Original Article

Design, Synthesis and Evaluation of 4-Aminopyridine Analogues as Cholinesterase Inhibitors for Management of Alzheimer's Diseases

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ABSTRACT

Introduction: Alzheimer's disease (AD) is a slowly progressive devasting neurodegenerative disorder of central nervous system manifested by deterioration of memory, cognitive functions, behaviour change and impairment in performing activities of daily life. Neurochemical studies of patients suffering from AD demonstrate selective loss of cholinergic neurons, low concentration of acetylcholine (Ach) in the selective areas of brain such as cortex and hippocampus. **Objective:** A series of new semicarbazones of 4-aminopyridine has been designed, synthesized and evaluated for cognition enhancing activities through the inhibition of acetylcholinesterase (AChE) and by passive avoidance model. **Material and methods:** In the present study, ten new 4-Aminopyridine analogues were synthesized and characterized by analytic methods such as UV, IR, NMR, elemental analysis and for their inhibitory role on Acetylcholinesterase activity by applying the molecular docking studies and performed enzyme kinetics study by Ellman's spectrophotometric method. The synthesized analogues were then evaluated for antiamnesic and cognition enhancing activities by passive avoidance test. **Results:** The results illustrated a significant cognition enhancing effect on passive avoidance test with a significant reversal of scopolamine-induced amnesia, which is comparable with standard drug rivastigmine. The *in-vitro* study of synthesized analogues showed maximum activity of compound-3 and compound-9 compared to standard drug rivastigmine, whereas its enzyme kinetic study revealed a non-competitive inhibition of acetylcholinesterase (AChE) is held responsible to a possible interaction of analogue with the peripheral anionic site (PAS) of AChE and was also confirmed by molecular docking studies. **Conclusion:** On the basis of present study, we are concluding that hydroxyl substituted Compound 3 and 9 identified as most potent drug which can leads to the discovery and development of new Cognition enhancers in near future.

Key words: 4-aminopyridine, Acetylcholinesterase, Passive avoidance test, Rivastigmine.

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INTRODUCTION

Alzheimer's disease (AD) is a slowly progressive devasting neurodegenerative disorder of central nervous system manifested by deterioration of memory, cognitive functions, behaviour change and impairment in performing activities of daily life.[1] Neurochemical studies of patients suffering from AD demonstrate selective loss of cholinergic neurons, low concentration of acetylcholine (Ach) and choline acetyltransferase in the selective areas of brain such as cortex and hippocampus.[2,3] According to World Alzheimer Report, around 47 million people were reported to have dementia in 2016 with a global cost of $818 billion. At present, it is considered to be the sixth largest cause of death in the United States. The most promising approach to design

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some new cognitive enhancer based on the function of cholinergic nervous system to improve cholinergic neurotransmission achieved by preventing biotransformation of Ach at the specific areas of brain, on the basis of this approach some acetylcholinesterase inhibitors which are capable of suppressing the normal degradation of Ach in the synoptic cleft, have been established to increase the concentration of Ach and used to treat the AD.[4,5] Collective interpretation of the different molecular docking and dynamic studies on different AChE inhibitors suggest that active centre of human acetylcholinesterase contains various domains like an esteratic site and an anionic site and the allosteric modulation of hAChE catalytic activity is possible through binding of some ligands at the peripheral anionic site (PAS) constituted by amino acid residues Tyr-341, Trp-286, Glu-285, Tyr-124 and Tyr-72.[6,7] Presently, 4-aminopyridine (4AP) analogues are under intensive investigation due to their antiacetylcholinesterase activity which has shown promising effects in eliminating memory related dysfunctions.[8] Several amides and imides derivatives of *m*-aminobenzoic acid and *p*-aminobenzoic acid have been synthesized and evaluated for their anticholinesterase activity, which suggested that, para-substituted derivatives are prominently active than their meta and ortho-substituted derivatives,[9,10] Numerous Schiff bases of styrylpyridine and carbamate analogues of 4AP and have been synthesized and evaluated for their anticholinesterase activity.[11,12] Some 2-indolinone analogues of 4AP and 4-aminobutyric acid (GABA) have been also reported to possess antiamnesic activity.[13] The hydrazone analogues of dihydropyridine and indolinones have also been reported to elicit potent anticholinesterase and antibutyrylcholinesterase activity.[14,15] Several N-benzylpiperidine–purine, 3-Methylpyridinium, Novel Oximes, Phenitidine Derivatives, Phenyl Benzamide Derivatives and 2-thionaphthol analogues of berberine have evaluated for AChE and BChE inhibitory activity.[16,17,18,19,20] AD is associated with aging and more prone to geriatric persons. Since, humans and rats exhibit similar age-related alterations therefore,[21,22,23] estimation of age-related cognitive impairment and spatial memory deficit in aged rats may provide more beneficial information associated to humans.[24] Keeping these facts in considerations, we synthesized and evaluated some new 4AP analogues as potential antiamnesic and cognition enhancing agents.

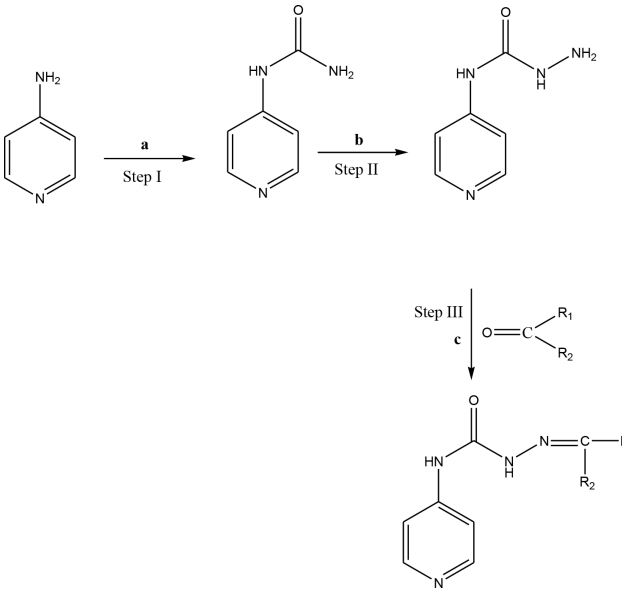
MATERIALS AND METHODS

All the chemicals used in the experiment were of analytical grade purity and were purchased from Sigma-Aldrich (India). Rivastigmine was obtained as a gift sample from Sun Pharmaceutical Industries Ltd (Silvassa, India). The Melting point of the synthesized analogues was determined in open capillary tube by using Stuart melting point apparatus and was uncorrected. The reaction progress was monitored by thin layer chromatography with chloroform: methanol (6:4) as the mobile phase on TLC silica gel 60 F254 aluminium sheets obtained from Merck Company and activated at 110°C for 10 min. Iodine was used for the colour visualization of the spots. UV spectral analysis was performed on JASCO (Model 7800) UV-VIS spectrophotometer. FTIR spectra were obtained on a Shimadzu FTIR Version 8400S at the scanning range of 400–4000 cm^{-1}. ^{1}H and ^{13}C NMR spectra were obtained in deuterated chloroform and deuterated dimethylsulfoxide as solvent and are recorded in parts per million (ppm) downfield from Tetramethylsilane (Me4Si) as internal reference by using JEOL AL Version 300 FT-NMR spectrophotometer. Elemental analysis was performed using Exeter CE-440 elemental analyzer.

Experimental details

The syntheses of semicarbazide of 4-aminopyridine analogues were carried out using the procedures as given in Scheme.

Scheme: Semicarbazones of 4-aminopyridine.



Scheme: The synthetic pathway of 1-10. (a) NACNO, glacial acetic acid, 4 h. (b) $NH_2NH_2.H_2O$, C_2H_5OH, NaOH, 3 h. (c) C_2H_5OH, glacial acetic acid, 2 h.

1-(pyridin-4-yl) urea (intermediate-1)

4-Aminopyridine (0.01mol) was dissolved in mixture containing 5ml of glacial acetic acid and 25ml with distilled water. Equimolar (0.01mol) quantity of NaCNO in 25ml

of warm water was added with continuous stirring, the reaction mixture was allowed to stand for 4 h and the product was obtained by filtration, washed with water, dried in an oven below melting point and recrystallized from ethanol to afford key intermediate-1,[25] Yield: 86.0%, mp:212–214°C, R_f 0.65, IR (KBr, υcm-^{1}): 3431, (NH), 3325, 3122 (doublet NH_2), 1678 (C=O), 1588, 1579 (C=N), 1474 (C=C, aromatic); ^{1}H NMR (DMSO-*d6*) (δ ppm): 8.91 (bs, 1H, NH), 8.39 (d, 2H, pyridine), 6.78 (d, 2H, pyridine), 6.54 (s, 2H, NH_2); ^{13}C NMR (δ ppm): 169.43 (C=O), 154.27, 150.10, 109.48 (pyridine); Anal. Calcd. (%) for $C_6H_7N_3O$: C 52.55, H 5.14, N 30.64; found (%) C 52.35, H 5.23, N 30.57.

4-(pyridin-4-yl) semicarbazide ((intermediate-2)

Intermediate-1 (0.01mol) and hydrazine hydrate (0.01mol) were dissolved in 5ml of ethanol and refluxed for 3h in presence of sodium hydroxide (0.01mol). The precipitate was obtained by filtration, washed with water, dried in an oven below melting point and recrystallized from ethanol to afford key intermediate-2,[26] Yield: 82.3%, mp: 230–232°C , R_f 0.50, IR (KBr, υcm-^{1}): 3431, (NH), 3325, 3122 (doublet NH_2), 1678 (C=O), 1588, 1579 (C=N), 1474 (C=C, aromatic); ^{1}H NMR (DMSO-*d6*) (δ ppm): 10.48, 9.31 (bs, 2H, NH), 8.36 (d, 2H, pyridine), 6.78 (d, 2H, pyridine), 6.54 (s, 2H, NH_2); ^{13}C NMR (δ ppm): 159.14 (C=O), 154.32, 150.66, 109.42 (pyridine); Anal. Calcd. (%) for $C_6H_8N_4O$: C 47.36, H 5.30, N 36.82; found (%) C 47.17, H 5.25, N 36.89.

General procedure for the synthesis of compounds (1-10)

Equal moles of intermediate-2 (0.456g, 0.003mol) in 5ml of ethanol mixed with equal moles of the different aldehyde or ketone was refluxed for 2hrs and glacial acetic acid was added to adjust the pH of the reaction between 5-6. The solid obtained after cooling was filtred, dried and crystallized from 95% ethanol to afford compounds (1-10).[27]

1-(2, 4, 6-trihydroxybenzylidene)-4-(pyridin-4-yl) semicarbazide (compound-1)

Yield: 82.3%, mp:230–232°C, R_f 0.50, IR (KBr, υcm-^{1}): 3515 (OH), 3432, 3325 (NH), 1677 (C=O), 1589, 1578 (C=N), 1473 (C=C, aromatic); ^{1}H NMR (DMSO-*d6*) (δ ppm): 11.5 (s, 3H, OH), 10.16, 10.10 (bs, 2H, NH), 8.38 (d, 2H, pyridine), 7.99 (s, 1H, N=CH), 7.38 (m, 2H, aromatic), 6.56 (d, 2H, pyridine); ^{13}C NMR (δ ppm): 167.54 (C=O), 153.15 (N=CH), 155.31, 150.48, 109.17 (pyridine), 137.70, 130.68, 129.39, 128.10 (aromatic); Anal. Calcd. (%) for $C_{11}H_9N_4O_3$: C 53.88, H 3.70, N 22.85; found (%)C 53.57, H 3.74, N 22.80.

1-(2, 4-dimethoxybenzylidene)-4-(pyridin-4-yl) semicarbazide (compound-2)

Yield: 82.3%, mp:230–232°C , R_f 0.50, IR (KBr, υcm-^{1}): 3431, 3325 (NH), 3062 (CH, CH_3), 1677 (C=O), 1589, 1578 (C=N), 1473 (C=C, aromatic); ^{1}H NMR (DMSO-*d6*) (δ ppm): 10.36, 9.48 (bs, 2H, NH), 8.40 (d, 2H, pyridine), 7.95 (s, 1H, N=CH), 7.29-7.53 (m, 3H, aromatic), 6.60 (d, 2H, pyridine), 4.20 (s, 6H, CH_3); ^{13}C NMR (δ ppm): 169.39 (C=O), 153.84 (N=CH), 155.34, 150.67, 109.17 (pyridine), 137.60, 130.29, 129.14, 128.13 (aromatic), 55.90 (CH_3); Anal. Calcd. (%) for $C_{15}H_{16}N_4O_3$: C 62.21, H 5.22, N 20.73; found (%) C 62.15, H 5.20, N 20.66.

1-(4-hydroxy-3, 5-dimethoxybenzylidene)-4-(pyridin-4-yl) semicarbazide (compound-3)

Yield: 82.3%, mp:230–232°C , R_f 0.50, IR (KBr, υcm-^{1}): 3515 (OH), 3431, 3325 (NH), 1677 (C=O), 1589, 1578 (C=N), 1473 (C=C, aromatic); ^{1}H NMR (DMSO-*d6*) (δ ppm): 10.80 (s, 1H, OH), 9.85, 9.52 (bs, 2H, NH), 8.36 (d, 2H, pyridine), 7.92 (s, 1H, N=CH), 7.66,7.59 (m, 2H, aromatic), 6.58 (d, 2H, pyridine), 4.26 (s, 6H, CH_3); ^{13}C NMR (δ ppm): 168.44 (C=O), 156.18 (N=CH), 154.68, 150.31, 109.44 (pyridine), 139.84, 130.44, 129.18, 128.11 (aromatic), 55.89 (CH_3); Anal. Calcd. (%) for $C_{15}H_{16}N_4O_4$: C 56.96, H 5.10, N 17.71; found (%) C 56.76, H 5.13, N 17.78.

1-(4-methoxyphenyl) ethylidene)-4-(pyridin-4-yl) semicarbazide (compound-4)

Yield: 82.3%, mp:230–232°C , R_f 0.50, IR (KBr, υcm-^{1}): 3431, 3325 (NH), 3063 (CH, CH_3), 1677 (C=O), 1589, 1578 (C=N), 1473 (C=C, aromatic); ^{1}H NMR (DMSO-*d6*) (δ ppm): 10.54, 8.56 (bs, 2H, NH), 8.34 (d, 2H, pyridine), 7.56-7.23 (m, 4H, aromatic), 6.57 (d, 2H, pyridine), 1.18, 4.34 (s, 6H, CH_3); ^{13}C NMR (δ ppm): 169.23 (C=O), 155.00 (N=C), 154.35, 150.45, 109.19 (pyridine), 139.66, 130.84, 129.77, 128.20 (aromatic), 24.34, 55.69 (CH_3); Anal. Calcd. (%) for $C_{15}H_{16}N_4O_2$: C 63.37, H 5.67, N 19.71; found (%) C 63.48, H 5.69, N 19.65.

1-(4-methoxyphenyl) propylidene)-4-(pyridin-4-yl) semicarbazide (compound-5)

Yield: 82.3%, mp:230–232 °C , R_f 0.50, IR (KBr, υcm-^{1}): 3431, 3325 (NH), 3063, 2940 (CH, CH_3), 1677 (C=O), 1589, 1578 (C=N), 1473 (C=C, aromatic); ^{1}H NMR (DMSO-*d6*) (δ ppm): 10.56, 8.55 (bs, 2H, NH), 9.54 (d, 2H, pyridine), 7.60-7.39 (m, 4H, aromatic), 6.58 (d, 2H, pyridine), 1.16, 4.36 (s, 6H, CH_3), 1.60 (s, 2H, CH_2); ^{13}C NMR (δ ppm): 169.25 (C=O), 155.10 (N=C), 154.34, 150.48, 109.17 (pyridine), 139.64, 130.82, 129.69, 128.00 (aromatic), 24.36 (CH_2), 8.20, 55.67 (CH_3); Anal. Calcd. (%) for $C_{16}H_{18}N_4O_2$: C 64.41, H 6.08, N 18.78; found (%)C 64.37, H 6.11, N 18.64.

1-(4-methoxyphenyl) (phenyl) methylene)-4-(pyridin-4-yl) semicarbazide (compound-6)

Yield: 82.3%, mp:230–232°C , R_f 0.50, IR (KBr, υcm-^{1}): 3431, 3325 (NH), 2940 (CH, CH_3), 1677 (C=O), 1589, 1578 (C=N), 1473(C=C, aromatic); ^{1}H NMR (DMSO-*d6*) (δ ppm): 10.58, 9.58 (bs, 2H, NH), 8.37 (d, 2H, pyridine), 7.68-7.10 (m, 9H, aromatic), 6.60 (d, 2H, pyridine), 4.32 (s, 3H, CH_3); ^{13}C NMR (δ ppm): 169.15 (C=O), 157.67 (N=C), 154.18, 150.32, 109.54 (pyridine), 139.24, 130.56, 128.10 (aromatic), 55.68 (CH_3); Anal. Calcd. (%) for $C_{20}H_{18}N_4O_2$: C 69.35, H 5.24, N 16.17; found (%) C 69.38, H 5.22, N 16.11.

1-bis (4-methoxyphenyl) methylene)-4-(pyridin-4-yl) semicarbazide (compound-7)

Yield: 82.3%, mp:230–232°C , R_f 0.50, IR (KBr, υcm-^{1}): 3431, 3325 (NH), 2941 (CH, CH_3), 1677 (C=O), 1589, 1578 (C=N), 1473(C=C, aromatic); ^{1}H NMR (DMSO-*d6*) (δ ppm): 10.86, 9.57 (bs, 2H, NH), 8.34 (d, 2H, pyridine), 7.70-7.12 (m, 8H, aromatic), 6.59 (d, 2H, pyridine), 4.34 (s, 6H, CH_3); ^{13}C NMR (δ ppm): 168.17 (C=O), 157.69 (N=C), 154.17, 150.30, 109.51 (pyridine), 139.26, 130.50, 128.13 (aromatic), 55.70 (CH_3); Anal. Calcd. (%) for $C_{21}H_{20}N_4O_3$: C 67.01, H 5.36, N 14.88; found (%)C 67.11, H 5.39, N 14.84.

1-(4-hydroxydiphenyl (phenyl)methylene)-4-(pyridin-4-yl) semicarbazide (compound-8)

Yield: 82.3%, mp: 230–232°C, R_f 0.50, IR (KBr, υcm-^{1}): 3516 (OH), 3431, 3326 (NH), 1677 (C=O), 1589, 1578 (C=N), 1473(C=C, aromatic); ^{1}H NMR (DMSO-*d6*) (δ ppm): 6.19 (s, 1H, OH), 10.51. 9.53 (bs, 2H, NH), 8.43 (d, 2H, pyridine), 7.54-7.40 (m, 9H, aromatic), 6.76 (d, 2H, pyridine); ^{13}C NMR (δ ppm): 169.71 (C=O), 156.54 (N=C), 154.30, 150.12, 109.11 (pyridine), 130.27, 129.61, 128.11 (aromatic); Anal. Calcd. (%) for $C_{19}H_{16}N_4O_2$: C 68.66, H 4.85, N 16.86; found (%)C 68.56, H 4.81, N 16.88.

1-bis (4-hydroxyphenyl) methylene)-4-(pyridin-4-yl) semicarbazide (compound-9)

Yield: 82.3%, mp: 230–232°C, R_f 0.50, IR (KBr, υcm-^{1}): 3515 (OH), 3431, 3326 (NH), 1677 (C=O), 1589, 1578 (C=N), 1473(C=C, aromatic); ^{1}H NMR (DMSO-*d6*) (δ ppm): 9.61 (s, 2H, OH), 10.51. 9.52 (bs, 2H, NH), 8.44 (d, 2H, pyridine), 7.52-7.48 (m, 8H, aromatic), 6.74 (d, 2H, pyridine); ^{13}C NMR (δ ppm): 169.73 (C=O), 156.49 (N=C), 154.31, 150.14, 109.13 (pyridine), 130.28, 129.59, 128.14 (aromatic); Anal. Calcd. (%) for $C_{19}H_{16}N_4O_3$: C 65.51, H 4.63, N 16.08; found (%)C 65.56, H 4.69, N 16.13.

1-(2, 4-dimethoxyphenyl) (4-hydroxyphenyl) methylene-4-(pyridin-4-yl) semicarbazide (compound-10)

Yield: 82.3%, mp: 230–232°C , R_f 0.50, IR (KBr, υcm-^{1}): 3516 (OH), 2943 (CH, CH_3), 3431, 3326 (NH), 1677 (C=O), 1589, 1578 (C=N), 1473 (C=C, aromatic); ^{1}H NMR (DMSO-*d6*) (δ ppm): 9.50 (s, 1H, OH), 10.52, 9.52 (bs, 2H, NH), 8.44 (d, 2H, pyridine), 7.53-7.42 (m, 7H, aromatic), 6.75 (d, 2H, pyridine), 4.34 (s, 6H, CH_3); ^{13}C NMR (δ ppm): 168.17 (C=O), 157.64 (N=CH), 154.16, 150.29, 109.56 (pyridine), 139.23, 130.54, 129.13, 128.11 (aromatic), 55.69 (CH_3); Anal. Calcd. (%) for $C_{21}H_{20}N_4O_4$: C 64.28, H 5.14, N 14.28; found (%) C 64.33, H 5.17, N 14.37.

Biological studies

Estimation of cholinesterase activity (*in-vitro*)

Ellman's spectrophotometric analysis was used to determine IC_{50} values.[28] This method is based on the reaction between synthetic substrate acetylthiocholine iodide (ATChI) and 5,5-dithio-bis-(2-nitrobenzoicacid) (DTNB) to produce a yellow colour (5-mercapto-2-nitrobenzoicacid) which was detected by Colorimeter. The IC_{50} values was determined by recording the rate of increase in the absorbance at 412 nm for 5 min. Prepared stock solution of AChE by dissolving AChE in 0.1 M phosphate buffer (pH 8). The final solution for assay is included 0.1 M phosphate buffer (pH 8.0) with the addition of 340 mM 5,5-dithio-bis (2-nitrobenzoic acid) (DTNB), 0.02 unit/mL of AChE and 550 mM of substrate (acetylthiocholine iodide, ATChI). Different concentrations of test compounds (inhibitors) between 20% and 80%) were selected in order to obtain inhibition of the enzymatic activity. From the inhibitors (synthesized analogues) solution (50 µL), increasing concentrations of the inhibitors were added to the assay solution. For this assay blank consisted of all the components except AChE. Further, the percent inhibition due to the presence of increasing concentrations of inhibitor was calculated after comparing the reaction rates which was analyzed in triplicate and determined IC_{50} values graphically by means of log concentration percent inhibition curves.[29,30]

Enzyme kinetics study

The Ellman's spectrophotometric analysis was used to identify the type of inhibition in this study. Acetylthiocholine iodide was used as a substrate in different concentrations, both below and above, near to K_m in a phosphate buffer at pH 8, with a definite concentration of acetylcholinesterase in the absence or presence of

various inhibitors. The concentration of the inhibitors was kept close to one that corresponds to IC_{50} value of enzyme inhibition. Further, their inhibitory kinetics were evaluated by the Lineweaver and Burk method.[31]

Animals

Charles foster rats of albino strain (4 to 5 months old and 150 to 200 g in weight) of either sex were procured from Central Animal House, Faculty of Pharmacy, Pacific academy of higher education and research university Udaipur (Registration No. 1622/PO/a/12/CPCSEA). Six animals were housed per cage with food and water available *ad-libitum* at constant temperature (25ºC ± 1ºC) and relative humidity (45-55%) under twelve h of light and dark cycles in a fully ventilated room.

Acute toxicity evaluation

The guidelines proposed by OECD (425) was used for determining acute toxicity studies of the analogues.[32] In this study, nulliparous, non-pregnant, healthy female albino rats weighing between 150-200 gm were fasted overnight with water *ad-libitum* prior to test. On the experimental day, analogues were administered at graded dose up to 100 mg/kg p.o. in 0.3% carboxymethyl cellulose as vehicle. The animals were constantly monitored for 30 min, 2 h and 48 h so that any change in autonomic or behavioural reactions could occur and also for convulsions, salivation, lacrimation, sleep, diarrhoea, heart rate, blood pressure, pulse rate, and feeding behaviour as a sign of acute toxicity.

Drug treatment

The synthesized analogues and standard rivastigmine were suspended in 0.3% carboxymethyl cellulose and was administered orally at a dose of 3 mg/kg and 6 mg/kg. All animals in the control groups were treated with 0.3% carboxymethyl cellulose equivalent to experimental drugs.

Passive avoidance task

The apparatus consists of two identical light and dark compartment with grid floors which can be electrified separately, where a guillotine door connects the two compartments. During the training trial, each rat was placed in the light compartment and after 10 s, the door was raised. As soon as the animal was placed with all four paws in the dark compartment, the door gets automatically closed and an electrical foot shock (0.02 mA/10 g body weight) lasting 2 s was delivered. The time elapsed by the rat being placed in light and entering the dark compartment was recorded as training trial entry latency time. Retention trial was performed 24 h after the training trial, following the similar procedure except that, the electric shock was not given and entry into the dark compartment was measured. The synthesized analogues and rivastigmine were suspended in 0.3% carboxymethyl cellulose and were administered orally 90 min before the training session and the amnesic drug was injected immediately after the conclusion of the training session. The maximum entry latency allowed in the retention session was 120 s.[33,34]

Molecular docking

All molecules of inhibitory activities were taken and sketched 3D structures using Maestro 9.3 and geometrically reduced with the help of Macromodel 9.9 based on OPLS-2005 force field. The crystal structure of AChE was obtained from the protein data bank, pdb code: 1B41 (Average R-value 0.234, Resolution 2.8 A^o).[35] The structure was prepared by using Maestro 9.3, including addition of hydrogens, assigning partial charges, protonation states, restrained, partial energy minimization and the resulting structure was used as the receptor model. Crystallographic and trajectory water molecules, ions and ligand compounds were removed from the receptor structure. Proteins were prepared by using Maestro 9.3 and the Glide XP algorithm was employed. All the structures were fitted in binding pocket and the lowest energy pose for each docking run was retained.[36]

RESULTS AND DISCUSSION

Chemistry

4-aminopyridine reacts with sodium cyanate in the presence of glacial acetic acid formed 4-aminopyridineurea (intermediate-1), which on condensed with hydrazine hydrate in the presence of sodium hydroxide yielded the 4-aminopyridine semicarbazide (intermediate-2). The Semicarbazones (1-10) were synthesized by treating with the various aldehyde or ketone with 4-aminopyridine semicarbazide as comprised in Scheme (Table1). The purity of synthesized analogues was confirmed by TLC and characterised by FT-IR, ^{1}H NMR, ^{13}C NMR and elemental analysis. The IR, peak of C=N and NH stretching vibrations was observed at 1588 cm^{-1} and 3431-3325 cm^{-1} respectively. In ^{1}H NMR spectra, intermediate-1 and intermediate-2 showed peak at δ 6.54 ppm due to the presence of -NH_2 proton. Compounds (1-3) showed peak at δ 7.92-7.99 ppm, reflecting the presence of N=CH proton, While a total disappearance of peak at δ 7.92-7.99 ppm in compounds (4-10) where this single proton was substituted by different groups, resulted in the formation of N=C bond due to which confirmed the substitution. The ^{13}C NMR values of δ 153-157 ppm also confirmed the formation of N=CH and N=C bond.

Table 1: Synthesized semicarbazide of 4-aminopyridine of Scheme.

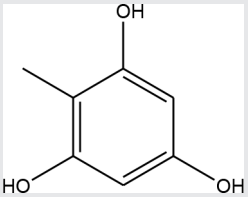
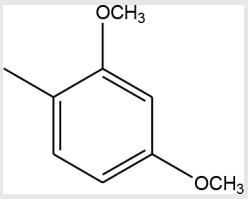
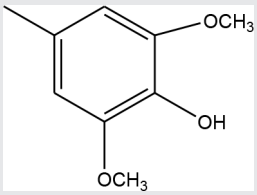
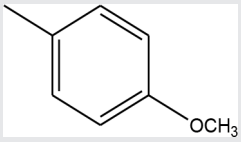
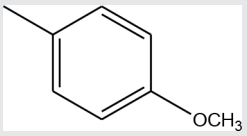
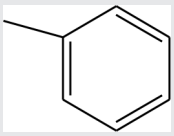
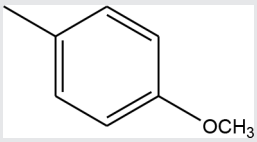
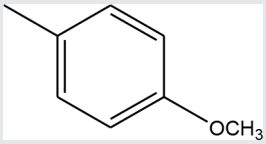
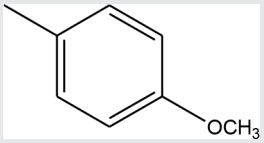
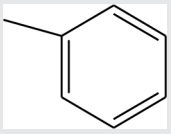
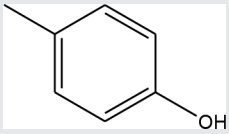
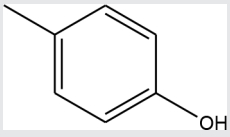
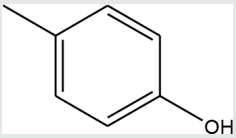
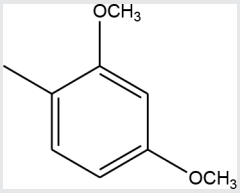
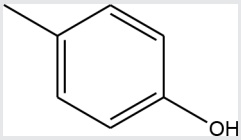
Compounds	R_1	R_2
1	—H	
2	—H	
3	—H	
4	—CH_3	
5	—C_2H_5	
6		
7		
8		
9		
10		

Table 2: Cholinesterase activity and Enzyme kinetics study of synthesized derivatives and Rivastigmine.

Compound	AChE	AChE	Inhibition
	IC_{50} (μM) ± SEM	Ki (μM) ± SEM	
1	10.2±1.75	18.27±1.480	*c*
2	8.24± 0.60	16.54± 0.66	*c*
3	6.32±1.50	11.23±0.84	*nc*
4	24.5±1.55	50.25±1.26	*c*
5	6.44± 0.60	7.84± 0.42	*c*
6	32.71±0.76	41.89±0.84	*nc*
7	22.6± 0.65	25.84± 0.80	*c*
8	11.7± 0.60	20.35± 0.94	*nc*
9	5.58±0.016	6.44± 0.65	*nc*
10	6.85±0.76	9.36± 0.76	*nc*
Rivastigmine*	6.15± 0.57	130.9 ± 0.6	*c*

c=competitive, nc=noncompetitive.

Biological activity

In vitro AChE Inhibition

AChE inhibitory activity of the all synthesized analogues was determined by using Ellman spectrophotometric method.[28] The nature of AChE inhibition was elucidated by performing enzyme kinetics study of all synthesized analogues. The inhibitory concentration of synthesized analogues (IC50) to inhibit acetylcholinesterase was calculated by using Graph Pad Prism. All the analogues exhibited moderate to excellent IC_{50} values. The IC_{50} values of compounds 3 and 9 are 6.32±1.50 μM and 5.58±0.016μM respectively with respect to standard rivastigmine (6.15± 0.57 μM). Further, enzyme kinetics study,[31] was also performed for all synthesized analogues to get information on the nature of their inhibition (Table 2). The most active compounds 3 and 9 demonstrated a non-competitive inhibition for AChE (Ki = 11.23±0.84 and 6.44± 0.65 respectively) enzyme (Table 2). The non-competitive inhibition,[34] is held responsible to a possible interaction of analogue with the peripheral anionic site (PAS) of AChE and it was also confirmed by docking studies.

Passive avoidance test

The synthesized analogues were then evaluated for anti-amnesic and cognition enhancing activities by passive avoidance test.[38] In this test, animals received punishment when it enters the dark room during the training session and thus remembers it in the session on the following day, unless their memory is impaired due to the amnesic drug. Pre-treatment with tested compounds resulted in elevate entry latency as compared to control group in significant and dose dependant manner, indicating convenient learning process. A prolonged latency indicates that the animal remembers that it has been punished and therefore, does avoid the darken chamber. The effect of inhibitors compound **3** and compound **9** on changes in entry latency in scopolamine-induced amnesia showed significant differences [$p < 0.05$] among treated groups (Table 3). Post-hoc analysis revealed that scopolamine (1.5 mg/kg) significantly [$p < 0.05$] decreased entry latency as compared to control group indicating amnesia. The inhibitors compound **3, 9** and rivastigmine, dose dependently reversed scopolamine-induced decrease in entry latency.

Molecular docking

Docking studies were carried out to provide a better interpretation of the biological profile of compound **3** and **9** toward AChE. It was found that compound **3** and **9** were properly positioned into the enzyme valley and showed interaction with the internal amino acid residue Tyr-334 and Trp-279 through a π–π interaction. The study clearly demonstrated that both compounds

Table 3: Effect of synthesized derivative and rivastigmine on cognition enhancing and scopolamine-induced amnesia on rat passive avoidance test.

Treatment [Dose(mg/kg)]	Entry latency (s)		
	Training trial	Retention trial	Δ
Control	17.83±0.60	95.50±0.76	77.67
3 (3.0)	20.72±0.49	153.62±0.66[a]	132.9
3 (6.0)	21.54±0.57	161.84±0.47[a]	140.3
5 (3.0)	18.33±0.66	118.68±0.66[a]	100.35
5 (6.0)	18.47±0.60	136.42±0.60[a]	117.95
9 (3.0)	16.60±0.60	175.62±0.76[a]	159.02
9 (6.0)	15.65±0.98	205.84±0.66[a]	190.19
Riva (3.0)	14.63±0.60	184.56±0.57[a]	169.93
Riva (6.0)	14.96±0.76	200.84±0.66[a]	185.88
SCP (1.5)	20.00±0.63	35.17±0.60[a]	15.17
3 (3.0) +SCP (1.5)	20.83±0.60	82.17±0.60[b]	61.34
3 (6.0) +SCP	20.17±0.60	86.33±0.66[b]	66.16
5 (3.0) +SCP	21.00±0.63	89.83±0.60[b]	68.83
5 (6.0) +SCP	20.33±0.80	84.83±0.60[b]	64.5
9 (3.0) +SCP	19.50±0.76	90.17±0.60[b]	70.67
9 (6.0) +SCP	16.67±0.49	98.00±0.63[b]	81.33
Riva (3.0) +SCP	19.00±0.63	89.83±0.60[b]	70.83
Riva (6.0) +SCP	17.17±0.60	97.67±0.49[b]	80.5

Data are expressed as mean ± SEM (n = 6). Data were statistically analyzed by one way ANOVA. [a]Significantly different from control $p < 0.05$. [b]Significantly different from scopolamine treated group $p < 0.05$. SCP=Scopolamine
Δ=Difference between Retention trial and Training trial, Riva= Rivastigmine.

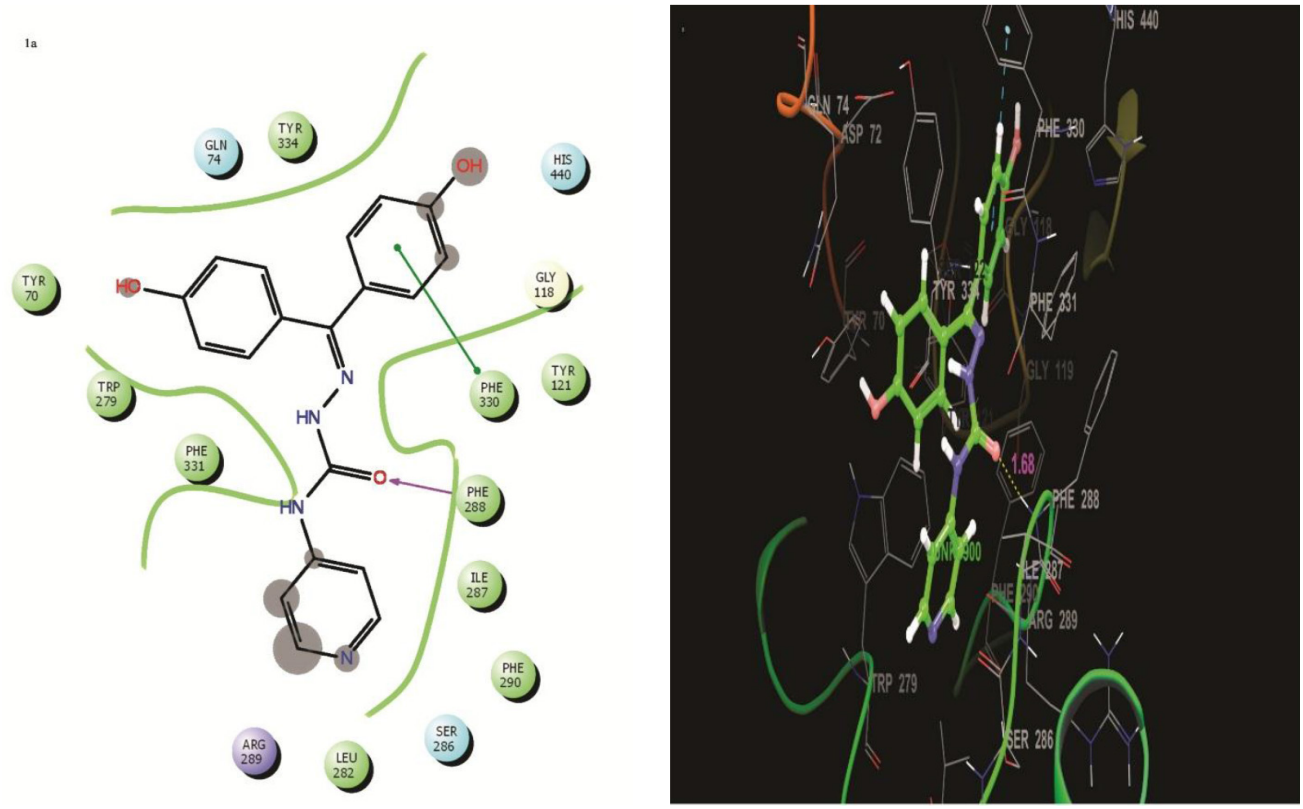


Figure 1: Molecular docking of compound 9 into the active sites of AChE (1a-2D, 1b-3D). Ligand is in green colour, dotted show H-bond interaction.

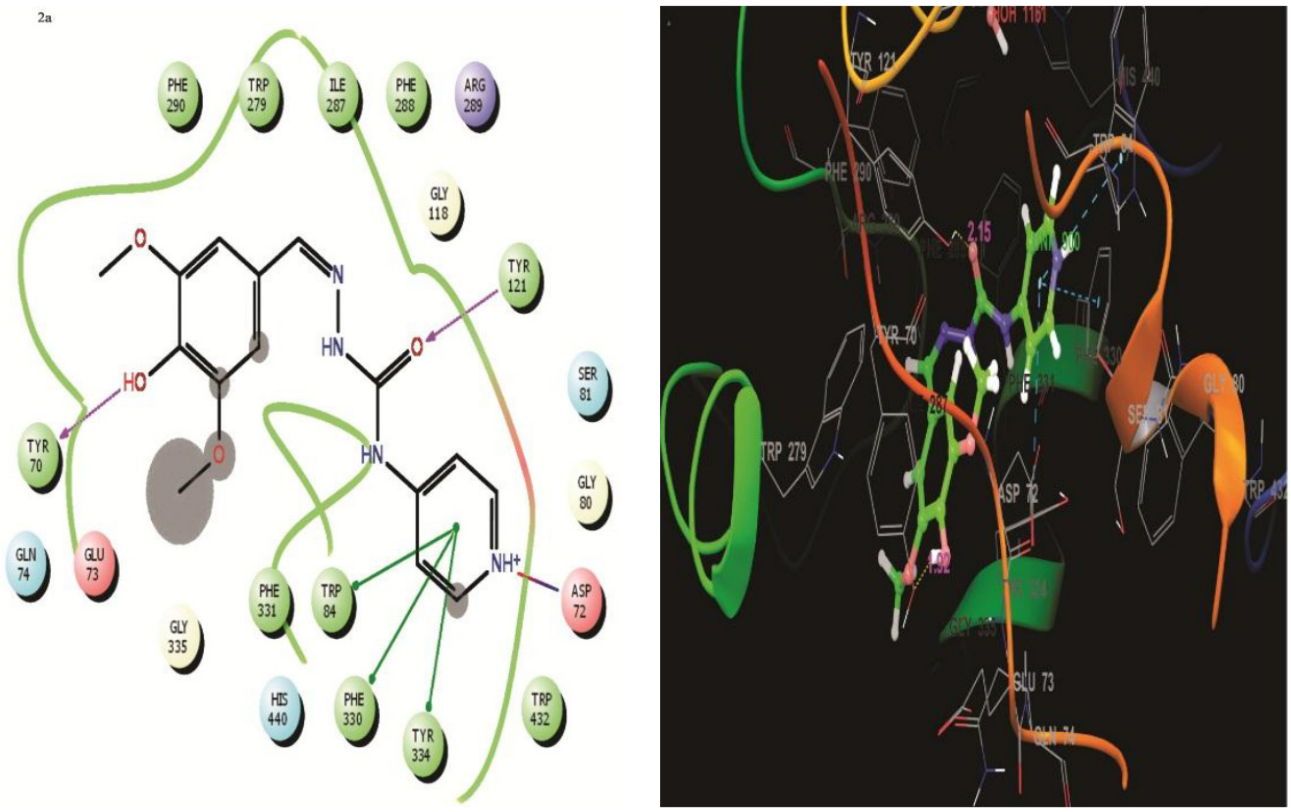


Figure 2: Molecular docking of compound 3 into the active sites of AChE (2a. 2D, 2b. 3D). Ligand is in green colour, dotted show H-bond interaction

were able to bind with the key peripheral anionic site (PAS) residue Trp-279, Tyr-334, Phe-330 and Phe-288. The carbonyl oxygen of compound **3** and **9** were involved in forming a hydrogen bond with Phe-290 and Phe-288 respectively (determine substrate specificity). The hydroxyl oxygen of compound **3** was involved in forming a hydrogen bond with Tyr-70 suggested that the compounds might probably act via the AChE inhibition. (Figure 1and 2)

CONCLUSION

From the above study, it was concluded that the hydroxyl substituted compounds **3** and **9** demonstrated a comparable activity with that of rivastigmine. In docking studies, the hydroxyl group of one of the phenyl rings of these compounds was observed establishing H-bond with Tyr-70 which plays a dual role in the active centre: (a) its hydroxyl appears to maintain the functional orientation of Phe-288 and Tyr-70 by hydrogen bonding and (b) its aromatic moiety maintains the functional orientation of the anionic subsite Trp-84. AChE inhibition and passive avoidance test of compound **3** and **9** identified as most potent drug which can leads to the discovery and development of new Cognition enhancers in near future.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

AChE: acetylcholinesterase; **BChE:** butyrylcholinesterase; **hAChE:** human acetylcholinesterase; **AD:** Alzheimer's disease; **PAS:** peripheral anionic site; IC_{50}, half-maximal inhibitory concentration.

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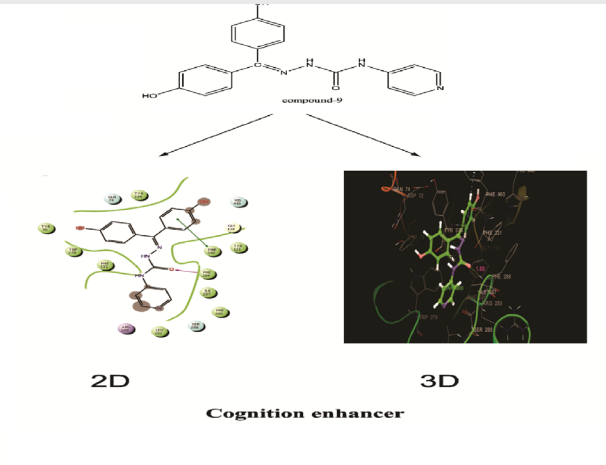
SUMMARY

- A novel series of 4-aminopyridine bearing semicarbazones derivatives were designed, synthesized, characterized and evaluated for cognition enhancing activity along with molecular docking studies. Out of the twenty compounds only two compounds 3 and 9 demonstrated a comparable activity with that of rivastigmine. In docking studies, the hydroxyl group of one of the phenyl rings of these compounds was observed establishing H-bond with Tyr-70 which plays a dual role in the active centre. These compounds are emerged as most potent of these series which can be considered for the future drug development in search of new cognition enhancers.

PICTORIAL ABSTRACT



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