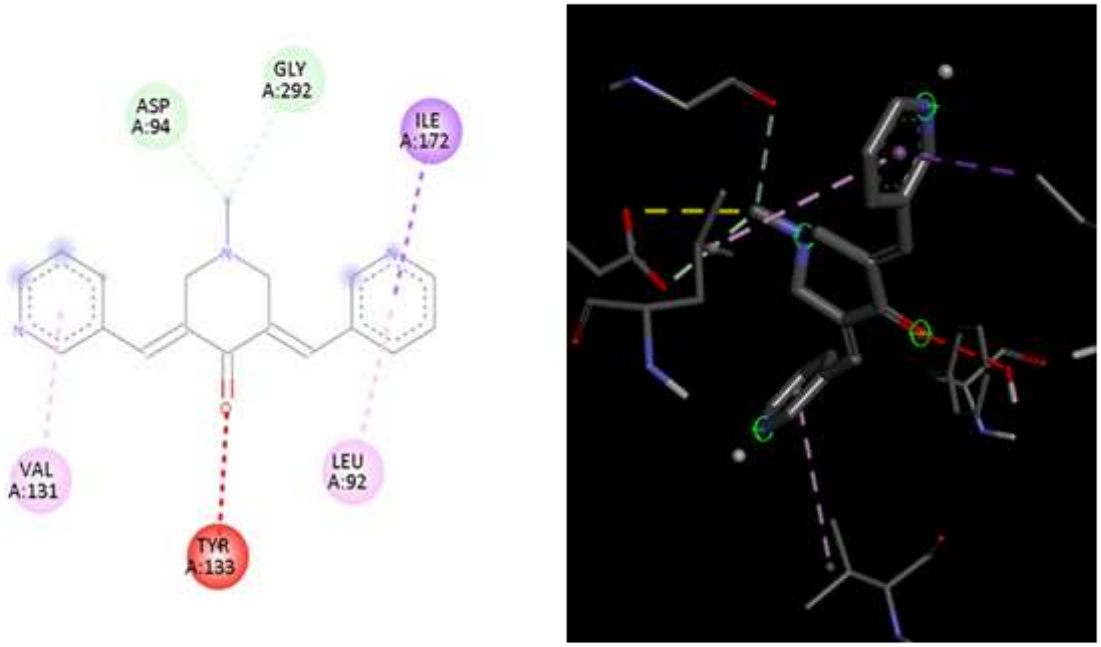
Table-6. Binding energy and enzyme inhibition of 1-Methyl-3,5-bis-(heteroaryl methylene)-piperidin-4- one

Ligand	Binding energy(kcal/mol)	Hydrogen bond		Interactions
		Amino acid	Bond length(Å)	Amino acids
PM1	-6.6	Valine: 56	2.35	Valine:426, Cysteine:397
PM2	-7.1	~	~	Leucine:462, Lysine:725
PM3	-6.8	Leucine:34	2.93	Isoleucine:56, Alanine:135

PM4	-7.1	~	~	Methionine:90, Valine:126, Cysteine:97
PM5	-6.3	Phenyl Alamine:78	2.52	Valine:132, Glycine:387, Alanine:498
PM6	-8.2	Glycine:292 Asparagine:94	3.21 3.34	Asparagine:94 Glycine:292 Leucine:92

All of the ligands were found to fit the receptor pockets perfectly. The ligand-receptor interaction was through alkyl bonds, Pi-Alkyl bonds, hydrogen bonds, and also through other Van der Walls forces of attraction. The ligand receptor interactions of PM 6 were portrayed in 3D and 2D form in Figure 3 below.

Figure-3. Ligand Interaction of 1-Methyl-3,5-bis-(heteroaryl methylene)-piperidin-4- one with 3IN3



1.3. Synthesis of 1-Methyl-3,5-bis-(heteroaryl methylene)-piperidin-4-one

Six synthetic analogues of 1-Methyl-3,5-bis-(heteroaryl methylene)-piperidin-4-one (PM1, PM2, PM3, PM4, PM5, PM6) were prepared by condensation of N-methyl piperidone with heterocyclic aldehydes using the method outlined[9] Eryanti, Y., et al., 2018 displayed in scheme-1.

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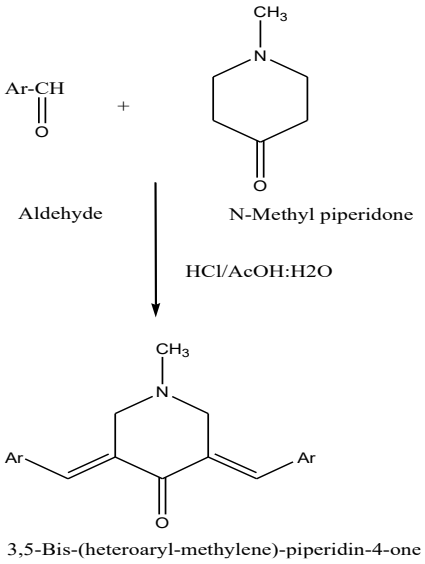
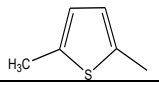
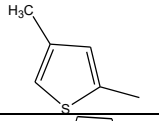
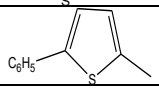
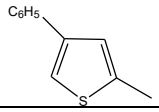
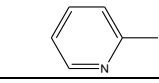
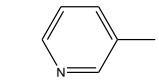
Scheme-1. Synthesis of 3,5-bis-(heteroaryl methylene)-piperidin-4-one



Compound	Ar
PM1	
PM2	
PM3	
PM4	
PM5	
PM6	

A mixture of the appropriate aldehyde (0.02mol) and the ketone (0.01mol) was heated in a water bath at 25-30°C until a clear solution was obtained, then 2M concentrated HCL was added while stirring for 5 min. The reaction mixture was then stirred at room temperature for 2hr. After two days, the mixture was treated with cold AcOH/water (1:1) and filtered. The solid obtained was then dried and crystallized from ethanol 95%.

1.3. Characterization of 1-Methyl-3,5-bis-pyridin-3-yl-methylene-piperidin-4-one

UV Spectra of 1-Methyl-3,5-bis-(heteroaryl methylene)-piperidin-4- one

Due to $\pi \rightarrow \pi^*$transitions, the compounds' UV spectra in 95% ethanol (10^{-3} M) displayed one broad band at 270 to 287 nm. Due to the double bond's conjugation to heterocyclic rings, these bands naturally moved to a higher wavelength. The electronic effects of various heteroaryl groups can be linked to the observed absorption maxima of this band.

IR Spectra of 1-Methyl-3,5-bis-(heteroaryl methylene)-piperidin-4- one

IR spectra of compounds exhibited prominent bands due to C-H stretching ranging from 2800 to 3300 cm^{-1}.C=0 stretching vibrational bands were observed at 1554 to 1734 cm^{-1}. Further, the alkenyl stretching vibrations observed at 1693 to 1714 cm^{-1} confirm the formation of the expected structure, and agree with reaction completion. The band at shorter wavelength region, ranging from 756 to 871.82 $cm^{-1,}$ is due to C-H bending vibrations. The characteristic IR spectral data of PM6 is given in Table 7.

Table-7. The characteristic IR data of 1-Methyl-3,5-bis-pyridin-3-yl-methylene-piperidin-4-one[10]

PM6	Probable Assignments
2056.12	CH-Stretching
1554.63	C=O Stretching
1693.50	C=C Stretching
871.82	CH Bending

^{1}H NMR Spectra of 1-Methyl-3,5-bis-pyridin-3-yl-methylene-piperidin-4-one[10]

The synthesized compounds' N-CH_3 signals were detected with δ values ranging from 2 to 2.12. The presence of aromatic CH proton is the cause of the multiplet signals seen in δ values 7.81 to 7.98. The signals of the aryl

CH proton are seen between 8.7 and 8.9. Table 8 displays the ^{1}H NMR spectra of. 1-Methyl-3,5-bis-pyridin-3-yl-methylene-piperidin-4-one. The ^{1}H NMR spectrum δ values are outlined in table-8

Table-8. ^{1}H NMR Spectra of 1-Methyl-3,5-bis-pyridin-3-yl-methylene-piperidin-4-one

Chemical shift (δ ppm)	Probable Assignment
2.1	Amino N-CH_3
7.8-7.9	Alkenyl- CH
8.7-8.9	Aryl-CH
3.1	Aliphatic CH

1.4. *In vitro* Neuroprotective Effect Determination by MTT Assay

SHSY-5Y (Neuroblastoma cells) cell line was purchased from NCCS Pune was maintained in Dulbecco's modified eagles' media (HIMEDIA) from National Centre for Cell Sciences (NCCS), Pune, India and maintained Dulbecco's Modified Eagles medium (DMEM) (Sigma Aldrich, USA). The cell line was cultured in a 25 cm^2 tissue culture flask with DMEM supplemented with 10% FBS, L-glutamine, sodium bicarbonate, and antibiotic solution containing: Penicillin (100U/ml), Streptomycin (100µg/ml), and Amphotericin B (2.5µg/ml). Cultured cell lines were kept at 37°C in a humidified 5% CO_2 incubator (NBS Eppendorf, Germany).

The viability of cells was evaluated by direct observation of cells by Inverted phase contrast microscope and followed by MTT assay method[11-12]. Two days old confluent monolayer of cells was trypsinized, and the cells were suspended in 10% growth medium. 100µl cell suspension ($5x10^4$ cells/well) was seeded in 96 96-well tissue culture plate and incubated at 37°C in a humidified 5% CO_2 incubator. 1mg of the sample was weighed and completely dissolved in 1mL DMEM using a cyclomixer.

The extract solution was filtered through a 0.22 µm Millipore syringe filter to ensure sterility. Beta amyloid(10µM) was added to induce toxicity.

Cytotoxicity Evaluation:

After attaining sufficient growth, Beta amyloid(10µM) was added to induce toxicity and incubated for one hour. After 1 hour, freshly prepared each compound was added at concentrations of 25µg/ml, 12.5 µg/ml, 6.25µg/ml, 3.1 µg/ml, and 1.5 µg/ml of DMEM. Each concentration was added in triplicate to the respective wells and incubated at 37°C in a humidified 5% CO_2 incubator. Untreated control cells and Beta amyloid alone-treated wells were also maintained.

Cytotoxicity Assay by Direct Microscopic Observation:

Entire plate was observed after 24 hours of treatment in an inverted phase contrast tissue culture microscope (Olympus CKX41 with Optika Pro5 CCD camera), and microscopic observations were recorded as images. Any detectable changes in the morphology of the cells, such as rounding or shrinking of cells, granulation, and vacuolization in the cytoplasm of the cells, were considered as indicators of cytotoxicity. Phase contrast microscopic image of morphology of cells given in Figure 4.

Cytotoxicity Assay by MTT Method:

Fifteen mg of MTT (Sigma, M-5655) was reconstituted in 3 ml PBS until completely dissolved and sterilized by filter sterilization. After 24 24-hour incubation period, the sample content in wells was removed, and 30µl of reconstituted MTT solution was added to all test and cell control wells. The plate was gently shaken well, then incubated at 37°C in a humidified 5% CO_2 incubator for 4 hours. After the incubation period, the supernatant was removed, and 100µl of MTT Solubilization Solution (DMSO was added and the wells were mixed gently by pipetting up and down in order to solubilize the formazan crystals. The absorbance values were measured by using a microplate reader at a wavelength of 540 nm (Laura B. Talarico et al., 2004).

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The percentage of growth inhibition was calculated using the formula:

$$\% \text{ of viability} = \frac{\text{১ OD Samples x } 100}{\text{OD of control group}}$$

Statistical analysis of MTT assay for neuroprotective action of PM 6 and standard drug curcumin for comparison was displayed in Tables 9 and 10.

Table-9. Percentage viability produced by PM6 for Beta amyloid induced cytotoxicity in Neuroblastoma cells

Sample PM6	OD1	OD2	OD3	Percentage viability 1	Percentage viability 2	Percentage viability 3	Average	St. dev.	Std. error
Control	0.35	0.36	0.36	100	100	100	100	0	0
Beta amyloid	0.17	0.17	0.18	50.281	47.534	49.236	49.01	1.386	0.46
1.5	0.19	0.19	0.20	55.665	54.126	54.934	54.90	0.769	0.25
3.1	0.22	0.23	0.22	64.374	62.980	62.131	63.16	1.132	0.37
6.25	0.25	0.26	0.26	71.082	71.206	71.128	71.13	0.062	0.02
12.5	0.28	0.27	0.27	80.439	75.565	74.918	76.97	3.018	1.00
25	0.30	0.31	0.30	85.682	85.017	84.214	84.97	0.734	0.24

Table-10. Percentage viability produced by Curcumin for Beta amyloid induced cytotoxicity in Neuroblastoma cells

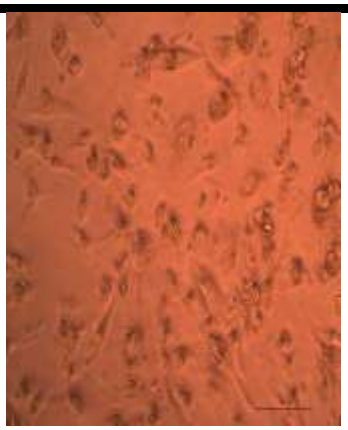
Sample Curcumin	OD1	OD2	OD3	Percentage viability 1	Percentage viability 2	Percentage viability 3	Average	St. dev.	Std. error
Control	0.35	0.36	0.36	100	100	100	100	0	0
Beta amyloid	0.17	0.17	0.18	50.28	47.53	49.23	49.01	1.38	0.46
1.5	0.19	0.19	0.20	53.60	51.94	54.66	53.40	1.36	0.45
3.1	0.20	0.21	0.21	56.93	57.53	58.23	57.56	0.65	0.21
6.25	0.23	0.24	0.24	64.93	65.59	65.83	65.45	0.46	0.15
12.5	0.26	0.25	0.25	73.36	69.08	69.87	70.77	2.27	0.75
25	0.27	0.28	0.28	78.10	76.57	77.67	77.44	0.78	0.262

Figure-4. Phase contrast microscopic image of morphology of cells

Beta amyloid-treated cells

Control

PM6 at 25µg/ml concentration



Curcumin at 25µg/ml concentration

RESULTS & DISCUSSION

The pharmacological actions of curcumin are mainly said to be mediated through a 1, 3-diketone system, in which the diketo function is attached directly to olefinic linkages. The strategy involved replacing the diketo system with mono-carbonyl analogues, securing a huge attention of medicinal chemists in the last decade. Further, these modifications were adopted in the justification of computer-aided drug design techniques. The drug likeness rules, ADME prediction, along with toxicity prediction conducted in this study, are in agreement with shortening the chain size to form mono carbonyl analogues of curcumin. The initial docking studies conducted in this direction also support the promising neuroprotective action of PM1-PM6. The compound code PM6 showed good binding affinity and strong hydrogen bond interactions in the *in-silico* part of the study. Hence, N-Methyl Piperidone with 3-Pyridyl substitution was found to be the lead molecule of the *in silico* study. The structure of 1-Methyl-3,5-bis-pyridin-3-yl-methylene-piperidin-4-one (PM6) was established by using UV, FTIR, ^{1}H NMR, and mass spectroscopic techniques. The alkenyl bond formation in conjugation with heterocyclic ring pi bonds. The new alkenyl bond formation is in agreement with the peaks and signals observed in IR and ^{1}H NMR spectrum.

Further, the *in vitro* MTT assay conducted to assess the neuroprotective action of the PM6 came out with results comparable matched with *in silico* prediction. Beta amyloid-treated neuroblastoma cells show significant deduction (49.01%) in cell viability. Whereas PM6 and curcumin, when treated with beta amyloid, induced neuroblastoma cells to show significant recovery with increased cell viability. The percentage of cell viability observed with PM6 for beta amyloid induced cytotoxicity was found to be 84.97% at a concentration of 25µg/ml. This was more than curcumin (77.44%) treated in the same manner for cytotoxic action.

This study suggests that monocarbonyl analogues of curcumin demand more attention in the future. These monocarbonyl analogues with terminal substitution with pyridine are a promising drug candidate. Our research suggests this may be due to the extended conjugation of alkenyl bond with pyridine ring. The heteroaryl substitution improves overall polarity and ionizability of the drug could be a reason for significant action of PM6. This study also proves the effective use of drug design tools to predict neuroprotective action of monocarbonyl curcumin. Further work can be performed on lead molecule to check its toxicity and bioavailability.

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