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2-Pyrrolidinone moiety is not critical for the cognition-enhancing activity of piracetam-like drugs

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Abstract

Following the indications of previous work, 2-pyrrolidinone moiety of piracetam and piracetam-like compounds has been opened to the corresponding amide derivatives. As found previously in the case of 1,4-diazabicyclo[4.3.0]nonan-9-one compounds, the cognition-enhancing activity of 2-pyrrolidinone compounds is maintained in most cases, suggesting that this moiety is not crucial for activity.

Keywords: 2-Pyrrolidinone; Moiety; Cognition-enhancing activity; Piracetam-like drugs

1. Introduction

Cognitive properties of piracetam were disclosed in 1967 [1] and after that a number of structurally related molecules were found to be endowed with a similar pharmacological profile. This class of cognition-enhancing drugs is often referred to as nootropics and it has been thoroughly reviewed [2–5].

Piracetam-like nootropics revert amnesia induced by scopolamine and other amnesing drugs, electroconvulsive shock and hypoxia with an unknown mechanism. In general, they show no affinity for the most important central receptors ($Ka > 10$ μM), but are able to modulate the action of most central neurotransmitters, in particular acetylcholine [6,7] and glutamate [8]. A great deal of different biochemical and behavioural findings have been presented for piracetam-like nootropics, but so far, they have been unable to indicate a common molecular mechanism of action. On the other hand, generalisation is made difficult by the fact that, quite often, piracetam-like nootropics do not share the same behaviour on many biological assays [9,10].

This has been a major problem in accepting them as cognition enhancers, even though some of them have been found effective in clinical trials [11,12] and show excellent tolerability and safety [13]. Nevertheless, a few have been introduced in therapy (piracetam, oxiracetam, aniracetam and pramiracetam) and others are currently in development to treat cognitive disorders (nebracetam, nefiracetam and fasoracetam) [5].

The lack of a common mechanism at a molecular level allows sound structure–activity correlations only with in vivo behavioural assays, leading to frustrating consequences in drug design. In fact, the resulting activity is the consequence of both pharmacokinetic and pharmacodynamic properties that may be differently affected by structural modifications. Moreover, the protocols for behavioural tasks that are commonly used vary widely between investigators [14], making general comparison of the results difficult. This fact limits the significance of the models of the pharmacophore that has been proposed for piracetam-like compounds [15]. However, most of the compounds containing a 2-oxopyrrolidine structure do present cognition-enhancing activity, suggesting that this feature plays a critical role.

Recently, we have described a new class of compounds with a 1,4-diazabicyclo[4.3.0]nonan-9-one struc-

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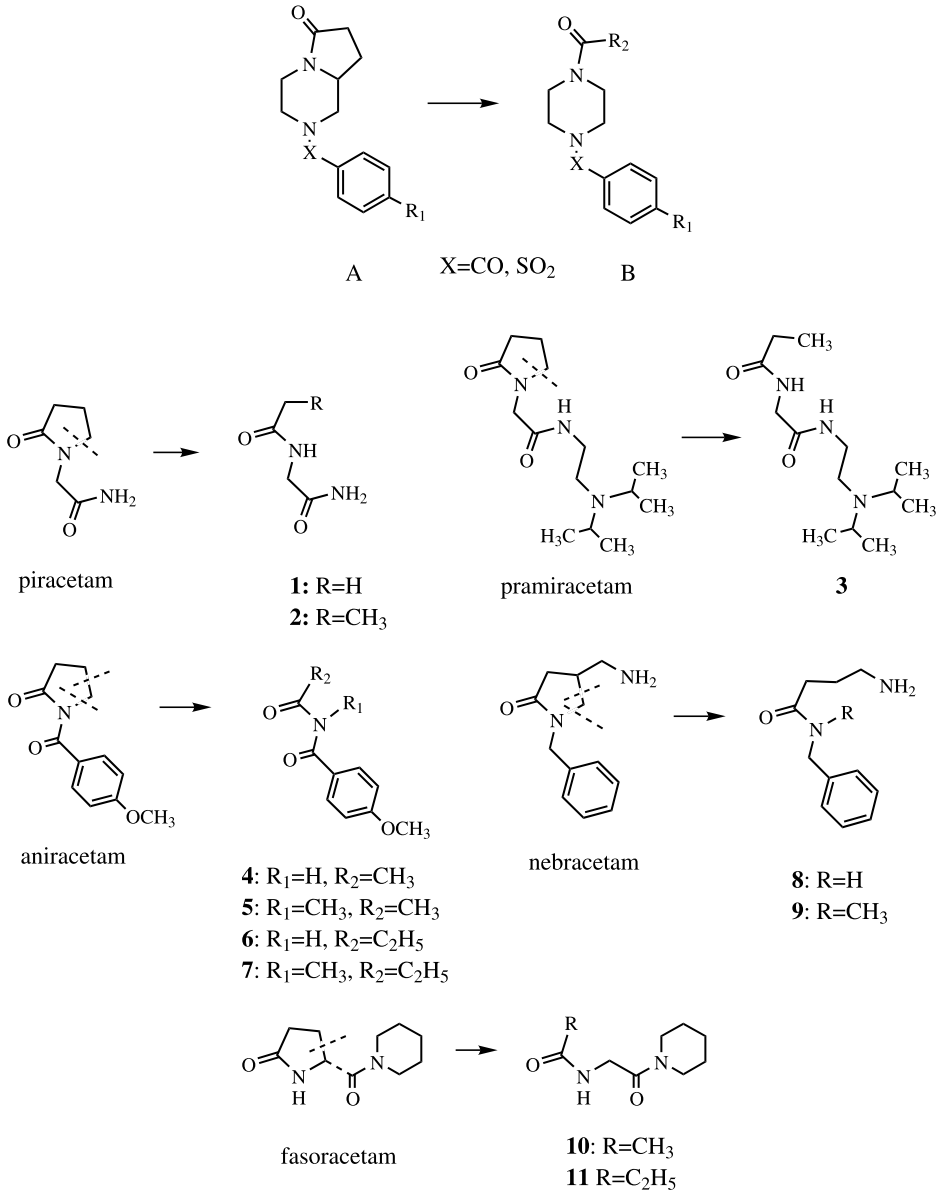


Plate 1.

ture [16] (Plate 1A) that showed a very potent cognition-enhancing activity on mouse-passive-avoidance assay. High activity was maintained when the pyrrolidinone ring present in this class of compounds was opened to give the corresponding piperazine derivatives [17] (Plate 1B), suggesting that in this class of compounds 2-oxopyrrolidine moiety is not critical for pharmacological action.

Continuing our work in this field, we started research to verify if these findings applied also to other piracetam-like compounds. Therefore, we have chosen some of the most widely used and studied compounds of this class and have prepared and studied their *seco* derivatives, i.e., the analogues where the pyrrolidinone nucleus has been opened at the level of bonds 1–5 (piracetam and pramiracetam), 4–5 (nebracetam and fasoracetam) or both (nebracetam and aniracetam) of the pyrrole nucleus. Formally, in the first case, cleavage would produce a butyrylamide. However, since we found [17] that increasing the acyl chain produced less potent and less soluble compounds, we have limited the choice to acetyl and propionyl derivatives.

The compounds studied and their relationships with the parent drug are indicated in Plate 1. To the best of our knowledge, there is only one poorly characterised example of a piracetam-like nootropic where the pyrrolidinone nucleus has been opened in a similar way: aloracetam [5,18]. On the other hand, it is well known that aniracetam's main metabolite, 4-(anysoylamino)butyric acid, maintains nootropic properties, although

with lower potency [19,20]. However, in this case the cleavage of the pyrrolidinone ring occurred at a different site.

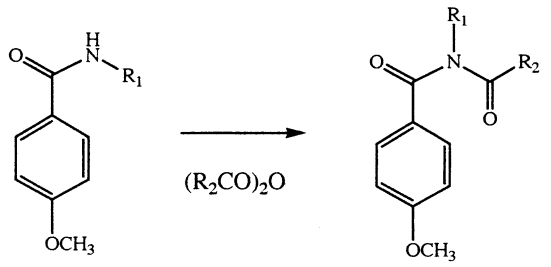
2. Chemistry

Compound **1** is commercially available. Compounds **2**, **3**, as well as **11**, were synthesised, as reported in Scheme 1, by amidation of the methyl ester of propionylaminoacetic acid **12**, obtained from glycine methyl ester and propionic acid anhydride, following the procedure described by Applewhite and Nieman [21] for a similar compound.

Compounds **4**–**7** were obtained as reported in Scheme 2. Of them, **4** [22] and **5** [23] have been previously described, while **6** and **7** were obtained starting from commercially available 4-methoxybenzamide and 4-methoxy-*N*-methylbenzamide [22], respectively.

Compounds **8** and **9** were obtained as reported in Scheme 3. 4-Amino-*N*-benzylbutyramide (**8**) was obtained from benzyl-pyrrolidin-2-ylidene-amine (**16**) [24]. However, under the conditions (H_2O, room temperature) reported in the literature [25], hydrolysis did not occur and 1 equiv. of NaOH at 80 °C was necessary to obtain the final product. Compound **9** was obtained from compound **15**, obtained from γ-butyrolactone and benzylmethylamine, through standard methods.

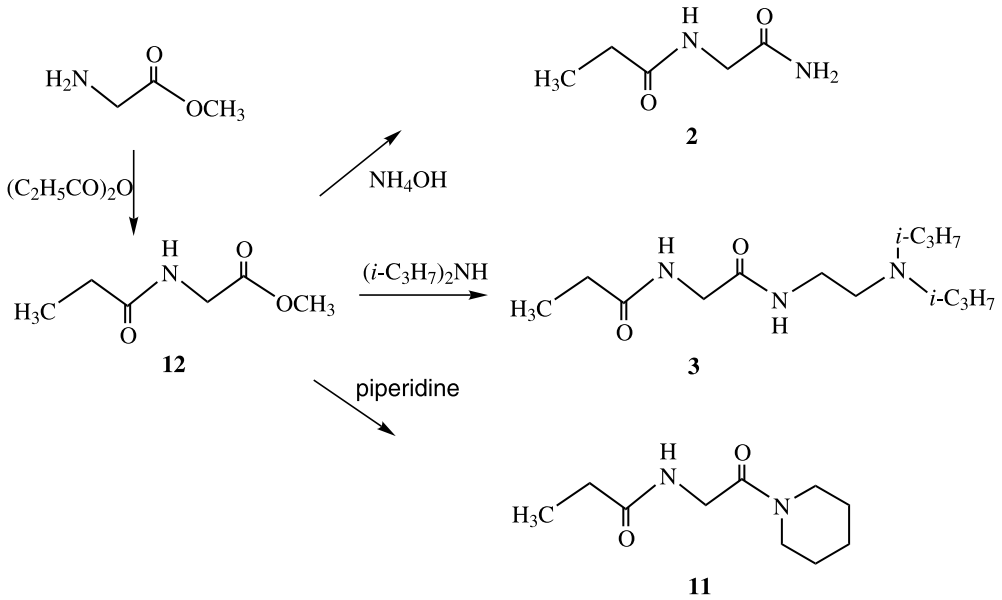
Finally, compound **10** was obtained starting from acetylglycine and piperidine according to the literature [26].



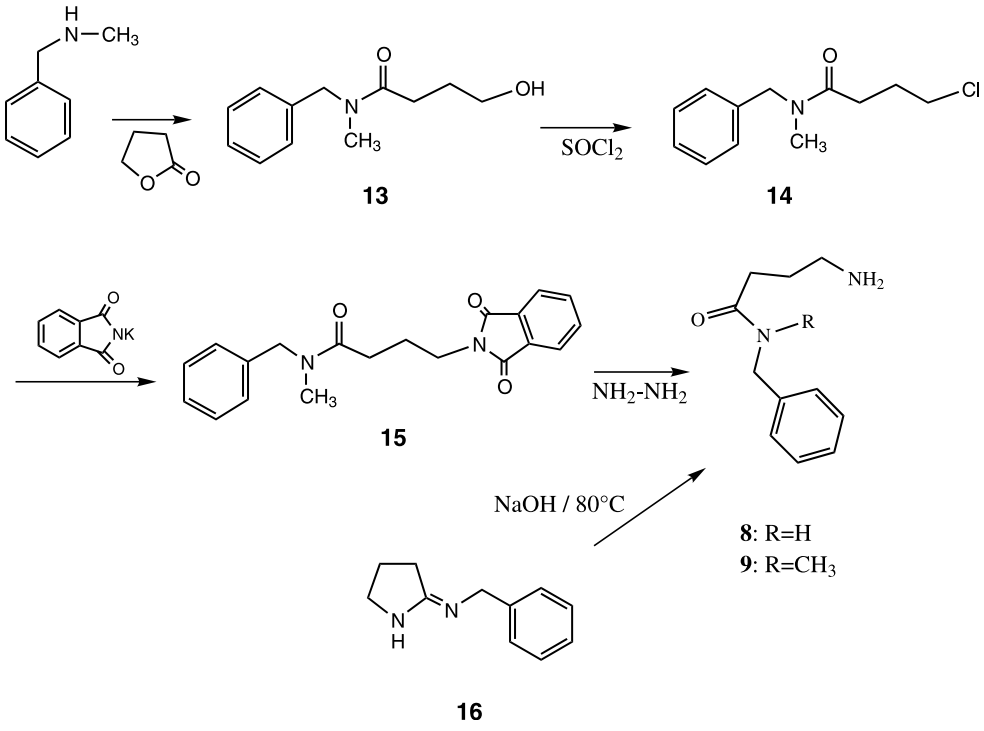
Scheme 2.

3. Pharmacology

The compounds studied were tested as cognition enhancers in the mouse-passive-avoidance test of Jarvik and Kopp [27], slightly modified by us (see Section 5). In short, mice receive a punishment when entering a dark room in the training session and remember it in the session on the following day, unless their memory is impaired by the amnesic drug. The parameter measured is the entry latency time (expressed in seconds) occurring between the time the mouse is placed in the light and the time it enters the dark room. On the first day, there is the training session, while on the second day the mice are placed again in the light and the new latency time is measured on animals treated or untreated with the nootropic drug. Investigated drugs were injected, in a 1–10 dilution sequence, 20 min before the training session, while amnesic drug was injected immediately after



Scheme 1.



Scheme 3.

termination of the training session. Scopolamine hydrobromide (i.p. 1.5 mg/kg) was used as amnesic drug and piracetam, pramiracetam, aniracetam, nebracetam and fasoracetam as reference drugs. Comparison of the latency times of saline-treated animals with those of mice that received both scopolamine and the investigated drug gives a measure of the cognition activity of the compounds tested. As a rule the compounds were injected intraperitoneally (i.p.), however, to compare the data with the literature some of them have also been administered per os (p.o.). The highest active doses of the investigated compounds did not altered animals' gross behaviour and were devoid of any behavioural side effect, as demonstrated by Irwing test [28].

4. Results and discussion

The results obtained performing the mouse-passive-avoidance test are reported in Table 1, in comparison with the results obtained with the parent compounds. Since some of the reference compounds were quite insoluble in physiological solution, they were given per os (p.o.). When necessary, new compounds have also been tested in this way to make a comparison possible.

Examining the results reported, it can be observed that **3** is less active than its parent compound pramiracetam and that nebracetam-derived **8** shows the same behaviour. On the contrary, compound **9** seems equipotent with the parent drug. This seems to be the case also for fasoracetam derivatives: compound **10** is much less potent, but compound **11**, when given p.o., maintains most of the potency of the parent drug, thus showing a remarkable potency (1 mg/kg) when given i.p. Also in the case of the derivatives of aniracetam, we obtained mixed results: compounds **6** and **7** are less potent, while compounds **4** and **5** are more potent than the parent drug. Particularly interesting is compound **4**, which is active at doses of 1 mg/kg i.p. and 3 mg/kg p.o., some 30 times lower than that of the parent compound.

Both derivatives of piracetam maintain the nootropic activity on passive-avoidance test, while **2** is equipotent with piracetam, compound **1** is quite more potent being active at the dose of 1 mg/kg i.p.

In general, as was found in the simplification of 1,4-diazabicyclo[4.3.0]nonan-9-ones [16] to the corresponding piperazine derivatives [17], even for the classical piracetam-like nootropics the cleavage of 2-pyrrolidinone ring is not critical for the nootropic activity. As a matter of fact, in the *seco* derivatives, in most cases the potency of the parent compounds is maintained or even improved.

As regards 2-pyrrolidinone bond that is cleaved, two of the most potent compounds (**1** and **4**) derived from the opening of the N–C bond 1–5. On the other hand, compounds **5** and **11**, that at least maintain the potency of parent compounds, were formally obtained by the opening of the C–C bond 4–5. In general, it can be said that 2-pyrrolidinone ring can be replaced by a linear amide and that an NH group is more useful than an N–CH_3 for high potency in the passive-avoidance test.

Table 1
Nootropic effect of compounds **1**–**11** on mouse-passive-avoidance test, using scopolamine (S) as amnesing drug

Reference/drug	Drug (number of animals)	Minimal effective dose mg kg^{-1} (p.o.)	Minimal effective dose mg kg^{-1} (i.p.)	Entry latency		
				1st day	2nd day	*Δ*
	Saline (15)		–	14.4±4.1	106.5±10.3 *	92.1
	Scopolamine (20)		1.5	13.9±3.2	51.7±7.4 **	37.8
Piracetam (P)	S+P (56)	–	30	15.9±2.1	105.3±7.8 ***	89.4
1	S+**1** (11)	–	1.0	20.6±3.8	104.0±8.7 ***	83.4
2	S+**2** (16)	> 10	30	14.9±4.1	87.8±9.6 ***	72.4
Pramiracetam	–	–	100 [a]	–	–	–
3	S+**3** (11)	–	> 100	–	–	–
	S+**3** (11)	> 100	–	–	–	–
Aniracetam (A)	S+A (31)	100	[b]	15.4±3.6	102.7±7.5 ***	87.1
4	S+**4** (18)	–	1.0	13.6±3.2	97.5±9.5 ***	83.9
	S+**4** (18)	3	–	16.0±2.2	89.8±9.5 ***	73.8
5	S+**5** (10)	–	10	14.5±4.3	98.4±8.5 ***	83.9
	S+**5** (16)	30		16.3±2.6	95.3±9.9 ***	79.0
6	S+**6** (12)	–	30	15.3±3.8	96.2±9.5 ***	80.9
	S+**6** (13)	> 100	–	–	–	–
7	S+**7** (15)	–	30	13.9±4.5	95.1±7.7 ***	81.2
	S+**7** (16)	> 100	–	–	–	–
Nebracetam (N)	S+**N** (12)	30		15.5±3.7	91.2±9.1 ***	75.7
	S+**N** (15)	–	10	15.0±3.8	95.2±7.5 ***	80.2
8	S+**8**(13)	–	> 10	–	–	–
	S+**8** (16)	> 30	–	–	–	–
9	S+**9** (20)	–	> 10	–	–	–
	S+**9** (20)	30	–	16.9±3.0	92.5±11.3 ***	75.6
Fasoracetam (F)	S+**F** (15)	3 [c]	[b]	15.0±3.2	97.3±9.0 ***	82.3
10	S+**10** (10)		> 10	–	–	–
	S+**10** (15)	> 30	–	–	–	–
11	S+**11** (11)	–	1.0	12.9±4.1	95.7±10.3 ***	82.8
	S+**11** (18)	10	–	13.6±4.2	95.3±8.5 ***	81.7

[a] See Ref. [30].
[b] The compound cannot be tested i.p. for solubility problems.
[c] See also Ref. [29].
* $P < 0.01$ with respect to mice treated with saline.
** $P < 0.05$ with respect to mice treated with scopolamine.
*** $P < 0.01$ with respect to mice treated with scopolamine.

In conclusion, considering the present results, as well as those previously reported [16,17], we have shown that 2-pyrrolidinone structure is not critical for nootropic activity of piracetam-like compounds.

5. Experimental

5.1. Chemistry

All melting points were taken on a Büchi apparatus and are uncorrected. Infrared spectra were recorded with a Perkin–Elmer 681 spectrophotometer in Nujol mull for solids and neat for liquids. Unless otherwise stated, NMR spectra were recorded on a Gemini 200 spectrometer (200 MHz for ^{1}H NMR and 50.3 MHz for ^{13}C), and chromatographic separations were performed on a silica gel column by gravity chromatography (Kieselgel 40, 0.063–0.200 mm; Merck) or flash chromatography (Kieselgel 40, 0.040–0.063 mm; Merck). The composition of the eluting systems was the following: WE1 = NH_4OH:abs. EtOH:$CHCl_3$:petrol ether, 8:65:340:60. WE2 = NH_4OH:abs. EtOH:CH_2Cl_2:Et_2O:-petrol ether, 9.9:180:360:360:900. WE4 = NH_4OH:abs. EtOH:CH_2Cl_2:petrol ether, 45:225:600:90. WE7 = NH_4OH:abs. EtOH:CH_2Cl_2:Et_2O:petrol ether, 25:45:180:180:450. Yields are given after purification, unless differently stated. Where analyses are indicated by symbols, the analytical results are within ±0.4% of the theoretical values.

5.2. General procedure for the synthesis of compounds *2*, *3* and *11*

Compound **12** (1 equiv.), dissolved in the opportune solvent (EtOH for compounds **2** and **3** and MeOH for

11), was added with an excess of appropriate amine, and a catalytic amount of NH_4Cl, in a steel bomb at 110 °C for 24 h. The solvent was eliminated under reduced pressure and the residue treated with water and extracted with $CHCl_3$. The organic layer was made anhydrous and evaporated under reduced pressure to give a mixture that was purified by column chromatography with the suitable eluent.

*5.2.1. N-Carbamoylmethylpropionamide (**2**)*

30% yield. m.p. 137–141 °C. Eluent WE_4. ^1H NMR (D_2O) δ 0.96 (t, 3H, CH_3CH_2, $J = 8.0$ Hz), 2.17 (q, 2H, CH_3CH_2, $J = 8.0$ Hz), 3.70 (s, 2H, $NHCH_2$) ppm. ^{13}C NMR (D_2O) δ 8.99 (q, CH_3CH_2, $^1J = 127.1$ Hz), 28.6 (t, CH_3CH_2, $^1J = 130$ Hz), 41.86 (t, $NHCH_2$, $^1J = 138.4$ Hz), 174.22 (s, CH_2CO), 178.48 (s, $CONH_2$) ppm. Anal. $C_5H_{10}N_2O_2$.

*5.2.2. N-[(2-Diisopropylaminoethylcarbamoyl)methyl]propionamide (**3**)*

26% yield. m.p. 45–49 °C. Eluent WE_4. ^1H NMR ($CDCl_3$) δ 0.95 (d, 12H, $CH(CH_3)_2$, $^1J = 6.0$ Hz), 1.12 (t, 3H, CH_3CH_2, $^1J = 8.0$ Hz), 2.23 (q, 2H, CH_2CH_3, $J = 8.0$ Hz), 2.52 (t, 2H, CH_2CH_2N, $J = 6.0$ Hz), 2.90–3.00 (m, 2H, $CH(CH_3)_2$), 3.18 (t, 0.5 2H, CH_2NH), 3.20 (t, 0.5 2H, CH_2NH), 3.86 (s, 0.5 2H, $NHCH_2CO$), 3.88 (s, 0.5 2H, $NHCH_2CO$), 7.80 (bs, 1H, *NH*) ppm. ^{13}C NMR ($CDCl_3$) δ 10.08 (q, CH_3CH_2, $^1J = 127.45$ Hz), 21.12 (q, $CH(CH_3)_2$, $^1J = 124.75$ Hz), 29.65 (t, CH_3CH_2, $^1J = 125.65$ Hz), 39.07 (t, CH_2NH, $^1J = 138.4$ Hz), 43.53 (t, CH_2CH_2NH, $^1J = 138.4$ Hz), 48.34 (d, $CH(CH_3)_2$, $^1J = 132$ Hz), 169.23 (s, CH_3CH_2CO), 174.62 (s, *CO*NH) ppm. Anal. $C_{13}H_{27}N_3O_2$.

*5.2.3. N-(2-Oxo-2-piperidin-1-yl-ethyl)propionamide (**11**)*

50% yield. m.p. 84–86 °C. Eluent WE_1 ^1H NMR ($CDCl_3$) δ 1.13 (t, 3H, CH_3CH_2, $J = 8.0$ Hz), 1.53–1.62 (m, 6H, CH_2 piper.), 2.24 (q, 2H, CH_2CH_3, $J = 8.0$ Hz), 3.30 (t, 2H, CH_2 piper., $J = 6.0$ Hz), 3.35 (t, 2H, CH_2 piper., $J = 6.0$ Hz), 3.98 (s, 0.5 2H, CH_2NH), 3.99 (s, 0.5 2H, CH_2NH), 6.69 (bs, 1H, *NH*) ppm. ^{13}C NMR ($CDCl_3$) δ 10.23 (q, CH_3CH_2, $^1J = 127.45$ Hz), 24.74 (t, CH_2 piper., $^1J = 128.5$ Hz), 25.80 (t, CH_2 piper., $^1J = 127.8$ Hz), 26.54 (t, CH_2 piper., $^1J = 127.7$ Hz), 29.87 (t, CH_3CH_2, $^1J = 126.5$ Hz), 41.6 (t, CH_2 piper., $^1J = 140$ Hz), 43.53 (t, CH_2 piper., $^1J = 134.7$ Hz), 45.82 (t, CH_2NH, $^1J = 135.3$ Hz), 166.46 (s, *CO*NH), 174.18 (s, *CO*N) ppm. Anal. $C_{10}H_{18}N_2O_2$.

*5.3. 4-Methoxy-N-propionylbenzamide (**6**)*

An excess of propionic anhydride and a catalytic amount of H_2SO_4 concentrated were added to 1 equiv. of commercially available 4-methoxybenzamide. The mixture was refluxed under stirring for 3 h. Then the reaction was cooled and a solution of 5% $NaHCO_3$ was added. The solid formed was filtered off and re-crystallised from EtOH; 63% yield. m.p. 109–111 °C. ^1H NMR ($CDCl_3$) δ 1.21 (t, 3H, CH_2CH_3, $^1J = 7.4$ Hz), 3.03 (q, 2H, CH_2CH_3, $^1J = 7.4$ Hz), 3.88 (s, 3H, OCH_3), 6.98 (d, 2H, *CH* arom., $^2J = 8.8$ Hz), 7.85 (d, 2H, *CH* arom., $^2J = 8.8$ Hz) ppm. ^{13}C NMR ($CDCl_3$) δ 8.73 (q, CH_3CH_2, $J = 127.45$ Hz), 31.53 (t, CH_2CH_3, $^1J = 124.75$ Hz), 55.95 (q, OCH_3, $^1J = 143.9$ Hz), 114.48 (dd, *CH* arom., $^1J = 161$ Hz, $^2J = 4.55$ Hz), 125.25 (t, *C* arom.), 130.26 (dd, *CH* arom., $^1J = 159.3$ Hz, $^2J = 7.3$ Hz), 163.82 (s, *C* arom.), 165.35 (s, CH_2CONH), 177.86 (s, $PhCONH$) ppm. Anal. $C_9H_8NO_3$.

*5.4. 4-Methoxy-N-methyl-N-propionylbenzamide (**7**)*

A mixture of *N*-methylamide of anisic acid (1.47 g, 8.9 mmol) and propionic anhydride (5 ml) was warmed at 165 °C for 5 h and worked up as reported in Ref. [22]. The residue was submitted to four chromatographic separations (column chromatography eluent WE_2, CH_2Cl_2, $CHCl_3$:MeOH, 99:01 and finally preparative TLC using as eluting system $CHCl_3$:MeOH, 99:01) to obtain compound **7**; 10% yield. m.p. 34–39 °C. ^1H NMR ($CDCl_3$) δ 1.14 (t, 3H, CH_2CH_3, $^1J = 7.4$ Hz), 2.58 (q, 2H, CH_2CH_3, $^1J = 7.4$ Hz), 3.22 (s, 3H, NCH_3), 3.88 (s, 3H, OCH_3), 6.95 (d, 2H, *CH* arom., $^2J = 8.8$ Hz), 7.63 (d, 2H, *CH* arom., $^2J = 8.8$ Hz) ppm. ^{13}C NMR ($CDCl_3$) δ 10.10 (q, CH_2CH_3, $^1J = 127.5$ Hz), 31.24 (t, CH_2CH_3, $^1J = 128.4$ Hz), 34.95 (q, NCH_3, $^1J = 140.2$ Hz), 55.93 (q, OCH_3, $^1J = 143.85$ Hz), 114.36 (dd, *CH* arom., $^1J = 161$ Hz, $^2J = 4.55$ Hz), 127.53 (t, *C* arom., $^2J = 8.2$ Hz), 131.43 (dd, *CH* arom., $^1J = 160$ Hz, $^2J = 6.4$ Hz), 163.53 (s, *C* arom.), 174.18 (s, $NCOCH_2$), 177.72 (s, *CO*C arom.) ppm. Anal. $C_{12}H_{15}NO_3$.

*5.5. 4-Amino-N-benzylbutyramide (**8**) [25]*

The hydrolysis of compound **16** was performed under different conditions from that reported in literature [25]. In fact **16** (1.5 g, 8.6 mmol) was dissolved in H_2O and to this solution was added 1 equiv. of NaOH (0.35 g, 8.6 mmol), the mixture obtained was warmed at 80 °C for 5 days. The crude product was purified by flash chromatography using WE_2 as eluting system. 20% yield. m.p. 118–121 °C. IR ν(cm^{-1}): 3300 (*NH*), 1650 (*CO*). ^1H NMR ($CDCl_3$) δ: 1.52 (bs, 2H, NH_2), 1.76–1.83 (m, 2H, $CH_2CH_2CH_2NH_2$), 2.3 (t, 2H, CH_2CO, $J = 7.0$ Hz), 2.74 (t, 2H, CH_2NH_2, $J = 7.0$ Hz), 4.42 (s, 0.5 2H, $PhCH_2$), 4.44 (s, 0.5 2H, $PhCH_2$), 6.30 (bs, 1H, *NH*), 7.27–7.32 (m, 5H, *CH* arom.) ppm. ^{13}C NMR (D_2O) δ: 26.31 (t, $CH_2CH_2CH_2NH_2$), 32.92 (t, CH_2CO), 39.45 (t, CH_2NH_2), 42.84 (t, CH_2NH), 127.02 (d, *CH* arom.), 127.24 (d, *CH* arom.), 128.57 (d, *CH* arom.), 137.74 (s,

C arom.), 175.55 (s, *CO*) ppm. *m/z* 192 [M^+]. Anal. $C_{11}H_{16}N_2O$.

5.6. 4-Amino-N-benzyl-N-methylbutyramide (**9**)

0.25 g of **15** (0.8 mmol) was dissolved in a mixture of 1.2 ml of EtOH abs. and 1.85 ml of THF and added of 0.29 ml (5.9 mmol) hydrazine hydrate. The mixture was refluxed for 2 h and then acidified with 2 N HCl. The white solid was filtered off and the water washed with Et_2O, added with 10% NaOH and extracted with $CHCl_3$. The organic phase was made anhydrous and evaporated under reduced pressure. The residue was purified by column chromatography (WE_4 as eluent). 54% yield. ^{1}H NMR ($CDCl_3$) δ 1.3 (bs, 2H, NH_2), 1.7–1.85 (m, 2H, $CH_2CH_2CH_2$), 2.39 (t, 2H, CH_2CO, $J = 8.0$ Hz), 2.63–2.80 (m, 2H, CH_2NH_2), 2.90 (s, 0.5 3H, NCH_3), 2.93 (s, 0.5 3H, NCH_3), 4.49 (s, 0.5 2H, $PhCH_2$), 4.53 (s, 0.5 2H, $PhCH_2$), 7.05–7.3 (m, 5H, *CH* arom.) ppm.

The amine was then transformed into the corresponding oxalate (m.p. 91–94 °C). Anal. $C_{12}H_{18}N_2O$.

5.7. Propionylaminoacetic acid methyl ester (**12**)

Glycine methyl ester hydrochloride (2.24 g, 17.9 mmol) was dissolved in 45 ml of a 1M $NaHCO_3$ solution under stirring until there was no more gas evolution (CO_2). Then 20 ml of ethyl acetate and 2.57 ml of propionic anhydride (20 mmol) were added and the mixture was left for 3 h at r.t. under stirring. The organic layer was treated with NaCl saturated solution, made anhydrous with Na_2SO_4 and distilled under reduced pressure to obtain a low melting solid; 93% yield. ^{1}H NMR ($CDCl_3$) δ 1.16 (t, 3H, CH_2CH_3, $^1J = 5.0$ Hz), 2.21 (q, 2H, CH_2CH_3, $^1J = 5.0$ Hz), 3.70 (s, 3H, OCH_3), 3.98 (s, 2H, NCH_2CO) ppm. Anal. $C_6H_{11}NO_3$.

5.8. N-Benzyl-4-hydroxy-N-methylbutyramide (**13**)

γ-Butyrolactone (4.66 ml, 60 mmol) and *N*-methylbenzylamine (4.46 ml, 34.8 mmol) were warmed under stirring at 70 °C for 20 h. Flash chromatographic separation (eluent WE_7) gave the title compound with 89% yield. IR ν(cm^{-1}): 3140–3600 (*OH*), 1665 (*CO*). ^{1}H NMR ($CDCl_3$) δ 1.85–2.1 (m, 2H, $CH_2CH_2CH_2OH$), 2.55 (t, 2H, CH_2CO, $J = 7.0$ Hz), 2.42 (bs, 1H, *OH*), 2.93 (s, 0.5 3H, NCH_3), 2.95 (s, 0.5 3H, NCH_3), 3.70 (t, 2H, CH_2OH, $J = 7.0$ Hz), 4.56 (s, 0.5 2H, $PhCH_2$), 4.58 (s, 0.5 2H, $PhCH_2$), 7.10–7.37 (m, 5H, *CH* arom.) ppm. Anal. $C_{12}H_{17}NO_2$.

5.9. N-Benzyl-4-chloro-N-methylbutyramide (**14**)

Compound **13** (1.5 g, 7.4 mmol) was dissolved in 20 ml of $CHCl_3$ (stabilised with amylene) and to this solution was added an excess of $SOCl_2$ (5 ml); the reaction was left under stirring at r.t. for 2 h. The solvent was removed under reduced pressure, the residue alkalinised with 1 N NaOH and extracted with ethyl acetate. After drying, the solvent was removed under reduced pressure to give compound **16** which was used without purification for the next step; 91% yield. IR ν(cm^{-1}): 1650 (*CO*). ^{1}H NMR ($CDCl_3$) δ: 2.10–2.25 (m, 2H, $CH_2CH_2CH_2Cl$), 2.57 (t, 2H, CH_2CO, $J = 7.0$ Hz), 2.94 (s, 0.5 3H, NCH_3), 2.96 (s, 0.5 3H, NCH_3), 3.69 (t, 2H, CH_2Cl, $J = 7.0$ Hz), 4.57 (s, 0.5 2H, $PhCH_2$), 4.59 (s, 0.5 2H, $PhCH_2$), 7.15–7.40 (m, 5H, *CH* arom.) ppm. Anal. $C_{12}H_{16}ClNO$.

5.10. N-Benzyl-4-(1,3-dioxo-1,3-dihydro-isoindol-2-yl)-N-methylbutyramide (**15**)

Compound **14** (0.55 g, 2.5 mmol) was dissolved in anhydrous toluene (4.7 ml) and 18 crown 6 (0.156 g, 0.59 mmol) in 4.7 ml of anhydrous toluene and potassium phthalimide (0.72 g, 3.9 mmol) were added under N_2. The reaction was refluxed for 5 h. Then the organic phase was washed three times with water and made anhydrous with Na_2SO_4. The solvent was distilled off under reduced pressure and the yellow oil obtained purified by flash chromatography (using cyclohexane:ethyl acetate, 5:5, as eluting system); 32% yield. ^{1}H NMR ($CDCl_3$) δ 2.00–2.20 (m, 2H, $CH_2CH_2CH_2$), 2.45 (m, 2H, CH_2CO), 2.90 (s, 0.5 3H, NCH_3), 2.92 (s, 0.5 3H, NCH_3), 3.81 (t, 2H, CH_2N phthalimide), 4.52 (s, 0.5 2H, $PhCH_2$), 4.54 (s, 0.5 2H, $PhCH_2$), 7.10–7.30 (m, 5H, *CH* arom.), 7.70–7.74 (m, 2H, *CH* phthalimide), 7.80–7.85 (m, 2H, *CH* phthalimide) ppm. Anal. $C_{20}H_{20}N_2O_3$.

5.11. Pharmacology

5.11.1. Antiamnesic test (passive-avoidance test)

The test was performed according to the step-through method described by Jarvik and Kopp [27]. The apparatus consists of a two-compartment acrylic box with a lighted compartment connected to a darkened one by a guillotine door. In the original method, mice received a punishing electrical shock as soon as they entered the dark compartment, while in our modified method, after entry into the dark compartment, mice receive a non-painful punishment consisting of a fall (from 40 cm) into a cold water bath (10 °C). For this purpose, the dark chamber was constructed with a pitfall floor. The latency times for entering the dark compartment were measured in the training test (first day) and after 24 h in the retention test (second day). Mice who did not enter after 60 s latency were excluded from the experiment. For memory disruption, mice were i.p. injected with the amnesic drugs (scopolamine). All investigated drugs were given i.p. (dissolved in saline solution) or p.o. (dispersed in 1% carboxymethylcellu-

lose) 20 min before the training session, while amnesic drugs were injected immediately after termination of the training session. The maximum entry latency allowed in the retention session was 120 s. The degree of received punishment memory (fall into cold water) was expressed as the increase in seconds between training and retention latencies.

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