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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and evaluation of some new 4-aminopyridine derivatives as a potent antiamnesic and cognition enhancing drugs

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Abstract 4-Aminopyridine (4AP) potentiates acetylcholine (ACh) release by blocking potassium channel in axon terminal and can be used in the treatment of Alzheimer's type of dementia and cognitive disorder. It is reported that ACh is well related with memory and learning. On the basis of these fact, we decided to synthesis and evaluate some new Schiff bases of 4AP (SBAPs) for their putative cognition enhancing, antiamnesic, and anticholinesterase activity. The synthesized and purified SBAPs were characterized by elemental analysis, UV, FTIR, ^{1}H-, and ^{13}C-NMR. SBAPs facilitated the learning on elevated plus maze model and they also significantly reversed the scopolamine-induced amnesia on the same model. The effect of SBAPs on learning and memory was qualitatively similar to standard nootropic drug piracetam used. The SBAPs were found to inhibit acetylcholinesterase enzyme significantly in specific brain regions prefrontal cortex, hippocampus, and hypothalamus. Thus, SBAPs derivatives showed cognitive and antiamnesic activities in the model tested and these effects may probably be due to their anticholinesterase activity.

Keywords 4-Aminopyridine · Nootropic · Antiamnesic · Anticholinesterase · Elevated plus maze

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Introduction

Alzheimer's disease (AD), the most common form of dementia in elderly people, is a complex neurodegenerative disorder of the central nervous system, characterized by progressive impairment in memory, cognitive functions, and behavioral disturbances (Bartus *et al.*, 1982). Neurochemical studies of brain specimens from patients with AD demonstrate large reductions of choline acetyltransferase and acetylcholinesterase (AChE) in the cortex and hippocampus (Perry *et al.*, 1977; Giovannini *et al.*, 1997). One possible approach to treating this disease is to restore the ACh levels by inhibiting AChE (Hakansson, 2009). AChE modulates ACh to proper levels by degradation; accordingly, excessive AChE activity leads to constant ACh deficiency, memory, and cognitive impairments (Yamada *et al.*, 2004). Cognition enhancers often referred to as nootropics, can be defined as drugs able to facilitate attentional abilities and acquisition, storage, and retrieval of information and to attenuate the impairment of cognitive functions (Gualtieri *et al.*, 2002). In fact, it is the only target that has provided the few palliative drugs presently marketed for the treatment of the AD (Giacobini, 2001; Lahiri *et al.*, 2002). Unfortunately, the therapeutic applications of the most common AChE inhibitors has been restricted due to their higher toxicity, as tacrine (Galisteo *et al.*, 2000) and short half-life, as rivastigmine (Grossberg *et al.*, 2000). In general, compounds containing a quaternary ammonium group do not penetrate the cell membrane; hence, antiAChE agents in this category are absorbed poorly from the gastrointestinal tract and are prevented to enter central nervous system by the blood–brain barrier (Taylor, 2001). 4-Aminopyridine (4AP) has become an orphan drug in the United State that has been indicated as a treatment in selected cases of a number of neuromuscular

disorders including multiple sclerosis, botulism, spinal cord injury, Alzheirmer's disease, myasthenia gravis, and Eaton–Lambert syndrome. They are also used for reversal of neuromuscular blockade in anesthesia, and verapamil overdose. (Polman *et al.*, 1994; Stork and Hoffman, 1994; Solari *et al.*, 2001). 4AP blocks potassium channels in axon terminals (Glover, 1982), efflux of potassium determines the duration of the action potential, which is therefore prolonged by 4AP. The prolonged action potential allows more calcium to move into the cell, which in turn, is responsible for the enhanced release of ACh (Folgering *et al.*, 1979). Clinical evidence suggests that 4AP penetrates the blood–brain barrier (Murray and Newsome-Davis, 1981). Several carbamate derivatives of 4AP and Schiff bases of styrylpyridine were synthesized and their anticholinesterase activity was evaluated (Scipione *et al.*, 2008; Chester *et al.*, 1971). Some 4-aminobutyric acid (GABA) and 2-indolinone derivatives of 4AP have also showed antiamnesic activity (Andreani *et al.*, 2000). Keeping these facts in considerations, we synthesized and evaluated some new 4AP derivatives as potential antiamnesic and cognition enhancing agents.

Materials and methods

4AP, aldehydes and ketones, and scopolamine hydrobromide were purchased from Sigma Aldrich Chemical Ltd, USA; Methanol and ethyl acetate was purchased from CDH Laboratory, India. Piracetam was purchased from UCB India Pvt. Ltd, Gujarat. HEPES buffer, sucrose, and ethylenediaminetetraacetic acid (EDTA) were purchased from Himedia Ltd., Mumbai, India. 5:5′-dithio-bis-2-nitrobenzoic acid (DTNB), acetylthiocholine iodide were obtained from SD fine chemicals, India. Melting points were determined using a BI 9300 Bumstead/electrothermal Stuart (SMPIO) melting point apparatus. Thin layer chromatography (TLC) was performed on TLC silica gel 60 F254 aluminum sheets (Merck, India). UV spectra were obtained using a JASCO (Model 7800) UV–VIS spectrophotometer. FTIR spectra were recorded on a Shimadzu FT-IR 8400S spectrophotometer at the scanning range of 400–4,000 cm^{-1}. ^{1}H- and ^{13}C-NMR spectra were recorded on a JEOL AL 300 FT-NMR spectrophotometer in DMSO-d_6. Elemental analysis was carried out by EXETER CE-440 ELEMENTAL ANALYZER.

The Student's paired *t* test (two tailed) using the GraphPad Prism version 4.0 for Windows (GraphPad Software, Inc., 2009) was used to analyze significant differences between the control and experimental groups. Data are expressed as mean ± SEM.

Synthesis of compounds (SBAPs)

General procedure for synthesis of Schiff bases (**4APa–4APh**)

4AP (0.05 mol) was dissolved in 5 ml of methanol in a 250-ml conical flask and was stirred at room temperature for 15 min to get a clear solution. To this solution, equimolar quantity (0.05 mol) of each substituted aryl aldehydes (in methanol) were added with few drops of concentrated hydrochloric acid (catalyst) and reaction mixture was refluxed with stirring up to 12–18 h at 70°C on magnetic stirrer. The reaction progress was monitored by TLC using mobile phase as chloroform:methanol (6:4). On completion of reaction, solvent was evaporated to dryness and compounds were obtained by precipitation on addition of 10 ml ethyl acetate. For the recrystallization of the SBAPs, ethyl acetate and absolute methanol were used in different proportions depending on the nature of the compound (Pandey and Srivastava, 2010).

*N-Benzylidenepyridin-4-amine (**4APa**)*

Yield: 75.0%, m.p.: 90–92°C, Rf 0.75, IR (KBr, υcm^{-1}): 1630 (C=N), 1615 (C=N, pyridine), 1476 (C=C, aromatic); ^{1}H-NMR (DMSO-d_6) (∂ ppm): 8.3 (s, 1H, N=CH), 8.7–7.8 (m, 9H, aromatic); ^{13}C-NMR: 163.1 (=CH), 153.1, 151.4, 113.7 (pyridine), 135.2, 132.4, 131.1, 130.2 (benzene). Anal. calcd for $C_{12}H_{10}N_2$: C 79.10, H 5.53, N 15.37; found C 82.24, H 6.56, N 12.15.

*2-((Pyridin-4-ylimino)methyl)phenol (**4APb**)*

Yield: 66.3.0%, m.p.: 100–102°C, Rf 0.61, IR (KBr, υcm^{-1}): 3510 (OH, aromatic), 1630 (C=N), 1615 (C=N, pyridine), 1476 (C=C, aromatic); ^{1}H-NMR (DMSO-d_6) (∂ ppm): 8.3 (s, 1H, N=CH), 8.7–7.1 (m, 8H, aromatic), 5.1 (s, 1H, OH); ^{13}C-NMR: 163.4 (=CH), 153.8, 151.2, 113.1 (pyridine), 160.4, 132.6, 117.9, 118.8, 122.4 (benzene). Anal. calcd for $C_{12}H_{10}N_2O$: C 72.71, H 5.08, N 14.13; found C 75.68, H 6.24, N 12.28.

*4-((Pyridin-4-ylimino)methyl)benzene-1,3-diol (**4APc**)*

Yield: 82.6%, m.p.: 180–182°C, Rf 0.52, IR (KBr, υcm^{-1}): 3510 (OH, aromatic), 1630 (C=N), 1615 (C=N, pyridine), 1476 (C=C, aromatic); ^{1}H-NMR (DMSO-d_6) (∂ ppm): 8.3 (s, 1H, N=CH), 8.7–7.1 (m, 7H,aromatic), 5.1 (s, 2H, OH); ^{13}C-NMR: 163.1 (=CH), 153.2, 151.3, 113.8 (pyridine), 162.4, 133.6, 112.9, 109.1, 105.2 (benzene). Anal. calcd for $C_{12}H_{10}N_2O_2$: C 67.28, H 4.71, N 13.08; found C 70.14, H 5.86, N 12.65.

*N-(4-Methoxybenzylidene)pyridin-4-amine (**4APd**)*

Yield: 72.8%, m.p.: 110–112°C, Rf 0.45, IR (KBr, υcm^{-1}): 3031 (CH, CH_3), 1630 (C=N), 1615 (C=N, pyridine), 1476 (C=C, aromatic); ^{1}H-NMR (DMSO-d_6) (∂ ppm): 8.3 (s, 1H, N=CH), 8.7–7.1 (m, 6H, aromatic), 3.6 (s, 3H, OCH_3); ^{13}C-NMR: 163.2 (N=CH), 153.2, 151.6, 113.4 (pyridine), 165.2, 131.1, 127.4, 115.1 (benzene), 56.2 (OCH_3). Anal. calcd for $C_{13}H_{12}N_2O$: C 73.56, H 5.70, N 13.20; found C 69.84, H 6.52, N 15.36.

*N-(3,4-Dimethoxybenzylidene)pyridin-4-amine (**4APe**)*

Yield: 88.4%, m.p.: 125–127°C, Rf 0.58, IR (KBr, υcm^{-1}): 3031 (CH, CH_3), 1630 (C=N), 1615 (C=N, pyridine), 1476 (C=C, aromatic); ^{1}H-NMR (DMSO-d_6) (∂ ppm): 8.3 (s, 1H, N=CH), 8.7–7.1 (m, 7H, aromatic), 3.6 (s, 6H, OCH_3); ^{13}C-NMR: 163.2 (=CH), 153.1, 151.6, 113.1 (pyridine), 153.9, 150.4, 129.4, 115.8, 114.7 (benzene), 56.1 (OCH_3). Anal. calcd for $C_{14}H_{14}N_2O_2$: C 69.41, H 5.82, N 11.56; found C 72.56, H 7.42, N 14.25.

*2-Methoxy-4-((pyridin-4-ylimino)methyl)phenol (**4APf**)*

Yield: 68.0%, m.p.: 140–142°C, Rf 0.38, IR (KBr, υcm^{-1}): 3510 (OH, aromatic), 3031 (CH, CH_3), 1630 (C=N), 1615 (C=N, pyridine), 1476 (C=C, aromatic); ^{1}H-NMR (DMSO-d_6) (∂ ppm): 8.3 (s, 1H, N=CH), 8.7–7.1 (m, 7H, aromatic), 3.6 (s, 6H, OCH_3), 5.1 (s, 1H, OH); ^{13}C-NMR: 163.1 (N=CH), 153.3, 151.2, 113.6 (pyridine), 151.4, 150.9, 130.1, 117.2, 115.4 (benzene), 56.2 (OCH_3). Anal. calcd for $C_{13}H_{12}N_2O_2$: C 68.41, H 5.30, N 12.27; found C 71.62, H 8.72, N 15.52.

*N-(2,2-Diphenylvinylidene)pyridin-4-amine (**4APg**)*

Yield: 92.4%, m.p.: 132–134°C, Rf 0.46, IR (KBr, υcm^{-1}): 1638 (C=N), 1615 (C=N, pyridine), 1476 (C=C, aromatic); ^{1}H-NMR (DMSO-d_6) (∂ ppm): 8.7–8.0 (m, 14H, aromatic); ^{13}C-NMR: 175.2 (N=C–(Ar) $_2$), 153.1, 151.3, 113.2 (pyridine), 133.4, 132.8, 131.6, 130.1, 129.0 (benzene). Anal. calcd for $C_{19}H_{14}N_2$: C 84.42, H 5.22, N 10.36; found C 86.45, H 7.25, N 12.36.

*(Z)-4-(1-(Pyridin-4-ylimino)ethyl)phenol (**4APh**)*

Yield: 80.6%, m.p.: 173–175°C, Rf 0.62, IR (KBr, υcm^{-1}): 3510 (OH, aromatic), 3133, 3031 (CH, CH_3), 1633 (C=N), 1615 (C=N, pyridine), 1476 (C=C, aromatic); ^{1}H-NMR (DMSO-d_6) (∂ ppm): 0.9 (s, 3H, CH_3), 8.7–7.1 (m, 7H, aromatic), 5.1 (s, 1H, OH); ^{13}C-NMR: 166.1 (N=C–CH_3), 153.4, 151.6, 113.1 (pyridine), 160.4, 131.8, 127.6, 117.2 (benzene), 17 (CH_3). Anal. calcd for $C_{13}H_{12}N_2O$: C 73.56, H 5.70, N 13.20; found C 76.52, H 8.12, N 16.11.

Animals

Charles Foster albino rats of either sex (weighing 120–150 g) were obtained from Central Animal House, Institute of Medical Sciences, Banaras Hindu University, Varanasi (Registration No. 542/02/ab/CPCSEA). They had free access to water ad libitum and were fed with semi-synthetic balanced diet, with occasional supply of green vegetables (salad leaves). Rats were caged six per cage at room temperature (22–25°C). Twelve hours of light and dark cycles was strictly followed in a fully ventilated room.

Acute toxicity evaluation

The mean lethal dose (LD50) was determined for the eight SBAPs in albino rats with weights varying between 120 and 150 g. SBAPs were given orally, using 0.3% carboxymethyl cellulose as a vehicle. The behavior of SBAPs as well as the cholinergic symptoms was observed during the first few minutes after the administration of these derivative and the number of deaths were registered up to 24 h.

Drug treatment

Animals were divided into 36 groups with six animals in each group, i.e., control-1 and experimental (2–36). In experimental groups rats were pretreated orally for 7 days with SBAPs (5 and 10 mg/kg once daily) suspended in 0.3% carboxymethyl cellulose (CMC). The treatment was continued till the end of the experiment procedures (Singh *et al.*, 2009). Piracetam and scopolamine were dissolved in water for injection before administration. Piracetam (200 mg/kg, i.p.) was administered 30 min before the start of experimental procedures (Kumar *et al.*, 2000). All animals of control groups were treated with 0.3 ml of vehicle (0.3% CMC suspension) equal to volume of experimental drugs. Refer to Table 1 for graphical time line of treatment schedule.

Elevated plus maze (EPM) task

The EPM test is regarded to be a simple method for evaluation of learning and memory processes by measuring transfer latency (Itoh *et al.*, 1990; Sharma and Kulkarni, 1992). The fabricated plus maze used in the study, consisted of two opposite open arms, 50 × 10 cm, crossed with two enclosed arms of same dimensions having walls with a height of 40 cm. The arms were connected with a central square (10 × 10 cm) to give the apparatus a plus sign appearance. The maze was kept in a dimly lit room at an elevated height of 50 cm above floor on day 7, 1 h after the last dose of SBAPs pretreatment and 30 min after

Table 1 Time line and treatment schedule of scopolamine-induced amnesic experiments

Treatment days	Treatment schedule for amnesic model on EPM				
	Control (0.3% CMC)	SBAPs (5, 10 mg) groups		Piracetam (PIRA; 200 mg/kg) treated groups	
		SBAPs	SBAPs + scopolamine (SCP 1 mg/kg)	PIRA	PIRA + SCP
Pretreatment period 1–6	Vehicle treated	SBAPs treatment	SBAPs (5, 10 mg)	Vehicle treatment	Vehicle treatment
Experimental manipulation seventh day was considered day 1 in EPM test	Vehicle treated	SBAPs treatment	SCP (1 mg/kg) given 1 h after SBAPs treatment	PIRA treatment	SCP (1 mg/kg) given 30 min after PIRA administration
Eighth day was considered day 2 in EPM test	Vehicle treated	SBAPs treatment	SBAPs treatment	PIRA treatment	PIRA treatment

piracetam treatment, individual rats from each group were placed on the end of one of the open arms, facing away from the centre. Time taken by the animal to enter one of the closed arms (transfer latency (TL) considered as day 1) was recorded with the help of a stop watch. The rat was left in the enclosed arm for 10–15 s and returned to its home cage. On day 8, i.e., second day of EPM exposure the same procedure was repeated and TL was recorded (Rakesh *et al.*, 2010).

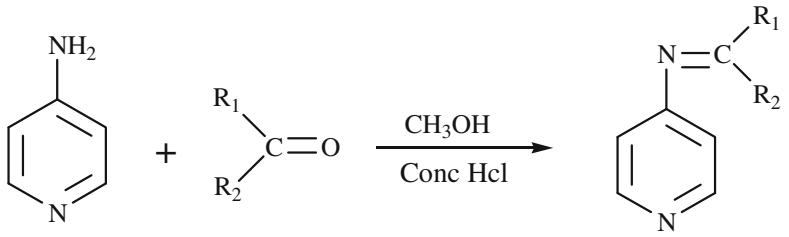
Estimation of AChE activity

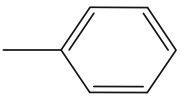
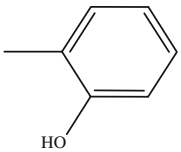
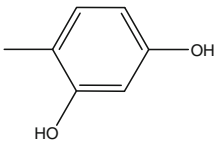
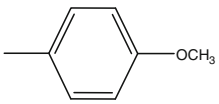
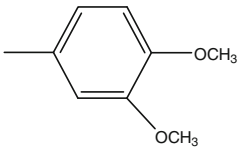
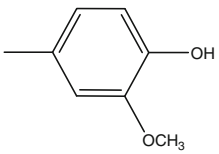
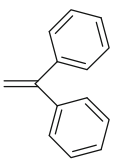
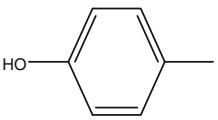
For determination of antiAChE activity of SBAPs, rats were distributed into 17 groups of six rats each: control (0.3% CMC, p.o.) and two doses of 8 SBAPs (5 and 10 mg/kg, p.o.). The rats were pretreated orally once with SBAPs for 7 days and on day 7, 1 h after the last doses of SBAPs, all rats were quickly killed using light ether anesthesia and brain was isolated from the skull immediately. The dissection for discrete regions of brain (prefrontal cortex, hippocampus, and hypothalamus) was carried out as described by Glowinski and Iversen (1966). AChE inhibitory activity of SBAPs in above brain regions was measured as described earlier (Ellman *et al.*, 1961). In this method, the discrete brain regions were homogenized in ice cold 0.1 M phosphate buffer (pH 8.0) which was centrifuged at 1,000×*g* for 10 min at 4°C, and supernatant was used for AChE assay. The obtained supernatant was mixed with phosphate buffer (pH 7.0) followed by addition of substrate acetyl thiocholine iodide and dithiobisnitrobenzoic acid (DTNB) reagent. On addition, acetylthiocholine iodide was hydrolyzed to thiocholine and acetate by AChE where thiocholine reacted with DTNB reagent to produce a yellow color. The rate of formation of thiocholine from ACh iodide in the presence of tissue cholinesterase was measured using a spectrophotometer. The principle of reaction is based on intensity of color development indicative of AChE activity. Change in absorbance per minute of the sample was read at 412 nm (Dhingra *et al.*, 2004). The enzyme activity is expressed as the "*n*" moles of substrate hydrolyzed/min/mg of protein. The protein contents were determined in the brain samples using Lowry *et al.* (1951) method.

Results

The Schiff bases from 4AP with various substituted aromatic aldehydes and ketones were synthesized according to reaction scheme (Table 2). All the SBAPs were characterized by FTIR, ^{1}H-, ^{13}C-NMR, and elemental analysis in order to verify their purity. All the spectral characterization data were found to support the SBAPs. FTIR data proved

Table 2 Reaction scheme: synthesis of Schiff bases of 4AP



Compound code	R1	R2	Mol. for	Mol. weight	Log *p*
4APa	H		$C_{12}H_{10}N_2$	182.22	2.56
4APb	H		$C_{12}H_{10}N_2O$	198.22	2.18
4APc	H		$C_{12}H_{10}N_2O_2$	214.22	1.79
4APd	H		$C_{13}H_{12}N_2O$	212.25	2.44
4Ape	H		$C_{14}H_{14}N_2O_2$	242.27	2.31
4APf	H		$C_{13}H_{12}N_2O_2$	228.25	2.05
4APg	-		$C_{19}H_{14}N_2$	270.33	2.54
4APh	CH_3		$C_{13}H_{12}N_2O$	212.25	1.74

the formation of Schiff's bases as the N=C peak appeared in the region of 1,615–1,638 cm^{-1} and diminished of the peaks for $–NH_2$ and –C=O groups. In case of ^{1}H- and ^{13}C-NMR, the ∂ values 8.3 and 163–175 for N=C group confirmed the formation of SBAPs, respectively. The SBAPs studied were tested as cognition enhancer in rat EPM test. The same test was used to evaluate the amnesic properties of SBAPs and in vivo AChE activity was also tested. The effect of SBAPs on changes in transfer latency in scopolamine-induced amnesia was analyzed by one-way ANOVA. Statistical analysis showed significant differences in transfer latency among treatment groups ($p < 0.001$). In the learning testing, pretreatment for 7 days with SBAPs significantly facilitated learning as evidenced by decrease in transfer latencies to reach the enclosed arms in training trials (Table 3). The scopolamine (1 mg/kg) significantly increased transfer latency as compared to control group on day 2, indicating amnesia which was significantly and dose dependently reversed by SBAPs and PIRA-200 treatment. Acute toxicity assays showed that no deaths were observed after applying SBAPs at a dose >35 mg/kg of body weight. The effect of SBAPs on cholinesterase activity in distinct brain regions are summarized in Table 4. The results obtained after one-way ANOVA showed significantly different AChE activity between groups ($p < 0.01$, $p < 0.05$). SBAPs (5 and 10 mg/kg) dose-dependently decreased AChE activity in prefrontal cortex, hippocampus, and hypothalamus compared to control (vehicle treated).

Table 3 Effect of synthesized derivative on cognition enhancing and scopolamine-induced amnesia on EPM in rats

Treatment [dose (mg/kg)]	Transfer latency (s)	
	Day 1	Day 2
Vehicle	67.17 ± 0.60	62.17 ± 0.47
4APa (5.0)	59.67 ± 0.71	51.83 ± 0.60*
4APa (10.0)	52.50 ± 0.76	47.17 ± 0.47*
4APb (5.0)	59.83 ± 0.79	50.17 ± 0.47*
4APb (10.0)	52.17 ± 0.60	42.50 ± 0.42*
4APc (5.0)	60.33 ± 0.76	51.17 ± 0.47*
4APc (10.0)	52.50 ± 0.76	48.67 ± 0.80*
4APd (5.0)	57.83 ± 0.79	49.50 ± 0.42*
4APd (10.0)	52.67 ± 0.71	46.33 ± 0.49*
4Ape (5.0)	58.00 ± 0.57	50.33 ± 0.49*
4Ape (10.0)	53.00 ± 0.57	47.50 ± 0.61*
4APf (5.0)	60.17 ± 0.65	55.67 ± 0.66*
4APf (10.0)	52.50 ± 0.76	47.67 ± 0.71*
4APg (5.0)	49.17 ± 0.60	44.00 ± 0.57*
4APg (10.0)	46.83 ± 0.60	40.17 ± 0.47*
4APh (5.0)	59.83 ± 0.60	56.17 ± 0.60*
4APh (10.0)	50.83 ± 0.60	46.83 ± 0.60*
Piracetam (200)	49.50 ± 0.76	45.67 ± 0.66*
SCP (1.0)	73.83 ± 0.60	81.17 ± 0.60**
4APa (5.0) + SCP(1.0)	53.83 ± 0.60	51.50 ± 0.42***
4APa (10.0) + SCP	48.83 ± 0.70	42.67 ± 0.49***
4APb (5.0) + SCP	53.50 ± 0.76	50.17 ± 0.47***
4APb (10.0) + SCP	48.17 ± 0.60	42.50 ± 0.42***
4APc (5.0) + SCP	51.67 ± 0.66	51.67 ± 0.49***
4APc (10.0) + SCP	46.50 ± 0.56	43.00 ± 0.36***
4APd (5.0) + SCP	52.00 ± 0.57	50.83 ± 0.60***
4APd (10.0) + SCP	48.67 ± 0.55	43.17 ± 0.47***
4Ape (5.0) + SCP	53.83 ± 0.60	50.67 ± 0.66***
4Ape (10.0) + SCP	48.50 ± 0.92	42.50 ± 0.42***
4APf (5.0) + SCP	54.17 ± 0.60	51.00 ± 0.57***
4APf (10.0) + SCP	48.50 ± 0.42	42.50 ± 0.42***
4APg (5.0) + SCP	46.83 ± 0.60	41.83 ± 0.60***
4APg (10.0) + SCP	45.67 ± 0.49	41.17 ± 0.47***
4APh (5.0) + SCP	53.17 ± 0.60	50.67 ± 0.49***
4APh (10.0) + SCP	47.00 ± 0.51	41.67 ± 0.66***
Piracetam (200) + SCP (1.0)	47.17 ± 0.60	43.33 ± 0.49***

Data are expressed as mean ± SEM ($n = 6$). Data were statistically analyzed by one-way ANOVA

* Significantly different from control (vehicle treated) group $p < 0.001$

** SCP treated group significantly different from control (vehicle treated) group $p < 0.001$

*** Significantly different from scopolamine-treated group $p < 0.001$

Discussion

In the plus maze test, rats showed a natural aversion to open and high spaces and therefore, spend more time in enclosed arms rather than the open arms. (Itoh *et al.*, 1990) suggested that transfer latency (the time in which the animal moves from the open arms to the enclosed arms) might be shortened. It is indicative if the animal had previous experience of entering the open arm and that the shortened transfer latency could be related to enhanced memory. Pretreatment with SBAPs resulted in reduction of transfer latency on second day of EPM exposure in significant and dose-dependant manner, which indicates that SBAPs facilitated learning process. Scopolamine in this study caused amnesia as observed by increased transfer latency in EPM. Pretreatment with SBAPs dose-dependently reversed scopolamine-induced amnesia. Piracetam also reversed scopolamine-induced amnesia in agreement with earlier reports (Kumar *et al.*, 2000). **4APg** at lower dose was equally potent and at higher dose was significantly effective than PIRA-200 in decreasing transfer latency and also in reversing scopolamine-induced memory deficits on all days tested. The better profile of **4APg** than other might be due to its benzophenone moiety which is reported as AChE inhibitor (Belluti *et al.*, 2009, 2011). The effect of

Table 4 Effect of synthesized derivatives on AchE activity on different region of rat brain

Treatment [dose (mg/kg)]	"*n*" moles of substrate hydrolyzed/min/mg protein		
	Prefrontal cortex	Hippocampus	Hypothalamus
Vehicle	45.83 ± 0.94	47.50 ± 0.84	40.50 ± 0.76
4APa (5.0)	39.00 ± 0.96**	43.83 ± 1.01*	29.33 ± 0.88**
4APa (10.0)	33.83 ± 0.70**	36.83 ± 0.94**	35.50 ± 0.76**
4APb (5.0)	37.50 ± 1.25**	41.83 ± 1.01**	34.33 ± 0.49**
4APb (10.0)	33.00 ± 1.06**	36.83 ± 0.94**	30.50 ± 0.76**
4APc (5.0)	41.00 ± 0.96*	42.83 ± 0.94**	34.50 ± 0.76**
4APc (10.0)	30.50 ± 0.76**	34.83 ± 1.07**	30.00 ± 0.57**
4APd (5.0)	36.00 ± 0.96**	41.50 ± 0.99**	35.67 ± 0.88**
4APd (10.0)	31.00 ± 1.06**	36.50 ± 0.76**	30.83 ± 0.83**
4Ape (5.0)	36.50 ± 1.17**	41.17 ± 1.07**	35.50 ± 0.76**
4Ape (10.0)	33.17 ± 1.01**	35.83 ± 0.94**	30.50 ± 0.76**
4APf (5.0)	36.00 ± 0.96**	40.83 ± 1.01**	35.17 ± 0.70**
4APf (10.0)	31.67 ± 0.88**	37.83 ± 0.94**	29.00 ± 0.50**
4APg (5.0)	37.00 ± 0.96**	42.17 ± 0.87**	34.67 ± 0.66**
4APg (10.0)	32.83 ± 0.94**	37.50 ± 0.99**	29.67 ± 0.88**
4APh (5.0)	39.33 ± 0.88**	40.50 ± 0.76**	35.17 ± 0.60**
4APh (10.0)	33.00 ± 1.23**	36.50 ± 0.76**	29.67 ± 0.88**

Data are expressed as mean ± SEM ($n = 6$). Data were statistically analyzed by one-way ANOVA. * $p < 0.05$, ** $p < 0.01$ as compared to control (vehicle treated) group

SBAPs was evaluated for antiAChE activity on three specific brain regions namely prefrontal cortex, hippocampus, and hypothalamus which are involved in processing of memory and are profusely endowed with cholinergic neurons. There is a 50–90% reduction in the content of ChAT, the enzyme that synthesizes ACh in hippocampus, cortex, and hypothalamus of dementia of Alzheimer's type (DAT) (Davies and Maloney, 1976; Harley *et al.*, 1983. Hippocampus is permanently considered to be involved with tasks in which memories have to be acquired and retrieved and is also temporarily involved in memory consolidation and an area for the temporary storage of the to-be-consolidated information (Knowlton and Fanselow, 1998; Squire and Zola-Morgan, 1991). Further, these informations are transferred into prefrontal cortex where short-term memory is converted into long-term memory, a process called as consolidation (Tronel *et al.*, 2004). Investigations have reported that stimulation of hypothalamus facilitates hippocampus dependent learning and memory processes in a wide variety of paradigms, in both young and aged rats (Milner, 1991; Soriano-Mas *et al.*, 2005). In this study, SBAPs pretreatment for 7 days inhibited AChE activity in the prefrontal cortex, hippocampus, and hypothalamus regions. These results suggest that 4AP derivatives due to its anticholinesterase activity significantly enhance cholinergic neurotransmission in these distinct brain regions and thus enhance learning and memory functions. In conclusion, we have identified a new class of potent cognition enhancing and antiamnesic drugs among which compound **4APg** deserves further study and can be a useful new lead to develop drugs with cognition enhancing and antiamnesic properties.

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