Design, Docking and Synthesis of Novel Carbamate derivatives as Acetyl-Cholinesterase inhibitors

Ekta Khare[1], Dr. Zeeshan Fatima*[1], Dr. O.P. Tiwari[2]

[1]Amity Institute of Pharmacy, Lucknow, Amity University Uttar Pradesh Lucknow, India, Sector 125, Noida, 201313, India

[2]Vindhya Gurukul College of Pharmacy,A.K.T.U. Uttar Pradesh Chunar-Mirzapur,India

Corresponding Author: zfatima@amity.edu

Abstract

A series of novel diphenyl methanol carbamate (DPM) derivatives as acetyl cholinesterase inhibitors for Alzheimer's disease were designed docked and synthesized. Molecular docking studies revealed that the binding of DPM derivatives were better as compared to standard drug rivastigmine. The synthesized compounds were further subjected to enzymatic assay for AChE inhibition where compound 3g exhibited potent in-vitro inhibitory activity against AChE having(20.65 μM) IC_{50} value. Further in silico screening was carried out to predict ADME parameters including drug likeliness. Compound 3g was found to be within drug likeliness range having 3.12 logP. Therefore, compound 3g can be considered as a promising molecule for further development of novel compound as anti-Alzheimer's agents.

.**Keywords:** Carbamate, acetylcholinesterase inhibitors, diphenyl methanol, Antialzheimer, synthesis

INTRODUCTION

Alzheimer's disease (AD) is a neuro-degenerative disorder that leads to gradual degeneration of the human brain which is being dramatically increasing worldwide. (Mina Saeedi et al., 2016) It is categorized under dementia. Dementia disease, is a group of mental disorders that effects memory, thinking, comprehension, and other essential brain functions.In Alzheimer's, memory loss is one of the earliest symptoms along with changes in personality and behavior. It is believed that in our brain, there is one specific protein beta-amyloid whose overproduction and accumulation in the brain leads to nerve cell death. It is characterized by aggregation of protein and tangles known as tau protein. Alois Alzheimer had discovered this disease in 1906 and so it is named. Over 25 million people worldwide currently experience AD. By 2050, this number could rise as high as 114 million. (Xiaoguang du et al. ,2018)

There are two main enzymes such as acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), are involved in AD. They act by hydrolyzing the neurotransmitter acetylcholine in cholinergic transmission. There is still much to learn about the genesis of AD.(Sara Azimia et al., 2017) However, scientists have shown that the pathophysiology of AD is characterized by hypo cholinergic function, amyloid-(A) deposition, intracellular neurofibrillary tangles (NFTs), oxidative stress, inflammation, hereditary factors, hormones, and metal ion deficit. The hypofunction of acetylcholine-containing neurons (ACh) in the brain is explained by the cholinergic theory. It triggers the cognitive deterioration in AD.

Only five FDA approved drugs such as Tacrine, Rivastigmine, Donepezil, Galantamine & Memantine are providing a symptomatic relief. Except for memantine, NMDA (N-Methyl D-Aspartate) receptor antagonist, the other four are cholinesterase inhibitors. While treating the elderly, many reports have come up with disadvantages and side effects for these drugs, which proved their unsuccessful application as medications.(Mona Mehta et al. 2012) Symptoms like hepatotoxicity, renal failure, abdominal pain, anorexia, nausea, and variation of blood levels of drugs led to discontinuation of treatment in AD patients. Apart from these facts, none of these drugs have proved their potential role in the effective treatment of mild cognitive impairment, 'symptomatic pre-dementia stage' which often progress mainly towards AD dementia. On the flip side, new drug development or better implementation of old drugs through clinical trials is challenging. Aβ peptide and the enzymes β and γ-secretase that generate Aβ peptide comprise the drug development targets. Clinical trials for these targets with developing drugs show frailty to get a successful outcome. (Francisco Javier et al., 2017) These findings have prompted us to explore new chemical entities having AChE inhibitory activity for Alzheimer's.

Methods:

Preliminary design

AChE inhibitors, Rivastigmine,has a carbamate moiety in its structure that is responsible for covalent interactions with the active site of the enzyme. Keeping this in view, a conformational restricted analog bearing a carbamate moiety were designed as shown in the figure 1.

The designed molecules were further subjected to docking studies in order to figure out the binding of the diphenyl methanol analogs with the receptor.

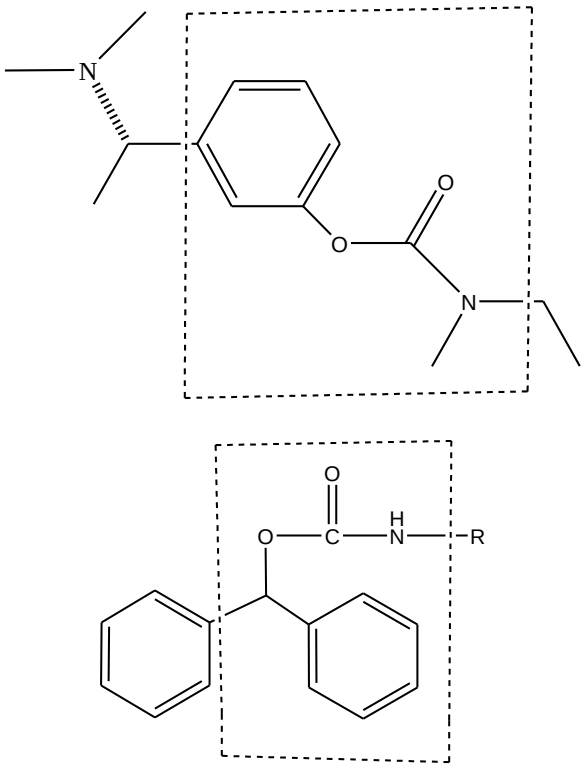


Fig. 1: Design of new Rivastigmine like analogs

Docking study:

The designed compounds were docked to the crystal structure of acetyl cholinesterase (PDB ID: 1GQR). Docking simulations were performed using Schrodinger Maestro software (version 11.1.012; Schrodinger LLC, New York).

The crystal structure of AChE (PDB ID:1GQR) was downloaded from PDB The protein structure was modified to conform to the pH 7 environment by adding hydrogen bonds and removing water molecules using a Maestro tool called Protein Prep Assistant. Default settings for docking small proteins were used throughout the simulations. Prepared proteins were docked at the junction using an extra precession mode. The top 10 poses were collected for each molecule with the highest docking score, compared to the co-crystal compound. Further on the basis of docking score, the compounds were synthesized. The synthesized compounds were tested for *in vitro* activity.

Experimental

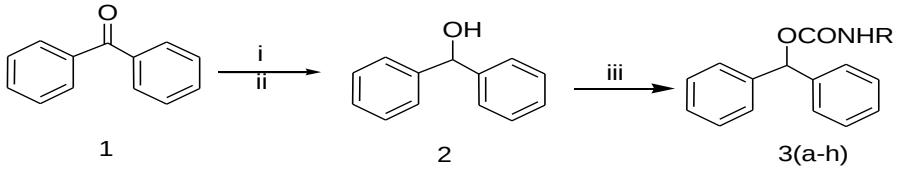
All chemicals including raw materials, reagents, and solvents were acquired from commercial vendors such as Merck and Sigma-Aldrich. On Merck silica gel (70-230 mesh), column chromatography was used to purify intermediates and finished chemicals. Using potassium bromide (KBr) discs and a Shimadzu 8400 S spectrophotometer, FTIR spectra were captured. Mass spectra were acquired on a positive mode ESI-MS spectrophotometer using water OPLC-TQDMS, and H^1NMR spectra were recorded on a JEOL AL 300 MHz spectrometer using TMS as an internal standard in DMSO/$CDCl_3$.

General method for the Preparation of diphenyl methanol

Benzophenone (2.0 mmol) was dissolved in 25 ml of ethanol and the solution was stirred continuously. The stirred benzophenone solution was treated with sodium borohydride (2.2 mmol). The progress of reaction was monitored through TLC by using mobile phase consisting of petroleum ether: ethyl acetate .Spots on the slides were visualized in iodine chamber.The final work up of the solution was done by pouring it in an ice-bath and adding 4M Hydrochloric acid (3ml). The precipitated product thus obtained by suction filtration was washed with cold water several times and dried. The compound was obtained as colorless powder having 81 %, yield.

General method for the Preparation of substituted benzhydryl phenyl carbamates

Diphenyl methanol was dissolved in dry THF at 10°C temperature and to it sodium hydride was added. After stirring for 15-20 minutes, a substituted isocyanate derivative was added to the stirring reaction mixture. The progress was monitored through TLC & spots on the slides were visualized in U.V. chamber/iodine chamber. After completion of reaction THF was removed and water was added. The result was a solid product that was filtered and dried. All the title compounds were synthesized by adopting the same procedure with different substituent having variation in reaction time.



Reagents : (I) Ethanolic KOH, $NaBH_4$ (ii) Substituted Isocyanates ,NaH ,Dry DHF

No	**R**
3a	3-Cl
3b	4-NO_2
3c	2,4 dichloro
3d	3-NO_2
3e	4-OMe
3f	4-Cl
3g	4-F
3h	4-Me

Table 1: R values for the compounds

3a Benzhydryl-3-chloro phenyl carbamate (3): Creamish yellow powder**,** Yield-76.6 % ;I.R.(KBr) cm^{-1}: 3502 (NH carbamate stretch),1746 (C=O ester stretch), 1285 (C-O ester stretch), 760 (C-Cl stretch); H^1NMR [500 MHz, $CDCl_3$,δ (ppm)] 8.96 (s, 1H) NH, 7.03-7.70 (m, 14 H) Aromatic,5.86 (d, 1 H) CH; EIMS:m/z (m^+)= 338

3b Benzhydryl-4-nitro phenyl carbamate(4): Turmeric yellow; Yield-80%; I.R.(KBr) cm-1:3322(NH Stretch) ,3031,2924(Aromatic C-H Stretch), 1743(Carbamate ester C=O Stretch),1660,1610(Aromatic C=C Bending), 1547(C-NO2),1302(Aromatic ester C-O Stretch), 852 (p-substituted)**;** H^1 NMR [500 MHz, CDCl3 , δ (ppm)] 8.25 (d, 1 H) NH, 7.36-7.79 (m;14 H) Aromatic, 6.9 (s;1 H) CH**;** EIMS:m/z (M^+, 100)=347

3c Benzhydryl-2,4-dichloro phenyl carbamate (5): White colored powder solid, yield-70%;I.R.(KBr) cm^{-1}: 3396 (NH carbamate stretch),1738(C=O ester stretch),1254(C-O ester stretch), 566, 599 (C-Cl stretch));H^1NMR[500 MHz CDCl3, δ (ppm)] 9.5 (s, 1H) NH, 7.6-7.7 (d, 2H) , 7.2-7.5 (m, 11H)Aromatic, 6.86 (s, 1 H) CH**;** EIMS:m/z (M^-, 100)=370

3d Benzhydryl-3-nitro phenyl carbamate (6): Brown colored powder, Yield-60.41%,I.R.(KBr) cm-1; 3423 (NH carbamate stretch) ,1732 (C=O ester stretch), 1286(C-O ester stretch), 1545(NO2 Stretch)**;** H^1NMR [500 MHz, DMSO, δ (ppm)] 10.5 (s, 1H) NH,8.46 (d, 1H),7.03-7.8 (m, 13H)Aromatic, 6.86 (s, 1 H) CH; EIMS:m/z (M+, 100)=347

3e Benzhydryl-4-Methoxy Phenyl Carbamate (7): White colored powder, Yield- 65 %**,** I.R.(KBr) cm-1: 3369 (NH carbamate stretch) ,1663 (C=O ester stretch), 1270(C-O ester stretch)**;** H^1 NMR [500 MHz $CDCl_3$, δ (ppm)] 8.5 (s, 1H) NH, 7.2-7.5 (m, 14H) Aromatic , 6.86 (s, 1 H) CH, 3.7 (s, 3 H) Methoxy**;** EIMS:m/z(M-1, 100)=332

3f Benzhydryl-4-Chloro Phenyl Carbamate(8): Cream colored powder, Yield- 68 %, I.R.(KBr) cm^{-1}: 3367 (NH carbamate stretch) ,1663 (C=O ester stretch), 1254(C-O ester stretch)**;** H^1 NMR [500 MHz $CDCl_3$, δ (ppm)] 8.9 (s, 1H) NH, 7.3-7.6 (m, 14H) CH Aromatic, 6.8 (s, 1 H) CH**;** EIMS:m/z(M-1, 100)=336

3g Benzhydryl-4-fluro Phenyl Carbamate (9): White colored powder, Yield- 60 %, I.R.(KBr) cm^{1}: 3325 (NH carbamate stretch) ,1698 (C=O ester stretch), 1302(C-O ester stretch)**;** H^1 NMR [500 MHz $CDCl_3$, δ (ppm)] 8.8 (s, 1H) NH, 7.3-7.6 (m, 14H) CH Aromatic , 6.86 (s, 1 H) CH; EIMS:m/z(M-1, 100)=320

3h Benzhydryl-4-tolyl Phenyl Carbamate(10): White colored powder, Yield- 71 %, I.R.(KBr) cm^{-1}: 3295 (NH carbamate stretch) ,1705 (C=O ester stretch), 1310 (C-O ester stretch); H^1NMR [500 MHz DMSO, δ (ppm)] 9.4 (s,1H) NH, 7.3-7.8 (m, 14H) Aromatic ,6.1 (s,1H) CH, 2.1(s, 3 H) CH3 ; EIMS: m/z (M-1, 100)=387

Compound Code	R	Melting Point(◦c)	Molecular Weight	Molecular Formula	Percentage yield
3a	3-Cl	132-134	336	$C_{20}H_{16}N\,O_2Cl$	50%
3b	4-NO_2	125-130	347	$C_{20}H_{16}N_2O_4$	80%
3c	2,4 dichloro	278-280	370	$C_{20}H_{15}Cl_2NO_2$	70%
3d	3-NO_2	154-156	347	$C_{20}H_{16}N_2O_4$	60%
3e	4-OMe	230-232°C	333	$C_{21}H_{19}NO_3$	65%
3f	4-Cl	240-242°C	337	$C_{20}H_{16}\,ClNO_2$	68%
3g	4-F	211-213°C	321	$C_{20}H_{16}\,FNO_2$	60%
3h	4-Me	222-224°C	317	$C_{21}H_{19}NO_2$	71%

Table 2: Physical data of synthesized compounds

Pharmacological evaluation

Evaluation of the pharmacological activity was done by modified colorimetric method of Ellman's .The ability of each molecule to reduce AChE activity was assessed using rivastigmine as a standard drug. A pre-incubation period of 30 minutes was followed by the pre-mixing of 40 mL of the test solution at various concentrations, 50 mL of 50 mM Tris-HCl buffer (pH 8.0), 50 mL of 0.02 U/mL AChE, and 40 mL of the mixture. At 4° C., the reaction was initiated by adding 30 µL of 10 mM DTNB [5,5-dithio-bis-(2-nitrobenzoic acid)] and 30 µL of 12 mM ATChI (Acetylthiocholine iodide) . AChE activity was estimated by measuring the change in UV absorbance at 412 nm of test solutions over 10 minutes at 25°C using an Elisa reader. Experiments were carried out three times.

Rivastigmine was used as a positive control. By comparing enzyme activity with and without an inhibitor, the enzyme inhibition rate was determined.

$$\text{AChE inhibition} = [(\text{OD of control} - \text{OD of test})/ (\text{OD of control})]\times 100$$

Table 3 provides an IC_{50} values of compounds. The test compound was chosen to inhibit the aforementioned enzyme because doing so remains a key goal in reducing the progression of the disease.

S.N.	Compound code	R	IC_{50} [Rivastigmine] (11.79) µM	Docking score [Rivastigmine] (-4.9) µM
1.	3a	3-Cl	26.25	-8.45
2.	3b	4-NO_2	46.7	-7.94
3.	3c	2,4 dichloro	26.36	-8.56
4.	3d	3-NO_2	55.7	-7.99
5.	3e	4-OMe	36.9	-9.80
6.	3f	4-Cl	22.45	-7.99
7.	3g	4-F	20.65	-7.42
8.	3h	4-Me	37.6	-9.98

Table 3 Results of AChE inhibition

ADME Profile:

ADME prediction was performed by Swiss ADME which was available through. http://www.swissadme.ch the Swiss ADME programme. Structures were drawn and converted into SMILES notation to predict ADME.

Result & Discussion

Molecular Docking: Docking study indicated that the complex of AChE and compounds occupied catalytic anionic and peripheral anionic sites. The AChE's active site was docked with each of the eight variants. The docking score showed the binding energy of the docked compound as given in table 3.

The chemical interactions of 3 g with the AChE enzyme's active site amino acid residues are shown in Fig. 2. PDB ID 1GQR used for the molecular docking.Tyr 334 and Trp 84, two hydrophobic amino acids, interacted with the 3 g nucleus of the enzyme in the peripheral anionic site (PAS site) of the enzyme. The molecular docking investigation resulted in a dock score of.3 g that was 7.4 kcal/J1.

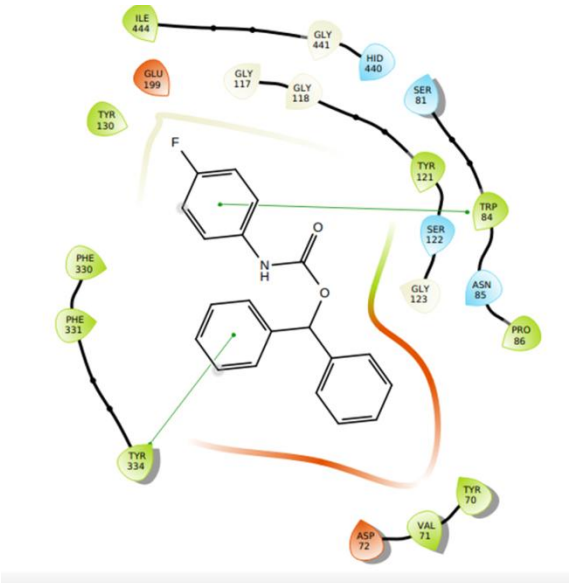


Fig.2: A Visual Representation of 3g Molecular docking study with AChE (PDB 1GQR)

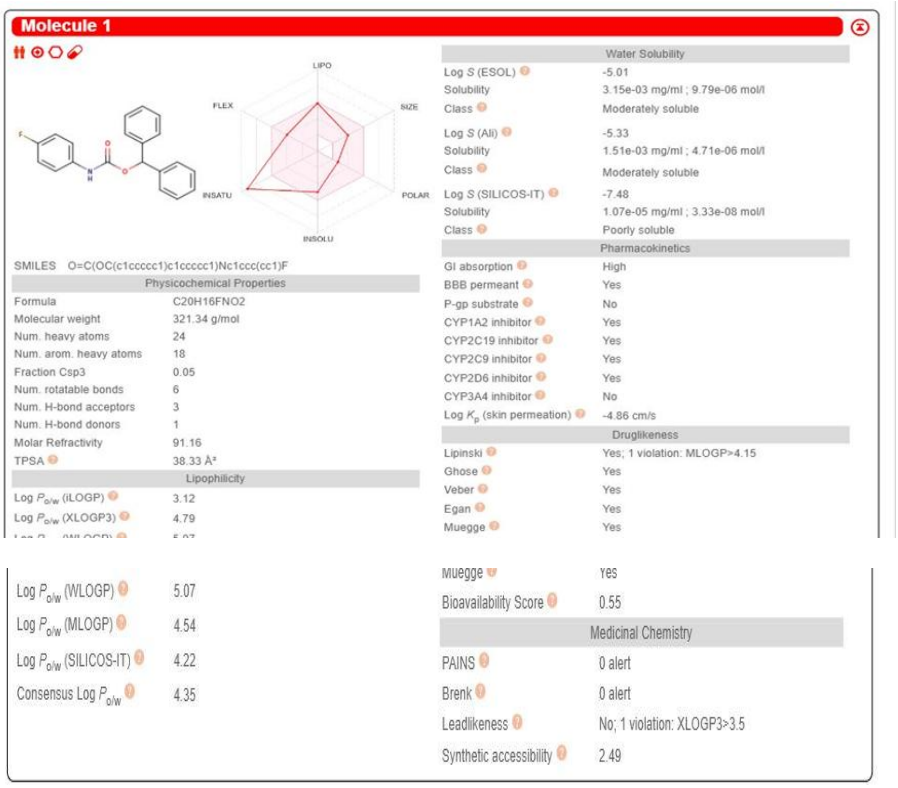
Chemistry:

The designed molecules showed better binding energy as compared to standard drug rivastigmine. Therefore, these compounds were synthesized having electron withdrawing and electron donating groups and were evaluated for AChE inhibitory activity. The pharmacological activity data revealed that electron withdrawing having halogens substituent contributed more towards the AChE inhibitory activity. Halogens are said to have a +R effect on aromatic rings, regardless of their position. When the effect of halogen was observed at the para-position, the fluorine substituent showed better effect compared with the Cl derivative. Since fluorine is a ring activator, the difference in inhibition rate may be due to the high electronegativity and small size of fluorine. Among the chloro-derivatives, changing the para-to-meta position proved unsuccessful in improving the potency of the chloro-substitution (Fig. 2a & f), as there was no such change in the IC_{50} values. The chloro substituent present at para was more favorable as compared to the meta position. We even tried to find out the effect of substitution when two positions on the aromatic ring are occupied with the halogen atoms. We thus included chlorine in 2nd and 4th position (2c). Inclusion of two chlorine atoms at both positions did not change potency. The activities of both meta- and di-substituted compounds were almost the same However, we found that nitro group present both at meta and para position contributed least towards the inhibitory activity. Among the electron donating substituent methoxy and methyl group activity were almost alike but it was less than the electron withdrawing substituent.

ADME Profile:

This free online tool was used to envisaged properties like BOILED EGG. absorption, distribution, metabolism, and excretion, along with physicochemical property of compounds. Compound 3 g which has shown significant activity was subject to ADME analysis. It was found that it has the ability to cross BBB. Figs.3a and 3b show the in silico outcome that software had exhibited for the molecule. It was found that the molecule 3g has favorable drug likeness characteristics.

(a)



(b)

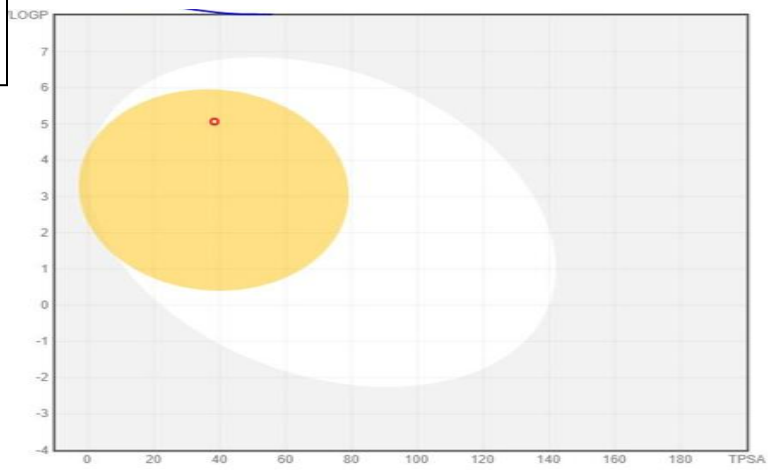


Fig. 3 (a) Predicted ADME properties of 3g as obtained from the report generated by Swiss ADME software. (b) BOILED EGG representation of 3g (denoted as Molecule 1 and represented by blue dot in the figure). From the BOILED EGG representation, the yolk denotes the points which allows passive blood–brain-barrier permeation, while the white region denotes points for passive gastrointestinal tract absorption. The graph is plotted against W log P [a log P(n-octanol/water partition coefficient) method developed by Wildman and Crippen] versus TPSA or Topological Polar Surface Area.

The BOILED EGG representation displays two parts, the white part representing those set of values which allows gastric permeation and the yolk or the yellow region representing those set of values which allows blood–brain-barrier permeation. The graph is plotted against W log P[a log P(n-octanol/water partition coefficient) method developed by Wildman and Crippen] versus TPSA or Topological Polar Surface Area in the BOILED EGG representation. The blue dot in the figure written as Molecule 1 is of 3g.

CONCLUSION

A series of novel carbamates of biological interest were synthesized and studied for their acetyl cholinesterase inhibitory activity after studying their binding pattern with the AChE enzyme. . The tested compounds showed the pharmacological activity with varying intensity against acetyl cholinesterase enzymes. Amongst all of the titled compound's,3g showed potent acetyl-cholinesterase inhibitory activity when compared with standard drug rivastigmine. The ADME profile showed that that the molecule has the tendency to cross blood brain barrier and possess all the requisite properties to be considered as a promising molecule. Based on these findings, it can be concluded that these derivatives might prove to be promising scaffolds for the development of new potent anti-AD drugs.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable

CONSENT FOR PUBLICATION

NO

FUND

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CONFLICT OF INTEREST

The author declares no conflict of interest.

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Availability of Data & Materials:

The author confirms that the data supporting figure, the finding of this study is available in the article.

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