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4-Aminopyridine derivatives with anticholinesterase and antiamnesic activity

Luigi Scipione,[a,*] Daniela De Vita,[a] Alessandra Musella,[a] Lisa Flammini,[b] Simona Bertoni[b] and Elisabetta Barocelli[b]

[a]*Dipartimento di ''Studi di chimica e tecnologia delle sostanze biologicamente attive'', Sapienza Università di Roma, Piazzale Aldo Moro, 5, 00185 Roma, Italia*
[b]*Dipartimento di ''Scienze Farmacologiche, Biologiche e Chimiche Applicate'', Università degli Studi di Parma, Viale G.P. Usberti 27/a, 43100 Parma, Italia*

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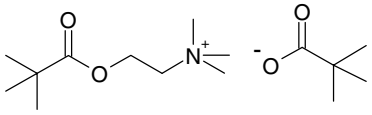
Abstract—Several carbamate derivatives of 4-aminopyridine were synthesized and their anticholinesterase activity was evaluated. Compound **4d** showed the highest inhibitory effect blocking non-competitively acetylcholinesterase and competitively butyrylcholinesterase. Furthermore, carbamate **4d** was able to revert the amnesic effects of scopolamine in the passive avoidance test in rats.

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.[1] The AD syndrome is associated with a severe deficit in the cholinergic neurotransmission due to a progressive degeneration in basal forebrain,[2] with the loss of neuronal projections to the cortex accompanied by a reduction of the levels of the acetylcholine (ACh), and biosynthetic enzyme choline acetyl transferase (ChAT) and of acetylcholinesterase (AChE).[3,4]

Among the different strategies investigated to improve cholinergic neurotransmission, the reduction of Ach synaptic hydrolysis by Cholinesterase inhibitors (ChEIs) and the increase in ACh synthesis are, up to date, the prevalent AD effective symptomatic treatment.

Unfortunately, the response to AD treatment with ChEIs shows a modest average degree of benefit.[5–7]

Recently, we have reported the synthesis of [2-(2,2-dimethylpropionyloxy)ethyl]trimethylammonium 2,2-dimethylpropionate (choline pivaloylester—CPE) (**1**)[8] and the evaluation of its biological effects on scopolamine-treated or nucleus basalis magnocellularis (NBM)-lesioned rats; CPE was able to restore object discrimination ability and improve spatial memory,[9] it was also able to induce electroencephalographic (EEG) desynchronisation and significant changes in the architecture of EEG tracings.[10] Furthermore, **1** was able to enhance the benefit effects of ChEIs, such as Tacrine and Galantamine, on EEG and cognitive performance in NBM lesioned and aged rats.[11]



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This synergic effect is probably due to the activity of **1**, or of its hydrolytic metabolite choline, as an agonist of pre- and postsynaptic α7 nicotinic acetylcholine receptors (α7 nAChRs).[12,13] These subtypes of functional nAChRs are highly expressed in the basal forebrain cholinergic neurons that project to the hippocampus and the cortex and are critically involved in cognitive and memory functions.[14]

Keywords: 4-Aminopyridine derivatives; Acetylcholinesterase; Butyrylcholinesterase; Antiamnesic effects.
* Corresponding author. Tel.: +39 0649913737; fax: +39 0649913133; e-mail: luigi.scipione@uniroma1.it

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On the basis of this positive example of synergism it seemed to be useful to associate **1** with 4-aminopyridine (4-AP), known as an enhancer of ACh in the intersynaptic space.[15] Notably, the molecular scaffold of 4-AP is also present in some carbamate cholinesterase inhibitors which proved to be effective in ameliorating behavioral performances in scopolamine treated animals.[16]

Previously, passive avoidance performance test was carried out on scopolamine-treated rats receiving, respectively, **1**, 4-AP and **1** + 4-AP.[17]

The scopolamine-treated rats exhibited significantly shorter Retention Trial latency time (RT) in comparison with vehicle-treated controls (51.1 ± 8.1 vs 152.0 ± 7.0, $P < 0.01$). 4-AP improved the memory deficit induced by scopolamine in rats showing a U-shaped dose–response curve. A significant antiamnesic effect was detected only at 0.005 mg/kg (92.3 ± 9.0 vs 51.1 ± 8.1, $P < 0.05$). In contrast with these results, **1** failed to affect the scopolamine-dependent cognitive deficit up to 34.73 mg/kg. A synergic protective effect on memory impairment was detected when **1** was associated to the lowest dose (0.001 mg/kg) (99.6 ± 23.6 vs 51.1 ± 8.1, $P < 0.05$) but not to the effective dose (0.005 mg/kg) of 4-AP.

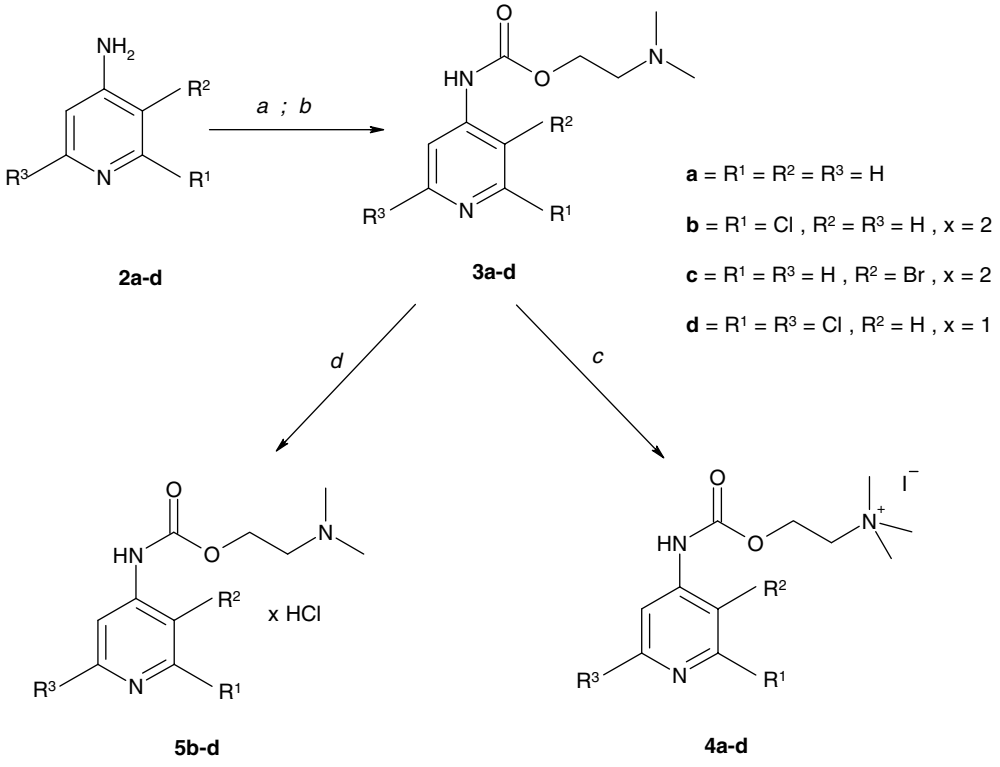
These results led us to investigate the activity of compounds generated by linking 4-AP derivatives with choline, by means of a carbamate function. This kind of molecules is developed expressly, through a dualistic approach, to target multiple brain systems for the treatment of memory and cognition impairment.[18]

First we carried out the synthesis of the carbamate **4a** following the pathway depicted in Scheme 1.

4-AP was refluxed with triphosgene in presence of TEA; at the end of reaction dimethylaminoethanol was added and the solution stirred overnight at room temperature.[19] The obtained carbamate **3a** was used to prepare choline derivative **4a**, which was assessed for its ability to inhibit acetyl and butyrylcholinesterase.

First, compounds **3a** and **4a**, dissolved in D_2O, pH 7.4, phosphate buffer containing acetyl or butyrylcholinesterase, were analyzed every 15 min for 1.5 h, by means of ^1H NMR spectroscopy, to evaluate possible enzymatic hydrolysis. Blank assays without enzymes were executed. Neither spontaneous nor catalyzed hydrolysis was observed.

Subsequently the spectrophotometric method of Ellman was used to determine the type of inhibition and K_i values.[20] Briefly, 3 mL of 0.1 M (pH 7.4) phosphate buffer containing 0.75 mmol of DTNB and 0.083 U AChE or 0.25 U of BChE was mixed with one of studied compounds (10–1000 μmol) in a polystyrene cuvette of 1 cm path length. The reaction was started by the addition of acetylthiocholine iodide or butyrylthiocholine iodide (75–900 μmol) and the changes in the absorbance at 412 nm were recorded at 25 °C between 0.5 and 1.5 min after reagent addition. Each determination was per-



Scheme 1. Reagents: (a) Compounds **2a–d** (2 mmol), TEA (6 mmol), triphosgene (1 mmol), benzene; (b) dimethylaminoethanol; (c) iodomethane, ethanol; (d) $HCL_{(g)}$ ethanol.

Table 1. Cholinesterase inhibition data of compounds **3a**, **4a–d** and **5b–d**

	AChE			BChE		
		$K_i \pm$ SEM (μM)	r^2		$K_i \pm$ SEM (μM)	r^2
3a	nc	313.7 ± 27.7	0.978	c	20.4 ± 0.7	0.994
4a	nc	159.0 ± 11.7	0.934	nc	754.2 ± 33.9	0.963
4b	nc	150.9 ± 5.9	0.984	c	161.3 ± 10.2	0.986
4c	nc	115.9 ± 6.8	0.967	nc	273.8 ± 11.5	0.994
4d	nc	64.4 ± 2.3	0.988	c	19.5 ± 2.0	0.991
5b	nc	207.7± 12.6	0.983	nc	379.7 ± 14.2	0.989
5c	nc	316.4 ± 23.8	0.989	c	273.4 ± 14.6	0.991
5d	nc	140.2 ± 4.6	0.989	nc	174.5 ± 8.2	0.986

nc, non-competitive; c, competitive.

formed at least in triplicate. The recorded data were analyzed with the enzyme kinetic module of SigmaPlot, version 8.02a (Systat Software, Inc.) in order to find the best fitting model of inhibition. K_i values were obtained according to Dixon's method.[21]

The obtained data are summarized in Table 1.

Carbamate **4a** showed non-competitive inhibition towards AChE and BChE with moderate potency. Compound **3a** inhibited both AchE and BChE, in a non-competitive and a competitive mode, respectively, with K_i value of 20.4 ± 0.7 μM versus BChE.

It is known that the introduction in some Tacrine derivatives of chlorine atoms provides more active AChE inhibitors.[22] This finding suggested us to modify the structure of carbamates **3a** and **4a** by inserting halogen substituents at 2, 3, and 6 position of the pyridine ring. Thus, we have synthesized 2-chloro, 3-bromo, and 2,6-dichloro substituted carbamates following the above-described procedure.[19] Compounds **4b–d** and **5b–d** were analyzed by ^{1}H NMR to evaluate the possible spontaneous or cholinesterase catalyzed hydrolysis; in no case hydrolysis was observed.

Afterwards, compounds **4b–d** and **5b–d** were also tested to determine their ability to inhibit acetyl and butyrylcholinesterase. The kinetic data, reported in Table 1, show that all studied carbamates are non-competitive inhibitors towards AChE with the lowest K_i (64.4 ± 2.3 μM) value for the derivative **4d**, which also resulted the most potent BChE competitive inhibitor (K_i = 19.5 ± 2.0 μM).

Compounds **3a, 4a**, and **4d** were thus studied in passive avoidance test to evaluate their ability in ameliorating mnemonic and cognitive performances in impaired scopolamine-treated rats. The obtained data are summarized in Table 2.

The 2,6-dichloro derivative **4d,** at variance with compounds **3a** and **4a** which were substantially inactive, showed a significant antiamnesic activity. Despite the moderate affinity exhibited by the compound towards AChE and BChE in in vitro test, it was able to reverse scopolamine-induced amnesia in a U-shaped dose–response way, showing maximal activity when administered at 1.2 mg/kg. This effectiveness led us to speculate the involvement of mechanisms additional to the simple inhibition of cholinesterase; the activity of compound **4d** could be also due to the above-mentioned cholinergic properties of 4-AP derivatives.

Table 2. Effects of studied compounds on rat passive avoidance test, using scopolamine (0.5 mg/kg sc) as amnesic drug

Drugs (dose, mg/kg ip)	Entry latency (s)	
	TT	RT
Saline	20.8 ± 0.3	152.0 ± 7.0
Scopolamine	18.4 ± 0.4	51.1 ± 8.1[a]
+4-AP (0.001)	23.9 ± 6.5	38.5 ± 2.5
+4-AP (0.005)	22.1 ± 4.9	92.3 ± 9.0[b]
+4-AP (0.01)	46.3 ± 22.7	63.5 ± 10.5
+4-AP (0.1)	20.3 ± 10.3	33.6 ± 5.2
+**1** (17.37)	38.8 ± 8.3	36.8 ± 10.8
+**1** (34.73)	14.5 ± 2.5	45.8 ± 20.1
+4-AP (0.001) + **1** (17.37)	13.2 ± 0.8	86.5 ± 22.2
+4-AP (0.001) + **1** (34.73)	29.8 ± 3.1	99.6 ± 23.6[b]
+4-AP (0.005) + **1** (17.37)	18.9 ± 2.0	18.7 ± 4.3
+4-AP (0.005) + **1** (34.73)	20.1 ± 2.0	51.1 ± 12.3
+**3a** (0.01)	20.3 ± 12.6	12.1 ± 0.9
+**3a** (0.5)	43.6 ± 16.4	49.7 ± 6.2
+**3a** (50)	25.5 ± 6.1	39.6 ± 3.5
+**4a** (0.02)	37.3 ± 17.7	40.4 ± 4.9
+**4a** (1.0)	21.4 ± 5.1	21.0 ± 4.7
+**4a** (100)	30.6 ± 8.4	21.3 ± 3.1
+**4d** (0.024)	14.4 ± 1.3	36.8 ± 13.5
+**4d** (0.24)	19.7 ± 5.2	56.9 ± 21.1
+**4d** (1.2)	21.4 ± 9.8	92.9 ± 19.4[b]
+**4d** (2.4)	19.0 ± 5.9	32.3 ± 10.1

Data are expressed as means ± SEM. Ten animals for each group. TT, training trial; RT, retention trial.
Data were statistically analyzed by one way ANOVA followed by Dunnett's post-test. Differences are considered to be statistically significant if the probability has a value of 0.05 or less.
[a] Significantly different from saline-treated group $P < 0.01$.
[b] Significantly different from scopolamine-treated group $P < 0.05$.

It is noteworthy that all synthesized carbamates showed non-competitive inhibition of AChE, suggesting for these compounds a possible interaction with the peripheral anionic site (PAS) of the enzyme. This site, besides its ability to regulate esterasic activity, is considered to be responsible for the β-amyloid aggregation and represents a putative target for novel drugs;[23] new molecules should be able to enhance cholinergic tone by reducing the hydrolytic activity of AChE and decreasing the deposition of Aβ fibrils.

The evidence of the positive behavioral effects of **4d** and the possible correlation with its anticholinesterase activity suggest the possibility for an in depth study to evaluate its amyloid anti-aggregating properties. Results could provide information for further structural optimization of these 4-aminopyridine derivatives, in order to obtain more potent cholinesterase inhibitors potentially useful in the treatment of AD.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.10.077.

References and notes

1. Bartus, R. T.; Dean, R. L., III; Beer, B.; Lippa, A. S. *Science* **1982**, *217*, 408.
2. Whitehouse, P. J.; Price, D. L.; Struble, R. G. *Science* **1982**, *215*, 1237.
3. Perry, E. K.; Perry, R. H.; Blessed, G.; Tomlinson, B. E. *Neuropath. Appl. Neuro.* **1978**, *4*, 273.
4. Gil-Bea, F. J.; Garcia-Alloza, M.; Dominguez, J.; Marcos, B.; Ramirez, M. J. *Neurosci. Lett.* **2005**, *375*, 37.
5. Mohs, R. C.; Doody, R. S.; Morris, J. C.; Ieni, J. R.; Rogers, S. L.; Perdomo, C. A.; Pratt, R. D. *Neurology* **2001**, *57*, 481.
6. Olin, J.; Schneider, L. *Cochrane database of systematic reviews* **2001**, *4*, CD001747.
7. Birks, J. *Cochrane Database of Systematic Reviews* **2006**, *1*, CD005593.
8. Carelli, V.; Liberatore, F.; Scipione, L.; Rotiroti, D.; Rispoli, V.; Cardellini M, Patent IT MI 2000 A000898 (2000), EP 1925883.(2001); Carelli, V.; Liberatore, F.; Scipione, L.; Rotiroti, D.; Rispoli, V.; Cardellini, M. *Chem. Abst.* **2001**, *135*, 344212m.
9. Rispoli, V.; Rotiroti, D.; Carelli, V.; Liberatore, F.; Scipione, L.; Marra, R.; Giorgioni, G.; Di Stefano, A. *Neurosci. Lett.* **2004**, *356*, 199.
10. Rispoli, V.; Rotiroti, D.; Carelli, V.; Liberatore, F.; Scipione, L.; Marra, R.; Tortorella, S.; Bi Rienzo, B. *Pharmacol. Biochem. Behav.* **2004**, *78*, 667.
11. Rispoli, V.; Marra, R.; Costa, N.; Scipione, L.; Rotiroti, D.; De Vita, D.; Liberatore, F.; Carelli, V. *Pharmacol. Biochem. Behav.* **2006**, *84*, 453.
12. Papke, R. L.; Bencherif, M.; Lippiello, P. *Neurosci. Lett.* **1996**, *213*, 201.
13. Alkondon, M.; Pereira, E. F. R.; Cortes, W. S.; Maelicke, A.; Albuquerque, E. X. *Eur. J. Neurosci.* **1997**, *9*, 2734.
14. Breese, C. R.; Adams, C.; Logel, J.; Drebing, C.; Rollins, Y.; Barnhart, M.; Sullivan, B.; Demasters, B. K.; Freedman, R.; Leonard, S. *J. Comp. Neurol.* **1997**, *387 3*, 385.
15. Davidson, M.; Zemishlany, J. H.; Mohs, R. C. *Biol. Psychiatry* **1988**, *23*, 485.
16. Smith, C. P.; Bores, G. M.; Petko, W.; Li, M.; Selk, D. E.; Rush, D. K.; Camacho, F.; Winslow, J. T.; Fishkin, R.; Cunningham, D. M.; Brooks, K. M.; Roehr, J.; Hartman, H. B.; Davis, L.; Vargas, H. M. *J. Pharmacol. Exp. Ther.* **1997**, *280*, 710.
17. Passive avoidance performance test was carried out according to the step-through method described by Giovannini (Behaviour Brain Research 1999, 104: 147–155). The time elapsed between the rat was placed in the light and it entered the dark compartment is recorded as Training Trial latency time (TT). Amnesia was induced in rats with scopolamine (0.5 mg/kg) given subcutaneously 1 h before Training Trial. Rats received intraperitoneally the compounds under study (4-AP, **3a**, **4a**, **4d**) or vehicle (saline) 30 min after scopolamine treatment. Compound **1** was given 15 min before Training Trial. In a second set of experiments **1** was combined with 4-AP following the same experimental protocol. Retention trial was performed 24 h later, following a similar procedure, except that the electric shock was not given. The latency time to enter the dark compartment was measured up to a cut off time of 180 s and indicated as Retention Trial latency time (RT). Experiments were done in compliance with the recommendations of the EEC 886/609/CEE and in accordance with the national laws (DL 116/92).
18. Youdim, M. B. H.; Buccafusco, J. J. *J. Neural. Transm.* **2005**, *112*, 519.
19. Compounds **3a–d** were prepared following a common procedure: 4-AP (5.2 mmol) was dissolved in 50 mL of anhydrous benzene, then 18.7 mmol of anhydrous TEA was added. The solution was kept under stirring at rt and 10 mL of benzene, containing 3.2 mmol of triphosgene, was added dropwise. The solution was refluxed for 5 h. The produced TEA HCl was filtered off, the solution was added with 5.2 mmol of freshly distilled 2-dimethylaminoethanol and stirred overnight. To the benzene solution, 50 mL of saturated Na_2CO_3 was added under stirring; the organic layer was separated and the alkaline solution was extracted with 5 portions (20 mL each) of chloroform. The recombined organic fractions were evaporated to give an oily residue which was chromatographed on silica gel column using 1:1 methanol/ethylacetate as eluent, collecting the product with lower R_f. Compounds **4a–d** were prepared following a common procedure: 3.0 mmol of compounds **3a–d** was dissolved in 30 mL of anhydrous ethanol and 6.0 mmol of iodomethane was added. The solution was stirred for 5–6 days at rt and then Et_2O was added (100 mL). The obtained yellow solid was separated and crystallized from MeOH/Et_2O. Compounds **5b–d** were prepared following a common procedure: compounds **3b–d** (1.0 mmol) were dissolved in 15 mL of anhydrous ethanol and cooled in an ice bath. The solution was saturated for 15 min with gaseous HCl and then 10 mL of Et_2O was added. The white crystalline precipitate was collected, washed with Et_2O and crystallized from MeOH/Et_2O. For all compounds elemental analyses are within 0.4%. ^1H, ^{13}C NMR and analytical data are reported in supplementary material.
20. Ellman, G. L.; Courtney, K. D.; Andres, V.; Featherstone, R. M. *Biochem. Pharmacol.* **1961**, *7*, 88.
21. Dixon, M. *Biochem. J.* **1953**, *55*, 170.
22. Recanatini, M.; Cavalli, A.; Belluti, F.; Piazzi, L.; Rampa, A.; Bisi, A.; Gobbi, S.; Valenti, P.; Andrisano, V.; Bartolini, M.; Cavrini, V. *J. Med. Chem.* **2000**, *43*, 2007.
23. Bartolini, M.; Bertucci, C.; Cavrini, V.; Andrisano, V. *Biochem. Pharmacol.* **2003**, *65*, 407.