Bioorganic & Medicinal Chemistry Letters 23 (2013) 2984–2989

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Design, synthesis and evaluation of some new 4-aminopyridine derivatives in learning and memory

Saurabh K. Sinha, Sushant K. Shrivastava *

Pharmaceutical Chemistry Research Laboratory, Department of Pharmaceutics, Indian Institute of Technology, Banaras Hindu University, Varanasi, UP 221005, India

ARTICLE INFO

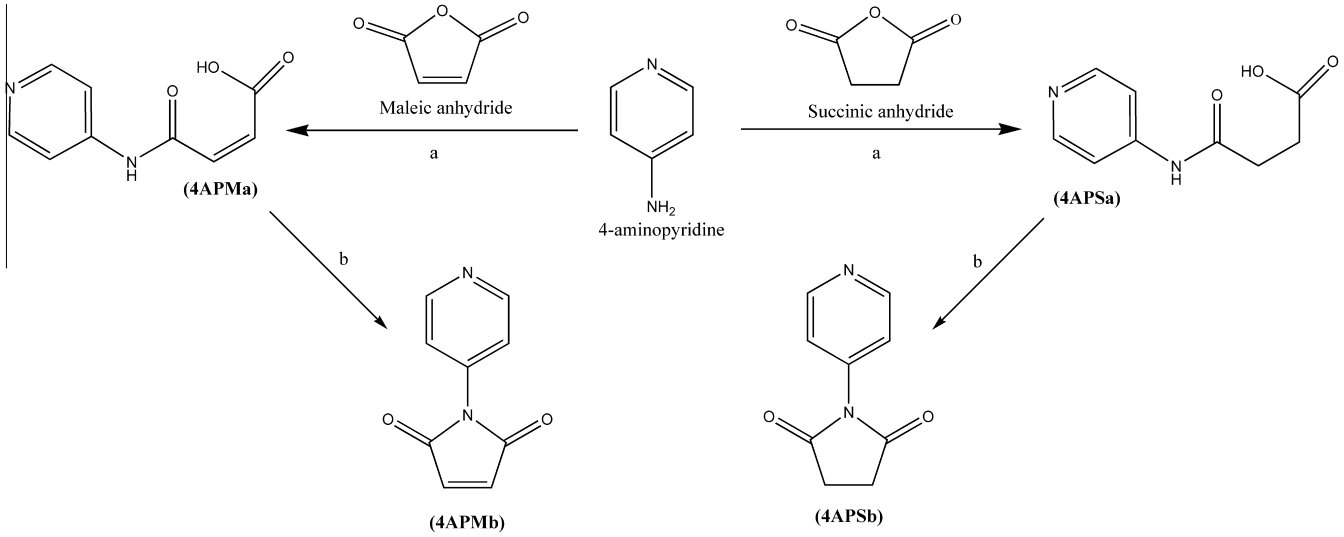
Article history:
Received 1 October 2012
Revised 21 February 2013
Accepted 8 March 2013
Available online 16 March 2013

Keywords:
4-Aminopyridine
Nootropic
Antiamnesic
Anticholinesterase
Elevated plus maze

ABSTRACT

Some new anilide and imide derivatives of 4-aminopyridine (4AP) were synthesized and evaluated against antiamnesic, cognition enhancing and anticholinesterase activity through their respective in vitro and in vivo models. These newly synthesized derivatives have illustrated an enhanced cognition effect on elevated plus maze model and also demonstrated a significant reversal in scopolamine-induced amnesia in same model. The IC_{50} value of synthesized compounds showed maximum activity of **4APMb** compared to standard drug donepezil and other derivatives, whereas its enzyme kinetic study revealed a non-competitive inhibition of acetycholinesterase (AChE) and a competetive inhibition of butyrylcholinesterase (BChE). Significant inhibitions in AChE activity by all the synthesized compounds were found in specific brain regions that is prefrontal cortex, hippocampus and hypothalamus. The docking study confirmed their consensual interaction with AChE, showed an affinity and binding with the key peripheral anionic site residues Trp-286, Tyr-124 and Tyr-341 of AChE.

Progressive impairment in memory, cognitive functions and behavioral disturbance has always been a major area of concern for complex neurodegenerative disorders of the central nervous system such as Alzheimer's disease (AD).[1] Patients suffering with AD have low level of acetylcholine (ACh) and biosynthetic enzyme choline acetyltransferase (ChAT) in the cortex and hippocampus.[2,3]



Scheme 1. The synthetic pathway of the compounds here presented. Reagents and conditions: (a) THF, 25 °C, 3 h; (b) Ac_2O, AcONa, 80 °C 5 h.

* Corresponding author. Tel.: +91 9452156527; fax: +91 5422368428.
E-mail addresses: sksinha.rs.phe@itbhu.ac.in, skshrivastava.phe@itbhu.ac.in (S.K. Shrivastava).

0960-894X/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmcl.2013.03.026

Table 1
Concentration of synthesized derivatives and donepezil required for 50% inhibition of AChE and BChE

Compound	IC_{50}(μM) ± SEM		Selectivity for AChE[a]
	AChE	BChE	
4APSa	40.33 ± 0.88	56.45 ± 0.60	1.39
4APSb	0.49 ± 0.02	3.82 ± 0.83	7.79
4APMa	48.67 ± 1.20	656.85 ± 1.60	13.49
4APMb	0.03 ± 0.014	11.02 ± 0.76	367
Donepezil	0.04 ± 0.012	15.24 ± 0.88	381

[a] Selectivity for AChE is defined as IC_{50} (BChE)/IC_{50} (AChE).

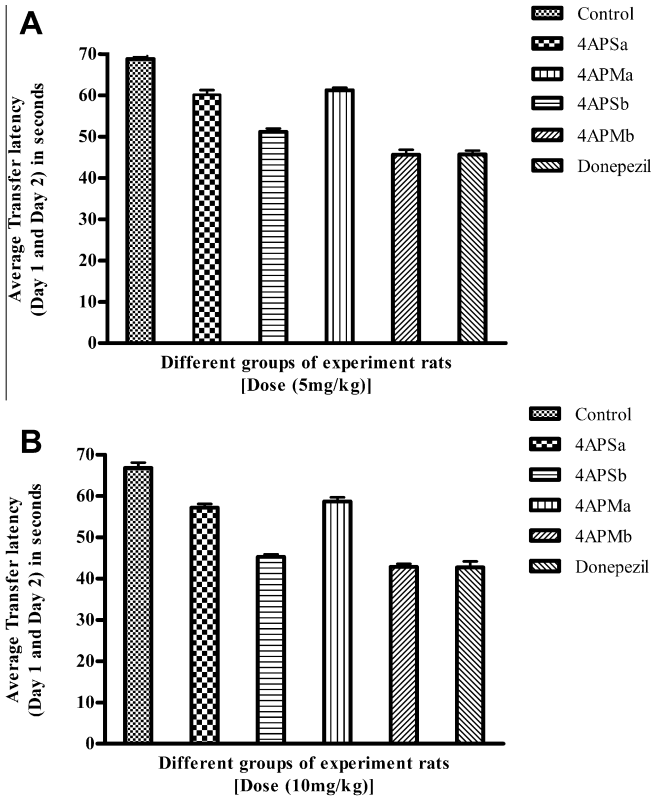


Figure 1. Effect of synthesized derivatives and Donepezil on learning impairment on elevated plus maze in wistar rats [A] at dose (5 mg/kg), [B] at dose (10 mg/kg).

Table 2
Reverse effect of synthesized derivative on scopolamine-induced amnesia on elevated plus maze in rats

Treatment [dose (mg/kg)]	Transfer latency (s)	
	Day 1	Day 2
VEHICLE	67.67 ± 0.88	62.50 ± 0.76
SCP (1.0)	86.83 ± 0.90	81.50 ± 0.76[a]
4APSa (5.0) + SCP (1.0)	77.67 ± 0.76	71.67 ± 0.66[b]
4APSa (10.0) + SCP	74.83 ± 0.60	70.00 ± 0.57[b]
4APSb (5.0) + SCP	66.83 ± 0.60	60.67 ± 0.66[b]
4APSb (10.0) + SCP	65.50 ± 0.76	58.33 ± 0.88[b]
4APMa (5.0) + SCP	80.50 ± 0.76	75.33 ± 0.80[b]
4APMa (10.0) + SCP	77.67 ± 0.66	72.50 ± 0.76[b]
4APMb (5.0) + SCP	64.67 ± 0.84	56.33 ± 0.88[b]
4APMb (10.0) + SCP	61.33 ± 0.61	53.83 ± 0.60[b]
Donepezil (5.0) + SCP	65.33 ± 0.66	56.67 ± 0.66[b]
Donepezil (10.0) + SCP	62.50 ± 0.76	54.50 ± 0.76[b]

Data are expressed as mean ± SEM ($n = 6$). Data were statistically analyzed by one way ANOVA.
[a] Significantly different from control (vehicle treated) group $p < 0.001$.
[b] Significantly different from scopolamine treated group $p < 0.001$.

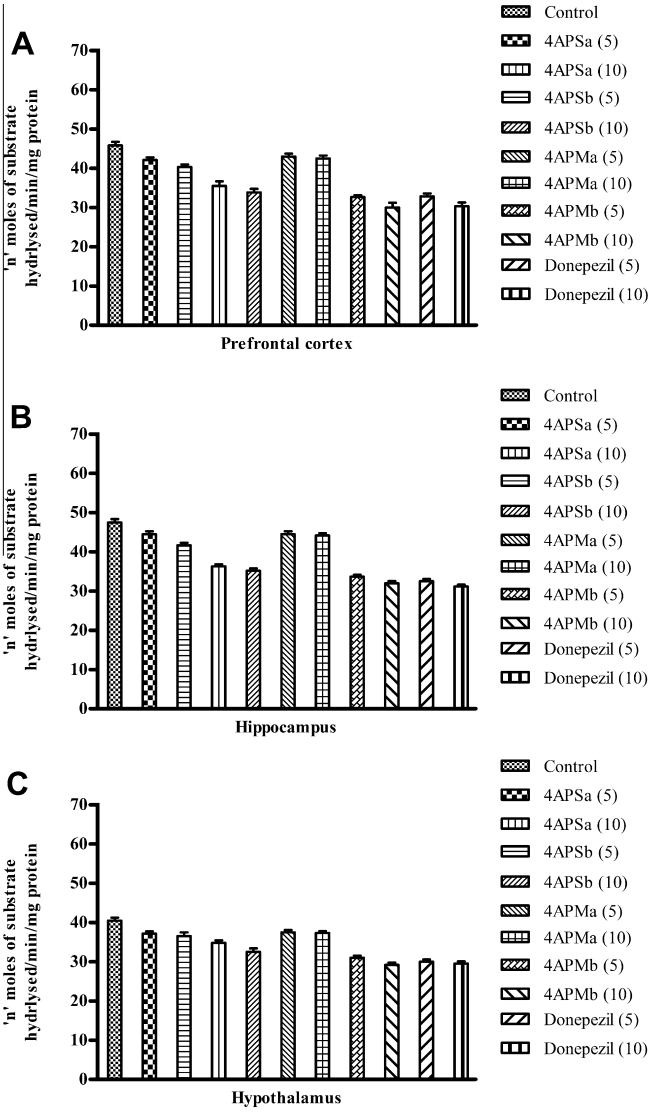
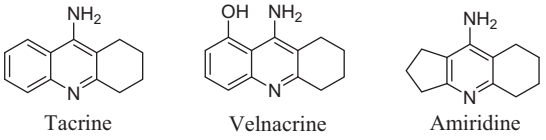


Figure 2. Effect of synthesized derivatives and donepezil (5 and 10 mg/kg) on acetylcholinesterase (AchE) activity on different region of rat brain [A] prefrontal cortex [B] hippocampus [C] hypothalamus. Results are expressed as mean ± SEM ($n = 6$).

These parameters have a vital role in learning and memory therefore, in order to treat this specific disease it is necessary to improve cholinergic neurotransmission which may be achieved by preventing the biotransformation of acetylcholine into the inactive metabolites choline and acetate at the specific sites of brain.[4] Based on this approach some drugs have been established and used to treat the AD.[5,6] The utility of 4AP derivatives in treating various neuromuscular disorders such as multiple sclerosis, botulism, spinal cord injury, Alzheimer's disease and myasthenia gravis have already been reported in previous investigations[7,8]which may be attributed to its capacity to penetrate the blood–brain barrier[9] but the therapeutic applications of the most common 4AP derivatives such as tacrine[10] and velnacrine[11] have been restricted due to their high toxicity. Another tacrine derivative amiridine is now under Phase III study in Japan.[12]



Several carbamate derivatives of 4AP and Schiff bases of styrylpyridine have been synthesized and evaluated for their anticholinesterase activity.[13,14] This activity has also been evaluated in some amides and imides derivatives of *m*-aminobenzoic acid and *p*-aminobenzoic acid.[15,16] Some 4-aminobutyric acid (GABA) and 2-indolinone derivatives of 4-Aminopyridine have been reported to possess antiamnesic activity.[17] Docking study of different AChE inhibitors revealed that the amino acid residues Tyr-72, Tyr-124, Glu-285, Trp-286, and Tyr-341 constitute the peripheral anionic site (PAS) in human acetylcholinesterase enzyme (hAChE). Binding of ligands with these residues may be the key to the allosteric modulation of hAChE catalytic activity.[18] In our previous study, some Schiff bases of 4-aminopyridine have been reported as cognition enhancing, antiamnesic and anticholinesterase activity.[19] In view of these facts we have designed and synthesized some new anilide and imide derivatives of 4AP and evaluated for the same activity as discussed earlier.

The reaction carried out of 4AP with succinic anhydride as well as 4AP with maleic anhydride in tetrahydrofurane at room temperature resulted the respective **4APSa** and **4APMa** compounds. These derivatives were transformed into **4APSb** and **4APMb**, by heating it in acetic anhydride with an equimolecular amount of sodium acetate (Scheme 1).[20] The reaction was monitored by TLC and formation of these compounds was confirmed by clear observation of shifting of value of $-NH_2$ group of 4AP in IR and ^1H NMR. In IR doublet values of 3456 and 3395 cm^{-1} shifted to 3369 cm^{-1} (**4APSa** and **4APMa**) which disappeared in case of compounds **4APSb** and **4APMb**. Likewise in ^1H NMR 4.5 δ ppm shifted to downfield 8.9 and 9.3 δ ppm for **4APSa** and **4APMa** while a total disappearance of the peak was observed in **4APSb** and **4APMb**.

The 50% inhibitory concentration (IC_{50}) of all the derivatives on acetycholinesterase (AChE) and butyrylcholinesterase (BChE) was determined by adopting Ellman method.[21] The results showed a comparable activity and selectivity of **4APMb** (0.03 ± 0.014 μM), (367) was observed with reference standard donepezil (0.04 ± 0.012 μM), (381) for AChE inhibition respectively (Table 1). Further, enzyme kinetics study[22] was also perform for the most active compound **4APMb**, which demonstrated a non-competitive inhibition of AChE (K_i = 0.042 ± 0.016) and competitive inhibition of BChE (K_i = 15.64 ± 0.66) enzymes. The non-competitive inhibition[23] suggested a possible interaction of compound with the peripheral anionic site (PAS) of AChE and was also confirmed by docking studies. The synthesized compounds were then evaluated for antiamnesic and cognition enhancing property in rat elevated plus maze (EPM) model[24] which is a simple method for evaluation of learning and memory that depends upon measuring the transfer latency of rats. A natural revulsion in rats to open and high spaces have been observed, where in these types of studies animals usually spend more time in enclosed arms rather than open arms in plus maze test.[25] This suggests a reduced transfer latency, where animals moves from the open arms to the enclosed arms that depends on previous experience of rats entering the open arm which may be attributed to an enhanced memory. Pre-treatment with tested compounds resulted in reduced transfer latency as compared to control group on first and second day of EPM exposure in significant and dose dependant manner, indicating facilitated learning process (Fig. 1). The scopolamine (1 mg/kg) significantly increased transfer latency as compared to control group [p <0.001], resulting in amnesia which was reversed by tested compounds and donepezil significantly[26] [p <0.001] (Table 2). The three specific brain regions that are involved in processing of memory and high innervations of cholinergic neurons include prefrontal cortex, hippocampus and hypothalamus. It is reported that 50–90% reduction in ChAT, an enzyme which synthesizes acetylcholine

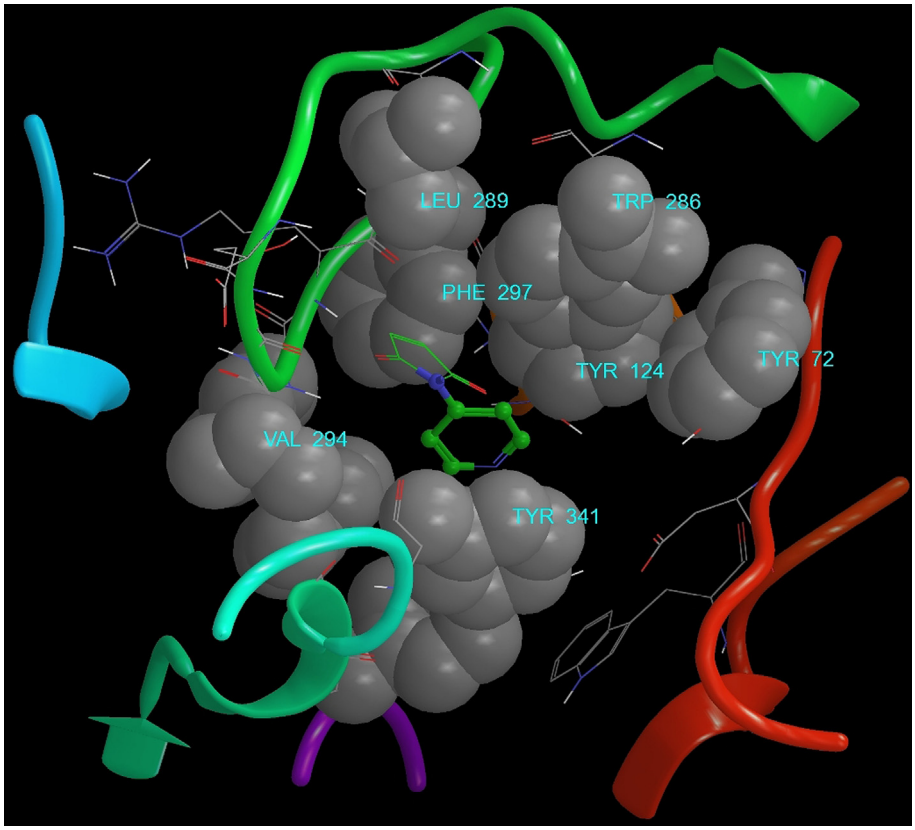


Figure 3. Hydrophobic interactions: 4AP ring of **4APMb** are well placed in the hydrophobic pockets.

is observed in hippocampus, cortex and hypothalamus in patients with dementia of Alzheimer's Type (DAT).[27,28] Hippocampus is permanently involved in memories that have to be acquired and retrieved. It is also temporarily involved in memory consolidation and as an area for the temporary storage of the to-be-consolidated information.[29] This information is further transferred into prefrontal cortex where short-term memory is converted into long term memory (a process called as consolidation).[30] It has also been reported through several Investigations that, hypothalamus stimulation facilitates hippocampus dependent learning and memory processes in both young and aged rats.[31] Therefore, in vivo AChE activity was determined which showed that all the synthesized compounds inhibited the AChE activity in distinct brain regions prefrontal cortex, hippocampus and hypothalamus compared to control [p <0.01, p <0.05 and p <0.001], respectively (Fig. 2). These results also confirmed that anilide and imide derivatives of 4AP significantly enhanced cholinergic neurotransmission in these distinct brain regions and attributed to enhanced learning and memory functions due to their anti-cholinesterase activity. Acute toxicity studies didn't show any signs of toxicity and mortality

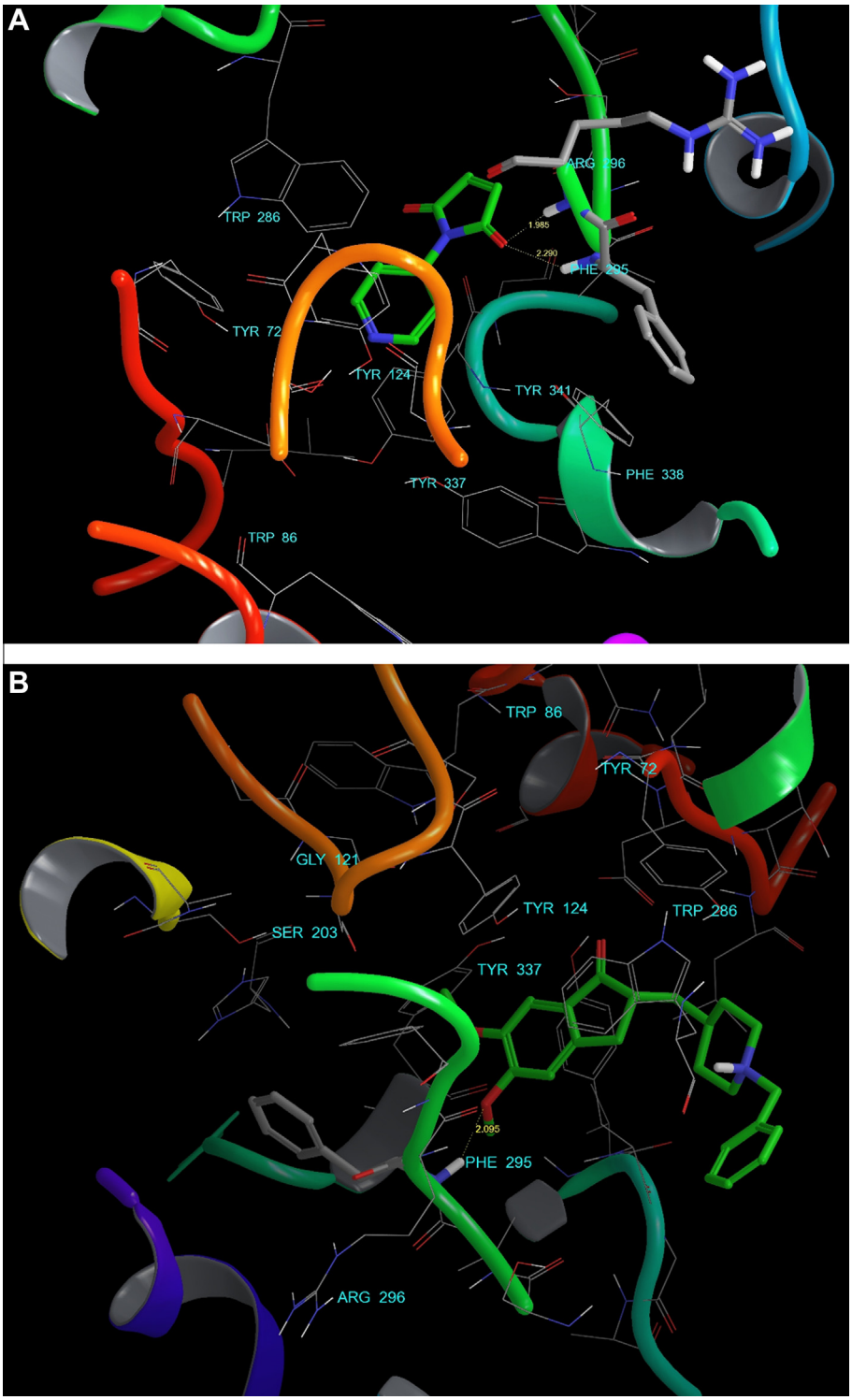


Figure 4. Docking model of compound [A] **4APMb** and [B] Donepezil at the active sites of AChE. The hydrogen bonds are displayed as dashed yellow.

up to a dose of 100 mg/kg of body weight as evident through a normal behavioral pattern of rats up to the desired period of observation (details in Supplementary data).

The synthesized compounds **4APSb** and **4APMb** showed much better AChE and BChE inhibitory activity, reversing acute memory loss and learning impairment as compared to **4APSa** and **4APMa**. On first day **4APMb** produced lesser transfer latency (61.33 ± 0.61 s) compared to scopolamine treated group (86.83 ± 0.90 s). However, on second day transfer latency (53.83 ± 0.60 s) was lesser than first day, which illustrated the beneficial role of **4APMb** in reversing acute memory loss and learning impairment compared to other derivatives and donepezil in both dose level.

The crystal structure of AChE with high resolution was retrieved from the protein data bank (pdb code: 1B41).[32] The structure was prepared in the following procedures by protein preparation wizard in Maestro 9.2 (Schrödinger, LLC, 2011), including adding hydrogens, assigning partial charges using the OPLS-2005 force field and assigning protonation states, and a restrained, partial energy minimization. Finally, the cocrystal ligand (snake-venom toxin fasciculin-II) was removed, and the resulting structure was used as the receptor model in docking studies. Docking studies were carried out to provide a better interpretation of the biological profile of **4APMb** and donepezil toward AChE. It was observed that **4APMb** and donepezil were properly positioned into the enzyme gorge and showed interaction with the internal amino acid residue Tyr-341 and Trp-286 by means of a π–π interaction. The 4AP ring of **4APMb** was well placed in the hydrophobic pockets formed by Tyr-341, Trp-286, Val-294, Phe-297, Leu-289, Tyr-124 and Tyr-72 and exhibited strong hydrophobic interactions (Fig. 3). The study clearly demonstrated that both compounds were able to bind with the key peripheral anionic site (PAS) residue Trp-286, Tyr-124 and Tyr-341. The carbonyl oxygen of **4APMb** was involved in forming a bifurcated hydrogen bond with Phe-295 (determine substrate specificity) and Arg-296. Introduction of carbon-carbon unsaturation in case of **4APMb** results high G-score (−7.16) indicates the high binding ability in comparison of other derivatives suggested the molecular flexibility on biological activity. The methoxy group of donepezil was observed in establishing the H-bond with Phe-295 backbone, suggested that the compounds might probably act via the AChE inhibition (Fig. 4).[33,34] ADME or Pharmacokinetic properties were also predicted for all the compounds by using the QikProp 3.4 module of the software consisting of principal descriptors and physiochemical properties with analysis of the log*P* (octanol/water), % human oral absorption, Lipinski's rule of five violation, CNS activity and permeability through MDCK (Madin-Darby Canine Kidney) cells in nm/s etc. (MDCK cells are considered to be a good mimic for the blood–brain barrier).The compounds **4APSb** and **4APMb** illustrated good oral absorption, CNS activity and permeability through blood–brain barrier and also satisfy Lipinski's rule for drug likeliness of the synthesized compounds.[35]

Conclusions and future directions

Thus, from the above study we have successfully identified a new class of potent cognition enhancing and antiamnesic drugs. Among the identified compounds, compound **4APMb** deserves further clinical studies which can lead to a discovery of a new lead having a potent cognition enhancing and antiamnesic properties.

Acknowledgments

The authors gratefully acknowledge the University Grants Commission (UGC), New Delhi, India for the financial support to Mr. Saurabh K Sinha. Grant No. R/Dev./IX-Sch./(SRF-JRF) Pharm./15402.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.03.026. These data include MOL files and InChiKeys of the most important compounds described in this article.

References and notes

1. Bartus, R. T.; Dean, R. L.; Beer, B.; Lippa, A. S. *Science* **1982**, *217*, 408.
2. Perry, E. K.; Perry, R. H.; Blessed, G.; Tomlinson, B. E. *Neuropathol. Appl. Neurobiol.* **1978**, *4*, 273.
3. Gil-Bea, F. J.; Garcia-Alloza, M.; Dominguez, J.; Marcos, B.; Ramirez, M. J. *Neurosci. Lett.* **2005**, *375*, 37.
4. Hakansson, L. *Acta Neurol. Scand.* **1993**, *88*, 7.
5. Giacobini, E. *Aging (Milano)* **2001**, *13*, 247.
6. Lahiri, D. K.; Farlow, M. R.; Greig, N. H.; Sambamurti, K. *Drug Dev. Res.* **2002**, *56*, 267.
7. Polman, C. H.; Bertelsmann, F. W.; van Loenen, A. C.; Koeteier, J. C. *Arch. Neurol.* **1994**, *51*, 292.
8. Stork, C. M.; Hoffman, R. S. *J. Toxicol. Clin. Toxicol.* **1994**, *32*, 583.
9. Murray, N. M. F.; Newsome-Davis, J. *Neurology* **1981**, *31*, 265.
10. Galisteo, M.; Rissel, M.; Sergent, O.; Chevanne, M.; Cillard, J.; Guillouzo, A.; Lagadic-Gossmann, D. *J. Pharmacol. Exp. Ther.* **2000**, *294*, 160.
11. Birks, J.; Wilcock, G. K. *Cochrane Database Syst. Rev.* **2004**. http://dx.doi.org/10.1002/14651858.CD004748. Art. No.: CD004748.
12. Moskovskij, N. I.; Grigorieva, I. V.; Sokolchik, E. I. *Zh. Nevrol. Psikhiatr. Im. S S Korsakova* **1991**, *91*, 53.
13. Scipione, L.; De Vita, D.; Musella, A.; Flammini, L.; Bertoni, S.; Barocelli, E. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 309.
14. Cavallito, C. J.; Yun, H. S.; Edwards, M. L.; Foldes, F. F. *J. Med. Chem.* **1971**, *14*, 130.
15. Trujillo-Ferrara, J.; Montoya Cano, L.; Espinoza-Fonseca, M. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1825.
16. Correa-Basurto, J.; Alcantara, I. V.; Espinoza-Fonseca, L. M.; Trujillo-Ferrara, J. G. *Eur. J. Med. Chem.* **2005**, *40*, 732.
17. Andreani, A.; Leoni, A.; Locatelli, A.; Morigi, R.; Rambaldi, M.; Pietra, C.; Villetti, G. *Eur. J. Med. Chem.* **2000**, *35*, 77.
18. Barak, D.; Kronman, C.; Ordentlich, A.; Ariel, N.; Bromberg, A.; Marcus, D.; Lazar, A.; Velan, B.; Shafferman, A. *J. Biol. Chem.* **1994**, *269*, 6296.
19. Sinha, S. K.; Shrivastava, S. K. *Med. Chem. Res.* **2012**, *19*. http://dx.doi.org/10.1007/s00044-012-9982-4.
20. General procedure for the synthesis of compounds
*4-oxo-4-(pyridin-4-ylamino)butanoic acid (**4APSa**)*
4AP (0.470 g, 5 mmol) was dissolved in 5 ml of tetrahydrofurane in a 25-ml conical flask. To this solution equimolar quantity (0.5 g, 5 mmol) of succinic anhydride was added and reaction mixture was stirring up to 3 h at 25 °C. The reaction progress was monitored by TLC using mobile phase as chloroform:methanol (6:4). On completion of reaction the resultant product **4APSa** was collected by filtration then washed three times with 0.001 M HCl and crystallized from 95% ethanol. Yield: 81.6%, mp: 232–234 °C, R_f 0.50, IR (KBr, ν cm^{-1}): 3413 (OH), 3369 (NH), 2950 (CH, CH_2), 1725 (CO, COOH), 1680 (CO, CONH); ^{1}H NMR (DMSO-d_6) (δ ppm): 11.3 (s, 1H, COOH), 8.9 (s, 1H, NH), 8.3–8.5 (m, 4H, pyridine ring), 2.9 (t, 2H, CH_2–COOH), 2.5 (t, 2H, CH_2–CONH); ^{13}C NMR (δ ppm): 179 (CONH), 173 (COOH), 153, 149, 109 (pyridine ring), 30, 28 (CH_2); Anal. Calcd for $C_9H_{10}N_2O_3$: C, 55.67; H, 5.19; N, 14.43; Found: C, 54.78; H, 5.26; N, 14.10.
*1-(Pyridin-4-yl)pyrrolidine-2,5-dione (**4APSb**)*
A solution of **4APSa** (0.970 g, 5 mmol) in anhydrous tetrahydrofuran (5 ml) and equimolecular amount of sodium acetate (0.410 g) and acetic anhydride (0.5 ml) was heated and stirred at 60 °C for 5 h, followed by evaporation of the solvent under vacuum. The residue was washed three times with 0.001 M HCl, yielding **4APSb**. Yield: 72.6%, mp: 186–188 °C, R_f 0.52, IR (KBr, ν cm^{-1}): 2950 (CH, CH_2), 1690 (CO); ^{1}H NMR (DMSO-d_6) (δ ppm): 8.3–8.5 (m, 4H, pyridine ring), 2.7 (dt, 4H, CH2); ^{13}C NMR (δ ppm): 185 (CO); 153, 149, 109 (pyridine ring), 35 (CH_2); Anal. Calcd for $C_9H_8N_2O_2$: C, 61.36; H, 4.58; N, 15.90; Found: C, 60.28; H, 4.32; N, 15.48.
*(Z)-4-oxo-4-(pyridin-4-ylamino)but-2-enoic acid (**4APMa**)*
4AP (0.470 g, 5 mmol) was dissolved in 5 ml of tetrahydrofurane in a 25-ml conical flask. To this solution equimolar quantity (0.490 g, 5 mmol) of maleic anhydride was added and reaction mixture was stirring up to 3 h at 25 °C. The reaction progress was monitored by TLC using mobile phase as chloroform:methanol (6:4). On completion of reaction the resultant product **4APMa** was collected by filtration then washed three times with 0.001 M HCl and crystallized from 95% ethanol. Yield: 78.6%, mp: 222–224 °C, R_f 0.42, IR (KBr, ν cm^{-1}): 3413 (OH), 3369 (NH), 3103 (CH, CH=CH), 1730 (CO, COOH), 1685 (CO, CONH); ^{1}H NMR (DMSO-d_6) (δ ppm): 11.5 (s, 1H, COOH), 9.3 (s, 1H, NH), 8.3–8.5 (m, 4H, pyridine ring), 6.9 (d, 1H, CH–COOH), 6.7 (t, 1H, CH–CONH); ^{13}C NMR (δ ppm): 179 (CONH), 173 (COOH), 153, 149, 109 (pyridine ring), 122 (CH=CH); Anal. Calcd for $C_9H_8N_2O_3$: C, 56.25; H, 4.20; N, 14.58; Found: C, 56.80; H, 4.36; N, 14.23.
*1-(Pyridin-4-yl)-1H-pyrrole-2,5-dione (**4APMb**)*
A solution of **4APMa** (0.960 g, 5 mmol) in anhydrous tetrahydrofuran (5 ml)

and equimolecular amount of sodium acetate (0.410 g) and acetic anhydride (0.5 ml) was heated and stirred at 60 °C for 5 h, followed by evaporation of the solvent under vaccum. The residue was washed three times with 0.001 M HCl, yielding **4APMb**. Yield: 74.8%, mp: 202–204 °C, R_f 0.38, IR (KBr, ν cm^{-1}): 3127 (CH, CH=CH), 1685 (CO); ^1H NMR (DMSO-d_6) (δ ppm): 8.3–8.5 (m, 4H, pyridine ring), 6.9 (dd, 2H, CH=CH); ^{13}C NMR (δ ppm): 190 (CO); 153, 149, 109 (pyridine ring), 125 (CH=CH); Anal. Calcd for $C_9H_6N_2O_2$: C, 62.07; H, 3.47; N, 16.09; Found: C, 61.85; H, 3.38; N, 16.22.

21. In vitro and in vivo estimation of acetylcholinesterase activity carried out according to method described by Ellman (*Biochem. Pharmacol.*, **1961**, *7*, 88–95) and Zdrazilova (*Z. Naturforsch.*, **2004**, *59*, 293–296). Detailed procedures are described in Supplementary data.
22. Lineweaver, H.; Burk, D. *J. Am. Chem. Soc.* **1934**, *56*, 658.
23. Bartolini, M.; Bertucci, C.; Cavrini, V.; Andrisano, V. *Biochem. Pharmacol.* **2003**, *65*, 407.
24. Sharma, A. C.; Kulkarni, S. K. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **1992**, *16*, 117.
25. Itoh, J.; Nabeshima, T.; Kameyama, T. *Psychopharmacology (Berl.)* **1990**, *101*, 27.
26. Snyder, P. J.; Bednar, M. M.; Cromer, J. R.; Maruff, P. *Alzheimers Dement.* **2005**, *1*, 126.
27. Davies, P.; Maloney, A. J. *Lancet* **1976**, *25*, 1403.
28. Harley, C. W.; Lacaille, J. C.; Galway, M. *Brain Res.* **1983**, *270*, 335.
29. Knowlton, B. J.; Fanselow, M. S. *Curr. Opin. Neurobiol.* **1998**, *8*, 293.
30. Tronel, S.; Matthijs, G. P. F.; Susan, J. S. *Learn. Mem.* **2004**, *11*, 453.
31. Soriano-Mas, C.; Redolar-Ripoll, D.; Aldavert-Vera, L.; Morgado-Bernal, I.; Segura-Torres, P. *Behav. Brain Res.* **2005**, *160*, 141.
32. Kryger, G.; Harel, M.; Giles, K.; Toker, L.; Velan, B.; Lazar, A.; Kronman, C.; Barak, D.; Ariel, N.; Shafferman, A.; Silman, I.; Sussman, J. L. *Acta Crystallogr. Sect. D* **2000**, *56*, 1385.
33. Friesner, R. A.; Banks, J. L.; Murphy, R. B.; Halgren, T. A.; Klicic, J. J.; Mainz, D. T.; Repasky, M. P.; Knoll, E. H.; Shaw, D. E.; Shelley, M.; Perry, J. K.; Francis, P.; Shenkin, P. S. *J. Med. Chem.* **2004**, *47*, 1739.
34. Halgren, T. A.; Murphy, R. B.; Friesner, R. A.; Beard, H. S.; Frye, L. L.; Pollard, W. T.; Banks, J. L. *J. Med. Chem.* **2004**, *47*, 1750.
35. Moorthy, N. S. H. N.; Karthikeyan, C.; Trivedi, P. *Med. Chem.* **2009**, *5*, 549.