Eur. J. Med. Chem. 35 (2000) 77−82

S0223523400001033/FLA

Original article

4-Aminopyridine derivatives with antiamnesic activity[#]

Aldo Andreani[a]*, Alberto Leoni[a], Alessandra Locatelli[a], Rita Morigi[a], Mirella Rambaldi[a], Claudio Pietra[b], Gino Villetti[b]

[a]Dipartimento di Scienze Farmaceutiche, Universita' di Bologna, Via Belmeloro 6, 40126 Bologna, Italy
[b]Chiesi Farmaceutici S.p.A., Via Palermo 26/A, 43100 Parma, Italy

Received 25 May 1999; revised 25 August 1999; accepted 7 September 1999

Abstract – Acetylcholine (Ach) enhancement, useful in the treatment of Alzheimer's disease (AD), may be obtained by means of ion channel modulators such as 4-aminopyridine (4-AP). 4-AP is also the central ring of tacrine, the first drug approved for the treatment of AD. The synthesis and pharmacological activity of three 4-AP derivatives, prepared with the aim of improving their antiamnesic activity, is here described. In two of these compounds 4-AP is connected to 4-aminobutyric acid (GABA), whereas in the third it is connected to 2-indolinone, i.e., the skeleton of linopirdine, another Ach enhancing agent. The new compounds showed potent antiamnesic activity in comparison with piracetam. © 2000 Édition scientifiques et médicales Elsevier SAS

4-aminopyridine / 4-aminobutyric acid / 2-indolinone / tacrine / linopirdine / antiamnesic activity / Alzheimer's disease

1. Introduction

The improvement of cholinergic transmission is a rational and well documented approach to the treatment of Alzheimer's disease (AD), which is considered the most common form of dementia. This target may be achieved with acetylcholine (Ach) precursors, muscarinic agonists or acetylcholinesterase inhibitors. The enhancement of Ach release leads to an increase in neuronal activity, thus restoring central cholinergic tone and improving attention and cognition. Besides acetylcholinesterase inhibitors, Ach enhancement may be obtained by modulating both ligand and voltage-gated (Ca^{++} and K^+) ion channels. Agents with this latter activity, such as the potassium channel modulators 4-aminopyridine (4-AP) [1] and linopirdine [2] (*figure 1*) have been clinically evaluated as Ach release enhancing agents. In AD patients, 4-AP, an A-type K^+ channel blocker, elicited inconsistent and unremarkable effects [3, 4].

On the other hand, tacrine (*figure 1*), the first drug approved by the FDA for the symptomatic treatment to mild cognitive disturbances associated with AD, may be considered a 4-AP derivative.

The disappointing clinical results of linopirdine, whose enhancement of Ach release correlates with its ability to block M-type K^+ channels, are probably related to its suboptimal pharmacokinetic profile.

On the basis of these considerations we designed and synthesized two 4-AP derivatives as potential antiamnesic agents. For the first one we planned to bind 4-AP to a molecule which could improve the diffusion into the brain and for this purpose we chose 4-aminobutyric acid (GABA). For the second one we selected 2-indolinone as the supporting moiety, since it is the skeleton of linopirdine. These two molecules and one precursor were subjected to an antiamnesic test (CO_2-induced amnesia in mice) in comparison to piracetam, the compound selected as a positive reference, whose antiamnesic activity in rodent species is well documented [5].

*Correspondence and reprints: aldoandr@alma.unibo.it
[#]Presented in part at the Italian-Hungarian-Polish Joint Meeting on Medicinal Chemistry, Giardini Naxos-Taormina, September 28th–October 1st 1999.

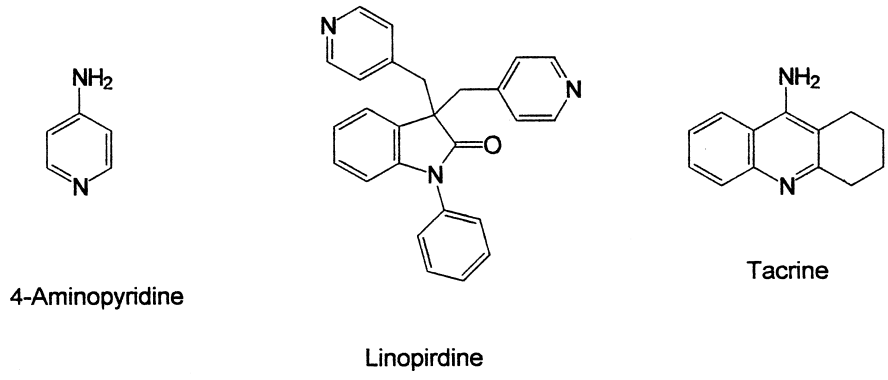

Figure 1. Structure of 4-aminopyridine, linopirdine and tacrine.

2. Chemistry

Compounds bearing 4-AP and GABA moieties (**2–3**, *figure 2*) were prepared from GABA, protected according to the literature method [6]. The reaction between 4-*tert*-butoxycarbonylamino-butyric acid **1** and 4-amino-pyridine in the presence of ethyl chloroformate gave [3-(pyridin-4-ylcarbamoyl)-propyl]carbamic acid *tert*-butyl ester **2**, which was easily deprotected under mild conditions to afford 4-amino-N-pyridin-4-yl-butyramide **3**. For the synthesis of 3-(4-aminopyridin-3-yl-methylene)1,3-dihydroindol-2-one **13**, 4-aminopyridine-3-carbaldehyde **12** was the starting material (*figure 3*). It is reported in the literature at least twice [7, 8], starting from 4-aminonicotinic acid hydrazide **11** which, in turn, may be prepared from 3-picoline-N-oxide **4** [7, 9]. These methods are very similar, we checked both of them and we modified the procedure according to *figure 3*. The most relevant difference from the reported methods is the oxidation of N-(3-methylpyridin-4-yl)acetamide **7**: we did not obtain directly the expected 4-aminonicotinic acid **9**, as reported, but its acetyl derivative **8** which could be converted into **9** by treatment with concentrated hydrochloric acid. Even with repeated attempts to optimize every step, the overall yield of the aldehyde **12** was very low. For this reason we studied another approach described in *figure 4*. We prepared dimethyl-[2-(4-nitro-1-oxypyridin-3-yl)vinyl]amine **14** by reaction of the activated methyl group of 3-methyl-4-nitropyridine 1-oxide **5** with N,N-dimethylformamide dimethylacetal. The enamine **14** was subjected to oxidation with $NaIO_4$ [10] to produce 4-nitro-1-oxypyridine-3-carbaldehyde **15** (*figure 4*). After hydrogenation of the nitro compound we obtained the expected aminoaldehyde **12** in good yield.

The reaction of **12** with 2-indolinone in the presence of piperidine gave only one isomer of **13** which was subjected to a series of NOE experiments. In the first one, the NH_2 group was irradiated (6.43 ppm) and NOE was observed at 6.66 ppm (py-5) and 7.48 ppm (CH). When py-2 was irradiated (8.35 ppm) the effect was evident at 7.33 ppm (ind-4) and a final irradiation at 7.33 ppm (ind-4) gave NOE at 6.85 ppm (ind-5) and 8.35 ppm (py-2). On the basis of these results we may conclude that compound **13** in our hands belongs to the E configuration.

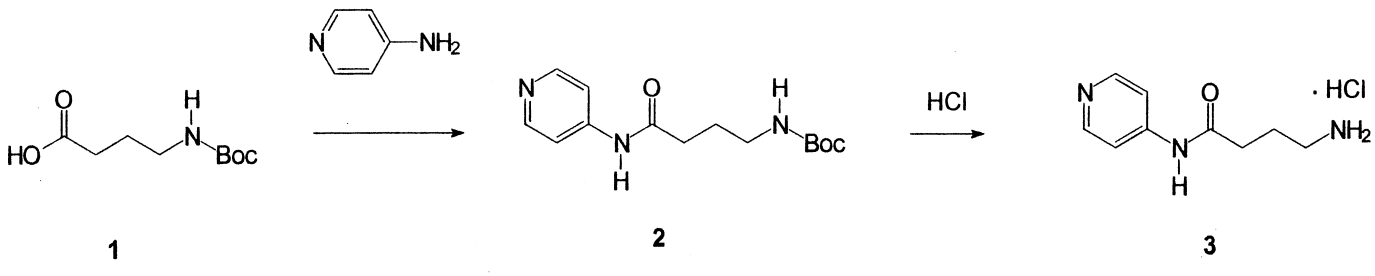

Figure 2. Synthesis of 4-amino-N-pyridin-4-yl-butyramide hydrochloride.

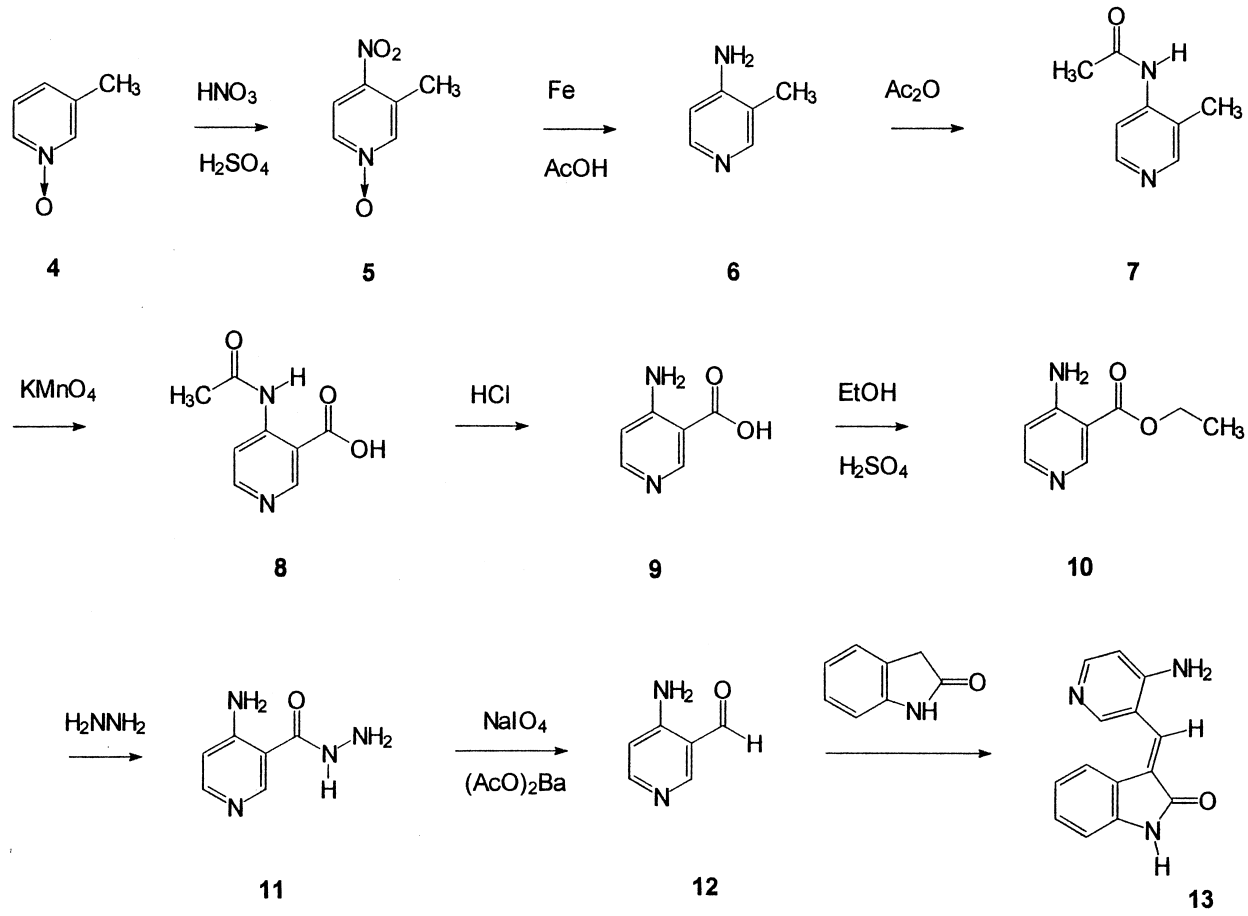

Figure 3. Synthesis of 3-(4-aminopyridin-3-ylmethylene)1,3-dihydroindol-2-one.

3. Pharmacological results and discussion

The effects of the synthesized compounds in antagonizing the CO_2-induced amnesia in mice are reported in *table I*. For comparison purposes, LD_{50} values and therapeutic indexes are also reported.

All 4-AP derivatives, compared to piracetam, showed an antiamnesic effect, but with different potencies. A significant ($P < 0.01$ vs. controls) activity was observed from 0.4, 107.4 and 42.1 μmol/kg p.o. after administration of **2**, **3** and **13**, respectively. Therefore, **2** was almost

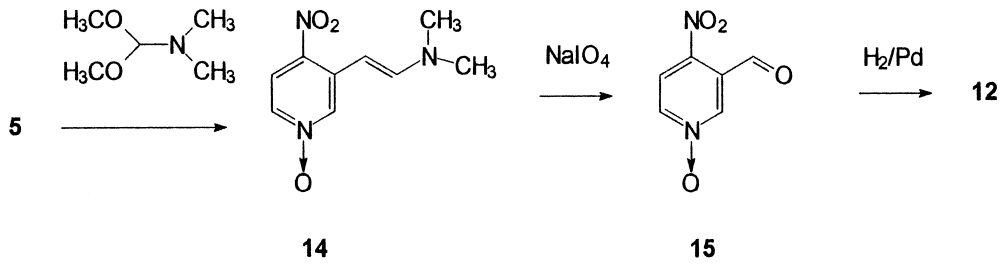

Figure 4. Alternative synthesis of the intermediate 4-aminopyridine-3-carbaldehyde.

Table I. Antiamnesic activity of compounds **2**, **3** and **13**.

Compound	Passive avoidance test		LD_{50} (μmol/kg p.o.)	Therapeutic index*
	Dose (μmol/kg p.o.)	Latency (% of control)		
2	0.4	+ 841**	87	73.10
	1.2	+ 3 748**		
	4.0	+ 2 823**		
3	10.7	+ 234	3 150	29.33
	35.8	+ 350		
	107.4	+ 2 386**		
13	12.6	+ 114	> 4 215	–
	42.1	+ 1 503**		
	126.4	+ 2 517**		
Piracetam	2 110	+ 1 723**	> 7 034	–

*: Ratio between LD_{50} and the dose inducing the maximal antiamnesic effect. **: $P < 0.01$ in comparison with the amnesic control group.

100–300-fold more potent than other compounds. The efficacy of the antiamnesic effects shown by **3** and **13** at their maximal dose was similar.

In acute toxicity studies, at doses higher than those endowed with antiamnesic activity, treatment of mice with **2** and **3** resulted in a typical behaviour of hyperexcitability followed by agitation, facial clonus, stretching and tonic convulsions followed by death. By contrast, the administration of **13** at 4 215 μmol/kg determined, in only 1 out of 5 mice, the appearance of the following behaviour: sedation, hypoactivity, palpebral closure, ataxia and dyspnea followed by death. Therefore, **13** was distinctly less toxic than other compounds. Due to this property it was impossible to calculate the LD_{50} value for this compound and, consequently, an appropriate therapeutic index.

The reference compound (piracetam at 2 110 μmol/kg) exhibited a significant antiamnesic effect, but the latency increase was lower than maximal values obtained after the administration of compounds **2**, **3** and **13**. Piracetam did not show a behavioural profile of hypolocomotion or ataxia. Also for this compound endowed with low toxicity, it was impossible to calculate an appropriate therapeutic index.

In conclusion, the new synthesized compounds showed a significant antiamnesic activity when compared to the reference drug piracetam. According to the pharmacology of the 4-aminopyridine derivatives, the activity of the compounds could also be due to an increase of the cerebral Ach, the primary neurotransmitter involved in cognitive processes. In fact, derivatives of the K^+ channel blocker linopirdine were reported to be active in increasing the rat brain Ach levels after oral administration when tested in microdialysis experiments [11]. Further studies of this type need to be performed in order to clarify whether compounds **2**, **3** and **13** also have a similar activity.

4. Experimental protocols

4.1. Chemistry

The melting points are uncorrected. Analyses (C, H, N) were within ± 0.4% of the theoretical values. TLC was performed on Bakerflex plates (Silica gel IB2-F): the eluent was a mixture of petroleum ether/acetone in various proportions. Kieselgel 60 (Merck) was used for column chromatography. The IR spectra were recorded in nujol on a Perkin-Elmer 683. The ^{1}H-NMR spectra were recorded in d_6-DMSO on a Varian Gemini (300 MHz), and were referenced to solvent signals. Chemical shifts are expressed in δ and *J* in Hz (py = pyridine, ind = indole). The mass spectra were recorded on a VG 7070E.

4.1.1. Synthesis of [3-(pyridin-4-ylcarbamoyl)-propyl] carbamic acid tert-butyl ester **2**

A mixture of 4-*tert*-butoxycarbonylamino-butyric acid **1** (1.5 g, 7.4 mmol) in THF (50 mL) and Et_3N (1 mL, 7.4 mmol) was cooled to –10 °C. Ice-cooled ClCOOEt (0.7 mL, 7.4 mmol) was added and stirring continued for 20 min at –10 °C. A solution of 4-aminopyridine (0.9 g, 9.6 mmol) and THF (25 mL) was then added and the heterogeneous mixture was allowed to gradually warm to room temperature and was stirred overnight. The crude product, obtained by evaporation under reduced pressure, was purified by column chromatography. Eluent: acetone/petroleum ether 1/1. A white solid was obtained (2.0 g, yield 97%). $C_{14}H_{21}N_3O_3$ (279.3), m.p. 145–149 °C. IR:

3 300–3 100, 1 660, 1 585, 1 290, 1 160 cm^{-1}. ^{1}H-NMR: 1.38 (9H, s, t-Bu), 1.70 (2H, m, CH_2–*CH_2*–CH_2), 2.36 (2H, m, CH_2–CO), 2.98 (2H, m, CH_2–N), 6.82 (1H, t, NH–CH_2), 7.57 (2H, d, py, *J* = 5), 8.41 (2H, d, py, *J* = 5), 10.29 (1H, s, CONH).

4.1.2. Synthesis of 4-amino-N-pyridin-4-yl-butyramide hydrochloride **3**

A solution of compound **2** (0.5 g, 1.8 mmol) in acetonitrile (20 mL) and HCl 3 N (5 mL) was stirred at room temperature for 36 h. The suspension was filtered and the precipitate crystallized (methanol/acetonitrile) to obtain a white solid (0.25 g, yield 63%). $C_9H_{14}ClN_3O$ (215.7), m.p. 267–270 °C dec. IR: 3 500–3 000, 1 700, 1 600, 1 510, 1 175 cm^{-1}. ^{1}H-NMR: 1.92 (2H, m, CH_2–*CH_2*–CH_2), 2.69 (2H, m, CH_2CO), 2.83 (2H, m, CH_2–N), 8.15 (3H, m, NH_3^+), 8.18 (2H, d, py, *J* = 6.9), 8.70 (2H, d, py, *J* = 6.9), 12.15 (1H, s, NH).

4.1.3. Synthesis of 4-acetylamino-nicotinic acid **8**

To a suspension of N-(3-methylpyridin-4-yl)acetamide **7** (10 g, 66.6 mmol) in water (200 mL) was added $KMnO_4$ (15 g, 94.9 mmol). The addition was made in small portions at 0 °C. The suspension was stirred for 2 h at room temperature, heated for 5 h at 70 °C and then filtered. The precipitate was washed with hot water. The solution was concentrated to a small volume (100 mL) and treated at 0 °C with HCl 12 N until pH 2 was reached. After 10 min the suspension was filtered to obtain a white solid (2.5 g, yield 20%). $C_8H_8N_2O_3$ (180.2), m.p. 247–250 °C dec. IR: 3 470, 1 700, 1 645, 1 365, 1 210 cm^{-1}. ^{1}H-NMR: 2.19 (3H, s, CH_3), 8.46 (1H, d, py-5), 8.58 (1H, m, py-6), 9.00 (1H, s, py-2), 11.92 (1H, broad s, NH). MS: 180 (M^+, 62), 138 (82), 120 (100), 43 (84).

4.1.4. Synthesis of 4-aminonicotinic acid **9**

A suspension of compound **8** (3.7 g, 20.5 mmol) in water (60 mL) was treated with HCl 37% (3 mL) and heated to reflux for 6 h. After cooling, the solution was concentrated to a small volume (30 mL) and NH_4OH (30%) was added at 0 °C until pH 5–6 was reached. After 30 min the white solid obtained was filtered (1.8 g, yield 63%, m.p. 330–333 °C dec.: lit. [7] m.p. 335–336 °C dec.).

4.1.5. Synthesis of 4-aminopyridine-3-carbaldehyde **12**

Methanol (40 mL) was mixed with 10% palladium on charcoal (0.03 g) and 4-nitro-1-oxypyridine-3-carbaldehyde **15** (0.7 g, 4.16 mmol) was added. The mixture was hydrogenated at atmospheric pressure and room temperature. After the calculated amount of hydrogen was taken up, the catalyst was removed by filtration and methanol was evaporated under reduced pressure. Chromatography of the residue on a silica gel column using methanol as the eluent led, after evaporation of the appropriate fraction, to the isolation of a white solid (0.3 g, yield 59%) m.p. 108–110 °C: lit. 113–114 °C [7], 110–112 °C [8].

4.1.6. Synthesis of 3-(4-aminopyridin-3-ylmethylene)1,3-dihydroindol-2-one **13**

A mixture of 2-indolinone (0.1 g, 0.75 mmol), compound **12** (0.092 g, 0.7 mmol), methanol (20 mL) and piperidine (0.1 mL) was refluxed for 5 h. After cooling at room temperature the precipitate thus formed was removed by filtration to obtain a yellow solid (0.1 g, yield 55%). $C_{14}H_{11}N_3O$ (237.2), m.p. 229–231 °C dec. IR: 3 500–2 500, 1 685, 1 600, 810, 720 cm^{-1}. ^{1}H-NMR: 6.43 (2H, s, NH_2), 6.66 (1H, d, py-5, *J* = 5.8), 6.85 (1H, t, ind-5, *J* = 7), 6.87 (1H, d, ind-7, *J* = 7), 7.21 (1H, t, ind-6, *J* = 7), 7.33 (1H, d, ind-4, *J* = 7), 7.48 (1H, s, CH), 8.04 (1H, d, py-6, *J* = 5.8), 8.35 (1H, s, py-2), 10.57 (1H, s, NH).

4.1.7. Synthesis of dimethyl-[2-(4-nitro-1-oxypyridin-3-yl)vinyl]amine **14**

A solution of 3-methyl-4-nitropyridine-1-oxide **5** (6 g, 38.9 mmol), dry DMF (30 mL) and N,N-dimethylformamide dimethylacetal (4 mL, 46.7 mmol), was warmed to 150 °C for 3 h under nitrogen. After cooling at room temperature the suspension was diluted with water (20 mL). A bright brown solid was obtained by filtration (4 g, yield 49%). $C_9H_{11}N_3O_3$ (209.2), m.p. 207–210 °C dec. IR: 1 585, 1 530, 1 220, 1 090, 1 060 cm^{-1}. ^{1}H-NMR: 2.97 (6H, s, CH_3), 5.84 (1H, d, CH, *J* = 13), 7.60 (1H, dd, py-6, *J* = 1.8, *J* = 7.2), 7.85 (1H, d, CH, *J* = 13), 7.89 (1H, d, py-5, *J* = 7.2), 8.67 (1H, d, py-2, *J* = 1.8). MS: 209 (M^+, 85), 86 (66), 53 (67), 42 (100).

4.1.8. Synthesis of 4-nitro-1-oxypyridine-3-carbaldehyde **15**

Dimethyl-[2-(4-nitro-1-oxypyridin-3-yl)vinyl]amine **14** (4 g, 19.1 mmol) and $NaIO_4$ (12 g, 56.1 mmol) were mechanically stirred in 50% aqueous THF (200 mL) at room temperature until the reaction was judged complete by TLC. The insolubles were removed and the filtrate was extracted with ethyl acetate. The organic layer was dried over Na_2SO_4 and concentrated. The crude product was purified by column chromatography (eluent: acetone/petroleum ether 2/8). A yellow solid was obtained (2 g, yield 62%). $C_6H_4N_2O_4$ (168.1), m.p. 154–155 °C dec. IR: 1 690, 1 505, 1 290, 1 245, 1 080 cm^{-1}. ^{1}H-NMR: 8.24 (1H, d, py-5, *J* = 7.1), 8.45 (1H, d, py-2, *J* = 2), 8.58 (1H, dd, py-6, *J* = 2, *J* = 7.1), 10.26 (1H, s, CHO). MS: 168 (M^+, 75), 121 (7), 110 (34), 63 (100).

4.2. Pharmacology

4.2.1. Acute toxicity

For acute toxicity in mice, groups of 5 male Crl:CD-1 (ICR)BR mice (Charles River, Italy) weighing 21–30 g were used. Mice fasted for 16 h were treated orally with various doses of the compounds and observed for 1 week after treatment. Deaths were recorded daily. The maximum dose administered, for compounds not showing clear signs of toxicity, was referred to the value of 1 000 mg/kg p.o.

The median lethal doses (LD_{50}) were then calculated according to the method described by Litchfield and Wilcoxon [12].

4.2.2. Effects on CO_2-induced memory impairment of passive avoidance task in mice

Hypercapnia, as well as hypoxia or ischaemia, is a condition in which the reduction of the energy supply to the brain results in an amnesia or in a learning deficit [13]. It has been reported that the CO_2-induced memory impairment might be a manifestation of a transient cerebral ischaemia [14]. Therefore, the above method can be considered in evaluating new compounds that might improve cognitive deficiencies due to cerebral insufficiencies.

In our experimental conditions we used the method described by Van Eys et al. [15] with some modifications. Groups of 10 male Crl:CD-1 (ICR)BR mice (Charles River, UK) weighing 19–25 g were used. The one-trial passive avoidance apparatus consisted of a 16 × 15 × 15 cm chamber with opaque walls and a metal grid floor. A 4 cm wide, 11.5 cm long runway protruded from the front wall of the chamber. The runway was illuminated, whereas the chamber was dark. When placed on the runway, a mouse could enter the chamber through a 3 × 3 cm opening. A scrambled footshock could be delivered through the metal grid floor of the chamber. On day 1 of the experiment each mouse was subjected to three training trials and could visit the chamber without receiving the footshock. Mice were fasted overnight prior to the experiment with water available ad libitum. On day 2 of the experiment mice were dosed orally with vehicle or tested compounds and 1 h after the acquisition trial was performed. During this trial mice received a footshock of 0.25 mA for 10 s, beginning 10 s after entering the chamber. Immediately after application of the footshock, mice were put in a box filled with carbon dioxide until respiratory arrest occurred; mice were then revived by artificial respiration using oxygen. Twenty-four hours after the acquisition trial, a single retrieval trial was given to each mouse and the step-through latency determined. If a mouse did not enter the chamber within 180 s it was removed from the runway and a score of 3 min was recorded. The retrieval times (the times on day 3 of the experiment) were submitted to a Kruskall-Wallis analysis of ranks. Other relevant comparisons between treatments and controls were made using Student's *t* distribution.

Acknowledgements

This work was supported by the University of Bologna (funds for selected research topics).

References

[1] Thesleff S., Neuroscience 5 (1980) 1413–1419.

[2] Maciag C.M., Logue A.R., Tinker W.J., Saydoff J.A., Tam S.W., Zaczek R., J. Pharmacol. Exp. Ther. 271 (1994) 891–897.

[3] Wesseling H., Agoston S., Van Dam G.B., Pasma J., DeWit D.J., Havinga H., N. Engl. J. Med. 310 (1984) 988–989.

[4] Davidson M., Zemishlany Z., Mohs R.C., Horvath T.B., Powchik P., Blass J.P., Davis K.L., Biol. Psychiatry 23 (1988) 485–490.

[5] Lenegre A., Chermat R., Avril I., Steru L., Porsolt R.D., Pharmacol. Biochem. Behav. 29 (1988) 625–629.

[6] Houssin R., Bernier J.L., Hénichart J.P., Synthesis (1988) 259–261.

[7] Armarego W.L.F., J. Chem. Soc. (1962) 4094–4103.

[8] Hawes E.M., Gorecki D.K.J., J. Heterocycl. Chem. 9 (1972) 703–706.

[9] Taylor E.C., Crovetti A.J., J. Org. Chem. 19 (1954) 1633–1640.

[10] Vetelino M.G., Coe J.W., Tetrahedron Lett. 35 (1994) 219–222.

[11] Earl R.A., Zaczek R., Teleha C.A., Fisher B.N., Maciag C.M., Marynowski M.E. et al., J. Med. Chem. 41 (1998) 4615–4622.

[12] Litchfield Jr. J.T., Wilcoxon F., J. Pharmacol. Exp. Ther. 96 (1949) 99–113.

[13] Lewis J.D., Psycol. Rev. 76 (1969) 461–472.

[14] Jensen T.S., De Fine Olivarius B., Acta Neurol. Scand. 61 (1980) 115–124.

[15] Van Eys G., Rigter H., Leonard B.E., Pharmacol. Biochem. Behav. 3 (1975) 787–793.