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New *N*-(benzhydryloxyalkyl)-4-(carboxy/carbamoylmethyl) piperidine derivatives with antidepressant activity

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Summary — Several benzhydryloxylalkylpiperidine derivatives were prepared with the aim of obtaining new antidepressant compounds. The influence of the length of the aliphatic chain and of aromatic and piperidine ring substitutions was studied. The pharmacological activity of compounds was investigated in vivo by means of a screening comprising four pharmacological tests: antagonism of reserpine and apomorphine hypothermia; increase of yohimbine induced mortality; and antagonism of immobility in tail suspension test. An in-depth pharmacological study was performed with the more active compounds and a binding study to serotonin (5-HT), norepinephrine (NE) and dopamine (DA) reuptake sites was performed for the preferred compounds. The most active compounds [+], [−]-*cis*-**31**, **32** and **46** exhibited an interesting psychopharmacological profile after intraperitoneal administration. This profile was confirmed by the oral route for **31** and **46**. In vitro, these compounds showed a non-selective inhibition of DA, NE and 5-HT uptake.

3-alkylpiperidine / benzhydryloxyalkyl / antidepressant activity / uptake inhibitor

Introduction

During the last three decades, the relationship between monoamine uptake and a variety of diseases and conditions has been appreciated and investigated. Initially, the monoamine reuptake blockers, which are used to treat depression, were either potent inhibitors of 5-HT and NE reuptake (imipramine and amitryptiline) or more specifically against NE (desipramine). However, a new class of reuptake inhibitors has been developed, which exhibits a more selective action on 5-HT uptake and still effectively alleviates the symptoms of depression. Recently, venlafaxine, which inhibits the reuptake of 5-HT, NE, and to a lesser extent DA [1], was shown to be more effective than imipramine in the treatment of major depression [2].

There is also evidence that, similarly to serotoninergic and noradrenergic neurons, dopaminergic systems are broken down in depression [3]. The involvement of dopaminergic mechanisms in depression has been considered with interest, and various results suggest that antidepressant activity is associated with an increase in dopaminergic transmission [3] and, furthermore, repeated electroconvulsive shocks induce supersensitivity of dopamine-mediated behaviour [4–6]. Duloxetine, a new 5-HT and NE uptake inhibitor, causes an increase in the output of 5-HT, NE and DA in the rat frontal cortex [7].

On the other hand, the combination of fluoxetine and D-amphetamine, as well as the combination of fluoxetine and desipramine, proved of interest in the management of refractory and major depression [8, 9]. In major depressions, the antidepressant response to tricyclics is also accelerated with adjunctive use of methylphenidate [10]. Bogeso [11] reported the importance of non-selective reuptake inhibitors in a phenylindanamine series. These compounds have the same potency on the three monoamine uptake sites.

In a benzhydryloxyethylpiperazine series, GBR 12935, a specific inhibitor of DA uptake (IC_{50} DA = 3.7 nM, NE = 289 nM, 5-HT = 1261 nM) [12] possesses non-amphetaminic psychostimulant properties in animals [13]. LR 1111, a homopiperazine analog, which differs from GBR 12935 by the addition of a methylene group into the piperazine ring, shows a high affinity for DA and NE uptake sites but appears less selective than the parent compound [12]. On the other hand, replacement of the phenylalkyl moiety in GBR series by an alkylcarboxylic group leads to compounds (I), which possess the same pharmacological profile [14].

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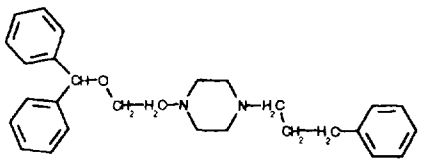
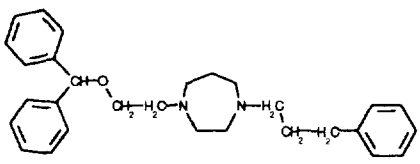
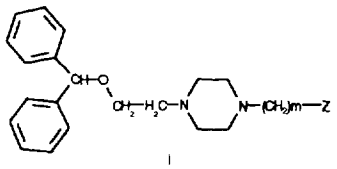


GBR 12935

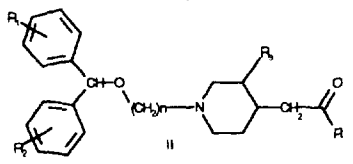


LR 1111



I

m = 1-4 Z = ON, $CO_2C_2H_5$, COOH, $CONR_2$



II

n = 2, 3, 4, 5 R_1 = H, OCH_3, CF_3, F

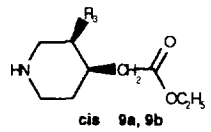
R_2 = H, F R_3 = CH_3, C_2H_5 R = OH, OC_2H_5, $N(C_2H_5)_2$

In order to investigate whether we could keep the affinity for DA and NE uptake inhibition and increase the affinity for 5-HT uptake inhibition, we synthesized a series of benzhydryloxyalkylpiperidines (II). In this work, we study the influence of the modification of the heterocycle, the replacement of a nitrogen atom of the piperazine by a methylene group, the introduction of an alkyl substituent on the 3-position of the piperidine ring, the length of the aliphatic chain between the piperidine ring and the benzhydrol moiety, the aromatic ring substitution and the nature of carboxylic function, as well as the influence of *cis–trans* configuration and the resolution of racemic mixture.

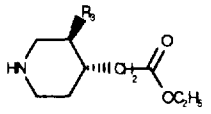
Chemistry

All esters **II** (**13–44**) were prepared according to the general procedure illustrated in scheme 1, by condensation of benzhydryloxyalkyl chlorides **6a–n** and substituted piperidines **9a** and **9b** in the presence of potassium carbonate and catalytic amount of sodium iodide (process A).

a: $R_3 = CH_3$
b: $R_3 = C_2H_5$



cis **9a, 9b**

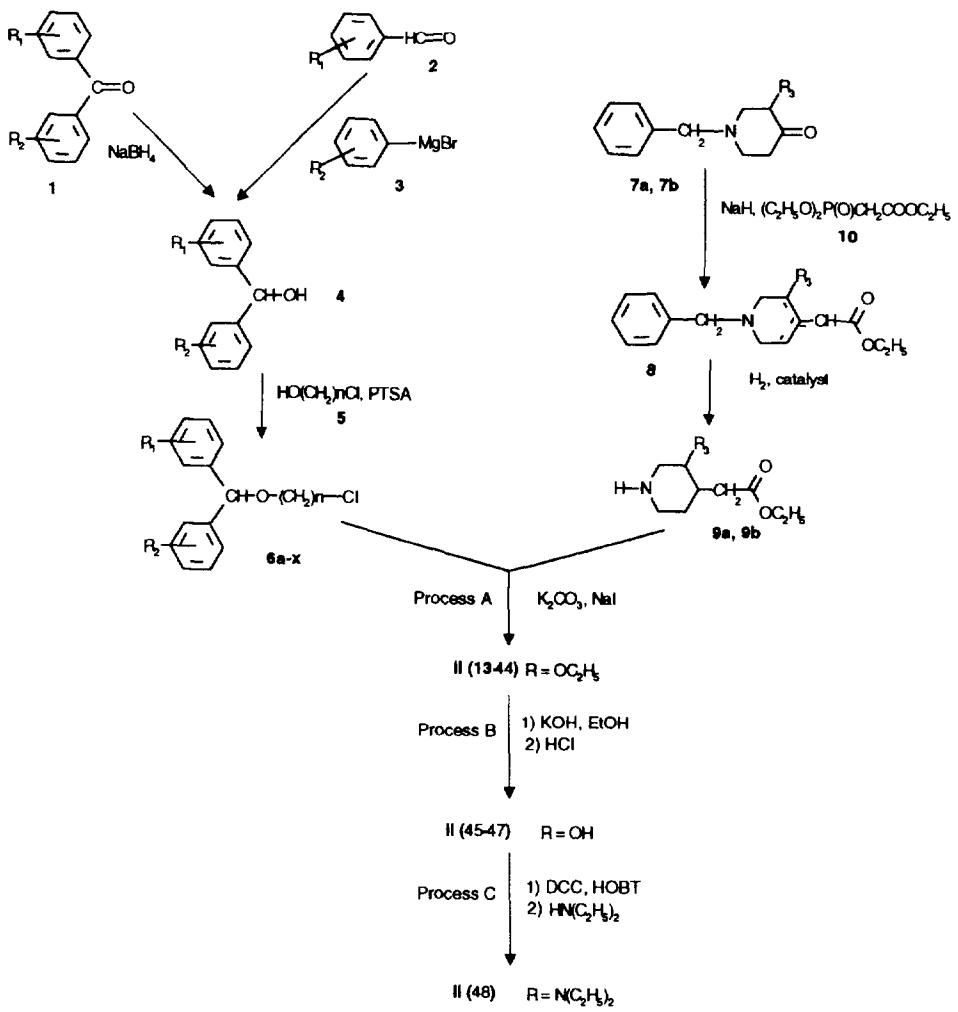


trans **9a, 9b**

The reaction with 3-ethyl or 3-methyl piperidine-4-acetic acid ethyl ester (**9a** $R_3 = CH_3$; **9b** $R_3 = C_2H_5$) gave a mixture of *cis* and *trans* isomers enriched in the *cis* isomer (2:1 or 3:1 ratio), which was separated by flash chromatography.

Treatment of the crude esters **II** (**19**, **31** and **32**) with a 10% alcoholic solution of potassium hydroxide, followed by acidification to pH 5.4 by hydrogen chloride led to the corresponding acids **II** (**45–47**) (process B). The amidification of the acid **II** (**46**) with diethylamine in the presence of dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole (HOBT) in chloroform, according to the method described by Genin et al [15] (process C), gave the amide derivative **II** (**48**). Condensation of 1-chloro-ω-alcohols **5** and related benzhydrols **4**, in the presence of catalytic amount of *p*-toluenesulfonic acid (PTSA), in benzene gave the intermediates **6a–n** in quantitative yields. The benzhydrols **4** were commercially available or prepared by reduction of the corresponding benzophenones **1** by sodium borohydride as described by Bogeso [16], or by reaction of substituted phenyl magnesium **3** with the benzaldehydes **2**. On the other hand, piperidines **9a** and **9b** could be synthesized by the general procedure described in scheme 2. Wittig–Horner [17] reaction between the carbanion of phosphonoacetic acid triethyl ester (**10**) and *N*-benzylpiperidones **7a** and **7b** in toluene gave ethylenic derivatives **8** which were debenzylated and hydrogenated under catalytic conditions with platinum oxide or palladium. The nature of the ethylenic compounds **8** depended on the experimental Wittig–Horner reaction conditions (scheme 2). At 20 °C, when two equivalents of sodium hydride were used, the four ethylenic derivatives **8a1,8b1**, **8a2,8b2**, **8a3,8b3** and **8a4,8b4** were obtained.

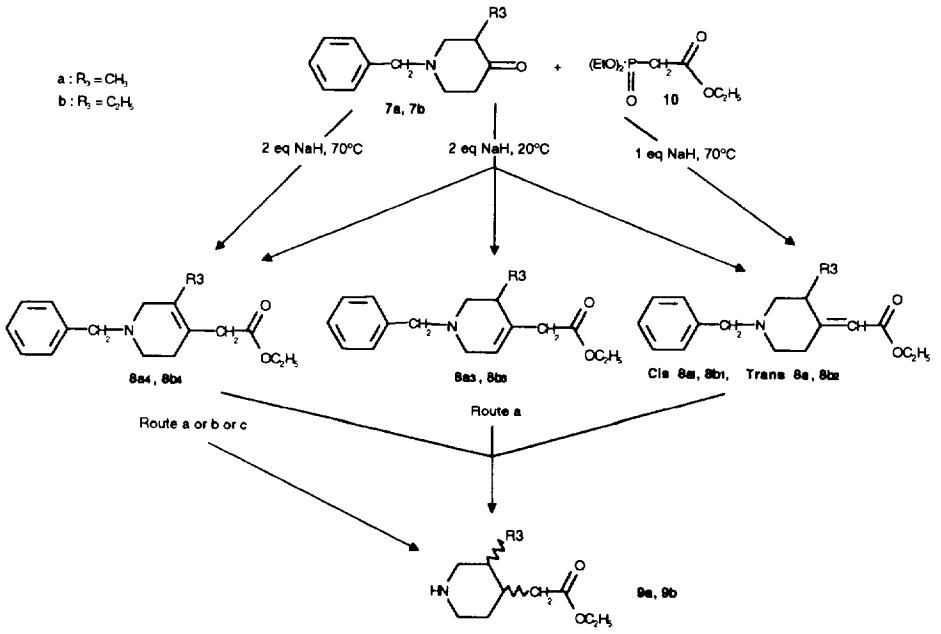
At 70 °C, with one equivalent of sodium hydride, the products formed were the *cis* and *trans* isomers of 1-benzyl-3-alkylpiperid-4-ylidene acetic acid ethyl esters **8a1,8b1** and **8a2,8b2**; at this temperature, in the presence of two equivalents of NaH, the ethylenics **8a4,8b4** was obtained with a good yield. The assignment of the *trans* doble bound configuration to the major isomer of **8** was inferred from the coupling constant of the doublet due to the vinyl proton in the ^{1}H-NMR spectrum (J = 0.5 Hz; compare with J = 1.8 Hz in the minor *cis* isomer), taking into account that transoid allylic coupling constants are smaller



Scheme 1.

than cisoid ones [18, 19]. The relative percentage of *cis* and *trans* isomers of piperidines **9a** and **9b** depended on the nature of the ethylenic compounds **8** used, and on the hydrogenation conditions. However, reduction and debenzylation of the mixture of the four products **8a1,8b1**, **8a2,8b2**, **8a3,8b3** and **8a4,8b4**, using palladium on carbon as a catalyst, gave a mixture of *cis* (67%) and *trans* (33%) 3-alkyl-4-piperidineacetic acid ethyl esters **9a** and **9b** (route a). Debenzylation of **8a4** or **8b4** by formic acid in the presence of palladium on carbon [20] or by chloroethyl chloroformate [21], followed by catalytic hydrogenation with platinum oxide in alcohol, gave selectively the *cis* isomers **9a** and **9b** (*cis* 98%, *trans* 2%) (route c); at the same conditions, when reduction was conducted with Pd/C the *trans* isomer percentage (28%) was higher (route b). *cis*-3-Methyl-4-piperidineacetic acid ethyl ester (**9a**) can also selectively be prepared by the method described by Ustokovic et al on 3-ethyl-4-methylpyridine [22] (scheme 3). 3,4-Lutidine (**11**) was lithiated with LDA in tetrahydrofuran at –60 °C and the formed carbanion was condensed with diethylcarbonate. The monosubstituted derivative **12** was purified by distillation.

Hydrogenation of the pyridine ring under platinum oxide in ethanol gave the crude *cis* isomer of **9c** (*cis* 96%, *trans* 4%); when Pd/C was used as catalyst, the percentage of the *trans* isomer (18%) was higher. The enantiomers of the most interesting product **31** were prepared by condensation of the optically pure piperidines **9a** with chloride **6t**. Dextrorotatory and levorotatory piperidines were separated by crystallization

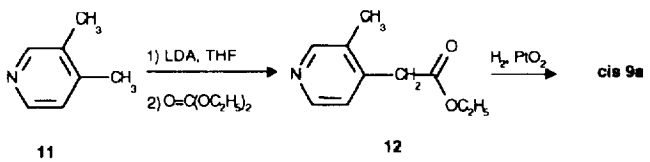


Scheme 2.

of 2,3,4,6-di-*O*-isopropylidene-2-keto-L-gluconic acid salt for the 3(*R*)-methyl-4(*S*)-piperidineacetic acid ethyl ester (**3R, 4S-9a**) and *d*(–)tartric acid salt for the 3(*S*)-methyl-4(*R*)-piperidineacetic acid ethyl ester (**3S, 4R-9a**). For **3S, 4R-9a**, the best obtained enantiomeric excess was ee = 81%. The absolute configuration (3*R*, 4*S*) and (3*S*, 4*R*) were determined by X-ray crystallography study.

Pharmacology. Results and discussion

Thirty-eight *N*-(benzhydryloxyalkyl)-4-(carboxy/carbamoylmethyl) piperidine derivatives were designed, prepared, and assessed for in vivo biological activity in mice using four assays predictive of antidepressant activity: antagonism of reserpine and apomorphine hypothermia; increase of yohimbine-induced mortality; and antagonism of immobility in tail suspension test. Most of the classical monoamine uptake inhibitors showed activity in one or more of these assays, in relation to their specificity for NE, 5-HT and DA uptake sites [23–27]. The results obtained are reported in table I.



Scheme 3.

In this work, we studied the influence of the following modifications of the structure on the activity in this screening: (a) substitutions on the piperidine moiety (R_3); (b) length of the aliphatic chain (*n*); (c) substitutions on the aromatic rings (R_1 and R_2); (d) conformation of the heterocycle; and (e) nature of the R substituent.

Influence of substituents on the aromatic rings (R_1 and R_2)

Unsubstituted compounds on the benzene ring (R_1 = R_2 = H; compounds **21**, **22**, **39** and **40**) were inactive in the tests. Except for *trans* isomers **28** and **29**, substitution on the *para* position of one of the aromatic rings by an atom of fluorine (R_1 = 4-F, R_2 = H; compounds **27**, **37** and **38**) or by a trifluoromethyl moiety (R_1 = 4-CF_3, R_2 = H; compound **30**), enhanced the activity in these assays. Replacement of fluorine by a methoxy group (R_1 = 4-OCH_3, R_2 = H; compounds **41** and **42**) led to inactive compounds. For the disubstituted derivatives, except for benzhydryloxyethyl derivatives (*n* = 2; compounds **13–16**), the best

Table I. Benzhydryloxyalkylpiperidine derivatives **II** (**13–48**).

Compound	cis/trans	n	R1	R2	R3	R	Method	Salt	Formula[b]	Mp (°C)	Last step yield (%)	ED_{50}, mg/kg ip, mice			
												Reserpine hypothermia antagonism	Apomorphine hypothermia antagonism	Potentiation yohimbine toxicity	TST immobility time reduction
13	*cis*	2	4-F	4-F	CH_3	OC_2H_5	A	O	$C_{25}H_{31}F_2NO_3 \cdot C_2H_2O_4$	152	60	>32	>32	>32	>32
14	*cis*	2	4-F	2-F	CH_3	OC_2H_5	A	O	$C_{25}H_{31}F_2NO_3 \cdot C_2H_2O_4$	152	50	>32	>32	>31	>15
15	*cis*	2	4-F	4-F	C_2H_5	OC_2H_5	A	O	$C_{26}H_{33}F_2NO_3 \cdot C_2H_2O_4$	144	47	>32	>32	>32	>32
16	*trans*	2	4-F	4-F	C_2H_5	OC_2H_5	A	O	$C_{26}H_{33}F_2NO_3 \cdot C_2H_2O_4$	120	21	>32	>32	>32	>32
17	*cis*	3	4-F	4-F	CH_3	OC_2H_5	A	O	$C_{26}H_{33}F_2NO_3 \cdot C_2H_2O_4$	152	36	6.1 (4.4–8.2)	1[a]	6.5 (5–8.5)	10.2 (7.7–13.4)
18	*trans*	3	4-F	4-F	CH_3	OC_2H_5	A	O	$C_{26}H_{33}F_2NO_3 \cdot C_2H_2O_4$	153	20	63 (53–74.6)	60.3 (47–77.2)	>64	16[a]
19	*cis*	3	4-F	2-F	CH_3	OC_2H_5	A	O	$C_{26}H_{33}F_2NO_3 \cdot C_2H_2O_4$	168	37	>32	>32	10[a]	3.4 (1–11.3)
20	*trans*	3	4-F	2-F	CH_3	OC_2H_5	A	O	$C_{26}H_{33}F_2NO_3 \cdot C_2H_2O_4$	169	12	32[a]	10[a]	5.1 (1.7–8.2)	4.1 (2.5-6.8))
21	*cis*	3	H	H	C_2H_5	OC_2H_5	A	O	$C_{27}H_{37}NO_3 \cdot C_2H_2O_4$	146	50	>64	>64	NT	36 (24.7–54.1)
22	*trans*	3	H	H	C_2H_5	OC_2H_5	A	O	$C_{27}H_{37}NO_3 \cdot C_2H_2O_4$	101	23.5	>32	>32	NT	35.7 (8.8–43.9)
23	*cis*	3	4-F	4-F	C_2H_5	OC_2H_5	A	O	$C_{27}H_{35}F_2NO_3 \cdot C_2H_2O_4$	162	45	30[a]	>64	20.2 (14.1–28.8)	14 (11–17.9)
24	*trans*	3	4-F	4-F	C_2H_5	OC_2H_5	A	O	$C_{27}H_{35}F_2NO_3 \cdot C_2H_2O_4$	144	37	10[a]	6[a]	33.7 (25.6–42.4)	>32
25	*cis*	3	4-F	3-F	CH_3	OC_2H_5	A	O	$C_{26}H_{33}F_2NO_3 \cdot C_2H_2O_4$	155	44	NT	14.5 (11.9–17.7)	23.3 (17.7–30.2)	19.7 (9.4–31.3)
26	*trans*	3	4-F	3-F	CH_3	OC_2H_5	A	O	$C_{26}H_{33}F_2NO_3 \cdot C_2H_2O_4$	149	24.7	>32	>32	>32	>32
27	*cis*	3	4-F	H	CH_3	OC_2H_5	A	O	$C_{26}H_{34}FNO_3 \cdot C_2H_2O_4$	130	45	36 (31.1–41.6)	16[a]	12.3 (6.7–22.6)	51.5 (35.2–75.4)
28	*trans*	3	4-F	H	CH_3	OC_2H_5	A	O	$C_{26}H_{34}FNO_3 \cdot C_2H_2O_4$	150	27.6	>64	NT	>64	>64
29	*trans*	3	4-CF_3	H	CH_3	OC_2H_5	A	O	$C_{26}H_{34}F_3NO_3 \cdot C_2H_2O_4$	120	24.7	>32	>32	>32	>32
30	*cis*	3	4-CF_3	H	CH_3	OC_2H_5	A	O	$C_{26}H_{34}F_3NO_3 \cdot C_2H_2O_4$	122	39	16[a]	16[a]	17.4 (6.9–43.2)	8[a]
31	*cis*	4	4-F	4-F	CH_3	OC_2H_5	A	O	$C_{27}H_{35}F_2NO_3 \cdot C_2H_2O_4$	132	46	4[a]	6.3 (5.2–7.6)	8.1 (4.8–13.9)	0.5 (0.2–1.4)
(–)-**31**[c]	*cis*	4	4-F	4-F	CH_3	OC_2H_5	A	O	$C_{27}H_{35}F_2NO_3 \cdot C_2H_2O_4$	122	66	5.1 (4.1–6.3)	12 (9.2–15.5)	10 (5.8–7.4)	4.1 (3.2–5.2)
(+)-**31**[c]	*cis*	4	4-F	4-F	CH_3	OC_2H_5	A	O	$C_{27}H_{35}F_2NO_3 \cdot C_2H_2O_4$	127	80.6	3.2 (3.5–4.1)	22 (16.9–28.7)	8.1 (4.7–13.8)	1 (0.5–2.1)
32	*trans*	4	4-F	4-F	CH_3	OC_2H_5	A	O	$C_{27}H_{35}F_2NO_3 \cdot C_2H_2O_4$	127	22	13 (11–15.5)	6.2 (5–7.7)	33.7 (10.6–42.4)	9.9 (8–12.2)
33	*trans*	4	4-F	2-F	CH_3	OC_2H_5	A	O	$C_{27}H_{35}F_2NO_3 \cdot C_2H_2O_4$	122	19	16[a]	6.8 (5.7–8.9)	27.5 (23.4–32.6)	4[a]
34	*cis*	4	4-F	2-F	CH_3	OC_2H_5	A	O	$C_{27}H_{35}F_2NO_3 \cdot C_2H_2O_4$	109	39	14.5 (11–19.2)	7.6 (6.3–9.3)	23.9 (17.2–33.3)	8.5 (6.3–11.5)
35	*trans*	4	4-F	4-F	C_2H_5	OC_2H_5	A	O	$C_{28}H_{37}F_2NO_3 \cdot C_2H_2O_4$	133	11	NT	NT	NT	7.9 (5.2–10.3)
36	*cis*	4	4-F	4-F	C_2H_5	OC_2H_5	A	O	$C_{28}H_{37}F_2NO_3 \cdot C_2H_2O_4$	146	62.3	2.7 (2.0–3.7)	6 (4.7–7.7)	5 (2.2–11.2)	3.6 (2.9–4.5)
37	*cis*	4	4-F	H	CH_3	OC_2H_5	A	O	$C_{27}H_{36}FNO_3 \cdot C_2H_2O_4$	107	44.5	11.8 (9.9–13.9)	12.4 (10.2–15)	9 (4.7–17.2)	11.9 (9.2–17.4)
38	*trans*	4	4-F	H	CH_3	OC_2H_5	A	O	$C_{27}H_{36}FNO_3 \cdot C_2H_2O_4$	104	24.4	8[a]	21 (17.3–25.3)	21.7 (–10.2–46)	30.8 (24.7–38.5)
39	*cis*	4	H	H	CH_3	OC_2H_5	A	O	$C_{27}H_{37}NO_3 \cdot C_2H_2O_4$	104	45	>64	>64	>64	16[a]
40	*trans*	4	H	H	CH_3	OC_2H_5	A	O	$C_{27}H_{37}NO_3 \cdot C_2H_2O_4$	99	31.8	>64	>64	>64	64[a]
41	*cis*	4	4-OCH_3	H	CH_3	OC_2H_5	A	O	$C_{28}H_{39}NO_4 \cdot C_2H_2O_4$	98	32	>64	>64	>64	64[a]
42	*trans*	4	4-OCH_3	H	CH_3	OC_2H_5	A	O	$C_{28}H_{39}NO_4 \cdot C_2H_2O_4$	91	31	>64	>64	>64	4[a]
43	*cis*	5	4-F	4-F	CH_3	OC_2H_5	A	O	$C_{28}H_{37}F_2NO_3 \cdot C_2H_2O_4$	101	42.6	NT	NT	20.9 (14.5–26.9)	5.4 (4–7.2)
44	*trans*	5	4-F	4-F	CH_3	OC_2H_5	A	O	$C_{28}H_{37}F_2NO_3 \cdot C_2H_2O_4$	93	30	NT	NT	18.9 (6.6–50)	14 (10.7–18.4)
45	*cis*	3	4-F	2-F	CH_3	OH	B	O	$C_{24}H_{29}F_2NO_3 \cdot C_2H_2O_4$	168	84	NT	NT	9.6 (2.8–16.1)	9.6 (8.1–11.3)
46	*cis*	4	4-F	4-F	CH_3	OH	B	O	$C_{24}H_{31}F_2NO_3 \cdot C_2H_2O_4$	98	97	2.4 (1.8–3.1)	15.7 (11.5–21.4)	17.9 (10.3–31)	2.4 (1.7–3.3)
47	*trans*	4	4-F	4-F	CH_3	OH	B	O	$C_{24}H_{31}F_2NO_3 \cdot C_2H_2O_4$	107	90	8.7 (6.2–12.3)	9.9 (7.4–13.1)	14.3 (8.1–20.1)	10.1 (7.9–12.9)
48	*cis*	4	4-F	4-F	CH_3	$N(C_2H_5)_2$	C	O	$C_{29}H_{40}F_2N_2O_3 \cdot C_2H_2O_4$	137	82.7	8[a]	7.5 (6.1–9.2)	8[a]	1.7 (0.8–3.5)

[a]No definite dose–effect relationship was obseved at this dose level; [b]satisfactory elemental analyses (±0.4%) for C, H, N were obtained for all compounds; [c](–)-**31**, αD^{22} = –6.17, enantiomeric excess = 100%, (*c* = 0.1, MeOH); (+)-**31**, αD^{22} = –5.1, enantiomeric excess = 82.6%, (*c* = 0.1, MeOH).

responses were observed when the aromatic rings were substituted by a fluorine. 4,4'-Difluoro (n = 3, 4, 5, $R_1 = R_2$ = 4-F; compounds **17**, **18**, **23**, **24**, **31**, (–)-**31**, (+)-**31**, **32**, **35**, **36**, **43**, **44** and **46–48**) and 4,2'-difluoro (R_1 = 4-F, R_2 = 2-F; compounds **19**, **20**, **33**, **34** and **45**) derivatives antagonized at low doses immobility induced by tail suspension test in mice.

Influence of the length of the aliphatic chain (n)

Benzhydryloxyethyl derivatives (n = 2; compounds **13–16**) were all inactive in these assays. The response appeared only for fluorosubstituted derivatives when aliphatic chain was a propyl (n = 3; compounds **17**, **20**, **23**, **25**, **27** and **45**), was maximal for n = 4 (compounds **31–38** and **46–48**), and was also present with the pentyl chain (n = 5; compounds **43**, **44**).

Influence of the substitution in the 3-position of the piperidine ring (R_3)

Except when n = 2, 3-methyl fluorosubstituted derivatives (n = 3, 4, 5; $R_3 = CH_3$; compounds **17**, **20**, **25**, **27**, **30–34**, **37** and **43–48**) were active compounds. Replacement of the methyl by an ethyl group (n = 3, 4; $R_3 = C_2H_5$; compounds **21–24** and **35**) led to less active compounds. Only 3-ethyl derivative **36** presented a good activity in the four tests of this screening.

Influence of the piperidine ring conformation

3-Substituted piperidinyl compounds possessed two conformations. Racemic, *cis* and *trans* derivatives were compared in this screening. The results reported in table I showed that, in vivo, *cis* isomers were generally more active than *trans* isomers **17** vs **18**, **19** vs **20**, **25** vs **26**, **27** vs **28**, **30** vs **29**, **31** vs **32**, **34** vs **33**, **37** vs **38**, **43** vs **44**, and **46** vs **47**.

These compounds also had asymmetric carbons. The purification of the most active derivative **31** was performed and enantiomers were tested. Dextrorotatory form (+)-**31** appeared slightly more active than levorotatory (–)-**31** form but identical with the racemate **31** in these assays in vivo.

Influence of R substituent

The acids and the diethylamide derivatives were as potent as the corresponding esters, **46** and **48** vs **31**, **47** vs **32**, in all assays of this screening.

Discussion

These structure–activity relationship studies showed that the best chemical modifications carried out were the fluorine substitution on the benzene ring (R_1 = 4-F, R_2 = H or $R_1 = R_2$ = 4-F or R_1 = 4-F, R_2 = 2-F), the presence of a butyl (n = 4) chain between benzhydryl and piperidine moieties, and a methyl substitution in the 3-position of the piperidine ring ($R_3 = CH_3$). The nature of the R group had no real influence on the pharmacological activity. Among the most active compounds, **31**, (–)-**31**, (+)-**31**, **32** and **46** were selected and studied in a wider pharmacological screening.

These active compounds have been chosen in order to (i) confirm in vivo (ip or po) their antidepressant activities in a screening containing behavioral tests and antidepressant assays [28, 29] (increase or decrease of locomotion, antagonism of reserpine hypothermia and ptosis, antagonism of apomorphine hypothermia, antagonism of oxotremorine hypothermia, antagonism of immobility in the swimming test and the tail suspension test, antagonism of tryptamine convulsions and increase of yohimbine toxicity); (ii) compare in vivo their activities to those observed with known antidepressant compounds (selective 5-HT uptake inhibitors (fluoxetine and fluvoxamine), NE and DA uptake inhibitor (nomifensine), selective NE uptake inhibitor (amitriptyline), and non-selective monoamine uptake inhibitors (imipramine and clomipramine)); and (iii) confirm in vitro their mechanism of action in binding study of the uptake inhibition on the DA, NE and 5-HT uptake sites. The results are shown in table II.

In the locomotion test, like nomifensine, the compounds tested induced an increase of locomotion, this activity was confirmed po for compounds **31** and **46**. This may reflect the DA reuptake inhibition component of benzhydryloxybutylpiperidine derivatives.

In the reserpine assay, except compound **32** which was less active, the tested products antagonized at low doses the hypothermia induced by reserpine. Compounds (+)-**31** and **46** are as potent as nomifensine which presented the best activity. The hypothermia antagonism was comparable to that observed with compounds which possessed NE uptake inhibition component, such as amitriptyline, nomifensine and imipramine. Tested products also antagonized the palpebral ptosis induced by reserpine. The 5-HT uptake inhibitors fluvoxamine, clomipramine and fluoxetine appeared in vivo less active on the reserpine test. The activity of compound **31** was confirmed po.

In the apomorphine test, the compounds antagonized apomorphine hypothermia, and compounds **31** and **32** had the same potency and were slightly more active than reference compounds.

In the yohimbine test, selective compounds enhanced yohimbine toxicity but were less active than reference compounds possessing good NE uptake inhibitory effect (nomifensine and amitryptiline).

In the oxotremorine test, except imipramine, none of the tested compounds showed any activity on peripheral symptoms induced by oxotremorine at

Table II. In vivo and in vitro results of **31**, (+)-**31**, (–)-**31**, **32**, **46** and reference compounds.

Compound	*ED_{50} (mg/kg, mice)*									*LD_{50} Lorke*	*Synaptosomamal uptake inhibition*		
	Locomotion[a]	*Reserpine*		*Apomorphine*[d]	*Oxotremorine*[d]	*Forced swimming immobility*	*TST immobility*	*Tryptamine convulsions*	*Yohimbine toxicity potentiation*		*5-HT*	*NE*	*DA*
		A[d]	*B*[e]										
31 (ip)	+4[b]	4[b]	16[b]	6.3 (5.2–7.6)	4[b]	4[b]	0.5 (0.2–1.4)	1.7 (1.2–2.4)	8.1 (4.8–13.8)	158	12 ± 0.05	41 ± 0.13	22.9 ± 0.1
31 (po)	+8[b]	13.2 (11.5–15.2)		+4[b]		+4[b]	+4[b]		12.7 (5.7–28.1)				
(–)-**31**	+16[b]	5.1 (4.1–6.3)	4[b]	12 (9.3–15.5)	NT[c]	0.5[b]	4.1 (3.2–5.2)	1[b]	10.1 (5.8–17.4)	134	9 ± 0.02	58.6 ± 0.4	22.4 ± 0.1
(+)-**31**	+8[b]	3.2 (2.5–4.1)	64[b]	22 (16.9–28.7)	NT	0.5[b]	1 (0.5–2.1)	2[b]	8.1 (4.7–13.8)	222	51.8 ± 0.4	66 ±.0.4	10.6 ±.0.1
32	+16[b]	12.9 (10.7–15.5)	8[b]	6.2 (5–7.7)	4[b]	4[b]	9.9 (8–12.3)	NT	23.7 (16.6–37.4)	282	7.3 ± 0.5	12 ±.0.4	7.7 ±.0.04
46 (ip)	+8[b]	2.4 (1.8–3.1)	64[b]	15.7 (11.5–21.4)	NT	0.5[b]	2.4 (1.7–3.3)	NT	17.9 (10.3–31)	243	NT	NT	NT
46 (po)	+8[b]			16.8 (14.7–19.3)		0.5[b]	1.3 (0.8–2.1)		11.7 (7.2–19.1)				
Fluoxetine	>64	8[b]	32[b]	>64	32[b]	32[b]	50.3 (40–63.3)	2.4 (1.8–3.2)	5.8 (2.6–13)	111	11.9 [32]	143 [35]	14100 [38]
Imipramine	>64	5.1 (3.8–6.6)	64[b]	0.5[b]	0.5[b]	4[b]	28.8 (20–41.5)	NT	1.5 (0.6–3.6)	111	42 [33]	50 [36]	16050 [39]
Nomifensine	+2[b]	2.2 (1.7–2.8)	16[b]	2[b]	0.5[b]	0.5[b]	2.3 (1.9–2.9)	NT	1.8 (0.8–4)	243	1280 [33]	4.7 [37]	134 [40]
Clomipramine	–32[b]	32[b]	32[b]	22.3 (17.4–28.5)	4[b]	16[b]	4[b]	NT	8.2 (4.4–15.2)	382	7.1 [34]	24 [25] (IC_{50})	4600 [41]
Fluvoxamine	>64	32[b]	32[b]	34.8 (29.2–41.4)	NT	4[b]	64[b]	NT	29.2 (19.1–44.6)	282	3.1 [32]	41 [25] (IC_{50})	–
Amitryptiline	– 4[b]	10.1 (8.3–12.3)	32[b]	11.7 (10.5–13.2)	4[b]	4[b]	20.3 (11.6–35.6)	NT	1.5 (0.4–6.3)	96	262 [34]	13.9 [35]	9700 [42]

[a]+: Increase, –: decrease; [b]no definite dose–effect relationship was observed at this minimum significant active dose; [c]NT = not tested; [d]hypothermia antagonism; [e]ptosis antagonism.

64 mg/kg ip. Compounds **31** and **32**, as well as imipramine, nomifensine, clomipramine and amitryptiline, antagonized oxotremorine hypothermia. This central activity, as specified by Bourin [27], appears to be due to a postsynaptic NE interaction. Nomifensine, which presented a nanomolar affinity for NE uptake sites, was the most potent in this test.

In the behavioral despair tests, forced swimming and tail suspension tests (TST), which had a good predictive value for antidepressant potency in man as reported by Willner [30] and Bourin [27], benzhydryloxybutyl derivatives significantly diminished the duration of immobility. In the TST, compounds **31** and (+)-**31** were more potent than nomifensine which exhibited the best activity among the reference products. This reduction of immobility duration may reflect the NE and DA components of our compounds. The specific 5-HT uptake inhibitors did not appear active in these tests [27, 31].

In tryptamine test, compound **31** and its enantiomers (+)-**31** and (–)-**31** enhanced serotoninergic firing induced by tryptamine, and appeared to be as active as the reference compound fluoxetine. This test confirmed that 4,4'-difluorobenzhydryloxybutyl derivatives also acted by a 5-HT mechanism.

In binding studies, compounds **31**, (–)-**31**, (+)-**31**, and **32** were potent inhibitors of NE, DA and 5-HT uptake. On 5-HT uptake sites, compound **32** presented more affinity than compounds (–)-**31**, **31** and (+)-**31**, and was intermediate between the two selective 5-HT uptake inhibitors, fluoxetine and fluvoxamine. On DA uptake sites, compound (+)-**31** was more potent than compounds **31** and (–)-**31**; compound **32** had the best affinity. The dopaminergic component of all our derivatives was higher than that of the reference compounds. On NE uptake sites, racemic **31** and its enantiomers (–)-**31** and (+)-**31** had approximately the same potency, but were less active than *trans* isomer **32**. Their affinity was comparable to that of imipramine and amitryptiline, but inferior to that of nomifensine.

Taken as a whole, the results of the in vivo and in vitro studies reported in table II clearly demonstrate that compound **31**, its enantiomers (–)-**31** and (+)-**31**, its *trans* isomer **32**, and its acid derivative **46**, have a potent antidepressant activity and acted as nonselective monoamine uptake inhibitors with comparable levels of potency on 5-HT, NE or DA uptake. In binding studies, compound **32** appeared more potent than the other tested 4,4'-difluorobenzhydryloxybutyl derivatives, but compound (+)-**31** presented the best activity in vivo. The low differences in activity between dextrorotatory, levorotatory enantiomers and the racemic compounds could be explained by the fact that conformation of the piperidine moiety has no influence on the interaction with the monoamine uptake sites.

Conclusion

The structure–activity relationships we have established with our compounds proved the importance of benzhydryloxyalkylpiperidine derivatives in depression. Except compound **32**, optimal antidepressive activity was obtained with *cis*-4,4'-difluorobenzhydryloxybutyl-3-methylpiperidine derivatives ($R_1 = R_2 = 4$-F, $n = 4$, $R_3 = CH_3$). Neither the piperidine conformation nor the nature of the R substituent influenced the level of antidepressant activity. Binding studies clearly demonstrated that these compounds acted by a nonselective uptake inhibitory mechanism, and were devoid of any anticholinergic activity. Compound (+)-**31** presented the best compromise between in vitro and in vivo studies.

Experimental protocols

Chemistry

Melting points were determined on a Büchi 535 apparatus and are uncorrected. ^{1}H-NMR spectra of crude bases were recorded on a Hitachi 1500 FT spectrometer (60 MHz). Chemical shifts are given as δ values with reference to Me_4Si as internal standard. Thin layer chromatography was performed on Merck silica gel 60 plates with fluorescent indicator. The plates were visualized with UV light (254 nM). Flash chromatography was conducted on Merck Kieselgel 60 (0.040–0.063). Elemental analyses were performed by the microanalysis laboratory at the Faculty of Pharmacy, Chatenay-Malabry, France, and agree to within ±0.4% of calculated values. Optical purity of enantiomers was determined by an HPLC method using a chiral column SCI Ultron (ES-OVM 4.6 x 150) performed on a L4500 Hitachi spectrometer. Structural determination of pure enantiomers was conducted on Nonius CAD-4 diffractometer. The intermediates 3(*R*)-methyl-4(*S*)-piperidineacetic acid ethyl ester (**3R**, **4S-9a**) and 3(*S*)-methyl-4(*R*)-piperidineacetic acid ethyl ester (**3S**, **4R-9a**) were analyzed after derivatization with benzoyl chloride in ether in the presence of triethylamine. Optical rotation (α) of the pure enantiomers was measured by a AA10 optical activity polarimeter.

*Preparation of benzhydryloxyalkyl chlorides **6a–n***

In a flask fitted with Dean–Stark collector, a mixture of benzhydrol **4** (100 mmol), hydroxyalkylchloride **5** (110 mmol) and *para*-toluenesulfonic acid (1 g) in benzene (150 mL) was refluxed until the separation of the theoretical water quantity (1.8 mL). After cooling, the reaction mixture was washed with water and the organic layer was dried and concentrated under vacuum to give **6a–n** in quantitative yields. ^{1}H-NMR ($CDCl_3$) δ **6a** ($n = 2$, $R_1 = R_2 = 4$-F): 3.70 (m, 4H), 5.35 (s, 1H), 6.80–7.50 (m, 8H); **6b** ($n = 2$, $R_1 = 4$-F, $R_2 = 2$-F): 3.40–3.80 (m, 4H), 5.30 (s, 1H), 6.80–7.40 (m, 8H); **6c** ($n = 3$, $R_1 = R_2 =$ H): 1.80–2.20 (m, 2H), 3.6 (t, 2H, $J = 6.5$ Hz), 3.70 (t, 2H, $J =$ 6.5 Hz), 5.30 (s, 1H), 7.25 (m, 10H); **6d** ($n = 3$, $R_1 = R_2 = 4$-F): 1.80–2.25 (m, 2H), 3.6 (t, 2H, $J = 6.5$ Hz), 3.70 (t, 2H, $J =$ 6.5 Hz), 5.27 (s, 1H), 6.80–7.40 (m, 8H); **6e** ($n = 3$, $R_1 = 4$-F, $R_2 = 2$-F): 1.80–2.20 (m, 2H), 3.6 (t, 2H, $J = 6.5$ Hz), 3.70 (t, 2H, $J = 6.5$ Hz), 5.27 (s, 1H), 6.80–7.40 (m, 8H); **6f** ($n = 3$,

R_1 = 3-F, R_2 = 4-F): 1.80–2.20 (m, 2H), 3.6 (t, 2H, J = 6.5 Hz), 3.70 (t, 2H, J = 6.5 Hz), 5.30 (s, 1H), 6.80–7.40 (m, 8H); **6g** (n = 3, R_1 = 4-F, R_2 = H): 1.80–2.25 (m, 2H), 3.6 (t, 2H, J = 6.5 Hz), 3.70 (t, 2H, J = 6.5 Hz), 5.32 (s, 1H), 6.80–7.30 (m, 9H); **6h** (n = 3, R_1 = 4-CF_3, R_2 = H): 1.90–2.25 (m, 2H), 3.6 (t, 2H, J = 6.5 Hz), 3.70 (t, 2H, J = 6.5 Hz), 5.39 (s, 1H), 7.25–7.55 (m, 9H); **6i** (n = 4, R_1 = R_2 = H): 1.75–2.10 (m, 4H), 3.6 (t, 2H, J = 6.5 Hz), 3.70 (t, 2H, J = 6.5 Hz), 5.32 (s, 1H), 7.30 (m, 10H); **6j** (n = 4, R_1 = R_2 = 4-F): 1.75–2.1 (m, 4H), 3.6 (t, 2H, J = 6.5 Hz), 3.70 (t, 2H, J = 6.5 Hz), 5.3 (s, 1H), 6.8–7.4 (m, 8H); **6k** (n = 4, R_1 = 4-F, R_2 = 2-F): 1.75–2.10 (m, 4H), 3.6 (t, 2H, J = 6.5 Hz), 3.70 (t, 2H, J = 6.5 Hz), 5.27 (s, 1H), 6.80–7.40 (m, 8H); **6l** (n = 4, R_1 = 4-F, R_2 = H): 1.80–2.10 (m, 4H), 3.6 (t, 2H, J = 6.5 Hz), 3.70 (t, 2H, J = 6.5 Hz), 5.30 (s, 1H), 6.85–7.40 (m, 9H); **6m** (n = 4, R_1 = 4-OCH_3, R_2 = H): 1.70–1.90 (m, 4H), 3.6 (t, 2H, J = 6.5 Hz), 3.70 (t, 2H, J = 6.5 Hz), 3.80 (s, 3H), 5.28 (s, 1H), 6.80–7.40 (m, 9H); **6n** (n = 5, R_1 = R_2 = 4-F): 1.40–1.80 (m, 6H), 3.6 (t, 2H, J = 6.5 Hz), 3.70 (t, 2H, J = 6.5 Hz), 5.28 (s, 1H), 6.85–7.40 (m, 8H).

*3-Alkyl-4-piperidineacetic acid ethyl ester **9a** and **9b**. Method A. Wittig–Horner reaction*

Two equivalents of NaH at room temperature. Triethylphosphonacetate (**10**) (7.95 g, 35 mmol) was added dropwise to a suspension of sodium hydride (1.47 g, 60 mmol) in toluene (20 mL) cooled to 16 °C. During the addition, the temperature was kept <20 °C. Then the reaction mixture was stirred for 1 h at room temperature. This was followed by addition dropwise, at <20 °C, of 1-benzyl-3-alkyl-4-piperidone (**7a, 7b**) (29 mmol) dissolved in toluene (20 mL). The reaction was stirred at room temperature. The reaction was monitored by thin layer chromatography. After the starting material had disappeared, ice-water (5 mL) was added. The organic layer was separated, dried and evaporated, and the residue was chromatographed on silica column (eluent: AcOEt/cyclohexane/Et_3N 5:95:0.2) to yield **8a** (R_3 = CH_3) global yield 75% (**8a1** 15%, **8a2** 19%, **8a3** 9%, **8a4** 57%); **8b** (R_3 = C_2H_5) global yield 68% (**8b1** 14.5%, **8b2** 17%, **8b3** 10.7%, **8b4** 57.8%).

Two equivalents of NaH at 70 °C. This was the same process as above, but after the addition of 1-benzyl-3-alkyl-4-piperidone (**7a, 7b**), the reaction mixture was heated to 70 °C. **8a4** (R_3 = CH_3) yield 70%; **8b4** (R_3 = C_2H_5) yield 64%.

One equivalent of NaH at 70 °C. This was the same process as above, but one equivalent of NaH was used. **8a** (R_3 = CH_3) global yield 65% (**8a1** 35%, **8a2** 65%); **8b** (R_3 = C_2H_5) global yield 62% (**8b1** 30%, **8b2** 70%). ^{1}H-NMR ($CDCl_3$) δ (*cis*) **8a1** (R_3 = CH_3): 1.00 (d, 3H, J = 7 Hz), 1.16 (t, 3H, J = 7 Hz), 1.50–3.20 (m, 7H), 3.45 (s, 2H), 4.10 (q, 2H, J = 7 Hz), 5.57 (d, 1H, J = 1.8 Hz), 7.30 (m, 5H); (*trans*) **8a2** (R_3 = CH_3): 1.05 (d, 3H, J = 7 Hz), 1.25 (t, 3H, J = 7 Hz), 1.80–2.85 (m, 7 H), 3.48 (s, 2H), 4.10 (q, 2H, J = 7 Hz), 5.61 (d, 1H, J = 0.5 Hz), 7.30 (m, 5H); **8a3** (R_3 = CH_3): 1.00 (d, 3H, J = 7 Hz), 1.23 (t, 3H, J = 7 Hz), 2.10–2.70 (m, 3H), 3.00 (m, 4H), 3.55 (s, 2H), 4.12 (q, 2H, J = 7 Hz), 5.51 (t, 1H, J = 5 Hz), 7.30 (m, 5H); **8a4** (R_3 = CH_3): 1.23 (t, 3H, J = 7 Hz), 1.61 (s, 3H), 2.20 (m, 2H), 2.51 (m, 2H), 2.88 (s, 2H), 3.02 (s, 2H), 3.56 (s, 2H), 4.10 (q, 2H, J = 7 Hz), 7.30 (m, 5H); (*cis*) **8b1** (R_3 = C_2H_5): 0.84 (t, 3H, J = 7 Hz), 1.25 (t, 3H, J = 7 Hz), 1.6 (m, 2H), 1.80–3.10 (m, 7H), 3.44 (s, 2H), 4.10 (q, 2H, J = 7 Hz), 5.63 (d, 1H, J = 1.8 Hz), 7.30 (m, 5H); (*trans*) **8b2** (R_3 = C_2H_5): 0.80 (t, 3H, J = 7 Hz), 1.25 (t, 3H, J = 7 Hz), 1.6 (m, 2H), 1.80–3.20 (m, 7H), 3.45 (s, 2H), 4.10 (q, 2H, J = 7 Hz), 5.61 (d, 1H, J = 0.5 Hz), 7.30 (m, 5H); **8b3** (R_3 = C_2H_5): 0.80 (t, 3H, J = 7 Hz), 1,24 (t, 3H, J = 7 Hz), 1.55 (m, 2H), 1.8–2.8 (m, 3H), 3.00 (m, 4H), 3.55 (s, 2H), 4.12 (q, 2H, J = 7 Hz), 5.55 (t, 1H, J = 5 Hz), 7.30 (m, 5H); **8b4** (R_3 = C_2H_5): 0.95 (t, 3H, J = 7 Hz), 1.24 (t, 3H, J = 7 Hz), 1.6–2.6 (m, 6H), 2.90 (s, 2H), 3.05 (s, 2H), 3.57 (s, 2H), 4.12 (q, 2H, J = 7 Hz), 7.30 (m, 5H).

*cis and trans 3-Alkyl-4-piperidineacetic acid ethyl esters **9a** and **9b***

Route a. To a solution of **8a1**, **8a2**, **8a3**, **8a4** or **8b1**, **8b2**, **8b3**, **8b4** hydrochloride (64 mmol) in ethanol (150 mL), was added Pd/C (10%) (2 g), and the mixture was hydrogenated at 70 atm and 60 °C. The reaction was monitored by thin layer chromatography and GC-MS. After the starting material had disappeared, the catalyst was removed by filtration and the filtrate was evaporated under vacuum. The residue was dissolved in ice-water (50 mL), neutralized with aqueous sodium hydroxide, and the mixture was extracted with ether (3 x 100 mL). The ether extract was dried, filtered, and evaporated. The product thus obtained was purified by distillation. **9a** (R_3 = CH_3): bp_{9mm} 112–114 °C, yield 67% of *cis* isomer and 33% of *trans* isomer (global yield 78%); **9b** (R_3 = C_2H_5): $bp_{9.5mm}$ 118–120 °C, yield 70% of *cis* isomer and 30% of *trans* isomer (global yield 72%). The hydrogenation of 1-benzyl-3-methyl-1,2,5,6-tetrahydro-4-pyridineacetic acid ethyl ester hydrochloride **8a4** in the same conditions yielded a mixture of 72% of *cis* isomer and 28% of *trans* isomer.

Routes b and c. 3-Methyl-1,2,5,6-tetrahydro-4-pyridineacetic acid ethyl ester. Method 1. Debenzylation by HCOOH, Pd/C. A mixture of 1-benzyl-3-methyl-1,2,5,6-tetrahydro-4-pyridineacetic acid ethyl ester **8a4** (36.7 g, 130 mmol), ethanol (730 mL), formic acid (36.7 g) and Pd/C (10%) (36.7 g) was stirred at room temperature. The reaction was monitored by thin layer chromatography and GC-MS. After the starting material had disappeared, the catalyst was removed by filtration and the filtrate was evaporated under vacuum. The residue was dissolved in 100 mL of ice-water and neutralized with aqueous sodium hydroxide, and the mixture was extracted with ether (3 x 150 mL). The ether extract was dried, filtered, and evaporated to yield 24g (98%). ^{1}H-NMR ($CDCl_3$) δ 1.26 (t, 3H, J = 7 Hz), 1.61 (s, 3H), 2.10 (t, 2H, J = 6.5 Hz), 2.45 (s, NH), 2.90 (t, 2H, J = 6.5 Hz), 3.10 (s, 2H), 3.25 (s, 2H); 4.15 (q, 2H, J = 7 Hz).

Method 2. Debenzylation by chloroethylchloroformate. To a solution of **8a4** (R_3 = CH_3) (0.5 g, 1.7 mmol) in 1,2-dichloroethane (DCE) (10 mL) cooled to 0 °C, was added dropwise 1-chloroethylchloroformate (0.29 g, 2 mmol) in DCE (1 mL). Then the reaction was refluxed for 4 h. The solvent was evaporated under vacuum, ethanol (15 mL) was added, and the reaction mixture was refluxed for 2 h. The solvent was evaporated and the crude product was taken up in ice-water (10 mL) and neutralized with aqueous sodium hydroxide. The mixture was extracted with ether (3 x 20 mL). The ether extract was dried, filtered and evaporated to yield 0.2 g (60%).

*cis-3-Methyl-4-piperidineacetic acid ethyl ester cis-**9a***

To a solution of 3-methyl-1,2,5,6-tetrahydro-4-pyridineacetic acid ethyl ester hydrochloride (23 g, 100 mmol) in ethanol (100 mL), the catalyst was added (route b: Pd/C; route c: PtO_2), and the mixture was hydrogenated at 50 atm at room temperature. The reaction was monitored by GC-MS. The catalyst was removed and the filtrate was evaporated under vacuum. The

residue was dissolved in ice-water (70 mL), neutralized with aqueous sodium hydroxide, and the mixture was extracted with ether (3 x 150 mL). The ether extract was dried, filtered, evaporated and distilled to yield **9a**. Route b (Pd/C): yield 74% (72% of *cis* isomer and 28% of *trans* isomer); route c (PtO_2): yield 77% (98% of *cis* isomer and 2% of *trans* isomer).

*3-Methyl-4-piperidineacetic acid ethyl ester **9a**. Method B. 3-Methyl-4-pyridineacetic acid ethyl ester **12***
Diisopropylamine (229 mL, 165.3 g, 1.63 mol) was added dropwise at –60 °C to a solution of *n*-butyllithium (1.6 M) in hexane (931 mL, 1.49 mol). Then, 3,4-lutidine (**11**) (80 g, 0.75 mol), dissolved in 1000 mL of anhydrous tetrahydrofuran, was added, and the reaction mixture was stirred for 30 min. This was followed by addition of diethylcarbonate (219 mL, 213.5 g, 1.81 mol), dissolved in anhydrous tetrahydrofuran (1 L). An extensive red precipitate was formed during the last addition. Stirring was continued at –60 °C for another 30 min; then the dry-ice bath was removed and the reaction was allowed to warm up for 2 h with stirring. 1500 mL of toluene was added, followed by 150 mL of saturated aqueous sodium sulfate solution. Stirring was continued until the organic precipitate was dissolved, and the solution was then dried over anhydrous sodium sulfate. Sodium sulfate was removed by filtration, and the filtrate evaporated to give a crude product which was dissolved in ether (1 L); this solution was first shaken with ice-water (200 mL) and concentrated hydrochloric acid (150 mL) and then twice with 1 N hydrochloric acid (200 mL). The aqueous acidic layer was neutralized with concentrated ammonium hydroxide at ice temperature and extracted with dichloromethane (3 x 500 mL). The extract was dried over anhydrous sodium sulfate, filtered and evaporated to give 233 g of crude product **12**. The product was further purified by distillation to yield 112 g (83.7%) of pure product **12**. $bp_{2.5mm}$ 104–106 °C; ^{1}H-NMR ($CDCl_3$) δ 1.25 (t, 3H, J = 7 Hz), 2.03 (s, 3H), 3.60 (s, 2H), 4.20 (q, 2H, J = 7 Hz), 7.15 (d, 1H, J = 5 Hz), 8.40 (m, 2H).

*Racemic cis-3-methyl-4-piperidineacetic acid ethyl ester cis **9a***
To the solution of 3-methyl-4-pyridineacetic acid ethyl ester (**12**) hydrochloride (50 g, 0.23 mol), dissolved in water (75 mL), ethanol (75 mL) and concentrated hydrochloric acid (0.75 mL), was added platinum oxide (0.6 g), and the mixture was hydrogenated at 70 atm. The catalyst was removed by filtration, and the filtrate was evaporated under vacuum. The residue was dissolved in ice-water (150 mL), neutralized with aqueous sodium hydroxide and the mixture was extracted with ether (3 x 150 mL). The ether extract was dried, filtered and evaporated. The product thus obtained was purified by distillation to yield 36 g (84%) of 96% pure *cis* isomer and 4% of *trans* isomer (if Pd/C was used as catalyst the purity was 82% of *cis* and 18% of *trans*); bp 112–114 °C (9 mmHg); ^{1}H-NMR ($CDCl_3$) δ 0.90 (d, 3H, J = 7 Hz), 1.15 (t, 3H, J = 7 Hz), 1.56 (s, NH), 1.30–3.20 (m, 10H), 4.15 (q, 2H, J = 7 Hz).

*cis-3(R)-Methyl-4(S)-piperidineacetic acid ethyl ester (**cis-3R, 4S-9a**)*
Racemic *cis*-3-methyl-4-piperidineacetic acid ethyl ester (**cis 9a**) (30 g, 0.16 mol) and 2,3:4,6-di-*O*-isopropilidene-2-keto-L-gluconic acid monohydrate (DAG) (47.25 g, 0.16 mol) were dissolved in hot ethyl acetate (440 mL) and hot ethanol (96%) (70 mL) and cooled to room temperature. Several crops of *cis*-3(*R*)-methyl-4(*S*)-piperidineacetic acid ethyl ester mono L-DAG were obtained. After three recrystallizations from ethyl acetate/ethanol (96%) (86:14), the yield was 6.18%; mp 117–178 °C; ee = 100%.

*cis-3(S)-Methyl-4(R)-piperidineacetic acid ethyl ester cis-**3S, 4R-9a***
The L-DAG mother liquors were converted into the free base. This base (9.5 g, 51 mmol) was then reacted with *d*(–)tartric acid (7.7 g, 51 mmol) in hot ethanol (96%) (517 mL) and cooled to room temperature. Several crops of *cis*-3(*S*)-methyl-4(*R*)-piperidineacetic acid ethyl ester mono *d*(–)-tartrate were obtained. After seven recrystallizations from ethanol (96%) the yield was 2.58 g (5%); mp 161–162 °C; ee = 81.5%.

*Compounds **II** (**13–48**)*

*Process A. General procedure. cis and trans Benzhydryloxyalkyl-4-piperidineacetic acid ethyl ester **13–69**.* A mixture of benzhydryloxyalkyl chloride **6a–n** (29 mmol), 3-alkyl-4-piperidine ethyl acetate **9a**, **9b** (29 mmol), potassium carbonate (8.8 g, 64 mmol) and sodium iodide (1 g, 6 mmol) in 150 mL of acetonitrile was refluxed for 24 h. The solvent was evaporated to dryness, the residue was taken up with water and extracted with dichloromethane. The organic layer was separated, dried and evaporated and the residue was chromatographed on a silica column (eluent, 20% ethyl acetate/cyclohexane) to give two products *cis* and *trans* **II** (**13–44**).

Preparation of the oxalate. The base was dissolved in alcohol (ethanol or isopropanol), an alcoholic solution of oxalic acid (1 equiv) was added and the salt crystallized out. Physicochemical and spectral data are shown in tables I and III.

*Process B. General procedure. cis and trans Benzhydryloxyalkyl-4-piperidineacetic acid **45–47**.* The ester **II** (**19**, **31**, **32**) (3 mmol) in a solution of 10% alcoholic potassium hydroxide was stirred at room temperature. The reaction was monitored by thin layer chromatography. After the starting material had disappeared, alcohol was evaporated off and the residual oil was taken up with water (30 mL). Hydrochloride acid (6 N) was then added to pH 5.4. The product was extracted with dichloromethane.

Preparation of the oxalate. The obtained product was dissolved in 2-butanone, one equivalent of oxalic acid dissolved in 2-butanone was added and the salt crystallized out. Physicochemical and spectral data are reported in tables I and III.

*Process C. cis 1-{1-{bis(4-Fluorophenyl)methoxy}-4-butyl}-3-methyl-4-diethylaminocarbonylmethylpiperidine **48**.* Dicyclocarbodiimide (DCC) (2 g, 9.7 mmol) in $CHCl_3$ (10 mL) was added dropwise to a mixture of acid **II** (**46**) (10 mmol), diethylamine (0.73 g, 10 mmol) and 1-hydroxybenzotriazole (HOBT) (1.35 g, 10 mmol) in $CHCl_3$ (30 mL). The reaction was stirred at room temperature under N_2 overnight. The DCU was removed by filtration. This solution was washed with 1 N NaOH, the organic layer was dried and concentrated to give a residue which was chromatographied on flash column (eluent: ethyl acetate).

Preparation of the oxalate. The base was dissolved in 2-propanol, oxalic acid (1 equiv) in 2-propanol was added and the salt crystallized out. Physichochemical and spectral data are shown in tables I and III.

Pharmacology

Animals and experimental conditions
The animals used in this study were male NMRI mice (18–22 g) obtained from IFFA-CREDO (France). They were delivered at least 6 days before the experiments and housed in

Table III. ^{1}H-NMR data of compounds **13–74**.

Compound	*Chemical shifts vs TMS*
13	0.95 (d, 3H, J = 7 Hz), 1.25 (t, 3H, J = 7 Hz), 1.40–2.80 (m, 12H), 3.55 (t, 2H, J = 6 Hz), 4.15 (q, 2H, J = 7 Hz), 5.38 (s, 1H), 6.85–7.50 (m, 8H)
14	0.95 (d, 3H, J = 7 Hz), 1.25 (t, 3H, J = 7 Hz), 1.40–2.80 (m, 12H), 3.57 (t, 2H, J = 6 Hz), 4.15 (q, 2H, J = 7 Hz), 5.42 (s, 1H), 6.8–7.6 (m, 8H)
15	0.90 (t, 3H, J = 7 Hz), 1.17 (t, 3H, J = 7 Hz), 1.40–2.75 (m, 14H), 3.55 (t, 2H, J = 6 Hz), 4.13 (q, 2H, J = 7 Hz), 5.35 (s, 1H), 6.80–7.40 (m, 8H)
16	0.96 (t, 3H, J = 7 Hz), 1.24 (t, 3H, J = 7 Hz), 1.40–3.10 (m, 14H), 3.62 (t, 2H, J = 6 Hz), 4.12 (q, 2H, J = 7 Hz), 5.35 (s, 1H), 6.80–7.40 (m, 8H)
17	0.85 (d, 3H, J = 7 Hz), 1.24 (t, 3H, J = 7 Hz), 1.40–2.70 (m, 14H), 3.46 (t, 2H, J = 6 Hz), 4.12 (q, 2H, J = 7 Hz), 5.27 (s, 1H), 6.80–7.40 (m, 8H)
18	0.90 (d, 3H, J = 7 Hz), 1.25 (t, 3H, J = 7 Hz), 1.40–3.00 (m, 14H), 3.46 (t, 2H, J = 6 Hz), 4.13 (q, 2H, J = 7 Hz), 5.28 (s, 1H), 6.80–7.50 (m, 8H)
19	0.88 (d, 3H, J = 7 Hz), 1.22 (t, 3H, J = 7 Hz), 1.40–2.70 (m, 14H), 3.51 (t, 2H, J = 6 Hz), 4.12 (q, 2H, J = 7 Hz), 5.26 (s, 1H), 6.80–7.50 (m, 8H)
20	0.90 (d, 3H, J = 7 Hz), 1.24 (t, 3H, J = 7 Hz), 1.40–3.00 (m, 14H), 3.50 (t, 2H, J = 6 Hz), 4.13 (q, 2H, J = Hz), 5.26 (s, 1H), 6.80–7.60 (m, 8H)
21	0.90 (t, 3H, J = 7 Hz), 1.23 (t, 3H, J = 7 Hz), 1.40–2.60 (m, 16H), 3.49 (t, 2H, J = 6 Hz), 4.12 (q, 2H, J = 7 Hz), 5.32 (s, 1H), 7.30 (m, 10H)
22	0.93 (t, 3H, J = 7 Hz), 1.23 (t, 3H, J = 7 Hz), 1.40–3.10 (m, 16H), 3.51 (t, 2H, J = 6 Hz), 4.11 (q, 2H, J = 7 Hz), 5.33 (s, 1H), 7.30 (m, 10H)
23	0.90 (t, 3H, J = 7 Hz), 1.24 (t, 3H, J = 7 Hz), 1.40–3.10 (m, 16H), 3.45 (t, 2H, J = 6 Hz), 4.13 (q, 2H, J = 7 Hz), 5.29 (s, 1H), 6.80–7.40 (m, 8H)
24	0.93 (t, 3H, J = 7 Hz), 1.24 (t, 3H, J = 7 Hz), 1.40–3.10 (m, 16H), 3.45 (t, 2H, J = 6 Hz), 4.13 (q, 2H, J = 7 Hz), 5.29 (s, 1H), 6.80–7.40 (m, 8H)
25	0.88 (d, 3H, J = 7 Hz), 1.25 (t, 3H, J = 7 Hz), 1.30–2.70 (m, 14H), 3.48 (t, 2H, J = 6 Hz), 4.12 (q, 2H, J = 7 Hz), 5.29 (s, 1H), 6.80–7.40 (m, 8H)
26	0.94 (d, 3H, J = 7 Hz), 1.24 (t, 3H, J = 7 Hz), 1.30–3.00 (m, 14H), 3.48 (t, 2H, J = 6 Hz), 4.13 (q, 2H, J = 7 Hz), 5.28 (s, 1H), 6.80–7.40 (m, 8H)
27	0.88 (d, 3H, J = 7 Hz), 1.25 (t, 3H, J = 7 Hz), 1.30–2.60 (m, 14H), 3.47 (t, 2H, J = 6 Hz), 4.12 (q, 2H, J = 7 Hz), 5.27 (s, 1H), 6.8–70.40 (m, 9H)
28	0.90 (d, 3H, J = 7 Hz), 1.23 (t, 3H, J = 7 Hz), 1.30–3.00 (m, 14H), 3.47 (t, 2H, J = 6 Hz), 4.11 (q, 2H, J = 7 Hz), 5.29 (s, 1H), 6.80–7.40 (m, 9H)
29	0.90 (d, 3H, J = 7 Hz), 1.25 (t, 3H, J = 7 Hz), 1.30–3.10 (m, 14H), 3.51 (t, 2H, J = 6 Hz), 4.13 (q, 2H, J = 7 Hz), 5.37 (s, 1H), 7.20–7.60 (m, 9H)
30	0.87 (d, 3H, J = 7 Hz), 1.25 (t, 3H, J = 7 Hz), 1.30–2.60 (m, 14H), 3.51 (t, 2H, J = 6 Hz), 4.13 (q, 2H, J = 7 Hz), 5.37 (s, 1H), 7.20–7.60 (m, 9H)
31	0.87 (d, 3H, J = 7 Hz), 1.25 (t, 3H, J = 7 Hz), 1.40–2.70 (m, 16H), 3.43 (t, 2H, J = 6 Hz), 4.13 (q, 2H, J = 7 Hz), 5.28 (s, 1H), 6.80–7.40 (m, 8H)
32	0.90 (d, 3H, J = 7 Hz), 1.25 (t, 3H, J = 7 Hz), 1.40–3.00 (m, 16H), 3.43 (t, 2H, J = 6 Hz), 4.13 (q, 2H, J = 7 Hz), 5.28 (s, 1H), 6.80–7.40 (m, 8H)

Table III. *Continued.*

Compound	*Chemical shifts vs TMS*
33	0.95 (d, 3H, J = 7 Hz), 1.25 (t, 3H, J = 7 Hz), 1.40–3.00 (m, 16H), 3.47 (t, 2H, J = 6 Hz), 4.13 (q, 2H, J = 7 Hz), 5.26 (s, 1H), 6.80–7.50 (m, 8H)
34	0.87 (d, 3H, J = 7 Hz), 1.24 (t, 3H, J = 7 Hz), 1.40–2.60 (m, 16H), 3.46 (t, 2H, J = 6 Hz), 4.12 (q, 2H, J = 7 Hz), 5.25 (s, 1H), 6.80–7.50 (m, 8H)
35	0.94 (t, 3H, J = 7 Hz), 1.25 (t, 3H, J = 7 Hz), 1.30–3.00 (m, 18H), 3.42 (t, 2H, J = 6 Hz), 4.13 (q, 2H, J = 7 Hz), 5.28 (s, 1H), 6.80–7.40 (m, 8H)
36	0.90 (t, 3H, J = 7 Hz), 1.25 (t, 3H, J = 7 Hz), 1.30–2.60 (m, 18H), 3.43 (t, 2H, J = 6 Hz), 4.13 (q, 2H, J = 7 Hz), 5.28 (s, 1H), 6.80–7.40 (m, 8H)
37	0.89 (d, 3H, J = 7 Hz), 1.24 (t, 3H, J = 7 Hz), 1.30–2.60 (m, 16H), 3.44 (t, 2H, J = 6 Hz), 4.13 (q, 2H, J = 7 Hz), 5.30 (s, 1H), 6.80–7.30 (m, 9H)
38	0.92 (d, 3H, J = 7 Hz), 1.24 (t, 3H, J = 7 Hz), 1.30–3.00 (m, 16H), 3.44 (t, 2H, J = 6 Hz), 4.13 (q, 2H, J = 7 Hz), 5.30 (s, 1H), 6.80–7.40 (m, 9H)
39	0.90 (d, 3H, J = 7 Hz), 1.25 (t, 3H, J = 7 Hz), 1.30–2.70 (m, 16H), 3.50 (t, 2H, J = 6 Hz), 4.13 (q, 2H, J = 7 Hz), 5.30 (s, 1H), 7.35 (m, 10H)
40	0.93 (d, 3H, J = 7 Hz), 1.25 (t, 3H, J = 7 Hz), 1.30–3.00 (m, 16H), 3.50 (t, 2H, J = 6 Hz), 4.13 (q, 2H, J = 7 Hz), 5.30 (s, 1H), 7.13 (m, 10H)
41	0.90 (d, 3H, J = 7 Hz), 1.25 (t, 3H, J = 7 Hz), 1.40–2.70 (m, 16H), 3.45 (t, 2H, J = 6 Hz), 3.78 (s, 3H), 4.15 (q, 2H, J = 7 Hz), 5.30 (s, 1H), 6.80–7.40 (m, 9H)
42	0.93 (d, 3H, J = 7 Hz), 1.25 (t, 3H, J = 7 Hz), 1.40–3.00 (m, 16H), 3.45 (t, 2H, J = 6 Hz), 3.76 (s, 3H), 4.15 (q, 2H, J = 7 Hz), 5.30 (s, 1H), 6.75–7.40, m, 9H)
43	0.90 (d, 3H, J = 7 Hz), 1.25 (t, 3H, J = 7 Hz), 1.30–2.70 (m, 18H), 3.40 (t, 2H, J = 6 Hz), 4.13 (q, 2H, J = 7 Hz), 5.30 (s, 1H), 6.85–7.40 (m, 8H)
44	0.95 (d, 3H, J = 7 Hz), 1.25 (t, 3H, J = 7 Hz), 1.30–2.70 (m, 18H), 3.40 (t, 2H, J = 6 Hz), 4.13 (q, 2H, J = 7 Hz), 5.30 (s, 1H), 6.80–7.50 (m, 8H)
45	0.98 (d, 3H, J = 7 Hz), 1.40–2.80 (m, 14H), 3.50 (t, 2H, J = 6 Hz), 5.25 (s, 1H), 6.90–7.50 (m, 8H), 10.80 (s, 1H)
46	0.95 (d, 3H, J = 7 Hz), 1.50–3.00 (m, 16H), 3.41 (t, 2H, J = 6 Hz), 5.27 (s, 1H), 6.80–7.40 (m, 8H), 11.19 (s, 1H)
47	0.98 (d, 3H, J = 7 Hz), 1.10–3.00 (m, 16H), 3.40 (t, 2H, J = 6 Hz), 5.28 (s, 1H), 6.70–7.50 (m, 8H), 11.60 (s, 1H)
48	0.90–1.25 (m, 11H), 1.40–2.80 (m, 14H), 3.20–3.50 (m, 6H), 5.30 (s, 1H), 6.80–7.40 (m, 8H)

macrolon cages with free access to solid food and water. Animals were maintained in a non-inverted light/dark cycle. The ambient temperature in animal rooms and laboratory was maintained at 22 ± 2 °C and humidity at 55 ± 15%.

Drugs

All drugs were dissolved in distilled water or were suspended in 0.5% aqueous arabic gum solution when not hydrosoluble. The drugs were administered ip or po at a volume of 1 mL/100 g body weight.

Statistical analysis of results

For the in vivo tests, results were expressed as ED_{50} (confidence limits), dose producing 50% response, compared to control groups. For non-linear responses results were given as minimal significant dose.

For the in vitro tests, the receptor binding assays were conducted using methods and conditions reported in table IV. The affinity of the tested ligands on muscarinic receptors and uptake sites was expressed as k_i ± standard errors and calculated using Graphpad software.

Table IV. Binding conditions.

Receptor	*5-HT uptake*	*Dopamine uptake*	*Dopamine uptake*	*Noradrenaline uptake*
Radioligand	[^{3}H]Paroxetine 0.12 nM	[^{3}H]GBR 12935 1 nM	[^{125}I]RTI 0.1 nM	[^{3}H]Nisoxetine 0.8 nM
Non-specific binding	Fluoxetine 10^{-5} M	GBR 12909 10^{-5} M	GBR 12909 10^{-5}M	Desipramine 10^{-5} M
Structure	Rat brain without cerebellum	Pig striatum	Pig striatum	Rat cortex
Protein (mg•mL^{-1})	0.50	0.25	0.40	0.80
Reference compounds	Fluoxetine, imipramine	GBR 12909, GBR 12935	GBR 12909, GBR 12935	Maprotiline desipramine
Binding buffer	Tris HCl 50 mM pH 7.4 NaCl 120 mM KCl 5 mM	Tris HCl 50mM pH 7.4 NaCl 120 mM KCl 5 mM	Tris HCl 50 mM pH 7.4 NaCl 120 mM KCl 5 mM	Tris HCl 50 mM pH 7.4 NaCl 300 mM KCl 5 mM
Incubation time	60 min	90 min	90 min	240 min
Temperature	22 °C	4 °C	4 °C	4 °C

Spontaneous motor activity [43]
Animals were individually placed in Plexiglas cages placed in the actimeter either 30 min after ip dosing or 60 min after oral administration of tested compounds. Motor activity was recorded 30 and 60 min after introduction of Plexiglas boxes into the actimeter. Control groups received only distilled water.

Antagonism of hypothermia and palpebral ptosis induced by reserpine [44]
Animals were treated with tested compounds 60 min before injection of 2 mg•kg^{-1} of reserpine. Palpebral ptosis was quoted from 0 to 4 for each eye and rectal temperature was recorded 4, 4.5, 5, 5.5 and 6 h after the final administration. Control groups received only reserpine.

Antagonism of apomorphine induced hypothermia [23]
Tested compounds were administered ip or po 30 or 60 min before ip injection of apomorphine 16 mg•kg^{-1}. Rectal temperatures were recorded 30 min later. Treated groups were compared with control groups that received only apomorphine.

Antagonism of oxotremorine hypothermia [46]
Rectal temperatures were recorded in animals treated ip or po with tested compounds; 30 min later animals received 0.5 mg•kg^{-1} ip of oxotremorine and temperatures were recorded 30 min after this injection. Treated groups were compared with control groups that received only oxotremorine.

Forced swimming test [47]
Animals were treated by ip or po route with tested compounds or with distilled water for control groups 30 min before testing. They were individually placed in a beaker filled with water and duration of immobility was recorded over a period of 5 min. Duration of immobility was then compared with control groups. Potential antidepressant drugs decrease duration of immobility of immersed mice.

Tail suspension test (TST) [48]
Animals were treated ip or po 30 or 60 min before testing. They were suspended by the tail and duration of immobility was recorded via a computerized device. Duration of immobility of treated groups was compared with a control group only treated with distilled water.

Increase of tryptamine convulsions [49]
Rats received ip either tested compounds or distilled water for the control group, and 30 min after an intravenous injection of 10 mg•kg^{-1} of tryptamine. Duration of convulsions was stopwatched and symptoms were quoted from 0 to 3.

Increase of yohimbine toxicity [50]
Animals were treated ip or po with tested compounds 30 or 60 min after ip injection of 25 mg•kg^{-1} of yohimbine. In each group the number of dead animals was recorded after 24 h and mortality was compared with control group only treated with distilled water.

Approximate LD_{50} according to Lorke [51]
Compounds were administered to three groups of three mice ip or po in doses of 5, 50 and 500 mg•kg^{-1}. The number of dead animals was recorded 24 h later. A computerized method allowed determination of four new doses that were administered to four groups of a single mouse. Mortality rate at 24 h enabled the calculation of an approximate LD_{50}.

Binding studies
Compounds were assayed for each type or reuptake site and/or receptor with 11 concentrations ranging from 10^{-4} to 10^{-10} M. Two reference products were simultaneously used. [^{3}H]Paroxetine [52, 53], [^{3}H]nisoxetine [54], and [^{3}H]GBR 12935 [55, 56] were respectively used for labelling serotonin, noradrenaline, dopamine reuptake sites. Binding conditions are reported in table IV.

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