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Molecular Simplification of 1,4-Diazabicyclo[4.3.0]nonan-9-ones Gives Piperazine Derivatives That Maintain High Nootropic Activity

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Several 4-substituted 1-acylpiperazines, obtained by molecular simplification of 4-substituted 1,4-diazabicyclo[4.3.0]nonan-9-ones, have been synthesized and tested in vivo on the mouse passive avoidance test, to evaluate their nootropic activity. The results show that, apparently, an *N*-acylpiperazine group can mimic the 2-pyrrolidinone ring of 1,4-diazabicyclo[4.3.0]nonan-9-one, as the compounds of the new series maintain high nootropic activity. Moreover molecular simplification produces more clear-cut structure−activity relationships with respect to the parent series. The mechanism of action also appears to be similar in the two series. In fact, although the molecular mechanism remains to be elucidated, the most potent compound of each class (DM232 and **13**, DM235) is able to increase acetylcholine release in rat brain. Piperazine derivatives represent a new class of nootropic drugs with an in vivo pharmacological profile very similar to that of piracetam, showing much higher potency with respect to the reference compound. Among the compounds studied, **13** (DM235) shows outstanding potency, being active at a dose of 0.001 mg kg^{-1} sc.

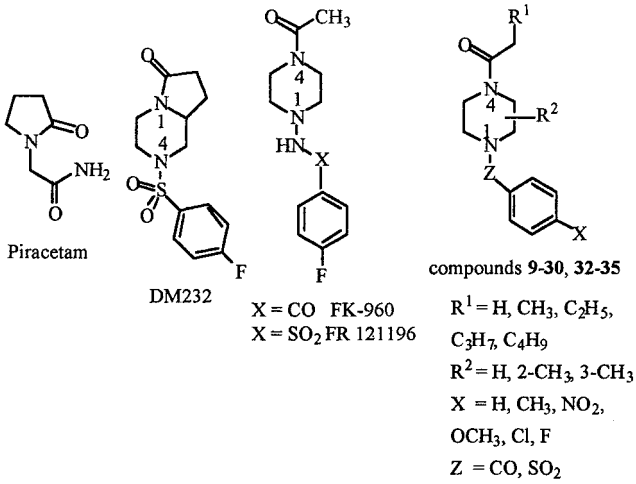
Introduction

Piracetam-like nootropic compounds,[1] through some as yet unidentified mechanism(s) of action,[2,3] act by increasing neuronal sensitivity toward stimulation. The members of this class present very low toxicity and lack the serious side effects associated with analeptics or psychostimulants, and a few have reached therapeutic use, now being on the market as nootropics. This makes them quite safe[4] for the treatment of age-related neurodegenerative pathologies, like mild to moderate Alzheimer's and Parkinson's diseases.[5,6]

In a preceding paper[7] we described a series of 1,4-diazabicyclo[4.3.0]nonan-9-ones that were extremely potent nootropic drugs in the mouse passive avoidance test. The most potent of the series, identified as DM232 (Chart 1), was able to prevent amnesia at doses as low as 0.001 mg kg^{-1} sc, being several thousand times more potent than the reference compound piracetam, which, in the same test, showed a minimum active dose of 30 mg kg^{-1} ip, being totally inactive at a dose of 10 mg kg^{-1} ip. In the structure of this new class of nootropics, condensed with a piperazine cycle, a 2-oxopyrrolidine ring is present, which is the constant feature of most nootropic compounds.

During the research we became aware of the nootropic action of two compounds: *N*-(4-acetyl-1-piperazinyl)-*p*-fluorobenzamide (FK 960)[8] and *N*-(4-acetyl-1-piperazinyl)-*p*-fluorobenzensulfonamide (FR 121196)[9] somehow related to our compounds (Chart 1). Their structure and activity prompted us to synthesize the simple

Chart 1



piperazinyl derivatives of general structure reported in Chart 1, which can be considered open analogues of 1,4-diazabicyclo[4.3.0]nonan-9-ones.

Chemistry

Compounds **9**−**30** were obtained according to Scheme 1, by treatment of the commercially available *N*-benzylpiperazine with the appropriate acyl chloride, reduction with H_2/Pd/C, and coupling of the base with the proper acyl and sulfonyl chloride, or by treatment of the commercially available *N*-acetylpiperazine with the same reagents. For the sake of clearness, since nomenclature rules may in this case introduce some confusion, we have numbered the critical positions of the ring throughout all figures. Among the compounds of this series (reported in Table 1), compound **10** is mentioned

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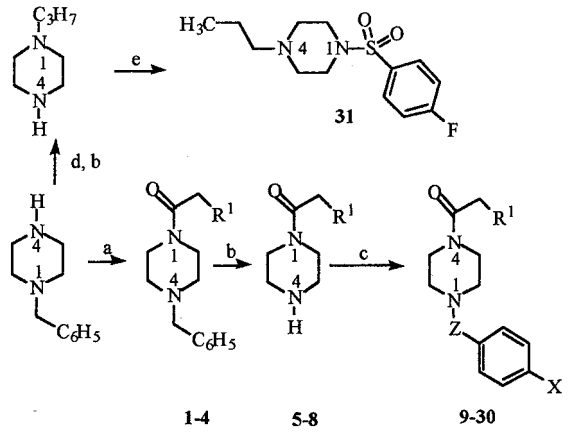
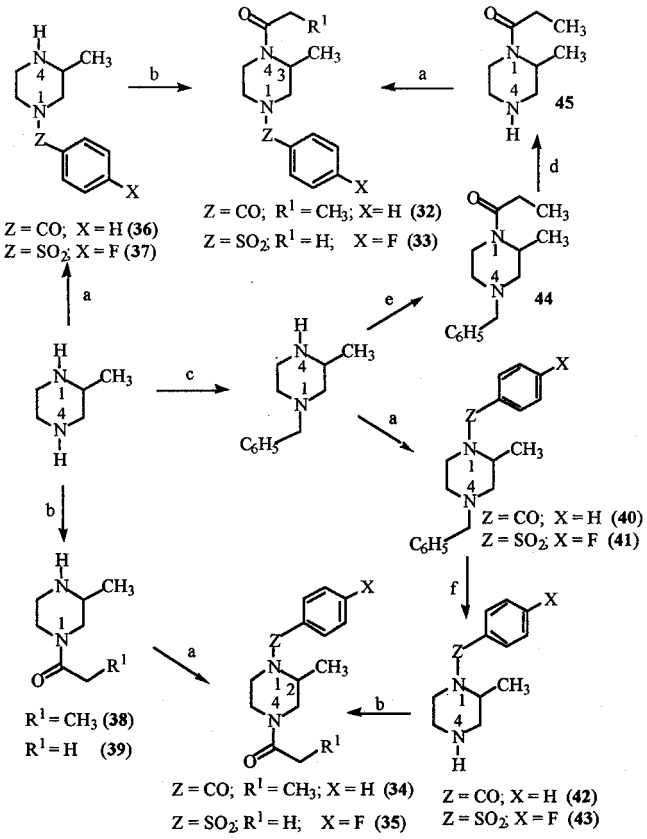
Scheme 1[a]



[a] (a) Acyl chloride; (b) H_2, Pd/C; (c) benzoyl or benzenesufonyl chloride; (d) C_3H_7Br; (e) 4-fluorobenzenesulfonyl chloride.

Scheme 2[a]



[a] (a) Benzoyl chloride or *p*-fluorobenzenesulfonyl chloride; (b) acetyl chloride or propionyl chloride; (c) benzyl chloride; (d) H_2, Pd/C; (e) propionic anhydride; (f) ammonium formate/Pd/C.

as a reaction intermediate in a patent of 1993.[10] Compound **31** was obtained from *N*-propylpiperazine,[11] obtained by us as reported in Scheme 1, by reaction with *p*-fluorobenzenesulfonyl chloride.

Compounds **32** and **33** were obtained according to Scheme 2. Commercially available 2-methylpiperazine was treated with benzoyl or *p*-fluorobenzenesulfonyl chloride to give **36** and **37**, respectively. Performing a similar reaction, Bolòs and co-workers[12] had previously shown that the substituents enter on the distal nitrogen with respect to the methyl. Compounds **36** and **37** were then treated, respectively, with propionyl or acetyl chloride to give the expected products **32** and **33**. Simple inversion of the sequence of reactions gave the isomeric 2-methyl derivatives **34** and **35** via the intermediates **38** and **39**.

However, the final compounds obtained in this way showed complex NMR spectra that, among other reasons, could be due to an incomplete regioselectivity of the first step of the reaction sequence. For this reason, compounds **32**, **34**, and **35** were synthesized also through an independent pathway from the already described[13] 1-benzyl-3-methylpiperazine, as shown in Scheme 2. The compounds obtained from these two pathways were identical, thus proving that the complexity of NMR spectra was not due to the presence of isomers. In the case of compound **33** it was not necessary to spend time in a new synthesis, since TLC chromatography showed that the compound is pure and different from its isomer **35**.

As mentioned above, the spectra of the final compounds are complex (see the Experimental Section). For instance, compound **33** shows two doublets for the 3-methyl, respectively, at $\delta = 1.28$ and 1.39, while compound **35** shows two doublets for the 2-methyl ($\delta =$ 0.97 and 1.05, respectively) and two singlets for the methyls of the *N*-acetyl group ($\delta = 2.04$ and 2.09, respectively). The reasons for this complexity of the NMR spectra of the final compounds are very likely due to conformational problems, but we did not investigate this any further.

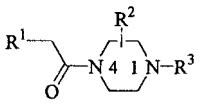
The chemical and physical properties of compounds **9**−**30** and **32**−**35** are reported in Table 1.

Pharmacology

Since the mechanism of action of piracetam-like cognition enhancers is still under investigation, the search for new drugs in this class generally relies on behavioral tests. Among them, the reversal of scopolamine-induced amnesia in passive avoidance test has been widely used. Therefore, our compounds were tested as cognition enhancers in the mouse passive avoidance test of Jarvik and Kopp, slightly modified by us (see the Experimental Section).[14] In short, mice receive punishment when entering a dark room in the training session and remember it in the following day's session, 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. Different amnesic drugs were used: scopolamine, diphenhydramine, and baclofen were injected immediately after the termination of the training session, while clonidine was injected 60 min before. Piracetam was used as the reference drug. Comparison of the latency times of saline-treated animals with those of mice that received both the amnesing drug and the investigated drug gives a measure of the cognition activity of the compounds tested.

All compounds elicited their antiamnesic effect without changing either gross behavior or motor coordina-

Table 1. Chemical and Physical Characteristics of Compounds **9**−**30** and **32**−**35**



no.	R^1	R^2	R^3	elut solv[a]	mp (°C)[b]	analysis[c]
9	H	H	COC_6H_5	B	94−95	$C_{13}H_{16}N_2O_2$
10	H	H	COC_6H_4-*p*-F	D	50−52	$C_{13}H_{15}FN_2O_2$[d]
11	H	H	$SO_2C_6H_5$	F	150−151	$C_{12}H_{16}N_2O_3S$
12	H	H	$SO_2C_6H_4$-*p*-F	D	100−102	$C_{12}H_{15}FN_2O_3S$
13	CH_3	H	COC_6H_5	B	57−58	$C_{14}H_{18}N_2O_2$
14	CH_3	H	COC_6H_4-*p*-CH_3	I	75−77	$C_{15}H_{20}N_2O_2$
15	CH_3	H	COC_6H_4-*p*-OCH_3	I	82−84	$C_{15}H_{20}N_2O_3$
16	CH_3	H	COC_6H_4-*p*-Cl	I	69−70	$C_{14}H_{17}ClN_2O_2$
17	CH_3	H	COC_6H_4-*p*-F	D	90−91	$C_{14}H_{17}FN_2O_2$
18	CH_3	H	COC_6H_4-*p*-NO_2	I	117−119	$C_{14}H_{17}N_3O_4$
19	CH_3	H	$SO_2C_6H_5$	G	110−112	$C_{13}H_{18}N_2O_3S$
20	CH_3	H	$SO_2C_6H_4$-*p*-CH_3	F	94−95	$C_{14}H_{20}N_2O_3S$
21	CH_3	H	$SO_2C_6H_4$-*p*-OCH_3	H	118−120	$C_{14}H_{20}N_2O_4S$
22	CH_3	H	$SO_2C_6H_4$-*p*-Cl	H	98−99	$C_{13}H_{17}ClN_2O_3S$
23	CH_3	H	$SO_2C_6H_4$-*p*-F	D	79−80	$C_{13}H_{17}FN_2O_3S$
24	CH_3	H	$SO_2C_6H_4$-*p*-NO_2	H	163−164	$C_{13}H_{17}N_3O_5S$
25	C_2H_5	H	COC_6H_5	C	38−40	$C_{15}H_{20}N_2O_2$
26	C_2H_5	H	COC_6H_4-*p*-F	B	56−57	$C_{15}H_{19}FN_2O_2$
27	C_2H_5	H	$SO_2C_6H_5$	C	71−73	$C_{14}H_{20}N_2O_3S$
28	C_2H_5	H	$SO_2C_6H_4$-*p*-F	F	122−123	$C_{14}H_{19}FN_2O_3S$
29	C_3H_7	H	$SO_2C_6H_4$-*p*-F	B	120−121	$C_{15}H_{21}FN_2O_3S$
30	C_4H_9	H	$SO_2C_6H_4$-*p*-F	F	133−134	$C_{16}H_{23}FN_2O_3S$
32	CH_3	3-CH_3	COC_6H_5	H	*e*	$C_{15}H_{20}N_2O_2$
33	H	3-CH_3	$SO_2C_6H_4$-*p*-F	H	98−100	$C_{13}H_{17}FN_2O_3S$
34	CH_3	2-CH_3	COC_6H_5	E	*e*	$C_{15}H_{20}N_2O_2$
35	H	2-CH_3	$SO_2C_6H_4$-*p*-F	E	86−88	$C_{13}H_{17}FN_2O_3S$

[a] Eluting system for column chromatography: A = $CHCl_3$/MeOH, 90:10; B = $CHCl_3$/MeOH, 97:3; C = $CHCl_3$/absolute EtOH/petroleum ether/NH_4OH, 360:65:60:8; D = cyclohexane/ethyl acetate, 50:50; E = $CHCl_3$/MeOH, 95:5; F = $CHCl_3$/MeOH/hexane, 65:5:30; G = CH_2Cl_2/MeOH, 98:2; H = CH_2Cl_2/MeOH, 95:5. [b] After crystallization from petroleum ether. [c] ^1H NMR and IR spectra are in accord with the proposed structures. [d] See ref 10. [e] Low-melting solid.

tion, 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 were unmodified by the administration of the compounds studied as revealed by the hole-board test in comparison with saline-treated mice (data not shown).

The most potent compound of the series (**13**, DM235) was also tested in a social learning paradigm in which adults rats with unimpaired memory were used.[15]

Compound **13** and its previously described cyclic analogue DM232[7] have been tested by microdialysis studies on rat cerebral cortex to evaluate their ability to facilitate acetylcholine release, in accordance with the method of Giovannini et al.[16] The antinociceptive activity of the same compounds has been evaluated on the mouse hot-plate test according to O'Callaghan.[17]

Results

The results obtained by screening the compounds studied on scopolamine-induced amnesia are reported in Table 2. The minimal effective dose (MED) for each compound is reported, compared with that of the reference compound, piracetam. Most of the compounds

Table 2. Nootropic Effect of Piperazine Derivatives **1** and **9**−**35** on Mouse Passive Avoidance Test, Using Scopolamine as Amnesic Drug

		entry latency (s)		
drug[a] (no. of animals)	MED (mg/kg, sc)	1st day	2nd day	Δ
saline (13)		15.0 ± 5.9	95.6 ± 8.8	80.6
vehicle (10)[b]		20.3 ± 5.3	108.5 ± 10.5	88.2
vehicle (10)[c]		15.7 ± 3.3	101.4 ± 9.6	85.7
scopolamine (6)	1.5[d]	16.6 ± 4.7	44.5 ± 8.3°	27.9
1 (10)	10	12.8 ± 4.1	93.9 ± 8.7*	81.1
9 (12)	10[e]	11.3 ± 5.3	119.0 ± 11.2*	107.7
10 (12)	10	17.5 ± 6.1	91.1 ± 9.5∧	72.6
11 (6)	0.01	17.5 ± 5.3	104.0 ± 14.5*	86.5
12 (10)	0.01	19.8 ± 4.1	89.0 ± 18.3∧	69.2
13 (31)	0.001	20.5 ± 3.4	91.5 ± 8.0*	71.0
14 (10)	>1[e]			
15 (10)	10	17.6 ± 3.5	96.1 ± 13.5*	78.5
16 (10)	>1[e]			
17 (25)	30	16.9 ± 3.7	77.1 ± 12.1∧	60.2
18 (10)	1.0	18.8 ± 5.0	104.3 ± 9.3*	85.5
19 (10)	0.1	19.0 ± 4.0	132.0 ± 11.1*	113.0
20 (10)	10	16.6 ± 3.9	88.6 ± 8.5∧	72.0
21 (10)	>1[e]			
22 (10)	>0.1[e]			
23 (34)	0.01	15.9 ± 3.2	90.6 ± 8.2*	74.7
24 (28)	>0.1[e]			
25 (8)	1.0	13.8 ± 3.7	103.6 ± 10.5*	89.8
26 (9)	10	19.8 ± 3.2	112.0 ± 8.7*	92.2
27 (10)	1.0	15.0 ± 3.6	85.4 ± 7.2∧	70.4
28 (10)[b]	>10[e]			
29 (15)[f]	>0.1[e]			
30 (12)	1.0	16.6 ± 3.6	81.5 ± 9.1∧	64.9
31 (10)	1.0	15.3 ± 3.9	96.7 ± 9.9*	81.4
32 (10)[c]	>10[e]			
33 (10)	1.0	12.5 ± 3.9	96.4 ± 10.1*	83.9
34 (23)	0.1	21.0 ± 5.3	81.2 ± 9.6∧	60.2
35 (13)	0.1	16.6 ± 4.1	99.2 ± 8.5*	82.6
scopolamine + piracetam (34)	30[d]	17.6 ± 3.6	108.8 ± 10.4*	91.2

[a] All compounds were dissolved in saline unless differently stated. [b] DMSO/H_2O (1:3) used as vehicle when the compound was not soluble in saline. [c] DMSO/H_2O (1:2) used as vehicle when the compound was not soluble in saline. [d] Dosed ip. [e] Dose limited by solubility problems. [f] Suspension. °$P < 0.01$ with respect to mice treated with saline; ∧$P < 0.05$; *$P < 0.01$ with respect to mice treated with scopolamine.

synthesized are active in preventing amnesia. Among them, compound **13** is the most potent as it is able to prevent amnesia at doses as low as 0.001 mg kg^{-1} sc, being some thousand times more potent than the reference compound piracetam. This latter compound, in the same test, shows a minimum active dose of 30 mg kg^{-1} ip, being totally inactive at a dose of 10 mg kg^{-1} ip. Several other compounds of the series are also active at MEDs below 1 mg kg^{-1} sc, showing that this series represents a new, very potent class of nootropic drugs. In addition to scopolamine (antimuscarinic), these compounds were also able to revert amnesia induced by diphenhydramine (antihistaminic), baclofen ($GABA_B$ agonist), and clonidine (α_2 agonist) with a potency pattern similar to that reported for scopolamine. The results regarding compound **13** and its previously reported cyclic analogue DM232 are reported in Table 3.

Compounds **11** and **13** are also active when given po (data not shown) although in this case the MED increases (from 0.001 to 0.1 mg kg^{-1} sc for **13**; from 0.01 to 0.1 mg kg^{-1} sc for **11**). However it should be considered that for aniracetam, used as a reference, the MED po is 100 mg kg^{-1}.

Table 3. Effect of Compound **13** and DM232 on Amnesia Induced by Diphenhydramine (20 mg/kg ip), Baclofen (2 mg/kg ip), and Clonidine (0.125 mg/kg ip) in Mouse Passive Avoidance Test

drug[a] (no. of animals)	dose (mg/kg, ip)	entry latency (s) 1st day	2nd day	Δ
saline (19)		16.1 ± 2.2	89.3 ± 10.4	73.2
DM232 + saline (10)	0.1	14.3 ± 3.7	94.5 ± 11.0	80.2
13 + saline (9)	0.1	17.2 ± 3.4	86.3 ± 9.9	69.1
saline + diphenhydramine (26)		15.7 ± 2.9	47.7 ± 8.3°	32.0
DM232 + diphenhydramine (13)	0.1	15.6 ± 2.8	95.2 ± 10.7*	79.6
13 + diphenhydramine (11)	0.01	15.8 ± 3.3	84.3 ± 14.1*	68.5
13 + diphenhydramine (12)	0.1	14.4 ± 2.8	81.4 ± 12.2*	67.0
saline + baclofen (29)		16.6 ± 2.1	38.5 ± 7.8°	21.9
DM232 + baclofen (11)	0.1	12.3 ± 3.5	76.4 ± 9.3^	64.1
13 + baclofen (11)	0.1	17.2 ± 3.3	73.2 ± 12.5^	56.0
saline + clonidine (22)		16.3 ± 4.1	46.8 ± 8.1°	30.5
DM232 + clonidine (14)	0.1	14.5 ± 3.6	81.2 ± 10.9*	66.7
13 + clonidine (11)	0.1	15.0 ± 2.7	78.8 ± 12.6*	63.8

[a] Compound **13** and DM232 were administered 20 min before the training session while clonidine 60 min before. Diphenhydramine and baclofen were injected immediately after the training session. °$P < 0.01$ in comparison with saline controls; *$P < 0.01$ and ^$P < 0.05$ in comparison with diphenhydramine-, baclofen-, or clonidine-treated mice.

At higher doses, all compounds maintain their antiamnesic activity without any effect on behavior (data not shown). As a matter of fact, rota-rod and hole-board tests did not evidence any effect on motor coordination, spontaneous motility, and curiosity of the animal treated. Moreover, the compounds did not exhibit any collateral symptoms when injected at a dose 1000-fold higher than the MED preventing amnesia.

At doses comparable to that resulted effective in the passive avoidance test, compound **13** was active in the social learning test in which adults rats with unimpaired memory were used. In fact **13**, as well as the reference drug piracetam, improved cognitive performance by prolonging the time physiologically spent by rats to delete mnemonic information (Figure 1).

Compound **13** (DM235) and its cyclic analogue DM232 are both able to facilitate the release of acetylcholine from rat cerebral cortex at doses of 0.01 mg kg^{-1} ip as shown in Figure 2. It is remarkable that at doses of 1.0 mg kg^{-1} ip there is no release facilitation, as reported also for DM232 in the previous paper.[7]

Coherently with their acetylcholine-releasing properties, both compounds possess atropine-sensitive analgesic activity[18] at a dose of 0.01 mg kg^{-1} ip, as shown in Figure 3.

Discussion

From the following discussion it will be clear that the molecular simplification, carried out on 1,4-diazabicyclo-[4.3.0]nonan-9-ones, results in much clearer structure–activity relationships (SARs), notwithstanding the uncertainties inherent to an in vivo test, like passive avoidance, that frequently complicate the elaboration of SARs. In fact, the resulting activity is the consequence of both pharmacokinetic and pharmacodynamic properties that can be differently affected by structural modifications.

If our initial reasonings on the correspondence of 1,4-diazabicyclo[4.3.0]nonan-9-one with 4-acylpiperazines

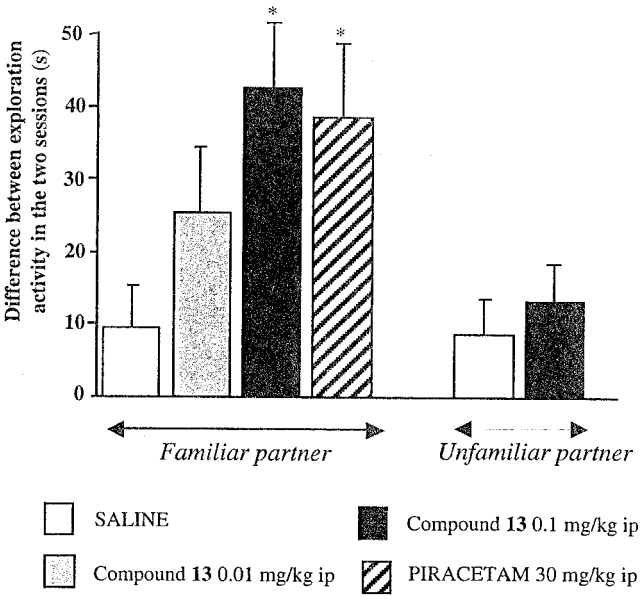


Figure 1. Effect of compound **13** in rat social learning test in comparison with piracetam. Each column represents the mean of 6 rats. *$P < 0.01$ in comparison with the corresponding saline-treated rats. Compound **13** and piracetam were administered 20 min before the first session. Vertical lines show SEM.

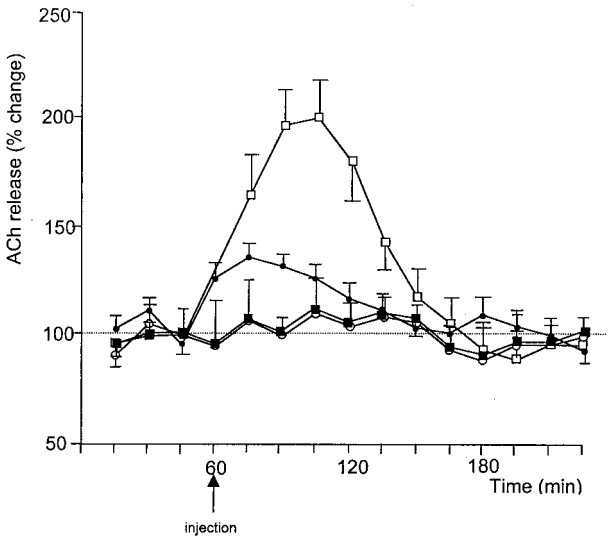


Figure 2. Dose–response curve for compound **13** (open symbols) and compound DM232 (closed symbols) on ACh release from rat cerebral cortex. Compounds were injected ip after 60 min as shown by the arrow. All values are expressed as changes over basal output. Vertical lines give SEM. Each point represents the mean of 3 experiments: open squares, compound **13** (DM235) 0.01 mg/kg; open circles, compound **13** (DM235) 1 mg/kg; closed circles, DM232 0.01 mg/kg; closed squares, DM232 1 mg/kg.

were correct, one would expect the 1-propionyl derivatives **13**–**24** to be the most potent nootropics of the series. This is indeed the case: all compounds of the series show nootropic activity comparable to that of the corresponding 1,4-diazabicyclo[4.3.0]nonan-9-ones.[7] However, unlike what happens in the bicyclic compound series, here the most potent member is compound **13**, which carries an unsubstituted benzoyl group on the piperazine nitrogen 4, suggesting slightly different SARs in the two series, or a critical difference in the influence of pharmacokinetics on their activity.

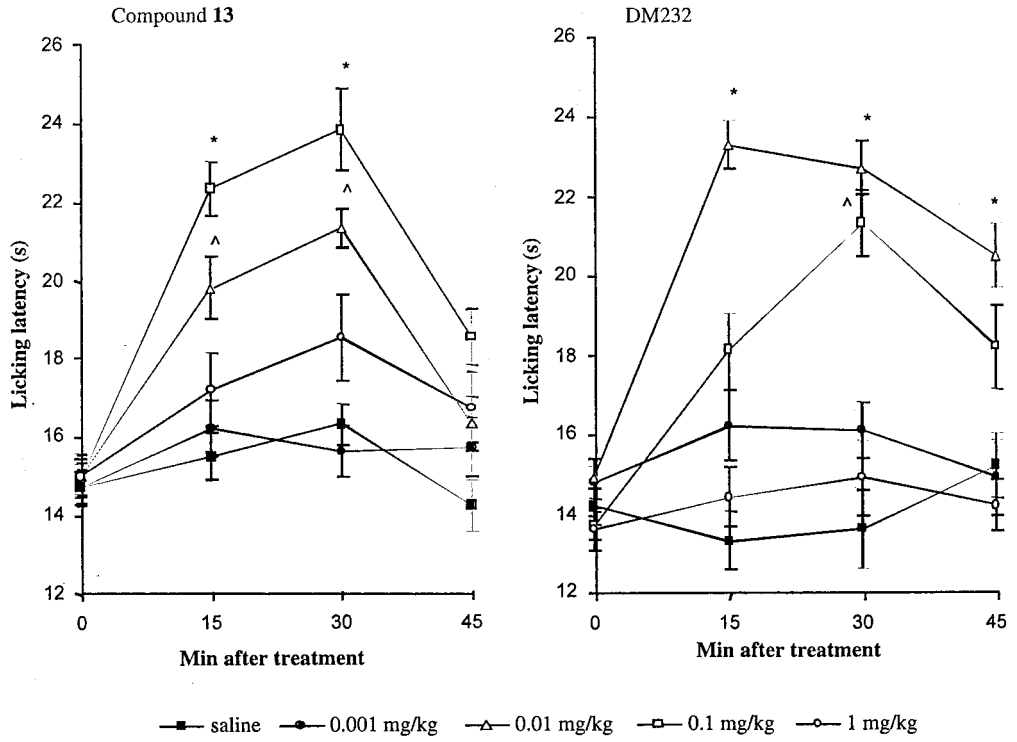
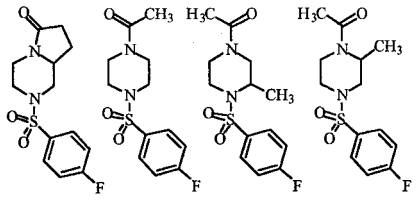


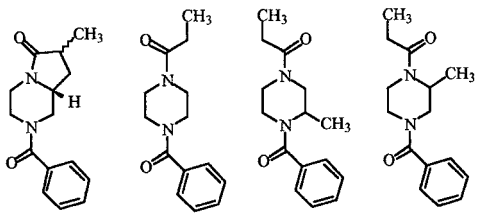
Figure 3. Dose−response curve of compound **13** (DM235, left) and DM232 (right) in the mouse hot-plate test. Compounds were injected ip. Each point represents the mean of at least 10 mice. Vertical lines show SEM. $*P < 0.01$; $\wedge P < 0.05$ in comparison with saline controls.

Chart 2



	DM232	**12**	**35**	**33**
Minimal Effective Dose (MED) mg/kg sc	0.001	0.01	0.1	1.0

Chart 3



	DM 265 (*exo*) DM 254 (*endo*)	DM 235 (**13**)	DM 325 (**34**)	DM 312 (**32**)
Minimal Effective Dose (MED) mg/kg sc	1 1	0.001	0.1	>10

Considering homogeneously 4-substituted sets (**9**, **13**, **25** or **12**, **23**, **28**, **29**, **30**) it appears that increasing the length of the 1-acyl group is not useful, and the best nootropics are obtained with the acetyl and propionyl substituents. Moreover, higher acyl groups introduce solubility problems.

The contribution of carbonyl and sulfonyl functions to the nootropic activity was checked by testing 4-benzyl-1-propionylpiperazine (**1**). The compound is some 4 orders of magnitude less potent than the corresponding benzoyl derivative **13** and 2 orders of magnitude less potent than the phenylsulfonyl derivative **19**, thus showing that, unlike what happens in the 1,4-diazabicyclo[4.3.0]nonan-9-one series,[7] the chemical nature of the substituent on nitrogen 4 is critical. Accordingly, to check the relevance of the chemical nature of the substituent in position 1, compound **31** (1-(4-fluorobenzenesulfonyl)-4-propylpiperazine) was synthesized. This compound is 2 orders of magnitude less potent than its acyl analogue **23**, thus showing that an amidic linkage on nitrogen 1 is critical for activity.

The 3-methyl derivatives **32** and **33** were synthesized to mimic more closely the pyrrolidinone ring of the 1,4-diazabicyclo[4.3.0]nonan-9-one series.[7] With respect to their demethyl derivatives **13** and **12,** they show a definitely lower nootropic activity. On the contrary, when the methyl group is in position 2, as in the corresponding compounds **34** and **35**, the activity, although lower with respect to compounds **13** and **12,** is still high. The reasons for this may be suggested by comparison of the compounds shown in Chart 2. DM232[7] is the most potent, **12** and **35** are 1 and 2 orders of magnitude weaker, while **33** is definitely less potent. It can be speculated that the active conformation of the acetyl group of **12** is optimized in DM232, which can be regarded as its frozen analogue,[19] and highly disturbed by the 3-methyl of **33**.

A similar analysis of the bicyclic and methyl analogues of **13** (see Chart 3) does not give such clear-cut results. In this case, the activity of the most potent compound **13** is reduced, although to a different extent, both by cyclization to the diastereomeric DM254 and DM265[7] and by substitution with a methyl group. Apparently, even in this case, the 3-methyl (**32**) disturbs the correct conformation, which on the other hand, is not achieved by the rigid bicyclic analogues. According

to what happens in the other case, the 2-methyl substitution is less disturbing and compound **34** is still very potent.

These results strongly suggest that compounds of both series interact with a specific target. It is well-known that nootropic effects can originate from an enhancement of central cholinergic transmission;[1,20,21] however, our compounds do not show any affinity for cholinergic receptors. Therefore, we examined the possibility of an indirect enhancement of cholinergic central tone through acetylcholine release.[22,23] Indeed, compounds **13** and DM232 do increase acetylcholine release in the central nervous system, as shown by microdialysis experiments on rat brain (Figure 2). Most likely, the increased level of acetylcholine in the central nervous system is responsible for the nootropic effect of the drugs as well as for their antinociceptive activity[18] (Figure 3). Therefore, even if the precise molecular mechanism is still unknown, it seems likely that our compounds elicit their nootropic action through an indirect enhancement of the central cholinergic tone.

Work is in progress to collect more information on the in vitro action of the compounds of both series, and it is hoped that this will help clarify the yet elusive[2] mechanism of action of piracetam-like compounds. However, it must be kept in mind that the results of a more complete pharmacological characterization of these compounds could lead to the conclusion that they, despite the chemical structure and the in vivo pharmacological profile so similar to that of piracetam, belong to a different class of drugs. Also, it can be added that these compounds do not interact with somastatin receptors as has been reported for FR 121196[24] (tested on DM232 and **13**).

For now, our results indicate that 4-substituted 1-acylpiperazines represent a new class of fairly simple nootropic drugs with a pharmacological profile very similar to that of piracetam, showing much higher potency with respect to the reference. Among the compounds studied, **13** (DM235) shows outstanding potency, being active at a dose of 0.001 mg kg^{-1} sc, not only by preventing amnesia induced by pharmacological treatment (passive avoidance) but also by producing a procognitive activity in a social learning task, a paradigm where there is no deficit in the cognitive functions. Compound **13** is also active, on passive avoidance, when given po at a MED of 0.1 mg kg^{-1}. Moreover, this compound did not show any impairment of motor coordination and spontaneous activity, at doses 1000-fold higher than that active in the passive avoidance test.

Work is in progress to collect more information on the molecular mechanism of action of the compounds and to evaluate the effect of other modifications of their chemical structure.

Experimental Section

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, 50.3 MHz for ^{13}C). Chromatographic separations were performed on a silica gel column by gravity chromatography (silica gel 40, 0.063−0.200 mm; Merck) or flash chromatography (silica gel 40, 0.040−0.063 mm; Merck). Yields are given after purification, unless otherwise stated. Where analyses are indicated by symbols, the analytical results are within ±0.4% of the theoretical values.

4-Benzyl-1-propionylpiperazine (1). Propionyl chloride (0.09 mL, 1 mmol) and $N(C_2H_5)_3$ (0.2 mL, 1.5 mmol) were added to commercially available 1-benzylpiperazine (0.17 mL, 1 mmol). After 2 h at room temperature, 50 mL of Et_2O were added and the solution washed with H_2O. Evaporation under reduced pressure of the dried solvent and purification of the residue by flash chromatography (using petroleum ether/CH_2Cl_2/Et_2O/absolute EtOH/NH_4OH 900:360:360:180:9.9 as the eluting system) gave title compound **1** that was used as such in the following reaction: IR (neat) ν 1660 (C═O) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.13 (t, J = 7.4 Hz, 3H, CH_3), 2.32 (q, J = 7.4 Hz, 2H, CH_2), 2.40−2.45 (m, 4H, CH_2), 3.43−3.48 (m, 2H, CH_2), 3.53 (s, 2H, CH_2Ph), 3.61−3.66 (m, 2H, CH_2), 7.25−7.33 (m, 5H, aromatics).

In the same way, starting from the suitable acyl chloride, **4-benzyl-1-butyrylpiperazine (2), 4-benzyl-1-pentanoylpiperazine (3),** and **4-benzyl-1-hexanoylpiperazine (4)** were prepared and purified by flash chromatography using $CHCl_3$/MeOH 90:10 as eluent. Their IR and ^{1}H NMR spectra are consistent with their structures. These compounds were used as such in the following reaction.

1-Propionylpiperazine (5). 4-Benzyl-1-propionylpiperazine (**1**) (0.22 g, 1 mmol) was hydrogenated over Pd/C 10% (0.10 g) in absolute ethanol at 47 psi for 12 h. After filtration, the solvent was removed under vacuum to give **5** (98% yield) as a nearly pure compound that was used as such in the following reaction: IR (neat) ν 3300−3500 (NH), 1670 (C═O) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.13 (t, J = 8 Hz, 3H, CH_3), 2.32 (q, J = 8 Hz, 2H, CH_2), 2.44 (bs, 1H, NH), 2.82−2.89 (m, 4H, CH_2), 3.42−3.49 (m, 2H, CH_2), 3.59−3.68 (m, 2H, CH_2).

In the same way, starting from the suitable 4-benzyl-1-acylpiperazine, **1-butyrylpiperazine (6), 1-pentanoylpiperazine (7),** and **1-hexanoylpiperazine (8)** were prepared, whose IR and ^{1}H NMR spectra are consistent with their structures, and were used as such in the following reaction.

General Procedure for the Synthesis of Benzoyl and Arenesulfonyl Derivatives 9−30. Benzoyl or an arenesulfonyl chloride (1 mmol) and $N(C_2H_5)_3$ (1.5 mmol) were added to the appropriate amine (commercially available 1-acetylpiperazine, **5**−**8**) dissolved in few milliliters of CH_3CN. After 2 h at room temperature, 50 mL of ether was added and the solution washed with H_2O. Evaporation under reduced pressure of the dried solvent gave mixtures that were purified by flash chromatography and crystallization from petroleum ether. Yields ranged from 60% to 95%. Chemical and physical characteristics of the compounds obtained are reported in Table 1.

1-Propylpiperazine.[11] 1-Bromopropane (0.13 mL, 1 mmol) was added to a solution of commercially available 1-benzylpiperazine (0.17 mL, 1 mmol) in a few milliliters of CH_3CN. After refluxing for 3 h, the mixture was alkalinized with $NaHCO_3$, extracted with $CHCl_3$ and dried. Removal of the solvent gave a mixture that was purified by column chromatography using $CHCl_3$/absolute EtOH/petroleum ether/NH_4OH 340:65:60:8 as eluent. Yield = 80%. The compound obtained (**1-benzyl-4-propylpiperazine)** was used as such in the following reaction: ^{1}H NMR ($CDCl_3$) δ 0.94 (t, J = 7.2 Hz, 3H, CH_3), 1.68−1.80 (m, 2H, CH_2), 2.55−2.70 (m, 2H, CH_2N), 2.77−2.87 (m, 8H, CHN), 3.58 (s, 2H, CH_2Ph), 7.24−7.31 (m, 5H, aromatics).

1-Benzyl-4-propylpiperazine (0.22 g, 1 mmol) was hydrogenated over Pd/C 10% (0.10 g) in absolute ethanol at 75 psi for 12 h. After filtration, the solvent was removed under vacuum to give title compound (83% yield) as a nearly pure compound that was used as such in the following reaction: ^{1}H NMR ($CDCl_3$) δ 0.91 (t, J = 7.3 Hz, 3H, CH_3), 1.47−1.59 (m, 2H, CH_2), 2.44 (t, J = 7.2 Hz, 2H, CH_2), 2.65 (bs, 1H, NH), 2.78−2.83 (m, 4H, CH_2), 3.23−3.28 (m, 4H, CH_2).

1-(4-Fluorobenzenesulfonyl)-4-propylpiperazine (31). Following the general procedure described above and starting

from 1-propylpiperazine and 4-fluorobenzenesulfonyl chloride, title compound was obtained in 85% yield after column chromatography purification (eluting solvent: petroleum ether/$CHCl_3$/Et_2O/absolute EtOH/NH_4OH, 450:180:180:45:2.5): mp 50−51 °C from petroleum ether; IR (neat) ν 1600 (aromatic CH), 1350 and 1160 (SO_2) cm $^{-1}$; ^{1}H NMR ($CDCl_3$) δ 0.86 (t, J = 7.2 Hz, 3H, CH_3), 1.38−1.50 (m, 2H, CH_2), 2.30 (t, J = 7.4 Hz, 2H, CH_2), 2.50−2.55 (m, 4H, CH_2), 3.02−3.12 (m, 4H, CH_2), 7.17−7.28 (m, J_{H-F} = 8.6 Hz, 2H, aromatics), 7.74−7.81 (m, J_{H-F} = 5.0 Hz, 2H, aromatics); ^{13}C NMR ($CDCl_3$) δ 13.76 (q), 21.82 (t), 47.99 (t), 54.07 (t), 62.02 (t), 118.26 (d, J_{C-F} = 22.0 Hz), 132.50 (d, J_{C-F} = 9.0 Hz), 133.34 (d, J_{C-F} = 3.2 Hz), 167.21 (d, J_{C-F} = 255.1 Hz). Anal. ($C_{13}H_{19}FN_2O_2S$) C, H, N.

1-Benzoyl-3-methylpiperazine (36). A solution of 1 g (0.01 mol) of commercially available 2-methylpiperazine and 3.78 g (0.045 mol) of $NaHCO_3$ in 13 mL of H_2O was diluted with 8 mL of acetone and cooled in an ice bath. Then a solution of benzoyl chloride (0.011 mol) in 4 mL of acetone was added dropwise and the mixture was allowed to warm to room temperature and stirred for further 1.5 h. The mixture was concentrated under vacuum and extracted with CH_2Cl_2. The product was purified by flash chromatography with CH_2Cl_2/MeOH 95:5 as eluent to give 59% yield of the desired product as a thick oil that was used as such in the following reaction: IR (neat) ν 3200−3500 (NH), 1630 (C=O) cm $^{-1}$; ^{1}H NMR ($CDCl_3$) δ 1.05 (d, J = 6.0 Hz, 3H, 3-CH_3), 1.74 (bs, 1H, NH), 2.51−3.06 (m, 6H, CH_2), 3.52−3.63 (m, 1H, CH), 7.33−7.42 (m, 5H, aromatics).

In the same way, using 4-fluorobenzenesulfonyl chloride**, 1-(4-fluorobenzenesulfonyl)-3-methylpiperazine (37)** was obtained in 64% yield as a thick oil that was used as such in the following reaction: IR (neat) ν 3200−3500 (NH), 1350 (SO_2), 1170 (SO_2) cm $^{-1}$; ^{1}H NMR ($CDCl_3$) δ 1.04 (d, J = 6.2 Hz, 3H, 3-CH_3), 1.56 (bs, 1H, NH), 1.91 (t, J = 10.0 Hz, 1H, CH), 2.25−2.36 (m, 1H, CH), 2.88−3.06 (m, 3H, CH), 3.59−3.65 (m, 2H, CH), 7.18−7.28 (m, J_{H-F} = 10.9 Hz, 2H, aromatics), 7.74−7.81 (m, J_{H-F} = 5.1 Hz, 2H, aromatics).

1-Benzoyl-3-methyl-4-propionylpiperazine (32). Method A: 0.2 g (1 mmol) of 1-benzoyl-3-methylpiperazine (**36**) and 0.4 mL of NEt_3 (3 mmol) in CH_3CN, cooled in an ice bath, were added of 0.95 mL (1.1 mmol) of propionyl chloride. After 1 h at 0 °C the mixture was washed with H_2O and extracted with Et_2O. The product was purified by flash chromatography with CH_2Cl_2/MeOH 95:5 as eluent (yield 70%): low-melting solid; (M^+) 260; IR (neat) ν 1630 (CON) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.00−1.18 (m, 6H, CH_3CH_2 and 3-CH_3), 2.15−2.29 (m, 2H, CH_3CH_2), 2.75−3.72 (m, 5H, CH), 4.26−4.67 (m, 2H, CH_3CH and CH), 7.27−7.36 (m, 5H, aromatics); ^{13}C NMR ($CDCl_3$) δ 9.79 (q), 18.75 (q), 26.78 (t), 27.09 (t), 44.95 (t), 49.03 (d), 58.31 (t), 127.29 (d), 127.80 (d), 128.69 (d), 128.93 (d), 130.28 (d), 135.50 (s), 171.56 (s), 172.72 (s). Anal. ($C_{15}H_{20}N_2O_2$) C, H, N.

Method B: To a solution of 0.150 g (0.96 mmol) of 2-methyl-1-propionylpiperazine (**45**) in CH_3CN (10 mL) were added 0.27 mL (1.92 mmol) of NEt_3 and 0.12 mL (0.96 mmol) of benzoyl chloride. After 3 h at room temperature, the mixture was washed with water, extracted with chloroform and purified by chromatography (Et_2O/petroleum ether/absolute EtOH/$CHCl_3$/NH_4OH 180/450/45/180/2.5) to give 0.21 g (70% yield) of the desired product.

4-Acetyl-1-(4-fluorobenzenesulfonyl)-3-methylpiperazine (33). Starting from **37** and acetyl chloride, the title compound was obtained following the procedure described for **32** (method A): yield 86%; mp 98−100 °C from petroleum ether; IR ν 1650 (CON), 1350,1160 (SO_2); ^{1}H NMR (mixture of conformers) ($CDCl_3$) δ 1.28 (d) and 1.39 (d) (J = 6.0 Hz, 3H, 3-CH_3), 2.03 (s, 3H, CH_3CO), 2.22−2.40 (m, 2H, CH), 2.93−3.05 (m) and 4.83−4.93 (m) (1H, CH), 3.57−3.76 (m, 3H, CH), 3.98−4.12 (m) and 4.43−4.55 (m) (1H, CH), 7.18−7.27 (m, J_{H-F} = 8.4 Hz, 2H, aromatics), 7.70−7.78 (m, J_{H-F} = 5.1 Hz, 2H, aromatics); ^{13}C NMR ($CDCl_3$) 15.41 (q), 16.49 (q), 21.60 (q), 22.06 (q), 35.84 (t), 41.22 (t), 43.87 (t), 46.42 (t), 49.43 (d), 50.78 (d), 116.68 (d, J_{C-F} = 21.9 Hz), 130.68 (d, J_{C-F} = 9.1 Hz), 131.89 (d, J_{C-F} = 2.7 Hz), 165.69 (d, J_{C-F} = 255.5 Hz), 169.10 (s). Anal. ($C_{13}H_{17}FN_2O_3S$) C, H, N.

1-Acetyl-3-methylpiperazine (39). Acetic anhydride (1.02 g, 10 mmol) in 4 mL of CH_2Cl_2 was added to a solution of 2-methylpiperazine (1 g, 10 mmol) in 8 mL of CH_2Cl_2, heated at 50 °C. The mixture was allowed to cool at room temperature, then the solvent was evaporated, the residue treated with 2 N HCl and extracted with $CHCl_3$. The aqueous layer was then made alkaline with 10% NaOH and extracted with $CHCl_3$. Evaporation of the dried solvent gave an oil that was used as such in the following reaction: IR (neat) ν 3300−3500 (NH), 1640 (C=O) cm $^{-1}$; ^{1}H NMR ($CDCl_3$) δ 1.07 (d, J = 5 Hz, 3H, 3-CH_3) 1.76 (bs, 1H, NH), 2.10 (s, 3H, CH_3CO), 2.57−2.87 (m, 3H, $CHCH_3$, CHN), 2.98−3.22 (m, 2H, CHN), 3.56−3.67 (m, 1H, CHN), 4.42−4.53 (m, 1H, CHN).

In the same way, starting from propionic anhydride, **3-methyl-1-propionylpiperazine (38)** was obtained.[25]

1-Benzoyl-2-methyl-4-propionylpiperazine (34). Method A: Starting from **38** and following the general procedure described above, the title compound was obtained in 67% yield as a low-melting solid, purified by silica gel chromatography using $CHCl_3$/MeOH 95:5 as eluent: IR (neat) ν 1650 (C=O) cm $^{-1}$; ^{1}H NMR (mixture of conformers) ($CDCl_3$) δ 1.16 (t) and 1.17 (t) (J = 7.4 Hz, 3H, CH_3CH_2), 1.28 (d, J = 6.6 Hz, 3H, 2-CH_3), 2.28−2.42 (m, 2H, CH_3CH_2), 2.61−2.87 (m, 1H, CH), 3.14−3.36 (m, 2H, CH), 3.61−3.84 (m, 2H, CH), 4.37−4.61 (m, 2H, CH_3CH + CH), 7.26−7.45 (m, 5H, aromatics); ^{13}C NMR ($CDCl_3$) δ 9.79 (q), 9.90 (q), 15.85 (q), 16.11 (q), 26.56 (t), 26.83 (t), 42.13 (t), 45.79 (t), 46.10 (t), 49.99 (d), 126.89 (d), 126.98 (d), 129.00 (d), 130.19 (d), 130.30 (d), 136.01 (s), 171.05 (s), 173.47 (s). Anal. ($C_{15}H_{20}N_2O_2$) C, H, N.

Method B: Starting from 1-benzoyl-2-methylpiperazine (**42**) and following the method described for **32**, title compound was obtained as a mixture that was purified by chromatography with Et_2O/petroleum ether/absolute EtOH/$CHCl_3$/NH_4OH 180:450:45:180:2.5 as eluent (92% yield).

4-Acetyl-1-(4-fluorobenzenesulfonyl)-2-methylpiperazine (35). Method A: Following the general procedure described above and starting from **39**, title compound was obtained in 50% yield after purification by silica gel chromatography with $CHCl_3$/MeOH 95/5 as eluent: mp 86−88 °C from petroleum ether; IR (neat) ν 1650 (C=O), 1350 (SO_2), 1150 (SO_2) cm $^{-1}$; ^{1}H NMR (mixture of conformers) ($CDCl_3$) δ 0.97 (d) and 1.05 (d) (J = 6.6 Hz, 3H, 2-CH_3), 2.04 (s) and 2.09 (s) (3H, CH_3CO), 2.64 (td, J = 13.0 and J = 6.2 Hz) and 2.82 (dd, J = 6.4 Hz) (1H, CH), 3.00−3.46 (m, 2H, CH), 3.47−3.74 (m, 2H, CH), 4.12−4.23 (m, 1H, CH_3CH), 4.26−4.37 (m) and 4.51−4.61 (m) (1H, CH), 7.16−7.28 (m, J_{H-F} = 8.9 Hz, 2H, aromatics), 7.78−7.88 (m, J_{H-F} = 4.8 Hz, 2H, aromatics); ^{13}C NMR ($CDCl_3$) δ14.50 (q), 14.72 (q), 21.44 (q), 21.57 (q), 40.14 (t), 40.52 (t), 46.22 (t), 49.30 (d), 49.70 (d), 51.45 (t), 116.88 (d, J_{C-F} = 21.9 Hz), 130.00 (d, J_{C-F} = 10.6 Hz), 136.56 (d, J_{C-F} = 3.6 Hz), 165.31 (d, J_{C-F} = 254.6 Hz), 169.88 (s). Anal. ($C_{13}H_{17}FN_2O_3S$) C, H, N.

Method B: A solution of 0.21 g (0.81 mmol) of 1-(4-fluorobenzenesulfonyl)-2-methylpiperazine (**43**) in $CHCl_3$ (15 mL) was heated at 50 °C and added with 0.08 g (0.81 mmol) acetic anhydride in $CHCl_3$ (5 mL). At the end of the addition the mixture was cooled, made alkaline by $NaHCO_3$ (solution in water) and extracted. After drying over sodium sulfate, the solvent was evaporated and purified by chromatography with Et_2O/petroleum ether/absolute EtOH/$CHCl_3$/NH_4OH 180/450/45/180/2.5 as eluent (53% yield). Compound **35** and its isomer **33** show different R_f using NH_4OH/Et_2O/petroleum ether/absolute EtOH/CH_2Cl_2 9.9/360/900/180/360 as eluent (R_f = 0.53 for **33** and 0.44 for **35**).

4-Benzyl-1-(4-fluorobenzenesulfonyl)-2-methylpiperazine (41). To a solution of 0.3 g (1.58 mmol) of 1-benzyl-3-methylpiperazine, obtained as reported in the literature[13] and 0.32 g (3.16 mmol) of triethylamine in 10 mL of CH_3CN was added 0.31 g (1.58 mmol) of 4-fluorobenzenesulfonyl chloride at 0 °C and the mixture was allowed to warm to room temperature. Then water was added and the mixture was extracted with Et_2O obtaining, in 63% yield, the desired product that was used as such in the following reaction: ^{1}H NMR ($CDCl_3$) δ 1.17 (d, J = 7.8 Hz, 3H, 2-CH_3), 1.97−2.20

(m, 2H, CH), 2.49−2.58 (m, 1H, CH), 2.67−2.78 (m, 1H, CH), 3.07−3.65 (m, 2H, CH), 3.36 (d, J = 14.0 Hz, 1H, CH_2Ph), 3.47 (d, J = 14.0 Hz, 1H, CH_2Ph), 4.01−4.11 (m, 1H, CH_3CH), 7.13−7.20 (m, J_{H-F} = 10.0 Hz, 2H, aromatics), 7.21−7.36 (m, 5H, aromatics), 7.79−7.86 (m, J_{H-F} = 5.0 Hz, 2H, aromatics).

Using the same procedure and starting from 1-benzyl-3-methylpiperazine and benzoyl chloride, **1-benzoyl-4-benzyl-2-methylpiperazine (40)** was obtained as a mixture that was purified by flash chromatography with NH_4OH/Et_2O/petroleum ether/absolute EtOH/CH_2Cl_2 9.9:360:900:180:360 as eluent (84% yield): ^{1}H NMR ($CDCl_3$) δ 1.36 (d, J = 6.9 Hz, 3H, 2-CH_3), 2.09−2.23 (m, 2H, CH), 2.63−2.93 (m, 2H, CH), 3.29−3.39 (m, 3H, CH), 3.41 (d, J = 12.4 Hz, 1H, CH_2Ph), 3.53 (d, J = 12.4 Hz, 1H, CH_2Ph), 7.24−7.43 (m, 10H, aromatics).

1-(4-Fluorobenzenesulfonyl)-2-methylpiperazine (43). To a solution of 0.4 g (1.1 mmol) 4-benzyl-1-(4-fluorobenzenesulfonyl)-2-methylpiperazine **(41)** in 15 mL of anhydrous methanol were added 0.36 g (5.7 mmol) of ammonium formate and 0.10 g of Pd/C 10% and the mixture refluxed for 2 h. Then the carbon was filtered off, the solvent evaporated and the residue made alkaline with $NaHCO_3$ and extracted with chloroform. The organic layer was dried over sodium sulfate; the solvent was evaporated to give, in 74% yield, the desired product that was used as such in the following reaction: ^{1}H NMR ($CDCl_3$) δ 1.15 (d, J = 7.0 Hz, 3H, 2-CH_3), 1.72 (bs, 1H, NH), 2.62−2.76 (m, 2H, CH), 2.85−2.97 (m, 2H, CH), 3.13 (td, J = 11.8 and 3.3 Hz, 1H, CH), 3.51−3.59 (m, 1H, CH), 3.98−4.04 (m, 1H, CH_3CH), 7.14−7.27 (m, J_{H-F} = 8.8 Hz, 2H, aromatics), 7.80−7.87 (m, J_{H-F} = 5.1 Hz, 2H, aromatics).

Starting from 1-benzoyl-4-benzyl-2-methylpiperazine **(40)**, the same procedure was used to obtain **1-benzoyl-2-methylpiperazine (42)**, in 45% yield: ^{1}H NMR ($CDCl_3$) δ 1.36 (d, J = 6.9 Hz, 3H, 2-CH_3), 2.47 (bs, 1H, NH), 2.74−3.05 (m, 6H, CH), 3.13−3.26 (m, 1H, CH), 7.27−7.43 (m, 5H, aromatics).

4-Benzyl-2-methyl-1-propionylpiperazine (44). Using the same procedure used to obtain **38** (method B) and starting from 1-benzyl-3-methylpiperazine and propionic anhydride, the title compound was obtained in 95% yield and used as such in the following reaction: ^{1}H NMR (mixture of conformers) ($CDCl_3$) δ 1.15 (t) and 1.16 (t) (J = 7.8 Hz, 3H, CH_3CH_2), 1.21 (d, J = 6.6 Hz, 3H, 2-CH_3), 2.01−2.18 (m, 1H, CH), 2.26−2.41 (m, 2H, CH_3CH_2), 2.42−2.53 (m, 1H, CH), 2.66−2.93 (m, 2H, CH), 3.48 (d, J = 12.4 Hz, 1H, CH_2Ph), 3.53 (d, J = 12.4 Hz, 1H, CH_2Ph), 3.50−3.63 (m, 1H, CH), 3.93−4.03 (m, 2H, CH_3CH+CH), 7.25−7.36 (m, 5H, aromatics).

2-Methyl-1-propionylpiperazine (45). Following the same procedure used to obtain **5** and starting from **44**, the desired product was obtained in 38% yield and used as such in the following reaction: IR ν 3200−3600 (NH), 1640 (CON); ^{1}H NMR (mixture of conformers) ($CDCl_3$) δ 1.15 (t, J = 7.4 Hz, 3H, CH_3CH_2), 1.27 (d, J = 6.8 Hz, 3H, 2-CH_3), 1.80 (bs, 1H, NH), 2.23−2.38 (m, 2H, CH_3CH_2), 2.60−3.06 (m, 4H, CH), 3.15−3.38 (m) and 3.86−4.06 (m) (1H, CH), 3.46−3.76 (m, 1H, CH), 4.26−4.55 (m, 1H, CH_3CH).

Pharmacology. Antiamnesic test (passive avoidance test): The test was performed according to the step-through method described by Jarvik and Kopp.[14] 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 nonpainful 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 that did not enter after 60 s latency were excluded from the experiment. For memory disruption, mice were ip injected with the amnesic drugs (scopolamine, diphenhydramine, baclofen and clonidine). All investigated drugs were injected 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 memory degree of received punishment (fall into cold water) was expressed as the increase in seconds between training and retention latencies.

Social learning test: The social learning test was performed according to Mondadori et al.[15] 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 4/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 behavior by the adult male within a 5-min fixed interval was recorded. Social investigatory behavior 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 behavior was recorded in a 5-min interval. Piracetam and **13** were ip injected 20 min before the first session of the experiment.

Acetylcholine release: Microdialysis was performed in rat parietal cortex as described by Giovannini et al.[16] The coordinates used for the implantation of the horizontal microdialysis tubing (AN 69 membrane, molecular weight cut off < 15 kDa; Dasco, Italy) were AP 0.5 mm and H 2.3 mm from the bregma.[26] One day after surgery the microdialyzing tubing was perfused at a constant flow rate (2 μL min^{-1}) with Ringer's solution (NaCl 147, KCl 4.0, $CaCl_2$ 1.2 mM) containing 7 μM physostigmine sulfate. After a 1-h settling period, the perfusate was collected at 15-min intervals in test tubes containing 5 μL of 0.5 mM HCl to prevent ACh hydrolysis. The samples were then assayed for ACh content either immediately or after freezing. Ach was detected and quantified by HPLC with an electrochemical detector as described by Damsma et al.[27] ACh release was expressed as percent change over the mean of the first three basal samples as controls.

Analgesic activity: Antinociceptive activity was evaluated on the mouse hot-plate test according to O'Callaghan.[17] The plate temperature was fixed at 52.5 ± 0.1 °C. An arbitrary cutoff time of 45 s was adopted. The number of mice treated in each test varied from 8 to 20. The level of analgesia reached was evaluated comparing the analgesic effect of the maximal effect dose of each compound to that of morphine, taken as the reference compound, and injected at 8 mg/kg sc, a dose that does not alter animal behavior.

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Supporting Information Available: Table 4 with NMR spectra of compounds **9**−**30**. This material is available free of charge via the Internet at http://pubs.acs.org.

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