JYP

Synthesis and Pharmacological Evaluation of Some Quinoline Derivatives as Potential Antiamnesic Agents

Piplani P[1], Rani A[2], Sandhir R[3], Kulkarni SK[1]

[1]University Institute of Pharmaceutical Sciences, Panjab University, Sector -14, Chandigarh 160014, [2]L R Institute of Pharmay, Ochghat, Solan, Himachal Pradesh-173212, [3]Department of Biochemistry, Panjab University, Sector -14, Chandigarh 160014, India

Address for correspondence: *Mrs. Anita Rani; E-mail: anita_chempharma@yahoo.co.in*

ABSTRACT

A deficit of cholinergic neurotransmission is considered to be one of the major causes of disturbance in learning and memory. Among the various therapeutic approaches investigated to enhance cholinergic transmission, acetylcholinesterase (AChE) inhibition is presently the most successful method to ameliorate cholinergic deficit. Keeping in view the activity of numerous quinoline derivatives reported as potential cognitive enhancers attempts were made to design and synthesize some 6-aminoquinoline derivatives. The title compounds were synthesized via an intermediate 6-nitroquinoline which was prepared by Skraup synthesis from 4-nitroaniline prepared from acetanilide by its nitration and subsequent hydrolysis. 6-Nitroquinoline after reduction was treated with various aldehydes (veratraldehyde, anisaldehyde, 3-hydroxybenzaldehyde, indole-3-carboxaldehyde etc.) to give the target compounds. Some of the compounds showed a significant memory enhancing activity using elevated plus maze at 5 mg/kg and 10 mg/kg doses. Biochemical studies have shown the compounds to possess unexpectedly good acetylcholinesterase inhibitory activity (max %inhibition 65.27).

Key words: Acetylcholinesterase inhibitors, Alzheimer's disease, antiamnesic agents, quinoline derivatives

DOI: 10.4103/0975-1483.59325

INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by progressive loss of memory, judgment, language, and motor functions. Approximately 12 million people are estimated Approximately 12 million people are estimated to suffer from AD in Japan, Europe and U. S. A., and a steady increase in the number of AD patients with increased life expectancy. It has become very important to develop a medicine effective against AD.[1]

The most comprehensive and integrated approach toward the discovery of memory and cognition facilitating drugs has been based on the functioning of the central cholinergic system.[2] According to the cholinergic hypothesis, memory impairments in patients with this senile dementia disease are due to a selective and irreversible deficiency in the cholinergic functions in the brain.[3] There is a selective loss of neurons containing choline acetyltransferase (CAT), the enzyme responsible for the synthesis of ACh, resulting in its decreased levels in the cortical tissue.[4] A recent study demonstrated that the presence of cerebral ACh is necessary for cognitive behavior and it can improve learning deficits and memory loss in rats that have incurred severe damage to the nucleus basalis of Meynert.[5] One approach to improve memory and cognition in patients with AD has been found by an increase in ACh levels through the use of cholinesterase (ChE) inhibitors.[6] These

agents enhance cholinergic neurotransmission by inhibiting acetylcholinesterase [AChE (EC 3.1.1.7)], the enzyme responsible for the breakdown of ACh[7] [Figure 1].

Dysfunction in cholinergic mechanisms may contribute to age-related memory impairments. The retrograde loss of the cholinergic system from the basal forebrain is the most common and the most severe neurochemical consequence of the disease.[8] The cholinergic neuron clusters of the basal forebrain innervate the hippocampus and areas of association in the cortex involved in higher processes such as long-term memory, working memory, and attention. In these structures, the concentration of choline acetyltransferase (ChAT) is decreased, accompanied by an impaired ability of high-affinity choline transport and synthesis of ACh.

Many attempts have been made to correct the cholinergic deficiency at various levels of cholinergic functioning to reduce, if not cure, some of the major cognitive disturbances of AD patients. Some cholinomimetic agents have been shown to improve age-related cognitive impairments. Among various cholinomimetic drugs, an AChE inhibitor as a palliative agent in the treatment of AD has been the most promising so far.[9] Thus, the search for a new cholinesterase inhibitor (ChEI) with properties that could overcome the limitations in the use of physostigmine and tacrine is still ongoing.[10,11] tacrine is still ongoing.[8,24]

Keeping in view the activity of numerous quinoline derivatives reported as potential cognitive enhancers.[12,13,14,15] attempts were made to synthesize quinoline derivatives having a therapeutic potential better than the existing ones. The screening of the literature has revealed that sixth position of the quinoline moiety has not been exploited as cognitive enhancers. It was thus envisaged to introduce different nitrogenous functionalities at this position in the quinoline ring using different aldehydes as veratraldehyde, anisaldehyde, 3-hydroxybenzaldehyde, 4-hydroxybenzaldehyde, *p*-dimethylaminobenzaldehyde etc. Thus, we planned to prepare a series of quinoline derivatives.

CHEMISTRY

A general synthesis of 6-aminoquinoline is shown in Scheme 1; the first compound 2 of the series was prepared by nitration of acetanilide (1) using sulphuric acid and concentrated nitric acid. The compound 2 was hydrolyzed with 70% sulphuric acid to yield 4-nitroaniline (3) .[16,17] Cyclization of 4–nitroaniline (3), using glycerol, iodine and concentrated sulphuric acid (Skraup synthesis) yielded 4.[18] Reduction of 6-nitroquinoline (4) with tin and concentrated hydrochloric acid afforded the desired 6-aminoquinoline (5).[19,20]

Condensation of amino compound **5** with different aldehydes 6a-e and 7 [Table 1], then followed by Schiff's base reduction with sodium borohydride under chilled conditions gave 6-aminoderivatives Scheme 2.

RESULTS

AChE inhibition determination

***In-vitro*:** To determine the AChE activity, compounds 8a-f was assayed by the method of[21] on AChE, isolated from

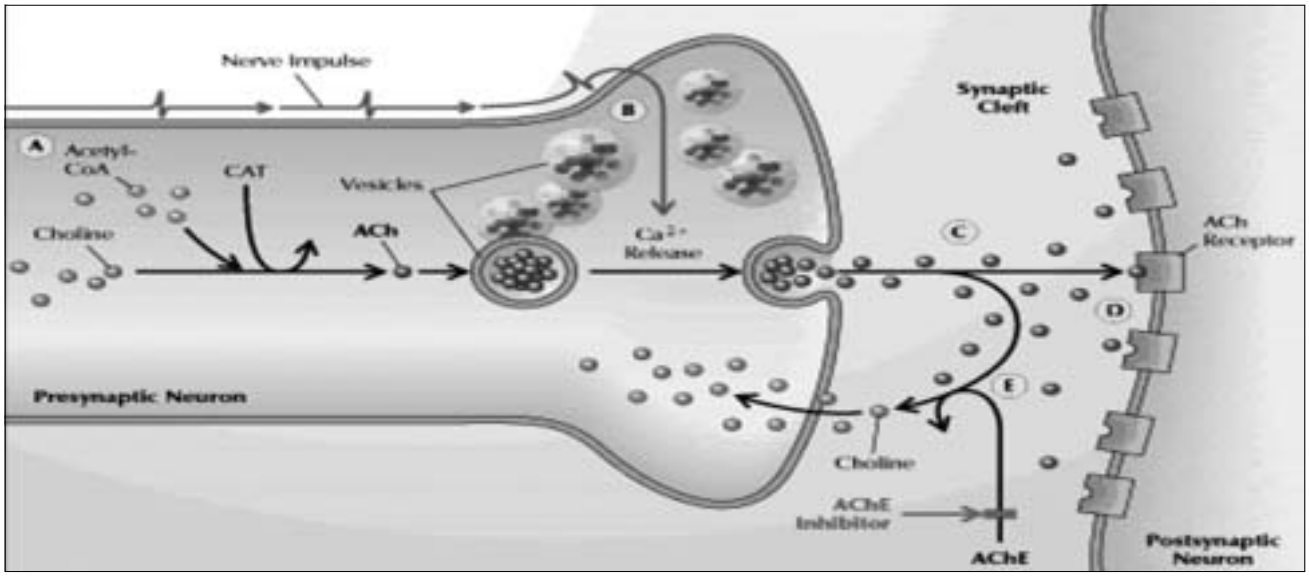
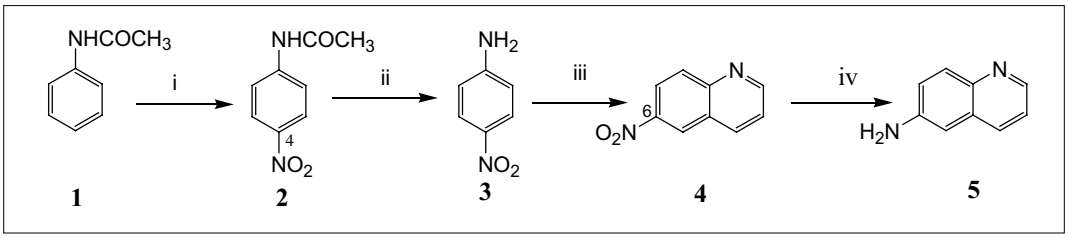


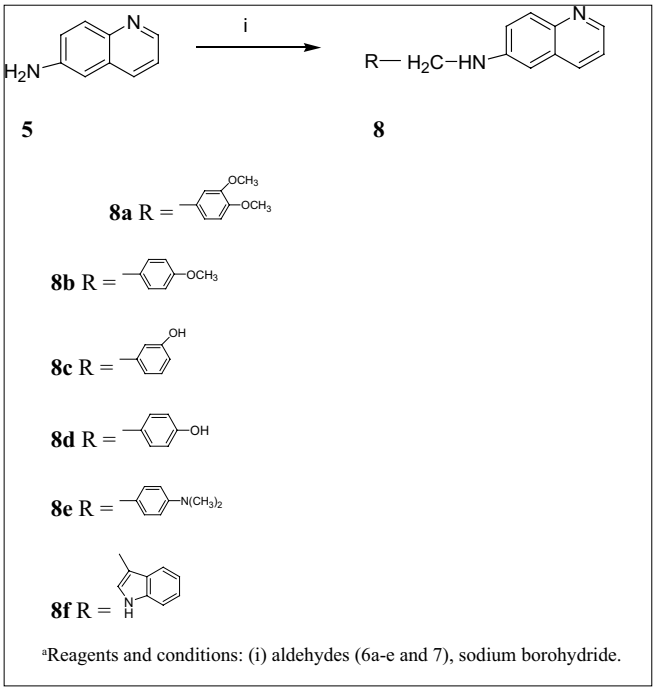
Figure 1: Synthesis of ACh by CAT and its degradation by AChE. Inhibition of action of AChE by AChE inhibitors



Scheme 1a: [a]Reagents and conditions: (i) H_2SO_4/HNO_3; (ii) 70% H_2SO_4; (iii) glycerol, iodine and conc. H_2SO_4; (iv) Sn/HCl Condensation of amino compound 5 with different aldehydes 6a-e and 7 (Table 1), then followed by Schiff's base reduction with sodium borohydride under chilled conditions gave 6-aminoderivatives Scheme 2.

Table 1: Aldehydes

Compound	R	R_1
6a	OCH_3	OCH_3
6b	OCH_3	H
6c	H	OCH_3
6d	OH	H
6e	$N(CH_3)_2$	H



[a]Reagents and conditions: (i) aldehydes (6a-e and 7), sodium borohydride.

Scheme 2b

the mice brain. Table 2 summarizes the results. As Table 2, Figure 2 shows some of the compounds that inhibited the AChE at 3 and 0.3 μM ranges; maximum inhibition was observed with 8d and 8e. Further, effects of varying concentration of 8d [Table 3, Figures 3, 4] and 8e [Table 4, Figures 5, 6] were seen on the AChE activity. It was seen that both drugs inhibited AChE activity in a dose-

Table 2: AChE inhibitory activity of 6-aminoderivatives

Drugs	Acetylcholinesterase (nmole product/min /mg protein)		% Inhibition	
	0.3μM	**3μM**	**0.3μM**	**3μM**
Control	138.14	138.14	00.00	00.00
8a	107.75	121.56	22.00	11.99
8b	122.06	101.22	11.64	26.73
8c	105.23	113.77	23.82	17.64
8d	124.57	047.97	09.82	65.27
8e	110.48	54.75	20.02	60.37
8f	105.49	100.21	23.63	27.45

Table 3: 8d

Drug concentration (μM)	Acetylcholinesterase (nmole product/min/ mg protein)	% Inhibition
Control	228.56	00.00
000.5	196.16	14.18
001.0	152.20	33.41
002.5	145.17	36.48
010.0	104.48	54.28
100.0	031.64	86.15

dependent manner. IC_{50} was found to be 3.1 and 0.8 μM, respectively for 8d and 8e. These results suggested that the drugs may be acting through AChE inhibition.

***In-vivo*:** In these studies, elevated plus maze was used to evaluate spatial long-term memory[22,23] and all the compounds were assessed, and Table 5 and Figure 7 show the results. From all the tested compounds, 8a-f compounds 8a, 8b, 8e showed significant memory-enhancing activity at 5 mg/kg and 10 mg/kg doses, which were found to be significant at ($P<0.05$) compared to control. However, compound 8d showed significant activity only at 5 mg/kg. Compound 8c did not show any memory-enhancing activity.

Furthermore, the compounds (8a, 8b, 8d) showed significant memory-enhancing activity as compared to piracetam (5 mg/kg). Besides, compound 8a showed significantly memory-enhancing activity when compared to piracetam (10 mg/kg).

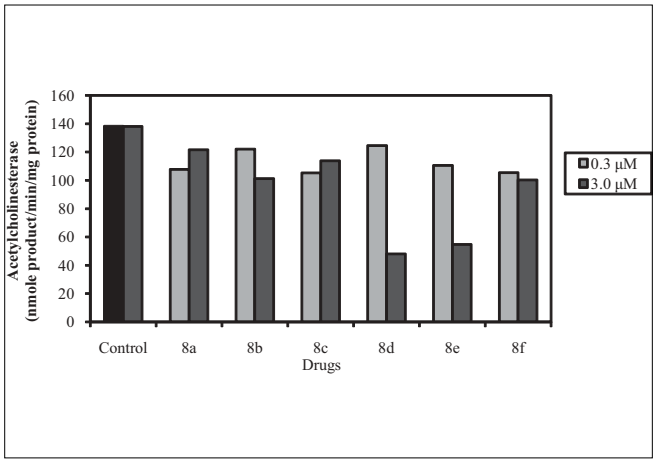


Figure 2: Effect of candidate drugs on activity of acetycholinesterase

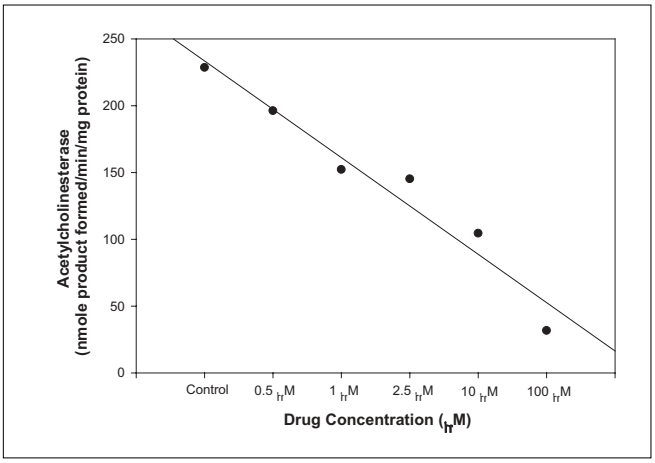


Figure 3: Regression plot for drug concentration (µM) versus acetylcholinesterase (nmole product formed/min/mg protein) of 8d

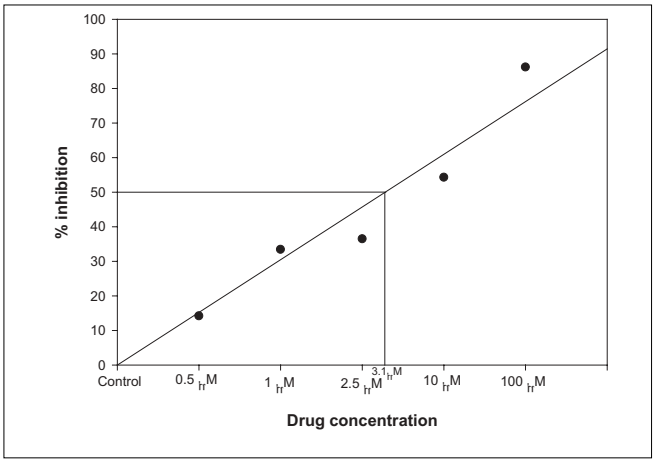


Figure 4: Regression plot for drug concentration (µM) versus % inhibition of 8d.

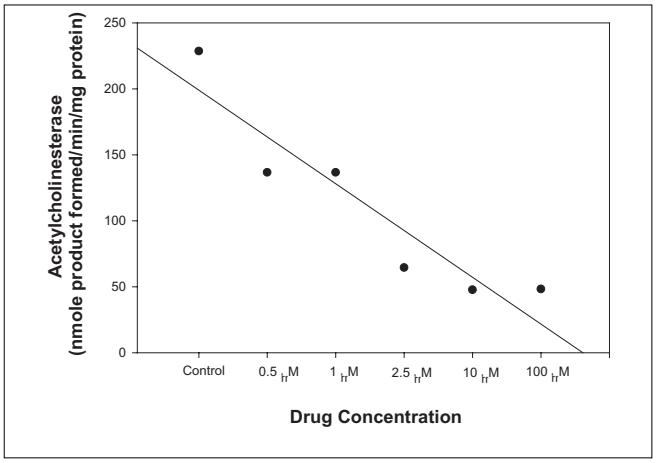


Figure 5: Regression plot for drug concentration (µM) acetylcholinesterase (nmole product formed/min/mg protein)

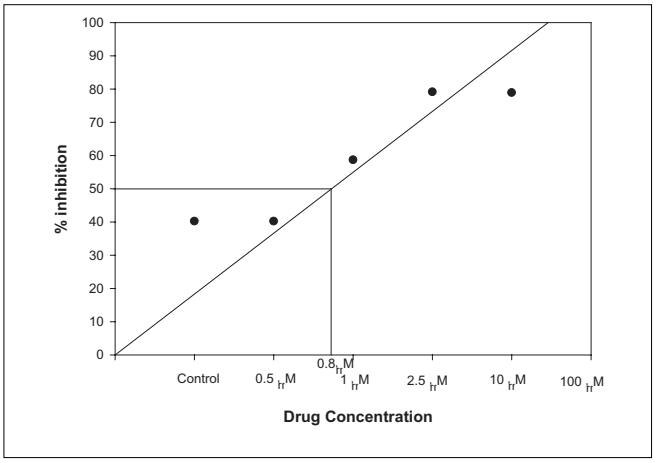


Figure 6: Regression plot for drug concentration (µM) versus % inhibition of 8e

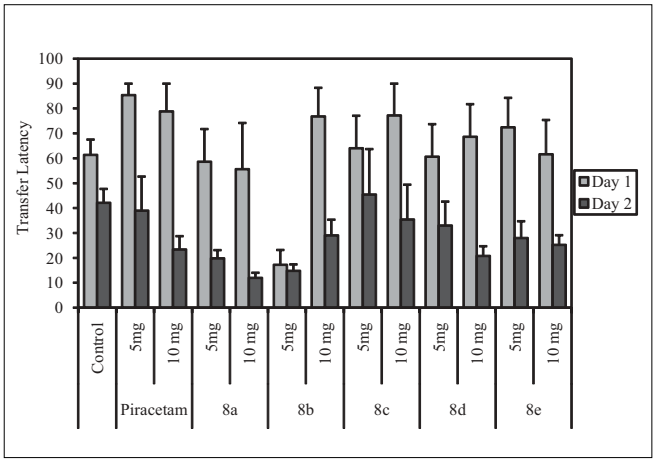


Figure 7: Effect of various compounds of 6-aminoquinoline series 8a-e) on elevated-plus maze performance task in mice. aP<0.05 as compared to control,bP<0.05 as compared to piracetam (5 mg/kg), cP<0.05 as compared to Piracetam (10 mg/kg)

Table 4: 8e

Drug concentrations (µM)	Acetylcholinesterase (nmole product/min/ mg protein)	% Inhibition
Control	228.56	00.00
000.5	136.64	40.22
001.0	136.64	40.22
002.5	094.44	58.68
010.0	047.22	79.13
100.0	048.22	78.90

CONCULSION

A novel family of inhibitors of AChE derived from 6-aminoquinoline moiety has been synthesized. From all the tested compounds 8a-e; 8d and 8e showed unexpectedly good AChE inhibitory activity by acting on AChE, and compound 8a showed significant memory-enhancing activity in *in vivo* evaluation. Therefore, it can be inferred that compound 8a and 8e may act by inhibiting AChE, whereas 8a may also have equipotent, affinity for muscarinic M_1 receptors.

The result found in the present study suggests that this 6-aminoquinoline series has been considered as new lead compound which has a significant memory-enhancing potential.

Experimental protocol

Chemical synthesis

MATERIALS AND METHODS

Chemistry

Melting points were determined on a Veego melting point apparatus and were uncorrected. Infrared (IR) and ultraviolet (UV) spectra were recorded on a Perkin-Elmer 882 and Lambda 15 spectrophotometer models, respectively. Proton (^{1}H) nuclear magnetic resonance spectroscopy was performed using a Brucker AC-300F, 300 MHz and Varian EM-360, 60 MHz instruments for solutions in deuteriochloroform or deuterated dimethyl sulfoxide containing tetramethylsilane (Me_4Si) as internal reference (chemical shifts in δ, ppm). Elemental analysis was carried out on a Perkin-Elmer-2400 model. The spin multiplicities are indicated by the symbols, s (singlet), d (doublet), t (triplet), p (pentet), m (multiplet), and br (broad). The purity of the compounds was established by thin layer chromatography (TLC). Plates for TLC were prepared with silica gel G according to Stahl (E. Merck) using ethyl acetate as solvent and activated at 110 ^{0}C for 30 min. Iodine vapors were used to develop the TLC plates. Anhydrous sodium sulfate was utilized as a drying agent.

N-(4-Nitrophenyl) acetamide (2)

To a well-stirred mixture of acetanilide (1) **(**1 g, 7.40 mmol) and glacial acetic acid (1 mL, 17.52 mmol), concentrated sulphuric acid (2 mL, 37.43 mmol) was added and slowly to the reaction mixture. Concentrated nitric acid (0.8 mL, 18.03 mmol) was then added drop-wise to the reaction mixture after cooling to 0 ^{o}C and the temperature was not allowed to exceed 20-25 ^{0}C. The reaction mixture was then poured into crushed ice after keeping it at room temperature for 20 min. The precipitated material was filtered and repeatedly washed with distilled water until free from acid, dried, and crystallized from ethanol to obtain **2** (0.65 g, 48.87%), mp 214 ^{0}C.

Analysis

IR (KBr): 3277, 3092, 2960, 1682, 1599, 1564, 1501, 1344, 1303, 1179, 1112, 1004, 849, 748, 690 cm^{-1}.

^{1}H NMR ($CDCl_3$): δ 2.18 (s, 3H, -CH_3), 7.84 (d, 2H, J_o = 9.3 Hz, aromatic protons ortho to acetamido function), 8.13 (d, 2H, J_o = 9.2 Hz, aromatic protons ortho to nitro group) and 10.23 ppm (s, 1H, -NHCOCH_3).

4-Nitroaniline (3)

N-(4-Nitrophenyl)acetamide **(2)** (1 g, 5.55 mmol) was refluxed with 70% sulphuric acid (6 ml, 112.30 mmol) on an oil bath for about 30 min. The reaction mixture was cooled to room temperature, poured into ice-cold water and 10% sodium hydroxide solution was added until precipitation stopped. The precipitated product was filtered, washed repeatedly with distilled water until free from acid, dried, and crystallized from a mixture of rectified spirit and water to obtain **3** (0.63 g, 82.17%), mp 147-148 ^{0}C.

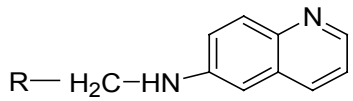
Analysis

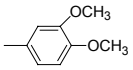
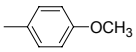
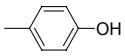
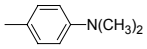
IR (KBr): 3481, 3361, 3107, 1632, 1599, 1505, 1470, 1395, 1300, 1182, 1135, 1113, 841, 753, 698, 633 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 4.40 (s, 2H, -NH_2), 6.63 (m, 2H, aromatic protons ortho to amino group) and 8.06 ppm (m, 2H, aromatic protons ortho to nitro group).

6-Nitroquinoline (4)

To a mixture of 4-nitroaniline **(3)** (1 g, 7.24 mmol), glycerol (1 mL, 13.70 mmol) and iodine (0.03 g, 0.236 mmol), concentrated sulphuric acid was added drop-wise with

Table 5: Physical data and acetylcholinesterase inhibition for 6-aminoquinoline derivatives



Compound	R	% yield	MP, °C	Molecular formula	Mean ± SEM			
					5mg/kg		10mg/kg	
					Day 1	Day 2	Day 1	Day 2
8a		67.09	135-140	$C_{18}H_{18}N_2O_2$	58.6 ± 13.15	19.8 ± 3.29	55.6 ± 18.6	12 ± 2
8b		74.74	135-140	$C_{17}H_{16}N_2O$	17.2 ± 5.96	14.8 ± 2.56	76.80 ± 11.53	29 ± 6.33
8c		78.91	140-145	$C_{16}H_{14}N_2O$	64 ± 13.1	45.4 ± 18.3	77.2 ± 12.8	35.4 ± 14.0
8d		78.91	195-200	$C_{16}H_{14}N_2O$	60.6 ± 13.11	33 ± 9.6	68.6 ± 13.15	20.8 ± 3.86
8e		71.21	125-130	$C_{18}H_{19}N_3$	72.4 ± 11.9	28 ± 6.7	61.6 ± 13.8	25.2 ± 3.89
Piracetam					85.4 ± 4.6	39 ± 13.66	78.8 ± 11.2	23.4 ± 5.3
Control					61.36 ± 6.17		42.1 ± 5.60	

constant stirring. The reaction temperature was increased to 80-90 °C and was maintained for 1 h and then at 160-170 °C for about 2 h. After cooling the contents to room temperature, they were treated with water and neutralized with solid sodium carbonate. The separated solid was collected by suction and washed with water (about 50 ml, portionwise), dried, and crystallized from petroleum ether to obtain **4** (0.9 g, 71.42%), mp 149-151 ^{0}C.

Analysis

IR (KBr): 3081, 1608, 1519, 1490, 1344, 1078, 902, 852, 792, 734 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 6.26 (d, 1H, J_o = 9.2 Hz, *H*-8), 7.60 (dd, 1H, *J* = 4.3, J_o = 8.2 Hz, *H*-3), 8.38 (dd, 1H, J_m = 1.3, J_o = 8.3 Hz, *H*-4), 8.50 (dd, 1H, J_m = 2.6, J_o = 9.2 Hz, *H*-7), 8.82 (d, 1H, J_m = 2.5 Hz, *H*-5) and 9.11 ppm (dd, 1H, J_m = 1.6, *J* = 4.3 Hz, *H*-2).

6-AminoquinolinE (5)

A granulated tin (2 g, 16.85 mmol) was added to an ice-cold solution of 6-nitroquinoline **(4)** (1 g, 5.74 mmol) in concentrated hydrochloric acid (15 ml, 485.46 mmol). The reaction mixture was heated on a water bath until the tin metal reacted and was then cooled and filtered. The filtrate was basified with a ammonia solution (25%). The precipitated material was extracted with chloroform (3 x 50 ml), washed, dried and chloroform was recovered to give a residue which was crystallized from ethanol to obtain **5** (0.5 g, 60.40%), mp 100-102 ^{0}C.

Analysis

IR (KBr): 3343, 3100, 2924, 1628, 1507, 1377, 1241, 1121, 834, 763 cm^{-1}.
^{1}H NMR ($CDCl_3$): δ 4.00 (s, 2H, -N*H*$_2$), 6.89 (d, 1H, J_m = 2.6 Hz, *H*-5), 7.15 (dd, 1H, J_m = 2.8, J_o = 6.4 Hz, *H*-7), 7.26 (dd, 1H, *J* = 4.0, J_o = 8.6 Hz, *H*-3), 7.89 (d, 1H, J_o = 8.2 Hz, *H*-8), 7.91 (dd, 1H, J_m = 1.8, J_o = 7.1 Hz, *H*-4) and 8.64 ppm (dd, 1H, J_m = 1.4, *J* = 4.3 Hz, *H*-2).

6-(3',4'-DimethoxybenzylAMINO)quinolinE (8a)

To a solution of 6-aminoquinoline **(5)** (1 g, 6.94 mmol) in methanol (10 ml) was added a solution of 3,4-dimethoxybenzaldehyde **(6a)** (1.45 g, 6.67 mmol) in methanol (10 ml) with constant stirring. The reaction mixture was allowed to stand overnight. Sodium borohydride (0.5 g) was added in small portions to the chilled and stirred reaction mixture over a period of 2 h. The reaction mixture was further stirred overnight. The methanol was recovered completely and crushed ice was added. The precipitate thus obtained was filtered, washed with water, dried, and crystallized from a mixture of ethyl acetate and methanol to obtain **8a** (1.37 g, 67.09%), mp 135-140 ^{0}C.

Analysis

UV_{max} (MeOH): 252.0 nm (log ε 4.25). IR (KBr): 3364, 3010, 2930, 2826, 1622, 1514, 1459, 1376, 1230, 1142, 1084, 1023, 923, 832, 761, 616 cm^{-1}. ^1H NMR ($CDCl_3$-DMSO-d_6): δ 3.89 (s, 6H, 2 x -OCH_3), 4.37 (s, 3H, -CH_2NH), 6.74 (s, 1H, *H*-5), 6.86 (d, 1H, J_o = 7.8 Hz, *H*-5'), 6.95 (s, 1H, *H*-2'), 6.98 (d, 1H, J_m = 1.6 Hz, *H*-6') 7.15 (dd, 1H, J_m = 2.8, J_o = 9.1 Hz, *H*-7), 7.26 (m, 1H, *H*-3), 7.89 (m, 1H, *H*-8), 7.91 (m, 1H, *H*-4) and 8.62 ppm (m, 1H, *H*-2). ^{13}C NMR ($CDCl_3$-DMSO-d_6): δ 47.26 (-*C*H_2NH), 55.38 (2 x –O*C*H_3), 119.19 (*C*-5, 7, 2' and 5'), 121.16 (*C*-6' and 3), 129.64-129.35 (*C*-8 and 4a), 131.05 (*C*-1'), 133.21 (*C*-4), 145.19 (*C*-3' and 4'), 147.68 (*C*-6 and 8a) and 153.61 ppm (*C*-2).

Calculated for $C_{18}H_{18}N_2O_2$: C, 73.44; H, 6.16; N, 9.52. Found: C, 73.12; H, 5.91; N, 9.38.

6-(4'-Methoxybenzylamino)quinoline (8b)

To a solution of 6-aminoquinoline (5) (1 g, 6.94 mmol) in methanol (10 ml) was added a solution of 4-methoxybenzaldehyde (6b) (1.4 ml, 11.54 mmol) in methanol (10 ml) with constant stirring. The reaction mixture was allowed to stand overnight. Sodium borohydride (0.5 g) was added in small portions to the chilled and stirred reaction mixture over a period of 2 h. The reaction mixture was further stirred overnight. Methanol was recovered completely and crushed ice was added to the contents to give an oily residue which solidified on cooling was filtered, washed with water, dried, and crystallized form ethyl acetate to obtain 8b (1.37 g, 74.74%), mp 135-140 ^{0}C.

Analysis

UV_{max} (MeOH): 252.8 nm (log ε 4.38).

IR (KBr): 3293, 3010, 2993, 2836, 1620, 1511, 1460, 1376, 1249, 1175, 1119, 1086, 1030, 827, 766, 731 cm^{-1}.

^1H NMR ($CDCl_3$–DMSO-d_6): δ 3.80 (s, 3H, -OCH_3), 4.34 (s, 3H, -CH_2NH), 6.72 (s, 1H, *H*-5), 6.88 (m, 2H, *H*-3' and 5'), 7.11 (m, 1H, *H*-7), 7.26 (m, 1H, *H*-3), 7.33 (m, 2H, *H* -2' and 6'), 7.87 (m, 1H, *H*-8), 7.91 (m, 1H, *H*-4) and 8.60 ppm (m, 1H, *H*-2).

^{13}C NMR ($CDCl_3$-DMSO-d_6): δ 47.44 (-*C*H_2NH), 55.08 (1 x –O*C*H_3), 113.96 (*C*-3' and 5'), 114.14 (*C*-5 and 7), 121.28 (*C*-3), 128.31 (*C*-4a), 129.92-129.99 (*C*-8, 2' and 6'), 130.56 (*C*-4), 133.7 (*C*-1'), 145.8 (*C*-8a), 146.03 (*C*-6), 153.61 (*C*-2) and 158.79 ppm (*C*-4').

Calculated for $C_{17}H_{16}N_2O$: C, 77.24; H, 6.10; N, 10.60. Found: C, 77.22; H, 5.89; N, 10.68.

6-(3'-Hydroxybenzylamino)quinoline (8c)

To a solution of 6-aminoquinoline (5) (1 g, 6.94 mmol) in methanol (10 ml) was added a solution of 3-hydroxybenzaldehyde (6c) (1.4 g, 11.46 mmol) in methanol (10 ml) with constant stirring. The reaction mixture was allowed to stand overnight. Sodium borohydride (0.5 g) was added in small portions to the chilled and stirred reaction mixture over a period of 2 h. The reaction mixture was further stirred overnight. Methanol was recovered completely and crushed ice was added. The precipitate thus obtained was filtered, washed with water, dried, and crystallized from a mixture of ethyl acetate and acetone to obtain 8c (1.37 g, 78.91%), mp 140-145 ^{0}C.

Analysis

UV_{max} (MeOH): 252.0 nm (log ε 4.56). IR (KBr): 3378, 3037, 2934, 1625, 1584, 1535, 1477, 1438, 1390, 1329, 1277, 1127, 827, 772, 692 cm^{-1}. ^1H NMR ($CDCl_3$–DMSO-d_6): δ 4.36 (s, 2H, -CH_2), 5.32 (s, 1H, -NH; disappeared on deuterium exchange), 6.66 (s, 2H, *H*-5 and 2'), 6.75 (dd, 1H, J_m = 1.8, J_o = 7.9 Hz, *H*-4'), 6.89 (d, 1H, J_o = 7.6 Hz, *H*-6'), 7.20 (m, 1H, *H*-7), 7.25 (m, 1H, *H*-3), 7.83 (d, 1H, J_o = 9 Hz, *H*-8), 7.89 (m, 1H, *H*-4), 8.55 (m, 1H, *H-2*), 7.19 (m, 1H, *H*-5') and 9.05 ppm (s, 1H, O*H*; disappeared on deuterium exchange). ^{13}C NMR ($CDCl_3$-DMSO-d_6): δ 47.21 (-*C*H_2NH), 114.02 (*C*-2' and 4'), 117.01 (*C*-5 and 7), 120.79 (*C*-6'), 121.76 (*C*-3), 129.88-128.42 (*C*-8, 5' and 4a), 134.19 (*C*-4), 139.94 (*C*-1'), 144.18 (*C*-8a), 146.36 (*C*-6), 150.29 (*C*-2) and 157.29 ppm (*C*-3'). Calculated for $C_{16}H_{14}N_2O$: C, 76.77; H, 5.63; N, 11.19. Found: C, 77.01; H, 5.32; N, 11.23.

6-(4'-Hydroxybenzylamino)quinoline (8d)

To a solution of 6-aminoquinoline (5) (1 g, 6.94 mmol) in methanol (10 mL) was added a solution of 4-hydroxybenzaldehyde 6d) (1.4 g, 11.46 mmol) in methanol (10 mL) with constant stirring. The reaction mixture was allowed to stand overnight. Sodium borohydride (0.5 g) was added in small portions to the chilled and stirred reaction mixture over a period of 2 h. The reaction mixture was further stirred overnight. Methanol was recovered completely and crushed ice was added to the residue. The precipitate thus obtained was filtered, washed with water, dried, and crystallized from the methanol to obtain 8d (1.37 g, 78.91%), mp 195-200 ^{0}C.

Analysis

UV_{max} (MeOH): 252.8 nm (log ε 4.52). IR (KBr): 3348, 3037, 2934, 1623, 1535, 1512, 1459, 1381, 1239, 1170, 1127, 1102, 1078, 819, 772 cm^{-1}. ^1H NMR ($CDCl_3$-DMSO-d_6): δ 4.29 (s, 2H, -CH_2), 5.75 (s, 1H, -N*H*; disappeared on deuterium exchange), 6.68 (s, 1H, *H*-5), 6.81 (m, 2H, *H*-3' and 5'), 7.20 (m, 1H, *H*-7), 7.21 (m, 2H, *H*-2' and 6'), 7.29 (m, 1H, *H*-3), 7.80 (m, 1H, *H*-4), 7.93 (d, 1H, J_o = 9.5 Hz, *H*-8), 8.51 (m, 1H, *H*-2), and 8.91 ppm (s, 1H, O*H*; disappeared on deuterium exchange). ^{13}C NMR ($CDCl_3$-DMSO-d_6): δ 46.91 (-*C*H_2NH), 115.09 (*C*-3' and 5'), 120.72 (*C*-5 and 7), 124.30 (*C*-3), 128.98-128.31 (*C*-8 and 4a), 129.71 (*C*-2' and 6'), 133.39 (*C*-4), 137.58 (*C*-1'), 144.75 (*C*-8a), 146.21 (*C*-6), 153.35 (*C*-2) and 156.15 ppm (*C*-4'). Calculated for $C_{16}H_{14}N_2O$: C, 76.77; H, 5.63; N, 11.19. Found: C, 76.21; H, 5.34; N, 11.11.

6-(p-Dimethylaminobenzylamino)quinoline (8e)

To a solution of 6-aminoquinoline (5) (1 g, 6.4 mmol) in methanol (10 ml) was added a solution of *p*-dimethylaminobenzaldehyde (6e) (1.4 g, 9.38 mmol) in methanol (10 ml) with constant stirring. Sodium borohydride (0.5 g) was added in small portions to the chilled and stirred reaction mixture over a period of 2 h. The reaction mixture was further stirred overnight. Methanol was recovered completely and crushed ice was added. The precipitate thus obtained was filtered, washed with water, dried and crystallized form the mixture of ether and ethyl acetate to obtain 8e (1.37 g, 71.21%), mp 125-130 ^0C.

Analysis

UV_{max} (MeOH): 257.2 nm (log ε 4.37). IR (KBr): 3291, 3110, 2862, 1616, 1522, 1450, 1379, 1345, 1235, 1184, 1063, 1014, 835, 803, 620 cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.94 [s, 6H, -N(CH_3)$_2$], 4.29 (s, 2H, -CH_2), 4.57 (s, 1H, -N*H*; disappeared on deuterium exchange), 6.72 (s, 1H, *H*-5), 6.72 (m, 2H, *H*-3' and 5'), 7.10 (dd, 1H, J_m = 2.6 Hz, J_o = 9.1 Hz, *H*-7), 7.25 (m, 1H, *H*-3), 7.25 ppm (m, 2H, *H*-2' and 6'), 7.85 (m, 1H, *H*-8), 7.91 (m, 1H, *H*-4) and 8.58 ppm (m, 1H, *H*-2). ^{13}C NMR ($CDCl_3$): δ 40.60 (2 x -CH_3), 47.76 (-*C*H_2NH), 112.75 (*C*-3' and 5'), 121.21 (*C*-5 and 7), 121.47 (*C*-3), 128.67 (*C*-4a), 129.89-129.34 (*C*-8, 2' and 6'), 130.15 (*C*-1'), 133.87 (*C*-4a), 145.76 (*C*-8a), 146.24 (*C*-6), 150.07 (*C*-2) and 150.18 ppm (*C*-4'). Calculated for $C_{18}H_{19}N_3$: C, 77.94; H, 6.90; N, 15.14. Found: C, 77.35; H, 6.57; N, 15.02.

6-(3'-Indolyl)methylaminoquinoline (8f)

To a solution of 6-aminoquinoline (5) (1 g, 6.94 mmol) in methanol (10 ml) was added a solution of indole-3-carboxaldehyde (7) (1.4 g, 9.64 mmol) in methanol (10 ml) with constant stirring. Sodium borohydride (0.5 g) was added in small portions to the chilled and stirred reaction mixture over a period of 2 h. The reaction mixture was further stirred overnight. Methanol was recovered completely and crushed ice was added, the contents were extracted with chloroform, the chloroform extract was washed with water, dried, and distilled to give an oily residue **8f**.

Analysis

IR (KBr): 3307, 3010, 2980, 1617, 1524, 1350, 1235, 835, 743, 620 cm^{-1}. ^1H NMR ($CDCl_3$-DMSO-d_6): δ 4.28 (s, 2H, -CH_2), 4.60 (s, 1H, -N*H*; disappear on deuterium exchange), 6.88 (s, 1H, *H*-5), 6.97-7.64 (m, 6H, aromatic and indolic protons, -C*H*), 7.16 (m, 1H, *H*-7), 7.28 (m, 1H, *H*-3), 7.76 (m, 1H, *H*-8), 7.92 (m, 1H, *H*-4) and 8.64 ppm (m, 1H, *H*-2).

6-(3'-Indolyl)methylaminoquinoline (8f)oxalate

To a solution of 6-(3'-indolyl)methylaminoquinoline **(8f)** (1.12 g, 4.098 mmol) in refluxing methanol (50 mL) was added oxalic and (1 g, 11.11 mmol) and refluxed for 2-3 h with stirring. The solvent was removed completely and residue was crystallized from methanol-acetone mixture to obtain oxalate of **8f** (1.12 g, 41.13%), mp 198-200 ^0C.

Analysis

UV_{max} (MeOH): 209 nm (log ε 3.07). IR (KBr): 3307, 3010, 2980, 1617, 1524, 1350, 1235, 835, 743, 620 cm^{-1}. ^1H NMR ($CDCl_3$-DMSO-d_6): δ 4.28 (s, 2H, -CH_2), 4.69 (s, 1H, -N*H*; disappeared on deuterium exchange), 6.97-7.64 (m, 6H, aromatic and indolic protons, -C*H*), 7.17 (s, 1H, *H*-5), 7.48 (m, 1H, *H*-7), 7.7 (m, 1H, *H*-3), 7.96 (m, 1H, *H*-8), 8.08 (m, 1H, *H*-4) and 8.63 ppm (m, 1H, *H*-2).

^{13}C NMR ($CDCl_3$-DMSO-d_6): δ 40.30 (-*C*H_2NH), 111.11 (*C*-5 and indolic proton, -*C*H), 116-135.772 (aromatic carbons), 117.85 (*C*-3), 121.06 (*C*-7), 128.92 (*C*-8 and 4a), 135.77 (*C*-4), 143.57 (*C*-6 and *C*-4a).

Calculated for $C_{20}H_{17}N_3$: C, 66.11; H, 4.72; N, 11.56. Found: C, 66.53; H, 4.96; N, 11.21.

BIOLOGICAL METHODS

In-vitro

Tissue homogenization

The animals were fasted overnight and sacrificed by

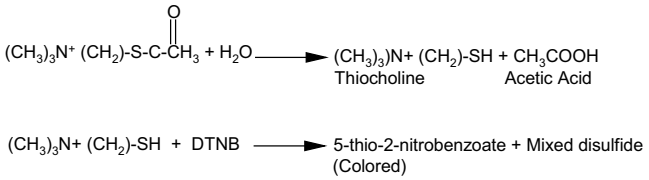
decapitation. The brains were removed, rinsed in ice-cold isotonic saline (0.9% w/v NaCl), blotted dry, weighed separately. A 10% (w/v) tissue homogenate was prepared in 50 mM phosphate buffered saline (pH 7.4) using Potter-Elvehjem glass homogenizer.

Assay of acetylcholinesterase

The activity of AChE was determined in the homogenate according to the method of.[21]

Principle

The substrate used in the assay system is acetylthiocholine iodide, the ester of thiocholine, and acetic acid. Acetylthiocholine is hydrolyzed into thiocholine and acetate by the enzyme AChE. Thiocholine forms mercaptan, which reacts with the oxidizing agent 5,5'-dithio-bis-2-nitrobenzoic acid (DTNB) to form 5-thio-2-nitrobenzoate, which has a maximum absorption at 412 nm. Thus, the activity of AChE can be measured by following the increase in absorbance at 412 nm.



Reagents

- 0.1 M Sodium phosphate buffer (pH 8.0)
- 10 mM DTNB (Ellman's reagent)
- 14.9 mM Acetylthiocholine iodide

Procedure

The sample consisted of 2.8 ml of 0.1 M phosphate buffer (pH 8.0), 0.1 ml of Ellman's reagent, and requisite amount of homogenate. The reaction was initiated by the addition of 0.1 ml acetylthiocholine iodide and the rate of change in absorbance was followed at 412 nm for 2 min.

Calculations

AChE activity was calculated using molar extinction coefficient of 5-thio-2-nitrobenzoic acid (13.6×10^3 M/cm). The results were expressed as nmole of acetylthiocholine iodide hydrolyzed/min/mg protein.

Estimation of protein content

The protein content was estimated according to the following method.[24]

Principle

This method is based on the color reactions of amino acids tryptophan and tyrosine with the Folin's phenol reagent. These amino acids react with phosphomolybdic acid and phosphotungstic acid (present in Folin's reagent) to give blue color, which is estimated colorimetrically. This color is the result of reduction of phosphomolybdic acid and phosphotungstic acid and biurette reaction of proteins with Cu^{2+} ions in the alkaline medium.

Reagents

- Reagent A-1% (w/v): Copper sulphate solution.
- Reagent B-2% (w/v): Sodium potassium tartarate.
- Reagent C-2% (w/v): Sodium carbonate in 0.1 N sodium hydroxide.
- Lowry's reagent: It was prepared just before use by mixing reagents A, B, and C in the ratio of 1:1:98.
- Folin-Ciocalteau reagent: It was prepared fresh by diluting the commercial 2N Folin's reagent with double distilled water (1:1, v/v).
- Standard BSA solution (1 mg/ml).

Procedure

0.1 ml of suitably diluted sample, BSA standard, and water (for blank), all were taken separately in different test tubes and the final volume was made 1.0 ml with double distilled water. A 3.0 ml of Lowry's reagent was added to all the tubes, vortexed, and allowed to stand for 10 min at room temperature. Further, 0.5 ml of Folin--Phenol reagent was added to each of the tubes, vortexed for 30 s, and allowed to stand for 30 min at room temperature. The absorbance was measured at 750 nm.

Calculations

The protein concentration was calculated from the standard curve made by taking different concentrations of the BSA standard.

In-vivo

Principle

Elevated plus-maze is the simplest apparatus to evaluate the antiamnesic activity of drugs in animals. Exposure of the animals to novel maze alley evokes an approach-avoidance conflict, which is stronger in open arm as compared to the enclosed arm. Rodents (rats and mice) have aversion for high and open space and prefer the enclosed arm, therefore, spend greater time in enclosed arm. When the animals enter the open arm, they freeze, become immobile, defecate, and show fear like movements. The plasma cortisol level has also been reported to be increased.

Requirements

Animals Mice (20-25 g).

Drugs — A stock solution was prepared containing different concentrations (5 mg/kg and 10 mg/kg) and injected according to the body weight of the mice. All the drugs were suspended in DMSO.

Equipment — Plus-maze consisted of two open arms (16 x 5 cm) and two enclosed arms (16 x 5 x 12 cm) with an open roof and was elevated to a height of 25 cm, stopwatch.

Procedure

1. The animals were weighed, numbered, and divided into different groups each of 4-5 mice.
2. Each animal was placed in the center of the maze, head facing toward open arm transfer latency (TL) and the following parameters were noted:
   - First preference of mice to open or enclosed arm.
   - Number of entries in open and enclosed arms (an arm entry defined as the entry of four paws into the arm).
   - Then drugs were injected (i.p.) to the test groups. After 30 min animals were placed individually in the center of the maze and all the parameters were noted as mentioned above.
   - After 24 h, the animals were placed on the elevated plus maze individually as before and TL was recorded again.
   - TL measured on the first and the second day served as a parameter for acquisition and retrieval, respectively. The results were expressed as mean ± SEM and analyzed by using one-way analysis of variance (ANOVA). In all the tests, the criterion for statistical significance was $P<0.05$.

ACKNOWLEDGEMENTS

We thank Dr**.** Rajat Sandhir, Dr. Shrinivas K Kulkarni for the *in vivo* and *iv vitro* studies.

REFERENCES

1. Tommo, S, Toshinao, U, Mitsuru, O, Tsunemasa, S, Tadashi, K. Synthesis and Muscarinic activity of a series of Quinolines and Naphthalenes with a 1-Azabicyclo[3.3.0]octane moiety. Chem. Pharm. Bull. 199;46:1265-1273.
2. Marta M, Castellano C, Oliverio A, Pavone F, Pagella PG, Brufani M, *et al.* New analogues of physostigmine: Alternative drugs for Alzheimer's disease? Life Sci 1988;43:1921-8.
3. Perry EK. The cholinergic hypothesis - ten years on. Br Med Bull 1986;42:63–9.
4. Davies P. Neurotransmitter-related enzyme in senile dementia of the Alzheimer type. Brain Res 1979;171:319–27.
5. Winkler J, Suhr ST, Gage FH, Thal LJ, Fisher LJ. Essential role of neocortical acetylcholine in spatial memory. Nature 1995;375:484–7.
6. Becker RE, Giacobini E. Mechanisms of cholinesterase inhibition in senile dementia of the Alzheimer type: Clinical, pharmacological and therapeutic aspects. Drug Dev Res 1988;12:163–95.
7. Saxena A, Fedorko JM, Vinayaka CR, Medhekar R, Radić Z, Taylor P, et al. Aromatic amino-acid residues at the active and peripheral anionic sites control the binding of E2020 (Aricept®) to cholinesterases. Eur J Bio Chem 2003;270:4447-58.
8. Susan DI. Behavioural evaluation of cholinergic drugs. Life Sci 1997;60:1145-52.
9. Parnetti L, Senin U, Mecocci P. Cognitive enhancement therapy for Alzheimer's disease. The way forward. Drugs 1997;53**:**752-68.
10. Giacobini E. From molecular structure to Alzheimer therapy. Jpn J Pharmacol 1997;74:225-41.
11. Ye JW, Cai JX, Wang LM, Tang XC. Improving effects of huperzine A on spatial working memory in aged monkeys and young adult monkeys with experimental cognitive impairment. J Pharmacol Exper Therap 1999;288:814-9.
12. Barril X, Kalko SG, Orozco M, Luque FJ. Rational design of reversible acetylcholinesterase inhibitors. Mini Rev in Med Chem 2002;2:27-36.
13. Recanatini M, Cavalli A, Belluti F, Piazzi L, Rampa A, Bisi A, *et al.* SAR of 9-Amino-1,2,3,4-tetrahydroacridine based acetylcholinesterase inhibitors: Synthesis, enzyme inhibitory activity, QSAR and structure-based COMFA of tacrine analogues. J Med Chem 2000;43:2007-18.
14. Camps P, Achab RE, Morral J, Munoz-Torrero O, Badia A, Banos JE, *et al.* New tacrine-hyperzine A hybrids (huprines): Highly potent tight-binding acetylcholinesterase inhibitors of interest for the treatment of Alzheimer's disease. J Med Chem 2000;43:4657-66.
15. Sarmek JJ, Frackiewicz EJ, Cutler NR. Reveiw of the acetylcholinesterase inhibtor galanthamine. Expert Opin Investig Durgs 2000;9:2393-402.
16. Furniss, BS, Hannafard, AJ, Smith, PG, Tatchell, AR. Nitration and hydrolysis, Vogel's textbook of practical organic chemistry. 5th ed. Longman Co.; 1994. p. 919-20.
17. Mann, FG, Saunders, BC. Nitration and hydrolysis, Practical organic chemistry. 4th ed. Orient Longman; 2003. P. 167-68.
18. Halama A, Kavalek J, Machacek V, Weidlich T. Aromatic nucleophilic subsititution of hydrogen: Mechanism of reaction of 6-nitroquinoline with cyanide ions, with and without participation of methyl cyanoacetate. J. Chem. Soc., Perkin Trans. 1999;1:1839-45.
19. Furniss, BS, Hannafard, AJ, Smith, PG, Tatchell, AR. Reduction of primary amine, Vogel's textbook of practical organic chemistry. 5th ed. Longman Co.; 1994. p. 1281-82.
20. Mann, FG, Saunders, BC. Nitration and hydrolysis, Practical organic chemistry. 4th ed. Orient Longman; 2003. P. 161-62.
21. Ellman GL, Courtney KD, Andress V, Featherston RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol 1961;7:88-95.
22. Itoh J, Nabeshima T, Kameyama T. Utility of an elevated plus-maze for dissociation of amnesic and behavioral effects of drugs in mice. Eur J Pharmacol 1991;194:71-6.
23. Sharma AC, Kulkarni SK. Evaluation of learning and memory mechanisms employing elevated plus-maze in rats and mice. Prog. Neuropsychopharmacol Biol Psychiatry 1992;16:117-25.
24. Lowry OH, Rosenberg NJ, Randall RJ. Protien measurement with the folin phenol reagent. J Biol Chem 1951;193:265-75.

Source of Support: Nil, **Conflict of Interest:** None declared.