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# Synthesis and efficacy of 1-[bis(4-fluorophenyl)-methyl]piperazine derivatives for acetylcholinesterase inhibition, as a stimulant of central cholinergic neurotransmission in Alzheimer's disease

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**Abstract**—The cholinergic hypothesis of Alzheimer's disease (AD) has spurred the development of numerous structural classes of compounds with different pharmacological profile aimed at increasing central cholinergic neurotransmission. Thus proving a symptomatic treatment for this disease are cholinomimetics with the pharmacological profile of acetyl cholinesterase (AchE) inhibitors. The novel bioactive 1-[bis(4-fluorophenyl)-methyl]piperazine derivatives were synthesized under mild conditions using different aryl/alkyl halides and heterocyclic alkyl halides with 1-[bis(4-fluorophenyl)-methyl]piperazine in the presence of powdered potassium carbonate in *N*,*N*-dimethylformamide. All the synthesized compounds were characterized by spectroscopic techniques, elemental analysis and were screened for their efficacy as AchE inhibitor. Some derivatives in this class showed good inhibition against AchE as compared to neostigmine as standard.


Alzheimer's disease (AD) is a progressive neurodegenerative disorder, responsible for over 50% of all cases of dementia, which affects up to 5% of people over 65 years, while its prevalence increases to more than 20% of those over 80 years.[1] Three main stages can be clinically characterized in AD.[2] The first stage is the so-called amnesia stage, which involves initial loss of short-term memory and lack of emotional spontaneity. In the second stage, the confusion stage, the patient exhibits time and space disorientation, severe mental confusion, and personality changes. The last stage, the dementia stage, involves the total mental incapacity and full dependence of the patient. While the disease itself is not fatal, medical complications associated with AD, usually viral or bacterial infections, lead to the death of the patient.[3] Thus, AD is the third largest cause of death in the western world after cardiovascular diseases and cancer. Taking into account the increase in life expectancy and the fact that the incidence of AD increases with advancing age, the devastating effects of this illness are found on rise. AD is currently a major public health problem and will presumably be the most important pathology of this century in developed/developing countries. Sustained efforts have been made in the last two decades to determine the etiopathogenesis of AD, and to carry out its early diagnosis and therapeutic control. Most relevant pathogenic events in AD can be classified into four main categories.[4] Primary events (genetic alterations, neuronal apoptosis-like processes leading to premature neuronal death and brain dysfunction), secondary events (β-amyloid deposition in senile plaques and brain vessels, neurofibrillary tangles due to hyperphosphorylation of tau proteins, synaptic loss), tertiary events (neuroimmune dysfunction, neuroinflammatory processes), and quaternary events (accelerated neuronal death due to excitotoxic reactions, cerebrovascular dysfunction). All of these pathogenic events are potential targets for treatment of AD. In spite of the multifactor nature of AD, most treatment strategies have been directed at two main targets: the β-amyloid peptide and the cholinergic neurotransmission. Parallel to the development of antidementia drugs, research efforts have been focused on the therapeutic potential of AchE inhibitors to slow the disorder progression.

*Keywords*: Piperazine; AchE; Rodent memory evaluator; Alzheimer's disease.
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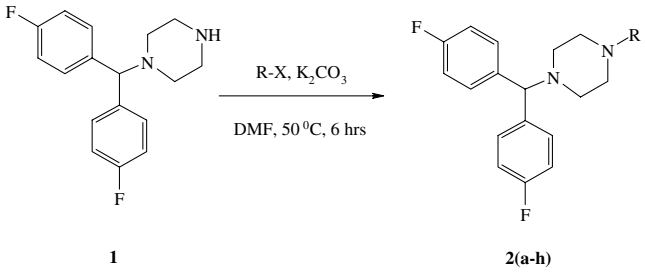
Piperazine derivatives are known to inhibit AchE in worms.[5] In this connection, piperazine derivatives were synthesized with condensing various pharmacologically active alkyl groups R by means of alkyl halides RX. The present study is undertaken with a view to find the efficacy of these piperazine derivatives (**2a**–**h**) as AchE inhibitors.

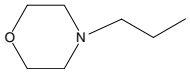
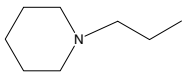
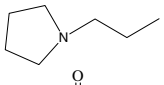
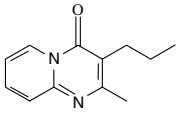
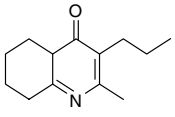
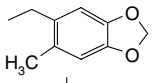
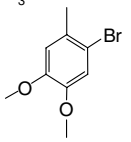
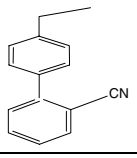
The novel 1-[bis(4-fluorophenyl)-methyl]piperazine derivatives (**2a**–**h**) were synthesized[14] by coupling with different aryl/alkyl and heterocyclic alkyl halides using dry potassium carbonate in DMF. (Scheme 1 and Table 1).

The effect of different doses of piperazine derivatives on AchE in rat brain is as shown in Figure 1. Activity was




**Scheme 1.**

**Table 1.** The physical characterization of the novel piperazine compounds **2**(**a**–**h**)

| Compound | R | Yield (%) | Mp (°C) |
|---|---|---|---|
| **2a** |  | 85 | 244 |
| **2b** |  | 84 | 221 |
| **2c** |  | 78 | 230 |
| **2d** |  | 75 | 226 |
| **2e** |  | 76 | 243 |
| **2f** |  | 78 | 212 |
| **2g** |  | 75 | 234 |
| **2h** |  | 76 | 218 |

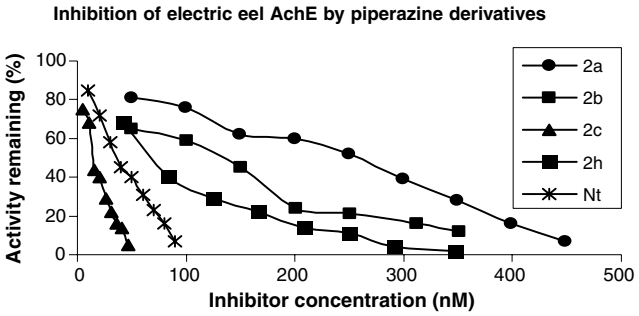



**Figure 1.** Inhibition of electric eel AchE by piperazine derivatives.

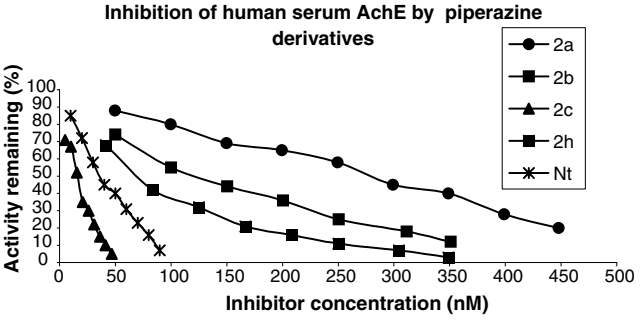



**Figure 2.** Inhibition of human serum AchE by piperazine derivatives.

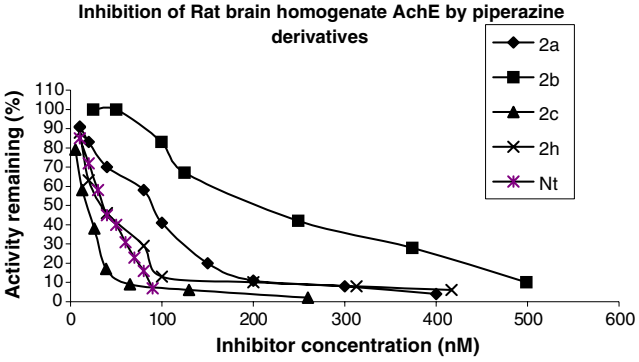



**Figure 3.** Inhibition of rat brain homogenate AchE by piperazine derivatives.

**Table 2.** Comparative inhibitory activities of 1-[bis(4-fluorophenyl)-methyl]piperazine derivatives against AchE from different sources

| Compound | $IC_{50}$ (nM) | | |
|---|---|---|---|
| | Rat brain homogenate | Human serum | Electric eel |
| **2a** | 200 | 284.2 | 252.9 |
| **2b** | 93.75 | 126.3 | 135.2 |
| **2c** | 18.75 | 21.0 | 17.6 |
| **2d** | NI | 3203.7 | 2127.4 |
| **2e** | NI | 1072.8 | 1210.2 |
| **2f** | NI | 1341.7 | 1136.2 |
| **2g** | NI | 1012.9 | 715 |
| **2h** | 37.5 | 73.6 | 70.5 |
| Neostigmine | 37.5 | 42.1 | 41.1 |

NI, no inhibition found.

**Table 3.** Study of antiamnesic effect of 1-[bis(4-fluorophenyl)-methyl]piperazine derivatives against scopolamine induced memory loss

| Sl. no | Experimental groups | Treatment (dose) mg/kg ip | Basal latency (s) of rat to reach shock-free zone (SFZ) | | | Memory parameters | |
|---|---|---|---|---|---|---|---|
| | | | I | II | III | Latency (s) | No. of mistakes |
| 1 | Control groups | — | 18 | 3 | 0.8 | 1 | 8 |
| 2 | Scopolamine treated groups | 0.4 | 36 | 10 | 8 | 4 | 35 |
| 3 | **2a** treated groups | 0.1 | 30 | 6 | 3 | 3 | 16 |
| 4 | **2b** | 0.1 | 28 | 7 | 6 | 3 | 12 |
| 5 | **2c** | 0.1 | 20 | 8 | 4 | 2 | 10 |
| 6 | **2d** | 0.1 | 34 | 9 | 8 | 4 | 32 |
| 7 | **2e** | 0.1 | 35 | 9 | 7 | 4 | 34 |
| 8 | **2f** | 0.1 | 36 | 10 | 7 | 4 | 33 |
| 9 | **2g** | 0.1 | 34 | 9 | 8 | 3 | 34 |
| 10 | **2h** | 0.1 | 20 | 7 | 5 | 3 | 11 |

50% at a dose of 200, 93.75, 18.75, and 37.5 nM for compounds **2a**, **2b**, **2c**, and **2h**, respectively. The significant potency of inhibition was shown by **2c** followed by **2h**, **2b**, and **2a** in increasing order.

The pharmacological conclusion is that compounds **2a**, **2b**, **2c**, and **2h** show anticholinesterase activity as confirmed by the biochemical finding of rat brain, human serum, and electric eel AchE. Compounds **2d**, **2e**, **2f**, and **2g** did not show any inhibitory activity. Compound **2c** is the most potent of all the inhibitory compounds tested. This leads to the suggestion that compounds like **2c** or further modified can lead to the development of a potent AchE inhibitor.

The inhibitory activity of the newly synthesized compounds against AchE was studied using the method of Ellman et al.[6] to determine the rate of hydrolysis of acetylthiocholine iodide in the presence of the inhibitor against different sources of AchE, electric eel AchE, human serum AchE, and rat brain homogenate AchE that are as shown in Figures 1–3, respectively.[15]

Activities of the synthesized compounds were compared with the inhibitory activity shown by the known standard inhibitor neostigmine. Different derivatives of 1-[bis(4-fluorophenyl)-methyl]piperazine having different heterocyclic rings were tested for their ability to block the AchE activity for the substrate acetylthiocholine iodide. The order of potency is **2c > 2h > 2b > 2a**. The other compounds screened failed to elicit any inhibition of acetylcholinesterase from rat brain homogenate. Among the molecules screened for the AchE inhibitory activity, pyrrolidine substituted piperazine **2c** ($IC_{50}$ = 12.4, 17.6, and 18.6 nM) was found effectively to block the enzyme as compared to the rest of the derivatives studied. Piperidinyl (**2b**) and morpholinyl (**2a**) derivatives are also effective in blocking the AchE enzyme activity ($IC_{50}$ = 93.75, 126.3, 135.2, 200, 284.2, and 252.9 nM), respectively (Table 2). The 2-cyanobiphenyl ring also shows good activity probably because of its bulkiness. Cinnarizine, a well-known cerebral vasodilator, passes through the blood–brain barrier (BBB)[7] because of the presence of lipophilic group (diphenyl methyl piperazine moiety) in the molecule. The molecule synthesized herein can be expected to enter the central nervous system (CNS) because of the structural similarity with cinnarizine. It is also known that piperazine analogues inhibit AchE.[8] The electron-donating effect is the most important factor on the benzyl benzene ring, suggesting a role in regulating the protonation equilibrium at the benzylic nitrogen of the piperazine skeleton. The smaller the substituent, the more favorable activity at diphenyl methyl site of the molecule. The aromatic fluoro substituent is slightly smaller than hydrogen in terms of molecular refractivity (MR), being 0.09 versus 0.10.[9]

From the SAR, it is observed that the piperazine basic ring containing single or non-fused heterocyclic moieties (R = **2a**, **2b**, and **2c**) showed a better activity than fused heterocyclic moieties (R = **2d**, **2e**, and **2f**). It is also observed from reversing amnesic effect of scopolamine induced memory loss in passive avoidance step-down task paradigm in rat.[10] Compound **2c** reverses the average number of mistakes done from 35 (scopolamine) to 10. Values for all the compounds are given in Table 3, which show that in vivo and in vitro results are fairly comparable.

It may be concluded from this study that, for effective binding and blocking of the AchE activity, molecule needs to bind with the peripheral site and the active site of the enzyme and it may be possible that the piperazine binds to the active site and the substituents containing heterocyclic rings **2(a–f)** bind to peripheral site of the enzyme. Therefore, it can be summarized that substitution of other heterocyclic rings on piperazine basic nucleus separated by two carbons needs to be studied for better AchE inhibitory activity.

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13. Sharma, A. C.; Kulkarni, S. K. *Methods Find. Exp. Clin. Pharmacol.* **1991**, *13*, 155.
14. *Experimental*. The melting points were determined on a SELACO-650 hot stage apparatus and are uncorrected. IR (KBr) spectra were recorded on a Jasco FT/IR-4100 Fourier transform infrared spectrophotometer, $^1$H NMR were recorded on Shimadzu AMX spectrophotometer by using $CDCl_3$ as solvent and TMS as an internal standard (chemical shift in ppm). Elemental analyses were obtained on a Vario-EL instrument. Thin-layer chromatography (TLC) was conducted on 0.25 mm silica gel plates ($60F_{254}$, Merck). Visualization was made with ultraviolet light. All extracted solvents were dried over anhydrous $Na_2SO_4$ and evaporated with a BUCHI rotary evaporator. Reagents were obtained commercially and used as received.*General procedures for the synthesis of* ***2(a–h)***. A mixture of 1-[bis(4-fluorophenyl)-methyl]piperazine **1**, aryl, alkyl, and heterocyclic alkyl halides was stirred in the presence of powdered potassium carbonate in *N*,*N*-dimethylformamide for about 6 h at 50 °C. The reaction was monitored by TLC (chloroform: methanol = 4.5:0.5). After completion of the reaction, the solvent was evaporated under reduced pressure. Demineralized water was added to the residue, extracted with ethyl acetate, and dried over anhydrous $Na_2SO_4$.*Synthesis of 4-(2-{4-[bis(4-fluoro-phenyl)-methyl]piperazine-1-yl}-ethyl)-morpholine* ***2a***. It was obtained from 1-[bis(4-fluorophenyl)-methyl]piperazine **1** (0.10 g, 0. 34 mmol), 4-(2-chloroethyl)morpholine hydrochloride (0.06 g, 0.34 mmol), and powdered $K_2CO_3$ (2.3 g, 0.0173 mmol). The product obtained was a pale yellow solid. IR (cm$^{-1}$ KBr): 1600, 1223. $^1$H NMR ($\delta$ ppm): 7.1 (dd, 4H, $J$ = 1.9 Hz, Ar–H), 7.3 (dd, 4H, $J$ = 1.9 Hz, Ar–H), 4.4 (s, 1H, –CH–), 2.1–2.8 (m, 16H, –$CH_2$–), 2.3 (t, 4H, –$CH_2$–) 3.6–3.7 (t, 4H, –$CH_2$–O–$CH_2$–).*Synthesis of 1-[bis(4-fluoro-phenyl)-methyl]-4-(2-piperidin-1-yl-ethyl)-piperazine* ***2b***. It was obtained from 1-[bis(4-fluorophenyl)-methyl]piperazine **1** (0.10 g, 0. 34 mmol), 1-(2-chloroethyl)piperidine hydrochloride (0.06 g, 0.34 mmol), and powdered $K_2CO_3$ (2.3 g, 0.0173 mmol). The product obtained was a pale yellow solid. IR (cm$^{-1}$ KBr): 1600, 1223. $^1$H NMR ($\delta$ ppm): 7.1 (dd, 4H, $J$ = 1.9 Hz, Ar–H), 7.3 (dd, 4H, $J$ = 1.9 Hz), 4.4 (s, 1H, –CH–), 2.3–2.5 (m, 12H, –$CH_2$–), 1.51 (s, 6H, –$CH_2$–), 2.2 (s, 4H, –$CH_2$–).*Synthesis of 1-[bis(4-fluoro-phenyl)-methyl]-4-(2-pyrrolidin-1-yl-ethyl)-piperazine* ***2c***. It was obtained from 1-[bis(4-fluorophenyl)-methyl]piperazine **1** (0.10 g, 0. 34 mmol), 1-(2-chloroethyl)pyrrolidine hydrochloride (0.06 g, 0.408 mmol), and powdered $K_2CO_3$ (2.3 g, 0.0173 mmol). The product obtained was a pale yellow solid. IR(cm$^{-1}$ KBr): 1600, 1223. $^1$H NMR ($\delta$ ppm): 7.2 (dd, 4H, $J$ = 1.92 Hz, Ar–H), 7.3 (dd, 4H, $J$ = 1.96 Hz, Ar–H), 4.4 (s, 1H, CH), 2.1–2.3 (t, 14H, –$CH_2$–), 1.5 (m, 4H, –$CH_2$–). *Synthesis of 3-(2-{4-[bis(4-fluoro-phenyl)-methyl]piperazine-1-yl}-ethyl)-2-methyl-pyrido[1,2-a]pyrimidin-4-one* ***2d***. It was obtained from 1-[bis(4-fluorophenyl)-methyl]piperazine **1** (0.10 g, 0. 34 mmol), 3-(2-bromoethyl)-2-methyl-pyrido[1,2-*a*]pyrimidin-4-one (0.070 g, 0.34 mmol), and powdered $K_2CO_3$ (2.3 g, 0.0173 mmol). The product obtained was a pale yellow solid. IR (cm$^{-1}$ KBr): 1600, 1223, 1750. $^1$H NMR ($\delta$ ppm): 7.15 (dd, 4H, $J$ = 1.92 Hz, Ar–H), 7.3 (dd, 4H, $J$ = 1.93 Hz, Ar–H), 4.4 (s, 1H, –CH–), 2.4 (m, 8H, –$CH_2$–), 2.9–3.15 (t, 2H, –$CH_2$–) 3.37–3.9 (t, 3H, –$CH_2$–), 1.6 (s, 3H, –$CH_3$), 2.3 (t, 1H, –CH–), 1.7 (s, 3H, –$CH_3$), 6.2 (d, 1H, Bz–H), 7.85–7.95 (t, 1H, Bz–H), 7.25–7.32 (t, 1H, Bz–H), 8.8–8.95 (d, 1H, Bz–H).*Synthesis of 3-(2-{4-[bis(4-fluoro-phenyl)-methyl]-piperazine-1-yl}-ethyl)-2-methyl-5,6,7,8-tetrahydro-4aH-quinolin-4-one* ***2e***. It was obtained from 1-[bis(4-fluorophenyl)-methyl]piperazine **1** (0.10 g, 0. 34 mmol), 3-(2-bromoethyl)-2-methyl-5,6,7,8-tetrahydro-4*aH*-quinolin-4-one (0.070 g, 0.34 mmol), and powdered potassium carbonate (2.3 g, 0.0173 mmol). The product obtained was a pale yellow solid. IR (cm$^{-1}$ KBr): 1600, 1223. $^1$H NMR ($\delta$ ppm): 7.1 (dd, 4H, $J$ = 1.93 Hz, Ar–H), 7.3 (dd, 4H, $J$ = 1.95 Hz, Ar–H), 4.4 (s, 1H, –CH–), 2.4 (s, 8H, –$CH_2$–), 2.92–3.15 (t, 2H, –$CH_2$–), 3.37–3.9 (t, 2H, –$CH_2$–), 1.6 (s, 3H, –$CH_3$–), 2.3 (t, 1H, –CH–), 1.3–1.5 (m, 8H, –$CH_2$–).*1-[Bis(4-fluoro-phenyl)-methyl]-4-(6-methyl-benzo[1,3]dioxol-5-ylmethyl)-piperazine* ***2f***. It was obtained from 1-[bis(4-fluorophenyl)-methyl]piperazine **1** (0.10 g, 0. 34 mmol), 5-1-(chloromethyl)6-methyl-benzo[1,3]dioxole (0.067 g, 0.408 mmol), and powdered potassium carbonate (2.3 g, 0.0173 mmol). The product obtained was a white yellow solid. IR (cm$^{-1}$ KBr): 1600, 1223. $^1$H NMR ($\delta$ ppm): 7.14 (dd, 4H, $J$ = 1.91 Hz, Ar–H), 7.31 (dd, 4H, $J$ = 1.9 Hz, Ar–H), 4.4 (s, 1H, –CH–), 2.4 (s, 8H, –$CH_2$–), 3.7 (s, 2H, –$CH_2$–), 2.23 (s, 3H, Ar–$CH_3$), 6.3 (s, 2H, Bz–H), 5.8 (s, 2H, –O–$CH_2$–O).*Synthesis of 1-[bis(4-fluoro-phenyl)-methyl]-4-(2-bromo-4,5-dimethoxy-benzyl)-piperazine* ***2g***. It was obtained from 1-[bis(4-fluorophenyl)-methyl]piperazine **1** (0.10 g, 0. 34 mmol), 1-bromo-2-bromomethyl-4,5-dimethoxybenzene (0.105 g, 0.34 mmol), and powdered potassium carbonate (2.3 g, 0.0173 mmol). The product obtained was a white yellow solid. IR (cm$^{-1}$ KBr): 1600, 1223, 2950. $^1$H NMR ($\delta$ ppm): 7.1 (dd, 4H, $J$ = 1.9 Hz, Ar–H), 7.3 (dd, 4H, $J$ = 1.92 Hz, Ar–H), 4.4 (s, 1H, –CH–), 2.4 (s, 8H, –$CH_2$–), 3.6 (s, 2H, –$CH_2$–), 6.35 (s, 1H, Ar–H), 6.7 (s, 1H, Ar–H), 3.8 (s, 6H, –$OCH_3$).*Synthesis of 1-[bis(4-fluoro-phenyl)-methyl]-4-1(benzyl)-4-(2-cyanophenyl)-piperazine* ***2h***. It was obtained from 1-[bis(4-fluorophenyl)-methyl]piperazine **1** (0.10 g, 0. 34 mmol), 4-(2-cyano-phenyl)-benzyl bromide (0.092 g, 0.34 mmol), and powdered potassium carbonate (2.3 g, 0.0173 mmol). The product obtained was a white yellow solid. IR (cm$^{-1}$ KBr): 1600, 1223, 2210. $^1$H NMR ($\delta$ ppm): 7.1 (dd, 4H, $J$ = 1.9 Hz, Ar–H), 7.3 (dd, 4H, $J$ = 1.9 Hz, Ar–H), 4.3 (s, 1H, –CH–), 2.2–2.4 (m, 8H, –$CH_2$–), 3.6 (s, 2H, –$CH_2$–),7.4–7.6 (m, 4H, Ar–H), 7.52–7.62 (m, 4H, Ar–H).
15. *Biology*. In vitro cholinesterase assay:The cholinesterase assay method of Ellman et al.[6] was used to determine the in vitro cholinesterase activity. The activity was measured by the increase in absorbance at 412 nm due

to the yellow color produced from the reaction of acetylthiocholine iodide with the dithiobisnitrobenzoate ion. AchE was obtained from the brain of Wistar rats by homogenizing under a Teflon blender for 10 min in 0.1 M $KH_2PO_4$ buffer, pH 8. A stock solution of the enzyme in 0.1 M $KH_2PO_4$ buffer (pH 8) was kept frozen. For each assay 300 μg of enzyme was used. Acetylthiocholine iodide was prepared daily using 0.1 M $KH_2PO_4$ buffer (pH 7). A 0.01 M solution of DTNB was prepared in 0.1 M $KH_2PO_4$ buffer (pH 7). Crude human AchE was prepared by mixing 9 ml of fresh blood (collected from healthy volunteers by vein puncture) with 1 ml of 3.8% (w/v) trisodium citrate and centrifuging at 3000 rpm at 0 °C for 20 min. The supernatant was used as a source of AchE. Electric eel AchE was obtained from Sigma Laboratory and similar procedure was employed for the assay as that of rat brain AchE.*Experimental conditions and kinetics*. Enzyme activity was measured using a Shimadzu Spectrophotometer. The assay medium contained phosphate buffer, pH 8.0 (2.6 ml), DTNB (0.1 ml), 5 μl of enzyme, and 20 μl of 0.075 M substrate. The activity was determined by measuring the increase in absorbance at 412 nm at 1 min intervals for 10 min at 37 °C. In dose-dependent inhibition studies, the substrate was added to the assay medium containing enzyme, buffer, and DTNB with inhibitor after 10 min of incubation time. Calculations were performed according to the method of the equation in Ellman et al.[6] All experiments were carried out in duplicate and the mean values are reported here. The relative activity is expressed as percentage ratio of enzyme activity in the absence of inhibitor.*Protein estimation*. Protein content was determined by the Lowry method[11] using bovine serum albumin as standard.*$IC_{50}$ determination*. AchE inhibitor neostigmine (a reversible cholinesterase inhibitor), used in the concentration range 10–90 nM, was used to inhibit AchE in electric eel, human serum, and rat brain homogenate. Inhibition by piperazine derivatives was studied in the presence of different concentrations of compounds and the percentage inhibition of enzyme activity was calculated. The inhibition of AchE by piperazine derivatives was analyzed with values obtained in comparison to that of neostigmine. Antiamnesic effect was carried out for synthesized 1-[bis(4-fluorophenyl)-methyl]piperazine derivatives against scopolamine induced memory loss using passive avoidance step-down task paradigm in rats according to the method of Sharma and Kulkarni.[12,13]