Eur. J. Med. Chem. 35 (2000) 107–121

S0223523400001136/FLA

Original article

Synthesis and structure–activity relationships of novel arylalkyl 4-benzyl piperazine derivatives as σ site selective ligands

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Received 5 January 1999; accepted 26 April 1999

Abstract – Continuing our previous work that established that some chromones substitued by an aryl alkyl piperazino alkyl side chain are potent and selective sigma ligands and could be interesting in the treatment of psychosis, we synthesized 60 new compounds, replacing the chromone moiety by various cyclic systems. Many derivatives bind to the sigma sites in the nanomolar range and are generally selective in comparison with $5HT_{1A}$ and the D_2 receptors. One of the most potent ligands of these series, 1-(2-naphthyl methyl)-4-benzyl piperazine **29**, has been studied in various pharmacological tests. Although it doesn't have potential in the treatment of psychosis, the results we obtained confirm the data which indicates that such derivatives could be interesting in the treatment of inflammatory diseases. © 2000 Éditions scientifiques et médicales Elsevier SAS

sigma ligand / benzylpiperazine / psychosis / inflammation

1. Introduction

ln a previous paper [1], we reported the reasons why the synthesis of selective sigma ligands interested us after the result of a random screening showing that 2-[(4-benzyl-piperazinyl)methyl]chromone **1** was a sigma ligand in the nanomolar range. In the first part of this work we carried out the synthesis of 28 new compounds keeping the chromone moiety of the molecule. Their pharmacological study proved that many of these derivatives are potent ligands to the sigma sites and that some of them are potentially interesting for the treatment of psychosis. Because of these encouraging results we decided to continue with the pharmacomodulation of the chromone ring.

First we replaced it by a chroman or a 2H-chromene. Then we decreased the steric hindrance of this structure and synthesized the phenoxy alkane analogues, and in a more general way, we introduced on the 1-benzyl piperazine moiety various heterocyclic or arylalkyl systems.

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For all these compounds the affinity for the sigma site is reported, as well as for the $5HT_{1A}$ and the D_2 receptors. For one of the most potent ligands, 1-(2-naphthylmethyl)-4-benzyl piperazine **29**, some complementary pharmacological tests directed to the central nervous system and inflammation areas have been carried out to check their potential in the treatment of psychosis and to examine the interest of such derivatives in the inflammatory diseases according to the results of previous works [2, 3].

2. Chemistry

Compounds have been synthesized according to *figure 1*.

The synthesis of N-arylalkyl-benzylpiperazine compounds has been carried out two ways. The first one includes the step of the acyl chloride that is treated with 1-benzylpiperazine optionally substituted (method 1) to give compounds **2**, **4–6**, **8**, **9**, **11**, **13**, **15**, **18**, **24**, **28**, **30**, **31**, **33**, **35**, **37**, **39**, **40**, **42**, **45**, **47**, **48**, **50**, **51**, **53**, **54**, **56**, **57** and **59**. Reduction of the amidic function of some of these compounds by lithium aluminium hydride (method 2)

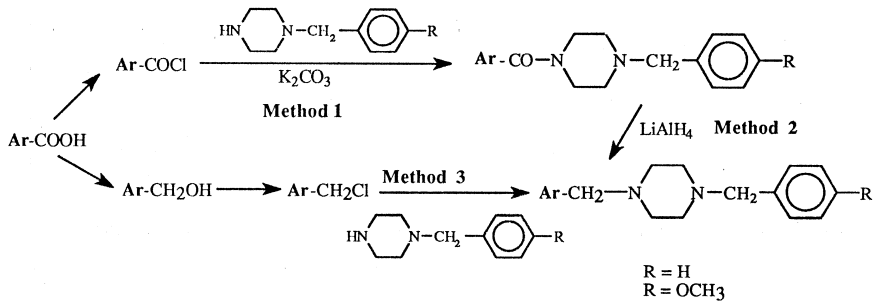
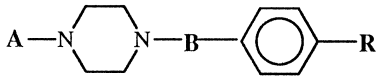


Figure 1

gives compounds **3**, **7**, **10**, **12**, **14**, **16**, **19**, **20**, **23**, **25**, **34**, **36**, **38**, **41**, **43**, **44**, **46**, **52**, **55** and **60** in base form. The third method consists of halogenating the primary alcoholic function by thionyl chloride. The halogenated compound is then reacted with 1-benzyl piperazine optionally substituted to obtain the desired tertiary amine in base form **1**, **17**, **21**, **22**, **26**, **27**, **29**, **32**, **49**, **58** and **61** (method 3). Bases are transformed into hydrochloride salts in hydrochloric isopropanol with almost quantitative yields.

Physico-chemical data for the hydrochlorides are summarized in *table I*.

Table I. Physico-chemical data of compounds **1**–**61**.



Compound	A	B	R	Prep[a]	Yield Base	M.p. (°C) HCl	Formula
1	2-chromone methyl	CH_2	H	3	36	238	$C_{21}H_{22}N_2O_2 \cdot 2HCl$
2	2-chromane carbonyl	CH_2	H	1	52	230	$C_{21}H_{24}N_2O_2 \cdot HCl$
3	2-chromane methyl	CH_2	H	2	83	> 230	$C_{21}H_{26}N_2O \cdot 2HCl$
4	2-chromane carbonyl	CH_2	OCH_3	1	65	198	$C_{22}H_{26}N_2O_3 \cdot HCl$
5	2-chromane carbonyl	CO	F	1	73	114	$C_{21}H_{21}FN_2O_3$
6	3-(5-methoxychromane)carbonyl	CH_2	H	1	63	237	$C_{22}H_{26}N_2O_3 \cdot HCl$
7	3-(5-methoxychromane)methyl	CH_2	H	2	85	240	$C_{22}H_{28}N_2O_2 \cdot 2HCl$
8	3-(5-methoxychromane)carbonyl	CH_2	OCH_3	1	31	222	$C_{23}H_{28}N_2O_4 \cdot HCl$
9	3-(5-methoxychromene)carbonyl	CH_2	H	1	72	234	$C_{22}H_{24}N_2O_3 \cdot HCl$
10	3-(5-methoxychromene)methyl	CH_2	H	2	85	216	$C_{22}H_{26}N_2O_2 \cdot 2HCl$
11	2-phenoxypropionyl	CH_2	H	1	82	258	$C_{20}H_{24}N_2O_2 \cdot HCl$
12	2-phenoxypropyl	CH_2	H	2	77	203	$C_{20}H_{26}N_2O \cdot 2HCl$
13	2-phenoxybutyryl	CH_2	H	1	79	210	$C_{21}H_{26}N_2O_2 \cdot HCl$
14	2-phenoxybutyl	CH_2	H	2	88	229	$C_{21}H_{28}N_2O \cdot 2HCl$
15	2-methyl-2-phenoxypropionyl	CH_2	H	1	75	212	$C_{21}H_{26}N_2O_2 \cdot HCl$
16	2-methyl-2-phenoxypropyl	CH_2	H	2	86	189	$C_{21}H_{28}N_20 \cdot 2HCl$
17	3-phenyl propyl	CH_2	H	3	70	218	$C_{20}H_{26}N_2 \cdot 2HCl$
18	2-phenoxyacetyl	CH_2	H	1	87	235	$C_{19}H_{22}N_2O_2 \cdot HCl$
19	2-phenoxyethyl	CH_2	H	2	81	234	$C_{19}H_{24}N_2O \cdot 2HCl$
20	2-benzofurylmethyl	CH_2	H	2	81	> 250	$C_{20}H_{22}N_2O \cdot 2HCl$
21	2-dihydrobenzofurylmethyl	CH_2	H	3	82	246	$C_{20}H_{24}N_2O \cdot 2HCl$
22	2-dihydrobenzofurylmethyl	CH_2	OCH_3	3	78	246	$C_{21}H_{26}N_20_2 \cdot 2HCl$
23	2-(5-fluorodihydrobenzofurylmethyl)	CH_2	H	2	85	214	$C_{20}H_{23}FN_2O \cdot 2HCl$

Table I. Continued.

Compound	A	B	R	Prep[a]	Yield Base	M.p. (°C) HCI	Formula
24	2-tetrahydronaphtoyl	CH_2	H	1	60	197	$C_{22}H_{26}N_2O\cdot HCl$
25	2-tetrahydronaphtylmethyl	CH_2	H	2	79	191	$C_{22}H_{28}N_2\cdot 2HCl$
26	7-methoxycoumarinylmethyl	CH_2	H	3	80	246	$C_{22}H_{24}N_2O_3\cdot 2HCl$
27	7-methoxycoumarinylmethyl	CH_2	OCH_3	3	83	208	$C_{23}H_{26}N_2O_4\cdot 2HCl$
28	2-naphthoyl	CH_2	H	1	73	238	$C_{22}H_{22}N_2O\cdot HCl$
29	2-naphthylmethyl	CH_2	H	3	76	230	$C_{22}H_{24}N_2\cdot 2HCl$
30	1-naphthoyl	CH_2	H	l	76	212	$C_{22}H_{22}N_2O\cdot HCl$
31	2-quinolylcarbonyl	CH_2	H	1	65	188	$C_{21}H_{21}N_3O\cdot 2HCl$
32	2-quinolylmethyl	CH_2	H	3	83	216	$C_{21}H_{23}N_3\cdot 3HCl$
33	α-methylcinnamoyl	XH_2	H	1	85	202	$C_{21}H_{24}N_2O.HCl$
34	α-methylcinnamyl	XH_2	H	2	78	246	$C_{21}H_{26}N_2\cdot 2HCl$
35	α-methylcinnamoyl	XH_2	OCH_3	1	89	238	$C_{22}H_{26}N_2O_2\cdot HCl$
36	α-methylcinnamyl	XH_2	OCH_3	2	82	248	$C_{22}H_{28}N_2O\cdot 2HCl$
37	4-chlorocinnamoyl	CH_2	H	1	70	220	$C_{20}H_{21}ClN_2O\cdot HCl$
38	4-chlorocinnamyl	CH_2	H	2	78	> 260	$C_{20}H_{23}ClN_2\cdot 2HCl$
39	4-methoxycinnamoyl	CH_2	H	1	81	250	$C_{21}H_{24}N_2O_2\cdot HCl$
40	3,4-dimethoxycinnamoyl	CH_2	H	1	56	211	$C_{22}H_{26}N_2O_3\cdot HCl$
41	3,4-dimethoxycinnamyl	CH_2	H	2	86	> 210	$C_{22}H_{28}N_2O_2\cdot 2HCl$
42	3,4-methylene dioxycinnamoyl	CH_2	H	1	81	256	$C_{2l}H_{22}N_2O_3\cdot HCl$
43	3,4-methylene dioxycinnamyl	CH_2	H	2	80	246	$C_{21}H_{24}N_2O_2\cdot 2HCl$
44	3,4,5-trimethoxycinnamyl	CH_2	H	2	81	238	$C_{23}H_{30}N_2O_3\cdot 2HCl$
45	2-(2-furyl)propenoyl	CH_2	H	1	75	254	$C_{18}H_{20}N_2O_2\cdot HCl$
46	2-(2-furyl)propenyl	CH_2	H	2	88	222	$C_{18}H_{22}N_2O\cdot 2HCl$
47	2-(2-thienyl)propenoyl	CH_2	H	1	86	230	$C_{18}H_{20}N_2OS\cdot HCl$
48	4-methoxyphenylacetyl	CH_2	H	1	65	240	$C_{20}H_{24}N_2O_2\cdot HCl$
49	4-methoxyphenylethyl	CH_2	H	3	71	250	$C_{20}H_{26}N_2O\cdot 2HCl$
50	4-chlorobenzoyl	CH_2	H	1	83	258	$C_{18}H_{19}ClN_2O\cdot HCl$
51	2-methoxybenzoyl	CH_2	H	1	88	> 260	$C_{19}H_{22}N_2O_2\cdot HCl$
52	2-methoxybenzyl	CH_2	H	2	86	248	$C_{19}H_{24}N_2O\cdot 2HCl$
53	4-methoxybenzoyl	CH_2	H	1	80	240	$C_{19}H_{22}N_2O_2\cdot HCl$
54	3,4-dimethoxybenzoyl	CH_2	H	1	64	154	$C_{20}H_{24}N_2O_3\cdot HCl$
55	3,4-dimethoxybenzyl	CH_2	H	2	80	> 220	$C_{20}H_{26}N_2O_2\cdot 2HCl$
56	3,4-methylene dioxybenzoyl	CH_2	H	1	82	> 260	$C_{19}H_{20}N_2O_3\cdot HCl$
57	2-pyridylcarbonyl	CH_2	H	1	75	213	$C_{17}H_{19}N_3O\cdot 2HCl$
58	2-pyridylmethyl	CH_2	H	3	68	248	$C_{17}H_{21}N_3\cdot 3HCl$
59	1-adamantane carbonyl	CH_2	H	1	66	250	$C_{22}H_{30}N_2O\cdot HCl$
60	2-furylmethyl	CH_2	H	2	82	> 250	$C_{16}H_{20}N_2O\cdot 2HCl$
61	6-uracyl methyl	CH_2	H	3	64	264	$C_{16}H_{20}N_4O_2\cdot 2HCl$

[a]Preparation = preparation method (see Experimental Section).

3. Pharmacology

All the compounds **1–61** were evaluated for their affinity for the sigma sites as well as for the $5HT_{1A}$ and the D_2 receptors.

For compounds **16**, **29** and **38**, having the highest affinity for the sigma sites, the binding for the two sigma subtypes was determined.

Compound **29** has also been studied on two tests predictive for antipsychotic activity.

— a biochemical one researching the effect on N-methyl-D-aspartate (NMDA).

— a behavioural one looking at the potential effect on hyperactivity induced by amphetamine and research for its potential cataleptic effect.

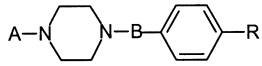
After the evidence that compound **29** had no antipsychotic activity we decided to research its anti-

inflammatory potential, since recent works mention this possibility for sigma ligands. The test we used is the carrageenan planta oedema.

4. Results and discussion

The results of binding of the 60 new compounds are indicated in *table II*. There are 30 amides and 30 amines according to the value of the A group. Some compounds have a binding affinity for the sigma site that is in the subnanomolar range. These are compounds **16**, **20**, **29**, **34**, **36** and **38**. The most potent compounds, **20** and **38**, have an IC_{50} between 0.3 and 0.4 nM. It appears that these six derivatives are all amines. In comparison, the most potent amides, **28** and **35**, have an IC_{50} which is 2 nM, that is to say almost ten-fold higher than that of the best amines. The most potent amines, except **29**, do not correspond exactly to the amides that best bind to the sigma sites.

Table II. Binding of 1-benzylpiperazines and related derivatives.



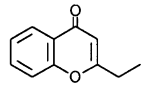
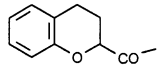
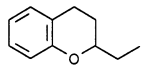
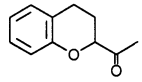
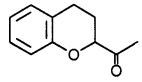
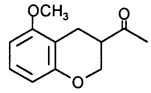
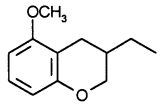
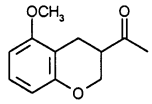
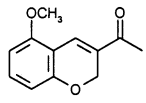
Compound	A	B	R	σ	$5HT_{1A}$	D_2
1		CH_2	H	3	$> 10^5$	$> 10^5$
2		CH_2	H	9	2×10^4	2 000
3		CH_2	H	10	10	100
4		CH_2	OCH_3	300	$> 10^5$	$> 10^5$
5		CO	F	3×10^4	$> 10^5$	$> 10^5$
6		CH_2	H	300	$> 10^5$	$> 10^5$
7		CH_2	H	20	50	400
8		CH_2	OCH_3	200	$> 10^5$	$> 10^5$
9		CH_2	H	800	$> 10^5$	$> 10^5$

Table II. Continued.

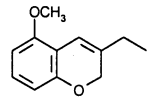
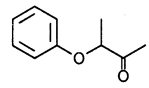
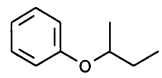
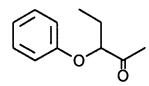
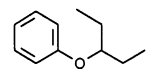
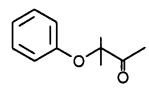
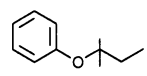
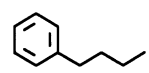
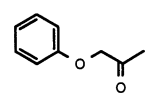
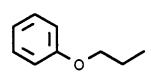
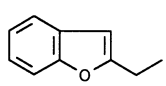
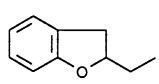
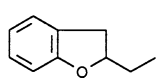
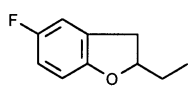
Compound	A	B	R	σ	$5HT_{1A}$	D_2
10		CH_2	H	10	300	300
11		CH_2	H	30	$> 10^5$	$> 10^5$
12		CH_2	H	4	1 000	700
13		CH_2	H	20	$> 10^5$	$> 10^5$
14		CH_2	H	30	300	$> 10^5$
15		CH_2	H	40	$> 10^5$	$> 10^5$
16		CH_2	H	0.9	$> 10^5$	$> 10^5$
17		CH_2	H	20	$> 10^5$	$> 10^5$
18		CH_2	H	40	$> 10^5$	$> 10^5$
19		CH_2	H	3	200	600
20		CH_2	H	0.4	5 000	1 000
21		CH_2	H	7	$> 10^5$	$> 10^5$
22		CH_2	OCH_3	9	80	600
23		CH_2	H	9	300	200

Table II. Continued.

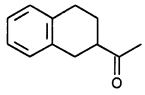
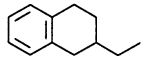
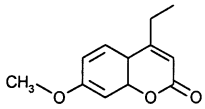
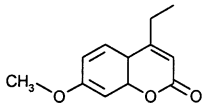
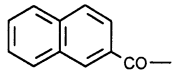
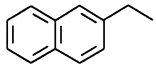
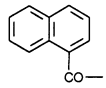
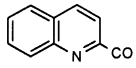
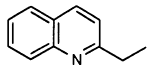
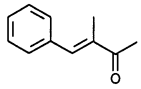
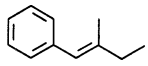
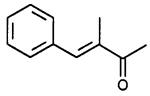
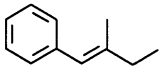
Compound	A	B	R	σ	$5HT_{1A}$	D_2
24		CH_2	H	400	$> 10^5$	$> 10^5$
25		CH_2	H	3	100	200
26		CH_2	H	1	10^4	2×10^4
27		CH_2	OCH_3	1	5×10^4	4 000
28		CH_2	H	2	8×10^4	900
29		CH_2	H	0.6	3 000	2 000
30		CH_2	H	7	4×10^4	3×10^4
31		CH_2	H	1 000	5 000	5 000
32		CH_2	H	1	2 000	9 000
33		CH_2	H	5	3×10^4	6 000
34		CH_2	H	0.7	300	400
35		CH_2	OCH_3	2	$> 10^5$	3 000
36		CH_2	OCH_3	0.8	900	500

Table II. Continued.

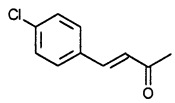
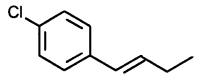
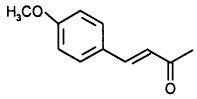
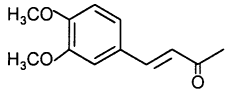
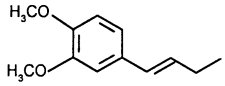
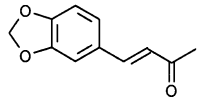
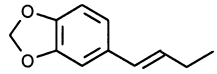
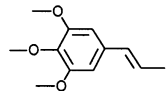
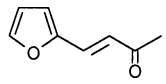
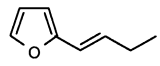
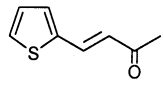
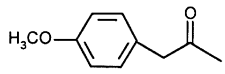
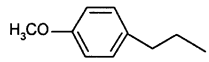
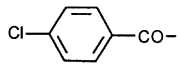
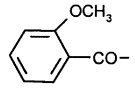
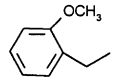
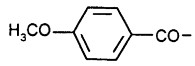
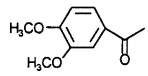
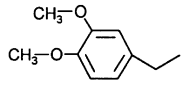
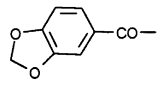
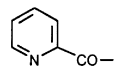
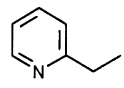
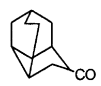
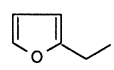
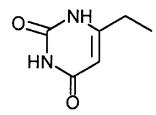
Compound	A	B	R	σ	$5HT_{1A}$	D_2
37		CH_2	H	70	$> 10^5$	2×10^4
38		CH_2	H	0.3	8 000	8×10^4
39		CH_2	H	3 000	$> 10^5$	$> 10^5$
40		CH_2	H	5 000	$> 10^5$	$> 10^5$
41		CH_2	H	40	$> 10^5$	$> 10^5$
42		CH_2	H	10	2×10^4	3×10^4
43		CH_2	H	30	$> 10^5$	$> 10^5$
44		CH_2	H	100	$> 10^5$	$> 10^5$
45		CH_2	H	1 000	$> 10^5$	$> 10^5$
46		CH_2	H	0.9	600	3 000
47		CH_2	H	1 000	$> 10^5$	$> 10^5$
48		CH_2	H	10^4	$> 10^5$	$> 10^5$
49		CH_2	H	100	$> 10^5$	$> 10^5$
50		CH_2	H	20	$> 10^5$	$> 10^5$
51		CH_2	H	40	$> 10^5$	$> 10^5$

Table II. Continued.

Compound	A	B	R	σ	$5HT_{1A}$	D_2
52		CH_2	H	2	2×10^4	800
53		CH_2	H	100	$> 10^5$	$> 10^5$
54		CH_2	H	1 000	$> 10^5$	$> 10^5$
55		CH_2	H	90	$> 10^5$	$> 10^5$
56		CH_2	H	200	$> 10^5$	$> 10^5$
57		CH_2	H	1 000	$> 10^5$	$> 10^5$
58		CH_2	H	50	2×10^4	$> 10^5$
59		CH_2	H	5 000	$> 10^5$	8 000
60		CH_2	H	4	5×10^4	10^4
61		CH_2	H	100	2×10^4	$> 10^5$

However, it appears that the replacement for an amine derivative of the methylene group by a carbonyl, i.e., the change from an amine to the corresponding amide almost always decreases the affinity for the sigma site, but the importance of the loss is very variable. The most important diminution noticed after this structural change concerns compounds **31** and **32**, and **45** and **46**. For these two paires, the loss is approximately 1 000-fold. In some particular cases this structural change does not modify, or even slightly increases the affinity for the sigma sites. See compounds **2** and **3**, **13** and **14**, and **42** and **43**.

The worst ligands for the sigma sites (IC_{50} higher than or equal to 1 000 nM) are compounds **5**, **31**, **39**, **40**, **45**, **47**, **48**, **54**, **57** and **59**. All are amides, this confirms that this chemical function is not as good as the amine one for obtaining a sigma ligand.

In a small homogeneous series it appears that the increase of the aromaticity of the A group enhances the affinity for the sigma sites. For instance the benzofuran derivative **20** has an IC_{50} that is 0.4 nM instead of 7 nM for the dihydrobenzofuran compound **21**. Tetralin derivatives **24** and **25**, respectively, have IC_{50} equal to 400 and

3 nM, instead of 2 and 0.6 nM for the corresponding naphthalenic analogues **28** and **29**.

Introduction of a carbonyl group in the 4 position of the piperazine moiety instead of a methylene group dramatically decreases the affinity for the sigma sites (compound **5**). Introduction of a methoxy group on the benzyl substituent of the piperazine modifies in various ways the affinity for the sigma sites (**4**, **8**, **22**, **27**, **35**, **36**).

Most of our sigma ligands are selective over $5HT_{1A}$ and D_2 receptors. If we consider only those of our sigma ligands that have an IC_{50} lower than 1 000 nM, it appears that among these 51 compounds only six have a ratio $[IC_{50}\ D_2]$:$[IC_{50}\ \sigma]$ and a ratio $[IC_{50}\ 5HT_{1A}]$:$[IC_{50}\ \sigma]$ lower than 100, they are compounds **3**, **7**, **10**, **22**, **23**, **25**; two more have only the ratio $[IC_{50}\ 5HT_{1A}]$:$[IC_{50}\ \sigma]$ lower than 100, they are **14** and **19**.

Except for the fact that all these nonselective derivatives are diamines, it seems quite hard to disclose other structure–activity relationships concerning the selectivity of our sigma ligands. Even the fact that all our nonselective σ ligands are amines is sufficient to predict nonselective derivatives, since many amines are very potent and selective sigma ligands over $5HT_{1A}$ and D_2. See for instance compounds **1**, **12**, **16**, **20**, **46**, **52** and **60**, and even the most selective **38**, which has a ratio 2.6×10^4 over $5HT_{1A}$ and 2.6×10^5 over D_2.

Compound **16** is a better σ_1 selective ligand since its IC_{50} for the σ_1 site is 0.69 nM and its IC_{50} for the σ_2 site is over 77 nM. Compounds **29** and **38** have IC_{50} values for the σ_1 site that are, respectively, 1.3 nM and 1.6 nM and for the σ_2 site, 23 nM and 15 nM. These compounds preferentially bind to the σ_1 over the σ_2 binding sites.

According to the binding data we decided to submit one compound to complementary studies in order to research its antipsychotic potential in accordance with our previous work [1]. We selected compound **29**, one of the most potent sigma ligands according to the results of *table II*.

4.1. Biochemical tests

Compound **29** was tested for its potential effect on N-methyl-D-aspartate (NMDA) induced release of [^{3}H]noradrenaline (NA) from preloaded hippocampal slices made from Sprague-Dawley (SD) rats. Compound **29** potentiated, in a concentration-dependent manner, NMDA-induced [^{3}H]NA release without affecting the basal out flows. The maximal response observed at the concentration of 1 mmol/L was an 800% increase of the NMDA response. Haloperidol, which did not modify NMDA-evoked [^{3}H]NA release by itself partially prevented the effects of compound **29** (63–78% prevention).

Gi/o proteins were inactivated with pertussis toxin (PTX), and the potentiation of NMDA response induced by compound **29** was completely abolished, showing that compound **29** interacted on the σ site coupled to Gi/o proteins, i.e. the σ_1 site.

Using electrophysiological methods compound **29** dose dependently potentiated the NMDA response after intravenous administration, the minimal effective dose was 10 mg/kg, while the dose of 3 mg/kg induced a slight but not significant increase of the NMDA response. The maximum effect was obtained with the dose of 100 mg/kg, which induced a 50% increase of the NMDA response. In about 70% of the neurones tested, compound **29** also increased the neuronal response to quisqualate. The effects described above were also partially reversed with a dose of haloperidol of 100 mg/kg iv.

4.2. Behavioural tests

Compound **29** was tested for its potential effect on hyperactivity induced by amphetamine, which is thought to result from dopaminergic activation in the limbic system. Selective antagonism of amphetamine induced hyperactivity is therefore considered to be predictive of an antipsychotic activity in the absence of extrapyramidal side-effects. Compound **29** poorly antagonized the hyperactivity induced by amphetamine in mice. At doses of 8, 16 and 32 $mg.kg^{-1}$ ip, the antagonism was, respectively, 3%, 14% and 21% (NS). In comparison, Haloperidol (0.125 $mg.kg^{-1}$ ip) completely antagonized the amphetamine induced hyperactivity.

4.3. Inflammation

In the carrageenan planta oedema test at a 100 $mg.kg^{-1}$ po dose, compound **29** significantly reduced the oedema volume. Two hours after administration, the percentage of reduction was 52.9% (42.3% with a 10 $mg.kg^{-1}$ po indomethacin dose) and 4 h after administration this value was 30.7% (39.2% with a 10 $mg.kg^{-1}$ indomethacin po dose).

Compound **29** showed a high affinity and selectivity for the σ sites and it reduces oedema volume. Therefore, owing to the selectivity of this compound **29** for σ sites, it can be assumed that oedema volume reduction and affinity are related. Compound **29** presents an original pharmacological profile of potential drug, at low doses, for treatment of inflammation disease.

5. Experimental protocols

5.1. Chemistry

All melting points were determined on a Kofler apparatus and are uncorrected. Infrared spectra were obtained on a Perkin-Elmer 983 G apparatus using films for liquids or inclusion in KBr pellets for solids. ^{1}H-NMR spectra were recorded using a Bruker AC-200 spectrometer using Me_4Si as an internal standard. Silica-gel TLC was performed on Merck 60 F-254 precoated sheets. Elemental analyses are in agreement with the accepted norms.

5.1.1. General procedure

Method 1:

A solution of acyl chloride (0.01 mol) in methylene chloride (100 mL) was added to a solution of 1-benzylpiperazine (0.01 mol) in the same solvent. The mixture was stirred at room temperature for 5 h or until thin layer chromatography showed the reaction to be complete. After filtration, the compound obtained in hydrochloride form was crystallized from ethanol. IR (KBr) ν cm^{-1}: 3 150, 3 090, 3 035, 2 810 (CH, CH_2); 2 600–2 400 (NH^+); 1 650 (CO); 1 610 (C=C).

Method 2:

0.03 mol of amide, obtained according to method 1, was dissolved in 150 mL of anhydrous tetrahydrofuran. 1 g (0.03 mol) of lithium aluminium hydride in pellet form was added. The mixture was stirred at room temperature for 24 h. Then, the excess hydride was destroyed by addition of 6 mL of ethyl acetate, 6 mL of methanol and 2 mL of water. Solvents were evaporated under reduced pressure and the residue was taken off with 150 mL of water and extracted by 3 × 30 mL of methylene chloride. After drying ($MgS0_4$) and concentration under reduced pressure, the residue was purified by silica gel column chromatography using methylene chloride as eluent. The isolated free base was further converted into its required hydrochloride salt with hydrochloric isopropanol and recrystallized from isopropanol.

Method 3:

0.03 mol of 4-benzylpiperazine optionally methoxylated, 0.03 mole of halogenomethylated compound and 0.03 mol (4.14 g) of potassium carbonate were added to 150 mL of anhydrous tetrahydrofuran. The reaction mixture was refluxed for 24 h or until thin layer chromatography showed the reaction to be complete. The solution was filtered and evaporated under reduced pressure. The solid so obtained was purified by silica gel column chromatography using methylene chloride as eluent. The isolated free base was further converted into its required hydrochloride salt with hydrochloric isopropanol and recrystallized from isopropanol. IR (KBr) ν cm^{-1}: 3 025, 3 000, 2 995, 2 850, 2 800 (CH, CH_2, CH_3); 1 600 (C=C).

5.1.1.1. 1-(2-Chromanylmethyl)-4-benzylpiperazine **1**

^{1}H-NMR ($CDCl_3$), δ ppm: 2.63 (m, 8H, piperaz.); 3.48 (s, 2H, Ar–CH_2–N); 3.52 (s, 2H, Ar–CH_2–N); 6.43 (s, 1H, H_3); 7.52 (m, 8H, Ar); 8.20 (dd, 1H, H_5).

5.1.1.2. 1-(2-Chromanylcarbonyl)-4-benzylpiperazine hydrochloride **2**

^{1}H-NMR (DMSO-d_6), δ ppm: 2.42 (m, 2H, CH_2–$\underline{CH}_2$–CH); 2.51–2.82 (m, 8H, piperaz.); 2.89 (t, 2H, $\underline{CH}_2$–CH_2–CH); 3.52 (s, 2H, CH_2–Ar); 4.74 (m, 1H, O–CH–CO); 7.54 (m, 9H, Ar); 11.62 (s broad, 1H, NH^+).

5.1.1.3. 1-(2-Chromanylmethyl)-4-benzylpiperazine **3**

^{1}H-NMR ($CDCl_3$), δ ppm: 1.66–1.82 (m, 2H, $\underline{CH}_2$–CH–O); 1.97–2.88 (m, 12H, 8H piperaz., N–CH_2 and Ar–CH_2); 3.52 (s, 2H, CH_2–N); 4.18 (m, 1H, CH_2–$\underline{CH}$–O); 6.78–7.33 (m, 9H, Ar).

5.1.1.4. 1-(2-Chromanylcarbonyl)-4-(4′-methoxybenzyl) piperazine hydrochloride **4**

^{1}H-NMR (DMSO-d_6), δ ppm: 2.60 (m, 4H, –$\underline{CH}_2$–$\underline{CH}_2$–CH–O); 3.40 (m, 6H, piperaz.); 3.90 (s, 3H, OCH_3); 4.17–4.26 (m, 4H, 2H piperaz. and $\underline{CH}_2$–Ar); 5.18 (m, 1H, CH–CO); 6.81–7.68 (m, 8H, Ar); 11.67 (s broad, 1H, NH^+).

5.1.1.5. 1-(2-Chromanylcarbonyl)-4-(4′-fluorobenzoyl) piperazine **5**

^{1}H-NMR ($CDCl_3$), δ ppm: 2.23 (m, 2H, $\underline{CH}_2$–CH_2–O); 2.88 (m, 2H, $\underline{CH}_2$–CH); 3.68 (m, 8H, piperaz.); 4.77 (t, 1H, O–CH–CO); 7.13 (m, 8H, Ar).

5.1.1.6. 1-[3-(5-Methoxychromanyl)carbonyl-4-benzylpiperazine hydrochloride **6**

^{1}H-NMR (DMSO-d_6), δ ppm: 2.84 (m, 3H, Ar–$\underline{CH}_2$ and CH–CO); 3.28 (m, 8H, piperaz.); 3.88 (s, 3H, OCH_3); 4.39 (m, 2H, OCH_2); 4.60 (m, 2H, CH_2–Ar); 6.51–7.72 (m, 8H, Ar); 11.64 (s broad, 1H, NH^+).

5.1.1.7. 1-[3-(5-Methoxychromanyl)methyl]-4-benzylpiperazine **7**

^{1}H-NMR ($CDCl_3$), δ ppm: 2.20–2.82 (m, 13H, $C\underline{H}_2$–N, 8H piperaz., $C\underline{H}_2$–CH–CH_2–O and CH_2–$\underline{CH}$–CH_2–O); 3.41 (s, 2H, N–$C\underline{H}_2$–Ar); 3.89 (s, 3H, O-CH_3); 4.27 (m, 2H, CH_2–CH–$C\underline{H}_2$–O); 6.37 (m, 8H, Ar).

5.1.1.8. 1-[3-(5-Methoxy-3-chromanyl]carbonyl-4-(4´-methoxybenzyl)piperazine hydrochloride **8**

^{1}H-NMR (DMSO-d_6), δ ppm: 2.77 (m, 3H, Ar–C<u>H</u>$_2$ and C<u>H</u>–CO); 3.34 (m, 8H, piperaz.); 3.78 (s, 6H, 2OC<u>H</u>$_3$); 4.09–4.12 (m, 4H, OC<u>H</u>$_2$ and N–C<u>H</u>$_2$–Ar); 6.40–7.50 (m, 7H, Ar); 11.14 (s, 1H, NH$^+$).

5.1.1.9. 1-[3-(5-Methoxy-Δ3-chromenyl)carbonyl-4-benzylpiperazine hydrochloride **9**

^{1}H-NMR (DMSO-d_6), δ ppm: 3.42 (m, 8H, piperaz.); 3.81 (s, 3H, OC<u>H</u>$_3$); 4.23 (s, 1H, C<u>H</u>=C–CO); 4.31 (s, 2H, N–C<u>H</u>$_2$–Ar); 4.74 (s, 2H, C<u>H</u>$_2$–O); 7.43 (m, 8H, Ar); 11.68 (s, 1H, NH$^+$).

5.1.1.10. 1-[3-(5-Methoxy-D3-chromenyl)methyl-4-benzylpiperazine **10**

^{1}H-NMR (CDCl$_3$), δ ppm: 2.46 (m, 8H, piperaz.); 3.06 (s, 2H, C<u>H</u>$_2$–N); 3.51 (s, 2H, N–C<u>H</u>$_2$–Ar); 3.80 (1s, 3H, O–C<u>H</u>$_3$), 4.70 (s, 2H, O–C<u>H</u>$_2$); 6.39–7.32 (m, 9H, Ar and H4).

5.1.1.11. 1-(2-Phenoxypropionyl)-4-benzylpiperazine hydrochloride **11**

^{1}H-NMR (DMSO-d_6), δ ppm: 1.65 (d, 3H, C<u>H</u>$_3$); 3.47–4.46 (m, 10H, piperaz. and C<u>H</u>$_2$–Ar); 5.37 (m, 1H, O–CH–CO) 6.90–7.68 (m, 10H, Ar); 11.11 (s broad, 1H, NH$^+$).

5.1.1.12. 1-(2-Phenoxy-n-propyl)-4-benzylpiperazine **12**

^{1}H-NMR (CDCl$_3$), δ ppm: 1.27 (d, 3H, C<u>H</u>$_3$–CH); 3.47 (s, 2H, CH$_2$–Ar); 3.51 (m, 10H, C<u>H</u>$_2$–N and 8H piperaz.); 4.52 (q, 1H, C<u>H</u>–CH$_3$); 6.84–7.30 (m, 10H, Ar).

5.1.1.13. 1-(2-Phenoxybutyryl)-4-benzylpiperazine hydrochloride **13**

^{1}H-NMR (DMSO-d_6), δ ppm: 1.13 (t, 3H, CH$_2$–C<u>H</u>$_3$); 1.89 (q, 2H, C<u>H</u>$_2$–CH$_3$); 3.55 (m, 8H, piperaz.); 4.44 (s, 2H, CH$_2$–Ar); 5.15 (t, 1H, C<u>H</u>–C$_2$H$_5$); 7.65 (m, 10H, Ar); 12.04 (s, 1H, NH$^+$).

5.1.1.14. 1-(2-Phenoxy-n-butyl)-4-benzylpiperazine **14**

^{1}H-NMR (CDCl$_3$), δ ppm: 0.94 (t, 3H, CH$_2$–C<u>H</u>$_3$); 1.70 (q, 2H, C<u>H</u>$_2$–CH$_3$) 2.43–2.70 (m, 10H, C<u>H</u>$_2$–N and 8H piperaz.); 3.46 (s, 2H, CH$_2$–Ar); 4.26–4.37 (m, 1H, C<u>H</u>–CH$_2$–N); 6.84–7.30 (m, 10H, Ar).

5.1.1.15. 1-(2-Phenoxyisobutyryl)-4-benzylpiperazine hydrochloride **15**

^{1}H-NMR (DMSO-d_6), δ ppm: 1.66 (s, 6H, 2CH$_3$); 3.47–4.68 (m, 10H, piperaz. and C<u>H</u>$_2$–Ar); 7 42 (m, 10H, Ar), 11.56 (s broad, 1H, NH$^+$).

5.1.1.16. 1-(2-Phenoxy-2-methylpropyl)-4-benzylpiperazine **16**

^{1}H-NMR (CDCl$_3$), δ ppm: 1.26 (s, 6H, –C(CH$_3$)$_2$); 2.46 (t, 4H, piperaz.); 2.52 (s, 2H, CH$_2$–N); 2.67 (t, 4H, piperaz.); 3.50 (S, 2H, N–C<u>H</u>$_2$–Ar); 6.95–7.34 (m, 10H, Ar).

5.1.1.17. 1-(2-Phenylethyl)-4-benzylpiperazine **17**

^{1}H-NMR (CDCl$_3$), δ ppm: 1.79 (m, 2H, Ar–CH$_2$–C<u>H</u>$_2$–CH$_2$–N); 2.35 (t, 2H, Ar–CH$_2$–CH$_2$–C<u>H</u>$_2$–N), 2.46 (m, 8H, piperaz.); 2.60 (t, 2H, Ar–C<u>H</u>$_2$–CH$_2$–CH$_2$–N); 3.48 (s, 2H, N–C<u>H</u>$_2$–Ar); 7.10–7.34 (m, 10H, Ar).

5.1.1.18. 1-(2-Phenoxyacetyl)-4-benzylpiperazine hydrochloride **18**

^{1}H-NMR (DMSO-d_6), δ ppm: 3.44 (m, 8H, piperaz.); 4.44 (s, 2H, C<u>H</u>$_2$–Ar); 4.99 (s, 2H, O–CH$_2$–CO); 7.54 (m, 10H, Ar); 11.73 (s broad, 1H, NH$^+$).

5.1.1.19. 1-(2-Phenoxyethyl)-4-benzylpiperazine **19**

^{1}H-NMR (CDCl$_3$), δ ppm: 2.75 (t, 2H, C<u>H</u>$_2$N); 3.45 (s, 2H, N–C<u>H</u>$_2$–Ar); 3.48 (m, 8H, piperaz.); 4.02 (t, 2H, C<u>H</u>$_2$–O–Ar); 7.54 (m, 10H, Ar).

5.1.1.20. 1-(2-Benzofurylmethyl)-4-benzylpiperazine **20**

^{1}H-NMR (CDCl$_3$), δ ppm: 2.57 (m, 8H, piperaz.); 3.36 (s, 2H, C<u>H</u>$_2$–N); 3.55 (s, 2H, N–C<u>H</u>$_2$–Ar); 6.43 (s, 1H, CH=C); 6.98 (m, 9H, Ar).

5.1.1.21. 1-[2-(2,3-Dihydrobenzofuryl)methyl-4-benzylpiperazine **21**

^{1}H-NMR (CDCl$_3$), δ ppm: 2.53 (m, 8H, piperaz.); 2.69–3.26 (m, 4H, C<u>H</u>$_2$–CH–C<u>H</u>$_2$–N); 3.49 (s, 2H, N–CH$_2$–Ar); 4.93 (m, 1H, O–C<u>H</u>–CH$_2$–N); 6.74–7.32 (m, 9H, Ar).

5.1.1.22. 1-[2-(2,3-Dihydrobenzofuryl)methyl]-4-(4´-methoxybenzyl)piperazine **22**

^{1}H-NMR (CDCl$_3$), δ ppm: 2.52 (m, 8H, piperaz.); 2.71–3.30 (m, 4H, –C<u>H</u>$_2$–CH–CH$_2$–N), 3.51 (1s, 2H, N–C<u>H</u>$_2$–Ar); 3.80 (s, 3H, Ar–O<u>CH</u>$_3$); 4.94 (m, 1H, O–C<u>H</u>–CH$_2$); 6.63–7.34 (m, 8H, Ar).

5.1.1.23. 1-[2-(5-Fluoro-2,3-dihydrobenzofuryl)methyl]-4-benzylpiperazine **23**

^{1}H-NMR (CDCl$_3$), δ ppm: 2.50–2.79 (m, 10H, C<u>H</u>$_2$–N and 8H piperaz.); 3.05 (2dd, 2H, C<u>H</u>$_2$–CH–O); 3.51 (s, 2H, N–C<u>H</u>$_2$–Ar); 4.94 (m, 1H, CH$_2$–C<u>H</u>–O); 6.63–7.34 (m, 8H, Ar).

5.1.1.24. 1-[2-(1,2,3,4-Tetrahydronaphthoyl)]-4-benzylpiperazine hydrochloride **24**

^{1}H-NMR (DMSO-d_6), δ ppm: 1.76 (m, 2H, Ar–CH$_2$–C<u>H</u>$_2$); 2.79 (m, 5H, Ar–C<u>H</u>$_2$–CH$_2$ and Ar–C<u>H</u>$_2$–

CH); 3.13 (m, 6H, piperaz.); 4.31 (m, 4H, 2H piperaz. and Ar–CH_2); 7.35 (m, 9H, Ar); 11.63 (s broad, 1H, NH^+).

5.1.1.25. 1-[2-(1,2,3,4-Tetrahydronaphthylmethyl)]-4-benzylpiperazine **25**

^{1}H-NMR ($CDCl_3$), δ ppm: 1.35 (m, 2H, Ar–CH_2–CH_2); 1.94 (m, 2H, Ar–CH_2); 2.25 (d, 2H, CH_2–N); 2.37 (m, 8H, piperaz.); 2.73–2.93 (m, 3H, CH_2–CH); 3.49 (s, 2H, N–CH_2–Ar); 7.05–7.33 (m, 9H, Ar).

5.1.1.26. 1-[4-(7-Methoxy-4-coumarinyl)methyl]-4-benzylpiperazine **26**

^{1}H-NMR ($CDCl_3$), δ ppm: 2.29 (m, 8H, piperaz.); 3.58–4.04 (2s, 4H, 2CH_2–N); 3.86 (s, 3H, O–CH_3); 4.70 (s, 1H, CO–CH); 6.85–7.70 (m, 8H, Ar).

5.1.1.27. 1-[4-(7-Methoxy-4-coumarinyl)methyl]-4-(4′-methoxybenzyl)piperazine **27**

^{1}H-NMR ($CDCl_3$), δ ppm: 2.50 (m, 8H, piperaz.); 3.45–3.48 (2s, 4H, 2CH_2–N); 3.78 and 3.81 (2s, 6H, 2OCH_3); 6.15 (s, 1H, CO–CH); 6.78–7.70 (m, 7H, Ar).

5.1.1.28. 1-(2-Naphthoyl)-4-benzylpiperazine hyrochloride **28**

^{1}H-NMR (DMSO-d_6), δ ppm: 3.53 (m, 8H, piperaz.); 4.34 (s, 2H, CH_2–Ar); 7.73 (m, 12H, Ar); 11.55 (s broad, 1H, NH^+).

5.1.1.29. 1-(2-Naphthylmethyl)-4-benzylpiperazine **29**

^{1}H-NMR ($CDCl_3$), δ ppm: 2.51 (m, 8H, piperaz.); 3.52 (s, 2H, N–CH_2–Ar); 3.66 (s, 2H, Ar–CH_2–N); 7.25–7.82 (m, 12H, Ar).

5.1.1.30. 1-(1-Naphthoyl)-4-benzylpiperazine hydrochloride **30**

^{1}H-NMR (DMSO-d_6), δ ppm: 3.47 (m, 8H, piperaz.); 4.34 (m, 2H, CH_2–Ar); 7.73 (m, 12H, Ar); 11.78 (s broad, 1H, NH^+).

5.1.1.31. 1-(2-Quinolinylcarbonyl)-4-benzylpiperazine hydrochloride **31**

^{1}H-NMR (DMSO-d_6), δ ppm: 3.70 (m, 8H, piperaz.); 4.38 (s, 2H, CH_2–Ar); 8.01 (m, 11H, Ar); 11.83 (s broad, 2H, NH^+).

5.1.1.32. 1-(2-Quinolinylmethyl)-4-benzylpiperazine **32**

^{1}H-NMR ($CDCl_3$), δ ppm: 2.53 (m, 8H, piperaz.); 3.53 and 3.84 (2s, 4H, 2CH_2); 7.25–8.00 (m, 11H, Ar).

5.1.1.33. 1-(3-Phenyl-2-methylacryloyl)-4-benzylpiperazine hydrochloride **33**

^{1}H-NMR (DMSO-d_6), δ ppm: 2.13 (s, 3H, CH_3); 3.33 (m, 6H, piperaz.); 4.40 (m, 4H, 2H piperaz. and N–CH_2–Ar); 6.71 (s, 1H, CH=C); 7.56 (m, 10H, Ar); 11.53 (s broad, 1H, NH^+).

5.1.1.34. 1-(α-Methylcinnamyl)-4-benzylpiperazine **34**

^{1}H-NMR ($CDCl_3$), δ ppm: 1.89 (S, 3H, CH_3–C=CH); 2.48 (m, 8H, piperaz.); 3.00 (s, 2H, C=C–CH_2); 3.52, (s, 2H, N–CH_2–Ar); 6.41 (s, 1H, CH=); 7.14–7.35 (m, 10H, Ar).

5.1.1.35. 1-[3-(3-Phenyl-2-methylacryloyl)-4-(4′-methoxybenzyl)]piperazine hydrochloride **35**

^{1}H-NMR (DMSO-d_6), δ ppm: 2.13 (s, 3H, CH_3–C=CH); 3.34 (m, 6H, piperaz.); 3.89 (s, 3H, OCH_3); 4.37 (m, 4H, 2H piperaz. and N–CH_2–Ar); 6.70 (s, 1H, CH=); 7.40 (m, 9H, Ar); 11.72 (s broad, 1H, NH^+).

5.1.1.36. 1-(α-Methylcinnamyl)-4-(4′-methoxybenzyl) piperazine **36**

^{1}H-NMR ($CDCl_3$), δ ppm: 1.87 (s, 3H, CH_3–C=CH); 2.46 (m, 8H, piperaz.); 3.00 (s, 2H, C=C–CH_2); 3.46 (s, 2H, N–CH_2–Ar); 3.79 (s, 3H, OCH_3); 6.41 (s, 1H, CH=); 6.81–7.36 (m, 9H, Ar).

5.1.1.37. 1-(4-Chlorocinnamoyl)-4-benzylpiperazine hydrochloride **37**

^{1}H-NMR (DMSO-d_6), δ ppm: 3.30 (m, 6H, piperaz.); 4.34 (s, 2H, –CH_2–Ar); 4.56 (m, 2H, piperaz.); 7.54 (m, 11H, Ar and CH=CH); 11.81 (s broad, 1H, NH^+).

5.1.1.38. 1-(4-Chlorocinnamyl)-4-benzylpiperazine **38**

^{1}H-NMR ($CDCl_3$), δ ppm: 2.61 (m 8H, piperaz.); 3.15 (d, 2H, CH_2–C=CH, *J* = 6 Hz); 3.54 (s, 2H, Ar–CH_2–N); 6.13 (dt, 1H, Ar–CH=CH–CH_2); 6.46 (d, 1H, Ar–CH=CH–CH_2); 6.92 (m, 9H, Ar).

5.1.1.39. 1-(4-Methoxycinnamoyl)-4-benzylpiperazine hydrochloride **39**

^{1}H-NMR (DMSO-d_6), δ ppm: 3.45–4.65 (m, 10H, piperaz. and CH_2–Ar); 3.91 (s, 3H, CH_3O); 7.08 (d, 2H, Ar); 7.23 (d, 1H, CH=CH–CO); 7.61 (d, 1H, CH=CH–CO); 7.63 (m, 5H, Ar); 7.72 (d, 2H, Ar); 11.14 (s broad, 1H, NH^+).

5.1.1.40. 1-(3,4-Dimethoxycinnamoyl)-4-benzylpiperazine hydrochloride **40**

^{1}H-NMR (DMSO-d_6), δ ppm: 3.57 (m, 6H, piperaz.); 3.90 and 3.93 (2s, 6H, 2OCH_3); 4.45 (s, 2H, CH_2–Ar); 4.66 (m, 2H, piperaz.); 7.25 (d, 1H, CH=CH–CO); 7.52 (d, 1H, CH–C=O); 7.56 (m, 8H, Ar); 11.87 (s broad, 1H, NH^+).

5.1.1.41. 1-(3,4-Dimethoxycinnamyl)-4-benzylpiperazine **41**

^{1}H-NMR ($CDCl_3$), δ ppm: 3.04 (m, 8H, piperaz.); 3.88 and 3.89 (2s, 6H, $2OCH_3$); 3.11 (d, 2H, CH=CH–C$\underline{H}_2$); 3.52 (s, 2H, N–C$\underline{H}_2$–Ar); 6.10 (td, 1H, Ar–CH=C$\underline{H}$–CH_2); 6.41 (d, 1H, Ar–C$\underline{H}$=CH–CH_2); 6.29–7.30 (m, 8H, Ar).

5.1.1.42. 1-(3,4-Methylenedioxycinnamoyl)-4-benzylpiperazine hydrochloride **42**

^{1}H-NMR (DMSO-d_6), δ ppm: 3.84 (m, 10H, piperaz. and $\underline{CH}_2$–Ar); 6.07 (s, 2H, $\underline{CH}_2O_2$), 7.18 (d, 1H, $\underline{CH}$=CH–CO); 7.50 (d, 1H, CH=$\underline{CH}$–CO); 7.24 (m, 8H, Ar); 11.04 (s broad, 1H, NH^+).

5.1.1.43. 1-(3,4-Methylenedioxycinnamyl)-4-benzylpiperazine **43**

^{1}H-NMR ($CDCl_3$), δ ppm: 2.25 (m, 8H, piperaz.); 3.14 (d, 2H, C$\underline{H}_2$–CH=CH); 3.52 (s, 2H, N–C$\underline{H}_2$–Ar); 6.01 (s, 2H, CH_2O_2); 6.12 (td, 1H, Ar–CH=C$\underline{H}$–CH_2); 6.45 (d, 1H, Ar–C$\underline{H}$=CH–CH_2); 6.37–7.32 (m, 8H, Ar).

5.1.1.44. 1-(3,4,5-Trimethoxycinnamyl)-4-benzylpiperazine **44**

^{1}H-NMR ($CDCl_3$), δ ppm: 3.04 (m, 8H, piperaz.); 3.13 (d, 2H, C$\underline{H}_2$–CH=CH); 3.48 (s, 2H, N–C$\underline{H}_2$–Ar); 3.70 (s, 3H, OCH_3); 3.85 (s, 6H, $2OCH_3$); 6.35 (s, 2H, Ar); 6.39 (d, 1H, Ar–C$\underline{H}$=CH–CH_2); 6.55 (td, 1H, Ar–CH=C$\underline{H}$–CH_2); 7.32–7.40 (m, 5H, Ar).

5.1.1.45. 1-[3-(2-Furyl)acryloyl]-4-benzylpiperazine hydrochloride **45**

^{1}H-NMR (DMSO-d_6), δ ppm: 3.13–4.44 (m, 10H, piperaz. and $\underline{CH}_2$–Ar); 6.73 (dd, 1H, $H_{4'}$); 7.00 (d, 1H, $H_{3'}$); 7.03 (d, 1H, C$\underline{H}$=CH–CO); 7.50 (d, 1H, CH–CO); 7.60 (m, 5H, Ar); 7.93 (d, 1H, $H_{5'}$); 11.30 (s broad, 1H, NH^+).

5.1.1.46. 1-[3-(2-Furyl-2-propenyl)]-4-benzylpiperazine **46**

^{1}H-NMR ($CDCl_3$), δ ppm: 2.51 (m, 8H, piperaz.); 3.11 (d, 2H, CH=CH–C$\underline{H}_2$); 3.51 (s, 2H, N–C$\underline{H}_2$–Ar); 6.18 (dt, 1H, Ar–CH=C$\underline{H}$–CH_2); 6.25 (d, 1H, H_2); 6.34 (dd, 1H, $H_{4'}$); 6.38 (d, 1H, Ar–C$\underline{H}$=CH–CH_2); 7.28–7.32 (m, 6H, Ar and $H_{5'}$).

5.1.1.47. 1-[3-(2-Thienyl)acryloyl]-4-benzylpiperazine hydrochloride **47**

^{1}H-NMR (DMSO-d_6), δ ppm: 3.16–4.57 (m, 10H, piperaz. and C$\underline{H}_2$–Ar); 7.04 (d, 1H, C$\underline{H}$=CH–CO); 7.61 (d, 1H, CH–C=O); 7.56–7.72 (m, 8H, Ar); 11.14 (s broad 1H, NH^+).

5.1.1.48. 1-(4-Methoxyphenylacetyl)-4-benzylpiperazine hydrochloride **48**

^{1}H-NMR (DMSO-d_6), δ ppm: 3.48–4.43 (m, 12H, piperaz. and $2\underline{CH}_2$–Ar); 3.91 (s, 3H, CH_3O); 7.35 (m, 9H, Ar); 11.82 (s broad, 1H, NH^+).

5.1.1.49. 1-[2-(4-Methoxyphenyl)ethyl)]-4-benzylpiperazine **49**

^{1}H-NMR ($CDCl_3$), δ ppm: 2.55 (m, 10H, piperaz. and C$\underline{H}_2$–N); 2.73 (t, 2H, Ar–C$\underline{H}_2$–CH_2–N); 3.49 (s, 2H, N–C$\underline{H}_2$–Ar); 3.72 (s, 3H, C$\underline{H}_3$O–Ar); 6.77–7.30 (m, 9H, Ar).

5.1.1.50. 1-(4-Chlorobenzoyl-4-benzylpiperazine hydrochloride **50**

^{1}H-NMR (DMSO-d_6), δ ppm: 3.71 (m, 10H, piperaz. and $\underline{CH}_2$–Ar); 7.49 (m, 9H, Ar); 11.27 (s broad, 1H, NH^+).

5.1.1.51. 1-(2-Methoxybenzoyl)-4-benzylpiperazine hydrochloride **51**

^{1}H-NMR (DMSO-d_6), δ ppm: 3.90 (s, 3H, OCH_3); 3.96 (m, 10H, piperaz. and $\underline{CH}_2$–Ar); 7.42 (m, 9H, Ar); 11.82 (s broad, 1H, NH^+).

5.1.1.52. 1-(2-Methoxybenzyl)-4-benzylpiperazine **52**

^{1}H-NMR ($CDCl_3$), δ ppm: 2.51 (m, 8 H, piperaz.); 3.51 and 3.58 (2s, 4H, CH_2N and C$\underline{H}_2$Ar); 3.80 (s, 3H, $\underline{CH}_3$O–Ar); 6.83–7.35 (m, 9H, Ar).

5.1.1.53. 1-(4-Methoxybenzoyl)-4-benzylpiperazine hydrochloride **53**

^{1}H-NMR (DMSO-d_6), δ ppm: 3.91 (s, 3H, OCH_3); 3.95 (m, 10H, piperaz. and $\underline{CH}_2$–Ar); 7.40 (m, 9H, Ar); 11.32 (s broad, 1H, NH^+).

5.1.1.54. 1-(3,4-Dimethoxybenzoyl)-4-benzylpiperazine hydrochloride **54**

^{1}H-NMR (DMSO-d_6), δ ppm: 3.50 (m, 8H, piperaz.); 3.90 (s, 6H, $2OCH_3$); 4.43 (m, 2H, $\underline{CH}_2$–Ar), 7.43 (m, 8H, Ar); 11.54 (s broad, 1H, N^+).

5.1.1.55. 1-(3,4-Dimethoxybenzyl)-4-benzylpiperazine **55**

^{1}H-NMR ($CDCl_3$), δ ppm: 2.49 (m, 8H, piperaz.); 3.50–3.55 (2s, 4H, CH_2N and C$\underline{H}_2$Ar), 3.68–3.80 (2s, 6H, $2CH_3O$); 6.80–7.31 (m, 8H, Ar).

5.1.1.56. 1-(3,4-Methylenedioxybenzoyl)-4-benzylpiperazine hydrochloride **56**

^{1}H-NMR (DMSO-d_6), δ ppm: 3.25 (m, 8H, piperaz.); 4.31 (s, 2H, N–$\underline{CH}_2$–Ar); 6.08 (s, 2H, CH_2O); 6.97 (m, 3H, Ar); 7.56 (m, 5H, Ar); 11.25 (s broad, 1H, NH^+).

5.1.1.57. 1-(2-Pyridylcarbonyl)-4-benzylpiperazine hydrochloride **57**

^{1}H-NMR (DMSO-d_6), δ ppm: 2.45 (m, 8H, piperaz.); 3.53 and 3.66 (2s, 4H, CH_2N and $C\underline{H}_2Ar$); 7.67 (m, 9H, Ar).

5.1.1.58. 1-(2-Pyridylmethyl)-4-benzylpiperazine **58**

^{1}H-NMR ($CDCl_3$), δ ppm: 2.45 (m, 8H, piperaz.); 3.53 (s, 2H, $C\underline{H}_2$–N), 3.66 (s, 2H, N–$C\underline{H}_2Ar$); 7.67 (m, 9H, Ar).

5.1.1.59. 1-(Adamantylcarbonyl)-4-benzylpiperazine hydrochloride **59**

^{1}H-NMR (DMSO-d_6), δ ppm: 1.79–2.08 (m, 15H, Ad–CO); 3.37 (m, 8H, piperaz.); 4.43 (s, 2H, $C\underline{H}_2$–Ar); 7.69 (m, 5H, Ar); 11.06 (s broad, 1H, NH^+).

5.1.1.60. 1-(2-Furylmethyl)-4-benzylpiperazine **60**

^{1}H-NMR ($CDCl_3$), δ ppm: 2.42 (m, 8 H, piperaz.); 3.41 and 3.45 (2s, 4H, CH_2N and $C\underline{H}_2Ar$); 6.10–6.22 (m, 3H, –C<u>H</u>–C<u>H</u>=C<u>H</u>–O); 7.28 (m, 5H, Ar).

5.1.1.61. 1-(6-Uracylmethyl)-4-benzylpiperazine **61**

^{1}H-NMR ($CDCl_3$), δ ppm: 2.52 (m, 8H, piperaz.); 3.31 (s, 2H, $C\underline{H}_2$–N); 3.53 (1s, 2H, N–$C\underline{H}_2$–Ar); 5.54 (1s, 1H, CO–C<u>H</u>=); 7.31 (m, 7H, 5H Ar and 2N<u>H</u>).

5.2. Pharmacology

5.2.1. Affinity to σ binding sites in vitro

The affinity of test compounds for σ binding sites was estimated by their ability to displace [^{3}H](+)(PPP) 3-(3-hydroxyphenyl)-N-(n-propyl) piperidine (3-PPP) from guinea-pig cortex homogenates, as described by Karbon et al. [4].

5.2.2. Specific σ_1 and σ_2 binding assays in vitro

The affinity of test compounds for σ_1 binding sites was estimated by their ability to displace [^{3}H](+)-pentazocine from Hartley guinea-pig brain homogenates minus cerebellum as described by De Haven-Hudkins et al. [5].

The affinity of test compounds for σ_2 binding sites was estimated by their ability to displace [^{3}H]1,3-di(o-tolyl)guanidine (DTG) from rat liver membranes, as described by Weber et al. [6].

5.2.3. Affinity to $5HT_{1A}$ binding sites in vitro

The affinity of test compounds for $5HT_{1A}$ binding sites was estimated by their ability to displace [^{3}H]-8-hydroxy-3-(di-n-propylamino)tetraline (8-OH DPAT) from bovine frontal cortex and hippocampus homogenates, as described by Hoyer et al. [7].

5.2.4. Affinity to D_2 binding sites in vitro

The affinity of test compounds for D_2 binding sites was estimated by their ability to displace [^{3}H]Raclopride from bovine striatum homogenates, as described by Köhler et al. [8].

5.2.5. Antagonism of amphetamine-induced hyperactivity in mice and rats

Swiss mice or Wistar rats were pretreated with d-amphetamine (4 mg/kg IP) and the compounds to be tested, and were placed 30 minutes later in an activity meter for a 30 minutes test according to Costall et al. [9].

5.2.6. Modulation by σ ligands of N-methyl-D-aspartate-induced [^{3}H]noradrenaline release in rat hippocampus

The experiment was carried out in hippocampal slices from Sprague-Dawley (SID) rats. The [^{3}H]NA release was evoked once by a 4 min exposure to NMDA, 40 min after the beginning of superfusion with an Mg^{2+}-free Krebs solution. Gi/o proteins were inactivated with pertussis toxin, injected locally from 3–11 days prior to the experiment, to assess the possible involvment of Gi/o proteins in the modulation of NMDA evoked [^{3}H]NA release.

Compound **29** was tested at different concentrations, ranging from 10–1 000 nmol, in continuous perfusion according to Monnet et al. [10, 11].

5.2.7. Modulation of the neuronal response to N-methyl-D-aspartate electrophysiological studies in the rat dorsal hippocampus

Electrophysiological experiments in Sprague-Dawley rats. Microiontophoretic applications and extracellular unitary recordings were performed with 5 barrelled glass micropipettes as described by Haigler and Aghajanian [12]. The central barrel, used for extracellular unitary recordings of the activity of the CA_1 or CA_3 hippocampal pyramidal neurones was filled with 2 M NaCl solution saturated with fast green FCF.

Compounds were dissolved in NaCl: compound **29**, 2 mM in 200 mM NaCl, pH 4; NMDA, 10 nM in 200 mM NaCl, pH 8; quisqualate, 1.5 mM in 400 mM NaCl, pH 8. The neuronal activity was monitored as described by Bergeron et al. [13]. Action potentials were detected and square pulses were fed to a computer which generated integrated firing rate histograms. Neurons from the CA_1 and CA_3 region of the hippocampus were identified according to the criteria of Kandal and Spencer [14]. The duration of the microiontophoretic applications of NMDA and Quisqualate was kept constant at 50 s.

The effect of compound **29**, administered intravenously (0.5–1 000 mg/kg) were assessed by determining

the ratio (N2/N1) of the number of spikes generated per nC by each excitatory substance (Quis or NMDA) before (N1) and after (N2) the injection. The effect of the microiontophoretic application of compound **29** was also assessed by determining the ratio (N2/N1) of the number of spikes generated per nC by each of the excitatory substances before (N1) and during (N2) the application. The potential 'antagonist' effect was assessed by comparing the number of spikes generated per nC of each excitatory substance following the administration of a σ agonist and after the injection of the putative σ antagonist.

Result were expressed as means + SEM. The means were compared using the paired Student's *t*-test. Each series of experiments was carried out in 8–12 rats.

5.2.8. Study of the anti-inflammatory activity

Study of the anti-inflammatory activity was carried out according to Winter's method [15]. Male Wistar rats weighing from 180–220 g were used in this study. A local oedema was created with an injection into the sole of the right paw of 0.1 mL of a 2% carrageenan suspension in 0.9% saline solution. The saline solution is also injected into the sole of the left paw which acts as a control.

Compound **29** is orally administrated at 100 $mg.kg^{-1}$ in a 0.05% suspension of mixture and water used before the oedemator induction.

Inflammation is characterized by an increase in the volume of the paw, which is determined using a plethysmometer (UGO Basile). An initial measurement of the volume of the paw is carried out just before oedemator induction, other measurements are carried out 2 and 4 h later. The oedemator volume (in mL) is obtained by the difference between the volume at 2 and 4 h and the initial volume. Indomethacin at $10mg.kg^{-1}$ po is used as a reference. Results are expressed as percentage of inhibition of the oedema.

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