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SYNTHESIS AND EVALUTION OF NEW TACRINE DERIVATIVES AS ANTI-ALZHEIMER'S AGENTS

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ABSTRACT

A new series of Tacrine and Piperzine conjugates were synthesized, characterized by spectral data (NMR, MASS and IR), and evaluated for acetyl cholinesterase inhibition activity. All most all the Tacrine and Piperzine conjugates exhibited significant potency to inhibit Cholinesterase enzyme with IC_{50} values between 0.98-1.34 µM, when compared to 11.07 µM shown by rivastigmine; Especially compound 5a and 5d displayed the potent activity to inhibit AChE with an IC_{50} Value of 0.983µM, 1.013 µM, respectively. SAR studies indicated that unsubstituted Piperzinyl Tacrine conjugate with six membered ring was more active than the substituted Piperzine derivatives and seven membered C ring (cycloheptanone) compounds.

KEY WORDS

Tacrine, Alzheimer's disease, cholinesterase inhibitors, Piperzines, neuro protection, Excitotoxicity,

INTRODUCTION:

Alzheimer's disease (AD) is a progressive, multifaceted neurodegenerative disorder affecting the aged population. It is characterized by memory loss, deficits in cognitive functions and behavioral abnormalities [Piazzi. L et al., 2003]. It is estimated that more than 36 million people suffer from AD and is reported to be one of the leading causes of death in developed countries [Prince. M et al., 2004]. Over the past decades, despite several efforts from various researchers across the globe, its pathogenesis still remains unclear.

Several factors including accumulation of amyloid β (Aβ), hyperphosphorylation of tau protein, oxidative stress, excitotoxicity, neuroinflammation, mitochondrial abnormalities and cholinergic dysfunction contribute to the progression of the disease [Walsh D.M et al., 2004]. The current clinical therapy for AD is mainly based on cholinergic hypothesis, which states that the loss of cholinergic neurotransmission in the cerebral cortex and other brain regions occur due to low levels of acetylcholine (ACh). Hence sustaining or recovering the cholinergic function can be clinically beneficial to AD patients [Munoz-Torrero.D et al., 2008]. Inhibition of acetyl cholinesterase (AChE), an enzyme involved in the hydrolysis of ACh is a practical method for the restoration of ACh level in the brain. AChE inhibition results in higher activation of post-synaptic ACh receptors and thereby enhances cholinergic neurotransmission. Four AChE inhibitors namely tacrine, donepezil, rivastigmine and galantamine (Figure 1) and an NMDA receptor antagonist, memantine, are the FDA approved drugs for the treatment of AD [Kurtz.A et al., 1998].

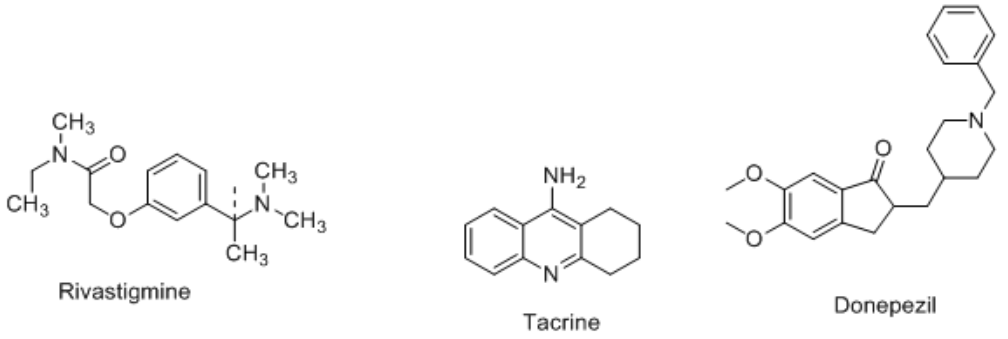


Figure 1: AChE inhibitors used as drugs for the treatment of AD.

Tacrine is the first cholinesterase inhibitor approved by FDA for the treatment of AD, but it has been withdrawn from the market due to its hepatotoxicity [Minarini. A et al., 2013]. Many tacrine derivatives/hybrids have been designed synthesized and evaluted in order to find compounds with reduced adverse side effects, while retaining its AChE inhibitory property [Spailovska.K et al.,2017]. Bis (7) tacrine [CarlierP.R et al., 2000], cystamine-tacrine dimer [Minarani. A et al.,2012], tacrine-ferulic acid hybrid [Zhang. Y et al.,2008], tacrine-lipoic acid hybrids [Packer.L et al., 1995], tacrine-melatonin hybrids [Reiter.R.J et al., 2003], tacrine-8-hydroxyquinoline derivatives [Fernadez-Bachiller.M.I et al., 2010], tacrine-donepezil [Alonso.L et al.,2005], NO donor tacrine hybrids [Fang.L et al., 2008], tacrine-chromen hybrids [Leong .S.W et al.,2016], tacrine-selegiline hybrids [Lu.C et al.,2013], tacrinecarbazole hybrids [Thiramatrakul . S et al., 2014], tacrine-trolox hybrids [Nepovimova.E et al., 2015], tacrine-pyrano[2,3-c] pyrazole hybrids [Pourabdi.L et al.,2016], tacrine-tianeptine hybrids [Ceschi. M.A et al.,2016], pyranotacrine [Garcia-font.N et al.,2016] and tacrine-multialkoxy benezene hybrids [Zhang.C et al., 2016] are some examples. Nonetheless, tacrine is still considered as a starting point for the development of drugs against AD.

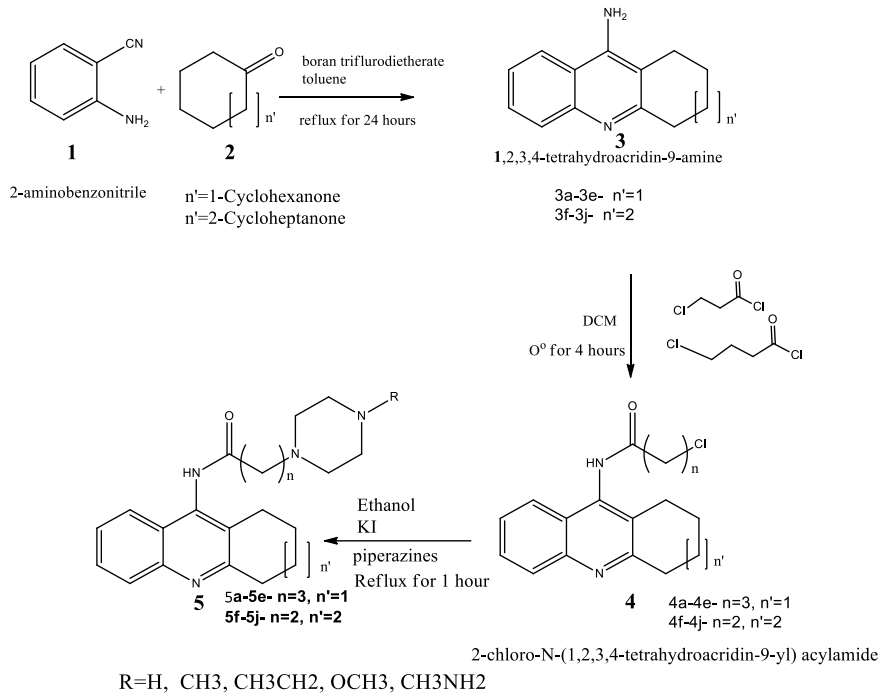
As a part of a project to design and develop novel AChE inhibitors a series of tacrine derivatives have been designed, synthesized and their AChE inhibitory potential has been tested [Reddy. E.K et al., 2016]. Conventionally, most of the tacrine derivatives were made by substituting its amine moiety by other functional groups. On the contrary, studies focus on the substitutions at C6 position of aromatic ring and C2 position of cyclohexyl ring of tacrine with various other functional groups, while retaining all the structural determinants required for the enzyme binding. The modifications at C2 position were aimed to achieve interaction with the peripheral anionic site (PAS) of AChE. Tacrine derivatives with a halogen substitution at C6 position is known to enhance AChE inhibition [Hu. M.K et al., 2002]. Literature survey also reveals that various tacrine derivatives with substituted pyridine or piperazines having alkylamino spacer were found to be possess potent AChE inhibition [Eeda Koti Reddy et al., 2016]. Tacrine piperazine conjugates via methelene spacers were also found to be potent antioxidants, tacrine with benzothiazole conjugation with amide linker were also to possess antioxidant, AChE inhibitory properties [Rangappa S et al., 2013]. Hence, it is proposed to synthesize tacrine-piperazine conjugates with amide linker as shown in the general structure (**I**) and evaluate them for their AChE inhibitory activity.

MATERIALS AND METHODS:

Chemistry:

Melting points were determined by open capillary tubes using VEEGO VMP-D Digital melting point apparatus. FTIR spectra of the powdered compounds were recorded using KBr on a JASCO FTIR 4100 series and are reported in $cm^{-1.}$ 1H NMR and ^{13}C NMR spectra were recorded on a BRUKER-II 400 (400 MHz NMR, ^{13}C NMR 100 MHz) spectrophotometer using TMS as an internal reference (Chemical shift represented in ppm). Purity of the compounds was checked on TLC plates using silica gel G as stationary phase and iodine vapors as the visualizing agent. All chemicals obtained from Sigma-Aldrich, HiMedia, Bangalore, India. The estimation of biochemical parameters was carried out using kits (Sigma-Aldrich). All solvents purchased from HiMedia laboratories, Mumbai, India.

Scheme-1:



Scheme1: Synthesis of compounds 5a-5j

General Synthetic Procedure:

General procedure for the synthesis of 1, 2, 3, 4-tetrahydroacridin-9-amine (3a-3e) / 7, 8, 9, 10-tetrahydro-6H-cyclohepta [b] quinolin-11-amine(3f-3j): To a solution of anthranonitrile (1.0mmol) and cyclohexanone (n'=1) / cycloheotanone (n'=2) (1.0mmol) in sodium dried toluene (8ml), boran triflurodietherate (1.28ml) was added slowly with syringe the mixture was then stirred and refluxed for 24 hours. After cooling the mixture toluene was decanted and the residue was treated with 2M NaOH (24ml) and refluxed for 24 hours. After cooling, the mixture was extracted with $CHCl_3$. The combined organic extracts were evaporated under reduced pressure to give yellow solid **(3a-3e, 3f- 3j).**

General procedure for the synthesis of N-chloro-N-(1,2,3,4 tetrahydro acridine-9-yl)acylamide (4a-4e)/ 7,8,9,10-tetrahydro-6H-cyclohepta[b]quinolin-9-yl) acylamide(4f-4j) : A solution of chloroacyl chlorides (0.5ml) in CH_2Cl_2 (10ml) was added dropwise to an ice-cold solution of triethylamine (2.5mmol or 1.8ml) and compound tacrine (3a-3e- n'=1, 3f-3j- n'=2) (5.0mmol or 1gm) in CH_2Cl_2 (30ml). After addition was completed reaction mixture was diluted with CH_2Cl_2 washed with water and brine solution. The organic layer was dried over anhydrous sodium sulphate and concentrated under reduced pressure to obtain the acylamide derivatives **(*4a-4e and 4f-4j*).**

General procedure for the synthesis of Substituted piperazinyl-N-(1, 2, 3, 4 tetrahydro acridine-9-yl)-acylamide (5a-5e) and Substituted piperazinyl-N-(7,8,9,10-tetrahydro-6H-cyclohepta[b]quinolin-9-yl) acylamide (4f-4j): The compound 4 (4a-ae & 4f-4j) (0.7mmol), substituted piperazines (10.5mmol) and potassium iodide (0.06gm or 0.4 mmol) in ethanol (30ml) was refluxed for 1 hour. After cooling, the solvent was removed under vaccum. The obtained residue was dissolved in $CHCl_2$ and then washed with water, dried over the anhydrous sodium sulphate and concentrated under vaccum to obtain the compound piperazinyl amino derivatives **(5a-5e / 5f-5j).**

RESULTS:

Physical data:

Compound	n	n'	R	Mobile Phase H: EA	Rf	Molecular formula	Molecular Weight	Melting point °C	% Yield
5a	3	1	H	7:3	0.9	$C_{21}H_{28}N_4O$	352	202-204	55
5b	3	1	Ethyl	7:3	0.5	$C_{23}H_{32}N_4O$	380	178-180	48
5c	3	1	Methyl	6:4	0.7	$C_{22}H_{30}N_4O$	366	160-162	62
5d	3	1	Methoxy	7:3	0.4	$C_{22}H_{30}N_4O_2$	382	138-140	36
5e	3	1	Methyl amino	7:3	0.9	$C_{22}H_{31}N_5O$	395	150-152	58
5f	2	2	H	7:3	0.6	$C_{22}H_{30}N_4O$	366	168-170	45
5g	2	2	Ethyl	7:3	0.7	$C_{24}H_{34}N_4O$	394	180-182	80
5h	2	2	Methyl	7:3	0.9	$C_{23}H_{32}N_4O$	380	196-198	65
5i	2	2	Methoxy	7:3	0.9	$C_{23}H_{32}N_4O_2$	396	148-150	54
5j	2	2	Methyl amino	7:3	0.6	$C_{23}H_{33}N_5O$	395	179-181	65

Table-1. Physical data of Synthesized compound 5a-5j

5.1. General procedure for the synthesis of 4-(piperazin-1-yl)-N-(1,2,3,4-tetrahydroacridin-9-yl)butanamide (5a): IR spectrum (KBr, cm^{-1}) 3438.02 (NH (str)), 3063.01– (C-H Aromatic (str)), 2950.09– (C-H Aliphatic (str)).1685.82– (C=O (str)), 1620.39– (C=N (str)), 1530.06– (C=C Aromatic(str)), 1365.26-(C-N (str)), ^{1}H NMR (400MHz CDCl3, δ ppm): 1.910-1.982 (m, 14H, aliphatic), 2.847-3.084 (m, 8H aliphatic), 4.689 (s, 1H, amine NH), 7.370-7.408 (t, J=7.2Hz, 1H, Ar-H), 7.567-7.607 (t, J=7.6Hz, 1H, Ar-H), 7.703-7.724 (d, J=8.4Hz, 2H, Ar-H), 7.927 (s, 1H, amide NH). ^{13}C NMR (100MHz, CDCl3) : 21.36, 22.19, 22.25, 26.06, 32.33, 34.67, 43.95, 53.54, 56.51, 120.07, 120.95, 121.88, 125.69, 127.42, 130.49, 141.80, 146.89, 157.63, 172.45. MASS spectrum of this compound showed $[M+H]^+$peak at m/z: 301(100.0%), 353.21 (23.1%).

5.2. General procedure for the synthesis 4-(4-ethylpiperazin-1-yl)-N-(1,2,3,4-tetrahydroacridin-9-yl)butanamide(5b): 3408.02 (NH (str)), 3055.01– (C-H Aromatic (str)), 2950.09– (C-H Aliphatic (str)).1685.82– (C=O (str)), 1619.39– (C=N (str)), 1528.06– (C=C Aromatic(str)), 1375.26-(C-N (str)),^{1}H NMR (400MHz CDCl3, δ ppm):1.569-1.638(m, 4H) , 1.794-1.806(m,2H aliphatic), 2.294(m, 9H aliphatic), 2.683-2.811(m, 9H aliphatic), 2.963-2.989(m, 3H aliphatic), 7.289-7.327(t, J=7.2Hz,1H aromatic), 7.470-7.508(t, J=7.6Hz, 1H aromatic), 7.639-7.659(d, J=8.0Hz, 2H aromatic), 8.112(s, amide NH). ^{13}C NMR (100 MHz, $CDCl_3$): 11.09,21.95, 22.25, 26.10, 32.30, 34.67, 51.29, 51.29, 51.64, 53.09, 56.02, 120.07, 120.95, 121.85, 125.69, 127.42, 130.20, 141.80, 146.89, 157.02, 171.42 MASS spectrum of this compound showed $[M+H]^+$peak at. m/z: 185(100.0%), 381.26 (25.3%), 382.26 (3.5%).

5.3. General procedure for the synthesis of 4-(4-methylpiperazin-1-yl)-N-(1,2,3,4-tetrahydroacridin-9-yl)butanamide(5c): IR spectrum (KBr, cm^{-1}) 3416.02 (NH (str)), 3083.01– (C-H Aromatic (str)), 2959.26– (C-H Aliphatic (str)).1680.52– (C=O (str)), 1620.17– (C=N (str)), 1525.86– (C=C Aromatic(str)), ^{1}H NMR (400MHz CDCl3, δ ppm):1.66-1.72(m, 2H, aliphatic), 1.87-1.92(m, 4H, aliphatic), 2.32-2.37(m, 5H), 2.53-2.59(m, 10H), 2.97-3.02(m, 2H), 7.45-7.47(t, J=7.2Hz, 1H), 7.77-7.80(t, J=7.6Hz, 1H), 7.91-7.93(d, J=8.0Hz, 1H), 7.97-7.99(d, J=7.8Hz, 1H), 9.6(s, 1H).^{13}C NMR (100MHz, CDCl3) :21.96, 22.19, 22.25, 32.31, 34.31, 45.40, 53.02, 56.52, 120.07, 120.97, 121.88, 125.69, 127.42, 141.80, 145.89, 157.67, 172.45. MASS spectrum of this compound showed $[M+H]^+$peak at m/z: 367.24 (100.0%), 367.25 (24.2%), 368.25 (3.0%).

5.4.General procedure for the synthesis of 4-(4-methoxypiperazin-1-yl)-N-(1,2,3,4-tetrahydroacridin-9-yl)butanamide (5d):): IR spectrum (KBr, cm^{-1}) 3456.12 (NH (str)), 3093.20– (C-H Aromatic (str)), 2949.42– (C-H Aliphatic (str)).1685.52– (C=O (str)), 1630.17– (C=N (str)), 1545.87– (C=C Aromatic(str)), ^{1}H NMR (400MHz CDCl3, δ ppm):):1.66-1.72(m, 2H, aliphatic), 1.87-1.92(m, 4H, cyclohexane), 2.34-2.37(t, 3H), 2.53-2.59(t, 2H, N-CH2), 2.75-2.78(t, 4H, Piperzinyl), 2.92-3.02(m, 6H, Piperzinyl), 3.40(s, 3H, OCH3), 7.45-7.47(t, J=7.6Hz, 1H, aromatic), 7.76-7.78(t,

J=7.6Hz , 1H, aromatic), 7.91-7.93(d, J=8.0Hz, 1H, aromatic), 7.97-7.99(d, 1H, aromatic), 9.6(s, 1H).^{13}C NMR (100MHz, CDCl3): 21.96, 22.19, 22.25, 26.10, 32.34, 34.67, , 53.02, 56.52, 120.07, 120.95, 121.88, 125.69, 127.42,130.29, 141.80, 145.89, 157.63, 172.45. MASS spectrum of this compound showed [M+H] $^{+}$peak at m/z: 382.24 (100.0%), 383.24 (24.2%), 384.24 (3.5%)

5.5.General procedure for the synthesis of 4-(4-(aminomethyl)piperazin-1-yl)-N-(1,2,3,4-tetrahydroacridin-9-yl)butanamide (5e): IR spectrum (KBr, cm^{-1}) 3476.12 (NH (str)), 3065.20– (C-H Aromatic (str)), 2950.52– (C-H Aliphatic (str)).1635.65– (C=O (str)), 1620.367– (C=N (str)), 1535.67– (C=C Aromatic(str)), ^{1}H NMR (400MHz CDCl3, δ ppm):1.51-1.53(s, 2H, NH2), 1.66-1.72(m, 2H), 1.85-1.89(m, 4H, cyclohexane), 2.34-2.38(t, 2H), 2.52-2.61(m, 10H, Piperzinyl), 2.97-3.02(m, 2H, cyclohexane), 3.08-3.10(m, 2H), 3.60-3.63(t, 2H), 7.45-7.47(t, J=7.2Hz, 1H, aromatic), 7.76-7.78(t, J=7.6Hz , 1H, aromatic), 7.91-7.93(d, J=8.0Hz, 1H, aromatic), 7.97-7.99(d, J=7.8 Hz 1H, aromatic), 9.6(s, 1H). ^{13}C NMR (100MHz, CDCl3): 21.96, 22.19, 22.25, 26.10, 32.31, 34.67, 51.24, 51.54, 56.52, 61.52, 120.07, 120.65, 121.88, 125.69, 127.42, 130.29, 141.80, 145.89, 157.63, 172.45. MASS spectrum of this compound showed [M+H] $^{+}$peak at m/z: 381.25 (100.0%), 382.26 (24.2%), 383.26 (3.0%).

sss5.6. General procedure for the synthesis of 4-(piperazin-1-yl)-N-(7,8,9,10-tetrahydro-6H-cyclohepta[b]quinolin-11-yl)butanamide (5f): IR spectrum (KBr, cm^{-1}) 3576.12 (NH (str)), 3096.63– (C-H Aromatic (str)), 2938.22– (C-H Aliphatic (str)).1652.75– (C=O (str)), 1654.67– (C=N (str)), 1587.47– (C=C Aromatic(str)), ^{1}H NMR (400MHz CDCl3, δ ppm):1.91-1.98(m, 15H), 2.28(s, 1H, NH), 2.39-2.42(m, 2H), 2.62 - 2.64(m, 2H), 3.03-3.06(m, 3H), 7.37-7.40(t, 1H,aromatic), 7.56-7.60(t, 1H, aromatic), 7.70-7.72(d, 1H, aromatic), 7.90-7.92(d, 1H, aromatic) 9.6(s, 1H, amide): ^{13}C NMR (100MHz, CDCl3): d, 1H, aromatic), 7.97-7.99(d, 1H, aromatic), 9.6(s, 1H). ^{13}C NMR (100MHz, CDCl3): 21.96, 22.19, 26.10, 32.30, 34.67, 43.54, 53.24, 56.54, 120.07, 120.95, 121.88, 125.69, 127.42, 130.29, 141.80, 146.89, 157.63, 172.45. MASS spectrum of this compound showed [M+H]$^{+}$peak at m/z: 366.24 (100.0%), 367.25 (24.2%), 368.25 (3.0%).

5.6. General procedure for the synthesis of 4-(4-ethylpiperazin-1-yl)-N-(7,8,9,10-tetrahydro-6H-cyclohepta[b]quinolin-11-yl)butanamide(5g): IR spectrum (KBr, cm^{-1}) 3423.12 (NH (str)), 3096.63– (C-H Aromatic (str)), 2945.32– (C-H Aliphatic (str)).1632.25– (C=O (str)), 1658.67– (C=N (str)), 1567.47– (C=C Aromatic(str)), ^{1}H NMR (400MHz CDCl3, δ ppm):1.01-1.05(t, 2H), 1.54-1.62(m, 8H), 2.34-2.37(t, 2H), 2.47-2.50(m, 2H), 2.52-2.56(m, 6H), 2.92-2.96(t, 2H), 3.01-3.06(t, 2H), 3.32-3.34(m, 6H) 7.37-7.40(t, 1H,aromatic), 7.56-7.60(t, 1H, aromatic), 7.70-7.72(d, 1H, aromatic), 7.90-7.92(d, 1H, aromatic) 9.6(s, 1H, amide): ^{13}C NMR (100MHz, CDCl3) : 11.09, 21.95, 22.25, 26.10, 27.56, 32.30, 34.67, 51.29, 51.29, 51.64, 53.09, 56.02, 120.07, 120.95, 121.85, 125.69, 127.42, 130.20, 141.80, 146.89, 157.02, 171.42. MASS spectrum of this compound showed [M+H] $^{+}$peak at m/z: 394.27 (100.0%), 395.28 (26.4%), 396.28 (3.6%).

5.7. General procedure for the synthesis of 4-(4-methylpiperazin-1-yl)-N-(7,8,9,10-tetrahydro-6H-cyclohepta[b]quinolin-11-yl)butanamide(5h): IR spectrum (KBr, cm^{-1}) 3416.02 (NH (str)), 3083.01– (C-H Aromatic (str)), 2959.26– (C-H Aliphatic (str)).1680.52– (C=O (str)), 1620.17– (C=N (str)), 1525.86– (C=C Aromatic(str)): ^{1}H NMR (400MHz CDCl3, δ ppm): 1.59-1.68(m, 8H), 2.32-2.37(m, 5H), 2.51-2.59(m, 10H), 2.92-2.95(t, 2H), 3.05-3.09(t, 2H), 7.37-7.40(t, 1H,aromatic), 7.56-7.60(t, 1H, aromatic), 7.70-7.72(d, 1H, aromatic), 7.90-7.92(d, 1H, aromatic) 9.6(s, 1H, amide): ^{13}C NMR (100MHz, CDCl3) : 22.25, 27.19, 27.45, 30.41, 34.67, 37.93, 45.40, 53.08, 53.83, 56.52, 121.47, 121.88, 125.69, 126.42,127.42, 130.29, 140.43, 146.89, 160.41, 172.45. MASS spectrum of this compound showed [M+H] $^{+}$peak at m/z: 380.26 (100.0%), 381.26 (25.3%), 382.26 (3.5%).

5.8. General procedure for the synthesis of 4-(4-methoxypiperazin-1-yl)-N-(7,8,9,10-tetrahydro-6H-cyclohepta[b]quinolin-11-yl)butanamide(5i): IR spectrum (KBr, cm^{-1}) 3456.12 (NH (str)), 3093.20– (C-H Aromatic (str)), 2949.42– (C-H Aliphatic (str)).1685.52– (C=O (str)), 1630.17– (C=N (str)), 1545.87– (C=C Aromatic(str)), ^{1}H NMR (400MHz CDCl3, δ ppm):1.59-1.68(m, 8H), 2.34-2.37(t, 2H), 2.55-2.58(t, 2H), 2.75-2.78(t, 4H), 2.92-2.97(m, 6H), 3.05-3.07(t, 2H), 3.40(s, 3H, OCH3), 7.37-7.40(t, 1H, aromatic), 7.56-7.60(t, 1H, aromatic), 7.70-7.72(d, 1H, aromatic), 7.90-7.92(d, 1H, aromatic) 9.6(s, 1H, amide): ^{13}C NMR (100MHz, CDCl3) : 22.25, 27.10, 27.42, 29.54, 30.41, 34.67, 37.93, 51.98, 55.02, 56.52, 61.99, 121.46, 121.88, 125.69, 126.02, 127.42, 130.29, 140.43, 146.89, 160.63, 172.45. MASS spectrum of this compound showed [M+H]$^{+}$peak at m/z: 396.25 (100.0%), 397.26 (25.3%), 398.26 (3.5%).

5.9. General procedure for the synthesis of 4-(4-(aminomethyl)piperazin-1-yl)-N-(7,8,9,10-tetrahydro-6H-cyclohepta[b]quinolin-11-yl)butanamide(5j): IR spectrum (KBr, cm^{-1}) 3476.12 (NH (str)), 3065.20– (C-H Aromatic (str)), 2950.52– (C-H Aliphatic (str)).1635.65– (C=O (str)), 1620.367– (C=N (str)), 1535.67– (C=C Aromatic(str)), ^{1}H NMR (400MHz CDCl3, δ ppm):1.59-1.68(m, 8H), 2.15(s, 2H, NH2), 2.34-2.37(t, 2H), 2.52-2.57(m, 10H), 2.92-2.93(t, 2H), 3.59-3.62(t, 2H), 7.37-7.40(t, 1H, aromatic), 7.56-7.60(t, 1H, aromatic), 7.70-7.72(d, 1H, aromatic), 7.90-7.92(d, 1H, aromatic) 9.6(s, 1H, amide): ^{13}C NMR (100MHz, CDCl3) : 22.25, 27.24, 27.42, 30.41, 34.67, 37.93, 51.24, 51.94, 56.52, 64.52, 121.46, 121.88, 125.69, 126.02, 127.42, 130.29, 141.43, 146.89, 160.41, 172.45. MASS spectrum of this compound showed $[M+H]^{+}$ peak at m/z: 395.27 (100.0%), 396.27 (27.1%).

Pharmacological evalution

6.0. Enzyme Inhibition Assay:

AMPLITe AChE assay kit (AAT Bioquest, Inc., Sunnyvale, CA) was used to identify the *in vitro* inhibitory effect of the newly synthesized tacrine derivatives. The assay system works on the basis of Ellman's method. AChE from electric eel (EC 3.1.1.7), assay buffer (pH 7.4), 5,5 dithiobis-2-nitrobenzoic acid (DTNB, known as Ellman's reagent) and the substrate acetylthiocholine (AChT) were there in the assay kit. 100 µL reaction mixture was prepared by mixing the enzyme (0.3U), 500 µM AChT solution in ddH2O and 500 µM DTNB in assay buffer. The enzyme activity was determined by measuring the increase in the absorbance at 412nm as a result of the reaction at 2-minute intervals at 37oC for 20 minutes. The tacrine derivatives are dissolved in DMSO and were preincubated at room temperature with the enzyme for 20 minutes, followed by the addition of the AChT and DTNB.

Acetylcholine → Thiocholine + Acetate

Thiocholine + Dithiobis nitrobenzoate → yellow colour

The enzyme inhibition (%) was calculated from the rate of absorbance change with time (V= Abs/Δt) the calculation as follows

Inhibition (%) = 100- Change of sample absorbance / Change of blank absorbance

The experiment was done in triplicate and concentrations of the test compound that inhibit the hydrolysis of the substrate (acetylcholine) by 50% (IC50) were determined by linear regression analysis between the inhibition percentage versus the concentration by using the Excel program.

S.NO	COMPOUND	IC_{50} (µM)
1	**5a**	**0.983-0.016**
2	5b	1.113-0.031
3	5c	1.085-0.011
4	**5d**	**1.013-0.024**
5	5e	1.234-0.032
6	5f	1.124-0.014
7	5g	1.324-0.041
8	5h	1.226-0.054
9	5i	1.296-0.026
10	5j	1.310-0.032
11	**Tacrine**	**0.0048-0.011**
12	Rivastigmine	11.070-0.010

Table-2. Inhibitory activity of synthesized compound 5a-5j against AChE

DISCUSSIONS:

Tacrine linked to substituted Piperzines and the cycloheptanone / cyclohexanone analogues were prepared by appropriate synthetic methodology. About 10 compounds were prepared from antharanitrile and cyclohexanone/ cycloheptanone has starting materials. All the title compounds were characterized by spectral data (NMR, MASS and IR) and evaluated for AChE inhibitory potency by Elman's method. All the tacrine derivatives were used at a concentration of 100µM, 150µM, 200µM, 300µM for the initial enzyme inhibition studies. All the compounds (5a, 5b, 5c, 5d)

have exhibited significant activity against acetyl cholinesterase and found to be more potent as AChE inhibitors when compared to the standard rivastigmine. Among all compounds 5a with amide linker and propylene spacer and simple piperazine moiety exhibited greater potency than its corresponding methyl, ethyl, methyl amino substituted derivatives.

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