Eur. J. Med. Chem. 33 (1998) 339–347

Synthesis and structure–activity relationships of novel 2-amino alkyl chromones and related derivatives as σ site-selective ligands

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(Received 9 July 1997; accepted 17 October 1997)

Abstract – Starting from a random screening showing that 2-[(4-benzylpiperazinyl)methyl] chromone was a selective and potent sigma ligand, a series of analogues were synthesized. Introduction of a substituent on the chromone moiety, replacement of methylenes by carbonyl groups and benzyl by aryl groups decrease the affinity for sigma sites. The result obtained after introduction of various substituents on the aromatic part of the benzyl is strictly depending on the size and on the position of these substituents. Stretching of the carbon chain between the phenyl and the piperazine does not strongly modify the affinity. 2-[4-(4'-methoxy benzyl)-1-piperazinyl methyl] chromone has been tested in behavioral tests that permit to believe that such derivatives could be interesting for the treatment of psychosis. © Elsevier, Paris

sigma ligand / chromone / benzylpiperazine / psychosis

1. Introduction

The existence of the σ sites has been postulated to account for the psychotomimetic effects of various opioid ligands such a *N*-allyl normetazocine (SKF 10047) and related racemic benzomorphans [1]. It readily appeared that they are not opioid receptor subtypes, according to their behaviour with opioid antagonists such as Naloxone and Naltrexone, because of the fact that (–)-enantiomers of benzomorphans bind to opioid receptors whereas (+)-enantiomers bind to σ ones; nevertheless, they are sensitive to haloperidol [2].

It is now clear that σ sites are different from other receptors such as dopaminergic, serotoninergic and histaminergic ones [3]. Their biochemical and physiological function have neither been clarified nor understood so far. Nevertheless, the existence of two σ_1 and σ_2 subtypes seems established [4–6] from biochemical and radiolabeled binding studies. So (+)-benzomorphans such as (+)-Pentazocine and (+)-SKF 10047 exhibit a high affinity [7] for the σ_1 subtype (K_1: 10^{-9} M) but only a weak one for the σ_2 subtype (10^{-6} M) whereas the (–)-isomers offer selectivity for the σ_2 sites and low affinities for both. More recently a σ_3 site has been disclosed [6]. In any case the discovery of new ligands besides the known ones e.g. butyrophenones, 3-phenylpiperidines, benzomorphans and disubstituted guanidines is of primary importance since:

– it should add to already known sigma ligands new pharmacological tools with high affinity and selectivity, and should permit the classification and the description of these biological functions;

– it should give new therapeutic agents.

Actually sigma sites have been disclosed as being involved in the mechanism of action of classical and non-classical antipsychotic drug [9, 10] such as Rimcazole, Remoxipride and BMY 14802. So it has been suggested in various papers that σ antagonist ligands would be convenient for such treatment. One of their special interests would remain the lack of extrapyramidal side effects and of tardive dyskinesia [3] that are on the contrary induced by typical neuroleptics. More particularly some chromone derivatives having an amino alkoxy side chain on the phenyl ring [10] were disclosed as selective σ ligands effective in two behavioral models predictive of an antipsychotic activity.

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In recent studies it appears that the cellular and anatomical localisation of these sites refers not only to central nervous system [12–14] but is also directed toward peripheric tissues such as blood vessels [15–18], surrenals, testis, ovaries [14], and toward the immune system [19]. During the past few years, a lot of research works were directed to the sigma sites because they seem implicated in various medical conditions such as anxiety, depression, schizophrenia, ischemic disorders, neuroprotection [3, 20–24], epilepsy [25] and a sigma ligand is currently proposed against autoimmune diseases in which both immune and inflammatory responses are involved [26]. The unexpected and high affinity for the sigma site ($K_i \cong 10^{-9}$ M) associated with the lack of affinity ($K_i > 10^{-5}$ M) for the other receptors observed from a random screening on 2-[(4-benzyl piperazinyl)methyl] chromone **1** [27] led us to undertake some pharmacomodulation from this lead compound in order to specify the structure–affinity relationships in this series, with the aim of determining the role and the influence of each structural part of the lead compound. In the present work, we report the synthesis of twenty nine new derivatives for which we kept the chromone moiety starting from the 2-[(4-benzyl piperazinyl)-methyl] chromone **1**.

In this way, we carried out various structural modifications such as: introduction of a methyl substituant on the chromone moiety, replacement of the methylene between the chromone and the piperazine moiety by a carbonyl, replacement of the piperazine by a piperidine, introduction of various substituents on the benzyl ring, modulation of the length of the carbon chain between the phenyl ring and the piperazine moiety, replacement of the phenyl ring by various systems.

2. Chemistry

Most of 1-benzyl piperazines as well as phenyl piperazines or 1-(2-pyrimidyl)piperazine are commercially available. In all other cases the piperazines **D** are obtained by reaction of ethyl 1-piperazine carboxylate **A** on an appropriated chlorinated derivative **B**. The ethyl 4-arylalkyl piperazino-1-carboxylate **C** previously formed (*figure 1*) by alkalin hydrolysis lead to the *N*-arylalkyl piperazine **D**.

All the compounds, but derivatives **2**, **17** and **19**, were prepared according to *figure 2* using 4-oxo-4H-1-benzopyran-2-carboxylic acid **E**, commercially available as starting material according to two synthetic routes function of their structure (*figure 2*).

In the first chemical way the carboxylic acid **E** is classically transformed into acylchloride **F** using PCl_5 and then condensed with the correctly substituted piperazine **D** to give the amides **G**.

Amide **17** was obtained according to the same route, but using 6-methyl 4-oxo-4H-1-benzopyran-2-carboxylic acid [28, 29]. Amide **19** was obtained using 3-methyl 4-oxo-4H-1-benzopyran-2-carboxylic acid as starting material [30, 31].

In the second one, the carboxylic acid **E** is esterified by methanol in presence of sulphuric acid to give the methylic ester **H** that is reduced by $NaBH_4$ to give 2-hydroxymethyl-4H-1-benzopyran-4-one **J** that is reacted with thionyl chloride to give 2-chloromethyl-4H-1-benzopyran-4-one **K** [26] which was reacted with various substituted piperazine to give the amines **L**.

Amine **2** was obtained using 6-methyl 4-oxo-4H-2-benzopyran-2-carboxylic acid as starting material.

Physicochemical data of compounds **1–29** are summarized in *table I*. The ^{1}H-NMR spectre are presented in *table II*.

3. Pharmacology

All the derivatives **1–29** were evaluated for their affinity for the sigma sites. For the most potent ones, the affinity for the $5HT_1A$ and the D_2 receptor was also researched.

Compound **5**, one of the compounds having the highest affinity for the sigma site has been studied on two models predictive for an antipsychotic activity:

– NMDA evoked release of [^{3}H]noradrenaline from preloaded hippocampal slices made from Sprague–Dawley rats;

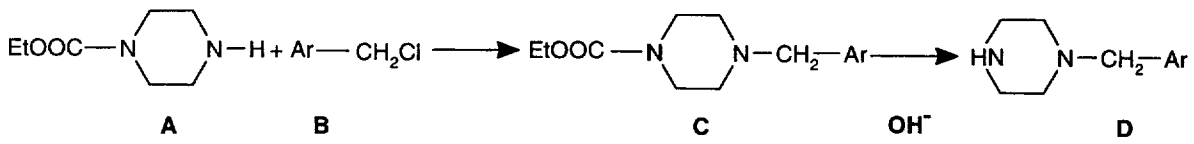


Figure 1.

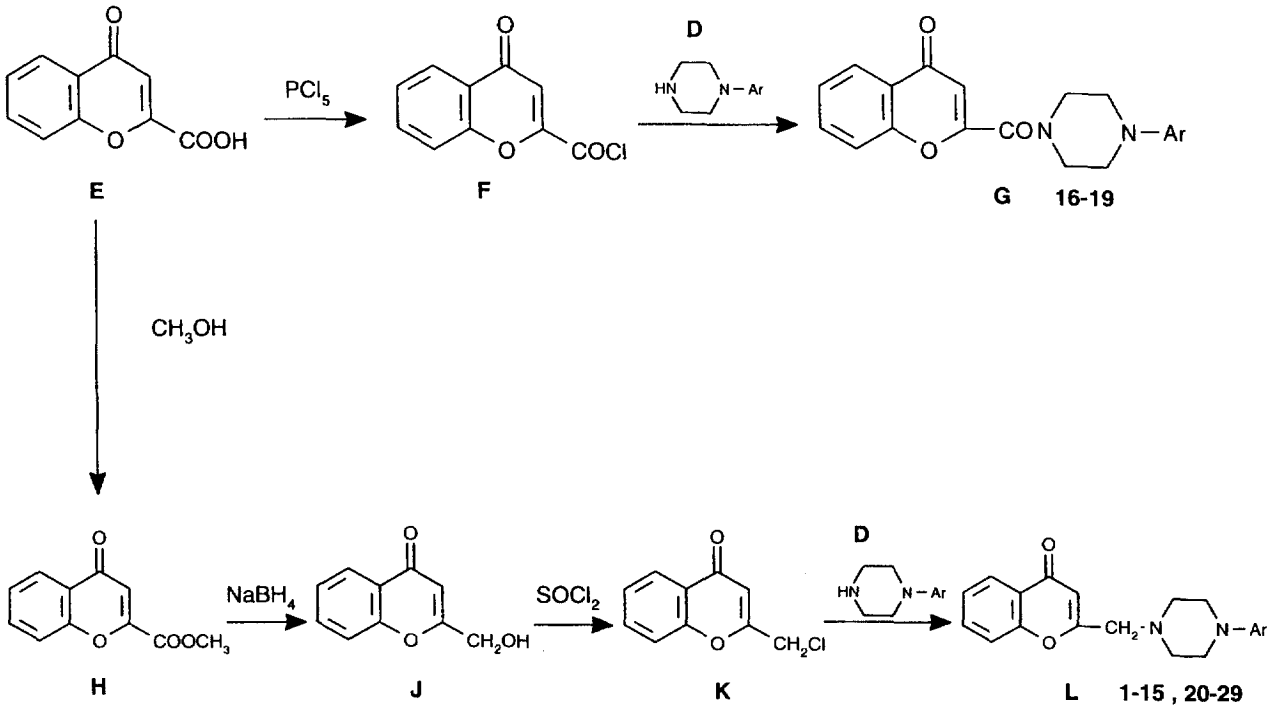


Figure 2.

– potential effect on hyperactivity induced by amphetamine and research for its potential cataleptic effect.

4. Results and discussion

4.1. Binding data

For all compounds, their chemical structure, their binding characteristics to sigma sites as well as for the most potent ones to the D_2 and $5HT_1A$ receptors are shown in *table III*. K_i are expressed in nanomoles.

The evaluation of these new ligands shows that the substitution of the chromone moiety by a methyl group slightly modifies the affinity of the corresponding compounds for the sigma site. The K_i of **2**, the 6-Me derivative of **1**, is 40 nM instead of 3 nM for **1**; **17**, the 6-Me derivative of **16** and **16** have both a 1000 nM K_i value and **19**, the 3-Me derivative of **18**, has a K_i value of 20 nM instead of 30 nM for **18**.

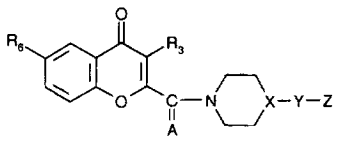
The replacement of the methylene located between the piperazine moiety and the chromone system by a carbonyl group decreases the affinity for all the studied compounds **16**, **17**, **18** in comparison to **1**, **2**, **5** (respectively 1000 nM, 1000 nM and 30 nM in comparison to 3.40 and 0.6 nM). The replacement of the piperazine by a piperidine (compound **20**) slightly decreases the affinity for the sigma sites (K_i = 30 nM instead of 3 nM for compound **1**).

The results obtained after substitution of the phenyl ring of the benzyl moiety are strictly depending on the nature of the substituent as well as its location on the phenyl ring. The best affinities (K_i = 0.6 nM) are obtained for the 4-OCH_3 derivative **5** as well as for the 4-F derivative **10**. The introduction of the methoxy substituent in the 3-position decreases the affinity to the K_i value of 20 nM; the 2-OCH_3 derivative **7** only has a K_i value of 800 nM. The same results appears concerning the dichlorobenzyl derivatives: the 3,4-dichloro isomer **12** exhibits a better σ affinity than its 2,4-analogue **13** in accordance with the conclusions of another report [32].

An increase of the length of the alkylene group between the phenyl group and the piperazine moiety, (**22**, **23**, **24**) slightly decreases the affinity for the sigma sites and the importance of this diminution is correlated to the increase of the length of this part of the molecule.

The replacement of the benzyl substituent of the piperazine moiety by an aryl group (phenyl **26**, optionnally substituted **27**, **28**, or 2-pyrimidyl **29**)

Table I. Physico-chemical data of compounds **1–29**.

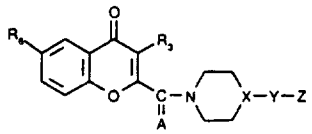


Compound	R_6	R_3	A	X	Y	Z	% yield	M.p. (°C) HCl	Formula
1	H	H	H_2	N	CH_2	phenyl	36	238–239	$C_{21}H_{22}N_2O_2$, 2HCl
2	CH_3	H	H_2	N	CH_2	phenyl	78	227–229	$C_{22}H_{24}O_2N_2$, 2HCl
3	H	H	H_2	N	CH_2	4-methylphenyl	15	283–284	$C_{22}H_{24}N_2O_2$, 2HCl
4	H	H	H_2	N	CH_2	4-trifluoromethyl	30	233–234	$C_{22}H_{21}F_3N_2O_2$, 2HCl
5	H	H	H_2	N	CH_2	4-methoxyphenyl	43	226–227	$C_{22}H_{24}N_2O_3$, 2HCl
6	H	H	H_2	N	CH_2	3-methoxyphenyl	42	208–209	$C_{22}H_{24}N_2O_3$, 2HCl
7	H	H	H_2	H	CH_2	2-methoxyphenyl	54	214–213	$C_{22}H_{24}N_2O_3$, 1HCl
8	H	H	H_2	N	CH_2	2,3,4-triOCH_3phenyl	54	181–182	$C_{24}H_{28}N_2O_5$, 2HCl
9	H	H	H_2	N	CH_2	4-isopropoxyphenyl	46	204–206	$C_{24}H_{28}N_2O_3$, 2HCl
10	H	H	H_2	N	CH_2	4-fluorophenyl	57	221–222	$C_{21}H_{21}FN_2O_2$, 2HCl
11	H	H	H_2	N	CH_2	4-chlorophenyl	41	204–205	$C_{21}H_{21}ClN_2O_2$, 2HCl
12	H	H	H_2	N	CH_2	3,4-dichlorophenyl	37	222–224	$C_{21}H_{20}Cl_2N_2O_2$, 2HCl
13	H	H	H_2	N	CH_2	2,4-dichlorophenyl	50	198–199	$C_{21}H_{20}Cl_2N_2O_2$, 2HCl
14	H	H	H_2	N	CH_2	3,4-methylenedioxy phenyl	59	214–215	$C_{22}H_{22}N_2O_4$, 2HCl
15	H	H	H_2	N	CH_2	2-naphtyl	51	185–186	$C_{25}H_{24}N_2O_2$, 1HCl
16	H	H	O	N	CH_2	phenyl	84	225–226	$C_{21}H_{20}N_2O_3$, 1HCl
17	CH_3	H	O	N	CH_2	phenyl	52	213–214	$C_{22}H_{22}N_2O_3$, 1HCl
18	H	H	O	N	CH_2	4-methoxyphenyl	52	258–259	$C_{22}H_{22}N_2O_4$, 1HCl
19	H	CH_3	O	N	CH_2	4-methoxyphenyl	52	230–231	$C_{23}H_{24}N_2O_4$, 1HCl
20	H	H	H_2	CH	CH_2	phenyl	10	208–209	$C_{22}H_{23}NO_2$, 2HCl
21	H	H	H_2	N	CO	4-fluorophenyl	36	204–206	$C_{21}H_{19}FN_2O_3$, 1HCl
22	H	H	H_2	N	$(CH_2)_2$	phenyl	60	223–224	$C_{22}H_{24}N_2O_2$, 2HCl
23	H	H	H_2	N	$(CH_2)_3$	phenyl	47	216–217	$C_{23}H_{26}N_2O_2$, 2HCl
24	H	H	H_2	N	$(CH_2)_4$	phenyl	63	187–188	$C_{24}H_{28}N_2O_2$, 2HCl
25	H	H	H_2	N	$(CH_2)_3CO$	4-fluorophenyl	55	198–199	$C_{24}H_{25}FN_2O_3$, 2HCl
26	H	H	H_2	N	phenyl	H	50	216–217	$C_{20}H_{20}N_2O_2$, 2HCl
27	H	H	H_2	N	phenyl	4-OCH_3	30	159–160	$C_{21}H_{22}N_2O_3$, 2HCl
28	H	H	H_2	N	phenyl	2-OCH_3	42	194–195	$C_{21}H_{22}N_2O_3$, 2HCl
29	H	H	H_2	N	pyrimidyl	H	15	198–199	$C_{18}H_{18}N_4O_2$, 2HCl

Table II. ^1H NMR ($CDCl_3$), δ ppm (bases).

1	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).
2	2.41 (s, 3H, Ar–CH_3); 2.50–2.61 (m, 8H, piperaz); 3.46–3.50 (2s, 4H, 2CH_2–N); 6.40 (s, 1H, H_3); 7.20–7.34 (m, 6H, Ar and H_8); 7.45 (dd, 1H, H_7, J = 2.1 Hz and J = 8.5 Hz); 7.95 (d, 1H, H_5, J = 2.1 Hz).
3	2.33 (s, 3H, Ar–CH_3); 2.56–2.65 (m, 8H, piperaz); 3.50 (s, 2H, N–CH_2); 3.67 (s, 2H, CH_2–N); 6.43 (s, 1H, H_3); 7.12–7.21 (m, 4H, Ar); 7.31–7.75 (m, 3H, H_6, H_7 and H_8); 8.18 (dd, 1H, H_5).
4	2.52–2.64 (m, 8H, piperaz); 3.49 (s, 2H, Ar–CH_2–N); 3.57 (s, 2H, CH_2–N); 6.44 (s, 1H, H_3); 7.14–7.67 (m, 7H, Ar and H_6, H_7, H_8); 8.18 (dd, 1H, H_5).
5	2.50–2.62 (m, 8H, piperaz); 3.52 (s, 2H, N–CH_2); 3.80 (s, 3H, OCH_3); 3.96 (s, 2H, CH_2–N); 6.43 (s, 1H, H_3); 6.85 (d, 2H, Ar, J = 8 Hz); 7.37 (d, 2H, Ar, J = 8 Hz); 7.46–7.94 (m, 3H, H_6, H_7 and H_8); 8.19 (dd, 1H, H_5).
6	2.52–2.64 (m, 8H, piperaz); 3.50 (s, 2H, N–CH_2); 3.80 (s, 3H, OCH_3); 4.02 (s, 2H, CH_2–N); 6.43 (s, 1H, H_3); 6.85–7.30 (m, 4H, Ar); 7.41–7.90 (m, 3H, H_6, H_7 and H_8); 8.19 (dd, 1H, H_5).
7	2.49–2.63 (m, 8H, piperaz); 3.50 (s, 2H, N–CH_2); 3.60 (s, 2H, CH_2–N); 3.80 (s, 3H, OCH_3); 6.43 (s, 1H, H_3); 6.90–7.33 (m, 4H, Ar); 7.45–7.92 (m, 3H, H_6, H_7 and H_8); 8.20 (dd, 1H, H_5).
8	2.56–2.60 (m, 8H, piperaz); 3.48 (s, 2H, N–CH_2); 3.76 (s, 3H, OCH_3); 3.82 (s, 3H, OCH_3); 3.87 (s, 3H, OCH_3); 4.21 (s, 2H, CH_2–N); 6.47 (s, 1H, H_3); 6.63 (d, 1H, Ar); 6.98 (d, 1H, Ar); 7.36–7.86 (m, 3H, H_6, H_7 and H_8); 8.20 (dd, 1H, H_5).
9	1.30 (d, 6H, $CH(CH_3)_2$, J = 6,05 Hz); 2.55–2.63 (m, 8H, piperaz); 3.46 and 3.49 (2s, 4H, N–CH_2 and CH_2–N); 4.49 (m, 1H, CH–$(CH_3)_2$); 6.43 (s, 1H, H_3); 6.79 (d, 2H, Ar, J = 8.90 Hz); 7.17 (d, 2H, Ar, J = 8.90 Hz); 7.27–7.68 (m, 3H, H_6, H_7, H_8); 8.17 (dd, 1H, H_5).
10	2.50–2.62 (m, 8H, piperaz); 3.48 (s, 2H, N–CH_2); 3.50 (s, 2H, CH_2–N); 6.43 (s, 1H, H_3); 7.01–7.35 (m, 4H, Ar); 7.41–7.91 (m, 3H, H_6, H_7 and H_8); 8.20 (dd, 1H, H_5).
11	2.58–2.68 (m, 8H, piperaz); 3.38 (s, 2H, N–CH_2); 3.53 (s, 2H, CH_2–N); 6.43 (s, 1H, H_3); 7.29–7.32 (m, 4H, Ar); 7.40–7.90 (m, 3H, H_6, H_7 and H_8); 8.18 (dd, 1H, H_5).
12	2.50–2.64 (m, 8H, piperaz); 3.46 (s, 2H, N–CH_2); 3.50 (s, 2H, CH_2–N); 6.43 (s, 1H, H_3); 7.15–7.36 (m, 3H, Ar); 7.41–7.86 (m, 3H, H_6, H_7 and H_8); 8.20 (dd, 1H, H_5).
13	2.53–2.62 (m, 8H, piperaz); 3.50 (s, 2H, N–CH_2); 3.59 (s, 2H, CH_2–N); 6.44 (s, 1H, H_3); 7.20–7.40 (m, 3H, Ar); 7.48–7.93 (m, 3H, H_6, H_7 and H_8); 8.18 (dd, 1H, H_5).
14	2.50–2.61 (m, 8H, piperaz); 3.56 (s, 2H, N–CH_2); 3.95 (s, 2H, CH_2–N); 5.93 (s, 2H, –OCH_2); 6.43 (s, 1H, H_3); 6.84–7.14 (m, 3H, Ar); 7.36–7.86 (m, 3H, H_6, H_7 and H_8); 8.20 (dd, 1H, H_5).
15	2.56–2.58 (m, 8H, piperaz); 3.44 (s, 2H, N–CH_2); 3.64 (s, 2H, CH_2–N); 6.42 (s, 1H, H_3); 7.25–7.79 (m, 10 H, Ar); 8.19 (dd, 1H, H_5).
16[a]	3.23–3.50 (m, 6H, piperaz); 4.35–4.45 (m, 4H, N–*CH_2*–Ar and piperaz); 6.60 (s, 1H, H_3); 7.42–7.98 (m, 8H, Ar); 8.18 (dd, 1H, H_5); 11.65 (s broad, 1H, NH^+).
17[a]	2.55 (s, 3H, CH_3); 3.28–3.72 (m, 6H, piperaz); 4.24–4.56 (m, 4H, N–*CH_2*–Ar and piperaz); 6.72 (s, 1H, H_3); 7.58 (m, 3H, Ar); 7.73 (m, 3H, Ar and H_8); 7.78 (m, 1H, H_7); 7.95 (dd, 1H, H_5, J = 2 and 8 Hz); 11.80 (s broad, 1H, NH^+).
18[a]	3.23–3.50 (m, 6H, piperaz); 3.89 (s, 3H, OCH_3); 4.25–4.63 (m, 4H, N–*CH_2*–Ar and 2H piperaz); 6.76 (s, 1H, H_3); 7.12 (d, 2H, Ar, J = 8.4 Hz); 7.62–7.70 (m, 2H, H_6, H_8); 7.80 (d, 2H, Ar, J = 8.4 Hz); 7.99 (m, 1H, H_7); 8.18 (dd, 1H, H_5, J = 2 and 8 Hz); 11.58 (s broad, 1H, NH^+).
19[a]	2.05 (s, 3H, CH_3); 3.20–3.56 (m, 6H, piperaz); 3.88 (s, 3H, OCH_3); 4.10–4.68 (m, 4H, N–*CH_2*–Ar and piperaz); 7.10–7.14 (m, 2H, Ar); 7.60–7.67 (m, 3H, Ar and H_6); 7.75 (m, 1H, H_8); 7.96 (m, 1H, H_7); 8.19 (d, 1H, H_5); 11.54 (s broad, 1H, NH^+).
20	1.47–2.10 (m, 9H, piperid); 2.54 (d, 2H, Ar–CH_2); 3.46 (s, 2H, N–CH_2); 6.42 (s, 1H, H_3); 7.11–7.47 (m, 5H, Ar); 7.14–7.64 (m, 3H, H_6, H_7, H_8); 8.18 (dd, 1H, H_5).
21	1.67–1.89 (m, 4H, piperaz); 2.60–2.65 (m, 4H, piperaz); 3.54 (s, 2H, CH_2–N); 6.45 (s, 1H, H_3); 7.06–7.37 (m, 4H, Ar); 7.47–7.88 (m, 3H, H_6, H_7 and H_8); 8.20 (dd, 1H, H_5).
22	2.57–2.86 (m, 12H, piperaz and 2 CH_2); 3.52 (s, 2H, CH_2–N); 6.45 (s, 1H, H_3); 7.15–7.35 (m, 5H, Ar); 7.39–7.70 (m, 3H, H_6, H_7, H_8); 8.20 (dd, 1H, H_5).
23	1.79 (m, 2H, N–CH_2–*CH_2*–CH_2–Ar); 2.04–2.65 (m, 12H, piperaz and N–*CH_2*–CH_2–*CH_2*–Ar); 3.50 (s, 2H, CH_2–N); 6.44 (s, 1H, H_3); 7.16–7.43 (m, 5 H, Ar); 7.47–7.70 (m, 3H, H_6, H_7, H_8); 8.15 (dd, 1H, H_5).
24	1.49–161 (m, 4H, 2 (CH_2)); 2.31–2.62 (m, 12H piperaz and 2 CH_2); 3.46 (s, 2H, CH_2–N); 6.41 (s, 1H, H_3); 7.12–7.32 (m, 5H, Ar); 7.38–7.68 (m, 3H, H_6, H_7, H_8); 8.18 (dd, 1H, H_5).
25	1.93 (m, 2H, N–CH_2–*CH_2*–CH_2–COAr); 2.45 (t, 2H, N–*CH_2*–CH_2–CH_2–COAr); 2.56–2.60 (m, 8H, piperaz); 2.94 (t, 2H, N–CH_2–CH_2–*CH_2*–COAr); 3.85 (s, 2H, CH_2–N); 6.41 (s, 1H, H_3); 6.88–7.94 (m, 4H, Ar); 7.38–7.88 (m, 3H, H_6, H_7, H_8); 8.20 (dd, 1H, H_5).
26	2.77–3.25 (m, 8H, piperaz); 3.57 (s, 2H, CH_2–N); 6.47 (s, 1H, H_3); 6.84–7.34 (m, 5H, Ar); 7.26–7.66 (m, 3H, H_6, H_7 and H_8); 8.23 (dd, 1H, H_5).
27	2.80–3.16 (m, 8H, piperaz); 3.70 (s, 2H, CH_2–N); 3.82 (s, 3H, OCH_3); 6.47 (s, 1H, H_3); 6.84–7.06 (m, 4H, Ar); 7.36–7.71 (m, 3H, H_6, H_7 and H_8); 8.20 (dd, 1H, H_5).
28	2.81–3.14 (m, 8H, piperaz); 3.58 (s, 2H, CH_2–N); 3.86 (s, 3H, OCH_3); 6.48 (s, 1H, H_3); 6.82–6.97 (m, 4H, Ar); 7.21–7.62 (m, 3H, H_6, H_7 and H_8); 8.18 (dd, 1H, H_5).
29	2.56–2.73 (m, 4H, piperaz); 3.55 (s, 2H, CH_2–N); 3.72–3.94 (m, 4H, piperaz); 6.42 (s, 1H, H_3); 6.47 (m, 1H, Ar); 7.30–7.68 (m, 3H, H_6, H_7 and H_8); 8.18 (dd, 1H, H_5); 8.31 (m, 2H, Ar).

Table III. Binding of 2-amino alkyl chromones and related derivatives.



Compound	R_6	R_3	A	X	Y	Z	σ K_i	$5HT_{1A}$ K_i	D_2 K_i
1	H	H	H_2	N	CH_2		3	$> 10^4$	10^4
2	CH_3	H	H_2	N	CH_2		40	$> 10^4$	10^4
3	H	H	H_2	N	CH_2		30	$> 10^4$	10^4
4	H	H	H_2	N	CH_2		50	$> 10^4$	10^4
5	H	H	H_2	N	CH_2		0.6	$> 10^4$	$> 10^4$
6	H	H	H_2	N	CH_2		20	$> 10^4$	$> 10^4$
7	H	H	H_2	N	CH_2		800	$> 10^4$	$> 10^4$
8	H	H	H_2	N	CH_2		500	$> 10^4$	$> 10^4$
9	H	H	H_2	N	CH_2		200	$> 10^4$	$> 10^4$
10	H	H	H_2	N	CH_2		0.6	$> 10^4$	$> 10^4$
11	H	H	H_2	N	CH_2		1	$> 10^4$	$> 10^4$
12	H	H	H_2	N	CH_2		0.8	$> 10^4$	5000
13	H	H	H_2	N	CH_2		20	6000	$> 10^4$
14	H	H	H_2	N	CH_2		10	8000	$> 10^4$
15	H	H	H_2	N	CH_2		5	$> 10^4$	$> 10^4$
16	H	H	O	N	CH_2		1000	10^4	$> 10^4$
17	CH_3	H	O	N	CH_2		1000	$> 10^4$	$> 10^4$
18	H	H	O	N	CH_2		30	$> 10^4$	4000
19	H	CH_3	O	N	CH_2		20	8000	$> 10^4$
20	H	H	H_2	CH	CH_2		30	$> 10^4$	$> 10^4$
21	H	H	H_2	N	CO		$> 10^4$	$> 10^4$	$> 10^4$
22	H	H	H_2	N	$(CH_2)_2$		5	$> 10^4$	$> 10^4$
23	H	H	H_2	N	$(CH_2)_3$		8	$> 10^4$	$> 10^4$
24	H	H	H_2	N	$(CH_2)_4$		10	$> 10^4$	$> 10^4$
25	H	H	H_2	N	$(CH_2)_3CO$		100	$> 10^4$	$> 10^4$
26	H	H	H_2	N		H	$> 10^4$	$> 10^4$	$> 10^4$
27	H	H	H_2	N		$-OCH_3$	5000	$> 10^4$	$> 10^4$
28	H	H	H_2	N		$-OCH_3$	$> 10^4$	$> 10^4$	$> 10^4$
29	H	H	H_2	N		H	$> 10^4$	900	$> 10^4$

dramatically lowers the affinity for the sigma sites. This is also the result obtained with compound **21** in which the benzyl group is replaced by a 4-fluoro benzoyl.

Concerning the affinity for the others receptors all the tested compounds do not have strong affinity neither for the $5HT_{1A}$ receptors nor for the D_2 ones.

If we compare our results to the previous disclosed compounds [10], it appears that our derivatives are more potent ligands to the sigma sites and, generally speaking, more selective in comparaison with D_2 receptors indicating that in this pharmacological field an amino alkyl side chain should be preferentially set on the pyrane ring.

In conclusion the present study has provided very potent and selective σ ligands. For many compounds the σ affinity is in the nanomolar range and the selectivity for the other receptors is very high.

According to these binding data, we decided to submit one compound to complementary studies in order to research their antipsychotic potentiel in accordance with the most recent works previously quoted about sigma ligands. We selected compound **5**, one of the most potent sigma ligands according to the result of *table III*.

4.2. Biochemical tests

Compound **5** was tested for its potential effect on *N*-methyl-D-Aspartate (NMDA) evoked release of [^{3}H] Noradrenaline (NA) from preloaded hippocampal slices made from Sprague–Dawley rats. Compound **5** potentiated in a concentration-dependent manner NMDA-induced [^{3}H] NA release, without affecting the basal out flows. The maximal response consisted of a 182% increase of NMDA response at a concentration of 300 nmol/L. The increase in NMDA evoked release of [^{3}H] noradrenaline was +97%, +158%, +160%, +182% and +81% respectively at 10, 30, 100, 300 and 1000 nM/L. Haloperidol, which did not modify NMDA-evoked [^{3}H] NA release by itself, completely prevented the effects of compound **5**. Gi/o proteins were inactivated with pertussis toxin (PTX) and the potentiation of NMDA response-induced by compound **5** was not modified, showing that the compound interacted on the σ site not coupled to Gi/o protein, i.e. σ_2 site and that this compound is a σ_2 agonist.

4.3. Behavioral tests

Compound **5** 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 antipsychotic activity in the absence of extrapyramidal side-effects.

Compound **5** dose-dependently reduced the hyperactivity induced by amphetamine in mice; the antagonism was 34%, 58%, $p < 0.001$ and 90%, $p < 0.001$ respectively for the doses of 8, 16 and 32 mg/kg IP.

In the same conditions, compound **5** did not antagonize the stereotypies induced by amphetamine, which are the results from dopaminergic activation in the striatum. In addition, compound **5** did not induce catalepsy at the doses of 16 and 64 mg/kg IP in rats which is considered to be a predictor of Parkinson-like extrapyramidal side effects. Compound **5** was thus active in tests predictive of antipsychotic activity and inactive in tests predictive of extrapyramidal effects that suggests it could be interesting in the treatment of psychosis.

5. Experimental protocols

5.1. Chemistry

Melting points were determined on a Kofler apparatus and are not corrected. When higher than 260 °C, they are determined on a Maquenne apparatus and not corrected. IR spectra were recorded on a Perkin ELMER 983 G spectrophotometer (all spectra were in accordance with the assigned structures). NMR spectra were recorded on a AC200 Brucker spectrometer. Chemical shifts are reported in δ units (parts per million) relative to $(CH_3)_4Si$. Elemental analysis for new substances were performed by CNRS Laboratories (Vernaison–France). Obtained results were within ± 0.4% of theoretical values.

*5.1.1. General procedure for the synthesis of the amidic derivatives G **16–19***

4-oxo-4H-1-benzopyran-2-carbonyl chloride **F** (*figure 2*) is prepared according to the previously disclosed method [33].

*5.1.2. Amidic derivatives G **16–19***

The method adopted for the synthesis of 2-(4-benzyl-1-piperazinyl carbonyl) 4H-1-benzopyran-4-one **16** is disclosed. To a solution of 1.76 g (0.01 mol) of 1-benzyl piperazine in 100 mL of methylene chloride, a solution of 2.09 g (0.01 mol) of 4-oxo-4H-1-benzopyran-2-carbonyl chloride in 100 mL of the same solvent is added. The mixture is stirred for 5 hours at room temperature. After filtration the compound obtained on the hydrochloride form is crystallized from ethanol affording 3.23 g (84%) of **16**: m.p. 225–226 °C.

*5.1.3. General procedure for the synthesis of the amine derivatives **L 1–15; 20–29** (figure 2)*

The method adopted for the synthesis of 2-(4-benzyl-1-piperazinylmethyl) 4H-1-benzopyran-4-one **1** is described.

*5.1.4. Methyl 4-oxo-4H-1-benzopyran 2-carboxylate **H** [34], 2-Hydroxymethyl 4H-1-benzopyran-4-one **J** [35], and 2-chloromethyl 4H-1-benzopyran-4-one **K** [27, 36]*

These compounds are prepared according to previously described methods.

*5.1.5. 2-(4-Benzyl-1-piperazinyl methyl) 4H-1-benzopyran-4-one **1***

5.28 g (0.03 mol) of 1-benzyl piperazine, 5.83 g (0.03 mol) of 2-chloromethyl-4H-1-benzopyran-4-one **K** and 6.91 g (0.05 mol) of potassium carbonate are added to 150 mL of anhydrous tetrahydrofuran. The reaction mixture was refluxed for 24 hours. After cooling, the solution is filtered and evaporated under reduced pressure. The solid so obtained is purified by silica gel column chromatography using methylene chloride as eluant. The product is obtained on the base form. IR ν cm^{-1} (film): 2937, 2913, 2879 (CH, CH_2); 1654 (C=O); 1607 (C=C).

To obtain the hydrochloride, 3.34 g (0.01 mol) of the piperazine derivative is dissolved in 10 mL of isopropanol. Then hydrochlorhydric isopropanol is added until a solid appears. The hydrochloride is crystallized from isopropanol m.p. 238–240 °C.

*5.1.6. General procedure for the synthesis of the 4-arylalkyl piperazine **D** (figure 1)*

The method adopted for the synthesis of 1-(4-trifluoromethylbenzyl)piperazine is disclosed.

5.1.7. Ethyl 4-(4-trifluoromethylbenzyl)-1-piperazine carboxylate

7.78 g (0.04 mol) of α-chloro-α,α,α-trifluoro-p xylene and 4.33 g (0.04 mol) of ethyl 1-piperazine carboxylate are added to 200 mL of anhydrous tetrahydrofuran. The reaction mixture is refluxed for 30 min. Then 5.53 g (0.04 mol) of potassium carbonate is then added and the mixture refluxed for 12 hours. After filtration and extraction by methylene chloride, the title product is purified by column chromatography on silica gel using methylene chloride as eluant.

IR (film) ν cm^{-1}: 3044, 2981, 2911, 2867 (CH, CH_2, CH_3); 1693 (COO); 1610 (C=C); ^1H NMR $CDCl_3$ δ ppm: 1.24 (t, 3H, CH_2–CH_3); 2.40 (m, 4H, $N(CH_2)_2$); 3.51 (m, 6H, $N(CH_2)_2$ and Ar–CH_2–N); 4.13 (q, 2H, CH_2–CH_3); 7.46 (d, 2H, H_2 and H_6, J = 8.3 Hz); 7.65 (d, 2H, H_3 and H_5, J = 8.3 Hz).

The previous method is also used for 1-(2-Phenylethyl)-piperazine, 1-(3-phenyl *n*-propyl piperazine) and 1-(naphthyl 2-methyl)piperazine, but THF is replaced by DMF.

5.1.8. 1-(4-Trifluoromethylbenzyl) piperazine

1.86 g (7 x 10^{-3} mol) of ethyl-4-(4-trifluoromethyl benzyl) 1-piperazinecarboxylate and 100 mL of sodium hydroxyde (10% w/w) are added to 50 mL of methanol. The reaction mixture is refluxed for 24 hours. After extraction with chloroforme, the compound is isolated by silica gel column chromatography using ethanol as eluant.

IR (film) ν cm^{-1}: 3391 (NH); 3002, 2942, 2914 (CH, CH_2); 1616 (C=C); ^1H NMR $CDCl_3$ δ ppm: 2.42 (m, 4H, $N(CH_2)_2$); 2.69 (s, broad, 1H, NH); 2.90 (m, 4H, $N(CH_2)_2$); 3.45 (s, 2H, Ar–CH_2–N); 7.44 (d, 2H, H_2 and H_6, J = 8 Hz); 7.60 (d, 2H, H_3 and H_5, J = 8 Hz).

5.1.9. Synthesis of 1-(4-fluorobenzoyl)piperazine

1-(4-fluorobenzoyl)-4-benzyl piperazine: To a solution of 1.76 g (0.01 mol) of 1-benzyl piperazine in 100 mL of methylene chloride, a solution of 1.60 g (0.01 mol) of 4-fluorobenzoylchloride in 100 mL of methylene chloride is added. The reaction mixture is stirred for 5 hours at room temperature. After filtration, the title compound on the hydrochloride form is crystallized from ethanol. IR (KBr) ν cm^{-1}: 3060, 2900, 2802 (CH, CH_2); 2630–2440 (NH^+); 1630 (CO); 1605, 1595 (C=C); ^1H NMR DMSO-d_6, δ ppm: 3.10–3.47 (m, 8H, $2N(CH_2)_2N$); 4.43 (s, 2H, N–CH_2–Ar); 7.48–7.60 (m, 9H, aromatics); 11.48 (s, broad, 1H, NH^+).

1-(4 fluorobenzoyl)piperazine: 6.70 g (0.02 mol) of 1-(4-fluorobenzoyl)-4-benzyl piperazine hydrochloride are dissolved in 200 mL of warm ethanol. Then 1 g of palladium on activated carbon (10% Pd) was added and the reaction mixture is introduced into a 500 mL hydrogenation apparatus. After flushing out, the necessary quantity of hydrogene is introduced and the reaction mixture is heaten up to 100 °C and stirred for 8 hours. The warm solution is filtered and evaporated under reduced pressure. The product so obtained, as hydrochloride, is crystallized from isopropanol. To obtain the base, the hydrochloride is treated with an aqueous solution of potassium carbonate. After filtration, the mixture is extracted three times with 30 mL of methylene chloride. The organic layer is evaporated under reduced pressure and the product is obtained. IR (KBr) ν cm^{-1}: 3386–3300 (NH); 2995, 2951, 2863, 2792 (CH, CH_2); 1643 (CO); 1600,1520 (C=C); ^1H NMR $CDCl_3$ δ ppm: 2.87–2.98 (m, 5H, $N(CH_2)_2$, and NH); 3.49–3.65 (m, 4H, $N(CH_2)_2$); 7.04–7.46 (m, 4H, aromatics).

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 [^3H](+) (PPP) 3-(3-hydroxyphenyl)-*N*-(*n*-propyl)piperidine (3-PPP) from Guinea pig cortex homogenates, as described by Karbon et al. [37].

5.2.2. 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 [^3H]-8-hydroxy-3-(di-*n*-propyl amino) tetraline (8-OH DPAT) from bovine frontal cortex and hippocampus homogenates, as described by Hoyer et al. [38].

5.2.3. 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 [^3H]-raclopride from bovine striatum homogenates, as described by Köhler et al. [39].

5.2.4. 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 [^3H](+)-Pentazocine from Hartley Guinea pig brain homogenates minus cerebellum as described by De Haven-Hudkins et al. [40]. The affinity of test compounds for σ_2 binding sites was estimated by their ability to displace [^3H] 1,3-di (0-tolyl) guanidine (DTG) from rat liver membranes, as described by Weber et al. [41].

5.2.5. Modulation by σ ligands of N-methyl D-aspartate-induced ^3H-noradrenaline release in rat hippocampus

The experiment was carried out in hippocampal slices from Sprague–Dawley rats. The [^3H] NA release was evoked once by a 4-minute exposure to NMDA, 40 minutes after the beginning of superfusion with a Mg^{2+} free Krebs solution.

G i/o proteins were inactivated with pertussis toxin, injected locally from 3 to 11 days prior to the experiment, to assess the possible involvment of G i/o proteins in the modulation of NMDA evoked [^3H] NA release.

Compound **5** was tested at different concentrations ranging from 10 to 1000 nmol, in continuous perfusion according to Monnet et al. [42, 43].

5.2.6. 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-minute test according to Costall et al. [44].

5.2.7. Catalepsy

Wistar rats were injected with the test compounds and tested for catalepsy at 30 min intervals. Catalepsy was assessed by three procedures: imposed crossing of the ipsilateral for- and hindlimbs, placing in the Buddha position and the tilting board, an automatic device which displaces the rat from a horizontal to vertical position and back while it clings to a wire grid with its front paws, according to Chermat and Simon [45].

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