MOLECULAR HYBRIDIZATION BASED DESIGN AND SYNTHESIS OF NOVEL 4-AMINO-1H-INDOLE-6-CARBOXAMIDES DERIVATIVES AS CHOLINESTERASE INHIBITORS

Kundavarapu Raju [1] L . Siva Sanker Reddy*

[1]Research Scholar, Department of Pharmaceutical Sciences, Jawaharlal Nehru Technological University Anantapur, Ananthapuramu, A.P., 515002.

*Professor, Department of Pharmaceutical Analysis & Chemistry, Santhiram College of Pharmacy, NH40, Nandyal, A.P., 518112.

Corresponding Author:

Dr. L . Siva Sanker Reddy ,

Professor,

Department of Pharmaceutical Analysis & Chemistry,

Santhiram College of Pharmacy,

NH40, Nandyal,

Andhra Pradesh, 518112.

ABSTRACT:

A Series of new novel 4-amino-1H-Indole -6-carboxamide derivatives were designed, synthesized, characterized by spectral data and evaluated for cholinesterase inhibitory activity to be useful in Alzheimer's disease. Most of the synthesized compounds showed good in vitro inhibitory activities towards acetyl cholinesterase (AChE), butyrylcholinestarse (BuChE) enzymes. Among them, compounds **7k** and **7o** with 3,4-diflouro, 3,4,5-trimethoxy substituents on the aromatic ring attached at the indole ring with carboxamide linker have shown equal potency to that of standard drug rivastigmine with IC_{50} values of 1.10±0.04 ; 1.32±0.12 and 0.89±0.03; 0.96±0.12 μM against AChE and BuChE respectively, Six compounds were selected based on significant potency in invitro AChE and BuChE inhibitory activity. Those Compounds screened against brain brain cholinesterase inhibitory activity. Compounds **7o** with IC_{50} of 26.32±0.25 and 31.24±0.18 μM and **7k** was further studied for brain cholinesterase inhibitory activity and exhibited potent activity with IC_{50} of 28.65±0.15 μM and 34.56±0.32 μM against AChE and BuChE and also demonstrated potent in vivo activities in Y-maze test, rectangular maze test and jumping box test which indicated our understanding from *in vitro* result that **7o** possess sufficient solubility a primary requirement for in vivo activities. Docking studies revealed that the designed molecules interacted with in nicotinic receptor with at list two hydrogen bond interactions barring few compounds.

Keywords: Alzheimer's disease, antioxidants, anti-inflammatory, cholinesterase inhibitor, Rivastigmine.

1. INTRODUCTION:

Alzheimer's disease (AD), also known as senile dementia of the Alzheimer's type (SDAT), is a neurological disorder that affects a significant number of elderly individuals. It is a progressive neurodegenerative disorder characterized by an initial deterioration in memory, followed by symptoms such as linguistic difficulties, visuospatial deficits, and cognitive impairment, which worsen with time and finally lead to death. Alzheimer's disease is currently the fourth primary cause of death in Western nations, in front of coronary heart disease, cancer, and cerebrovascular illness.[1] The World Health Organization estimated that in the next century, Alzheimer's disease would be more prevalent than AIDS, cancer, and CVS disorders.[2] In the United States, more than 17% of those aged over 75 are afflicted with Alzheimer's disease, incurring an estimated treatment cost of over USD 236 billion, which may exceed USD 700 billion by 2050.[3] The etiology of Alzheimer's disease (AD) continues to be inexplicable; however, numerous factors, including amyloid-β (Aβ) deposits, tau protein aggregation, excitotoxicity hypothesis, oxidative stress [4], and reduced acetylcholine levels [5], have been associated with the Patho mechanism of Alzheimer's disease.[6] Furthermore, it has been proposed that mitochondrial metabolism may be impaired by oxidative damage to intracellular structures, which is a contributing factor to aberrant cellular function and, it appears, cell mortality, as well as the onset of inflammatory symptoms. [7] Currently, there is no complete therapy for Alzheimer's disease, and clinical treatments only produce symptomatic effects. Several drugs have been used to reduce the levels of Aβ in the brain, either by reducing its production or its excretion. However, these methods have not been successful in improving the cognitive abilities of Alzheimer's disease patients in clinical trials. The most recent drug

discoveries in the Alzheimer's disease segment that target BACE (beta-site amyloid precursor protein cleaving enzyme) have not met the expectations due to the associated toxicities. Drugs such as tacrine, donepezil, galantamine, and rivastigmine, which inhibit acetylcholinesterase (AChE) and increase acetylcholine (ACh) levels, and memantine, an NMDA receptor antagonist.[8] 1H-indole was selected as a molecular fragment to design a novel 4-Amino-Indole-6- carboxamide hybrids with multi-target characteristic. It is an endogenous low-molecular-weight non-peptide compound that is utilized in the synthesis of a wide range of heterocyclic compounds and possesses numerous intriguing biological properties. [9] A number of studies have investigated the modulator function of indole, which was discovered in mammalian tissues and body fluids. Studies indicated that 1H-indole inhibited AChE activity, thereby increasing ACh levels.[10] Isatin as an endogenous inhibitor of monoamine oxidase, particularly MAO-B, may contribute to the control of brain levels of acetylcholine and dopamine, which are reduced in age-related neurodegenerative disorders such as Alzheimer's disease and Parkinson's disease.[11] The neuroprotective attribute of isatin is mostly associated with the suppression of monoamine oxidase. Indole functions as a bioregulator with a selective impact on certain protein-protein interactions. It may affect the relationship between amyloid-beta and its essential intracellular targets, hence creating new opportunities for the therapeutic prevention of amyloid-beta toxicity.[12-15] Indole ability to inhibit enzymes (cholinesterase, carbonic anhydrase, MAO-B), influence protein aggregations, exhibit neuroprotective effects, and demonstrate antioxidant activity positions it as a promising option for the development of multifunctional compounds targeting neurodegenerative illnesses.[16-18]

As the main purpose of the present study, a selection of Novel Design, synthesis of novel 4-amino-1H-Indole -6-carboxamide derivatives was consequently synthesized and their structures were clarified. The synthesized compounds were assessed for their in vitro efficacy as inhibitors of AChE, BuChE and amyloid-beta aggregation.

2. Design novel 4-amino-1H-Indole -6-carboxamide derivatives :

Bingul et al., 2020 [19] was reported the Methyl 7-[(E)-(2-benzoylhydrazinylidene)-methyl]-4,6-dimethoxy-1H-indole-2-carboxylates (**I**) were evaluated as anti-cholinesterase activity with highest inhibition was determined with the values of 83.31% and 73.55 % for AChE and BChE, respectively. **Prochnow et al. 2018** [20] developed the compound (2-fluoro-6-methoxyphenyl)(1-(2-phenylethynyl)-1H-indol-2-yl)methanol (**II**) were evaluated as anti-cholinesterase activity with the inhibitory effect of the AChE inhibition activity with IC_{50} values 7.16 µM.

Cheung et al. 2012 [21] investigated acetylcholinesterase inhibitors, donepezil (2-((1-benzylpiperidin-4-yl)-methyl)-5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-one) (**III**) is of most highly effective and well-known FDA-approved drug. Donepezil consists of dimethoxy indanone showed that the indanone moiety fuses with the PAS of AChE, and the benzylic part links with the catalytic anionic site CAS. Shahinda alsayed et al.,2021 [22] was reported the compound N-(1-(Adamantan-1-yl)ethyl)-4,6-dichloro-1H-indole-2- carboxamide (**IV**) displayed the highest activity (MIC - 0.32 µM) against the drug-sensitive Mycobacterium tuberculosis H37Rv strain. This compound also exhibited high selective activity towards over mammalian cells [IC_{50}- 40.9 µM], suggesting its minimal cytotoxicity. Among the series, compounds with a Carboxamide fused with indole ring and 4- amino substituted derivatives on indole ring were found to be potent AChEIs. In the present study, we used molecular hybridization and design, synthesis of novel 4-amino-1H-Indole -6-carboxamide as depicted in **Figure 1** and report the synthesis and AChE and BuChE inhibitory activity to be useful as anti-Alzheimer's agents.

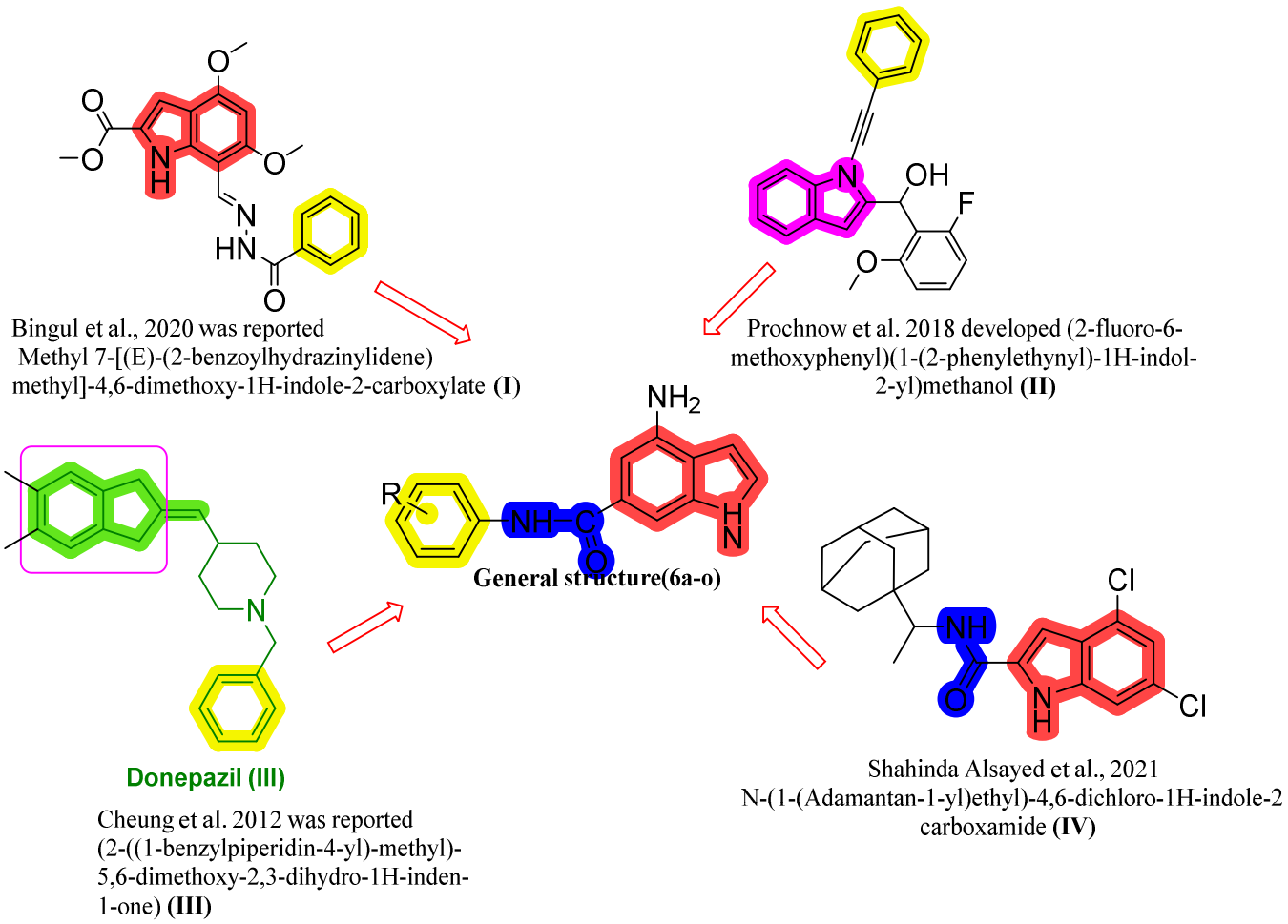


Figure:1: Design of novel 4-amino-1H-Indole -6-carboxamide derivatives

3.0 Materials and Methods:

3.1. Chemistry:

Melting points were ascertained using open capillary tubes with the VEEGO VMP-D Digital melting point device. The FTIR spectra of the powdered compounds were obtained using KBr on a JASCO FTIR 4100 series spectrometer and are shown in cm-1. The 1H NMR and 13C NMR spectra were acquired on a BRUKER-II 400 (400 MHz for 1H NMR and 100 MHz for 13C NMR) spectrophotometer, using TMS as an internal reference. The purity of the compounds was assessed on pre-coated TLC plates with silica G as the stationary phase, with iodine vapors and ultraviolet light serving as the visualization agents. All chemicals, including

conventional pharmaceuticals and solvents, were obtained from Sigma-Aldrich, Hi Media, Bangalore, India, and other suppliers. The assessment of biochemical parameters was conducted using kits from Sigma-Aldrich.

3.1. General Synthetic Procedure [23]:

3.1.1. Synthesis of 3, 5 di amino benzoic acid derivatives (2):

3, 5-dinitrobenzoic acid (**1**; 1.0 mmol) and methanol (10ml) was taken in to the dry round bottom flask and added with a Tin reacts with hydrochloric acid to form Tin (IV) chloride catalyst a reduction reaction is carried out for 2-10 hours under the reaction temperature 70°C thereby obtaining crude product; the crude product is separated and removed off the solvent to obtain the 3, 5-diaminobenzoic acid.

3.1.2. Synthesis of 3-amino-5-((cyanomethyl)amino)benzoic acid (4):

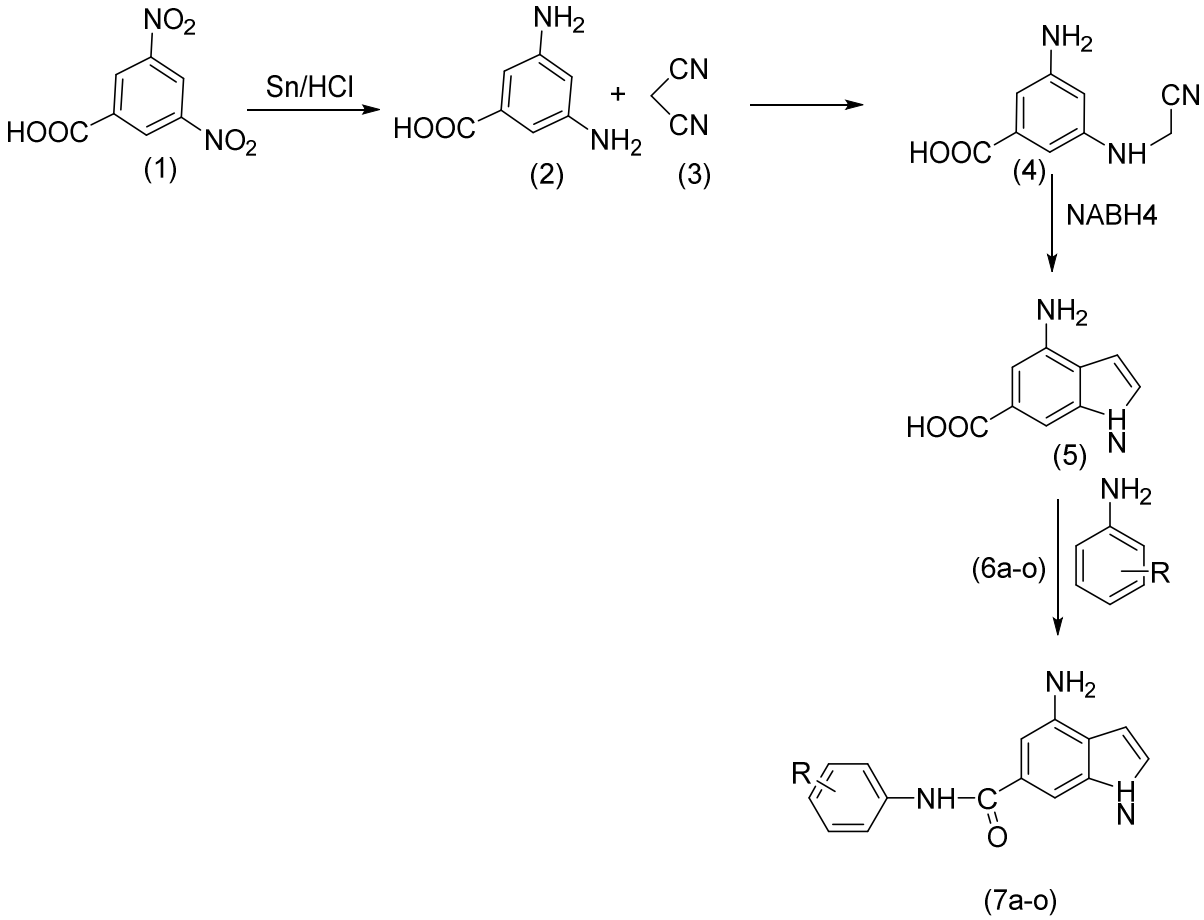
A mixture of 3, 5-diaminobenzoic acid. (**2**, 10 mmol), malononitrile (**3**, 10 mmol) were refluxed in ethanol for 10 hours with stirring in the presence of $AlCl_3$ and BCl_3 (0.2 eq.). The crude precipitate resulted in was filtered, washed with cold ethanol and dried to produce the desired compound 3-amino-5-((cyanomethyl)amino)benzoic acid (**4)**.

3.1.3. Synthesis of 4-amino-1H-indole-6-carboxylic acid (5): A solution of compound 3-amino-5-((cyanomethyl)amino)benzoic acid in toluene was added $NaBH_4$ Reagent (2 eq) and heated at 90 °C for 2 h. Then cooled to room temperature, water was added to the mixture, and the residue was extracted with EtOAc. The crude product obtained after concentration was purified with column chromatography to afford the compound 4-amino-1H-indole-6-carboxylic acid (5).

3.1.4. **Synthesis of 4-amino-N-phenyl-1H-indole-6-carboxamide (7):**

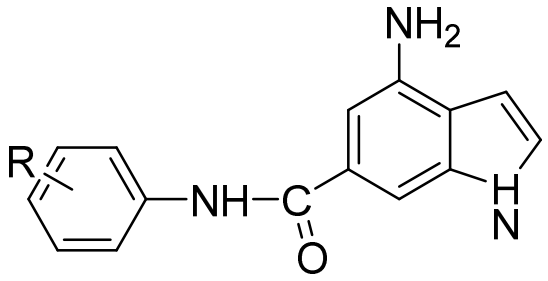
To a solution of compound 4-amino-1H-indole-6-carboxylic acid (5) 0.88 g, 2.39 mmol) in 70 mL K3PO4 (7.46 g, 35.14 mmol) and Substituted anilines (6a-o; 3.9 g, 17.56 mmol) were added in order. The reaction mixture was stirred at 90 °C for 6 h under argon atmosphere1.

Water was added to the mixture and extracted with EtOAc, then washed with brine and water. The crude product obtained after concentration was purified with column chromatography to give the title compounds 4-amino-N-substituted -phenyl-1H-indole-6-carboxamide (7a-o).



Scheme-1: Synthesis of 4-amino-N-substituted -phenyl-1H-indole-6-carboxamide (7a-o).

Table1.Physical data of New 4-amino-N-substituted -phenyl-1H-indole-6-carboxamide (7a-o)



General structure-I (7a-o)

Com	R	M. Form	M.Wt	M.P	Rf*	% Yield
7a	H	$C_{15}H_{13}N_3O$	251	176-178	0.6	42
7b	4-Cl	$C_{15}H_{12}ClN_3O$	285	156-158	0.9	50
7c	4-Br	$C_{15}H_{12}BrN_3O$	330	164-166	0.5	60
7d	4-F	$C_{15}H_{12}FN_3O$	269	240-242	0.8	40
7e	4-NO_2	$C_{15}H_{12}N_4O_3$	296	160-162	0.8	70
7f	4-CH_3	$C_{16}H_{15}N_3O$	265	158-160	0.8	45
7g	3-Cl	$C_{15}H_{12}ClN_3O$	285	189-192	0.4	38
7h	2,4 Di chloro	$C_{15}H_{11}Cl_2N_3O$	320	156-158	0.7	40
7i	3,4,5 tri chloro	$C_{15}H_{10}Cl_3N_3O$	355	160-162	0.9	30
7j	2,4 Di bromo	$C_{15}H_{11}Br_2N_3O$	409	188-190	0.5	48
7k	2,4 Di flouro	$C_{15}H_{11}F_2N_3O$	287	230-232	0.5	45
7l	2,4 Di nitro	$C_{15}H_{11}N_5O_5$	341	165-167	0.8	50
7m	2,4 Di methyl	$C_{17}H_{17}N_3O$	279	180-182	0.6	65
7n	2,4 dihydroxy	$C_{15}H_{13}N_3O_3$	283	190-192	0.4	81
7o	3,4,5 tri methoxy	$C_{18}H_{19}N_3O_4$	341	196-198	0.5	55

*** Mobile phase: hexane: ethyl acetate**

3.0 Characterization of compounds:

3.1.1. **4-amino-N-phenyl-1H-indole-6-carboxamide (7a):**

Compound **7a** obtained as yellowish orange solid (yield 27%); ^{1}H NMR (400 MHz DMSO, δ ppm): 10.550 (s, 1H, NH, indole),8.09-8.11 (d, 1H, *J*= 8.0 Hz, Ar-H), 7.81-7.82 (d, 1H, *J*=4.0 Hz, Ar-H) 7.55-7.56 (d, 1H, *J*=4.0 Hz, Ar-H), 7.51-7.53 (t, 1H, *J*=4.0 Hz Ar-H), 7.35-7.39 (t, 1H, *J*=8.0 Hz Ar-H), 7.23-7.33 (m,7H, Ar-H), 5.349(s, 2H, NH_2); ^{13}C NMR (100 MHz, DMSO): 191.15, 160.25, 158.15, 154.17, 145.12, 135.18, 130.25, 128.10, 127.38, 127.84 126.24, 125.18, 125.01, 124.25, 116.52; MASS spectrum m/z: 252.5 $[M+H]^+$ Calc. for $C_{15}H_{13}N_3O$; CHN: C, 71.70; H, 5.21; N, 16.72; O, 6.37; Found: C, 71.70; H, 5.21; N, 16.72; O, 6.37. IR (KBr, cm^{-1}): 3060.21 (C-H, Aromatic), 2968.10 (C-H, Aliphatic), 1716.96 (C=O), 1516.01 (C=C, Aromatic), 1265.51 (C-O).

3.1.2. 4-amino-N-(4-chlorophenyl)-1H-indole-6-carboxamide (7b): Compound **7b** obtained as yellowish white solid (yield 30%), m. p. 201-203 °C. ^{1}H NMR (400 MHz DMSO, δ ppm): 10.550 (s, 1H, NH, indole),8.09-8.11 (d, 1H, *J*= 8.0 Hz, Ar-H), 7.80-7.82 (d, 1H, *J*= 8.0 Hz, Ar-H), 7.76-7.78 (d, 1H, *J*= 4.0 Hz, Ar-H), 7.50-7.53 (t, 1H, *J*= 4.0 HzAr-H), 7.37-7.39 (t, 1H, *J*= 4.0 Hz, Ar-H), 7.32-7.36 (m, 4H, Ar-H), 7.23-7.31 (m, 2H, Ar-H), 5.349(s, 2H, NH_2), ^{13}C NMR (100 MHz, DMSO): 191.15, 160.25, 158.15, 154.17, 145.12, 135.18, 130.25, 128.10, 127.38, 127.84 126.24, 125.18, 125.01, 124.25, 116.52; MASS spectrum m/z: 287.20 $[M+H]^+$ Calc. for $C_{15}H_{12}ClN_3O$; CHN: C, 63.05; H, 4.23; Cl, 12.41; N, 14.71; O, 5.60; Found: C, 63.01; H, 4.20; Cl, 12.45; N, 14.76; O, 5.65. IR (KBr, cm^{-1}): 3103.33 (C-H, Aromatic), 2922.15 (C-H, Aliphatic), 1718.28 (C=O**),** 1590.56 (C=C, Aromatic), 1184.54 (C-O).

3.1.3. 4-amino-N-(4-bromophenyl)-1H-indole-6-carboxamide (7c): Compound **7c** obtained as white solid (yield 39%); ^{1}H NMR (400 MHz $CDCl_3$, δ ppm): 10.550 (s, 1H, NH, indole), 7.91-7.93 (d, 2H, *J*= 8.0 Hz Ar-H), 7.71-7.73 (t, 1H, *J*= 4.0 Hz, Ar-H) 7.71-7.73 (t, 1H, *J*= 4.0

Hz, Ar-H), 7.47-7.56 (m, 3H, Ar-H), 7.35-7.39 (t, 2H, Ar-H), 7.18-7.20 (d, 2H, Ar-H), 5.349(s, 2H, NH_2), ^{13}C NMR (100 MHz, $CDCl_3$): 193.09, 165.36, 160.20, 156.25, 145.12, 134.10, 131.10, 128.32, 128.04, 127.12, 126.58, 125.10, 125.51 123.24, 113.1520.14; MASS spectrum m/z: 332.12$[M+2]^+$ Calc. for $C_{15}H_{12}BrN_3O$; CHN: C, 54.56; H, 3.66; Br, 24.20; N, 12.73; O, 4.85; Found: C, 54.50; H, 3.60; Br, 24.25; N, 12.70; O, 4.85. IR (KBr, cm^{-1}): 3058.56 (C-H, Aromatic), 2976.58 (C-H, Aliphatic), 1724.84 (C=O**),** 1591.59 (C=C, Aromatic), 1080.34 (C-O).

3.1.4. 4-amino-N-(4-fluorophenyl)-1H-indole-6-carboxamide (7d): Compound **7d** obtained as orange solid (yield 26 %); ^1H NMR (400 MHz $CDCl_3$, δ ppm): 10.550 (s, 1H, NH, indole), 8.49-8.51 (d, 1H, *J*= 8.0Hz, Ar-H), 7.95-8.00 (t, 1H, *J*= 10 Hz, Ar-H) 7.89-7.91 (d, 1H, *J*= 8.0Hz, Ar-H), 7.77-7.81 (t, 2H, *J*= 8.0Hz, Ar-H), 7.63-7.67 (m, 2H, Ar-H), 7.38-7.47 (m, 2H, Ar-H), 7.19-7.23 (t, 1H, *J*= 8.0Hz, Ar-H), 6.80-6.82 (d, 1H, *J*= 9.6 Hz, Ar-H), 5.349(s, 2H, NH_2), ^{13}C NMR (100 MHz, $CDCl_3$): 193.09, 165.36, 160.20, 156.25, 145.12, 134.10, 131.10, 128.32, 128.04, 127.12, 126.58, 125.10, 125.51 123.24, 113.15, 105.10, 68.15, 58.17, 45.14, 36.14, 20.14; Mass spectrum m/z: 271.12 $[M+2]^+$ Calc. for $C_{15}H_{12}FN_3O$; CHN: C, 66.91; H, 4.49; F, 7.06; N, 15.60; O, 5.94; Found: C, 66.90; H, 4.40; F, 7.04; N, 15.65; O, 5.94; IR (KBr, cm^{-1}): 3071.66 (C-H, Aromatic), 2981.22 (C-H, Aliphatic), 1730.51 (C=O**),** 1591.45 (C=C Aromatic), 1294.70 (C-O).

3.1.5. 4-amino-N-(4-nitrophenyl)-1H-indole-6-carboxamide (7e): Compound 7e obtained as orange solid (yield 51 %); ^1H NMR (400 MHz $CDCl_3$, δ ppm): 10.550 (s, 1H, NH, indole), 8.49-8.51 (d, 1H, J= 8.0 Hz, Ar-H), 7.95-8.00 (t, 2H, J= 8.4Hz, Ar-H), 7.89-7.91 (d, 1H, J= 8.0 Hz, Ar-H), 7.77-7.81 (t, 1H, J= 6.0 Hz, Ar-H), 7.63-7.68 (m, 3H, Ar-H), 7.40-7.47 (m, 3H, J= 8.0Hz, Ar-H), 7.19-7.23 (t, 1H, J= 7.6 Hz, Ar-H), 6.81-6.83 (d, 1H, J= 7.2 Hz, Ar-H), 5.349(s, 2H, NH_2), ^{13}C NMR (100 MHz, $CDCl_3$): 196.34, 168.13, 164.53, 153.78, 152.34, 143.63, 142.44, 139.59, 138.74, 136.03, 129.96, 129.40, 127.96, 127.65, 126.73, 125.01, 119.69;

MASS spectrum m/z: 297.12 $[M+H]^+$ Calc. for $C_{15}H_{12}N_4O_3$; CHN: C, 60.81; H, 4.08; N, 18.91; O, 16.20; Found: C, 60.81; H, 4.08; N, 18.91; O, 16.20. IR (KBr, cm^{-1}): 3086.15(C-H, Aromatic), 2922.18(C-H, Aliphatic), 1722.81(C=O), 1549.75 (C=C, Aromatic), 1210.31 (C-O).

3.1.6. 4-amino-N-(p-tolyl)-1H-indole-6-carboxamide (7f): Compound **7f** obtained as orange solid (yield 49 %); ^{1}H NMR (400 MHz $CDCl_3$, δ ppm): 10.550 (s, 1H, NH, indole), 8.49-8.51 (d, 1H, *J*= 8.0 Hz, Ar-H), 7.95-8.00 (t, 2H, *J*= 8.4 Hz, Ar-H) 7.89-7.91 (d, 1H, *J*= 8.0Hz, Ar-H), 7.77-7.81 (t, 2H, *J*= 6.0 Hz Ar-H), 7.63-7.67 (m, 2H, Ar-H), 7.28-7.47 (m, 3H, Ar-H), 7.19-7.23 (t, 1H, *J*= 7.6 Hz, Ar-H), 6.80-6.82 (d, 1H, *J*= 7.2 Hz, Ar-H), 5.349(s, 2H, NH_2), ^{13}C NMR (100 MHz, $CDCl_3$): 196.6, 164.6, 161.3, 152.3, 138.4, 137.2, 135.2, 133.7, 132.9, 131.8, 129.9, 128.0, 127.6, 127.5, 127.4, 127.3, 127.0, 126.5,126.0, 125.1, 120.2, 117.6, 116.1; MASS spectrum m/z: 266.24 $[M+H]^+$ Calc. for $C_{16}H_{15}N_3O$; CHN: C, 72.40; H, 5.71; N, 15.80; O, 6.02; Found: C, 72.43; H, 5.70; N, 15.84; O, 6.03. IR (KBr, cm^{-1}): 3067.12 (C-H, Aromatic), 2938.02 (C-H, Aliphatic), 1720.32(C=O**)**, 1515.12(C=C, Aromatic), 1124.61(C-O).

3.1.7. 4-amino-N-(4-chlorophenyl)-1H-indole-6-carboxamide (7g): Compound **7g** obtained as yellow solid (yield 48 %); ^{1}H NMR (400 MHz $CDCl_3$, δ ppm): 10.550 (s, 1H, NH, indole), 8.09-8.11 (d, 1H, *J*= 8.0 Hz, Ar-H), 7.80-7.81 (d, 1H, *J*= 4.0 Hz, Ar-H), 7.76-7.77 (d, 1H, *J*= 4.0 Hz, Ar-H), 7.50-7.53 (t, 2H, *J*=6.0 Hz, Ar-H), 7.17-7.39 (m, 3H, Ar-H), 7.13-7.14 (d, 1H, *J*= 4.0 Hz, Ar-H), 7.11-7.12 (d, 1H, *J*= 4.0 Hz, Ar-H), 5.349(s, 2H, NH_2), ^{13}C NMR (100 MHz, $CDCl_3$):196.10, 160.25, 158.15, 154.17, 145.12, 135.18, 130.25, 128.10, 128.02, 127.38, 127.84 126.24, 126.10, 125.18, 125.01, 124.25, 116.52, 101.28, 60.21, 57.40, 48.58, 38.04, 29.29, 14.09; MASS spectrum m/z: 403.24 $[M+H]^+$ Calc. for $C_{15}H_{12}ClN_3O$; CHN: C, 63.05; H, 4.23; Cl, 12.41; N, 14.71; O, 5.60; Found: C, 63.01; H, 4.20; Cl, 12.40; N, 14.70; O, 5.60; IR (KBr, cm^{-1}): 3068.23 (C-H, Aromatic), 2980.18 (C-H, Aliphatic), 1720.13 (C=O**)**, 1516.04 (C=C, Aromatic), 1124.61 (C-O).

3.1.8. 4-amino-N-(2,4-dichlorophenyl)-1H-indole-6-carboxamide (7h): Compound **7h** obtained as cream solid (yield 38 %), ^{1}H NMR (400 MHz DMSO, δ ppm): 10.550 (s, 1H, NH, indole), 8.09-8.11(d, 1H, *J*= 8.0 Hz, Ar-H), 7.80-7.82 (d, 1H, *J*= 8.0 Hz, Ar-H), 7.76-7.77 (d, 1H, *J*= 4.0 Hz, Ar-H), 7.50-7.52 (t, 2H, *J*= 4.2 Hz, Ar-H), 7.29-7.38 (m, 3H, Ar-H), 7.25-7.26 (d, 1H, *J*= 4.0 Hz, Ar-H), 5.30 (s, 1H), 3.26-3.28 (t, 1H, *J*= 4.0 Hz), 3.07-3.11 (m, 2H), 1.82-1.84 (t, 1H, *J*= 4.2 Hz), 1.70-1.76 (m, 2H). ^{13}C NMR (100 MHz, DMSO): 196.10, 160.25, 158.15, 154.17, 145.12, 135.18, 130.25, 128.10, 128.02, 127.38, 127.84 126.24, 126.10, 125.18, 125.01, 124.25, 116.52; MASS spectrum m/z: 321.24 $[M+H]^{+}$ Calc. for $C_{15}H_{11}Cl_2N_3O$; CHN: C, 56.27; H, 3.46; Cl, 22.14; N, 13.12; O, 5.00; Found: C, 56.20; H, 3.41; Cl, 22.11; N, 13.17; O, 5.05. IR (KBr, cm^{-1}): 3089.23 (C-H, Aromatic), 2965.18 (C-H, Aliphatic), 1715.10 (C=O**),** 1535.14 (C=C, Aromatic), 1140.12 (C-O).

3.1.9. 4-amino-N-(3,4,5-trichlorophenyl)-1H-indole-6-carboxamide (7i): Compound **7i** obtained as light yellow solid (yield 45 %);^{1}H NMR (400 MHz DMSO, δ ppm): 10.550 (s, 1H, NH, indole), 8.10-8.12 (d, 1H, *J*= 8.0 Hz, Ar-H), 7.81-7.82 (d, 1H, *J*= 4.0 Hz, Ar-H), 7.76-7.78 (d, 1H, *J*= 8.0 Hz, Ar-H), 7.50-7.54 (t, 2H, *J*= 8.0 Hz, Ar-H), 7.20-7.34 (m, 3H, Ar-H), 7.14-7.16 (d, 1H, *J*= 8.0 Hz, Ar-H), 7.11-7.13 (d, 1H, *J*= 8.0 Hz, Ar-H), 5.349(s, 2H, NH_2), ^{13}C NMR (100 MHz, DMSO): 196.06, 164.06, 161.03, 151.08, 141.07, 134.80, 133.51, 130.16, 128.28, 128.15, 128.10, 128.04, 126.32, 126.12, 126.04, 123.08, 123.02, 122.10, 118.09, 109.3. MASS spectrum m/z: 355.32 $[M+H]^{+}$ Calc. for $C_{15}H_{10}Cl_3N_3O$; CHN: C, 50.81; H, 2.84; Cl, 29.99; N, 11.85; O, 4.51; Found: C, 50.81; H, 2.80; Cl, 29.90; N, 11.80; O, 4.51. IR (KBr, cm^{-1}): 3071.13(C-H, Aromatic), 2957.18 (C-H Aliphatic), 1718.10 (C=O**)**, 1524.12(C=C, Aromatic), 1132.14(C-O).

3.1.10. 4-amino-N-(2,4-dibromophenyl)-1H-indole-6-carboxamide (7j): Compound **7j** obtained as light orange solid (yield 21 %), ^{1}H NMR (400 MHz $CDCl_3$, δ ppm): 10.550 (s, 1H, NH, indole), 8.12 (s, 1H, Ar-H), 8.04-8.06 (d, 1H, *J*= 8.0 Hz, Ar-H), 7.80 -7.82 (d, 1H, *J*=

8.0 Hz, Ar-H), 7.70-7.72 (d, 1H, *J*= 8.0 Hz, Ar-H), 7.40-7.43 (t, 1H, *J*= 4.2 Hz, Ar-H), 7.29-7.31 (t, 1H, *J*= 6.8 Hz, Ar-H), 7.26-7.28 (d, 1H, *J*= 8.0 Hz, Ar-H), 6.70-6.71 (d, 1H, *J*= 4.0 Hz, Ar-H), 6.62-6.64 (d, 1H, *J*= 6.8 Hz, Ar-H), 5.349(s, 2H, NH_2), ^{13}C NMR (100 MHz, $CDCl_3$): 196.6, 164.6, 161.03, 152.06, 151.8, 148.04, 133.05, 132.5, 128.8, 128.3, 126.03, 123.08, 123.02, 122.04, 122.01, 119.04 ; MASS spectrum m/z: 411.48 $[M+H]^+$ Calc. for $C_{15}H_{11}Br_2N_3O$; CHN: C, 44.04; H, 2.71; Br, 39.07; N, 10.27; O, 3.91; Found: C, 44.04; H, 2.70; Br, 39.08; N, 10.21; O, 3.95. IR (KBr, cm^{-1}): 3071.05(C-H, Aromatic), 2988.18(C-H, Aliphatic), 1720.14 (C=O**)**, 1545.38 (C=C, Aromatic), 1148.20 (C-O).

3.1.11. 4-amino-N-(2,4-difluorophenyl)-1H-indole-6-carboxamide (7k); Compound **7k** obtained as white solid (yield 40%); ^1H NMR (400 MHz DMSO, δ ppm): 10.550 (s, 1H, NH, indole), 8.821-8.843 (d, 2H, Ar-H), 8.228-8.290 (m, 1H, Ar-H) 7.697-7.732 (m, 1H, Ar-H, amide NH), 7.550-7.559 (d,d, 2H, Ar-H), 7.451-7.455 (d, 1H, J= 4.0 Hz, Ar-H), 7.430-7.435(d, 1H, J= 8.0 Hz, Ar-H), 5.349(s, 2H, NH_2), ^{13}C NMR (100 MHz, DMSO): 168.65, 161.15, 153.78, 152.34, 143.63, 142.44, 139.59, 138.74, 136.03, 129.96, 129.40, 127.96, 126.73, 125.01, 119.04, MASS spectrum m/z: 289.21 $[M+2]^+$ Calc. for $C_{15}H_{11}F_2N_3O$; CHN: C, 62.72; H, 3.86; F, 13.23; N, 14.63; O, 5.57; Found: C, 62.70; H, 3.84; F, 13.22; N, 14.60; O, 5.54; IR (KBr, cm^{-1}): 3081.87 (C-H, Aromatic), 2924.43 (C-H, Aliphatic), 1638.12(C=O**)**, 1572.70 (C=C, Aromatic), 1136.97 (C-O).

3.1.12. 4-amino-N-(2,4-dinitrophenyl)-1H-indole-6-carboxamide (7l): Compound **7l** obtained as white solid (yield 36 %), ^1H NMR (400 MHz, $CDCl_3$, δ ppm): 10.550 (s, 1H, NH, indole), 8.14 (s, 1H, Ar-H), 8.02-8.04 (d, 1H, *J*= 8.0 Hz, Ar-H), 7.87 -7.88 (d, 1H, *J*= 4.0 Hz, Ar-H), 7.76-7.78 (d, 1H, J= 8.0 Hz, Ar-H), 7.46-7.48 (t, 1H, *J*= 4.0 Hz, Ar-H), 7.34-7.36 (t, 1H, *J*= 4.2 Hz, Ar-H), 7.30-7.32 (d, 1H, *J*= 8.0 Hz, Ar-H), 6.80-6.82 (d, 1H, *J*= 8.0 Hz, Ar-H), 5.349(s, 2H, NH_2), ^{13}C NMR (100 MHz, $CDCl_3$): 196.6, 164.6, 161.03, 152.06, 151.8, 148.04, 133.05, 132.5, 128.8, 128.3, 126.03, 123.08, 123.02, 122.04, 122.01, 119.04, MASS

spectrum m/z: 439.28 $[M+H]^+$ Calc. for $C_{15}H_{11}N_5O_5$; CHN: C, 52.79; H, 3.25; N, 20.52; O, 23.44; Found: C, 52.79; H, 3.25; N, 20.52; O, 23.44. IR (KBr, cm^{-1}): 3071.13 (C-H, Aromatic), 2957.18 (C-H Aliphatic), 1718.10 (C=O**)**, 1524.12 (C=C, Aromatic), 1132.14 (C-O).

3.1.13. 4-amino-N-(2,4-dimethylphenyl)-1H-indole-6-carboxamide (7m): Compound **7m** obtained as white solid (yield 48 %), ^{1}H NMR (400 MHz DMSO, δ ppm): 8.09-8.11 (d, 1H, J= 8.0 Hz, Ar-H), 7.80-7.82 (d, 1H, J= 8.0 Hz, Ar-H), 7.75-7.77 (d, 1H, J= 8.0 Hz, Ar-H), 7.49-7.53 (t, 1H, J= 8.2 Hz, Ar-H), 7.35-7.39 (t, 1H, J= 12.0 Hz, Ar-H), 7.25-7.26 (d, 1H, J= 8.0 Hz, Ar-H), 6.75-6.77 (d, 1H, Ar-H), 6.67 (s, 1H, Ar-H), 5.349(s, 2H, NH_2), ^{13}C NMR (100 MHz, DMSO): 196.6, 164.6, 161.3, 151.8, 147.6, 133.5, 129.8, 129.4, 128.6, 128.4, 128.2, 126.3, 126.2, 123.8, 123.8, 123.2, 122.1, 118.9; MASS spectrum peak m/z: 280.21$[M+H]^+$ Calc. for $C_{17}H_{17}N_3O$; CHN: C, 73.18; H, 6.15; N, 15.08; O, 5.70; Found: C, 73.10; H, 6.13; N, 15.04; O, 5.73; IR (KBr, cm^{-1}): 3604.23 (OH), 3067.12 (C-H, Aromatic), 2985.42 (C-H, Aliphatic), 1713.75 (C=O**),** 1523.20 (C=C, Aromatic), 1268.31(C-O).

3.1.14. 4-amino-N-(2,4-dihydroxyphenyl)-1H-indole-6-carboxamide (7n): Compound **7n** obtained as orange solid (yield 35 %), ^{1}H NMR (400 MHz DMSO, δ ppm): 10.550 (s, 1H, NH, indole), 8.10-8.12 (d, 1H, J= 8.0 Hz, Ar-H), 7.82-7.84 (d, 1H, J= 8.0 Hz, Ar-H), 7.72-7.73 (d, 1H, J= 4.2 Hz, Ar-H), 7.48-7.51 (t, 1H, J= 4.0 Hz, Ar-H), 7.30-7.33 (t, 1H, J= 4.0 Hz, Ar-H), 7.27-7.29 (d, 1H, J= 8.0 Hz, Ar-H), 6.72-6.74 (d, 1H, J= 8.0 Hz, Ar-H), 6.69 (s, 1H, Ar-H), 6.62-6.64 (d, 1H, J= 8.0 Hz, Ar-H), 5.349(s, 2H, NH_2),. ^{13}C NMR (100 MHz, DMSO): 195.6, 168.6, 163.3, 156.8, 148.2, 132.5, 129.6, 129.3, 128.2, 128.1, 128.0, 126.8, 126.4, 123.8, 123.2, 123.1, 122.8, 118.6; MASS spectrum m/z: 284.15 $[M+H]^+$ Calc. for $C_{15}H_{13}N_3O_3$; CHN: C, 63.60; H, 4.63; N, 14.83; O, 16.94; Found C, 63.64; H, 4.60; N, 14.82; O, 16.90. IR (KBr, cm^{-1}): 3082.10 (C-H, Aromatic), 2975.15 (C-H, Aliphatic), 1720.12(C=O**)**, 1541.20 (C=C, Aromatic), 1150.68 (C-O).

3.1.15. 4-amino-N-(3,4,5-trimethoxylphenyl)-1H-indole-6-carboxamide (7o): Compound **7o** obtained as cream white solid (yield 28 %), ^{1}H NMR (400 MHz $CDCl_3$, δ ppm): 10.550 (s, 1H, NH, indole) 7.864-7.917 (m, 3H, Ar-H, amide NH), 7.469-7.568 (m, 2H, Ar-H), 7.375-7.397 (d, 1H, J= 4.0 Hz, Ar-H), 6.70-6.72 (d, 1H, J= 8.0 Hz, Ar-H), 6.68 (s, 1H, Ar-H), 6.62-6.377(s, 2H, Ar-H), 5.349(s, 2H, NH_2), 3.755, 3.801 (s, 3H, 3OCH3), ^{13}C NMR (100 MHz, $CDCl_3$): 168.65, 161.15, 142.08, 132.54, 129.35, 129.09, 128.56, 128.14, 126.88, 125.27, 124.48, 114.02, MASS spectrum m/z: 342.30000 $[M+H]^+$ Calc. for $C_{18}H_{19}N_3O_4$; CHN: C, 73.60; H, 6.50; N, 14.30; O, 5.47; Found C, 73.69; H, 6.53; N, 14.32; O, 5.45. IR (KBr, cm^{-1}): 3058.56 (C-H, Aromatic), 2976.58 (C-H, Aliphatic), 1660 (C=N), 1591.59(C=C), 1222.35 (S=O), 1080.34 (C-N).

4.0. Pharmacological screening:

All of the studies were planned and carried out in compliance with the CPCSEA, New Delhi's standard guiding principles, and the Institutional Animal Ethical Committee accepted the protocol. For behavioral investigations, 5–6 week old male Swiss albino mice weighing 20–25 g were housed in normal settings with a temperature range of 20–25 °C, a 12-hour light/dark cycle, and an ad libitum supply of water and standard pellet food. Before the tests, the animals were given a week to become used to the lab setting. They were then randomly split into five groups of six animals each, as follows:

Group 1: (vehicle control): Animals received PBS (Phosphate Buffer Solution), p.o

Group 2: (negative control): Mice administered with β-amyloid peptide by cerebroventricular injection.

Group 3: Mice injected with β-amyloid peptide and rivastigmine (p.o.) 5 mg/kg.

Group 4: Mice injected with β-amyloid peptide and Tacrine (p.o.) 5mg/kg

Group 5: Mice injected with β-amyloid peptide and treated with 200 mg/kg and 400 mg/kg of test compounds (p.o) Using a stereotaxic device to locate the bregma position in the skull, 10

μL containing 10 μg Aβ (25-35) peptide was delivered to all groups except one in order to induce neurotoxicity using intracerebroventicular injection (INCO, India). Test compounds and standard medicines were delivered on the fourteenth day after β-amyloid peptide delivery. Following β-amyloid peptide therapy, behavioral and biochemical markers are examined on days 7 and 21.

4.1. In vitro AChE and BuChE inhibitory studies [24]:

The investigation of AChE and BuChE was conducted using conventional described procedures and ready-to-use kits, with all reagents supplied by M/s. Sigma Aldrich. The test chemicals were solubilized in DMSO and 1% bovine serum, then subsequently diluted in phosphate buffer. Enzyme solutions were formulated to provide a concentration of 2.0 units/ml, and a solution of Ellman's reagent (5, 5'-dithiobis-2-nitrobenzoic acid, DTNB) was made by dissolving 2 mg of DTNB in 200 mL of buffer with intermittent vortexing. The determination of IC50 for test compounds using the modified Ellman technique entails the generation of thiocholine via the activity of acetylcholinesterase (AChE). Thiocholine subsequently interacts with DTNB to produce a yellow hue, the strength of which correlates with enzyme activity in the presence of test chemicals, evaluated spectroscopically at 412 nm. A test tube was prepared with a combination of 10 μL AChE and 30 μL DTNB solution, which was thereafter agitated intermittently. A solution of suitable concentration of test compounds and standards was added and incubated for 20 minutes, followed by the addition of 30 μL DTNB reagent and 30 μL of the substrate acetyl thiocholine iodide. The intensity of the produced yellow hue was measured at 412 nm with a one-minute interval at 37 °C in triplicate. The intensity of the yellow hue produced in the stoichiometric reaction signifies the quantity of acetylthiocholine hydrolyzed during the enzymatic process. The comparative inhibitions of all test substances were assessed in relation to the native esterase activity. The previously indicated approach was used in a similar manner for BuChE activity with butyrylthiocholine iodide as the substrate. The linear

regression plot of % inhibition vs log concentration of test chemicals in MS Excel gives the IC50 of the substances. The data are expressed as the mean ± standard deviation of the triplicates.

4.2.. In vitro estimation of Brain Cholinesterases:

The improved Ellman's method was subsequently used to quantify brain AChE and BuChE levels in experimental subjects. Rat brain was used to determine the cholinesterase inhibitory activity of test compounds, whereby the animals were euthanized to harvest the brain, and a homogenate was made in 0.1M phosphate buffer (pH 8.0). 0.4 mL of brain homogenate was added to a test tube containing 2.6 mL of phosphate buffer, and the mixture was well combined. 100 µL of DTNB chromophore reagent was added to the aforementioned solution and agitated for thorough mixing with air bubbling. The absorbance of the resulting color was measured at 412 nm, and after it stabilized, it was recorded as the baseline measurement. Twenty microliters of acetyl thiocholine substrate were put into a test tube, and the variation in absorbance was recorded for a period of ten minutes at two-minute intervals; thereafter, the change in absorbance per minute was calculated.

4.3. In vivo Behavior Pharmacological Activities:

4.3.1. Jumping Avoidance Box (Conditioned Avoidance Test) [25-26]

A Plexiglas partition divided a box into two equal chambers, with a gate allowing access for the animals to the neighboring compartment via a 14 x 17 cm opening. The test subjects are subjected to 30 seconds of light, followed by a 10-second auditory stimulus and a single low-intensity foot shock (0.5 mA) lasting three seconds in each trial. Every mouse from all groups had 15 trials daily, with a 15-second interval between consecutive trials, conducted over a span of five days.

4.3.2. Rectangular Maze Test [27-28]**:**

The Rectangular maze test equipment has three linked chambers: A, B, and C, with chamber B serving as the labyrinth used to assess the memory ability of test animals. All deprived mice were permitted to go from room A to C via chamber B. Chamber C housed reward food for the animal that moved from chamber A to C, as shown by the pilot light. All test animals underwent daily training to get the incentive meal, and the length was meticulously observed. The animals were deemed trained when the time taken to complete each labyrinth remained consistent over three consecutive days. The time necessary for maze completion was subsequently recorded for each animal prior to and following medication with test compounds and a standard medication.

4.3.3. Y-Maze Test [29-30]:

The special recognition memory of mice may be assessed using a two-trial recognition Y-maze test that does not need a learning rule. The working memory of mice is assessed by spontaneous alternation behavior in a Y maze constructed from black-painted wood. A Y maze consisting of three arms, each measuring 40 cm in length, 12 cm in height, 3 cm in width at the base, and 10 cm in width at the apex, converging in an equilateral triangular middle region. In the eight-minute experiment, a mouse is positioned in one arm of a Y maze and let to navigate freely. Arm entries are visually recorded, defined as complete when the mouse's rear paw fully enters the arm. In the Y maze, the word "Alternation" is defined as consecutive entry into all three arms, and the percentage of Alternation is computed using the following calculation.

% alternation = {(No. of alternations) / (Total arm entries - 2)} x 100

The percentage of alternation serves as an indicator of the depth of working memory; a larger percentage reflects an enhanced degree of memory in rodents. All mice in groups, except group 1, were administered β-amyloid protein (10μg) on day one, and all groups underwent maze training. On the seventh day, maze counts were recorded, and on the fourteenth day, conventional medications tacrine and rivastigmine, together with test compounds, were

delivered. On the 21st day, the mice were let to navigate the maze freely, and the entrance counts were recorded.

5.0. MOLECULAR DOCKING [31]:

Schrödinger program (Version 2019-1, Schrödinger) (Glide module) was used for docking investigations. ChemDraw was used to draw the ligands that were utilized as docking inputs. Ligand preparation was done in Ligprep (Version 2019-1, Schrödinger) utilizing the OPLS3e force field. The insertion of hydrogens to the ligands and the assignment of bond ordering are made easier by this minimization. For docking experiments, the output file that was created (the best conformation of the ligands) was used. The protein preparation in Maestro Wizard 31 (Version 2019-1, Schrödinger) was used to prepare the protein. The proteins were treated to an atom of hydrogen, charges were applied, and het states were produced at pH 7.2 using epik. Since docking tests showed that water molecules and other heteroatoms had no bearing on the protein's function, they were left out of the crystal structure. Ultimately, the force field of optimum potential liquid simulations (OPLS3) was used to improve the protein. In the vicinity of the cocrystal ligand—the ligand's X-ray position inside the protein—a receptor grid was produced. With a partial atomic charge of 0.25, the centroid of the grid box and the Vander Waal radius of the receptor atoms were scaled to 1.00 Å. To identify the optimal docked structure from the result, the glide docking score was used. XP Visualizer (Version 2019-1, Schrödinger) was used to study the poses of the ligands that were created following docking.

6.0. RESULTS AND DISCUSSIONS:

6.1. Chemistry:

The FTIR spectra of intermediates **2** showed absorption bands between 3400-3300 cm^{-1} due to the asymmetric and symmetric stretching vibrations of primary amine group and also ^{1}H NMR spectrum of the same compounds showed a broad peak at δ ~5.349 which indicated the

presence of primary amine. FTIR spectra of compound **5** showed carbonyl stretching vibration around 1660-1740 cm^{-1}, whereas band for secondary amine was located at ~3200 cm^{-1}.

The title compounds New 4-amino-N-substituted -phenyl-1H-indole-6-carboxamide (7a-o) were characterized ^{1}H NMR spectrum of all the compounds showed resonance for NH proton of indole at around δ value at 10.550 ppm. 9-Methoxy protons located in the range of 3.755 and 3.801 ppm and FTIR spectrum of all final compounds **7a-o** indicated the presence of amidic carbonyl group showing a band at 1700-1600 cm^{-1} and also the absorption band of secondary NH of amide was found at ~3340 cm^{-1}. ^{1}H NMR spectrum of all the compounds showed resonance for primary NH proton at around δ value 5.349 ppm and all aromatic protons in the range of 6.377-7.917 ppm ppm. The ESI mass spectrum of all the compounds showed peaks at relevant M+H m/z and complemented the FTIR & ^{13}C NMR spectra for the confirmation of expected structures of all the compounds. All aromatic carbons observed at 114.02-161.15 ppm and amidic carbon observed at 168.65 The Physical data of the final compounds has been listed in **Table 1**.

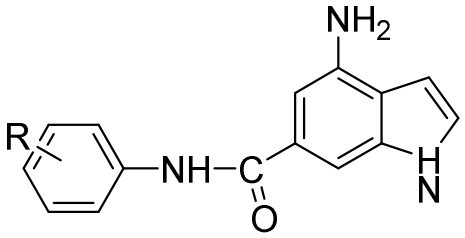
6.2. *In vitro* AChE and BuChE inhibitory activity

All the fifteen compounds have been screened against human AChE and BuChE by following reported methods and compared the potency with standards Rivastigmine. Six compounds (**7i,7j, 7h, 7k, 7n 7o**,) were reported earlier with similar in vitro cholinesterase inhibitory activity by following different methodology, here we have reported both cholinesterase and *in vivo* activities of these six compounds too. AChE and BuChE inhibitory activity can be tangibly correlated with structure of all compounds depending on the substitution on aromatic ring between indole ring . AChE and BuChE inhibitory activities of all the compounds have been listed in **Table 2** and depicted in **Figure 3** and **4**.

The compound **7a** Unsubstituted aromatic ring fused 5-amino indole ring with amidic bond has demonstrated significant activity with IC_{50} of 5.97±0.04 and 6.74±0.14μM against AChE

and BuChE respectively. Compounds **7m and 7e** showed inferior activity with methyl and nitro substituted on aromatic ring with IC_{50} value of 5.40±0.02, 5.95±0.23 and 4.09±0.02, 5.59±0.25μM against AChE and BuChE respectively. However, acetylcholinesterase inhibitory activity of four compounds **7b, 7c, 7d** were better than their respective methyl derivatives and unsubstituted compounds. Compound **7o** with tri methoxy moiety substituted on aromatic ring fused with 5-amino indole ring with amide linker demonstrated most potent acetylcholinesterase inhibitory activity among all the tested compounds with IC_{50} value of 0.89±0.03 μM and 0.96±0.12 μM respectively. Aforementioned structure activity relationship revealed that large hydrophobic space is available which accommodates semi-flat dicyclic ring and comparatively small void space could be available where substituents R occupy due to which substitution with methyl and nitro group reduced the potency of acetylcholinesterase inhibitory activity. In case of compounds with un substitution on aromatic ring (7a), replace with di halo substituted compounds (7h-7n) resulted increase in potency when compared to mono halo derivatives.

Table-2. *In vitro* AChE and BuChE inhibitory activity of New 4-amino-N-substituted -phenyl-1H-indole-6-carboxamide (7a-o)



General structure-I (7a-o)

Com	R	M. Form	AChE [a]	BuChE [a]
7a	H	$C_{15}H_{13}N_3O$	5.97±0.04	6.74±0.14
7b	4-Cl	$C_{15}H_{12}ClN_3O$	2.02±0.02	3.65±0.15
7c	4-Br	$C_{15}H_{12}BrN_3O$	2.85±0.02	4.35±0.25
7d	4-F	$C_{15}H_{12}FN_3O$	2.89±0.03	2.64±0.15
7e	4-NO_2	$C_{15}H_{12}N_4O_3$	4.09±0.02	5.59±0.25
7f	4-CH_3	$C_{16}H_{15}N_3O$	4.90±0.04	5.48±0.24
7g	3-Cl	$C_{15}H_{12}ClN_3O$	2.94±0.02	3.41±0.13
7h	2,4 Di chloro	$C_{15}H_{11}Cl_2N_3O$	2.61±0.02	2.35±0.13
7i	3,4,5 tri chloro	$C_{15}H_{10}Cl_3N_3O$	1.28±0.04	1.61±0.15
7j	2,4 Di bromo	$C_{15}H_{11}Br_2N_3O$	2.93±0.02	2.64±0.14
7k	2,4 Di flouro	$C_{15}H_{11}F_2N_3O$	1.10±0.04	1.32±0.12
7l	2,4 Di nitro	$C_{15}H_{11}N_5O_5$	3.14±0.03	6.24±0.03
7m	2,4 Di methyl	$C_{17}H_{17}N_3O$	2.40±0.02	3.45±0.23
7n	2,4 dihydroxy	$C_{15}H_{13}N_3O_3$	2.07±0.04	4.38±0.15
7o	3,4,5 tri methoxy	$C_{18}H_{19}N_3O_4$	0.89±0.03	0.96±0.12
Standard	Rivastigmine	-	0.56±0.02	0.46±0.12

Data expressed as IC_{50} μM (mean ± SD, n=3).

6.3. *In vivo* brain AChE/ BuChE inhibitory activity:

Six compounds with significant potency **7i, 7j, 7h, 7k, 7l, 7n** and **7o** were subjected to *in vivo* brain AChE and BuChE inhibitory activity and rivastigmine were included in the study as standards by following the reported methodology. *In vivo* brain AChE and BuChE inhibitory activities have been reported as IC_{50} in micro moles and presented in Table 3 and Figure 5.

Compound 7k displayed potent *in vivo* brain AChE inhibitory activity with IC_{50} of 28.65±0.15

μM and 34.56±0.32. Compound 7h resulted in decrease in the activity with IC_{50} of 43.34±0.18 and 45.43±0.26 μM against brain AChE and BuChE . Compound 7o was found to be most potent in in vivo model with IC_{50} of **26.32±0.25** and **31.24±0.18** against AChE which was comparable with both standard compounds rivastigmine. *In vivo* activity of 7i and 7o indicated the existence of conformational flexibility in active site.

Table 3: *In vivo* brain AChE/ BuChE inhibitory activity of compounds

No.	Group	AChE [a]	BuChE [a]
1.	7i	31.27±0.20	38.36±0.28
2.	7j	42.24±0.20	42.45±0.23
3.	7h	43.34±0.18	45.43±0.26
4.	7k	**28.65±0.15**	**34.56±0.32**
5.	7l	40.24±0.41	44.35±0.34
6.	7n	34.31±0.24	38.23±0.26
7.	7o	**26.32±0.25**	**31.24±0.18**
8.	Rivastigmine	21.48±0.20	25.25±0.28
9.	Negative Control	38.25±0.52	55.20±0.22

[a] Data expressed as IC_{50} μM (mean ± SD, n=3).

6.4. Behavioral studies *(In vivo)*:

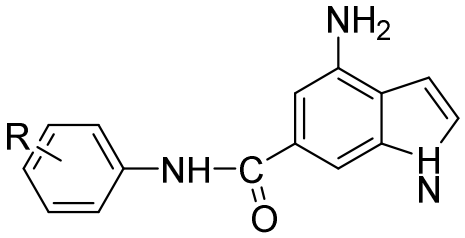
All fifteen synthesized compounds have been evaluated for their effects on behavior in experimental animals. The tests conducted were the Y-maze test, the rectangular maze, and the jumping box test, according to established in vivo protocols, with findings shown in Table 4. The Y maze test assesses the propensity of experimental animals, often rats, to explore novel habitats by examining one of the new arms of the Y maze instead of reverting to the original arm. The outcome of the Y maze experiment is shown as a percentage of alternation for both

pre- and post-administration of test substances. All compounds and standards of Rivastigmine were delivered at a dosage of 400 mg/kg.

All the compounds exhibited moderate to potent in Y-maze test which is comparable with rivastigmine. Compounds and **7o** were found to show equal potent activity alongside of rivastigmine and among all the compounds **7o** was noticed to be more potent than rivastigmine with statistical significance ($p<0.001$). Compound **7o** was most potent among all the synthesized compounds in acetylcholinesterase inhibitory activity which indicates that **7o** possesses both potent in vitro and in vivo activity along with sufficient solubility which is primary requirement for in vivo activity. Compounds **7i, 7j, 7h, 7k, 7l, 7n** demonstrated moderate activity whereas rest other compounds exhibited low activity.

In rectangular maze test there was an increase in maze traverse period in negative control group (148.87± 2.53) when compared to vehicle control and there was a decrease in traverse period in groups treated with **7o** (154.15±2.85; 96.50±2.85 at 400 mg/kg) with statistically significance ($p<0.001$). The jumping box test also considered as conditioned avoidance test (memory). The activity was expressed in latency periods with time in amnesia induced mice. In jumping box test, there was an increase in latency period in negative control group (40.12±1.09) and there was a decrease in latency period in groups treated with **7k,** and **7o** (11.24±1.62 and 13.42±3.18 sec) at 400 mg/kg) compared standard drug rivastigmine 9.30±0.71 sec which was statistical significant ($p<0.001$), indicating significant improvement in memory compared to control group.

Table 4. Behavioral effects in Y-maze test, rectangular maze, jumping box test



General structure-I (7a-o)

Comp	Y-maze test (% alternations)		Rectangular maze (sec)		Jumping box (sec)	
	Before treatment	**After treatment**	**Before treatment**	**After treatment**	**Before treatment**	**After treatment**
7a	28.15±1.24	41.15±1.24	154.15±2.65	118.50±2.65	32.6±1.65	20.3±1.65
7b	25.15±2.25	34.15±2.52	156.17±2.65	139.10±2.65	32.4±1.65	18.13±1.65
7c	24.32±1.03	35.65±1.46	150.18±1.42	146.24±2.65	30.8±1.20	27.33±1.20
7d	25.35±1.30	37.10±1.87	146.24±2.35	138.10±2.35	26.20±2.45	20.01±2.45
7e	23.15±1.42	33.14±2.65	146.26±2.40	131.25±2.40	30.23±2.54	18.43±2.54
7f	25.15±1.38	36.12±3.46	146.16±1.40	132.07±1.48	30.15±2.10	22.56±2.10
7g	23.32±1.03	40.20±2.14	150.25±3.24	122.56±3.24	31.6±3.56	19.35±3.56
7h	**26.62±1.30**	**39.32±1.87**	**148.28±2.35**	**136.20±2.35**	**26.15±2.45**	**18.18±2.45**
7i	**24.30±1.30**	**38.24±3.14**	**148.28±2.40**	**132.18±2.40**	**28.18±2.54**	**16.12±2.54**
7j	**29.12±1.25**	**40.20±2.10**	**146.21±2.98**	**134.07±2.35**	**26.20±2.15**	**14.43±2.15**
7k	**28.60±1.30**	**33.15±2.52**	**140.14±1.48**	**131.07±1.48**	**34.10±3.18**	**13.42±3.18**
7l	26.28±1.42	35.65±1.46	145.76±2.37	128.75±2.37	29.5±0.22	21.17±0.22
7m	24.02±1.38	37.13±1.87	143.12±2.40	128.25±2.40	30.23±2.54	20.12±2.54
7n	27.10±2.25	35.14±2.65	140.72±2.37	128.75±2.37	29.5±1.23	19.7±1.23
7o	**25.42±1.03**	**41.61±1.87**	**154.15±2.85**	**96.50±2.85**	**32.6±1.62**	**11.24±1.62**
Rivastigmine	27.28±2.42	49.32±2.58	145.42±2.21	81.61±2.21	28.32±0.71	9.30±0.71
-ve	21.19±2.18		148.87±2.53		40.12±1.09	
Vehicle control	47.61±3.15		47.83±2.53		12.5±2.18	

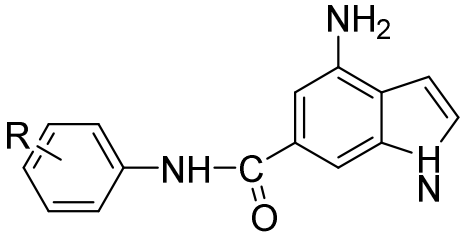
6.5. Molecular docking:

In vitro studies of synthesized compounds showed the potential cholinesterase inhibitory activity and among all, the compound **7o** showed promising cholinesterase inhibitory activity. These result encouraged us to perform docking studies to get the insight in to the binding mode of synthesized compounds within binding pocket of AChE and BChE. All structures of ligands were built using maestro and further prepared using LigPrep form Schrodinger package.

Protein structures were obtained from the Protein Data Bank (PDB ID: acetyl cholinesterase-1EVE and butylcholinesterase-4BDS) and necessary correction to the protein structure were done using Protein Preparation Wizard in Schrodinger package. Docking studies were performed using Glide docking software and docking protocol was validated by docking the cocrystal ligand which resulted with RMSD of docked conformation and cocrystal ligand pose was found to be 0.6. The binding interactions of compounds with AChE and BChE have been listed in **Table 6**

Binding poses of synthesized compounds with AChE have shown that these molecules bind well within binding pocket of enzyme. Among the all synthesized molecules, **7o** with potent cholinesterase inhibitory activity**,** has shown the highest binding score. However, 3,4,5-triimethoxyphenyl ring has occupied the empty additional space available in binding pocket. In binding pocket, **7o** was involved in π-π stacking interactions with Phe331 and His440. 3-methoxy group formed hydrogen bond with side chain of Ser122 while 4-methoxy group was involved in hydrogen bonding with side chain carbonyl carbon of Asn80 (**figure 8**). Indole ring was found to involve in hydrophobic contacts with Tyr334 and Phe331 and indole ring was in close proximity with Trp84, Glu199, His440 and Gly441. Moreover, docking studies on the BuChE showed the similar results as acetylcholinesterase. Compound **7o** has the highest binding score and 3,4,5-trimethoxyphenyl was involved in hydrogen bonding interactions with enzyme; 3-methoxy group with His148, whereas 4-methoxy was forming with Gly78 and Trp82 (**figure 9**).

Table-2. Molecular docking of New 4-amino-N-substituted -phenyl-1H-indole-6-carboxamide (7a-o)



General structure-I (7a-o)

Com	R	M. Form	Docking score of AChE (2WG1)	Docking score of BuChE (7Q1M)
7a	H	$C_{15}H_{13}N_3O$	-8.07	-7.57
7b	4-Cl	$C_{15}H_{12}ClN_3O$	-10.22	-6.61
7c	4-Br	$C_{15}H_{12}BrN_3O$	-9.66	-6.38
7d	4-F	$C_{15}H_{12}FN_3O$	-8.51	-7.33
7e	4-NO_2	$C_{15}H_{12}N_4O_3$	-8.73	-6.84
7f	4-CH_3	$C_{16}H_{15}N_3O$	-8.90	-7.52
7g	3-Cl	$C_{15}H_{12}ClN_3O$	-8.05	-7.46
7h	2,4 Di chloro	$C_{15}H_{11}Cl_2N_3O$	-9.73	-8.56
7i	3,4,5 tri chloro	$C_{15}H_{10}Cl_3N_3O$	-10.33	-10.55
7j	2,4 Di bromo	$C_{15}H_{11}Br_2N_3O$	-9.97	-7.44
7k	2,4 Di flouro	$C_{15}H_{11}F_2N_3O$	-11.71	-10.13
7l	2,4 Di nitro	$C_{15}H_{11}N_5O_5$	-8.62	-8.51
7m	2,4 Di methyl	$C_{17}H_{17}N_3O$	-8.50	-7.59
7n	2,4 dihydroxy	$C_{15}H_{13}N_3O_3$	-10.79	-6.15
7o	3,4,5 tri methoxy	$C_{18}H_{19}N_3O_4$	**-12.38**	**-11.16**

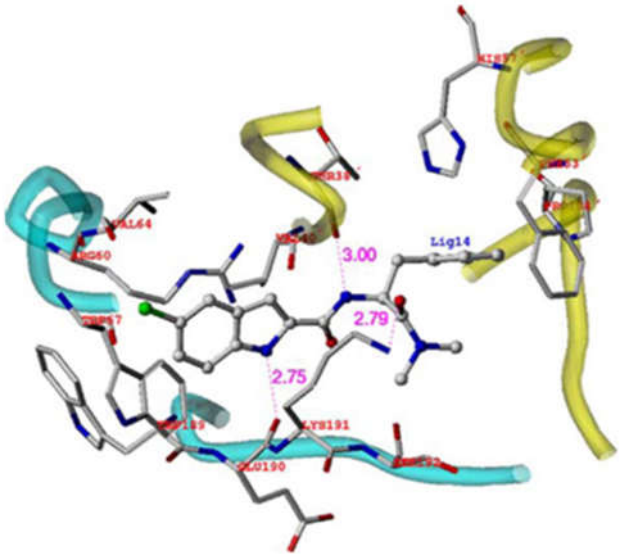


Figure: 4: The interacting mode of compound 7o with (2WG1)

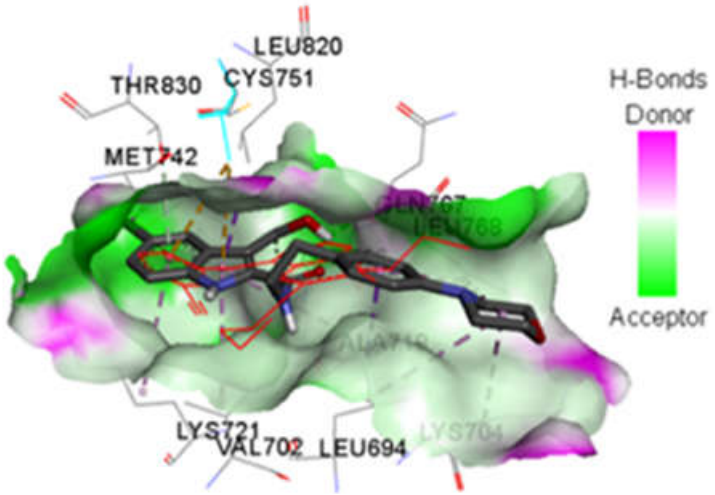


Figure:5: 3D binding mode of compound 7o (shown as sticks colored by element) into the active site of **2WG1** (shown as red lines), receptor shown as hydrogen bond surface

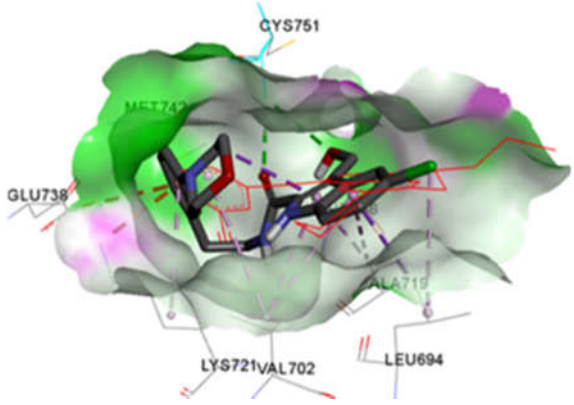


Figure:6: 3D binding mode of **7k** (shown as sticks colored by element), into the active site of **2WG1** overlapped with the co-crystallized receptor shown as hydrogen bond surface;

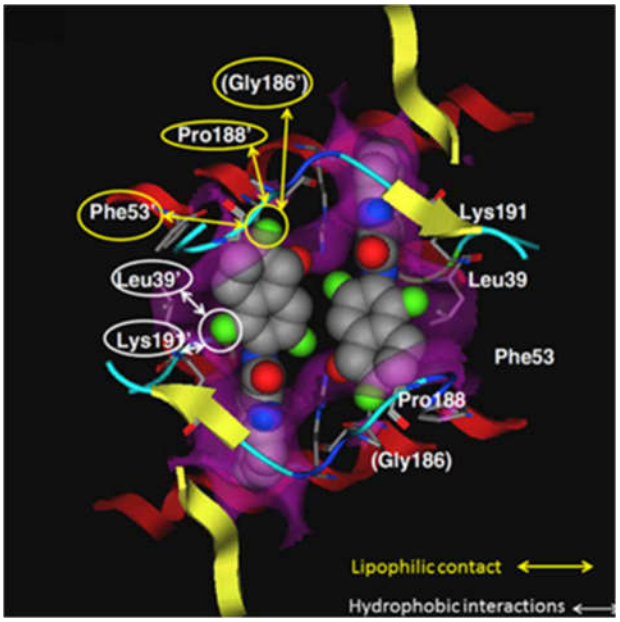


Figure:6: Predicted binding model for compound 7o with 2WG1.

7.0 Conclusions:

In conclusion, new compounds based **4-amino-N-substituted -phenyl-1H-indole-6-carboxamide (7a-o)** have been designed such molecules have been synthesized from 3, 5-dinitrobenzoic acid. All the synthesized compounds were characterized and compounds were subjected for *in vitro* acetyl and butyrylcholinesterase inhibitory activity and *in vivo* behavioral studies on experimental animals. Also, in order to understand the binding interactions within the active site of cholinesterases, compounds were subjected to molecular docking studies. Compounds 7k **and 7o** have exhibited potent acetyl and butyrylcholinesterase inhibitory activity with with IC_{50} values of 1.10±0.04 ; 1.32±0.12 and 0.89±0.03; 0.96±0.12 µM against AChE and BuChE respectively which are comparable with standard drugs like and rivastigmine. Compounds **7o** with IC_{50} of 26.32±0.25 and 31.24±0.18 µM and **7k** was further studied for brain cholinesterase inhibitory activity and exhibited potent activity with IC_{50} of

28.65±0.15 µM and 34.56±0.32 µM against AChE and BuChE and also demonstrated potent in vivo activities in Y-maze test, rectangular maze test and jumping box test which indicated our understanding from *in vitro* result that **7o** possess sufficient solubility a primary requirement for in vivo activities. Docking studies revealed that the designed molecules interacted with in nicotinic receptor with at list two hydrogen bond interactions barring few compounds.

Acknowledgement:

One of the authors KR expresses thank to the Department of Pharmaceutical Sciences, Jawaharlal Nehru Technological University Anantapur, Anantha providing necessary facilities.puramu, Authors thank the Principal, Professor, Department of Pharmaceutical Analysis & Chemistry, Santhiram College of Pharmacy, Nandyal for providing necessary facilities.

REFERENCES:

1. Goedert M, Spillantini MG (2006) A century of Alzheimer's disease. Sci 314(5800): 777-781. doi: 10.1126/science.1132814.

2. Wimo A, Prince M, Guerchet M, Ali GC, Wu YT, Prina M (2015) World Alzheimer Report 2015. http://www.alz.co.uk/world report 2015.

3. Sultana R, Butterfield DA (2010) Role of oxidative stress in the progression of Alzheimer's disease. J Alzheimer's Dis19(1):341-353. https://doi.org/10.3233/jad-2010-1222

4. Jucker M, Walker LC (2011) Pathogenic protein seeding in Alzheimer's disease and other neurodegenerative disorders. Annal. Neuro 70: 532-540. https://doi.org/10.1002/ana.22615.

5. Kaur R, Mehan S, Singh S (2019) Understanding multifactorial architecture of Parkinson's disease: pathophysiology to management. Neurological Sci 40(1): 13-23. https://doi.org/10.1007/s10072-018-3585-x.

6. Meng Q, Ru J, Zhang GL (2007) Re-evaluation of tacrine hepatotoxicity using gel entrapped hepatocytes. Toxicol Lett168: 140–147. **https://doi.org/10.1016/j.toxlet.2006.11.009**

7. Wu WY, Chendai YU, Gu T (2017) Novel multitarget-directed tacrine derivatives as potential candidates for the treatment of Alzheimer's disease. J Enzyme Inhib Med Chem 32(1): 572-587; https://doi.org/10.1080/14756366.2016.1210139.

8. Mohamed T, Rao PP (2011) Alzheimer's Disease: emerging trends in small molecule therapies. Cur Med Chem18(28): 4299–4320. **https://doi.org/10.2174/092986711797200435**

9. B. Bhrigu, D. Pathak, N. Siddiqui, et al., Search for biological active isatins: a short review, Int. J. Pharm. Sci. Drug Res. 2 (2010) 229–235.

10. V. Raj, Review on CNS activity of isatin derivatives, Int. J. Curr. Pharm. Res 4 (2012)1–9.

11. J.F. Da Silva, S.J. Garden, A.C. Pinto, The chemistry of isatins: a review from 1975 to 1999, J. Braz. Chem. Soc. 12 (2001) (1975) 273–324, https://doi.org/10.1590/ S0103-50532001000300002.

12. D.O. Ozgun, C. Yamali, H.I. Gul, et al., Inhibitory effects of isatin Mannich bases on carbonic anhydrases, acetylcholinesterase, and butyrylcholinesterase, J. Enzyme Inhib. Med. Chem. 31 (2016) 1498–1501, https://doi.org/10.3109/14756366. 2016.1149479.

13. P. Riederer, W. Danielczyk, E. Grünblatt, Monoamine oxidase-B inhibition in Alzheimer's disease, Neurotoxicology 25 (2004) 271–277, https://doi.org/10. 1016/S0161-813X(03)00106-2.

14. L. Huang, C. Lu, Y. Sun, et al., Multitarget-directed benzylideneindanone derivatives: anti-β-amyloid (Aβ) aggregation, antioxidant, metal chelation, and monoamine oxidase B (MAO-B) inhibition properties against Alzheimer's disease, J. med. chem. 55 (2012) 8483–8492, https://doi.org/10.1021/jm300978h.

15. A. Medvedev, O. Buneeva, O. Gnedenko, et al., Isatin interaction with glyceraldehyde-3-phosphate dehydrogenase, a putative target of neuroprotective drugs: partial agonism with

deprenyl, in: Oxidative Stress and Neuroprotection, 2006, Springer, pp. 97–103. https://doi.org.10.1007/978-3-211-33328-0_11.

16. P. Ershov, Y. Mezentsev, E. Yablokov, et al., Effect of Bioregulator Isatin on ProteinProtein Interactions Involving Isatin-Binding Proteins, Russ. J. Bioorgan. Chem. 44 (2018) 193–198, https://doi.org/10.1134/S1068162018010053.

17. A. Andreani, S. Burnelli, M. Granaiola, et al., New isatin derivatives with antioxidant activity, Eur. J. Med. Chem. 45 (2010) 1374–1378, https://doi.org/10. 1016/j.ejmech.2009.12.035.

18. Z. Omran, T. Cailly, E. Lescot, et al., Synthesis and biological evaluation as AChE inhibitors of new indanones and thiaindanones related to donepezil, Eur. J. Med. Chem. 40 (2005) 1222–1245, https://doi.org/10.1016/j.ejmech.2005.07.009.

19. Bingul M, Ercan S, Boga M (2020) The design of novel 4, 6-dimethoxyindole based hydrazide-hydrazones: Molecular modeling, synthesis and anticholinesterase activity. J Mol Struct 1213:128202 77.

20. Prochnow T, Maroneze A, Back DF, Jardim NS, Nogueira CW, Zeni G (2018) Synthesis and anticholinesterase activity of 2-substituted-N-alkynylindoles. Org Biomol Chem 16(42):7926–7934

21. Cheung J. Rudolph M. J. Burshteyn F. Cassidy M. S. Gary E. N. Love J. Franklin M. C. Height J. J. *J. Med. Chem.* 2012;55:10282–10286. doi: 10.1021/jm300871x.

22. Alsayed SS, Lun S, Bailey AW, Suri A, Huang CC, Mocerino M, Payne A, Sredni ST, Bishai WR, Gunosewoyo H. Design, synthesis and evaluation of novel indole-2-carboxamides for growth inhibition of Mycobacterium tuberculosis and paediatric brain tumour cells. RSC advances. 2021;11(26):15497-511.

23. Zhang H, Wang X, Wan Y, Liu L, Zhou J, Li P, Xu B. Discovery of *N*-Arylsulfonyl-Indole-2-Carboxamide Derivatives as Galectin-3 and Galectin-8 C-Terminal Domain Inhibitors. ACS

Med Chem Lett. 2023 Aug 15;14(9):1257-1265. doi: 10.1021/acsmedchemlett.3c00261. PMID: 37736168; PMCID: PMC10510525.

24. Ellman GL, Courtney KD, Anders V, Featherstone RM (1961) New and rapid colorimetric determination of acetyl cholinesterase activity. Biochem Pharmacol 7: 88-95. https://doi.org/10.1016/0006-2952(61)90145-9.

25. Barros DM, Izquierdo LA, Mello ST, Ardenghi P, Pereira P, Medina JH, Izquierdo I (2000) Molecular signaling pathways in the cerebral cortex are required for retrieval of one-trial inhibitory avoidance learning in rats. Behav Brain Res 114(1-2): 183-192. https://doi.org/10.1016/S0166-4328(00)00226-6.

26. Bornschein RL, Hastings L, Manson JM (1980) Behavioral toxicity in the offspring of rats following maternal exposure to dichloromethane. Toxic Appl Pharmacol 52(1): 29-37. https://doi.org/10.1016/0041-008X(80)90244-6.

27. Buresova O, Bures J, Oitzl MSA (1985) Radial maze in the water tank: an aversively motivated spatial working memory task Zahalka. Physiol Behav 34(6): 1003–1005. https://doi.org/10.1016/0031-9384(85)90028-9.

28. Shear DA, Dong J, Haik-Creguer KL, Bazzett TJ, Albin RL, Dunbar GL (1998) Chronic administration of quinolinic acid in the rat striatum causes spatial learning deficits in a radial arm water maze task. Exp Neurol 150(2): 305–311. https:// doi.org/ 10.1006/exnr.1998.6767.

29. Goodarzi Z, Mele B, Guo S, Hanson H, Jette N, Patten S, Pringsheim T, Holroyd-Leduc J (2016) Guidelines for dementia or Parkinson's disease with depression or anxiety, a systematic review. BMC Neurol 16(1) 244-248. https://doi.org/10.1186/s12883-016-0754-5.

30. Conrad CD, Lupien SJ, Thanasoulis LC, McEwen BS (1997) The effects of type I and type II corticosteroid receptor agonists on exploratory behavior and spatial memory in the Y-maze. Brain Res759(1): 76–83. https://doi.org/10.1016/S0006-8993(97)00236-9.