Design, Synthesis, and Preliminary Pharmacological Evaluation of 4-Aminopiperidine Derivatives as N-Type Calcium Channel Blockers Active on Pain and Neuropathic Pain

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Several compounds with a 4-aminopiperidine scaffold decorated on both nitrogen atoms by alkyl or acyl moieties containing the structural motifs of verapamil and of flunarizine, as well as those that are more frequent in known N-type calcium channel antagonists, have been synthesized. Antinociceptive activity on the mouse hot-plate test was used to select molecules to be submitted to further studies. Active compounds were tested in vitro on a PC12 rat pheochromocytoma clonal cell line, to evaluate their action on N-type calcium channels, and on a rat model of neuropathic pain. Two compounds that show N-type calcium channel antagonism and are endowed with potent action on pain and neuropathic pain (**3** and **18**) have been selected for further studies.

Introduction

Excitable cells possess voltage-dependent calcium channels (VDCC) that control ion flux in to and out of the cell, by opening and closing in response to changes in membrane potential.[1] They modulate many important physiological functions such as neurotrasmitter release, gene expression and muscle contraction. Biophysical and pharmacological studies have identified five different subtypes of high-voltage activated (HVA) calcium channels: L, N, P, Q and R and one low-voltage activated channel: the T-type. Functional calcium channels are formed by a combination of subunits, the largest of which, the α_1 subunit, forms the voltage-sensitive ion-conducting pore.

Most neurons express multiple calcium channel subtypes.[2] Apparently, they contribute to different extents to the physiology of neuronal cells depending on the types of cells and synapses formed by them. It is generally accepted that L-type channels, unlike N-type and P/Q-type, make only minor contributions to neurotransmitter release in the brain. Indeed, N-type calcium channels, which are located presynaptically and are selectively blocked by ω-conotoxin MVIIA,[3] a peptide toxin derived from *Conus magus*, seem to play a fundamental role in neuronal cell excitability and, as a consequence, in neuroprotection[4] and pain perception.[5,6] This suggests that N-type calcium channel blockers could be useful in pathological states such as stroke, pain and, in particular, neuropathic pain.[2,7] It is interesting that very recently T-calcium channels have also been found to play a role in neurophatic pain.[8]

The promising properties of ziconotide (SNX-111), the synthetic equivalent of ω-conotoxin MVIIA,[9] have fulfilled these expectations and have stimulated a large synthetic effort to individuate small molecules able to block N-type calcium channels, while showing improved pharmacokinetic properties. In fact, ziconotide has marked therapeutic benefits in both analgesia and in neuroprotection, is in phase III clinical trial and has been made available to patients for compassionate use.[10] Unfortunately, the drug has to be administered intrathecally to allow direct access to the spinal cord. This has prompted the design and the synthesis[11−13,14] of many small molecules that would block the N-type calcium channel, giving the same therapeutic benefits as ziconotide, with more acceptable pharmacokinetic properties.

For several years we have been involved in the design and synthesis of L-type calcium channel antagonists related to verapamil,[15−18] and we decided to take advantage of the experience accumulated during this researche to design and study N-type calcium channel antagonists. Indeed, during the past decade, it was found that the classic L-type antagonists are not that specific, as a few of them show some action also on other types of calcium channels, namely the N-type. As an example, Chart 1 reports some L-type classical antagonists that have also shown remarkable N-type antagonism: (*S*)-emopamil,[19] cilnidipine,[20] flunarizine.[21] More interestingly, structural motifs of L-type antagonists can be found in the new molecules designed as N-type antagonists such as compounds A[22] and B[23] shown in Chart 1. These results seem to suggest that it is possible to obtain N-type calcium channel antagonists by molecular manipulation of the structure of L-type antagonists and on this basis, we have designed the series of compounds shown in Chart 2. The 4-aminopiperidine scaffold, that has been already used as supporting structure for N-type antagonists,[13] has been decorated on both nitrogen atoms by alkyl or acyl moieties

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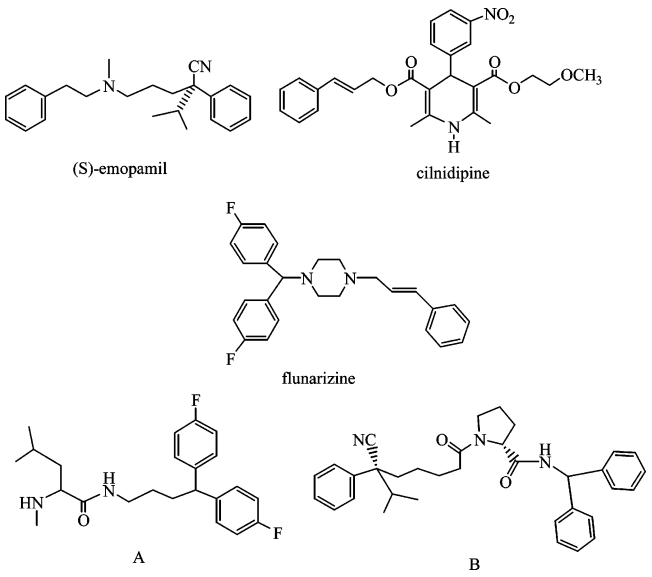
Chart 1



containing the structural motifs of verapamil and of flunarizine as well as those that are more frequent in known N-type calcium channel antagonists.[14] It must be stressed that we did not intend to synthesize all possible isomers corresponding to the X and Y groups indicated in Chart 2, as we rather wanted to explore the possibility of obtaining efficient and possibly selective N-type antagonists, using the approach described above.

Chemistry. The reaction pathways used to synthesise the designed compounds (**1**−**20**) are described in Schemes 1−4 and their chemical and physical characteristics are reported in Table 1.

The key intermediates 1-benzyl-4-alkylaminopiperidines (**1**, **21**−**23**) were obtained by alkylation of 1-benzyl-4-aminopiperidine with 5-bromo-2-(3,4-dimethoxyphenyl)-2-(methylethyl) pentanenitrile[24] or by reductive alkylation of 1-benzyl-4-piperidone with the suitable amine (commercially available 2-(3,4-dimethoxyphenyl)ethylamine, 2-phenoxyethylamine and butylamine), using titanium(IV) isopropoxide as Lewis acid catalyst and $NaBH_3CN$ as reducing agent, according to the Mattson procedure[25] (Scheme 1).

The 1-alkyl-4-alkylaminopiperidines **2**−**12** were synthesized from the corresponding 1-benzyl-4-alkylaminopiperidine (**1**, **21**−**23**) by alkylation, with the suitable alkyl halide, of compounds **24**−**27**, obtained by debenzylation of **1**, **21**−**23** (Scheme 2). The alkyl halides used are commercially available (butyl iodide, cinnamyl bromide) or synthesized according to the literature (1,1′-(4-bromobutylidene)bis(4-fluorobenzene),[26] 1-bromo-4,4-diphenylbutane,[27] (2-bromoethoxy)benzene,[28] 4-(2-bromoethyl)-1,2-dimethoxybenzene[29]). 5-Bromo-2-phenyl-2-(methylethyl)pentanenitrile (**33**), used to obtain compound **4**, was obtained with the same procedure described by Teodori[24] for the 3,4-dimethoxyphenyl derivative, starting from 3-methyl-2-phenylbutyronitrile.[30]

The 1-piperidine amide derivatives (**13**−**15**) were synthesized from the corresponding 4-alkylaminopiperidine (**24**−**27**) and 4-cyano-4-(3,4-dimethoxyphenyl)-5-methylhexanoic acid (**28**) using diethylcyanophosphonate as dehydrating agent. Compound **28** was obtained from the corresponding alcohol[31] by oxidation with $KMnO_4$ and tetrabutylammonium bromide in benzene (Scheme 2).

4,4′-Bis-(4-fluorophenyl)-*N*-piperidine-4-ylbutyramide **16** was obtained from 4,4′-bis-(4-fluorophenyl)-butyric acid[26] by reaction with 1-benzyl-4-aminopiperidine to give **29**, which was then debenzylated (Scheme 3). Reaction of **16** with chloroethanol in the presence of triethylamine gave **17**. 1-(4-Aminopiperidin-1-yl)-4,4′-bis-(4-fluorophenyl)butan-1-one **18** was synthesized by cleavage with Me_3SiI in MeOH of the *t*-BOC protected compound **30**. The latter was obtained from 4,4′-bis-(4-fluorophenyl)butyric acid[26] and 4-(*tert*-butyloxycarbo-

Chart 2

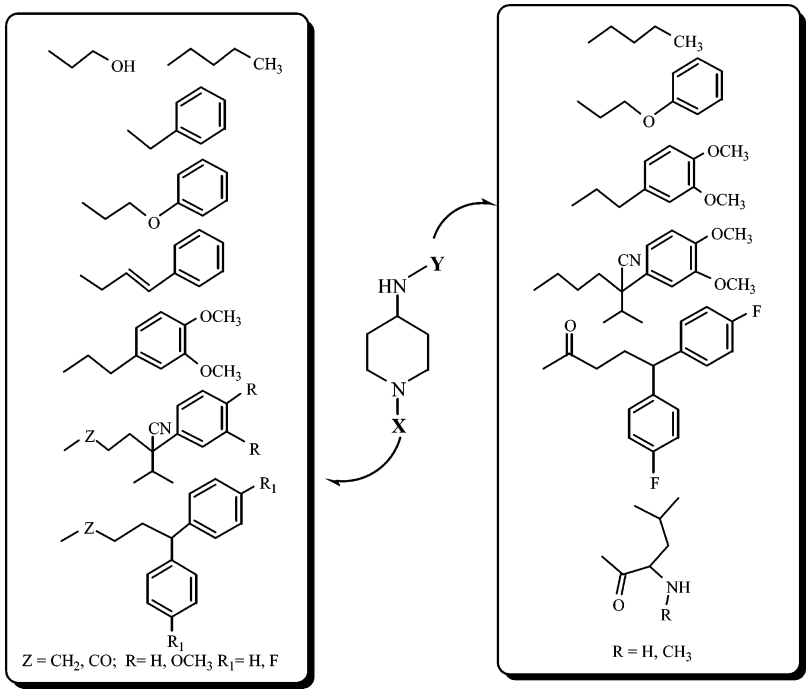
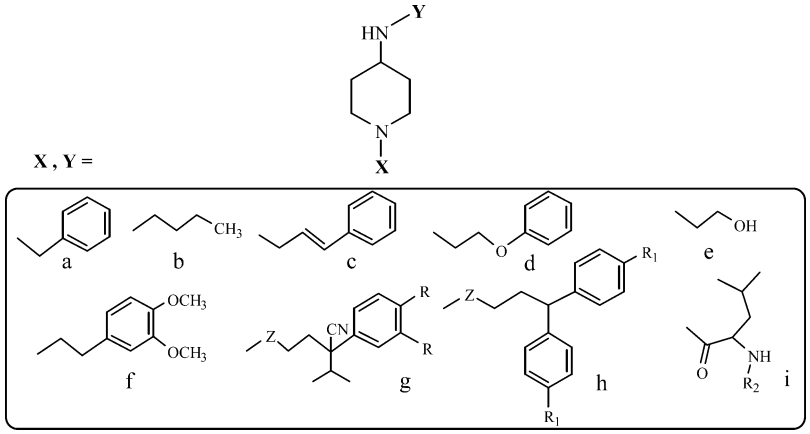


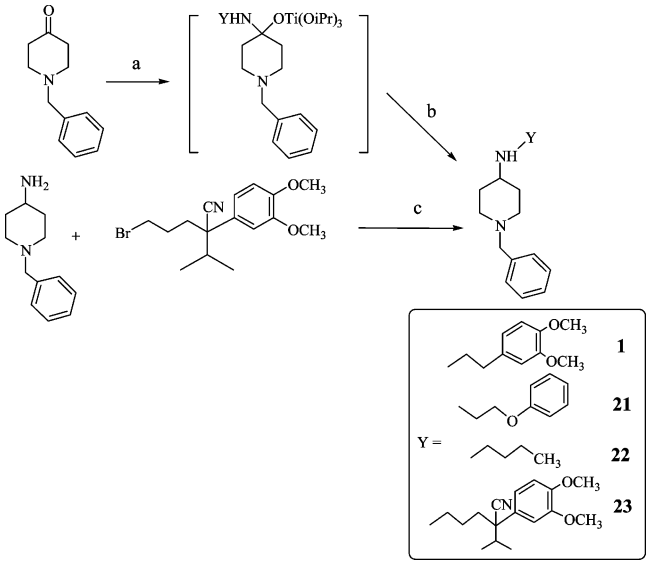
Table 1. Chemical and Physical Characteristics of Compounds **1**−**20**



N	X	Y	Z	R	R_1	R_2	yield, %	salt/mp (°C)	analysis[a]
1	a	f	-	-	-	-	88	HCl/260−265[b]	$C_{22}H_{31}ClN_2O_2$
2	g	b	CH_2	OCH_3	-	-	57	HCl/234−235[b]	$C_{25}H_{42}ClN_3O_2$
3	g	d	CH_2	OCH_3	-	-	31	oxalate/217−218[c]	$C_{31}H_{43}N_3O_7$
4	g	f	CH_2	H	-	-	83	oxalate/233−234[c]	$C_{31}H_{43}N_3O_6$
5	g	f	CH_2	OCH_3	-	-	56	oxalate/198−200[c]	$C_{33}H_{47}N_3O_8$
6	h	b	CH_2	-	H	-	80	oxalate/252−253[c]	$C_{27}H_{38}N_2O_4$
7	h	f	CH_2	-	H	-	35	oxalate/234−235[b]	$C_{31}H_{41}ClN_2O_2$
8	h	f	CH_2	-	F	-	64	HCl/190−192[b]	$C_{31}H_{39}ClF_2N_2O_2$
9	b	g	CH_2	OCH_3	-	-	34	HCl/130−132[b]	$C_{25}H_{42}ClN_3O_2$
10	d	g	CH_2	OCH_3	-	-	58	HCl/240−245[b]	$C_{30}H_{46}ClN_3O_3$
11	c	g	CH_2	OCH_3	-	-	10	HCl/210−215[b]	$C_{30}H_{42}ClN_3O_2$
12	f	g	CH_2	OCH_3	-	-	24	HCl/135−140[b]	$C_{31}H_{45}N_3O_4$
13	g	f	CO	OCH_3	-	-	32	oxalate/80−82[c]	$C_{33}H_{45}N_3O_9$
14	g	d	CO	OCH_3	-	-	43	oxalate/104−105[c]	$C_{31}H_{41}N_3O_8$
15	g	b	CO	OCH_3	-	-	32	HCl/90−92[b]	$C_{25}H_{40}ClN_3O_3$
16	none	h	CO	-	F	-	62	oxalate/100−102[c]	$C_{23}H_{26}F_2N_2O_5$
17	e	h	CO	-	F	-	44	HCl/170−175[b]	$C_{23}H_{29}ClF_2N_2O_2$
18	h	none	CO	-	F	-	45	HCl/138−141[b]	$C_{21}H_{25}ClF_2N_2O$
19	h	i	CO	-	F	H	69	HCl/165−167[b]	$C_{27}H_{36}ClF_2N_3O_2$
20	h	i	CO	-	F	CH_3	38	HCl/85−87[c]	$C_{28}H_{38}ClF_2N_3O_2$

[a] All compounds have been analyzed for C, H, N after vacuum-drying at a temperature below the melting point; the results obtained range within ±0.4% of the theoretical values. [b] Recrystallization solvent: absolute ethanol/ anhydrous diethyl ether. [c] Recrystallization solvent: ethyl acetate.

Scheme 1[a]



[a] Reagents and conditions: (a) $(iPrO)_4Ti$, 2-(3,4-dimethoxyphenyl)ethylamine or 2-phenoxyethylamine or butylamine. (b) $NaBH_3CN$. (c) Et_3N.

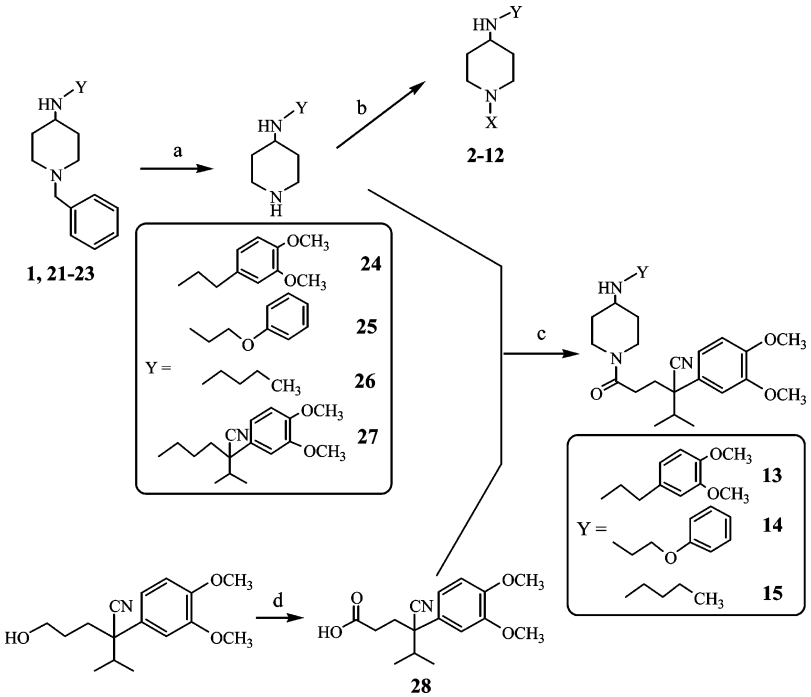
nylamino)piperidine[32] using diethylcyanophosphonate as dehydrating agent (Scheme 3). The leucine derivatives, **19** and **20**, were obtained from **18** by reaction with N-*t*-BOC-L-leucine (commercially available) and N-*t*-BOC-*N*-methyl-L-leucine[33] respectively, followed by cleavage of the protected intermediates **31** and **32** (Scheme 4).

Pharmacological Studies. Antinociceptive activity of compounds **1**−**20** was evaluated on the mouse hot-plate test according to O'Callaghan and Holtzman.[34] All compounds were administered icv at the maximal dose soluble in isotonic (NaCl 0.9%) saline solution (saline) or in a vehicle constituted by water and DMSO (2:1).

L-Type calcium channel binding affinity at the 1,4-dihydropyridine and phenylalkylamine binding sites of the compounds showing antinociceptive activity (**2**, **3**, **6**, **8**−**11**, **16**, **18**−**20**) was evaluated on rat ventricle membranes using [^{3}H]D888 and [^{3}H]PN200-110, respectively. N-Type calcium channel blocking activity of compounds **2**, **3**, **6**, **8**−**11**, **15**, **16**, **18**−**20** was determined in vitro on a PC12 rat pheochromocytoma clonal cell line, using a spectrophotofluorimetric method employing a fluorescent probe sensitive to intracellular calcium changes.[35,36] Different concentrations of the tested compounds (dissolved in DMSO) and ω-conotoxin MVIIA, as the reference compound, were used.

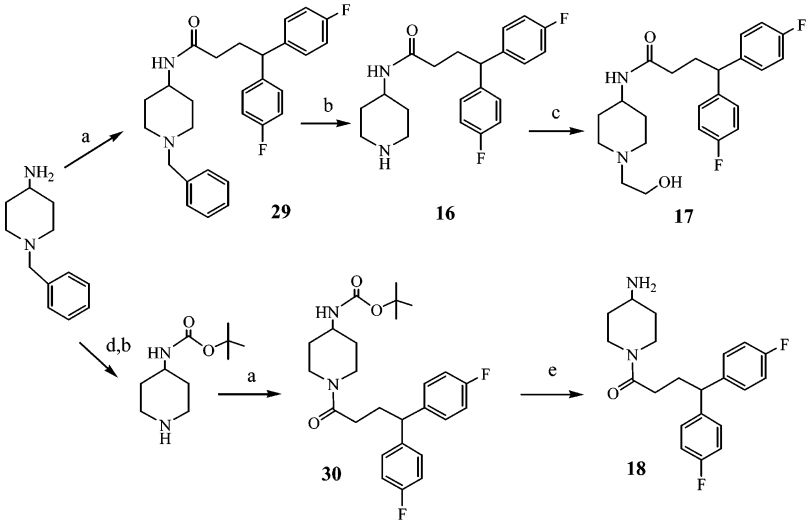
Compounds **2**, **3**, **6**, **10**, **15**, **18**−**20** were also tested on a neuropathic pain model. Toward this end, a peripheral mono neuropathy was produced in adult rats by loosely placing constrictive ligatures around the

Scheme 2[a]



[a] Reagents and conditions: (a) $HCOONH_4$, MeOH, Pd/C. (b) XBr or XI, Et_3N. (c) Diethylcyanophosphonate, DMF, Et_3N. (d) $KMnO_4$/But_4NBr, benzene. For the meaning of X and Y see Table 1.

Scheme 3[a]



[a] Reagents and conditions: (a) 4,4′-Bis(4-fluorophenyl)butyric acid,[26] diethylcyanophosphonate, DMF, Et_3N. (b) $HCOONH_4$, MeOH, Pd/C. (d) $ClCH_2CH_2OH$, Et_3N. (d) *t*-BOC, THF. (e) Me_3SiI, MeOH.

common sciatic nerve according to the method described by Bennett.[37] The nociceptive threshold in the rat was determined with an analgesimeter according to the paw pressure test.[38]

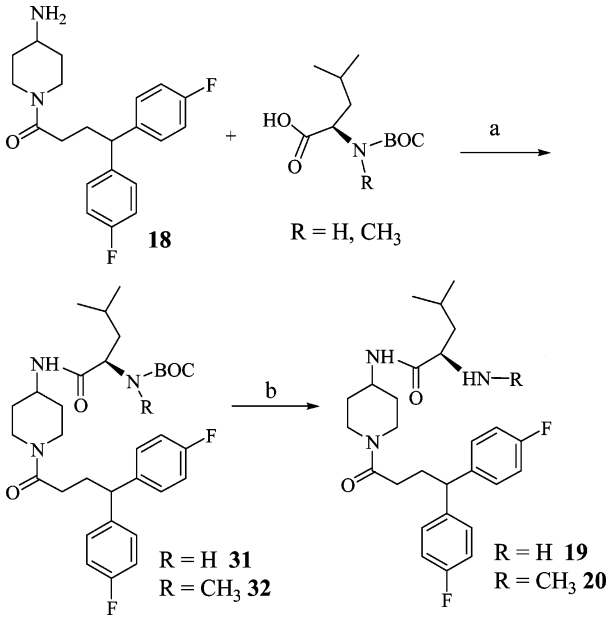
Results and Discussion

The mouse hot-plate test was used to identify, among the compounds synthesized the molecules able to induce analgesia and that, therefore, may possibly be endowed with activity on N-type calcium channels.

As it can be seen from Table 2, intracerebroventricular injection of several compounds of the series (**2**, **3**, **6**, **8**−**11**, **16**, **18**−**20**) induced a statistically significant antinociception in the hot-plate test. The analgesic efficacy of active compounds was evaluated and expressed as the % of the antinociception produced by morphine (10 μg per mouse icv). It must be emphasized that, due to the poor solubility of many of the compounds of the series, their antinociceptive activity has been evaluated at very low doses, and therefore their analgesic activity may be underestimated and some of the inactive compounds could indeed be active, at higher doses.

With the exception of compound **9**, all the active compounds (**2**, **3**, **6**, **8**, **10**, **11**, **16**, **18**−**20)** show an analgesic efficacy that is more than 50% of that exerted by morphine (Table 2). Three compounds are outstanding in the series: compounds **11**, **19** that possess 69%, 71% of morphine efficacy at the dose of 20, 40 μg/mouse icv, respectively, and compound **18** which, at the dose

Scheme 4[a]



[a] Reagents and conditions: (a) Diethylcyanophosphonate, DMF, Et_3N. (b) 2 N HCl/ethyl acetate.

of 50 μg/mouse icv, shows an analgesic efficacy higher than that of morphine (147%). At 25 μg/mouse icv, efficacy of **18** was still very good (80%) while at 10 μg/mouse icv the compound was ineffective. Its analgesic activity reached the maximum peak 15 min after administration, persisted almost unchanged up to 30 min, then progressively diminished, disappearing at 60 min (data not shown). The analgesic activity of **18** was not inhibited by naloxone, showing that opioid receptors are not involved in its action. Active compounds, at the effective doses, neither produced any alteration of the animals' gross behavior nor modified the spontaneous motility and inspection activity as revealed by the hole-board test (data not shown). The zero percent value, that appears in Table 2 in correspondence of some compounds, means that those molecules are devoid of any statistical significance versus the corresponding control group treated with saline or vehicle.

Since significant antinociception can be produced also by L-type calcium channel antagonists,[39,40] binding affinity at the 1,4-dihidropiridine and phenylalkylamine binding sites of L-type calcium channel of the compounds showing antinociceptive activity (**2**, **3**, **6**, **8**−**11**, **16**, **18**−**20**) was evaluated. On rat ventricle membranes, using [^{3}H]D888 and [^{3}H]PN200-110 as radioactive ligands, such compounds have shown very low affinity (K_D = 10−100 μM, data not shown) for the two binding sites of L-type calcium channels.

Therefore, compounds (**2**, **3**, **6**, **8**−**11**, **16**, **18**−**20**) which induced a statistically significant antinociception in the mouse hot-plate test and, as a control, compound **15** which is devoid of analgesic efficacy, were selected for testing their ability to block N-type calcium channels.

A number of studies have demonstrated that PC12 cells express N- and L-type calcium channels.[41,42] In preliminary experiments, we have tested whether the PC12 clone used in our studies (see Experimental Section), which is characterized by the ability to grow in suspension, expresses N-type calcium channels, by evaluating the increase of intracellular calcium stimulated by KCl (50 mM, final concentration) after complete blockade of L-type calcium channels with 10 μM nifedipine. As can be observed in Figure 1, in these conditions the residual Ca^{2+}-flux (shown in Figure 2) is abolished by ω-conotoxin MVIIA at concentrations (0.1−1 μM) known to specifically inhibit N-type calcium

Table 2. Analgesic Activity and Efficacy of Compounds **1**−**20** Evaluated in the Mouse Hot-Plate Test

treatment	dose icv, μg/mouse	before treatment	licking latency (s) after treatment[d] 15 min	30 min	45 min	% analgesic efficacy compared to morphine[a]
saline	-	14.4 ± 1.0	13.9 ± 1.6	14.8 ± 2.1	14.0 ± 1.9	0
vehicle	-	15.7 ± 1.2	14.8 ± 1.7	15.5 ± 1.6	16.0 ± 1.3	0
morphine	10	15.3 ± 1.1	28.6 ± 2.5*	27.7 ± 1.5*	22.4 ± 1.9*	-
1	50	13.7 ± 1.5	15.5 ± 1.5	16.7 ± 1.9	15.3 ± 1.8	0[b]
2	30	15.3 ± 1.3	23.1 ± 1.9*	21.3 ± 1.8∧	18.7 ± 2.2	59
3	50	14.2 ± 0.6	22.3 ± 2.2*	20.8 ± 1.5∧	18.9 ± 1.3	61
4	10	13.8 ± 1.1	16.6 ± 1.0	18.1 ± 1.7	17.5 ± 2.0	0[b]
5	10	15.0 ± 0.9	17.2 ± 1.9	18.3 ± 2.1	17.6 ± 2.0	0[b]
6	10	14.0 ± 1.2	22.6 ± 1.5*	22.4 ± 2.3*	15.8 ± 2.5	64
7	5	13.7 ± 0.9	17.7 ± 2.0	16.4 ± 1.6	16.8 ± 1.9	0[b]
8	10	14.7 ± 0.9	21.5 ± 1.5∧	16.3 ± 2.2	16.6 ± 2.3	51
9	25	15.3 ± 1.5	20.5 ± 1.6∧	21.6 ± 1.9∧	19.0 ± 2.0	47
10	30	14.7 ± 1.0	22.6 ± 2.1*	16.5 ± 1.4	15.2 ± 1.9	59
11	20	15.1 ± 1.6	24.3 ± 1.9*	20.2 ± 1.5∧	16.2 ± 1.6	69
12	15	13.5 ± 0.8	17.7 ± 1.2	18.3 ± 1.6	15.4 ± 1.4	0[b]
13	10	14.6 ± 0.8	16.5 ± 1.8	18.8 ± 2.2	17.3 ± 1.5	0[b]
14	10	14.9 ± 1.3	15.7 ± 1.7	18.5 ± 1.9	13.8 ± 1.6	0[b]
15	30	14.4 ± 1.0	18.7 ± 1.6	17.4 ± 2.1	17.0 ± 1.7	0[b]
16	20	13.3 ± 1.4	21.8 ± 1.1*	16.7 ± 1.3	17.1 ± 1.8	64
17	15	14.5 ± 1.0	16.9 ± 1.7	16.8 ± 1.5	17.3 ± 2.0	0[b]
18[c]	50	14.5 ± 0.9	34.1 ± 1.8*	31.7 ± 1.4*	29.5 ± 2.0*	147
	25	13.9 ± 1.0	24.6 ± 1.8*	23.9 ± 1.8*	20.5 ± 2.3∧	80
	10	14.3 ± 1.1	15.7 ± 2.0	16.1 ± 1.9	16.4 ± 1.8	0[b]
19	40	13.8 ± 1.5	23.2 ± 1.5*	22.6 ± 1.6∧	16.6 ± 1.6	71
20	40	14.1 ± 1.1	21.5 ± 1.8∧	20.7 ± 1.8∧	15.5 ± 1.9	56

[a] The % analgesic efficacy was evaluated at the maximal soluble dose in saline or in vehicle H_2O/DMSO (2:1) with respect to the maximal analgesic effect of morphine (10 μg/mouse icv) taken as 100%. [b] Zero percent value means that the molecule is devoid of any statistical significance versus the control group. [c] The analgesic effect was not blocked by naloxone at 1 mg/kg ip. [d] *P < 0.01; ∧P < 0.05 in comparison with controls treated with saline or vehicle.

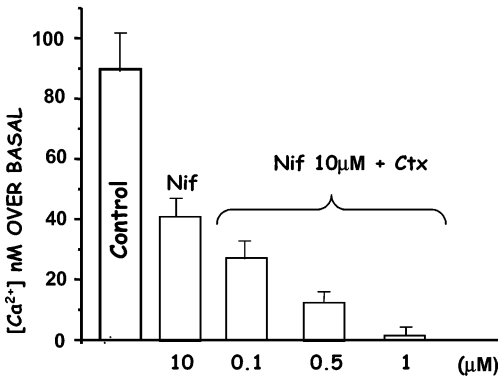


Figure 1. Increase in intracellular $[Ca^{2+}]_i$ in response to KCl (50 mM) in basal conditions (control) and following addition of nifedipine (10 μM) alone or in combination with ω-conotoxin (Ctx) (0.1−1 μM).

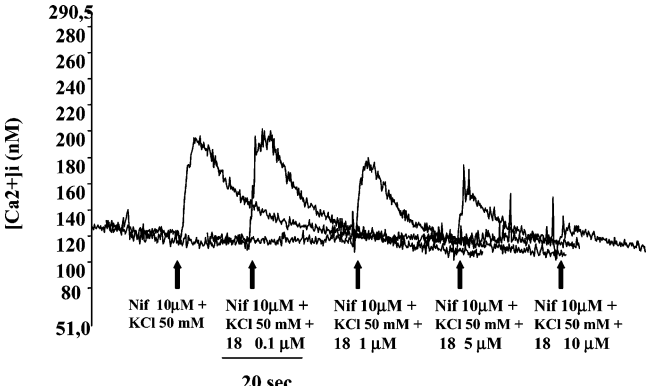


Figure 2. Intracellular calcium waves in response to KCl (50 mM) in the presence of nifedipine (10 μM) and different concentrations of compound **18** (0.1−10 μM).

channels.[43] Although in most studies PC12 cells expression of N-type calcium channels is induced by treatment with NGF,[41] we argued from these experiments that the PC12 clone used expresses a significant amount of N-type calcium channels, even without treatment with this expensive factor. Subsequent experiments were performed evaluating the effect of our compounds on the residual calcium fluxes obtained with KCl after nifedipine block.

All the compounds tested, except **6** and **15**, which was inactive as analgesic, were able to reduce, to a different extent, the calcium flux in the presence of nifedipine, showing that they are inhibiting N-type calcium channels. The result of a typical experiment, performed with the compound **18** is reported in Figure 2.

The IC_{50} of the most potent compounds (**3**, **10**, **18**, **20**) was evaluated and compared to that of ω-conotoxin MVIIA (Table 3). Compound **3**, which is moderately analgesic, shows an IC_{50} comparable with that of ω-conotoxin MVIIA in this test while compound **18**, which is the most effective analgesic, shows an IC_{50} value 2.5 times greater. The discrepancy between the analgesic activity and N-channel inhibition may be explained, among other reasons, by different pharmacokinetics of the two drugs in the mouse.

Compound **18** is able to block the Ca^{2+}-flux in a dose dependent manner, and the Ca^{2+}-flux is completely blocked at 100 μM (Figure 3A). However, at this dose, the compound is very likely blocking also the L-type channels, according with its binding affinity for this

Table 3. N-Type Calcium Channels Antagonism of Compounds **3**, **10**, **18**, **20** Evaluated on PC12 Cells[a]

N	IC_{50} (μM)	IC_{50} (μM) in the presence of nifedipine
3	0.70 ± 0.30	0.40 ± 0.07
10	3.80 ± 1.20	3.10 ± 0.50
18	1.03 ± 0.10	2.40 ± 0.60
20	3.00 ± 0.90	nt[b]
ω-conotoxin	0.60 ± 0.30	0.50 ± 0.20

[a] Compounds **6** and **15** did not change calcium influx, while **2**, **8**, **9**, **11**, **16** and **19** were able to antagonize calcium influx, but their potency was too low to calculate an IC_{50}. [b] nt = not tested.

subtype of channels (K_D = 100 μM). Indeed, when **18** is tested in the presence of the L-type calcium channels blocker nifedipine (10 μM), Ca^{2+}-flux was almost completely antagonized at lower doses (Figure 3B), suggesting that compound **18** is an N-type calcium channel antagonist.

As regards structure−activity relationships (even if this was not the main goal of the present research), it can be said that the position of the residue on the 4-aminopiperidine moiety is not crucial and analgesic compounds are found in both series of regioisomers (compare **2** and **9**, **3** and **10**, **16** and **18**). Both 4,4′-bis-(4-fluorophenyl)butyramide and 4,4′-bis(4-fluorophenyl)-butylamine series possess analgesic activity (see **6**, **8**, **18**−**20**), while this is not true for the corresponding (3,4-dimethoxyphenyl)isopropylpentanenitrile derivatives, where only the amines show analgesic activity (amides **13**, **14**, **15** are inactive, whereas amines **2**, **3**, **9**, **10**, **11** are active). Finally, our efforts to increase the solubility of the most potent compound **18** were not fully satisfactory, as compound **17** is inactive as analgesic, while **19** and **20** show lower, although remarkable, efficacy with respect to the parent compound (71% and 56%, respectively, at 40 μg/mouse icv).

On the basis of the well documented involvement of N-type calcium channels in the neuropathic pain,[2,7,8,44] compounds **2**, **3**, **6**, **10**, **15**, **18**−**20** were tested on the model described by Bennett[37] (Table 4). Experiments were performed on rats submitted to paw-pressure test 14 days after the operation since at this time a significant reduction of the pain threshold of the injured paw (dx) was observed. By contrast, in the controlateral paw the pain perception remained unchanged. Compounds **2**, **3**, **6**, **18** and **19** showed appreciable antihyperalgic activity on this model when compared with the saline-treated group 30, 45 and 60 min after administration. All active compounds did not modify pain threshold in controlateral, nonoperated paw and at the doses administered were devoid of any antihyperalgic effect. Rats treated with compounds **2**, **3**, **6**, **18** and **19** at the highest doses showed a normal behavior compared to saline control rats.

It can be observed that the results obtained from neuropathic pain model do not completely overlap those obtained in the hot plate test. The discrepancy existing between the results obtained in the two analgesic tests can be due to the different stimulus applied (thermic in the hot-plate and mechanical in the paw-pressure) or to the different animal species. As a matter of fact, the pharmacokinetic profile of the compounds could differ greatly in mouse and rat species. Furthermore, it has to be considered that in the Bennett model the rat has a chronic pain caused by the operation and

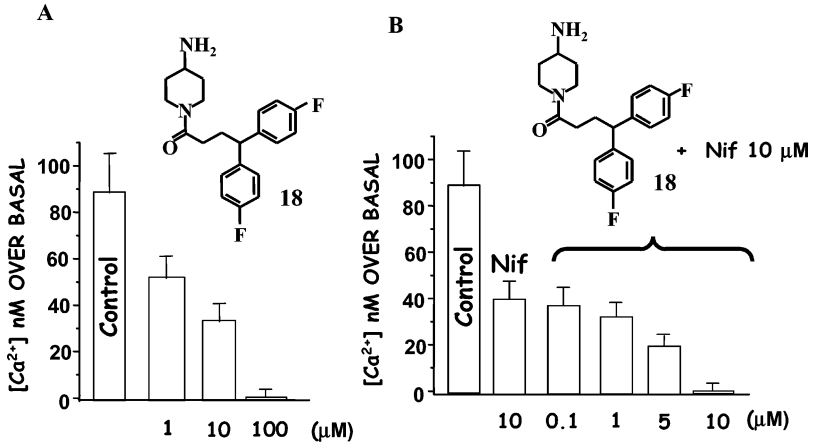


Figure 3. Increase in intracellular $[Ca^{2+}]_i$ in response to KCl (50 mM) in basal conditions (control) and following addition of increasing concentrations of compound **18** alone (panel A) or in combination with nifedipine (10 μM) (panel B).

Table 4. Antihyperalgic Effect of Compounds **2**, **3**, **6**, **10**, **13**, **18**, **19**, **20** in a Rat Model of Mononeuropathy dx Evaluated in the Paw Pressure Test[a]

				paw pressure in rats (g)[b]		
treatment	dose icv, μg/rat	paw	before treatment	30 min	45 min	60 min
saline	-	sn	64.7 ± 4.6	62.3 ± 5.7	58.1 ± 4.5	60.9 ± 6.1
saline	-	dx	27.3 ± 4.9	24.2 ± 6.3	25.1 ± 6.6	25.4 ± 5.5
2	30	sn	56.5 ± 3.7	59.1 ± 7.1	57.3 ± 6.4	59.2 ± 5.1
2	30	dx	28.7 ± 3.8	47.3 ± 4.8∧	43.5 ± 5.8∧	38.6 ± 6.2
3	50	sn	63.1 ± 5.0	59.5 ± 5.8	64.7 ± 6.3	60.6 ± 7.5
3	50	dx	29.4 ± 4.1	61.7 ± 5.3*	58.3 ± 7.9*	54.8 ± 6.8*
6	10	sn	60.1 ± 3.7	57.5 ± 4.2	61.2 ± 5.6	63.0 ± 3.9
6	10	dx	25.8 ± 2.8	50.3 ± 4.4*	52.6 ± 5.0*	46.5 ± 6.7∧
10	30	sn	60.5 ± 4.8	57.3 ± 5.4	55.6 ± 6.3	59.4 ± 5.3
10	30	dx	27.8 ± 3.6	33.4 ± 6.5	38.6 ± 7.1	40.5 ± 5.9
15	30	sn	62.3 ± 4.1	65.0 ± 7.2	56.9 ± 6.6	59.3 ± 6.3
15	30	dx	30.5 ± 3.8	28.4 ± 5.1	26.7 ± 7.0	32.0 ± 3.7
18	50	sn	57.3 ± 3.9	62.3 ± 7.3	55.6 ± 6.7	54.7 ± 6.8
18	50	dx	30.1 ± 4.0	55.1 ± 6.6*	54.8 ± 4.9*	59.3 ± 8.1*
19	50	sn	62.6 ± 5.2	55.9 ± 5.8	61.3 ± 6.4	63.2 ± 7.0
19	50	dx	32.2 ± 4.3	62.5 ± 8.7*	60.3 ± 5.3*	55.8 ± 3.2*
20	40	sn	56.9 ± 4.2	55.7 ± 4.9	61.3 ± 6.5	66.4 ± 4.9
20	40	dx	25.7 ± 3.0	28.1 ± 3.5	33.4 ± 5.1	30.1 ± 4.2

[a] There were 9 to 10 rats per group. Each value represents the mean of two separate experiments. All compounds were administered 30 min before test. Vehicle: H_2O/DMSO (2:1). [b] *$P < 0.001$; ∧$P < 0.05$ in comparison with controls.

therefore the experimental conditions are very different from that observable in acute pain. However, it is remarkable that compounds **3** and **18** are active in both models and are antagonist of the N-type calcium channels with a potency close to that of ω-conotoxin MVIIA.

In conclusion we have identified a new series of compounds that possess antinociceptive activity and very likely work as N-type calcium channel antagonists. Among them, compounds **3** and **18** are potent and efficacious molecules that can be used as leads to develop more potent and selective drugs.

Experimental Section

Chemistry. All melting points were taken on a Büchi apparatus and are uncorrected. Infrared spectra were recorded with a Perkin-Elmer 681 or a Perkin-Elmer Spectrum RX I FT-IR spectrophotometer in Nujol mull for solids and neat for liquids. 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. Compounds were named following IUPAC rules as applied by Beilstein-Institut AutoNom (version 2.1), a software for systematic names in organic chemistry. When reactions were performed in anhydrous conditions, the mixtures were maintained under nitrogen.

(**1-Benzylpiperidin-4-yl)-[2-(3,4-dimethoxyphenyl)ethyl]-amine (1).** A mixture of commercially available 1-benzyl-4-piperidone (0.5 g, 2.64 mmol), 2-(3,4-dimethoxyphenyl)-ethylamine (0.45 mL, 2.64 mmol) and titanium(IV) isopropoxide (1 mL, 3.22 mmol) was stirred with a drying tube at room temperature. After 4 h, the IR spectrum of the mixture showed the absence of the carbonyl band, and the viscous solution was diluted with absol ethanol (2 mL). Sodium cyanoborohydride (0.3 g, 5.0 mmol) was added, and the solution was stirred for 20 h. Water (0.5 mL) was then added, the resulting inorganic precipitate was filtered and washed with ethanol, and the filtrate was concentrated in vacuo. The crude product was dissolved in chloroform, filtered to remove the solids, and washed with a solution of $NaHCO_3$ and water; after drying with Na_2SO_4, the solvent was removed under reduced pressure, and the crude substance was purified by flash chromatography using $CHCl_3$/MeOH (90:10) as eluting system. Title compound (620 mg, 88% yield) was obtained as an oil. IR (neat) ν cm^{-1} 3300 (NH). ^1H NMR ($CDCl_3$) δ 1.21−1.59 (m, 3H, NH, CH_2); 1.66−2.12 (m, 4H, $2CH_2$); 2.38−2.45 (m, 1H, CH); 2.65−2.88 (m, 6H, $3CH_2$); 3.45 (s, 2H, CH_2); 3.84 (s, 6H, $2OCH_3$); 6.64−6.76 (m, 3H, aromatics); 7.28 (s, 5H, aromatics) ppm.

The oily product was transformed into the hydrochloride, treating the free base with HCl/absol EtOH and recrystallized

from absol EtOH/anhydrous ether. Mp: 260−265 °C. Anal. ($C_{22}H_{31}ClN_2O_2$) C, H, N.

Compounds **21** and **22** were obtained in the same way using the suitable commercially available amine (2-phenoxyethylamine and butylamine, respectively). Their IR and ^{1}H NMR spectra are consistent with the proposed structures.

5-(1-Benzylpiperidin-4-ylamino)-2-(3,4-dimethoxyphenyl)-2-(methylethyl)pentanenitrile (23). Commercially available 4-amino-1-benzylpiperidine (0.46 mL, 2.23 mmol), 5-bromo-2-(3,4-dimethoxyphenyl)-2-(methylethyl)pentanenitrile[24] (760 mg, 2.23 mmol) and 0.26 mL of dry triethylamine were heated at 60 °C for 24h. The reaction mixture was cooled to room temperature and treated with CH_2Cl_2, and the organic layer was washed with 10% NaOH solution. After drying with Na_2SO_4, the solvent was removed under reduced pressure and the residue purified by flash chromatography using CH_2Cl_2/Et_2O/absol EtOH/NH_4OH (900:360:180:9.9) as eluting system. Title compound (560 mg, 56% yield) was obtained as an oil. IR (neat) ν cm^{-1} 3300 (NH), 2240 (CN). ^{1}H NMR ($CDCl_3$) δ 0.78 (d, J = 6.6 Hz, 3H, CH_3); 1.21 (d, J = 6.6 Hz, 3H, CH_3); 1.25−1.60 (m, 6H, 3CH_2); 1.65−2.21 (m, 6H, NH, CH, 2CH_2); 2.25−2.69 (m, 3H, CH, CH_2); 2.75−2.89 (m, 2H, CH_2); 3.47 (s, 2H, CH_2); 3.89 (s, 6H, 2OCH_3); 6.79−6.85 (m, 3H, aromatics); 7.26 (s, 5H, aromatics) ppm. Anal. ($C_{28}H_{39}N_3O_2$) C, H, N.

[2-(3,4-Dimethoxyphenyl)ethyl]piperidin-4-ylamine (24). To 310 mg (0.87 mmol) of **1** dissolved in dry MeOH were added Pd/C 10% (0.15 g) and $HCOONH_4$ (276 mg, 4.37 mmol). The reaction mixture was heated to reflux for 8 h; after cooling, the solvent was filtered and evaporated. The residue was dissolved in CH_2Cl_2 and washed with a solution of $NaHCO_3$; after drying with Na_2SO_4, the solvent was removed under reduced pressure and the residue purified by column chromatography using $CHCl_3$/absol EtOH/petroleum ether/NH_4OH (340:65:60:8) as eluting system. Title compound (140 mg, 61% yield) was obtained as an oil. IR (neat) ν cm^{-1} 3300 (NH). ^{1}H NMR ($CDCl_3$) δ 1.09−1.29 (m, 2H, CH_2); 1.71−1.89 (m, 2H, CH_2); 2.09 (bs, 2H, 2NH); 2.42−3.09 (m, 9H, 4CH_2, CH); 3.81 (s, 6H, 2OCH_3); 6.68−6.81 (m, 3H, aromatics) ppm. Anal. ($C_{15}H_{24}N_2O_2$) C, H, N.

Compounds **25**, **26** and **27** were obtained in the same way from the corresponding benzyl derivative (**21**, **22** and **23,** respectively). Their IR and ^{1}H NMR spectra are consistent with the proposed structures.

2-(3,4-Dimethoxyphenyl)-5-(4-[2-(3,4-dimethoxyphenyl)-ethylamino]piperidin-1-yl)-2-(methylethyl)pentanenitrile (5). Compound **24** (0.11 mg, 0.41 mmol), 5-bromo-2-(3,4-dimethoxyphenyl)-2-(methylethyl)pentanenitrile[24] (140 mg, 0.41 mmol), 0.1 mL of dry triethylamine and 1 mL of acetonitrile were heated at 60 °C for 8 h. The reaction mixture was cooled to room temperature and treated with CH_2Cl_2, and the organic layer was washed with 10% NaOH solution. After drying with Na_2SO_4, the solvent was removed under reduced pressure and the residue purified by column chromatography using $CHCl_3$/absol EtOH/petroleum ether/NH_4OH (340:65:60:8) as eluting system. Title compound (120 mg, 56% yield) was obtained as an oil. IR (neat) ν cm^{-1} 3300 (NH), 2240 (CN). ^{1}H NMR ($CDCl_3$) δ 0.81 (d, J = 6.6 Hz, 3H, CH_3); 1.18 (d, J = 6.6 Hz, 3H, CH_3); 1.10−1.37 (m, 2H, CH_2); 1.43−1.67 (m, 2H, CH_2); 1.71−1.96 (m, 6H, NH, 2CH_2, CH); 1.99−2.13 (m, 2H, CH_2); 2.16−2.29 (m, 2H, CH_2); 2.32−2.49 (m, 1H, CH); 2.59−2.96 (m, 6H, 3CH_2); 3.83 (s, 6H, 2OCH_3); 3.90 (s, 6H, 2OCH_3); 6.68−6.98 (m, 6H, aromatics) ppm.

The oily product was transformed into the oxalate that was recrystallized from ethyl acetate. Mp: 198−200 °C. Anal. ($C_{33}H_{47}N_3O_8$) C, H, N.

Compounds **2**, **3**, **4**, **6**, **7**, **8**, **9**, **10**, **11**, **12** were obtained in the same way using the suitable halide (5-bromo-2-(3,4-dimethoxyphenyl)-2-(methylethyl)pentanenitrile,[24] 5-bromo-2-(methylethyl)-2-phenylpentanenitrile (**33**), 1-bromo-4,4-diphenylbutane,[27] 1,1′-(4-bromobutylidene)bis(4-fluorobenzene),[26] butyl iodide (commercially available), (2-bromoethoxy)benzene,[28] cinnamyl bromide (commercially available), 4-(2-bromoethyl)-1,2-dimetoxybenzene[29]). Their chemical and physical characteristics are reported in Table 1 and IR and ^{1}H NMR spectra in Table 5.

4-Cyano-4-(3,4-dimethoxyphenyl)-5-methylhexanoic Acid (28). To a solution of $KMnO_4$ (310 mg, 1.96 mmol) in 3.2 mL of H_2O at 0 °C, were added a solution of 2-(3,4-dimethoxyphenyl)-5-hydroxy-2-(methylethyl)pentanenitrile[31] (200 mg, 0.72 mmol), dissolved in 2 mL of benzene, and 30 mg of tetrabutylammonium bromide. The mixture was left at room temperature for 12 h, and then a solution of $NaHSO_4$ (3 mL) was added. The mixture was then acidified with 6 N HCl and extracted with diethyl ether. The organic layer was dried with Na_2SO_4, and the solvent was removed under reduced pressure. Title compound (190 mg, 90% yield) was obtained as a white oil. IR (neat) ν 2500−3000 (OH), 2240 (CN), 1700 (CO) cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 0.80 (d, J = 6.5 Hz, 3H, CH_3); 1.21 (d, J = 6.2 Hz, 3H, CH_3); 1.98−2.21 (m, 3H, CH, CH_2); 2.36−2.58 (m, 2H, CH_2); 3.89 (s, 6H, 2OCH_3); 6.83−6.91 (m, 3H, aromatics); 7.98 (bs, 1H, OH) ppm. Anal. ($C_{16}H_{21}NO_4$) C, H, N.

2-(3,4-Dimethoxyphenyl)-5-(4-[2-(3,4-dimethoxyphenyl)-ethylamino]piperidin-1-yl)-2-(methylethyl)-5-oxopentanenitrile (13). Compound **24** (140 mg, 0.53 mmol), 5-cyano-4-(3,4-dimethoxyphenyl)-5-methylhexanoic acid **28** (155 mg, 0.53 mmol), diethyl cyanophosphonate (0.1 mL, 0.66 mmol), dry triethylamine (0.7 mL) and dry DMF (1.5 mL) were mixed at 0 °C and then stirred at room temperature for 12 h. Brine was added, the reaction mixture was extracted with ethyl acetate, and the organic layer was washed first with brine and then with a solution of $NaHCO_3$. After drying with Na_2SO_4, the solvent was removed under reduced pressure and the residue purified by column chromatography using $CHCl_3$/absol EtOH/petroleum ether/NH_4OH (340:65:60:8) as eluting system. Title compound (90 mg, 32% yield) was obtained as an oil. IR (neat) ν cm^{-1} 3300 (NH), 2240 (CN), 1650 (CO). ^{1}H NMR ($CDCl_3$) δ 0.81 (d, J = 6.6 Hz, 3H, CH_3); 1.18−1.32 (m, 6H, CH, CH_2, CH_3); 1.70−2.95 (m, 16H, 7CH_2, NH, CH); 3.83 (s, 6H, 2OCH_3); 3.92 (s, 6H, 2OCH_3); 6.67−6.98 (m, 6H, aromatics) ppm. The oily product was transformed into the oxalate that was recrystallized from ethyl acetate. Mp: 80−82 °C. Anal. ($C_{33}H_{45}N_3O_9$) C, H, N.

Compounds **14** and **15** were obtained in the same way starting from (2-ethoxyphenyl)piperidin-4-ylamine **25** and butylpiperidin-4-ylamine **26**, respectively. Their chemical and physical characteristics are reported in Table 1 and IR and ^{1}H NMR spectra in Table 5.

***N*-(1-Benzylpiperidin-4-yl)-4,4-bis(4-fluorophenyl)butyramide (29).** Following the procedure described for compound **13,** starting from 1-benzyl-4-aminopiperidine (0.6 g, 3.16 mmol) and 4,4′-bis-(4-fluorophenyl)butyric acid[26] (0.86 g, 3.16 mmol), compound **29** (430 mg, 30% yield) was obtained as a colorless oil. IR (neat) ν cm^{-1} 3300 (NH), 1650 (CO). ^{1}H NMR ($CDCl_3$) δ 1.20−1.56 (m, 2H, CH_2); 1.81−1.98 (m, 2H, CH_2); 2.01−2.18 (m, 4H, 2CH_2); 2.22−2.41 (m, 2H, CH_2); 2.75−2.95 (m, 2H, CH_2); 3.45 (s, 2H, CH_2); 3.63−3.78 (m, 1H, CH); 3.83 (t, J = 9.5 Hz, 1H, CH); 5.24 (bs, 1H, NH); 6.98−7.03 (m, 4H, aromatics); 7.06−7.14 (m, 4H, aromatics); 7.25 (s, 5H, aromatics) ppm. Anal. ($C_{28}H_{30}F_2N_2O$) C, H, N.

4,4-Bis(4-fluorophenyl)-*N*-piperidin-4-ylbutyramide (16). Following the procedure described for compound **24**, starting from **29** (430 mg, 0.99 mmol), compound **16** (220 mg, 62% yield) was obtained. IR (neat) ν cm^{-1} 3300 (NH), 1650 (CO). ^{1}H NMR ($CDCl_3$) δ 1.18−1.28 (m, 2H, CH_2); 1.78−1.85 (m, 2H, CH_2); 1.98−2.09 (m, 2H, CH_2); 2.20−2.34 (m, 3H, CH_2, NH); 2.59 (t, J = 13.0 Hz, 2H, CH_2); 2.94−3.02 (m, 2H, CH_2); 3.64−3.79 (m, 1H, CH); 3.83 (t, J = 9.5 Hz, 1H, CH); 5.67 (bs, 1H, NH); 6.84−6.90 (m, 4H, aromatics); 7.08−7.23 (m, 4H, aromatics) ppm.

The oily product was transformed into the oxalate that was recrystallized from ethyl acetate. Mp: 100−102 °C. Anal. ($C_{23}H_{26}F_2N_2O_5$) C, H, N.

4,4-Bis(4-fluorophenyl)-*N*-[1-(2-hydroxyethyl)piperidin-4-yl]butyramide (17). Following the procedure described for compound **5**, starting from **16** (240 mg, 0.67 mmol) and chloroethanol (0.04 mL, 0.75 mmol), compound **17** (120 mg,

Table 5. IR and ^{1}H NMR Spectra of Compounds **2**−**4**, **6**−**15**

N	IR ν (cm^{-1})	^{1}H NMR ($CDCl_3$) δ
2	3300 (NH), 2240 (CN)	0.77 (d, J = 6.6 Hz, 3H, CH_3); 0.91−0.94 (m, 3H, CH_3); 1.17 (d, J = 6.6 Hz, 3H, CH_3); 1.22−1.67 (m, 9H, CH, 4CH_2); 1.69−2.44 (m, 9H, NH, 4CH_2); 2.54−2.61 (m, 5H, CH, 2CH_2); 3.86 (s, 6H, 2OCH_3); 6.82−6.88 (m, 3H, aromatics) ppm
3	3300 (NH), 2240 (CN)	0.74 (d, J = 6.6 Hz, 3H, CH_3); 1.28 (d, J = 6.6 Hz, 3H, CH_3); 1.27−1.66 (m, 7H, CH, 3CH_2); 1.73−1.98 (m, 5H, NH, 2CH_2); 1.98−2.14 (m, 2H, CH_2); 2.20−2.34 (m, 2H, CH_2); 2.36−2.57 (m, 1H, CH); 2.64−2.85 (m, 2H, CH_2); 3.86 (s, 6H, 2OCH_3); 4.05 (t, J = 6.3 Hz, 2H, CH_2O); 6.60−7.00 (m, 6H, aromatics); 7.19−7.33 (m, 2H, aromatics) ppm
4	3300 (NH), 2240 (CN)	0.76 (d, J = 6.6 Hz, 3H, CH_3); 1.18 (d, J = 6.6 Hz, 3H, CH_3); 1.25−1.63 (m, 4H, 2CH_2); 1.65−1.98 (m, 6H, NH, CH, 2CH_2); 2.05−2.31 (m, 4H, 2CH_2); 2.36−2.57 (m, 1H, CH); 2.78−2.84 (m, 6H, 3CH_2); 3.85 (s, 6H, 2OCH_3); 6.72−6.76 (m, 3H, aromatics); 7.27−7.36 (m, 5H, aromatics) ppm
6	3300 (NH)	0.94 (t, J = 6.3 Hz, 3H, CH_3); 1.35−1.46 (m, 8H, 4CH_2); 1.76−2.15 (m, 7H, NH, 3CH_2); 2.50−2.60 (m, 3H, CH, CH_2); 2.61 (t, J = 8.6 Hz, 2H, CH_2); 2.88−2.55 (m, 2H, CH_2); 3.90 (t, J = 9.5 Hz, 1H, CH); 7.25−7.28 (m, 10H, aromatics) ppm
7	3300 (NH)	1.12−1.53 (m, 5H, NH, 2CH_2); 1.73−2.12 (m, 6H, 3CH_2); 2.26−2.38 (m, 3H, CH, CH_2); 2.66−2.93 (m, 6H, 3CH_2); 3.85 (s, 7H, CH, 2OCH_3); 6.69−6.85 (m, 3H, aromatics); 7.09−7.33 (m, 10H, aromatics) ppm
8	3300 (NH)	1.25−1.53 (m, 5H, NH, 2CH_2); 2.73−2.06 (m, 6H, 3CH_2); 2.19−2.53 (m, 3H, CH, CH_2); 2.66−2.92 (m, 6H, 3CH_2); 3.86 (s, 7H, CH, 2OCH_3); 6.73−6.78 (m, 3H, aromatics); 6.91−7.11 (m, 4H, aromatics); 7.12−7.18 (m, 4H, aromatics) ppm
9	3300 (NH), 2240 (CN)	0.76 (d, J = 6.6 Hz, 3H, CH_3); 0.87 (t, J = 7.1 Hz, 3H, CH_3); 1.16 (d, J = 6.6 Hz, 3H, CH_3); 1.19−2.60 (m, 8H, NH, CH, 3CH_2); 1.69−2.15 (m, 8H, 4CH_2); 2.17−2.42 (m, 4H, 2CH_2); 2.44−2.69 (m, 1H, CH); 2.76−2.92 (m, 2H, CH_2); 3.86 (s, 6H, 2OCH_3); 6.82−6.87 (m, 3H, aromatics) ppm
10	3300 (NH), 2240 (CN)	0.79 (d, J = 6.6 Hz, 3H, CH_3); 1.19 (d, J = 6.6 Hz, 3H, CH_3); 1.28−1.69 (m, 3H, CH, CH_2); 1.76−2.25 (m, 9H, NH, 4CH_2); 2.34−2.69 (m, 3H, CH, CH_2); 2.78 (t, J = 5.9 Hz, 2H, CH_2); 2.85−3.01 (m, 2H, CH_2); 3.87 (s, 6H, 2OCH_3); 4.06 (t, J = 6.1 Hz, 2H, CH_2O); 6.84−6.91 (m, 6H, aromatics); 7.21−7.25 (m, 2H, aromatics) ppm
11	3300 (NH)	0.80 (d, J = 6.6 Hz, 3H, CH_3); 1.19 (d, J = 6.6 Hz, 3H, CH_3); 1.36−1.65 (m, 3H, CH, CH_2); 1.79−2.30 (m, 8H, 4CH_2); 2.41−2.73 (m, 4H, NH, CH, CH_2); 2.92−3.09 (m, 2H, CH_2); 3.23 (d, J = 8.1 Hz, 2H, CH_2); 3.89 (s, 6H, 2OCH_3); 6.19−6.39 (m, 1H, CH); 6.54 (d, J = 16.1 Hz, 1H, CH); 6.85−6.90 (m, 3H, aromatics); 7.27−7.36 (m, 5H, aromatics) ppm
12	3300 (NH), 2240 (CN)	0.78 (d, J = 6.6 Hz, 3H, CH_3); 1.18 (d, J = 6.6 Hz, 3H, CH_3); 1.28−1.63 (m, 3H, CH,CH_2); 1.73−2.28 (m, 8H, 4CH_2); 2.30−2.80 (m, 8H, NH, CH, 3CH_2); 2.85−3.01 (m, 2H, CH_2); 3.83 (m, 6H, 2OCH_3); 3.89 (m, 6H, 2OCH_3); 6.73−6.90 (m, 6H, aromatics) ppm
14	3300 (NH), 2240 (CN), 1650 (CO)	0.78 (d, J = 6.6 Hz, 3H, CH_3); 1.23 (d, J = 6.6 Hz, 3H, CH_3); 1.15 1.39 (m, 3H, CH, CH_2); 1.76−2.15 (m, 5H, CH, 2CH_2); 2.22−2.85 (m, 5H, CH, 2CH_2); 2.90−3.03 (m, 2H, CH_2); 3.50−3.69 (m, 1H, CH); 3.79 (s, 6H, 2OCH_3); 4.06 (t, J = 6.1 Hz, 2H, CH_2); 4.37−4.50 (m, 1H, CH); 6.92−6.93 (m, 5H, aromatics); 7.22−7.27 (m, 3H, aromatics) ppm
15	3300 (NH), 2240 (CN), 1650 (CO)	0.77 (d, J = 6.6 Hz, 3H, CH_3); 0.89 (t, J = 6.4 Hz, 3H, CH_3); 1.18 (d, J = 6.6 Hz, 3H, CH_3); 1.10−1.53 (m, 5H, CH, 2CH_2); 1.73−2.50 (m, 9H, CH, 4CH_2); 2.53−2.73 (m, 4H, 2CH_2); 2.80−3.03 (m, 1H, CH); 3.50−3.69 (m, 1H, CH); 3.87 (s, 6H, 2OCH_3); 4.37−4.50 (m, 1H, CH); 6.82−6.84 (m, 3H, aromatics) ppm

44% yield) was obtained. IR (neat) ν cm^{-1} 3500 (OH), 3300 (NH), 1650 (CO). ^{1}H NMR ($CDCl_3$) δ 1.17−1.46 (m, 2H, CH_2); 1.89−1.99 (m, 2H, CH_2); 2.02−2.24 (m, 4H, 2CH_2); 2.26−2.28 (m, 2H, CH_2); 2.50 (t, J = 6.0 Hz, 2H, CH_2); 2.60−2.84 (m, 3H, CH_2, OH); 3.57 (t, J = 5.0 Hz, 2H, CH_2); 3.65−3.83 (m, 1H, CH); 3.90 (t, J = 9.0 Hz, 1H, CH); 5.42 (bs, 1H, NH); 6.91−7.00 (m, 4H, aromatics); 7.12−7.27 (m, 4H, aromatics) ppm.

The oily product was transformed into the hydrochloride by treating the free base with HCl/absol EtOH and recrystallized from absol EtOH/anhydrous ether Mp: 170−175 °C. Anal. ($C_{23}H_{29}ClF_2N_2O_2$) C, H, N.

(1-[4,4-Bis(4-fluorophenyl)butyryl]piperidin-4-yl)carbamic Acid *tert*-Butyl Ester (30). Following the procedure described for compound (**13**), starting from 4-(*tert* butyloxycarbonylamino)piperidine[32] (600 mg, 3.03 mmol) and 4,4′-bis-(4-fluorophenyl)butyric acid[26] (824 mg, 3.03 mmol), compound **30** (1.36 g, 98% yield) was obtained as an oil. IR (neat) ν cm^{-1} 3300 (NH), 1720 (CO). ^{1}H NMR ($CDCl_3$) δ 1.03−1.25 (m, 2H, CH_2); 1.33−1.41 (s, 9H, 3CH_3); 1.83−1.98 (m, 2H, CH_2); 2.12−2.28 (m, 4H, 2 CH_2); 2.68 (t, J = 12.8 Hz, 1H, CH); 2.83−3.03 (m, 2H, CH_2); 3.42−3.65 (m, 2H, CH_2); 3.92 (t, J = 9.0 Hz, 1H, CH); 4.45 (bs, 1H, NH); 6.86−6.99 (m, 4H, aromatics); 7.04−7.31 (m, 4H, aromatics) ppm. Anal. ($C_{26}H_{32}F_2N_2O_3$) C, H, N.

1-(4-Aminopiperidin-1-yl)-4,4-bis(fluorophenyl)butan-1-one (18). To a solution of **30** (1.36 g, 2.97 mmol) dissolved in $CHCl_3$ was added Me_3SiI (1.9 mL, 13.3 mmol). The reaction mixture was left at room temperature for 6 h, the MeOH (8 mL) was added, the organic layer was removed under reduced pressure and the residue was dissolved in $CHCl_3$ and washed with $NaHCO_3$ solution. After drying with Na_2SO_4, the solvent was removed under reduced pressure and the residue purified by flash chromatography using $CHCl_3$ as eluent. Title compound (480 mg, 45% yield) was obtained as a green oil. IR (neat) ν cm^{-1} 3300−3400 (NH_2) 1650 (CO). ^{1}H NMR ($CDCl_3$) δ 0.92−1.19 (m, 2H, CH_2); 1.54−1.65 (m, 4H, NH_2, CH_2); 2.02−2.35 (m, 4H, 2CH_2); 2.46 (t, J = 12.8 Hz, 1H, CH); 2.62−2.85 (m, 2H, CH_2); 3.37−3.59 (m, 1H, CH); 3.84 (t, J = 9.0 Hz, 1H, CH); 4.25−4.32 (m, 1H, CH); 6.75−6.98 (m, 4H, aromatics); 7.01−7.15 (m, 4H, aromatics) ppm.

The oily product was transformed into the hydrochloride, treating the free base with HCl/absol EtOH and recrystallized from absol EtOH/anhydrous ether. Mp: 138−141 °C. Anal. ($C_{21}H_{25}ClF_2N_2O$) C, H, N.

(1-{1-[4,4-Bis(4-fluorophenyl)butyryl]piperidin-4-yl-carbamoyl}-3-methylbutyl)carbamic Acid *tert*-Butyl Ester (31). Following the procedure described for **13**, starting from **18** (100 mg, 0.28 mmol) and *N*-*t*-BOC-L-leucine (70 mg, 0.28 mmol), compound **31** (110 mg, 70% yield) was obtained as an oil. IR (neat) ν cm^{-1} 3300 (NH), 1650 (CO). ^{1}H NMR ($CDCl_3$) δ 0.82−0.98 (m, 6H, 2CH_3); 1.12−1.41 (m, 4H, 2CH_2); 1.40 (s, 9H, 3CH_3); 1.58−1.66 (m, 2H, CH_2); 1.82−1.98 (m, 2H, CH_2); 2.18−2.39 (m, 3H, CH, CH_2); 2.64−2.85 (m, 1H, C*H*H); 2.92−3.12 (m, 1H, C*H*H); 3.53−3.65 (m, 1H, CH); 3.83−4.10 (m, 3H, CH_2, NH); 4.38−4.44 (m, 1H, CH); 4.84−4.98 (m, 1H, CH); 6.25 (bs, 1H, NH); 6.90−7.04 (m, 4H, aromatics); 7.15−7.22 (m, 4H, aromatics) ppm. Anal. ($C_{32}H_{43}F_2N_3O_4$) C, H, N.

(1-{1-[4,4-Bis(4-fluorophenyl)butyryl]piperidin-4-yl-carbamoyl}-3-methylbutyl)methylcarbamic Acid *tert*-Butyl Ester (32). Following the procedure described for **13**, starting from **18** (110 mg, 0.30 mmol) and *N*-*t*-BOC-*N*-methyl-L-leucine[33] (120 mg, 0.48 mmol), compound **32** (130 mg, 40% yield) was obtained as an oil. IR (neat) ν cm^{-1} 3300 (NH), 1650 (CO). ^{1}H NMR ($CDCl_3$) δ 0.82−0.98 (m, 6H, 2CH_3); 1.38−1.51

(m, 2H, CH_2); 1.45 (s, 9H, $3CH_3$); 1.58−1.98 (m, 4H, $2CH_2$); 2.18−2.39 (m, 5H, CH, $2CH_2$); 2.65 (s, 3H, CH_3); 2.71−2.70 (m, 1H, C*H*H); 2.82−3.10 (m, 1H, C*H*H); 3.53−3.65 (m, 1H, CH); 3.83−4.10 (m, 2H, CH_2); 4.38−4.54 (m, 2H, CH_2); 6.20−6.33 (m, 1H, NH); 6.90−7.04 (m, 4H, aromatics); 7.15−7.22 (m, 4H, aromatics) ppm. Anal. ($C_{33}H_{45}F_2N_3O_4$) C, H, N.

2-Amino-4-methylpentanoic Acid {1-[4,4-bis(4-fluorophenyl)butyryl]piperidin-4-yl}amide (19). To a solution of **31** (110 mg, 0.19 mmol) dissolved in ethyl acetate was added 2 N HCl (4 mL). The reaction mixture was left at room temperature for 24 h, and then the solution was basified with 10% NaOH solution and extracted with CH_2Cl_2. After drying with Na_2SO_4, the solvent was removed under reduced pressure and the residue purified by column chromatography using $CHCl_3$/CH_3OH (90:10) as eluting system. Title compound (60 mg, 69% yield) was obtained as a colorless oil. IR (neat) ν cm^{-1} 3300−3400 (NH and NH_2); 1630−1650 (2CO). ^1H NMR ($CDCl_3$) 0.82−0.98 (m, 6H, $2CH_3$); 1.02−1.41 (m, 2H, CH_2); 1.45−1.78 (m, 4H, NH_2, CH_2); 1.80−1.98 (m, 2H, CH_2); 2.18−2.41 (m, 5H, CH, $2CH_2$); 2.61−2.82 (m, 1H, C*H*H); 2.95−3.19 (m, 1H, C*H*H); 3.33−3.39 (m, 1H, CH); 3.55−3.65 (m, 1H, CH); 3.81−4.05 (m, 2H, CH_2); 4.38−4.56 (m, 1H, CH); 6.86−7.04 (m, 4H, aromatics); 7.15−7.22 (m, 4H, aromatics); 7.25−7.38 (m, 1H, NH) ppm.

The oily product was transformed into the hydrochloride, treating the free base with HCl/absol EtOH and recrystallized from absol EtOH/anhydrous ether. Mp 165−167 °C. Anal. ($C_{27}H_{36}ClF_2N_3O_2$) C, H, N.

4-Methyl-2-methylaminopentanoic Acid {1-[4,4-bis(4-fluorophenyl)butyryl]piperidin-4-yl}amide (20). Following the procedure described for **19**, starting from **32** (130 mg, 0.22 mmol), compound **20** (40 mg, 38% yield) was obtained as an oil. IR (neat) ν cm^{-1} 3300−3400 (NH and NH_2); 1630−1650 (2CO). ^1H NMR ($CDCl_3$) 0.82−1.05 (m, 6H, $2CH_3$); 1.08−1.41 (m, 3H, CH,CH_2); 1.45−1.78 (m, 3H, NH, CH_2); 1.80−1.98 (m, 2H, CH_2); 2.18−2.41 (m, 7H, CH_3, $2CH_2$); 2.62−2.85 (m, 1H, C*H*H); 2.91−3.12 (m, 2H, CH, C*H*H); 3.53−3.69 (m, 1H, CH); 3.85−4.05 (m, 2H, CH_2); 4.41−4.58 (m, 1H, CH); 6.86−7.04 (m, 4H, aromatics); 7.15−7.22 (m, 4H, aromatics); 7.25−7.38 (m, 1H, NH) ppm.

The oily product was transformed into the hydrochloride, treating the free base with HCl/absol EtOH and recrystallized from absol EtOH/anhydrous ether. Mp 85−87 °C. Anal. ($C_{28}H_{38}ClF_2N_3O_2$) C, H, N.

5-Bromo-2-(methylethyl)-2-phenylpentanenitrile (33). 3-Methyl-2-phenylbutyronitrile[30] (0.5 g, 3.14 mmol) was dissolved in 10 mL of anhydrous THF and cooled to −78 °C; 2.7 mL (4.32 mmol) of *n*-butyllithium (1.6 M in hexane) was added, and the mixture was left at −78 °C for 1.5 h. Then 0.64 mL (6.37 mmol) of 1,3-dibromopropane was added, and the mixture was allowed to warm to room temperature, treated with a saturated solution of NH_4Cl and extracted with diethyl ether. The organic layer was dried over Na_2SO_4, the solvent removed under reduced pressure, and the residue purified by column chromatography using CH_2Cl_2 (100) as eluting system. Title compound (520 mg, 59% yield) was obtained as an oil. IR (neat) ν cm^{-1} 2240 (CN). ^1H NMR ($CDCl_3$) δ 0.79 (d, J = 5.5 Hz, 3H, CH_3); 1.23 (d, J = 5.5 Hz, 3H, CH_3); 1.40−1.59 (m, 1H, CH); 1.88−2.42 (m, 4H, $2CH_2$); 3.33 (t, J = 3.6 Hz, 2H, CH_2); 7.27−7.40 (m, 5H, aromatics) ppm. Anal. ($C_{14}H_{18}BrN$) C, H, N.

Pharmacology. Analgesic Activity. Hot-Plate Test. The method adopted was described by O'Callaghan and Holzman.[34] Mice were placed inside a stainless steel container, thermostatically set at 52.5 ± 0.1 °C in a precision water-bath from KW Mechanical Workshop, Siena, Italy. Reaction times (s) were measured with a stop-watch before icv injections and at regular intervals (15 min) up to a maximum of 60 min after treatment (cutoff) in order to prevent tissue damage. The endpoint used was the licking of the fore or hind paws. Antinociception was seen as increased latencies to the responses evaluated, while increased nociception was seen by shorter latencies. Those mice scoring below 12 and over 18 s in the pretest were rejected (30%). An arbitrary cutoff time of 45 s was adopted. Morphine hydrochloride (SALARS) was used as a reference drug. Other chemicals were of the highest quality commercially available. The percentage value inserted in the table has been calculated as follows: A = maximum analgesic effect of morphine on the hot plate expressed as latency in s (peak of analgesia) minus the latency of pretest (about 15 s); B = maximum analgesic effect of compound X on the hot plate expressed as latency in s (peak of analgesia) minus the latency of pretest (about 15 s); percentage = $B/A \times 100$.

Animals. Male Swiss albino mice (23−30 g) from Morini (San Polo d'Enza, Italy) and male rats from Harlan-Nossan (Correzzana, Milano, Italy) breeding farm were used. Fifteen mice or four rats were housed per cage. The cages were placed in the experimental room 24 h before the test for acclimatization. The animals were kept at 23 ± 1 °C with a 12 h light/dark cycle, light at 7 a.m., with food and water ad libitum. All experiments were carried out according to the guidelines of the European Community Council.

Intracerebroventricular Injections. Icv administration was performed during brief anaesthesia according to the method described for mice by Haley and McCormick[45] that we have adapted also for rats. Briefly, during anaesthesia, mice (22−25 g) or rats (150−200 g) were grasped firmly by the loose skin behind the head. A 0.4 mm external diameter, hypodermic needle attached to a 10 μL syringe was inserted perpendicularly through the skull no more than 2 mm into the brain of mouse and 4 mm into the brain of rat, where 5 μL (for mouse) and 10 μL (for rat) of solution (H_2O/DMSO 2:1) were injected. The injection site was 1 mm (for mouse) or 2 mm (for rat) to the right or left of the midline along a line drawn through the anterior base of the ears. To ascertain that the drugs were administered exactly into the cerebral ventricle, after the test some assayed mice and rats were icv injected with 5−10 μL of diluted 1:10 India ink and their brains examined macroscopically after sectioning.

Analgesic Action on Chronic Constriction Injury. A peripheral mono neuropathy was produced in adult rats by placing loosely constrictive ligatures around the common sciatic nerve according to the method described by Bennett and Xie.[37] Rats were anaesthetised with chloral hydrate. The common sciatic nerve was exposed at the level of the middle of the thigh by blunt dissection through biceps femoris. Proximal to sciatic nerve trifurcation, about 1 cm of the nerve was freed of adhering tissue and four ligatures (3/0 silk tread) were tied loosely around it with about 1 mm spacing. The length of the nerve thus affected was 4−5 mm long. Great care was taken to tie the ligatures such that the diameter of the nerve was seen to be just barely constricted when viewed with 40× magnification. In every animal, an identical dissection was performed on the opposite side except that the sciatic nerve was not ligated. The left paw was untouched.

Paw Pressure Test. The nociceptive threshold in the rat was determined with an analgesimeter (Ugo Basile, Varese, Italy), according to the method described by Leighton.[38] The instrument exerts a force which is applied at a costant rate (32 g/s) with a cone-shaped pusher on the upper surface of the rat hind paw. The force is continuously monitored by a pointer moving along a linear scale. The pain threshold is given by the force which induces the first struggling from the rat. Pretested rats which scored below 40 g or over 75 g during the test before drug administration (25%) were rejected. An arbitrary cut-off value of 250 g was adopted.

Statistical Analysis. All experimental results are given as the mean ± SEM. Analysis of variance (ANOVA), followed by Fisher's Protected Least Significant Difference (PLSD) procedure for post-hoc comparison, was used to verify significance between two means. Data were analyzed with the StatView software for the Macintosh (1992). P values of less than 0.05 were considered significant.

Binding Assays. Reversible, saturable and high affinity binding sites were detected for [^{3}H]D888 and [^{3}H]PN200-110 in rat ventricle membranes: the K_D amounted to 4.75 ± 1.05 nM for [^{3}H]D888 and 98.10 ± 2.55 pM for [^{3}H]PN200-110 and the maximal binding capacities (B_{max}) were 972.73 ± 223.73

fmol/mg protein and 220.41 ± 34.08 fmol/mg protein for [^{3}H]-D888 and [^{3}H]PN200-110, respectively.

(−)-[*N*-Methyl-^{3}H]desmethoxyverapamil ((−)-[^{3}*H*]-D-888) Binding Assays. Membrane Preparation. Hearts were rapidly removed from male Sprague- Dawley rats (200−250 g), the ventricles placed in ice-cold 20 mmol/L $NaHCO_3$ supplemented with 0.1 mmol/L phenylmethylsulfonyl-fluoride (PMSF), pH 7.4, and the wet weight-to-volume ratio was 1:10. The membrane preparations were performed as described by Goll et al.[46] (with modification). Briefly, the tissue was homogenized by 3 × 20 s with ultraturrax, and the crude homogenate was centrifuged at 300*g* for 15 min. The resultant pellet was resuspended 50 mmol/L Tris-HCl, 0.1 mmol/L PMSF (pH 7.4) and centrifuged again. The supernatants of both runs were collected and spun at 45 000*g* for 15 min. The pellets were then suspended in the same buffer and sedimented twice at 45 000*g* for 15 min. The final crude membrane was stored at −80 °C until use.

Binding Experiments. Binding assays for (−)-[^{3}H]D-888 (86 Ci/mmol, Amersham Life Science) were performed in a final volume of 0.5 mL; the frozen pellet was suspended in 50 mmol/L Tris-HCl supplemented with 0.1 mmol/L PMSF (pH 7.4) to a final concentration of 0.3−0.6 mg/mL and was incubated at 25 °C for 60 min. In homologous competition curves, the marker ligand was present at 0.5 nM in tubes containing increasing concentrations of unlabeled D-888 (0.5 nM to 0.1 mM) and at 0.0625−0.5 nM in tubes without unlabeled ligand. In heterologous competition curves fixed concentrations of the tracer (0.5 nM) were displaced by increasing concentrations of several unlabeled ligands (0.1 nM-0.1 mM). The incubation was termined by filtration through Whatman GF/C glass filter presoaked in buffer 20 mmol/L Tris-HCl plus 10 mmol/L $MgCl_2$ and 10% (poly(ethylene glycol)) PEG 4000, pH 7.4, using a Brandel M-48R well cell harvester. Filters were washed twice with 5 mL of 20 mmol/L Tris-HCl. In all experiments, the radioactivity retained by filters was measured in a liquid scintillation counter (TRI-CARB 1900TR, Packard) after the addition of 4 mL of scintillation fluid (Filter Count, Packard), and all measurements were obtained in duplicate. Protein was measured by the method of Bradford,[47] using bovine serum albumine as standard. The binding data were evaluated quantitatively with nonlinear least-squares curve fittings using the computer program LIGAND:[48] this analysis provides optimal estimates of "binding parameters" (such as affinity constants, binding capacities and nonspecific binding).

(+)-[^{3}H]-PN 200-110 Binding Assays. Membrane Preparation. Preparation of the membrane was carried out according to Matucci[49] with minor modification: hearts of male Sprague−Dawley rats (200−250 g) were removed and placed in ice cold solution containing 10 mmol/L Tris-HCl, 250 mmol/L saccarose and 0.2 mmol/L PMSF, pH 7.4; the tissue was homogenized by ultraturrax and filtered through a single layer of cheesecloth and centrifuged at 1000*g* for 10 min. The supernatant was centrifuged at 48 000*g* for 25 min. The final pellet was stored at −80 °C until assayed.

Binding Experiments. For the binding experiments, the defrozen membranes (0.3−0.6 mg/mL) were incubated in 50 mmol/L Tris-HCl plus 1.2 mmol/L $MgCl_2$, pH 7.4, in a final volume of 1 mL at 37 °C for 20 min. In homologous competition curves, (+)-[^{3}H]-PN 200-110 (85.1 Ci/mmol, Perkin-Elmer Life Science) was present at 0.1 nM in tubes containing increasing concentrations of unlabeled isradipine (0.1 nM to 0.1 mM) and at 0.025−0.1 nM in tubes without unlabeled ligand. In heterologous competition curves fixed concentrations of the tracer (0.1 nM) were displaced by increasing concentrations of the tested compounds that were used from 0.1 nM to 0.1 mM. At the end of incubation period, the samples were filtered and processed as described above. All measurements were obtained in duplicate.

N-Channel Inhibition. Cell Culture. PC12 cells (a rat pheochromocytoma cell line) were obtained from ATTC (Interlab Cell Line Collection (ICLC) Genova, Italy). The cells were grown in suspension in RPMI medium supplemented with 5% fetal bovine serum, 10% horse serum, 2 mM L-glutamine. Before each experiment the cells were kept in serum-free medium overnight.

Measurement of [Ca^2] Fluxes. PC12 cells, prepared as described above, were loaded with 2 μM Fura-2/AM for 45 min at 37 °C, washed, resuspended in Krebs Henseleit HEPES (KHH) medium (140 mM Na^+, 5.3 mM K^+, 132.4 mM Cl^-, 1 mM Ca^{++}, 0.81 mM Mg^{2+}, 5.5 mM glucose, 20.3 mM HEPES, 2.5 mM Na-pyruvate, 2 mM HEPES, 0.1% BSA, pH 7.5). $[Ca^{2+}]_i$ was measured as described previously using a spectrofluorimetric method[35,36] employing a Perkin-Elmer LS50B instrument equipped with a fast rotary filter shuttle for alternate 340 and 380 nm excitation. KCl (50 mM) was added directly in the cuvette either in the absence or presence of the different concentrations of the tested compounds. In addition the effect of the different compounds was tested also in the presence of the L-type calcium channel inhibitor nifedipine at the concentration of 10 μM. Each compound was initially dissolved in DMSO (100%) at the concentration of 10 mM, and further dilutions were performed in BSA-containing KHH medium. Fluorescence measurements were converted to $[Ca^{2+}]_i$ by determining maximal fluorescence (F_{max}) with digitonin (0.01% final concentration) followed by minimal fluorescence (F_{min}) with 10 mM EGTA (pH 10). $[Ca^{2+}]_i$ was calculated according to Grynkiewicz[50] using the ratio 340/380 and assuming a dissociation constant of Fura-2 for calcium of 224 nM.

IC_{50} were evaluated, for each compound, using the computer program ALLFIT,[51] which allows the simultaneous analysis of different families of dose−responses curves.

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