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Structure–activity relationship studies on unifiram (DM232) and sunifiram (DM235), two novel and potent cognition enhancing drugs

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Abstract—Structure–activity relationships on two novel potent cognition enhancing drugs, unifiram (DM232, **1**) and sunifiram (DM235, **2**), are reported. Although none of the compounds synthesised reached the potency of the parent drugs, some fairly active compounds have been identified that may represent new leads to develop other cognition enhancing drugs. An interesting result of this research is the identification of two compounds (**13** and **14**) that are endowed with amnesing activity (the opposite of the activity of the original molecules) and are nearly equipotent to scopolamine. Moreover, two compounds of the series (**5** and **6**) were found endowed with analgesic activity on a rat model of neuropathic pain at the dose of 1 mg/kg.

1. Introduction

One of the main symptoms accompanying ageing, stroke, head injury and neurodegenerative diseases like Alzheimer's disease is the decline of cognitive functions. Cognition enhancers,[1] are defined as drugs able to facilitate attentional abilities and acquisition, storage and retrieval of information, and to attenuate the impairment of cognitive functions associated with age and age-related pathologies.[2] By definition, this class of drugs improves the decline of cognitive functions but does not change the rate of progression of neurodegeneration.[3]

Previously, we described a new class of compounds with a 1,4-diazabicyclo[4.3.0]nonan-9-one structure[4] (Chart 1) related to the family of piracetam-like nootropics[1,5–7] by the presence of the 2-pyrrolidinone ring (DM 232 (**1**); Chart 1). Most of the compounds of this class showed a very potent cognition enhancing activity in a mouse passive avoidance assay. Activity was maintained at the same level when the 2-pyrrolidinone ring was opened to give the corresponding piperazine derivatives[8] (DM 235 (**2**); (Chart 1), suggesting that in this class of compounds the 2-oxopyrrolidine moiety is not critical for pharmacological action. Both series of compounds were effective also in other behavioural tests such as social learning and Morris water maze.[9,10] Further research confirmed and extended this finding, as it was observed that the *seco* derivatives of many classical piracetam-like drugs behave like the parent compounds.[11] Finally, potent cognition enhancing activity was also found in a series of closely related 4-aminopiperidines.[12]

We have continued exploring structure–activity relationships in these series of compounds and we now report the cognition enhancing activity of compounds where (Chart 2): (i) The 1,4-diazabicyclo[4.3.0]nonan-9-one moiety has been expanded to a 1,4-diazabicyclo[4.4.0]decan-10-one by enlarging the pyrrolidinone cycle. (ii) The 1,4-diazabicyclo[4.3.0]nonan-9-one, 1,4-diazabicyclo[4.4.0]decan-10-one and piperazine scaffolds have been decorated with other groups, in particular those present in a series of compounds acting as allosteric modulators of the AMPA receptor.[13–15] As a matter of fact, there are indications that AMPA-recep-

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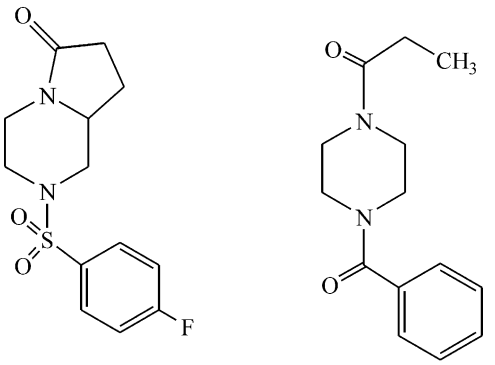
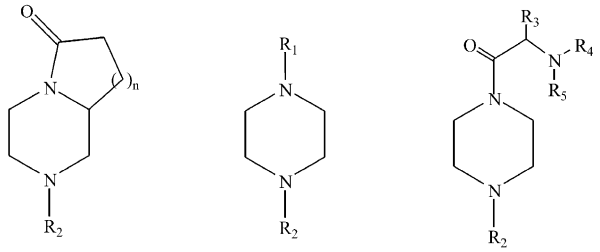


DM 232 (**1**) DM 235 (**2**)

Chart 1.



3-14 **15-27** **28-37**

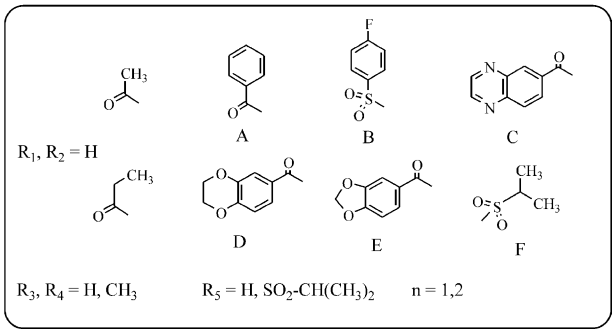


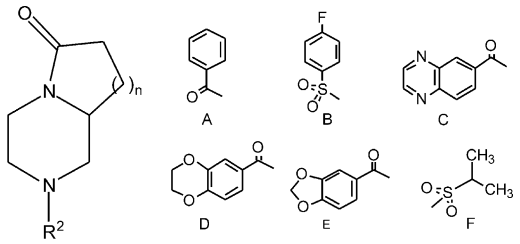
Chart 2.

tor activation is involved in the antiamnesic effect of DM232 and DM235.[16] (iii) An amino group has been introduced into the acyl side chain of the piperazine compound DM 235 (**2**), mimicking the basic side chain of nebracetam.[1] Cognition enhancing activity of the compounds obtained has been evaluated through the passive avoidance test and confirmed with the social learning test[17] in the case of one of the most potent compounds. Moreover, following the report of Rashid et al.[18] on the neuropathy-specific analgesic action of nefiracetam, the compounds have been also screened in the ligated sciatic nerve model.

2. Chemistry

Compounds **3**–**14**, whose chemical and physical characteristics are reported in Table 1, were synthesized as reported in Scheme 1. 1,4-Diazabicyclo[4.4.0]decan-10-one **4** was obtained by metallation of commercially

Table 1. Chemical and physical characteristics of compounds **3**–**14**

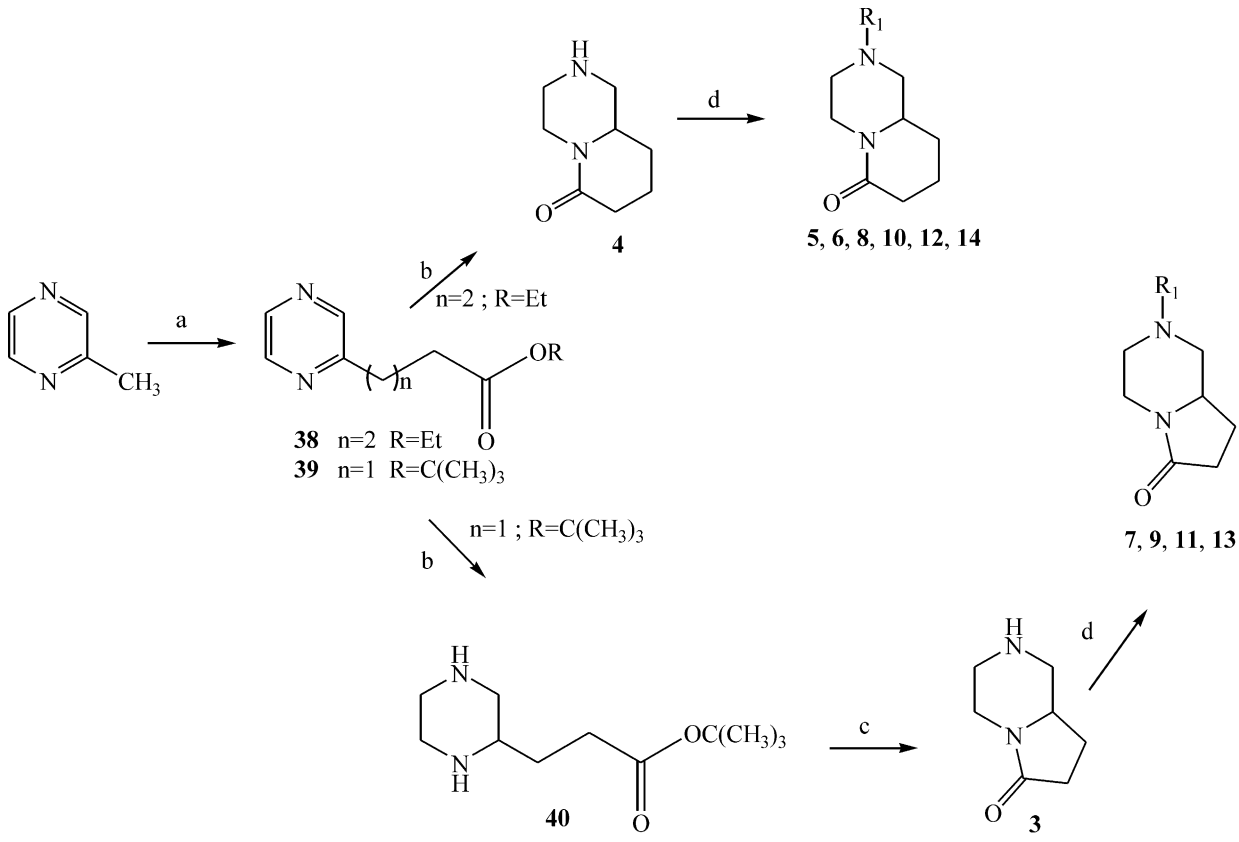


N	*n*	R_2	Mp °C (Petrol ether)	Purification eluent[a]	Analysis
3[b]	1	Hydrogen	—	—	—
4	2	Hydrogen	Oil	II	$C_8H_{14}N_2O$
5	2	A	130–133	[c]	$C_{15}H_{18}N_2O_2$
6	2	B	130–133	[c]	$C_{14}H_{17}FN_2O_3S$
7	1	C	166–169	III	$C_{16}H_{16}N_4O_2$
8	2	C	170–172	III	$C_{17}H_{18}N_4O_2$
9	1	D	181–184	III	$C_{16}H_{16}N_2O_4$
10	2	D	86–89	I	$C_{17}H_{20}N_2O_4$
11	1	E	140–142	IV	$C_{15}H_{16}N_2O_4$
12	2	E	147–150	VI	$C_{16}H_{18}N_2O_4$
13	1	F	114–117	VI	$C_{10}H_{18}N_2O_3S$
14	2	F	113–116	VI	$C_{11}H_{20}N_2O_3S$

[a] See Experimental.
[b] See ref 4.
[c] Crystallized directly from abs EtOH/petrol ether.

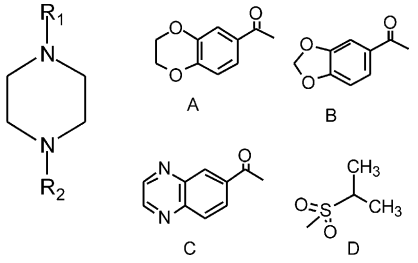
available methylpyrazine with LDA at −78 °C.[19] The metallic species obtained, was then reacted with ethyl 2-bromopropionate to give compound **38.** Hydrogenation of **38** gave **4** directly which was then reacted with the appropriate acyl or sulfonyl chloride to give compounds **5**, **6**, **8**, **10**, **12**, **14**. A similar pathway allowed the synthesis of 1,4-diazabicyclo[4.3.0]nonan-9-one **3** with a new, more efficient, synthetic route[19] with respect to our original synthesis.[4] In fact, compound **39** obtained from metallated methylpyrazine and *t*-butyl bromoacetate, was reduced to ester **40** that, when heated at 200 °C, gave **3**. This compound was then reacted with the appropriate acyl or sulfonyl chloride to give compounds **7**, **9**, **11**, **13**.

Compounds **15**–**27,** whose chemical and physical characteristics are reported in Table 2, were synthesized as shown in Scheme 2. 1-Benzoyl-4-benzylpiperazine[20] and **41**, synthesized from 1-benzylpiperazine and isopropylsulfonyl chloride, were hydrogenated with $HCOONH_4$, Pd/C 10% in MeOH[21] to give **24**[22] and **42**. Commercially available 1-acetylpiperazine, 1-propionylpiperazine (**16**), **24** and **42**,[8] were then treated with commercially available piperonyloyl chloride, isopropylsulfonyl chloride, 4-F-benzenesulfonyl chloride or home made 1,4-benzodioxan-6-carbonyl chloride and 6-quinoxaloyl chloride, to give the desired compounds **15**, **17**–**23**, **25**–**27**. 1,4-Benzodioxan-6-carbonyl chloride was obtained by oxidation of commercially available 1,4-benzodioxan-6-carboxaldehyde with $KMnO_4$[23,24] followed by treatment with $SOCl_2$ by standard methods.[25] 6-Quinoxaloyl chloride was synthesised from commercially available 3,4-diaminobenzoic acid with potassium carbonate and glyoxal sodium bisulfite hydrate[26] followed by reaction with $SOCl_2$.



Scheme 1. (a) LDA, −78 °C; $Br(CH_2)_2COOC_2H_5$ or $BrCH_2COOC(CH_3)_3$; (b) H_2, Pd/C; (c) 200 °C; (d) CH_3CN, Et_3N, R_1Cl.

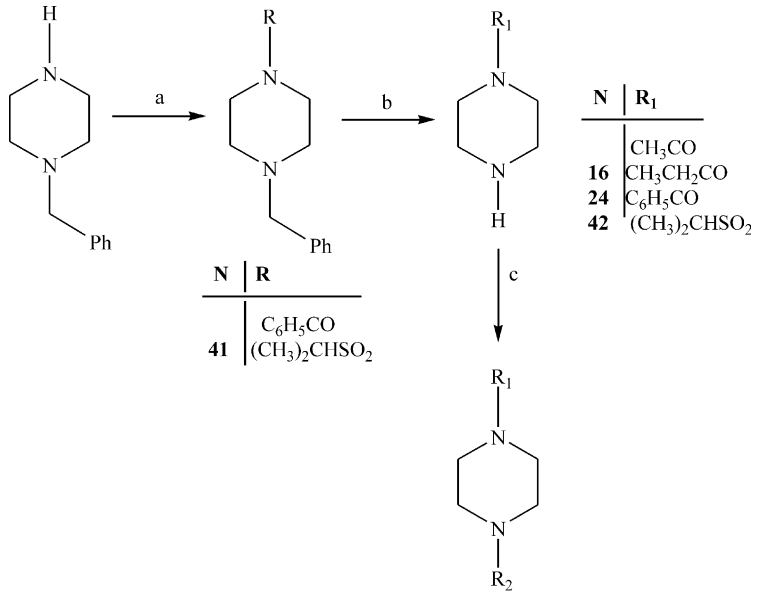
Table 2. Chemical and physical characteristics of piperazine derivatives **15**–**27**



N	R_1	R_2	Mp °C (Petrol ether)	Purification eluent[a]	Analysis
15	CO–CH_3	A	60–61	IV	$C_{15}H_{18}N_2O_4$
16[b]	CO–CH_2–CH_3	Hydrogen	—	—	—
17	CO–CH_2–CH_3	A	65–66	IV	$C_{16}H_{20}N_2O_4$
18	CO–CH_3	B	50–51	IV	$C_{14}H_{16}N_2O_4$
19	CO–CH_2–CH_3	B	60–62	V	$C_{15}H_{18}N_2O_4$
20	CO–CH_3	C	55–56	IV	$C_{15}H_{16}N_4O_2$
21	CO–CH_2–CH_3	C	43–44	IV	$C_{16}H_{18}N_4O_2$
22	CO–CH_3	D	45–46	IV	$C_9H_{18}N_2O_3S$
23	CO–CH_2–CH_3	D	60–61	IV	$C_{10}H_{20}N_2O_3S$
24	CO–C_6H_5	Hydrogen	Oil	IV	$C_{11}H_{14}N_2O$
25	CO–C_6H_5	D	142–144	VI	$C_{14}H_{20}N_2O_3S$
26	$SO_2C_6H_4$-*p*-F	CO–C_6H_5	105–106	VI	$C_{17}H_{17}FN_2O_3S$
27	SO_2–$CH(CH_3)_2$	$SO_2C_6H_4$-*p*-F	161–163	VI	$C_{13}H_{19}FN_2O_4S_2$

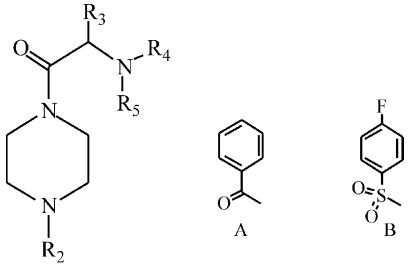
[a] See Experimental.
[b] See ref 8.

Compounds **28**–**37,** whose chemical and physical characteristics are reported in Table 3, were synthesised as shown in Scheme 3. The commercially available *N*-(*tert*-butoxycarbonyl)-L-alanine, *N*-(*tert*-butoxycarbonyl) glycine and *N*-(*tert*-butoxycarbonyl)sarcosine (**43**), obtained with standard methods[27] were activated with *N*-hydroxysuccinimide[28] and *N*,*N*′-dicyclohexylcarbodiimide. These derivatives were then treated with benzylpiperazine[28] to



Scheme 2. (a) $(CH_3)_2CHSO_2Cl$, Net_3, CH_3CN; (b) $HCOONH_4$, Pd/C 10%, MeOH; (c) R_2COCl or $(CH_3)_2CHSO_2Cl$ or 4-F-C_6H_4–SO_2Cl, Net_3, CH_3CN.

Table 3. Chemical and physical characteristics of compounds **28–37**



N	R_2	R_3	R_4	R_5	Mp °C (Petrol ether)	Purification eluent[a]	Analysis
28	A	H	H	H	72–74	IV	$C_{13}H_{17}N_3O_2$
29	A	H	H	SO_2-$CH(CH_3)_2$	90–91	IV	$C_{16}H_{23}N_3O_4S$
30	A	H	CH_3	H	65–66	VII	$C_{14}H_{19}N_3O_2$
31	A	H	CH_3	SO_2-$CH(CH_3)_2$	80–81	IV	$C_{17}H_{25}N_3O_4S$
32	A	CH_3	H	H	78–79	IV	$C_{14}H_{19}N_3O_2$
33	B	H	H	H	136–137	IV	$C_{12}H_{16}FN_3O_3S$
34	B	H	H	SO_2-$CH(CH_3)_2$	190–195	IV	$C_{15}H_{22}FN_3O_5S_2$
35	B	H	CH_3	H	64–65	IV	$C_{13}H_{18}FN_3O_3S$
36	B	H	CH_3	SO_2-$CH(CH_3)_2$	65–66	IV	$C_{16}H_{24}FN_3O_5S_2$
37	B	CH_3	H	H	131–132	IV	$C_{13}H_{18}FN_3O_3S$

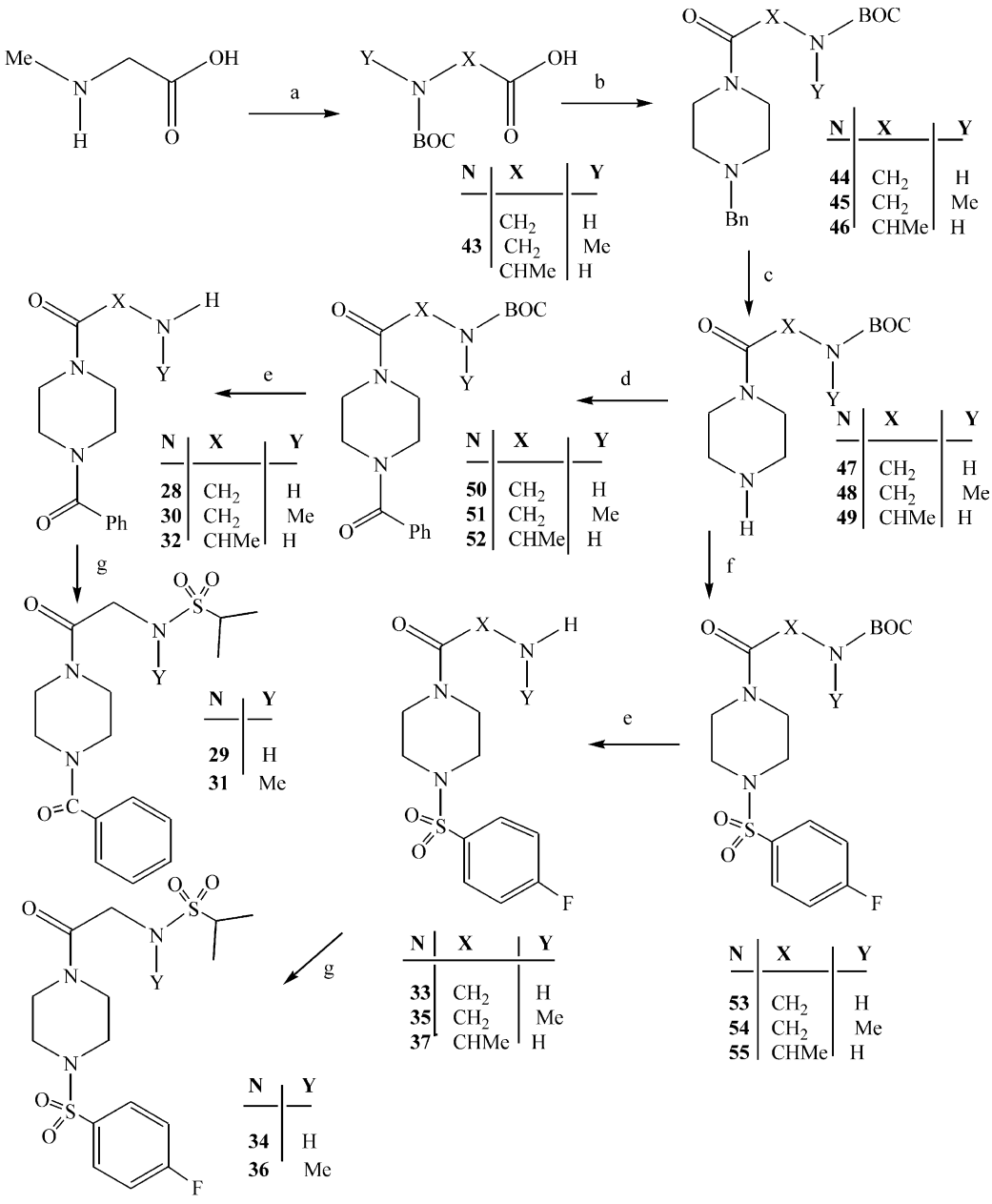
[a] See Experimental.

give **44–46** that, in turn, were hydrogenated with $HCOONH_4$, Pd/C 10% in MeOH[21] to give **47–49**. Compounds **46** and **49** have already been described in literature,[29,30] but were synthesised with a different method. Compounds **47–49** were treated with benzoyl chloride and NEt_3 (**50–52**) and de-protected with HCl to afford **28**, **30**, **32**. Analogously, the same compounds were reacted with 4-fluorobenzenesulfonyl chloride (**53–55**) and then de-protected to give **33**, **35** and **37**. Finally, compounds **29**, **31**, **34**, **36** were synthesised from **28**, **30**, **33** and **35** with isopropylsulfonyl chloride by the method already described.

3. Pharmacology

3.1. Cognition enhancing activity

The compounds studied were tested as cognition enhancers in the mouse passive avoidance test of Jarvik and Kopp,[31] slightly modified by us (see the Experimental). The same test was used to evaluate the amnesing properties of compounds **13** and **14**. The cognition enhancing properties of compound **22** were also tested in the rat social learning test.[17]



Scheme 3. (a) $(tBuOCO)_2O$, NEt_3; (b) DCC, *N*-hydroxysuccinimide, benzylpiperazine; (c) $HCOONH_4$, Pd/C 10%, MeOH; (d) PhCOCl, NEt_3; (e) HCl 2M; (f) 4-F-C_6H_4-SO_2Cl, NEt_3; (g) $(CH_3)_2CH$–SO_2Cl, CH_3CN, NEt_3.

3.2. Analgesic activity

A peripheral mono neuropathy was produced in adult rats by placing loosely constrictive ligatures around the common sciatic nerve according to the method described by Bennett.[32] The nociceptive threshold in the rat was determined with an analgesimeter according to the paw pressure test.[33]

4. Results and discussion

4.1. Cognition enhancing activity

To establish structure–activity relationships, all the synthesised compounds were evaluated in the mouse passive avoidance test. The compounds showing anti amnesic activity below the 10 mg/kg minimal effective dose are reported in Table 4; the remaining compounds did not show any activity up to the same dose. It must be kept in mind that the tests are made in vivo and that pharmacokinetic factors could play a role on the potency of the drugs. For instance, the structural modifications introduced to study SARs may affect critical pharmacokinetic properties such as bioavailability. The minimal effective doses of piracetam, DM232 and DM235 are reported in the same table. In general, even if some molecules show much better potency with respect to piracetam, none of them reached the potency of unifiram (DM232, **1**) and sunifiram (DM235, **2**).

Cycle enlargement was clearly detrimental for nootropic activity, as shown by compounds **5** and **6,** that are some hundred times less potent than **1** and **2**. However, the

Table 4. Compounds active on mouse passive avoidance test, using scopolamine (S) as amnesing drug. All other compounds are inactive up to 10 mg/kg

Drug (number of animals)	Minimal effective dose mg/kg (ip)	Entry latency		
		1st day	2nd day	Δ
Saline (13)	—	15.0±5.9	95.6±8.8	80.6
Scopolamine(S) (6)	1.5	16.6±4.7	44.5±8.3[a]	27.9
S+**1**(35)	0.01	15.2±3.2	98.7±9.1[b]	83.5
S+**2**(31)	0.01	14.9±3.6	95.4±8.7[b]	80.5
S+**piracetam** (56)	30	15.9±2.1	105.3±7.8[b]	89.4
S+**3** (9)	0.1	18.6±6.0	106.8±12.5[b]	88.2
S+**4** (18)	0.1	15.3±3.1	77.6±11.6[b]	62.3
S+**5** (16)	1	15.2±3.3	108.3±10.6[b]	93.2
S+**6** (18)	1	16.3±2.7	99.4±11.1[b]	83.1
S+**8** (16)	10	13.9±3.2	92.52±11.5[b]	78.6
S+**10** (20)	10	15.5±4.1	105.6±10.5[b]	90.1
S+**11** (12)	1	14.6±3.4	99.3±8.3[b]	84.7
S+**16** (10)	0.1	22.6±4.5	130.3±11.2[b]	107.7
S+**17** (12)	1	16.4±3.2	108.2±9.3[b]	91.8
S+**22** (19)	0.1	13.8±3.6	87.1±9.6[c]	73.3
S+**23** (19)	1	13.7±3.9	88.9±10.3[c]	75.2
S+**24** (10)	0.1	16.3±4.6	105.7±9.3[b]	89.4
S+**30** (11)	1	17.0±3.5	87.8±9.1[b]	70.8
S+**35** (11)	1	13.8±3.2	83.4±8.4[b]	69.6

[a] $p<0.01$ with respect to mice treated with saline.
[b] $p<0.01$.
[c] $p<0.05$ with respect to mice treated with scopolamine.

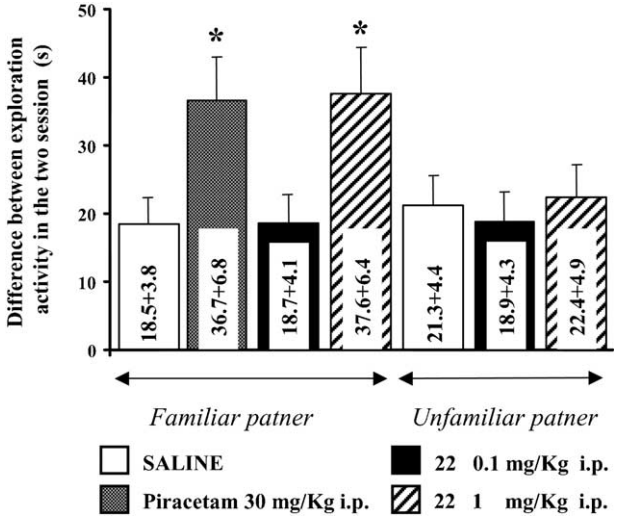


Figure 1. Effect of compound **22** in a rat social learning test in comparison with piracetam. Each column represents the mean of six rats. *$p<0.01$ in comparison with the corresponding saline-treated rats. Piracetam and **22** were administered 20 min before the first session. Vertical lines show SEM.

unsubstituted 1,4-diazabicyclo[4.4.0]decan-10-one **4** is active at the dose of 0.1 mg/kg ip which is close to the potency of the reference compounds. The same happens for the1,4-diazabicyclo[4.3.0]nonan-9-one analogue **3**, and for the mono substituted piperazines **16** and **24**. Apparently, the *N*-substitution (or the second *N*-substitution in the case of piperazines) while increasing in some cases nootropic potency, is not critical for high activity. It is possible that the unsubstituted or mono substituted compounds are indeed active metabolites of the parent drugs. Studies are in progress to evaluate this possibility.

Also the substitution of benzoyl (A) and 4-F-benzenesulfonyl (B) groups with the acyl groups present in some ampakines[13] such as 6-quinoxaloyl (C), 1,4-benzodioxan-6-carbonyl (D), piperonyloyl (E) was disappointing. As a matter of fact, the compounds obtained are inactive up to 10 mg/kg dose (**7**, **9**, **12**) or show much lower potency (**8**, **10**, **11**) with respect to the parent compounds, independently of the size of the cycle. Similar results were observed when the same substitution was performed on the piperazine scaffolding of DM235. Only compound **17** is active at the dose of 1 mg/kg, the other compounds (**15**, **18**–**21**, **25**–**27**) being inactive up to the dose of 10 mg/kg. On the contrary, the insertion of the isopropylsulfonyl group, present in a new series of AMPA receptor modulators,[14] gave better results as compound **23** is active at 1 mg/kg and **22** at 0.1 mg/kg, close to the potency of the parent drug. The cognition enhancing properties of this compound were confirmed by the results of the social learning test[17] as it is shown in Figure 1.

A very interesting result was obtained when the isopropylsulfonyl group was introduced into both 1,4-diazabicyclo[4.3.0]nonan-9-one and 1,4-diazabicyclo[4.4.0]decan-10-one scaffolding. Indeed, the resulting compounds **13** and **14** are endowed with an opposite action with respect to the other members of the series and appear to be potent amnesic drugs, as is shown in Table 5. Their potency and activity seem comparable to those of scopolamine and their amnesic action was overcome by compound **1** in a dose-dependent manner that parallels that of scopolamine. These results are intriguing and studies are under way to clarify the mechanism of action of **13** and **14**.

Finally, as regards the results of inserting an amino group into the acyl chain of the piperazine series, most of the molecules synthesised (**28**, **29**, **31**–**34**, **36**, **37**), are inactive up to the dose of 10 mg/kg. However, two compounds (**30** and **35**) show a minimal effective dose of 1 mg/kg and may represent new leads to develop other molecular species endowed with cognition enhancing properties.

4.2. Analgesic properties on neuropathic pain

Following the interesting report of Rashid et al.,[18] all compounds were tested on the model of neuropatic pain described by Bennett.[32] Experiments were performed on rats submitted to paw-pressure test 14 days after the operation since at this time a significant reduction of the pain threshold of the injured paw (dx) was observed. By contrast in the controlateral paw the pain perception remained unchanged. Only two compounds showed appreciable analgesic activity on this model: **5** (1 mg/kg ip) and **6** (1 mg/kg ip) exhibited an anti hyperalgic effect when compared with saline treated group 30, 45 and 60 min after administration. Both compounds did not modify pain threshold in controlateral, non operated, paw. Compounds **5** and **6** at the doses of 0.1 mg/kg ip

Table 5. Amnesia inducing effect of compounds **13** and **14**

Drug (number of animals)	Dose mg/kg (ip)	Entry latency		
		1st day	2nd day	Δ
Saline (13)	—	15.5±3.0	102.6±8.7	87.1
Scopolamine (6)	1.5	16.6±4.7	44.5±8.3[b]	27.9
13 (18)	10	13.7±3.2	20.5±9.4[b]	6.8
14 (19)	10	14.3±3.0	22.6±7.9[b]	8.3
13 (18)	1	13.6±3.9	61.5±10.4[b]	47.9
14 (19)	1	14.5±4.4	69.8±8.5[b]	55.3
13[a] + **1** (18)	0.001	16.0±4.2	36.4±9.5[b]	20.4
13[a] + **1** (16)	0.01	14.1±3.9	81.2±9.4[c]	67.1
13[a] + **1** (17)	0.1	13.8±3.3	111.6±10.3[c]	97.8
14[a] + **1** (14)	0.01	15.3±4.4	41.9±9.1[b]	20.4
14[a] + **1** (12)	0.1	17.2±3.4	79.8±9.3[c]	82.6
14[a] + **1** (14)	1.0	13.8±4.1	109.5±9.6[c]	95.7

[a] 10 mg/kg as amnesing drug.
[b] $P < 0.01$ with respect to mice treated with saline.
[c] $P < 0.01$; $P < 0.05$ with respect to mice treated with **13** or **14** (10 mg/kg).

were devoid of any anti hyperalgic effect. Rats treated with **5** and **6** at the highest doses showed a normal behaviour compared to saline control rats. It is interesting that the two active molecules belong to the 1,4-diazabicyclo[4.4.0]decan-10-one series and that the corresponding 1,4-diazabicyclo[4.3.0]nonan-9-one derivatives are inactive in this assay. The mechanism of action does not seem related to that determining cognition enhancement as, in this case the compounds of both series are active, although with different potencies (see Table 4 and ref 4). Compounds **5** and **6** represent two fairly potent analgesic compounds that can be useful leads to find new drugs for a pathology that is difficult to control.

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, 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: I) petroleum ether/CH_2Cl_2/Et_2O/EtOH abs. 900:360:360:180; II) NH_4OH/EtOH abs./$CHCl_3$/petroleum ether 45:225:600:90; III) NH_4OH/EtOH abs./CH_2Cl_2/petroleum ether 4:25:150:50; IV): $CHCl_3$/EtOH abs./petroleum ether/NH_4OH 340:65:60:8; V): $CHCl_3$/MeOH 90:10; VI): petroleum ether/CH_2Cl_2/Et_2O/EtOH abs./NH_4OH 900:360:360:180:9.9; VII): CH_2Cl_2/MeOH 95:5. 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. GC/MS was performed with a Perkin-ElmerAutosystem XLTurbomass.

5.1.1. 4-Pyrazin-2-yl butyric acid ethyl ester (38) and 3-pyrazin-2-yl propionic acid *tert*-butyl ester (39). One equivalent of butyllithium (1.6 M hexane solution) was added to 1 equiv of diisopropylamine in anhydrous THF at 0 °C. The mixture was stirred at 0 °C for 30 min, then cooled at −78 °C and added with 1 equiv of methylpyrazine. After 10 min the appropriate bromoester was added and the mixture maintained 1 h at −78 °C. Then the reaction was quenched with NH_4Cl and extracted with diethyl ether and the organic phase dried and evaporated under vacuum. Compound **38** was obtained after purification by column chromatography (eluent I) in 15% yield. ^{1}H NMR ($CDCl_3$) δ: 1.26 (t, J=6.0 Hz, 3H,CH_2CH_3); 2.10–2.20 (m, 2H, $CH_2CH_2CH_2COO$); 2.39 (t, J=6.0 Hz, 2H, $CH_2CH_2CH_2COO$); 2.88 (t, J=8 Hz, 2H, CH_2COO); 4.14 (q, J=8 Hz, 2H, OCH_2); 8.41–8.51 (m, 3H, *H pyrazine protons*) ppm. Anal. ($C_{10}H_{14}N_2O_2$) C,H,N.

Compound **39**[19] was obtained, in the same way, after purification by flash chromatography (eluent I) in 20% yield. ^{1}H NMR ($CDCl_3$) δ: 1.42 (s, 9H, $C(CH_3)_3$; 2.72 (t, J=8 Hz, 2H, CH_2CH_2COO); 3.08 (t, J=8.0 Hz, 2H, CH_2COO); 8.39–8.49 (m, 3H, *H pyrazine protons*) ppm. Anal. ($C_{11}H_{16}N_2O_2$) C,H,N.

5.1.2. 1,4-Diazabicyclo[4.4.0]decan-10-one (4). Compound **38** was hydrogenated over Pd/C 10% in abs. EtOH, at 60 psi for 60 h. After filtration, the solvent was removed under vacuum to give a residue that was purified by flash chromatography (eluent II). 60% yield. ^{1}H NMR ($CDCl_3$) δ: 1.33–1.50 (m, 1H); 1.50–1.73 (m, 1H); 1.73–2.03 (m, 3H, 2CH and NH); 2.25–2.53 (m, 3H); 2.57–2.78 (m, 2H); 2.91–3.08 (m, 2H); 3.20–3.38 (m, 1H); 4.50–4.66 (m, 1H) ppm. ^{13}C NMR ($CDCl_3$) δ : 19.60 (t); 27.67 (t); 33.14 (t); 42.40 (t); 46.13 (t); 53.27 (t); 57.28 (d); 169.50 (s). m/z 154 (M^+). Anal. ($C_8H_{14}N_2O$) C,H,N.

5.1.3. 3-Piperazin-2-yl propionic acid *tert*-butyl ester (40). Compound **39** (2 mmol, 0.43 g) was hydrogenated over Pd/C10% in abs. EtOH at 70 psi for 24 h. After filtration, the solvent was removed under vacuum to give compound **40** that was used without purification in the next step. 93% yield. ^{1}H NMR ($CDCl_3$) δ: 1.2 (s, 9H, $C(CH_3)_3$; 1.48–1.67 (m, 2H); 2.18–2.43 (m, 5H); 2.57–3.00 (m, 6H) ppm; ^{13}C NMR ($CDCl_3$) δ: 28.31 (q); 29.80 (t); 32.00 (t); 46.66 (t); 47.17 (t); 52.33 (t); 55.71 (d); 80.31(s); 172.81 (s). Anal. ($C_{11}H_{22}N_2O_2$) C,H,N.

5.1.4. 1,4-Diazabicyclo[4.3.0]nonan-9-one (3). Compound **40** (1.9 mmol, 0.41 g)was heated to 200 °C; after 1 h the reaction was cooled to rt and $CHCl_3$ (100 mL) was added. The solid residue was eliminated by filtration and the organic phase evaporated under vacuum. Compound **3**[4,19] was purified by flash chromatography (eluent II) and obtained in 43% yield.

5.1.5. *N*-(*tert*-Butoxycarbonyl)sarcosine (43). NEt_3 (2.3 mL; 0.016 mol) and di-*tert*-butyl dicarbonate (1.23 g;

5.61 mmol), in a minimal amount of THF, were added to sarcosine (0.5 g; 5.61 mmol) in 1.5 mL of H_2O and 15 mL of THF, cooled to 0 °C. After 24 h at rt, the solvent was removed and the aqueous layer was extracted with $CHCl_3$. After drying and evaporation of the solvent, the product was used as such for the next reaction. ^1H NMR ($CDCl_3$) δ: 1.28 (s, 3H, CH_3N), 1.34 (s, 9H, $(CH_3)_3C$), 3.83 (s, 2H, CH_2N), 9.2 (bs, OH) ppm. Anal. ($C_8H_{15}NO_4$) C,H,N.

5.2. Activation of protected aminoacids with N-hydroxysuccinimide

1 equiv of *N*-(*tert*-butoxycarbonyl)aminoacid in 5 mL of CH_2Cl_2 was cooled to 0 °C and then added with 1 equiv of *N*-hydroxysuccinimide and 1.1 equiv of *N*,*N*′-diciclohexylcarbodiimide in a minimal amount of CH_2Cl_2. After 1 h at 0 °C, the mixture was left at room temperature for 20 h. After filtration, the solution was cooled to 0 °C and 1 equiv Benzylpiperazine was added. After 24 h at room temperature, CH_2Cl_2 was added, the solution washed with H_2O and the dried solvent evaporated under reduced pressure. Compounds **44** and **46**[29] were purified by flash chromatography with eluent I, while the derivative 45 was obtained pure. Yields and spectroscopic characteristics of compounds **44**–**46** are reported in Table 7 (supplementary material).

5.3. Removal of the benzyl group

To 1 equiv of 1-benzoyl-4-benzylpiperazine, **41** and **44**–**46** in MeOH anhydrous and under N_2 of 0.5 equiv of Pd/C/10% and 5 equiv of $HCOONH_4$ were added. The mixture was refluxed for 8 h, then filtered and the solution was evaporated. The residue was made alkaline with $NaHCO_3$, extracted with $CHCl_3$ and dried. After removal of the solvent compounds **24**, **42, 47**, **48** and **49**[30] were obtained. Their yields and spectroscopic characteristics are reported in Table 7 (supplementary material).

5.4. Synthesis of acyl or sulfonyl derivatives

To a CH_3CN solution of 1 equiv of the suitable piperazine derivative (1-benzylpiperazine, 1-acetylpiperazine, 1-propionylpiperazine, **3**, **4**, **24**, **28**, **30**, **33**, **35**, **42**, **47**–**49**) 3 equiv of NEt_3 were added. The mixture was refrigerated to 0 °C and then 1 equiv of the proper arylsulfonyl chloride, alkylsulfonyl chloride or acyl chloride was added. After 24 h at room temperature, the solvent was removed, the mixture made alkaline with $NaHCO_3$, extracted with $CHCl_3$ and dried. Removal of the solvent gave compounds that were used as such for the next step (**41**, **50**–**55**), or purified by column chromatography using the eluent indicated in Table 1 (**5**–**14**), **2** (**15**–**27**) and **3** (**29**, **31**, **34**, **36**). Their yields and spectroscopic characteristics are reported in Table 7 (supplementary material).

5.5. Deprotection of 4-substituted-1-acyl-piperazine

A solution of HCl 2 M (2 equiv) was added to intermediates **50**–**55** and the mixture left at room temperature for 24 h. Then $NaHCO_3$ was added and the solution extracted with $CHCl_3$ and dried. Removal of the solvent gave compounds that were purified with column chromatography using eluent IV (**28**, **32**, **33**, **35**, **37**) or eluent VII for compound **30**. Their yields and spectroscopic characteristics are reported in Table 7 (supplementary material).

6. Pharmacology

6.1. Antiamnesic test: passive-avoidance test

The test was performed according to the step-through method described by Jarvik and Kopp.[31] 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. Mice receive the punishment when entering the 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. Mice who did not enter after 60 s latency were excluded from the experiment. For mem-

Table 6. Antihyperalgic effect of **5** and **6** in a rat model of mononeuropathic dx evaluated in the paw pressure test

Treatment	Dose mg/kg (s.c.)	Paw	Paw pressure in rats			
			Before treatment.	After analgesic treatment.		
				30 min	45 min	60 min
Saline		sn	51.1±2.5	54.5±4.6	48.2±5.2	53.6±5.4
Saline		dx	26.5±4.3	25.2±5.1	29.7±4.8	30.6±4.9
5	0.1	sn	50.2±3.9	48.6±4.3	53.1±3.9	51.7±4.4
5	0.1	dx	28.5±3.5	29.5±3.6	31.3±5.1	28.9±3.7
5	1.0	sn	49.4±4.8	52.1±6.0	50.8±4.2	47.2±3.5
5	1.0	dx	24.8±3.5	49.2±3.1*	49.7±3.3*	51.6±4.0*
6	0.1	sn	48.7±3.9	51.3±4.5	54.2±5.1	49.6±3.7
6	0.1	dx	31.9±3.1	32.5±3.3	35.3±4.5	38.4±3.1
6	1.0	sn	52.5±4.5	54.1±4.2	52.7±3.7	47.3±5.0
6	1.0	dx	26.8±3.3	48.3±3.7*	48.9±3.5*	45.1±4.5*

There were 5–7 rats per group. *$p < 0.001$ versus the saline dx paw.

Table 7. Supplementary material to 'Structure–activity relationship studies in a class of potent cognition enhancing drugs'

Sigla	*N*	Yield (%)	IR ν (cm^{-1})	^1H NMR ($CDCl_3$) δ	^{13}C NMR ($CDCl_3$) δ
EM107	**15**	35	—	2.12 (s, 3H, CH_3CO); 3.42–3.77 (m, 8H, CH_2 piperazine); 4.27 (s, 4H, CH_2O); 6.81–6.98 (m, 3H, aromatics) ppm.	21.79 (q); 41.84 (t); 46.55 (t); 64.65 (t); 64.82 (t); 117.09 (d); 117.68 (d); 121.03 (d); 128.35 (s); 143.73 (s); 145.56 (s); 169.50 (s); 170.43 (s).
MN69	**17**	49	—	0.98 (t, $J=7.2$ Hz, 3H, CH_3CH_2); 2.18 (q, $J=7.2$ Hz, 2H, CH_2CH_3); 3.18–3.36 (m, 8H, CH_2 piperazine); 4.05 (s, 4H, CH_2O); 6.63–6.80 (m, 3H, aromatics) ppm.	9.79 (q); 26.89 (t); 43.45 (t); 45.66 (t); 64.63 (t); 64.81 (t); 117.08 (d); 117.66 (d); 121.01 (d); 128.38 (s); 143.73 (s); 145.54 (s); 170.41 (s); 172.80 (s).
EM105	**18**	74	—	2.11 (s, 3H, CH_3CO); 3.48–3.66 (m, 8H, CH_2 piperazine); 6.00 (s, 2H, CH_2O); 6.83–6.89 (m, 3H, aromatics) ppm.	21.77 (q); 41.76 (t); 46.48 (t); 101.89 (t); 108.30 (d), 108.57 (d); 121.99 (d); 128.89 (s); 148.00 (s); 149.40 (s); 169.46 (s); 170.36 (s).
MN62	**19**	61	—	1.15 (t, $J=7.3$ Hz, 3H, CH_3CH_2); 2.36 (q, $J=7.3$ Hz, 2H, CH_2CH_3); 3.44–3.76 (m, 8H, CH_2 piperazine); 6.00 (s, 2H, CH_2O); 6.78–6.96 (m, 3H, aromatics) ppm.	10.97 (q); 26.92 (t); 43.45 (t); 43.60 (t); 102.05 (t); 108.35 (d); 108.83 (d); 122.05 (d); 129.02 (s); 148.07 (s); 149.45 (s); 170.45 (s); 172.83 (s).
EM108	**20**	48	—	2.10 (s, 3H, CH_3CO); 3.55–3.66 (m, 8H, CH_2 piperazine); 7.78 (d, $J=8.8$ Hz, 1H, aromatic); 8.10–8.18 (m, 2H, aromatics); 8.88 (s, 2H, aromatics) ppm.	21.81(q); 41.76 (t); 46.50 (t); 128.44 (d); 128.89 (d); 130.77 (d); 136.87 (s); 142.59 (s); 143.53 (s); 146.32 (d); 146.43 (d); 169.32 (s); 169. 41 (s).
EM109	**21**	37	—	1.15 (t, $J=7.3$ Hz, 3H, CH_3CH_2); 2.37 (q, $J=7.3$ Hz, 2H, CH_2CH_3); 3.49–3.70 (m, 8H, CH_2 piperazine); 7.81 (dd, $J=8.4$ Hz, 1.8 Hz, 1H, aromatic); 8.12 (d, $J=1.8$ Hz, 1H, aromatic); 8.19 (d, $J=8.8$ Hz, 1H, aromatic); 8.90 (s, 2H, aromatic) ppm.	9.70 (q); 26.78 (t); 41.80 (t); 42.69 (t); 45.48 (t); 47.90 (t); 128.37 (d); 128.84 (d); 130.64 (d); 136.87 (s); 142.50 (s); 143.41 (s); 146.27 (d); 146.37 (d); 169.21 (s); 172.63 (s).
EM106	**22**	66	—	1.33 (d, $J=7.0$ Hz, 6H, $(CH_3)_2CH$);2.10 (s, 3H, CH_3CO); 3.11–3.27 (m, 1H, $CH(CH_3)_2$); 3.29–3.42 (m, 4H, CH_2 piperazine); 3.48–3.53 (m, 2H, CH_2 piperazine); 3.62–3.68 (m, 2H, CH_2 piperazine) ppm.	17.11 (q); 21.79 (q); 42.16 (t); 46.50 (t); 46.77 (t); 47.17 (t); 53.89 (d); 169.34 (s).
DM360	**23**	53	—	1.16 (t, $J=7.3$ Hz, 3H, CH_3CH_2); 1.35 (d, $J=7.0$ Hz, 6H, $(CH_3)_2CH$); 2.36 (q, $J=7.3$ Hz, 2H, CH_2CH_3); 3.13–3.26 (m, 1H, $CH(CH_3)_2$); 3.34–3.41 (m, 4H, CH_2 piperazine); 3.48–3.53 (m, 2H, CH_2 piperazine); 3.67–3.73 (m, 2H, CH_2 piperazine) ppm.	9.80 (q); 17.12 (q); 26.90 (t); 46.28 (t); 46.57 (t); 46.81 (t); 47.21 (t); 53.87 (d); 172.65 (s).
MN32	**24**	71	—	1.76 (bs, 1H, NH); 2.82–3.03 (m, 4H, CH_2 piperazine); 3.38–3.53 (m, 2H, CH_2 piperazine); 3.62–3.84 (m, 2H, CH_2 piperazine); 7.32–7.46 (m, 5H, aromatics) ppm.	–

(*Continued on next page.*)

Table 7 (*continued*)

Sigla	*N*	Yield (%)	IR ν (cm^{-1})	^{1}H NMR ($CDCl_3$) δ	^{13}C NMR ($CDCl_3$) δ
DM366	**25**	95	—	1.36 (d, J = 7.0 Hz, 6H, $(CH_3)_2CH$); 3.17–3.28 (m, 1H, $CH(CH_3)_2$); 3.33–3.42 (m, 8H, CH_2 piperazine); 7.39–7.47 (m, 5H, aromatics) ppm.	25.37 (q); 54.48 (t); 55.04 (t); 62.17 (d); 135.63 (d); 137.23 (d); 138.69 (d); 143.57 (s); 179.10 (s).
DM368	**26**	26	—	2.98–3.18 (m, 4H, CH_2 piperazine); 3.53–3.91 (m, 4H, CH_2 piperazine); 7.30–7.44 (m, 5H + 2H, aromatics); 7.74–7.81 (m, 2H, aromatics) ppm.	45.93 (t); 46.46 (t); 117.03 (d, J_{C-F} = 22.9 Hz); 127.46 (d); 129.00 (d); 130.64 (d); 130.75 (d, J_{C-F} = 11.0 Hz); 131.64 (d, J_{C-F} = 2.8 Hz); 135.01 (s); 165.73 (d, J_{C-F} = 256.4 Hz); 170.74 (s).
DM369	**27**	35	—	1.32 (d, J = 7.0 Hz, 6H, $(CH_3)_2CH$); 3.05–3.12 (m, 4H, CH_2 piperazine); 3.10–3.19 (m, 1H, $CH(CH_3)_2$); 3.46–3.51 (m, 4H, CH_2 piperazine); 7.21–7.29 (m, 2H, aromatics); 7.73–7.80 (m, 2H, aromatics) ppm.	17.09 (q); 46.15 (t); 46.92 (t); 54.20 (d); 117.05 (d, J_{C-F} = 22.8 Hz); 130.75 (d, J_{C-F} = 9.1 Hz); 131.64 (d, J_{C-F} = 2.7 Hz); 165.76 (d, J_{C-F} = 255.5 Hz).
MN45	**28**	43	3300–3380 (NH_2); 1670 (CO); 1630 (CO).	1.72 (s, 2H, NH_2); 3.18–3.43 (m, 4H, CH_2 piperazine); 3.42 (s, 2H, CH_2CO); 3.47–3.72 (m, 4H, CH_2 piperazine); 7.33–7.41 (m, 5H, aromatics) ppm.	42.38 (t); 43.59 (t); 44.46 (t); 127.30 (d); 129.00 (d); 130.60 (d); 135.20 (s); 170.91 (s, CO); 171.93 (s, CO)ppm.
EM112	**29**	24	—	1.37 (d, J = 6.6 Hz, 6H, $(\underline{CH_3})_2CH$); 3.09–3.24 (m, 1H, $CH(CH_3)_2$); 3.40–3.73 (m, 8H, CH_2 piperazine); 3.97 (s, 2H, CH_2CO); 5.40 (bs, 1H, NH); 7.27–7.45 (m, 5H, aromatics) ppm.	16.98 (q); 44.65 (t); 44.75 (t); 44.93 (t); 54.18 (d), 127.40 (d); 129.04 (d); 130.57 (d); 135.19 (s); 166.99 (s, CO); 170.92 (s, CO).
MN58	**30**	42	3340 (NH); 1670 (CO); 1620 (CO).	2.00 (bs, 1H, NH); 2.42 (s, 3H, CH_3N); 3.39 (s, 2H, CH_2CO); 3.45–3.67 (m, 8H, CH_2 piperazine); 7.27–7.41 (m, 5H, aromatics) ppm.	37.00 (q); 42.43 (t); 44.99 (t); 52.83 (t); 127.40 (d), 129.00 (d); 130.50 (d); 135.30 (s); 170.10 (s, CO); 170.90 (s, CO) ppm.
EM123	**31**	20	—	1.40 (d, J = 6.9 Hz, 6H, $(CH_3)_2CH$); 3.03 (s, 3H, CH_3N); 3.28–3.35 (m, 1H, $CH(CH_3)_2$); 4.15 (s, 2H, CH_2CO); 7.40–7.47 (m, 5H, aromatics) ppm.	17.23 (q); 37.14 (q); 42.38 (t); 45.22 (t); 51.98 (t); 58.84 (d); 127.40 (d); 129.04 (d); 130.55 (d); 135.27 (s); 166.88 (s, CO); 170.94 (s, CO).
MN40	**32**	67	3300–3380 (NH); 1650 (CO); 1640 (CO).	1.12 (d, J = 6.6 Hz, 3H, CH_3); 1.79 (bs, 2H, NH_2); 3.18–3.60 (m, 8H, CH_2 piperazine); 3.61–3.64 (m, 1H, $CHCH_3$); 7.19–7.30 (m, 5H, aromatics) ppm.	22.07 (q); 42.45 (t); 45.21 (t); 47.18 (d); 127.30 (d); 128.90 (d); 130.41 (d); 135.29 (s); 170.70 (s,CO); 175.00 (s,CO) ppm.
MN44	**33**	52	3340–3400 (NH_2); 1660 (CO).	1.53 (bs, 2H, NH_2); 2.98 (t, J = 5.1 Hz, 4H, CH_2 piperazine); 3.37 (s, 2H, CH_2CO); 3.44–3.48 (m, 2H, CH_2 piperazine); 3.61–3.71 (m, 2H, CH_2 piperazine); 7.16–7.27 (m, 2H, aromatics); 7.69–7.79 (m, 2H, aromatics) ppm.	41.64 (t); 43.64 (t); 44.04 (t); 46.25 (t); 46.34 (t); 116.90 (dd, J_{C-F} = 21.9 Hz); 130.70 (d, J_{C-F} = 9.2 Hz); 131.60 (d, J_{C-F} = 3.6 Hz); 165.70 (d, J_{C-F} = 254.0 Hz); 171.90 (s, CO) ppm.

(*Continued on next page.*)

Table 7 (*continued*)

Sigla	*N*	Yield (%)	IR ν (cm^{-1})	^1H NMR ($CDCl_3$) δ	^{13}C NMR ($CDCl_3$) δ
EM111	**34**	56	—	1.15 (d, $J = 6.9$ Hz, 6H, $(CH_3)_2CH$); 2.82–2.93 (m, 4H, CH_2 piperazine); 3.06–3.11 (m, 1H, $CH(CH_3)_2$); 3.42–3.52 (m, 4H, CH_2 piperazine); 3.81 (s, 2H, CH_2CO); 5.04 (bs, 1H, NH); 7.43–7.52 (m, 2H, aromatics); 7.75–7.82 (m, 2H, aromatics) ppm.	15.74 (q); 42.86 (t); 43.37 (t); 45.22 (t); 51.49 (d); 116.23 (d, $J_{C-F} = 22.9$ Hz); 130.15 (d, $J_{C-F} = 9.1$ Hz); 130.48 (d, $J_{C-F} = 3.6$ Hz); 164.21 (d, $J_{C-F} = 251.9$ Hz); 166.44 (s, CO).
MN57	**35**	46	3350 (NH); 1650 (CO).	2.08 (bs, 1H, NH); 2.35 (s, 3H, CH_3); 2.83–3.01 (m, 4H, CH_2 piperazine); 3.29 (s, 2H, CH_2CO); 3.47–3.58 (m, 2H, CH_2 piperazine); 3.62–3.71 (m, 2H, CH_2 piperazine); 7.16–7.27 (m, 2H, aromatics); 7.69–7.77 (m, 2H, aromatics) ppm.	36.89 (q); 41.32 (t); 44.37 (t); 52.71 (t); 116.90 (dd, $J_{C-F} = 22.7$ Hz); 130.70 (d, $J_{C-F} = 9.1$ Hz); 131.60 (s); 165.70 (d, $J_{C-F} = 254.0$ Hz); 169.70 (s, CO) ppm.
EM122	**36**	30	—	1.34 (d, $J = 6.9$ Hz, 6H, $(CH_3)_2CH$); 2.93 (s, 3H, CH_3N); 2.97–3.03 (m, 4H, CH_2 piperazine); 3.18–3.29 (m, 1H, $CH(CH_3)_2$); 3.55–3.60 (m, 2H, CH_2 piperazine); 3.62–3.69 (m, 2H, CH_2 piperazine); 4.05 (s, 2H, CH_2CO); 7.20–7.28 (m, 2H, aromatics); 7.73–7.80 (m, 2H, aromatics) ppm.	18.45 (q); 37.11 (q); 41.25 (t); 44.03 (t); 46.10 (t); 54.35 (d); 117.04 (d, $J_{C-F} = 22.9$ Hz); 130.78 (d, $J_{C-F} = 7.3$ Hz); 131.61 (s); 165.76 (d, $J_{C-F} = 255.5$ Hz); 166.61 (s, CO).
MN36	**37**	60	3450 (NH_2); 1650 (CO).	1.12 (d, $J = 6.9$ Hz, 3H, CH_3); 2.01 (bs, 2H, NH_2); 2.79–3.08 (m, 4H, CH_2 piperazine); 3.48–3.59 (m, 4H, CH_2 piperazine); 3.66 (q, $J = 6.8$ Hz, 1H, $CHCH_3$); 7.15–7.23 (m, 2H, aromatics); 7.68–7.74 (m, 2H, aromatics) ppm.	22.15 (q); 41.63 (t); 44.83 (t); 46.23 (t); 46.46 (t); 47.29 (d); 116.90 (dd, $J_{C-F} = 22.7$ Hz); 130.70 (d, $J_{C-F} = 9.2$ Hz); 131.5 (d, $J_{C-F} = 2.7$ Hz); 165.7 (d, $J_{C-F} = 254.0$ Hz); 174.8 (s, CO) ppm.
	41	91	—	1.36 (d, $J = 6.6$ Hz, 6H, $(CH_3)_2CH$); 2.42–2.56 (m, 4H, CH_2 piperazine); 3.09–3.22 (m, 1H, $CH(CH_3)_2$); 3.35–3.42 (m, 4H, CH_2 piperazine); 3.57 (s, 2H, CH_2Ph); 7.27–7.37 (m, 5H, aromatics) ppm.	—
	42	74	—	1.34 (d, $J = 7.0$ Hz, 6H, $(CH_3)_2CH$); 1.68 (bs, 1H, NH); 2.87–2.93 (m, 4H, CH_2 piperazine); 3.12–3.22 (m, 1H, $CH(CH_3)_2$); 3.31–3.35 (m, 4H, CH_2 piperazine) ppm.	—

(*Continued on next page*.)

Table 7 (*continued*)

Sigla	*N*	Yield (%)	IR ν (cm^{-1})	^{1}H NMR ($CDCl_3$) δ	^{13}C NMR ($CDCl_3$) δ
	44	70	—	1.37 (s, 9H, $(CH_3)_3C$); 2.33–2.38 (m, 4H, CH_2 piperazine); 3.31–3.38 (m, 2H, CH_2 piperazine); 3.44 (s, 2H, CH_2Ph); 3.54–3.66 (m, 2H, CH_2 piperazine); 3.86 (s, 2H, CH_2CO); 5.61 (bs, 1H, NH); 7.21–7.27 (m, 5H, aromatics) ppm.	—
	45	99	—	1.39 (s, 9H, $(CH_3)_3C$); 2.37–2.42 (m, 4H, CH_2 piperazine); 2.57 (s, 2H, CH_2CO); 2.79 (s, 3H, CH_3N); 3.28–3.41 (m, 2H, CH_2 piperazine); 3.43 (s, 2H, CH_2Ph); 3.52–3.62 (m, 2H, CH_2 piperazine); 7.17–7.31 (m, 5H, aromatics) ppm.	—
	46	97	—	1.22 (d, $J = 6.4$ Hz, 3H, CH_3CH); 1.37 (s, 9H, $(CH_3)_3C$); 2.41 (bs, 1H, NH); 2.20–2.58 (m, 4H, CH_2 piperazine); 3.25–3.66 (m, 4H, CH_2 piperazine); 3.5 (s, 2H, CH_2Ph); 4.51–4.60 (m, 1H, $CHCH_3$); 7.20–7.27 (m, 5H, aromatics) ppm.	—
	47	75	—	1.38 (s, 9H, $(CH_3)_3C$); 2.75–2.81 (m, 5H, CH_2 + NH piperazine); 3.27–3.32 (m, 2H, CH_2 piperazine); 3.50–3.55 (m, 2H, CH_2 piperazine); 3.88 (d, $J = 4.4$. Hz, 2H, CH_2NH); 5.56 (bs, 1H, NHCO) ppm.	—
	48	63	—	1.32 (s, 9H, $(CH_3)_3C$); 2.31 (bs, 1H, NH); 2.63–2.78 (m, 4H, CH_2 piperazine); 2.74 (s, 3H, CH_3N); 3.12–3.23 (m, 2H, CH_2 piperazine); 3.38–3.43 (m, 2H, CH_2 piperazine); 3.92 (s, 2H, CH_2N) ppm.	—
	49	46	—	1.20 (d, $J = 6.6$ Hz, 3H, CH_3CH); 1.34 (s, 9H, $(CH_3)_3C$); 2.20 (bs, 1H, NH); 2.73–2.82 (m, 4H, CH_2 piperazine); 3.38–3.61 (m, 4H, CH_2 piperazine); 4.45–4.58 (m, 1H, $CHCH_3$); 5.65 (bs, 1H, NHCO) ppm.	—
	50	99	—	1.38 (s, 9H, $(CH_3)_3C$); 3.21–3.41 (m, 4H, CH_2 piperazine); 3.42–3.63 (m, 4H, CH_2 piperazine); 3.85 (s, 2H, CH_2CO); 5.51 (bs, 1H, NH); 7.31–7.40 (m, 5H, aromatics)ppm.	—
	51	99	—	1.42 (s, 9H, $(CH_3)_3C$); 2.88 (s, 3H, CH_3N); 3.28–3.71 (m, 8H, CH_2 piperazine); 4.04 (s, 2H, CH_2CO); 7.27–7.43 (m, 5H, aromatics) ppm.	—

(*Continued on next page*.)

Table 7 (*continued*)

Sigla	*N*	Yield (%)	IR ν (cm^{-1})	^1H NMR ($CDCl_3$) δ	^{13}C NMR ($CDCl_3$) δ
	52	64	—	1.22 (d, $J = 7.3$ Hz, 3H, CH_3CH); 1.35 (s, 9H, $(CH_3)_3C$); 3.28–3.73 (m, 8H, CH_2 piperazine); 4.45–4.61 (m, 1H, $CHCH_3$); 5.58 (d, $J = 9.2$ Hz, 1H, NH); 7.33–7.35 (m, 5H, aromatics) ppm.	—
	53	99	—	1.38 (s, 9H, $(CH_3)_3C$); 2.90–2.98 (m, 4H, CH_2 piperazine); 3.45–3.47 (m, 2H, CH_2 piperazine); 3.66–3.70 (m, 2H, CH_2 piperazine); 3.86 (d, $J = 4.4$ Hz, 2H, CH_2NH); 5.39 (bs, 1H, NH); 7.16–7.27 (m, 2H, aromatics); 7.68–7.76 (m, 2H, aromatics) ppm.	—
	54	99	—	1.37 (s, 9H, $(CH_3)_3C$); 2.81 (s, 3H, CH_3N); 2.94–2.97 (m, 4H, CH_2 piperazine); 3.41–3.52 (m, 2H, CH_2 piperazine); 3.59–3.68 (m, 2H, CH_2 piperazine); 3.92 (s, 2H, CH_2CO); 7.15–7.25 (m,2H, aromatics); 7.67–7.72 (m, 2H, aromatics) ppm.	—
	55	99	—	1.19 (d, $J = 6.6$ Hz, 3H, CH_3CH); 1.34 (s, 9H, $(CH_3)_3C$); 2.79–2.95 (m, 2H, CH_2 piperazine); 3.11–3.21 (m, 2H, CH_2 piperazine); 3.35–3.58 (m, 2H, CH_2 piperazine); 3.61–3.76 (m, 1H, CH_2 piperazine); 3.86.3.97 (m, 1H, CH_2 piperazine); 4.42–4.56 (m, 1H, $CH–CH_3$); 5.36 (d, $J = 8.1$ Hz, 1H, NH); 7.09–7.32 (m, 2H, aromatics); 7.68–7.82 (m, 2H, aromatics) ppm.	—

ory disruption, mice were ip injected with the amnesic drugs (scopolamine, **13** or **14**). All investigated drugs were given, ip, 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. Piracetam, DM232 (**1**), and DM235 (**2**) were used as the reference drugs.

All compounds elicited their antiamnesic effect without changing either gross behaviour or motor coordination, as revealed by the rota-rod test (data not shown). None of the drugs, at the active doses, increased the number of falls from the rotating rod in comparison with saline-treated mice. The number of falls in the rota-rod test progressively decreased since mice learned how to balance on the rotating rod. The spontaneous motility and inspection activity of mice was unmodified by the administration of the studied compounds as revealed by the hole-board test in comparison with saline-treated mice (data not shown).

6.2. Antiamnesic test: social learning

The social learning test was performed according to Mondadori et al.[17] Male wistar rats (350–450 g) were used throughout the experiments and juvenile males (90–110 g) were used as social stimuli. All the adult animals were housed individually and placed in the testing room at least 24 h before the experiment. On the day preceding the experiment, adult rats were handled to become familiar with the operator. Juvenile rats were housed four per cage and brought into the testing room the same day of the experiment. Each mature rat was tested in its home cage. The first day of the experiment, a juvenile rat was introduced into the adult male's cage and the time spent in social-investigatory behaviour by the adult male within a 5-min fixed interval was recorded. Social investigatory behaviour was defined as being proximally oriented to the juvenile or in direct contact while sniffing, following, nosing, grooming or generally inspecting any body surface of the juvenile. After 24 h, either the same juvenile or an unfamiliar one was placed again into the mature male's cage and social investigatory behaviour was recorded in a 5-min interval. Piracetam and **22** was ip injected 20 min before the first session of the experiment.

6.3. Analgesic action on chronic constriction injury

A peripheral mono neuropathy was produced in adult rats by placing loosely constrictive ligatures around the common sciatic nerve according to the method described by Bennett.[32] Rats were anaesthetised with chloral hydrate. The common sciatic nerve was exposed at the level of the middle of the thigh by blunt dissection through biceps femoris. Proximal to sciatica's tri furcation, about 1 cm of the nerve was freed of adhering tissue and four ligatures (3/0 silk tread) were tied loosely around it with about 1 mm spacing. The length of the nerve thus affected was 4–5 mm long. Great care was taken to tie the ligatures such that the diameter of the nerve was seen to be just barely constricted when viewed with 40× magnification. In every animal, an identical dissection was performed on the opposite side except that the sciatic nerve was not ligated. The left paw was untouched.

6.4. Paw pressure test

The nociceptive threshold in the rat was determined with an analgesimeter (Ugo Basile, Varese, Italy), according to the method described by Leighton et al.[33] Rats scoring below 40 g or over 75 g during the test before drug administration (25%) were rejected. An arbitrary cut-off value of 250 g was adopted.

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