Structure−Affinity Relationships of a Unique Nicotinic Ligand: N^1-Dimethyl-N^4-phenylpiperazinium Iodide (DMPP)

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Received April 17, 2001

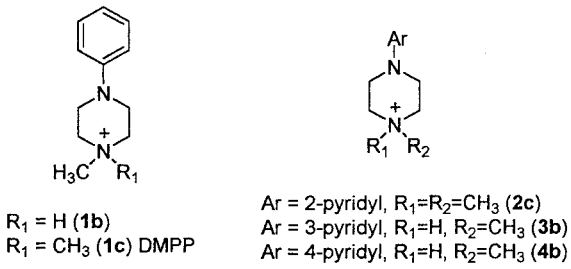
DMPP is a well-known nicotinic agonist that does not fit any proposed pharmacophore for nicotinic binding and represents a unique ligand among the hundreds of nicotinic agonists studied in the past decades. A systematic modulation of the chemical structure of DMPP, aimed to establish its structure−affinity relationships, is reported. The research has allowed to identify molecules such as **11c**, **13c**, **14c**, and **28c**, with affinities for $\alpha_4\beta_2$ receptors in the low nanomolar range, some 2 orders of magnitude lower than the lead compound. The agonistic properties of the most interesting compounds have been assessed by measuring their analgesic activity on mice (hot-plate test). Another result of the research was the identification of DMPP analogues, such as **3a** ($K_i = 90$ nM) and **14b** ($K_i = 180$ nM), that maintain affinity for the central nicotinic receptor when the ammonium function is changed into an aminic one and are therefore possible leads for drug development in neurodegenerative diseases.

Introduction

Nicotinic acetylcholine receptors (nAChR) are a subtype of acetylcholine-operated receptors and members of the superfamily of ligand-gated ion channel receptors.[1] Over the past 20 years, molecular biology techniques have greatly expanded our understanding of the structure and of the diversity of the subunits that form the nAChR channel.[2] Pentameric combination of several α and β subunits discovered makes possible the existence of a great number of nicotinic receptors, which complicates their study and characterization.[3] Paralleling these findings, nACh receptors have been and are the subject of ever increasing therapeutic interest[4] since they are widely distributed throughout the body, exert fundamental physiological functions,[5] and are presumed to be involved in the etiology of numerous pathological states.[6] As a matter of fact nicotine, the prototype ligand of nACh receptors, is responsible for a variety of peripheral and central pharmacological effects such as cardiovascular, gastrointestinal, and endocrine activity, cognition and attention enhancement, analgesia, neurotransmitters release, and neuroprotection.

Recently, most attention has been directed to characterize neuronal nAChRs,[7,8] to evaluate their role in neurodegenerative diseases such as Alzheimer's disease[9] and to define the therapeutic potential of drugs modulating such receptors.[10,11] Among the many neuronal nicotinic receptor subtypes, particular attention was given to those that have been considered of prominent importance in CNS: $\alpha_4\beta_2$ and α_7. As a consequence a myriad of new molecules, able to modulate central nicotinic receptors, has been synthesized and studied. They have been reported in details in several excellent reviews.[12−17] Among them, that of Schmitt,[18] where the fundamental literature concerning nicotinic receptors and their structure is reviewed, is the most recent and recommended for references.

Chart 1



Since the structure of the receptor has not been resolved at the atomic level, medicinal chemists have relied on classical structure−activity relationships of the ligands to elucidate the binding site of nicotinic drugs. As a result of these investigations, several pharmacophoric models to identify the essential features for molecular recognition of nicotinic ligands have been proposed.[14,19−24] Although none of them seems of general application, their value cannot be overemphasized, considering the present lack of a direct knowledge of the binding site of the receptor. A variety of molecules have been used to develop such models, but one of the best known nicotinic drugs, N^1-dimethyl-N^4-phenylpiperazinium iodide[25−27] (DMPP, **1c**, Chart 1), was never present in the driving set, due to its peculiar structure which is unique among nicotinic ligands and does not fit any proposed model.

Some time ago, intrigued by the peculiarity of DMPP, we started a molecular modulation of its structure, aiming to identify derivatives with improved potency

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10.1021/jm010901y CCC: $20.00
Published on Web 10/06/2001

and pharmacokinetic properties. In fact, in our hands DMPP presents a $K_i = 250$ nM (Boksa[28] reports 57 nM) for the nicotinic receptors of the rat brain labeled by [^{3}H]-cytisine (thought to be represented mainly by the $\alpha_4\beta_2$ subtype) and, being a quaternary salt, does not cross the blood brain barrier, as required for drugs useful to treat neurodegenerative diseases. We found that both properties could be improved by suitable modification of the lead as reported in Table 1 for compounds **3b** and **4b**[29] (Chart 1). As an extension and completion of the research, we report now the results of a systematic modulation of the lead molecule (Chart 2) aimed at defining structure−affinity relationships in the series. At the same time, we tried to identify molecules useful to characterize the nicotinic receptor and, eventually, to single out new leads for the development of drugs useful to treat neurodegenerative diseases. Toward this end, the benzene ring of DMPP was substituted with groups of different electronic and steric properties (**6**−**25**). We substituted both 3′ and 4′ positions in accord with the results obtained before with compounds **3b** and **4b**. The same ring was replaced by cyclohexenone and cyclopentenone (**26**, **27**) or heterocyclic rings (**28**−**30**). Finally, N^1-piperazine nitrogen was changed into the isosteric carbon of tetrahydropyridine (**31**−**34**) and the latter derivatives reduced to give the corresponding piperidine analogues (**35**−**38**).

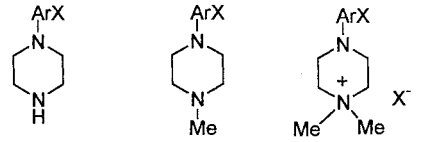
Chemistry

Preparation of the compounds studied was done according to literature methods for the compounds already described, while simple chemistry was involved in the synthesis of new compounds. For the sake of clarity we have adopted the same identification number for each family of compounds using the label **a** for secondary amines, **b** for tertiary amines, and **c** for ammonium salts.

Phenylpiperazines. The following compounds were prepared according to literature methods: 1-(3′-pyridyl)-piperazine (**3a**)[29] and the analogues 1-(4′-pyridyl)- (**4a**),[29] 1-(3′-fluorophenyl)- **8a**,[30] 1-(3′-nitrophenyl)- **17a**;[30] 1-(3′-methoxyphenyl)-4-methylpiperazine (**12b**)[31] and the analogues 1-(3′-cyanophenyl)- (**16b**),[32] 1-(4′-hydroxyphenyl)- (**20b**),[33] 1-(4′-nitrophenyl)- (**22b**),[34] and 1-(4′-aminophenyl)- (**23b**).[34] 1-(3′-Hydroxyphenyl)-4-methylpiperazine (**11b**) was prepared by hydrolysis of the methoxy derivative **12b**, and 1-(3′-acetoxyphenyl)-4-methylpiperazine (**13b**) was prepared by acetylation of **11b**. 1-(3′-Aminophenyl)-4-methylpiperazine (**14b**)[35] was prepared by reduction of the nitro derivative **17b**, and 1-(3′-acetamidophenyl)-4-methylpiperazine (**15b**) was prepared by acetylation of **14b**.

The already known (3′,4′-methylenedioxy)phenylpiperazine (**25a**)[36] and 1-(3′-methylphenyl)-4-methylpiperazine (**7b**)[31] were prepared by condensation of the suitable aniline with bis(2-chloroethyl)amine and bis-(2-chloroethyl)methylamine, respectively (Scheme 1). Tertiary amines **8b**−**10b**, **17b**−**19b, 21b,** and **24b** (Table 5,[37−41] Scheme 1) were prepared by methylation of the secondary amines using formaldehyde and formic acid. 1-(3′,4′-Methylenedioxyphenyl)-4-methylpiperazine (**25b**) was prepared by reaction of **25a** with 1 equiv of methyl iodide (Scheme 1). Compounds **6b**[42] and **6c** were prepared according to the synthetic pathway reported

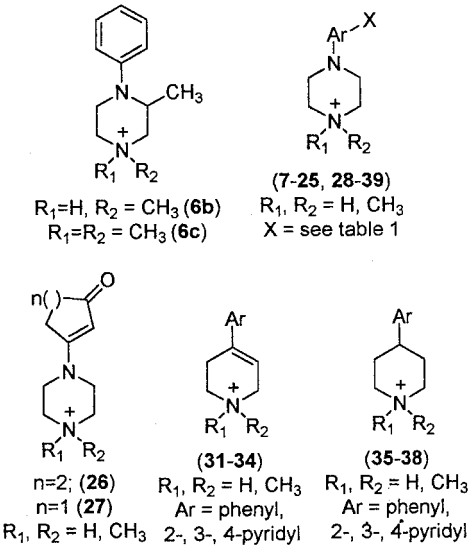
Table 1. Binding Affinity of Piperazine Derivatives **1**−**30** on the Nicotinic Receptors of Rat Brain



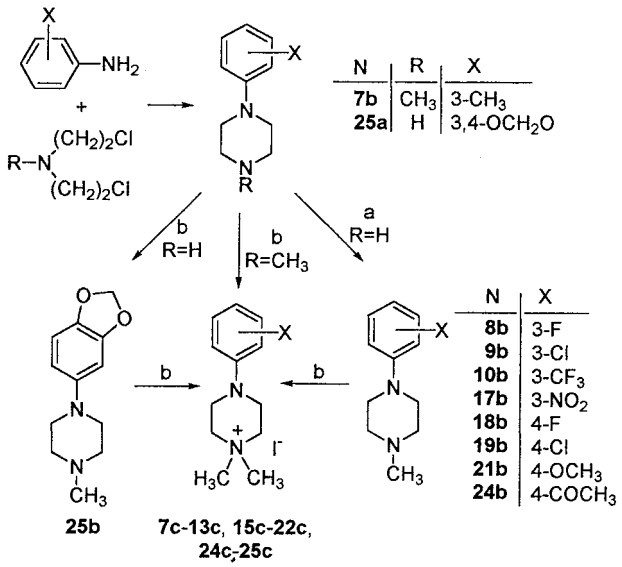
N	X	Ar	salt/X$^-$	binding[a] K_i nM (±SEM)	pK_i
1b[b]	H	phenyl	HCl	>10000	
1c[b]	H	phenyl	iodide	250 (30)	6.60
2b[b]	H	2-pyridyl	*c*	>10000	
2c[b]	H	2-pyridyl	iodide	500 (30)	6.30
3a	H	3-pyridyl	oxalate	90 (10)	7.05
3b[b]	H	3-pyridyl	*c*	90 (10)	7.05
4a	H	4-pyridyl	*c*	>10000	
4b[b]	H	4-pyridyl	*c*	170 (10)	6.77
5b[b]	2-CH_3	phenyl	oxalate	>10000	
5c[b]	2-CH_3	phenyl	iodide	>10000	
6b[d]	H	phenyl	HCl	>10000	
6c[d]	H	phenyl	iodide	>10000	
7b	3-CH_3	phenyl	oxalate	>10000	
7c	3-CH_3	phenyl	iodide	1900 (153)	5.72
8a	3-F	phenyl	oxalate	>10000	
8b	3-F	phenyl	oxalate	6500 (289)	5.19
8c	3-F	phenyl	iodide	22 (2)	7.66
9b	3-Cl	phenyl	HCl	>10000	
9c	3-Cl	phenyl	iodide	80 (5)	7.10
10a[e]	3-CF_3	phenyl	oxalate	>10000	
10b	3-CF_3	phenyl	oxalate	>10000	
10c	3-CF_3	phenyl	iodide	1200 (2)	5.92
11b	3-OH	phenyl	oxalate	>10000	
11c	3-OH	phenyl	iodide	8 (0.6)	8.10
12b	3-OCH_3	phenyl	HCl	>10000	
12c	3-OCH_3	phenyl	iodide	900 (80)	6.05
13b	3-$OCOCH_3$	phenyl	oxalate	800 (81)	6.10
13c	3-$OCOCH_3$	phenyl	iodide	6.8 (0.9)	8.17
14b	3-NH_2	phenyl	*c*	180 (20)	6.74
14c	3-NH_2	phenyl	bromide	2.3 (0.1)	8.64
15b	3-$NHCOCH_3$	phenyl	oxalate	>10000	
15c	3-$NHCOCH_3$	phenyl	iodide	850 (43)	6.07
16b	3-CN	phenyl	oxalate	>10000	
16c	3-CN	phenyl	iodide	300 (12)	6.52
17a	3-NO_2	phenyl	oxalate	>10000	
17b	3-NO_2	phenyl	*c*	1930 (124)	5.71
17c	3-NO_2	phenyl	iodide	22 (2)	7.66
18a[e]	4-F	phenyl	oxalate	>10000	
18b	4-F	phenyl	oxalate	>10000	
18c	4-F	phenyl	iodide	420 (2)	6.38
19b	4-Cl	phenyl	*c*	>10000	
19c	4-Cl	phenyl	iodide	2400 (150)	5.62
20b	4-OH	phenyl	oxalate	>10000	
20c	4-OH	phenyl	iodide	2390 (158)	5.62
21b	4-OCH_3	phenyl	*c*	>10000	
21c	4-OCH_3	phenyl	iodide	40.000	4.40
22b	4-NO_2	phenyl	*c*	>10000	
22c	4-NO_2	phenyl	iodide	>10000	
23b	4-NH_2	phenyl	*c*	>10000	
24a[e]	4-$COCH_3$	phenyl	oxalate	>10000	
24b	4-$COCH_3$	phenyl	oxalate	>10000	
24c	4-$COCH_3$	phenyl	iodide	>10000	
25a	3,4-OCH_2O-	phenyl	oxalate	>10000	
25b	3,4-OCH_2O-	phenyl	oxalate	>10000	
25c	3,4-OCH_2O-	phenyl	iodide	672 (52)	6.17
26b	3′-cyclohexenone		oxalate	>10000	
26c	3′-cyclohexenone		iodide	1450 (29)	5.84
27b	3′-cyclopentenone		oxalate	>10000	
27c	3′-cyclopentenone		iodide	853 (30)	6.07
28a	6′-Cl	3′-pyridazinyl	*c*	1030(40)	5.99
28b[b]	6′-Cl	3′-pyridazinyl	*c*	>10000	
28c	6′-Cl	3′-pyridazinyl	iodide	4.5 (0.38)	8.35
29a	H	3′-pyridazinyl	oxalate	644 (25)	6.19
29b	H	3′-pyridazinyl	oxalate	386 (35)	6.41
30b[b]	H	2′-pyrimidinyl	*c*	>10000	
30c	H	2′-pyrimidinyl	iodide	>10000	

[a] On rat brain homogenates. The nicotinic receptors were labeled by [^{3}H]-cytisine. See the pharmacological experimental part for details. [b] See ref 29. [c] Tested as free base. [d] Derivative of 2-methylpiperazine. [e] Commercially available.

Chart 2



Scheme 1[a]



[a] (a)HCHO, HCOOH; (b) CH_3I.

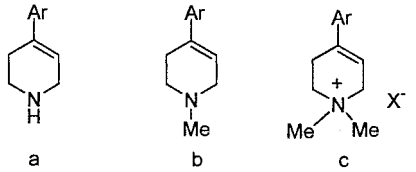
in Scheme 2. The keto derivatives **26b** and **27b** were prepared by condensation of the suitable 1,3-cycloalkyldienone with methylpiperazine (Scheme 3).

Methiodides **7c**−**13c**, **15c**−**22c**, **24c**−**27c** were prepared by reaction of the corresponding tertiary amines with methyl iodide (Schemes 1 and 3). Chemical and physical characteristics of the synthesized methiodides are reported in Table 6. The synthesis of 4-(3′-aminophenyl)-1,1-dimethylpiperazinium bromide hydrobromide **14c** is reported in Scheme 4.

Heteroarylpiperazines. 1-(6′-Chloro-3′-pyridazinyl)-piperazine (**28a**)[43] and its 4-methyl analogue **28b**[29] were prepared according to literature methods. 1-(3′-Pyridazinyl)piperazine (**29a**) and the 4-methyl derivative (**29b**) were prepared by hydrogenolysis of 1-(6′-chloro-3′-pyridazinyl)-4-benzylpiperazine (**40**) and of **28b,** respectively (Scheme 5).

Reaction of **28b**[29] and 1-(2′-pyrimidinyl)-4-methylpiperazine (**30b**)[29] with methyl iodide gave the piperazinium iodides **28c** and **30c** as the only products (Scheme 5).

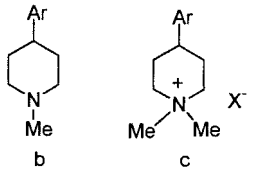
Table 2. Binding Affinity of Tetrahydropyridine Derivatives **31**−**34** on the Nicotinic Receptors of Rat Brain



N	Ar	salt/X^-	binding[a] K_i nM (±SEM)	pK_i
31b	phenyl	HCl	>10000	
31c	phenyl	iodide	750 (50)	6.12
32b	2′-pyridyl	HCl	>10000	
33a	3′-pyridyl	HCl	1100 (30)	5.96
33b	3′-pyridyl	HCl	1200 (10)	5.92
34b	4′-pyridyl	HCl	>10000	

[a] On rat brain homogenates. The nicotinic receptors were labeled by [^{3}H]-cytisine. See the pharmacological experimental part for details.

Table 3. Binding Affinity of Piperidine Derivatives **35**−**38** on the Nicotinic Receptors of Rat Brain



N	Ar	salt/X^-	binding[a] K_i nM (±SEM)	pK_i
35b	phenyl	HCl	>10000	
35c	phenyl	iodide	600 (40)	6.22
36b	2′-pyridyl	HCl	>10000	
37b	3′-pyridyl	HCl	1200 (60)	5.92
38b	4′-pyridyl	HCl	>10000	

[a] On rat brain homogenates. The nicotinic receptors were labeled by [^{3}H]-cytisine. See the pharmacological experimental part for details.

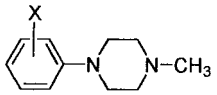
Table 4. Analgesic Activity of Selected Compounds on the Mouse Hot-Plate Test

	analgesic activity	
N	M.A.D. (μg/kg)[a]	efficacy (%)[b]
3a	50	51.1
8b	na	
8c	50	31.0
11c	1	54.7
13c	10	29.0
14c	10	28.0
16c	10	47.0
28a	na	
28c	0.1	66.2
33a	10	37.8
33b	1	36.5
37b	10	29.2

[a] Minimal analgesic dose of the compound injected intracerebroventricularly (icv). The analgesia was completely reverted by mecamylamine. [b] Effect (%) respect to that of 5 μg/kg of morphine icv. na: not active up to the dose of 100 μg/kg.

Tetrahydropyridines and Piperidines. 1-Methyl-4-phenyl-1,2,5,6-tetrahydropyridine (**31b**, MPTP),[44] 1-methyl-4-(2′-pyridyl)-1,2,5,6-tetrahydropyridine (**32b**),[45] and the 3′-pyridyl and 4′pyridyl analogues **33b**[46] and **34b**[47] were prepared according to the literature methods. 4-(3′-Pyridyl)-1,2,5,6-tetrahydropyridine (**33a**) was prepared starting from N-BOC-piperidone (Scheme 6). Piperidines **35b**−**38b** were obtained by catalytic hydro-

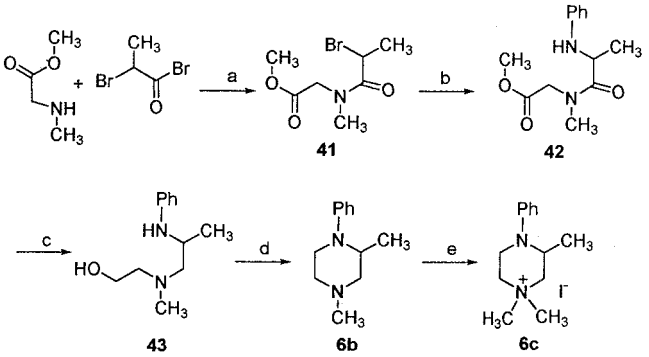
Table 5. Chemical and Physical Characteristics of Compounds **8b–10b**, **17b–19b**, **21b**, and **24b**



N	X	yield %	mp (°C)	purification[a]	anal/ref
8b	3-F	20	152–154[b]	A	$C_{11}H_{15}FN_2$
9b	3-Cl	84	214–215[c]		(ref 37)[d]
10b	3-CF_3	98	110[b]		(ref 38)[d]
17b	3-NO_2	98	260[b]		(ref 39)[d]
18b	4-F	99	125–128[b]		$C_{11}H_{15}FN_2$
19b	4-Cl	84	76–78[e]		$C_{11}H_{15}ClN_2$
21b	4-OCH_3	18	52–55[e]	B	(ref 40)[d]
24b	4-$COCH_3$	93	185–190[b]	A	(ref 41)[d]

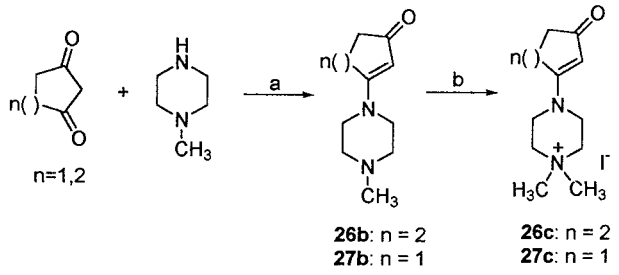
[a] Eluent A: CH_2Cl_2/absolute EtOH/petroleum ether/diethyl ether/NH_4OH 360/180/900/360/9.9. Eluent B: $CHCl_3/CH_3OH$ 7/3. [b] As oxalate salt. [c] As hydrochloride salt. [d] These compounds were known but they have been prepared by a different procedure. [e] As free base.

Scheme 2[a]



[a] (a) NEt_3; (b) $PhNH_2$; (c) $LiAlH_4$; (d) PPh_3, DEAD; (e) MeI.

Scheme 3[a]



[a] (a) *p*TSA; (b) MeI.

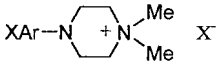
genation of the corresponding 1,2,5,6-tetrahydropyridines; compounds **35b**, **36b,** and **38b** have been already described.[48–50] Compounds **31b** and **35b** were transformed into the methiodides **31c**[51] and **35c**[52] by reaction with methyl iodide.

Pharmacology

The compounds obtained have been tested in vitro on rat brain homogenates, to evaluate their affinity for the central nicotinic receptors labeled by [^{3}H]-cytisine, that is believed to label $\alpha_4\beta_2$ which represent up to 90% of the high affinity agonist binding sites in rat brain.[13,53]

Since nicotinic agonists show analgesic activity which is peculiar of their central action,[54] the most affinitive compounds have been tested as analgesics on the hot-plate test to confirm their agonistic action on central cholinergic receptors.[55]

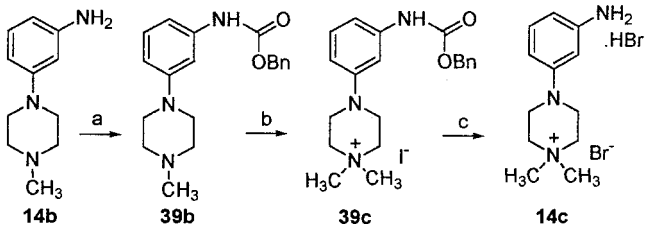
Table 6. Chemical and Physical Characteristics of the Piperazinium Derivatives



N	X	Ar	X^-	mp (°C)	anal. [ref]
6c	phenyl[a]		I	137–140	$C_{13}H_{21}IN_2$
7c	3-Me	phenyl	I	162–167	$C_{13}H_{21}IN_2$
8c	3-F	phenyl	I	188–191	$C_{12}H_{18}FIN_2$
9c	3-Cl	phenyl	I	195	[ref 63][b]
10c	3-CF_3	phenyl	I	218–223	[ref 63][b]
11c	3-OH	phenyl	I	230	$C_{12}H_{19}IN_2O$
12c	3-OMe	phenyl	I	205	$C_{13}H_{21}IN_2O$
13c	3-OCOMe	phenyl	I	160	$C_{14}H_{21}IN_2O_2$
15c	3-NHCOMe	phenyl	I	245	$C_{14}H_{22}IN_3O$
16c	3-CN	phenyl	I	200	$C_{13}H_{18}IN_3$
17c	3-NO_2	phenyl	I	190–192	$C_{12}H_{18}IN_3O_2$
18c	4-F	phenyl	I	200–203	$C_{12}H_{18}FIN_2$
19c	4-Cl	phenyl	I	220–222	$C_{12}H_{18}ClIN_2$
20c	4-OH	phenyl	I	230	$C_{12}H_{19}IN_2O$
21c	4-OMe	phenyl	I	218–220	[ref 40][b]
22c	4-NO_2	phenyl	I	220	$C_{12}H_{18}IN_3O_2$
24c	4-COMe	phenyl	I	205–208	$C_{14}H_{21}IN_2O$
25c	3,4-OCH_2O	phenyl	I	130	$C_{13}H_{19}IN_2O_2$
26c	3′-cyclohexenone		I	143	$C_{12}H_{21}IN_2O$
27c	3′-cyclopentenone		I	172	$C_{11}H_{19}IN_2O$
28c	6′-Cl-3′-pyridazinyl		I	250	$C_{10}H_{16}ClIN_4$
30c	2′-pyrimidinyl		I	255	$C_{10}H_{17}IN_4$
39c	3′-NHCBZ	phenyl	I	200	$C_{20}H_{26}IN_3O_2$

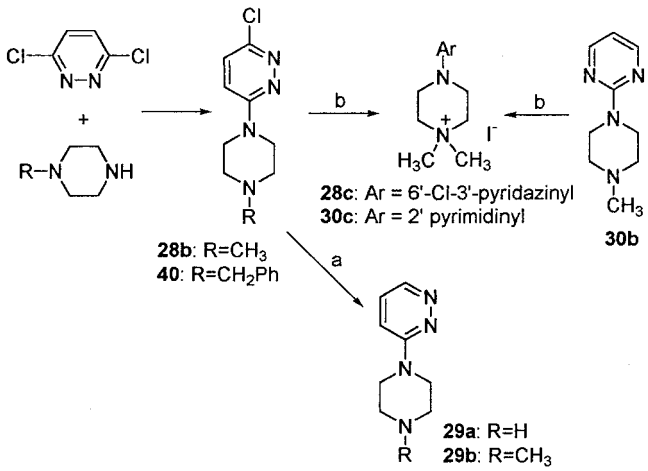
[a] This compound is the 2-methylpiperazine derivative. [b] These compounds are known but they have been prepared by a different procedure.

Scheme 4[a]



[a] (a) dibenzyldicarbonate; (b) MeI; (c) HBr, AcOH.

Scheme 5[a]

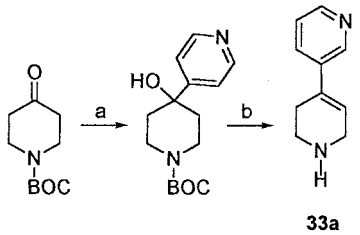


[a] (a) $HCOONH_4$, Pd/C; (b) MeI.

Results

The results of rat brain binding against [^{3}H]-cytisine of compounds **6–38** are reported in Tables 1–3, where the results for the already described compounds[29] are also reported for comparison.

Scheme 6[a]



[a] (a) 3-Lithium-pyridine; (b) H_2SO_4 80%.

It is apparent from the tables that, much alike the parent compound where the tertiary base **1b** is devoid of any affinity for the nicotinic receptors, generally, only the quaternary ammonium salts present nicotinic affinity. Thus, the ammonium functionality seems to be a critical feature for the interaction. The already mentioned **3b** and **4b** and, among the new compounds, **3a**, **14b**, **29a,** and **29b** represent a few exceptions to this general behavior.

The results reported in Table 1 also clearly show that substitution in the para position of the phenyl ring is definitely detrimental for affinity. On the contrary, substitution in position 3 gave very interesting results, affording several compounds with nanomolar affinity for the nicotinic receptor (**8c, 9c, 11c, 13c, 14c, 17c**). Among the compounds where the phenyl has been substituted with a heterocyclic ring, compound **28c** is the most affinitive, showing low nanomolar affinity.

Isosteric replacement of the piperazine nitrogen with a sp^2 carbon (Table 2) maintained the affinity for the receptor but generally at a lower level, as shown by compounds **31c** and **33b** with respect to compounds **1c** and **3b**. Finally, reduction of the double bond (Table 3) did not change substantially the situation, as shown by compounds **35c** and **37b** with respect to compounds **31c** and **33b**.

Analgesic activity, which is typical of centrally acting nicotinic agonists, was evaluated for a few selected compounds of the series. The results, reported in Table 4, confirm that the compounds showing affinity for the nicotinic receptor are able to induce mecamylamine-dependent analgesia and behave as central nicotinic agonists. Among the compounds tested, **28c**, which is one of the most affinitive, is also the most potent analgesic, showing a remarkable potency and efficacy.

Discussion

The results obtained with the series of compounds examined make it possible to individuate, in the structure of DMPP, a region which is critical for the interaction and whose manipulation can greatly improve affinity for the brain nicotinic receptors. This region corresponds to the *meta* position of the piperazine-linked benzene ring and to the 1-nitrogens of piperazine-linked pyridine and pyridazine (Figure 1). When atoms or groups of atoms able to form hydrogen bond are located in this region, the affinity for the nicotinic receptor results increased by some 2 orders of magnitude. The importance of this hydrogen bond seems confirmed by compounds **26c** and **27c** that, although lacking the aromatic ring, maintain a modest but definite affinity.

Introduction of substituents into the *para* position of the aromatic ring gives compounds with reduced affinity

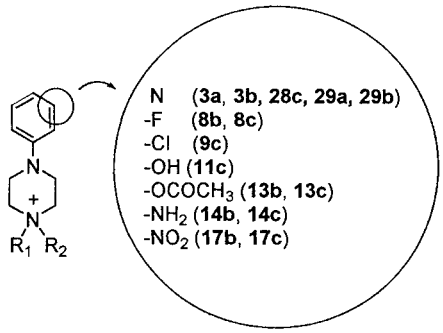


Figure 1. Structure of the most interesting compounds.

with respect to DMPP; this may be due to steric hindrance in the interaction with the receptor, since only the 4-pyridyl derivative **4b** is more active. The introduction of a chlorine atom in a similar position on the pyridazinyl ring (compounds **28a** and **28b**) reduces affinity with respect to the unsubstituted derivatives **29a** and **29b**; this is in contrast to what is reported in the literature for similar compounds[56] and does not hold for the quaternary derivative **28c**, which is the most potent compound of this series.

From a medicinal chemistry point of view, a particularly important consequence of the manipulation of the DMPP molecule is that interesting nicotinic affinity can be induced also in nonquaternary compounds, which are of course much more suitable for therapeutic use in neurodegenerative diseases. Even if they are exceptions in the series, compounds **4b** (K_i = 170 nM), **14b** (K_i = 180 nM), and **29b** (K_i = 386 nM) may represent new leads to develop therapeutically useful drugs. The substitution of the phenyl ring with a 3-pyridyl moiety, which locates a basic nitrogen in the critical region of binding, seems to be of particular interest in this respect. Indeed, this variation of the lead affords compounds that do not need to be quaternary salts to present affinity for the central nicotinic receptor, as is the case of **3b** (K_i = 90 nM), **33b** (K_i = 1200 nM), and **37b** (K_i = 1200 nM). Remarkably, the same substitution is able to induce nanomolar affinity also in a secondary amine like **3a** (K_i = 90 nM), in accord to what is found by Nielsen.[56]

To characterize our compounds we needed to be sure that they were actually nicotinic agonists and interacted with the active site of brain nicotinic receptors. Analgesic action, which is typical of centrally acting nicotinic agonists, was therefore evaluated for some selected compounds of the series. The results, reported in Table 4, confirm that these compounds, being able to induce mecamylamine-dependent analgesia, are indeed central nicotinic agonists.

From the inspection of the data reported in Tables 1−3 it can be seen that activity is mainly associated with a quaternary ammonium compounds and/or with the presence of a H-bond forming group in position 3 on the aromatic ring. Classical nicotinic agonists contain an H-bond acceptor group, at a certain distance from a cationic nitrogen. From our data it is possible to see that in the phenylpiperazine series an aromatic moiety is also important for affinity. In fact DMPP, which lacks the H-bond acceptor group, shows affinity, and unsaturated ketones **26** and **27**, whose ability of accepting H-bond is higher with respect to other atoms such as

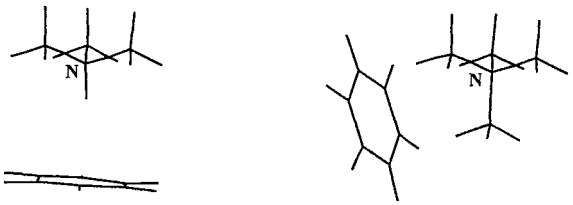


Figure 2. Geometry of interaction of the complexes between a phenyl ring and protonated trimethylamine (left) or trimethylammonium ion (right).

Cl or groups such as NH_2, are less active than compounds **9** or **14**. It may be possible that the aromatic ring contributes to affinity with $\pi-\pi$ interaction (such as, for instance, charge-transfer): a similar hypothesis has been proposed by Elliott, by inspection of the activity of a series of arylpyrrolidine structurally related to nicotine.[57]

Regarding the distance between the H-bond acceptor group and the cationic nitrogen, values of 4.8 Å[19] or 5.5 Å[22] were proposed on the basis of the possible conformations of nicotine and epibatidine. In our molecules, this distance is always higher, ranging from the 6.15 Å of the pyridazinyl derivative **28** to 8.37 Å of the cyano derivative **16**, indicating that this class of compounds may interact with the receptor in a different way, or with different residues of the active site, with respect to classical agonists. In addition groups such as NH_2 and OH, which are both H-bond donors and acceptors, may be considered atypical within nicotinic agonists: only a few examples of the presence of these groups are known,[18,56,58] some of which[18,56] regard pyridine derivatives.

Although acetylcholine possesses an ammonium function, many of the classical nicotinic agonists show higher affinity as secondary or tertiary amines than as quaternary ammonium compounds: this is the case, for instance, of anatoxin and nicotine, whose methiodides are 500 and 15 times less potent than the uncharged counterparts.[18] On the contrary, in our series of molecules, high affinity is practically confined into quaternary ammonium compounds. Charge distribution, beside the volume of the additional methyl group, is the main difference between a protonated amino group and a quaternary ammonium. In the protonated amine, a large part of the charge is located on the hydrogen atom, while, in the ammonium group, the charge is spread on the hydrogen atoms of the alkyl groups. The mode of binding of secondary and tertiary amines may then be driven by the strong electrostatic interaction between the NH and the aromatic residues of the receptor. On the contrary, the interaction between the same residues and the ammonium group, which is weaker because the partial charges are lower, may allow a different, more favorable orientation of the ligand in the active site. To support this hypothesis, we have done a semiempirical calculation on a complex between a phenyl ring and tetramethylammonium or protonated trimethylamine. As it can be seen from Figure 2, the geometry of the low-energy conformations of the complexes are different, suggesting that an ammonium group and a protonated amine can orient the aromatic residues of the active site in a different way. In other words, they may have a different interaction mode.

Conclusions

We have shown that manipulation of a nicotinic agonist such as DMPP (**1c**), with modest affinity for $\alpha_4\beta_2$ nicotinic brain receptors, can definitely improve its affinity for the same receptors. The best results are obtained when the phenyl ring of DMPP is substituted in position 3′ by atoms or groups of atoms able to form hydrogen bond and the molecules maintain an ammonium function. Of particular interest is the fact that substitution of the phenyl with a 3-pyridyl ring results in compounds that maintain remarkable affinity for the nicotinic receptors, even as secondary or tertiary amines. These studies have confirmed the peculiarity of this class of compounds, where low nanomolar affinity for the central nicotinic receptors is related to a pharmacophore somehow different from that of classical nicotinic ligands.

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 a Nujol mull for solids and neat for liquids. Unless otherwise stated, NMR spectra were recorded on a Gemini 200 spectrometer. 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). 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.

General Procedure for the Methylation of Secondary Amines. A mixture of the secondary amine, formic acid, and formaldehyde was kept under reflux for 4−8 h; then it was concentrated under vacuum, treated with a saturated solution of $NaHCO_3$, and extracted with chloroform. After anhydrification and removal of solvent, the residue was purified (when necessary) by flash chromatography and/or transformed into the oxalate salt by treatment with 1 equiv of oxalic acid in ethyl acetate or into the hydrochloride salt by treatment with a HCl saturated ethanolic solution. Compounds **8b**−**10b**, **17b**−**19b, 21b,** and **24b** were prepared by this procedure (Table 5).

General Procedure for the Synthesis of Methiodides. An ether solution of the tertiary amine was treated with an excess of methyl iodide and kept for one night at room temperature in the dark. The obtained solid was filtered, dried under vacuum, and recrystallized (when necessary) from absolute ethanol. Compounds **6c**−**13c**, **15c**−**22c**, **24c**−**28c**, **30c**, **31c**, **35c**, and **39c** have been prepared by this procedure (Table 6).

1-Phenyl-2,4-dimethylpiperazine (6b). To a solution of PPh_3 (0.42 g, 1.5 mmol) in anhydrous THF, DEAD (0.24 mL, 1.5 mmol) was added, and the mixture was kept under stirring at room temperature for 30 min; then compound **43** (0.33 g, 1.5 mmol) in anhydrous THF was added. After 24 h stirring at room temperature, the solvent was evaporated, leaving a residue which was purified by flash chromatography (eluent: CH_2Cl_2/abs EtOH/petroleum ether/diethyl ether 360/180/900/360); 0.17 g of **6b** were obtained as an oil (60% yield). [^{1}H]-NMR ($CDCl_3$, δ): 1.15 (d, 3H, J = 8.6 Hz, CCH_3); 2.30 (s, 3H, NCH_3); 2.32−2.42 (m, 1H), 2.43−2.60 (m, 2H), 2.71−2.82 (m, 1H) and 3.06−3.29 (m, 2H) (piperazine protons); 3.75−3.90 (m, 1H, CHN); 6.81−6.99 (m, 3H) and 7.20−7.32 (m, 2H) (aromatic protons) ppm. The compound was transformed into the oxalate salt with an equivalent of oxalic acid in ethyl acetate: mp 137−140 °C.

1-(3′-Methylphenyl)-4-methylpiperazine (7b). A mixture of 2.7 g (0.014 mol) of bis(2-chloroethyl)methylamine hydrochloride, 1.5 g (0.014 mol) of *m*-toluidine, and Na_2CO_3 (3.09 g, 0.0292 mol) in absolute EtOH was kept under reflux for 20 h. The solvent was removed under vacuum, and the mixture was partitioned between water and chloroform. The organic phase

was collected, anhydrified, and evaporated. The residue was purified by flash chromatography using $CHCl_3$/MeOH 95:5 as eluent, giving the title compound in 40% yield. [^{1}H]-NMR ($CDCl_3$, δ): 2.35 (s, 3H, CH_3); 2.37 (s, 3H, NCH_3); 2.56−2.63 (m, 4H) and 3.19−3.26 (m, 4H) (piperazine protons); 6.65−6.81 (m, 3H), and 7.12−7.26 (m, 1H) (aromatic protons) ppm. [^{13}C]-NMR ($CDCl_3$, δ): 22.28 (q), 46.57 (t), 49.56 (t), 55.61 (t), 113.62 (d), 117.26 (d), 121.00 (d), 129.32 (d), 139.02 (s), 151.70 (s) ppm. The oxalate salt melted at 105−110 °C.

1-(3′-Hydroxyphenyl)-4-methylpiperazine (11b). 1-(3′-Methoxyphenyl)-4-methylpiperazine **12b** (0.45 g, 2.18 mmol) in 4 mL of HBr 40% were kept at 100 °C for 60 h. After cooling at 0 °C, the mixture is basified to pH 8 with NaOH and extracted with $CHCl_3$. After anhydrification (Na_2SO_4) and removal of solvent, the residue was purified by column chromatography ($CHCl_3$/CH_3OH 90/10 as eluent) to obtain the title compound (0.37 g, 88% yield). [^{1}H]-NMR ($CDCl_3$, δ): 2.36 (s, 3H, NCH_3); 2.56−2.65 (m, 4H) and 3.10−3.20 (m, 4H) (piperazine protons); 6.25−6.38 (m, 2H, aromatics); 6.46 (d, 1H, J = 8.0 Hz), 6.62 (bs, 1H, OH); 7.08 (d, 1H, J = 8 Hz) (aromatic protons) ppm. [^{13}C]-NMR ($CDCl_3$, δ): 46.10 (q), 48.90 (t), 55.11 (t), 104.19 (d), 107.92 (d), 108.47 (d), 130.30 (d), 152.68 (s), 158.00 (s) ppm. Anal. $C_{11}H_{16}N_2O$ (C,H,N). The oxalate salt melted at 230 °C (after recrystallization from absolute ethanol).

1-(3′-Acetoxyphenyl)-4-methylpiperazine (13b). 1-(3′-Hydroxyphenyl)-4-methylpiperazine **11b** (0.20 g, 1.04 mmol) in $CHCl_3$ (1 mL) and acetic anhydride (1 mL) were kept at 60 °C for 4 h. After removal of solvent, the residue was treated with a saturated solution of $NaHCO_3$ and extracted with $CHCl_3$. After anhydrification (Na_2SO_4) and removal of solvent, the residue was purified by column chromatography ($CHCl_3$/petroleum ether/absolute EtOH/ NH_4OH 1200/180/450/9.9 as eluent); 0.21 g of the desired compound were obtained (86% yield). [^{1}H]-NMR ($CDCl_3$, δ): 2.27 (s, 3H, $COCH_3$); 2.34 (s, 3H, NCH_3); 2.47−2.55 (m, 4H) and 3.18−3.25 (m, 4H) (piperazine protons); 6.52−6.61 (m, 2H), 6.77 (d, 1H, J = 9 Hz) and 7.23 (dd, 1H, J = 9 Hz) (aromatic protons) ppm. [^{13}C]-NMR ($CDCl_3$, δ): 21.61 (q), 46.52 (t), 49.01 (t), 55.35 (t), 109.25 (d), 112.60 (d), 113.49 (d), 130.01 (d), 151.95 (s), 152.68 (s), 169.81 (s) ppm. Anal. $C_{13}H_{18}N_2O_2$ (C,H,N). The oxalate salt melted at 95 °C.

1-(3′-Aminophenyl)-4-methylpiperazine (14b).[35] A mixture of **17b** (0.5 g, 2.26 mmol), $SnCl_2 \cdot 2H_2O$ (3.1 g, 14 mmol), and concentrated HCl (4.3 mL) was stirred at room temperature for 24 h, then it was made alkaline with NaOH, and extracted with chloroform. After anhydrification (Na_2SO_4) and removal of solvent, the residue was purified by flash chromatography ($CHCl_3$/petroleum ether/absolute EtOH/NH_4OH 340/60/65/8 as eluent). The title compound was obtained in 58% yield. [^{1}H]-NMR ($CDCl_3$, δ): 2.39 (s, 3H, NCH_3); 2.55−2.68 (m, 4H) and 3.18−3.27 (m, 4H) (piperazine protons); 3.65 (bs, 2H, NH_2); 6.20−6.30 (m, 2H), 6.35 (dd, 1H, J = 8 and 2.5 Hz, 6′-H), and 7.06 (dd, 1H, J = 8 Hz, 5′-H) (aromatic protons) ppm.

4-(3′-Aminophenyl)-1,1-dimethylpiperazinium Bromide Hydrobromide (14c). A mixture of **39c** (0.2 g, 0.54 mmol) in a 33% acetic acid solution of HBr (3 mL) was kept at room temperature for 12 h, then the solvent was evaporated, and the residue was recrystallized twice from absolute ethanol. Mp 240 °C. [^{1}H]-NMR (D_2O, δ): 3.15 (s, 6H, $N(CH_3)_2$); 3.41−3.55 (m, 8H, piperazine protons); 6.43−6.51 (m, 2H), 6.58 (d, 1H, J = 8.4 Hz) and 7.18 (dd, 1H, J = 8.4 and 7.8 Hz, 5′-H) (aromatic protons) ppm. Anal. $C_{12}H_{20}BrN_3 \cdot HBr \cdot H_2O$: % calc C 37.47, H 6.02, N 10.91; found C 37.62, H 6.03, N 11.60.

1-(3′-Acetylaminophenyl)-4-methylpiperazine (15b). Following the same procedure as for **13b**, starting from **14b** (0.24 g, 1.25 mmol), $CHCl_3$ (1 mL), and acetic anhydride (2 mL), 0.15 g of the desired compound was obtained (51% yield). [^{1}H]-NMR ($CDCl_3$, δ): 2.11 (s, 3H, $COCH_3$); 2.32 (s, 3H, NCH_3); 2.51−2.58 (m, 4H) and 3.15−3.24 (m, 4H) (piperazine protons); 6.65 (d, 1H, J = 9.5 Hz), 6.85 (d, 1H, J = 10 Hz), 7.15 (dd, 1H, J = 10 and 9.5 Hz) and 7.27 (s, 1H) (aromatic protons); 7.68 (s, 1H, NH) ppm. [^{13}C]-NMR ($CDCl_3$, δ): 24.94 (q), 46.50 (q), 49.16 (t), 55.39 (t), 108.09 (d), 111.56 (d), 112.09 (d), 129.68 (d), 139.41 (s), 152.01 (s), 169.34 (s) ppm. Anal. $C_{13}H_{19}N_3O$ (C,H,N). The oxalate salt melted at 150 °C.

1-(3′,4′-Methylenedioxyphenyl)piperazine (25a). Following the same procedure used for **7b**, starting from (3,4-methylenedioxy)aniline (2 g, 0.0146 mol), bis(2-chloroethyl)-amine hydrochloride (2.6 g, 0.0146 mol), and Na_2CO_3 (3.09 g, 0.0292 mol), the title compound was obtained in 35% yield after purification by flash chromatography using $CHCl_3$/MeOH 85:15 as eluent. [^{1}H]-NMR ($CDCl_3$, δ): 1.90 (bs, 1H, NH); 3.05 (s, 8H, piperazine protons); 5.92 (s, 2H, OCH_2O); 6.37 (d, 1H, J = 9.2 and 3.1 Hz, H-6′), 6.57 (d, 1H, J = 3.1 Hz, H-2′) and 6.73 (d, 1H, J = 9.2 Hz) (aromatic protons). Compound **25a** was transformed into the oxalate salt by treatment with 1 equiv of oxalic acid in ethyl acetate: mp 253−258 °C.

1-(3′,4′-Methylenedioxyphenyl)-4-methylpiperazine (25b). A solution of **25a** (0.27 g, 1.3 mmol) and CH_3I (80 μL, 1 equiv) in diethyl ether was kept under stirring at room temperature for 40 h. The solvent was evaporated under vacuum, and the residue was treated with $NaHCO_3$ and extracted with $CHCl_3$. After anhydrification (Na_2SO_4) and removal of solvent, the residue was purified by column chromatography ($CHCl_3$/CH_3-OH 85/15 as eluent) to obtain 0.1 g of the desired compound (25% yield). [^{1}H]-NMR ($CDCl_3$, δ): 2.38 (s, 3H, NCH_3); 2.55−2.65 (m, 4H) and 3.04−3.13 (m, 4H) (piperazine protons); 5.91 (s, 2H, OCH_2O); 6.48 (dd, 1H, J = 9.6 and 3.2 Hz, H-6′), 6.56 (m, 1H, H-2′) and 6.72 (dd, 1H, J = 9.6 and 1.9 Hz) (aromatic protons) ppm. [^{13}C]-NMR ($CDCl_3$, δ): 46.37 (q), 51.04 (t), 55.53 (t), 100.29 (d), 101.20 (t), 108.50 (d), 109.36 (d), 141.88 (s), 147.58 (s), 148.51 (s) ppm. Anal. $C_{12}H_{16}N_2O_2$ (C,H,N). The oxalate salt melted at 125 °C.

3-(4′-Methylpiperazinyl)cyclohex-2-en-1-one (26b). A mixture of 1,3-cyclohexanedione (0.3 g, 2.7 mmol) and *N*-methylpiperazine (0.27 g, 2.7 mmol) in toluene (5 mL) was kept under reflux for 4 h, with a Dean−Stark trap. After removal of solvent, the residue was purified by column chromatography ($CHCl_3$/petroleum ether/absolute EtOH/NH_4OH 1200/180/450/9.9 as eluent). The title compound (0.3 g, 85% yield) was obtained as a white, low-melting solid. [^{1}H]-NMR ($CDCl_3$, δ): 1.95−2.05 (m, 4H); 2.30 (s, 3H, NCH_3); 2.25−2.35 (m, 2H, CH_2-CO); 2.40−2.50 (m, 4H) and 3.30−3.40 (m, 4H) (piperazine protons); 5.30 (s, 1H, C=CH) ppm. [^{13}C]-NMR ($CDCl_3$, δ): 22.42 (t), 27.24 (t), 35.90 (t), 46.19 (q), 46.20 (t), 54.71 (t), 100.27 (d), 165.19 (s), 197.62 (s) ppm. Anal. $C_{11}H_{18}N_2O$ (C,H,N). The oxalate salt, after crystallization from absolute ethanol, melted at 143 °C.

3-(4′-Methylpiperazinyl)cyclopent-2-en-1-one (27b). Following the same procedure as for **26b**, starting from 0.4 g (4 mmol) of 1,3-cyclopentanedione and 0.4 g (4 mmol) of *N*-methylpiperazine, the desired product (0.5 g, 70% yield) was obtained. [^{1}H]-NMR ($CDCl_3$, δ): 2.30 (s, 3H, NCH_3); 2.35−2.45 (m, 6H); 2.55−2.65 (m, 2H, CH_2CO); 3.30−3.45 (m, 4H, piperazine protons); 5.05 (s, 1H, C=CHCO) ppm. [^{13}C]-NMR ($CDCl_3$, δ): 27.60 (t), 34.25 (t), 46.34 (q), 47.57 (t), 54.62 (t), 100.73 (d), 176.68 (s), 204.03 (s) ppm. Anal. $C_{10}H_{16}N_2O$ (C,H,N). The oxalate salt, after recrystallization from absolute ethanol, melted at 172 °C.

1-(3′-Pyridazinyl)piperazine (29a). A mixture of 3,6-dichloropyridazine (0.7 g, 5 mmol) and 1-benzylpiperazine (0.9 g, 5 mmol) was kept at 90 °C for 4 h. After cooling, the mixture was extracted with $CHCl_3$, the solution was dried (Na_2SO_4), and the solvent was removed under vacuum. The residue, **1-(6′-chloro-3′-pyridazinyl)-4-benzylpiperazine** (**40**), was obtained in 74% yield and was used in the next step without further purification. [^{1}H]-NMR ($CDCl_3$, δ): 2.60 (t, 4H, J = 4 Hz, piperazine protons); 3.60 (s, 2H, N−CH_2−Ph); 3.63 (t, 4H, J = 4 Hz, piperazine protons); 6.87 (d, 1H, J = 10 Hz, pyridazine); 7.18 (d, 1H, J = 10 Hz, pyridazine); 7.33−7.50 (m, 5H, aromatic protons). This compound (1.0 g) was dissolved in methanol (10 mL), Pd/C (0.25 g) and ammonium formate (0.6 g) were added, and the mixture was kept under reflux for 12 h. The solvent was removed under vacuum, and the residue was dissolved in a saturated solution of $NaHCO_3$ and extracted with $CHCl_3$. After anhydrification (Na_2SO_4) and removal of solvent, the residue was purified by column chromatography

($CHCl_3$/petroleum ether/absolute EtOH/NH_4OH 1200/180/450/9 as eluent), obtaining **29a** as an oil (20% yield). [^{1}H]-NMR ($CDCl_3$, δ): 2.31 (s, 1H, NH); 2.89−3.02 (m, 4H) and 3.49−3.61 (m, 4H) (piperazine protons); 6.87 (d, 1H, J = 10 Hz, 4′-H), 7.15 (dd, 1H, J = 4.8 and 10 Hz, 5′-H) and 8.49 (d, 1H, J = 4.8 Hz, 6′-H) (aromatic protons). [^{13}C]-NMR ($CDCl_3$, δ): 46.08 (t), 46.27 (t), 112.69 (d), 127.53 (d), 143.61 (d), 160.51 (s) ppm. Anal. $C_8H_{12}N_4$ (C,H,N). The oxalate salt melts at 205 °C.

1-(3′-Pyridazinyl)-4-methylpiperazine (29b). A mixture of 1-(6′-chloro-3′-pyridazinyl)-4-methylpiperazine[29] (0.5 g), Pd/C 10% (0.125 g), ammonium formate (0.3 g), and methanol (15 mL) was kept under reflux for 9 h. The solvent was removed under vacuum, and the residue was dissolved in a saturated solution of $NaHCO_3$ and extracted with $CHCl_3$. After anhydrification (Na_2SO_4) and removal of solvent, the residue was purified by column chromatography ($CHCl_3$/petroleum ether/absolute EtOH/NH_4OH 1200/180/450/9 as eluent), obtaining the desired compound as an oil (38% yield). [^{1}H]-NMR ($CDCl_3$, δ): 2.24 (s, 3H, NCH_3); 2.38−2.52 (m, 4H) and 3.52−3.65 (m, 4H) (piperazine protons); 6.82 (d, 1H, J = 9.5 Hz, 4′-H), 7.10 (dd, 1H, J = 4.5 and 9.5 Hz, 5′-H) and 8.48 (d, 1H, J = 4.5 Hz, 6′-H) (aromatic protons). [^{13}C]-NMR ($CDCl_3$, δ): 45.13 (t), 46.57 (q), 55.00 (t), 112.69 (d), 127.55 (d), 143.67 (d), 160.31 (s) ppm. Anal. $C_9H_{14}N_4$ (C,H,N). The oxalate salt melts at 135 °C.

4-(3′-Pyridyl)-1,2,5,6-tetrahydropyridine (33a). To a solution of 3-bromopyridine (0.79 g, 5 mmol) in anhydrous THF (10 mL), cooled at −60 °C, was added 3.45 mL (5.5 mmol) of butyllithium (1.6 M) dropwise. After 15′ stirring at −60 °C, N-BOC-piperidone[46] (0.5 g, 2.5 mmol) was added, and the mixture was kept for a further 60 min at low temperature. The reaction was quenched with an aqueous solution of acetic acid and extracted with chloroform. After anhydrification and removal of solvent, the residue was purified by column chromatography (CH_2Cl_2/petroleum ether/diethyl ether/absolute EtOH/NH_4OH 36/90/36/18/1 as eluent) to give 0.2 g (29% yield) of **N-BOC-4-(3′-pyridyl)-4-piperidinol**. [^{1}H]-NMR ($CDCl_3$, δ): 1.5 (s, 9H, $(CH_3)_3$); 1.56−1.85 (m, 3H, CH_2 and OH); 1.85−2.12 (m, 2H, CH_2); 3.18−3.37 (m, 2H, CH_2); 3.98−4.16 (m, 2H, CH_2); 7.26−7.34 (m, 1H), 7.78−7.83 (m, 1H), 8.51−8.54 (m, 1H), 8.76 (s, 1H) (aromatic protons) ppm. This product was dissolved in 1 mL of methanol, treated with 2 mL of H_2SO_4 80%, and kept for 5 h at 85 °C. After cooling to room temperature, the mixture was made alkaline with $NaHCO_3$ and extracted with chloroform. The organic layer was anhydrified, and the solvent was removed, to yield the title compound (oil, 0.07 g, 60% yield). [^{1}H]-NMR ($CDCl_3$) δ: 1.94 (bs, 1H, NH); 2.43−2.65 (m, 2H); 3.12−3.18 (m, 2H); 3.56−3.58 (m, 2H); 6.17−6.21 (m, 1H, =CH); 7.22−7.29 (m, 1H), 7.64−7.68 (m, 1H), 8.47−8.50 (m, 1H), 8.65 (s, 1H) (aromatic protons) ppm. IR (neat) ν 3200−3400 (NH). Anal. $C_{10}H_{12}N_2$ (C,H,N). The product was transformed into the hydrochloride: mp 260−262 °C.

1-Methyl-4-(3′-pyridyl)piperidine (37b). 1-Methyl-4-(3′-pyridyl)-1,2,5,6-tetrahydropyridine[46] (**33b**), dissolved in absolute ethanol, was hydrogenated at 80 psi at room temperature with Pd/C 10% (30 mg) for 10 h. The solid was filtered off, and the solvent was removed to give the title compound in 95% yield. [^{1}H]-NMR ($CDCl_3$) δ 1.82−2.14 (m, 4H, CH), 2.21−2.38 (m, 2H, CH), 2.45 (s, 3H, CH_3N), 2.46−2.61 (m, 1H, CH), 3.12−3.21 (m, 2H, CH), 7.21−7.27 (m, 1H, aromatic), 7.57−7.61 (m, 1H, aromatic), 8.43−8.47 (m, 2H, aromatics) ppm. [^{13}C]-NMR ($CDCl_3$) δ 32.68 (t), 39.43 (q), 45.99 (t), 55.89 (d), 123.90 (d), 134.54 (d), 141.09 (d), 148.01 (d), 149.05 (s) ppm. Anal. $C_{11}H_{16}N_2$ (C,H,N). The product was transformed into the hydrochloride: mp 236−238 °C.

1-(3′-Benzyloxycarbonylaminophenyl)-4-methylpiperazine (39b). To a solution of **14b** (0.28 g, 1.48 mmol) in anhydrous dioxane (5 mL) was added at once dibenzyl dicarbonate (0.44 g, 1.48 mmol), and the mixture was kept under stirring at room temperature for 36 h. After removal of solvent under vacuum, the residue (0.5 g) was purified by flash chromatography ($CHCl_3$/CH_3OH 93/7 as eluent) to obtain 0.2 g of a yellow solid (41% yield). [^{1}H]-NMR ($CDCl_3$, δ): 2.36 (s, 3H, NCH_3); 2.50−2.62 (m, 4H) and 3.20−3.40 (m, 4H) (piperazine protons); 5.22 (s, 2H, O−CH_2); 6.61 (d, 1H, J = 10 Hz), 6.72 (d, 1H, J = 10 Hz) and 7.05 (s, 1H) (aromatic protons), 7.20−7.32 (m, 2H, 5′-H and NH); 7.40 (s, 5H, Ph) ppm. [^{13}C]-NMR ($CDCl_3$, δ): 46.25 (q, N−CH_3); 49.00 (t), 55.26 (t), 67.26 (t), 106.88 (d), 110.51 (d), 111.40 (d), 128.62 (d), 128.94 (d), 129.86 (d), 136.47 (s), 139.24 (s), 152.10 (s), 153.83 (s) ppm. Anal. $C_{19}H_{23}N_3O_2$ (C,H,N).

Methyl N-(2-Bromopropionyl)-*N*-methylaminoacetate (41). A solution of methyl sarcosinate (2.82 g, 27 mmol), 2-bromopropionyl bromide (5.8 g, 27 mmol), and triethylamine (3.8 mL) in 5 mL of chloroform was kept under stirring at 0 °C for 2 h. The mixture was then treated with ether, the solid was filtered off, and the solvent was evaporated. The desired compound was obtained in 70% yield and used for the next step without purification. [^{1}H]-NMR ($CDCl_3$, δ): 1.75 (d, 3H, J 8.1 Hz, C−CH_3); 3.10 (s, 3H, NCH_3); 3.65 (s, 3H, OCH_3); 3.95 (d, J = 18.9 Hz) and 4.25 (d, J = 18.9 Hz) (CH_2CO); 4.55 (q, 1H, J = 8.1 Hz, CHCO) ppm.

Methyl N-(2-Phenylaminopropionyl)-*N*-methylaminoacetate (42). Compound **41** (4.5 g, 19 mmol) and aniline (2 equiv) were kept at 60 °C for 3 h. The mixture was extracted with CH_2Cl_2; the solvent was evaporated to give the desired compound as a yellow oil (74% yield). [^{1}H]-NMR ($CDCl_3$, δ): 1.45 (d, 3H, J = 8.3 Hz, C−CH_3); 3.20 (s, 3H, NCH_3); 3.70 (s, 3H, OCH_3); 4.03 (d, 1H, J = 16.7 Hz) and 4.29 (d, 1H, J = 16.7 Hz) (CH_2CO); 4.45 (q, 1H, J = 8.3 Hz, CHMe); 6.65−6.90 (m, 3H) and 7.10−7.30 (m, 2H) (aromatic protons) ppm.

N-[(2-Phenylamino)propyl]-*N*-methylaminoethanol (43). To compound **42** (1.08 g, 4 mmol) dissolved in anhydrous ether, $LiAlH_4$ (0.6 g, 16 mmol) was added at 0 °C; the mixture was allowed to warm to room temperature and was kept under stirring for 3 h. The excess hydride was quenched with ethyl acetate and ice, and the mixture was extracted with ether. After anhydrification (Na_2SO_4) and removal of solvent, the residue was purified by flash chromatography (eluent: CH_2Cl_2/absolute EtOH/petroleum ether/diethyl ether/NH_4OH 360/180/900/360/9.9) obtaining the desired compound as oil (40% yield). [^{1}H]-NMR ($CDCl_3$, δ): 1.25 (d, 3H, J = 8.1 Hz, CCH_3); 2.35 (s, 3H, NCH_3); 2.60−2.70 (m, 3H, $NCHCH_2N$); 3.25 (bs, 2H, OH and NH); 3.55−3.70 (m, 4H, CH_2CH_2); 6.60−6.75 (m, 3H) and 7.10−7.30 (m, 2H) (aromatic protons) ppm.

Pharmacology

Binding Studies. Cerebral cortices of male Wistar rats (150−200 g) were dissected on ice. The tissue was homogenized in 50 mmol Tris-HCl buffer (pH = 7.4 at 2 °C) containing 120 mmol of NaCl, 5 mmol of KCl, 1 mmol of $MgCl_2$, and 2.5 mmol of $CaCl_2$. The homogenate was centrifuged at 40 000 ×g for 10 min; the pellet was resuspended in ice-cold buffer, recentrifuged, and resuspended again in buffer. Binding experiments[59] with [^{3}H]-cytisine (New England Nuclear, Boston, MA; 39.7 Ci/mmol) were performed in 250 μL of buffer which contained 2 nmol of [^{3}H]-cytisine, membranes from 15 mg (wet weight) of tissue, and the compound to be tested. After 75 min of incubation at 2 °C, separation of bound radioligands from the free ones was performed by rapid filtration through Whatman GF/C glass fiber filter, which were then washed three times with ice-cold buffer, dried, and counted in 5 mL of Aquassure (Packard, Downers Grove, USA). Binding in the presence of 10 mmol (−)-nicotine bitartrate was defined unspecific and was, routinely, about 10% of the total binding. K_i values were calculated from the Cheng-Prusoff equation[60] using 1.5 nmol as the K_d for [^{3}H]-cytisine, determined by previous saturation experiments.

Antinociceptive Activity. Analgesic activity was evaluated on mice with the hot-plate test,[55] and the

compounds were injected intracerebroventricularly (icv). Results are given as minimum analgesic dose (MAD) and efficacy (E%) with respect to the analgesic activity of 5 μg of morphine icv. More details on the protocol used can be found in previous publications.[61,62] The nicotinic origin of analgesia was checked by its reversion by mecamylamine at the dose of 2 mg/kg i.p. This dose is able to prevent nicotinic analgesia (1.5 mg/kg s.c.) but not the analgesia induced by morphine (7 mg/kg s.c.) or baclofen (4 mg/kg s.c.).

Semiempirical Calculations. Tetramethylammonium, protonated trimethylamine, and benzene were built and minimized with AM1 within Spartan (PC Spartan Pro, V1.01, Wavefunction, Inc., Irvine, CA); the complexes were assembled using InsightII (Accelrys Inc., San Diego, CA) in different starting geometries, which were then minimized with AM1. In Figure 2 are reported the geometry of the complexes showing lower energy.

Acknowledgment. This research was supported by funds of the Italian Ministry of University and Scientific Research (MURST).

Supporting Information Available: Tables 7−9 with NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org

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JM010901Y