Design and Evaluation of Naphthol- and Carbazole-Containing Fluorescent σ Ligands as Potential Probes for Receptor Binding Studies

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Some 3,3-dimethyl-1-(3-naphthylpropyl)piperidine and 1-cyclohexyl-4-(3-naphthylpropyl)piperazine derivatives, structurally containing naphthol as a fluorescent moiety, were prepared for being potentially used as fluorescent σ ligands. Structurally related analogs were also prepared, where the naphthalene nucleus was replaced by the fluorescent carbazole moiety and chain length was varied. For all compounds the in vitro affinities toward σ receptors and $\Delta_8-\Delta_7$ sterol isomerase site were measured, and the fluorescent properties were determined. Compound **19** gave the best results both for σ receptor affinities (σ_1, $K_i = 6.78$ nM and σ_2, $K_i = 26.4$ nM) and fluorescence features; thus, it was chosen for in vitro saturation binding analysis at σ receptors. The good results obtained in such assay suggested that the fluorescent compound **19** could be used instead of a radioligand in "green" binding assays.

Introduction

Sigma (σ) receptors are classified in two distinct subtypes, σ_1 and σ_2.[1] These receptors have been found in the central nervous system (CNS), in endocrine tissues, liver, kidney, and immune system cells, and their physiological functions are still under investigation.[2] In CNS the σ_1 receptor subtype is involved in modulation of K^+ and Ca^{2+} channels and in NMDA, serotonergic, dopaminergic, and muscarinic neurotransmission, suggesting a potential therapeutic role in the treatment of cognitive diseases, depression, and schizophrenia.[3] Human σ_1 receptor has been isolated and its cDNA has been cloned.[4] The expressed receptorial protein (25 kDa) displayed 30% homology with a yeast $\Delta_8-\Delta_7$ sterol isomerase (SI), but no enzymatic activity nor structural homology with the functional mammalian counterpart of yeast SI was found.[5]

σ_2 Receptors are involved in Ca^{2+} cytoplasmic modulation. In particular, it was demonstrated that σ_2 receptor agonists induced cell death by caspase-independent apoptosis, promoting Ca^{2+} depletion from endoplasmic and mitochondrial stores.[6,7] Moreover, σ_2 ligands down-modulate P-glycoprotein expression, suggesting their ability to revert drug resistance in several tumor cell lines.[8,9] Attempts have been carried out to isolate the σ_2 receptor more recently by our group. Characterization of the isolated proteins allowed supposition that σ_2 receptors are histone proteins.[10] Since σ receptors are overexpressed in many tumor cell lines and tumor tissues, the availability of radiolabeled σ ligands can potentiate in vitro and in vivo tumor diagnosis.[11,12]

To date, the most common σ_1 ligands reported are (+)-pentazocine (**1**), 1-(3,4-dimethoxyphenethyl)-4-(3-phenylpropyl)piperazine (SA4503 now AGY94806, **2**),[13] *N*,*N*-dipropyl-2-[4-methoxy-3-(2-phenylethoxy)phenyl]ethylamine (NE100, **3**)[14] (Chart 1). Structure−affinity relationship (SAfiR) studies on several arylalkylamine analogs[15,16] have been carried out, and a valid σ_1 pharmacophoric model has been suggested.[17] In recent years, our group developed a ligand consistent with such a σ_1 pharmacophoric model, 3,3-dimethyl-1-[3-(6-methoxynaphthalen-1-yl)propyl]piperidine (**12**), which displayed high σ_1 receptor affinity and good selectivity toward the σ_2 receptor.[18]

Among the σ_2 receptor ligands reported in the literature, 1,3-di-2-tolylguanidine (DTG, **4**) is the most used as σ_2 radioligand. A promising new agent for tumor therapy is the σ_2 receptor ligand 1′-[4-[1-(4-fluorophenyl)-1*H*-indol-3-yl]-1-butyl]spiro-[isobenzofuran-1(3*H*),4′-piperidine] (siramesine, **5**)[19,20] (Chart 1). At the present, a good σ_2 receptor ligand commercially available is 1-cyclohexyl-4-[3-(5-methoxy-1,2,3,4-tetrahydronaphthalen-1-yl)propyl]piperazine (PB28, **6**),[21] a potent pharmacological tool used for in vitro assays.[9,12,22,23] In order to better clarify the physiological and pathological involvement of σ_1 and σ_2 receptors in tumor cell growth, a fluorescent ligand could be a useful tool for investigating living cells. Fluorescent receptor ligands are usually obtained as covalent derivatives with a fluorescent moiety such as Bodipy (**7**) or Dansyl (**8**) (Chart 2).[24−27] As a unique example, Dansyl-conjugated ligands have been used to study the subcellular localization of σ_2 receptors with two-photon microscopy.[26] However, the development of these fluorescent molecular tools is limited by pharmacodynamic and pharmacokinetic aspects. Indeed, the receptor affinity, the selectivity, and the lipophilicity values of these functionalized compounds are likely different from the corresponding unlinked compounds. Furthermore, very sensitive detectors are required for the low concentrations used, and sometimes, interference by tissue autofluorescence or possible degradation by living tissue or cells has been observed.[24,25]

The aim of the present work was to obtain σ ligands having intrinsic fluorescent properties without linking any fluorescent additional moiety, in order to be used as alternative probes to radioligands. No intrinsically fluorescent σ ligand is known to have been used yet. Since 2-naphthol and 1-naphthol are used as fluorescent probes,[28−31] starting from compound **12** as a lead, we prepared a series of compounds structurally containing the 2-naphthol moiety, where the presence and position of a methoxyl or hydroxyl substituent have been investigated. Two different basic rings were linked by an alkyl chain at the substituted naphthalene nucleus: the 3,3-dimethylpiperidine as a moiety for σ_1 receptor affinity[32] and the 1-cyclohexylpiperazine for σ_2 receptor affinity.[33] In addition, some derivatives bearing fluorescent carbazole nucleus were prepared.[34−36] The spectrofluorimetric properties and the affinities toward σ_1, σ_2,

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Chart 1. Structures of σ Ligands **1**−**6** and **12**

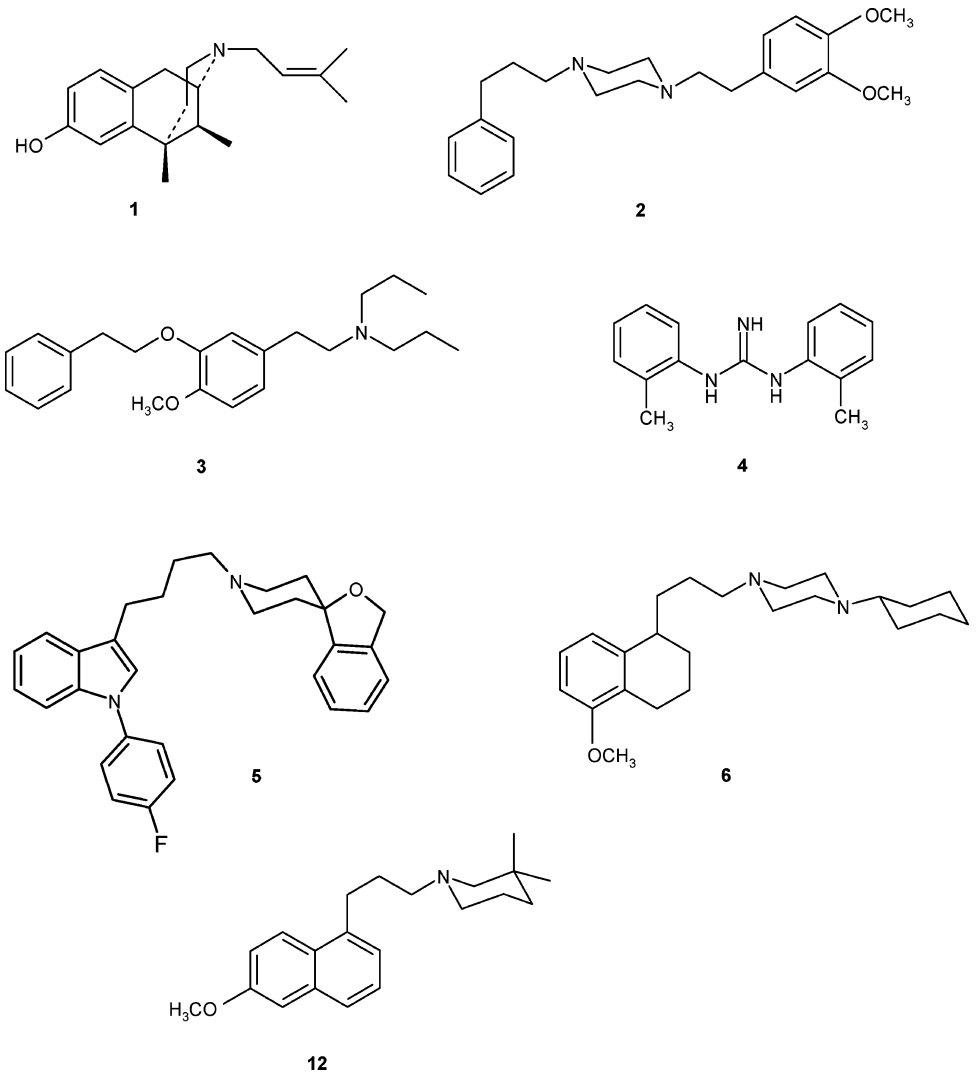
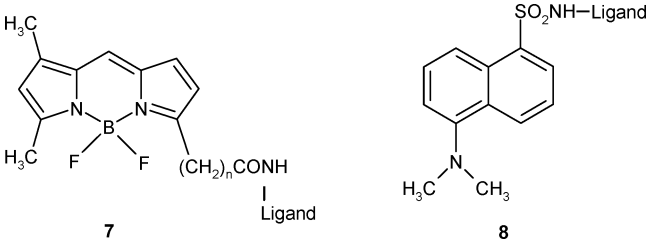


Chart 2. Structures of Fluorescent Moieties Usually Linked to Ligands



and SI sites were determined for each compound. In addition, σ receptor binding saturation analysis was carried out with compound **19**.

Chemistry

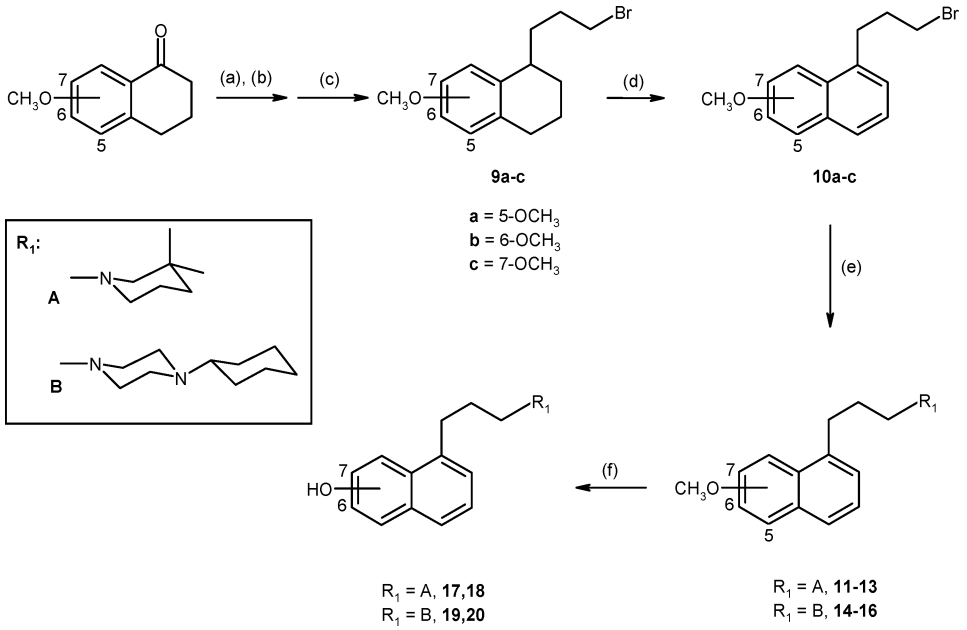
The synthesis of final compounds **11**−**20** is depicted in Scheme 1. The key intermediates **9a**−**c** were prepared via Grignard reaction starting from the corresponding methoxy-1-tetralones, as already reported.[37] Bromoalkyltetralins **9a**−**c** were treated for aromatization with DDQ (2,3-dichloro-5,6-dicyano-1,4-benzoquinone) to afford the corresponding bromoalkylnaphthalenes **10a**−**c**.[33] Final compounds **11**−**13** were obtained by alkylating 3,3-dimethylpiperidine with compounds **10a**−**c**. In the same manner, final compounds **14**−**16** were synthesized by alkylating *N*-cyclohexylpiperazine. The hydroxynaphthalene derivatives **17**−**20** were prepared from the corresponding methoxy derivatives **12**, **13**, **15**, and **16**, respectively, in the presence of BBr_3.

The preparation of final compounds **26**−**29** is depicted in Scheme 2. By reaction between carbazole and ethyl acrilate, the ester **21** was afforded and then reduced by $LiAlH_4$ to the already known alcohol **22**.[38] The corresponding mesyl derivative **23** was obtained by treating compound **22** with mesyl chloride. The chloroalkyl intermediates **24**[39] and **25** were prepared by reaction of carbazole with $NaNH_2$ and the appropriate bromochloroalkyl derivative.[40] The mesylate **23** and the chloroalkylcarbazoles **24** and **25** were treated with 3,3-dimethylpiperidine or cyclohexylpiperazine to give the final amine compounds **26**−**29**. All the final amine compounds were converted to the hydrochloride salts with gaseous HCl in the usual way. Their physical properties are listed in Table 1, along with the calculated values of the logarithm of the partition coefficient (ClogP) for the corresponding free bases.[41] The fluorescence spectra of the compounds **11**−**20** and **26**−**29** were recorded in EtOH. More spectra for compounds **17**−**20** were recorded also in 2 N NaOH. Each compound was studied at 10^{-7} M concentration.

Pharmacology

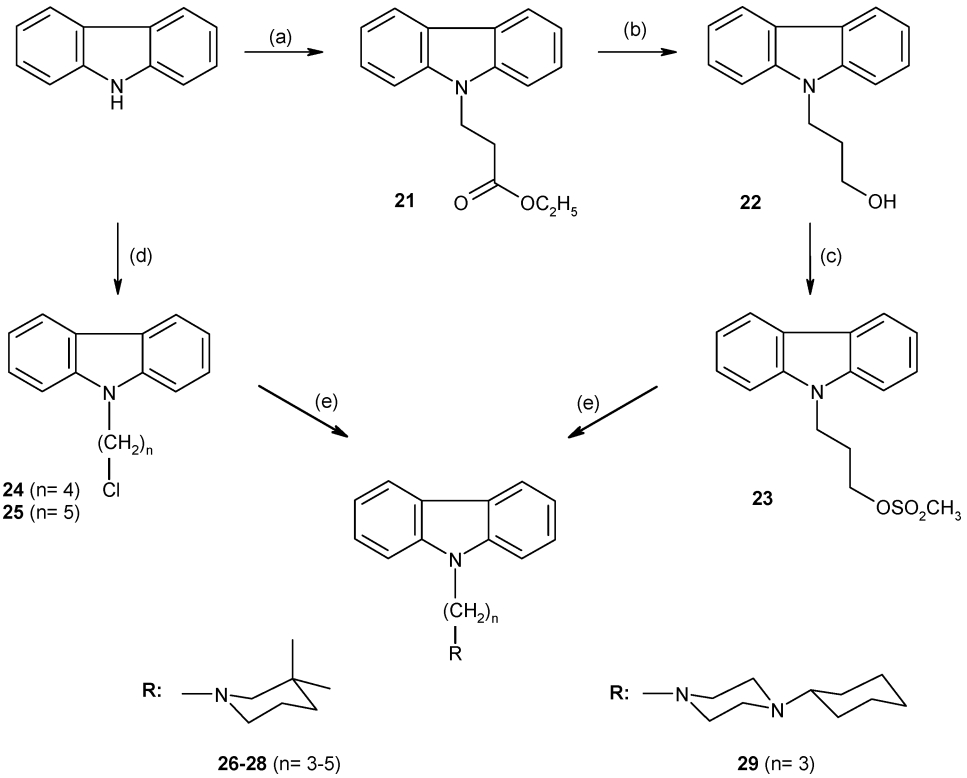
All the target compounds **11**−**20** and **26**−**29**, as hydrochloride salts, were evaluated for in vitro affinity at σ_1 and σ_2 receptors

Scheme 1[a]



[a] Reagents: (a) cyclopropylmagnesium bromide; (b) HBr; (c) H_2, 5% Pd/C; (d) DDQ; (e) 3,3-dimethylpiperidine or cyclohexylpiperazine; (f) BBr_3.

Scheme 2[a]



[a] Reagents: (a) ethyl acrylate; (b) $LiAlH_4$;(c) CH_3SO_2Cl; (d) $NaNH_2$, Br $(CH_2)_nCl$; (e) 3,3-dimethylpiperidine or cyclohexylpiperazine.

by radioreceptor binding assays. Compounds **12** and **14** were tested in previous works.[33] The specific radioligands and tissue sources were respectively (a) σ_1 receptor, (+)-[^{3}H]pentazocine ((+)-[2*S*-(2α,6α,11*R*)]-1,2,3,4,5,6-hexahydro-6,11-dimethyl-3-(3-methyl-2-butenyl)-2,6-methano-3-benzazocin-8-ol), guinea pig brain membranes without cerebellum; (b) σ_2 receptor, [^{3}H]-DTG (1,3-di-2-tolylguanidine) in the presence of 1 μM (+)-pentazocine to mask σ_1 receptors, rat liver membranes; (c) $\Delta_8-\Delta_7$ SI site, (±)-[^{3}H]emopamil [α-(1-methylethyl)-α-[3-[methyl-(2-phenylethyl)amino]propyl]benzeneacetonitrile], guinea pig liver membranes. The following compounds were used to define the specific binding reported in parentheses: (a) (+)-pentazocine (73−83%), (b) DTG (81−93%), (c) (±)-ifenprodil [2-(4-benzyl-piperidino)-1-(4-hydroxyphenyl)-1-propanol] (66−78%). Concentrations required to inhibit 50% of radioligand specific binding (IC_{50}) were determined by using six to nine different concentrations of the drug studied in two or more experiments with samples in duplicate. Scatchard parameters (K_d and B_{max}) and apparent inhibition constants (K_i) values were determined by nonlinear curve fitting, using Prism, version 3.0 (GraphPad).[42]

Table 1. Physical and Photophysical Properties

compd	formula	mp, °C[a]	ClogP[b]	excitation λ_{max} (nm) EtOH	excitation λ_{max} (nm) NaOH	emission λ_{max} (nm) EtOH	emission λ_{max} (nm) NaOH	QY[c]
11	$C_{21}H_{29}NO\cdot HCl\cdot {}^{3}/_{4}H_2O$	216−218	6.01	223		340		0.10
12[d]			6.01	225		352		0.16
13	$C_{21}H_{29}NO\cdot HCl\cdot {}^{1}/_{2}H_2O$	194−197	6.01	225		353		0.28
14[e]			4.77	222		340		0.14
15	$C_{24}H_{34}N_2O\cdot 2HCl$	282−285	4.77	226		352		0.20
16	$C_{24}H_{34}N_2O\cdot 2HCl\cdot {}^{1}/_{4}H_2O$	279−282	4.77	227		354		0.27
17	$C_{20}H_{27}NO\cdot HCl\cdot {}^{3}/_{4}H_2O$	208−210	5.23	225	240	356	415	0.25
18	$C_{20}H_{27}NO\cdot HCl\cdot {}^{3}/_{4}H_2O$	220−223	5.23	226	240	360	415	0.37
19	$C_{23}H_{32}N_2O\cdot 2HCl\cdot {}^{1}/_{2}H_2O$	285 (dec)	4.18	226	240	358	416	0.30
20	$C_{23}H_{32}N_2O\cdot 2HCl\cdot {}^{5}/_{4}H_2O$	302 (dec)	4.18	227	240	360	415	0.35
26	$C_{22}H_{28}N_2\cdot HCl$	212−214	6.41	228		352		0.41
27	$C_{23}H_{30}N_2\cdot HCl\cdot {}^{1}/_{4}H_2O$	206−208	6.59	232		352		0.30
28	$C_{24}H_{32}N_2\cdot HCl\cdot {}^{1}/_{4}H_2O$	242−243	7.12	232		352		0.38
29	$C_{25}H_{33}N_3\cdot 2HCl\cdot {}^{1}/_{4}H_2O$	290 (dec)	5.36	232		352		0.36

[a] Recrystallized from MeOH/Et_2O. [b] Referred to the corresponding free bases. [c] Fluorescence relative quantum yields. [d] Data already reported (ref 18). [e] Data already reported (ref 33).

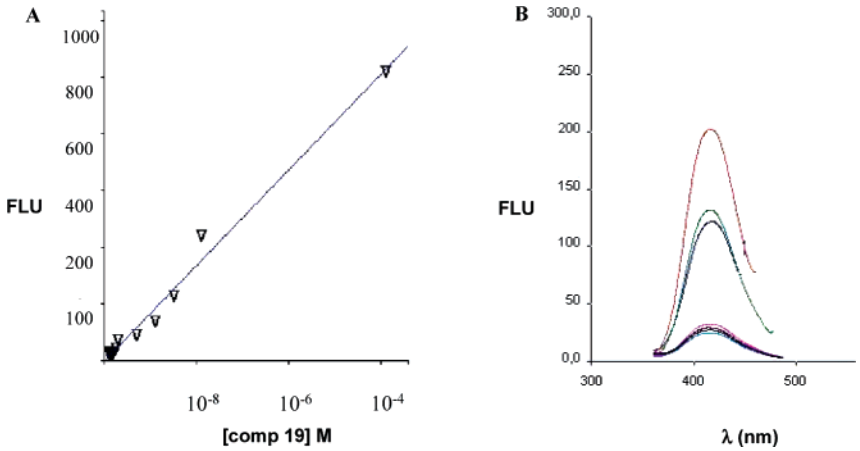


Figure 1. Calibration curve of compound **19** in 2 N NaOH (A). Fluorescence emission of compound **19** by exciting at $\lambda = 416 \pm 30$ nm shifting each wavelength 10 nm (B).

Results and Discussion

Fluorescent Ligand Studies. The fluorescent properties of tested compounds are listed in Table 1. The spectra recorded in EtOH showed the maximum excitation wavelengths (excitation λ_{max}) from 222 to 232 nm and the maximum emission wavelengths (emission λ_{max}) from 340 to 360 nm. For all compounds fluorescence relative quantum yields (QY) were calculated according to the general equation

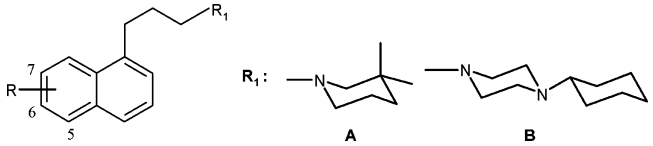
$$QY_u = (A_s/A_u)(F_u/F_s)(n_u/n_s)^2 QY_s$$

where A is the absorbance at the excitation wavelength, F is the area under the corrected emission curve, n is the refractive index of the solvents, and the subscripts u and s refer to the unknown and the standard, respectively.[43] 2-Aminopyridine in EtOH was used as a standard (excitation at $\lambda = 285$ nm; QY = 0.37).[44] Among compounds containing the 1-naphthol moiety, the 5-methoxy derivatives **11** and **14** displayed the worst fluorescence properties. Although carbazole derivatives **26**−**29** displayed high QYs (0.30−0.38), they had an emission λ_{max} in the UV range, and this finding discouraged their evaluation as fluorescent probes in biological assays. All phenolic derivatives **17**−**20** containing the 2-naphthol moiety presented an excitation λ_{max} at 240 nm in their fluorescence spectra recorded in 2 N NaOH. The emission λ_{max} was at 415 nm for compounds **17**, **18**, and **20** and 416 nm for compound **19**. The best emission intensities were recorded for hydroxyl derivatives **17**−**20** (QY ranging from 0.15 to 0.39), and among them compound **19** (QY = 0.30) displayed the best spectroscopic properties (Figure 1) and high σ_1 and σ_2 receptor affinities at the same time (Table 2). On the basis of these evaluations, compound **19** was selected as fluorescent probe in saturation binding analysis at σ_1 and σ_2 receptors.

Radioligand Binding Assays. The results of radioligand binding experiments for examined compounds **11**−**20** and **26**−**29** are listed in Tables 2 and 3, respectively. The new compounds tested did not reach very high affinities toward σ_1, σ_2, and SI sites. The lead compound **12** still showed the highest σ_1 receptor affinity ($K_i = 0.35$ nM) and the best selectivity relative to the σ_2 receptor (σ_2/σ_1 K_i ratio = 680) (Table 2). Among all the other examined compounds, the 7-methoxy derivative **16** displayed the best affinity for both σ_1 ($K_i = 0.90$ nM) and σ_2 receptors ($K_i = 8.27$ nM). Although σ_2 receptor affinities were only moderate, a significant selectivity relative to σ_1 receptor was demonstrated by compound **29**. As for the SI site, compounds **13** and **14** showed good affinities ($K_i \sim 4$ nM), and compound **13** presented a good selectivity relative to σ_1 and σ_2 receptors (28- and 163-fold, respectively). The ClogP values of the examined compounds **11**−**29** (Table 1) ranged from 4.18 for phenolic compounds **19** and **20** to 7.12 for carbazole derivative **28** and displayed no correlation with the affinities for σ_1, σ_2, and SI sites. This lower hydrophobicity of phenolic compounds is added to their better fluorescent properties as another positive feature for their use as biological probes.

In the 3,3-dimethylpiperidine series, 5-methoxy derivative **11** and 7-methoxy derivative **13** displayed a decreased σ_1 receptor

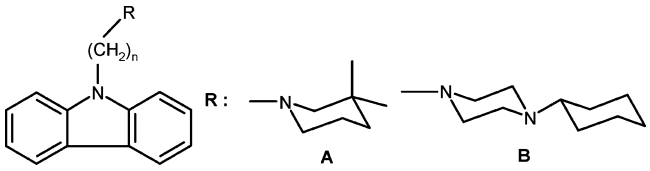
Table 2. Binding Affinities and Selectivities



compd	R	R_1	$K_i \pm$ SEM, (nM) σ_1	$K_i \pm$ SEM, (nM) σ_2	$K_i \pm$ SEM, (nM) $\Delta_8-\Delta_7$ SI	K_i ratio σ_2/σ_1
11	5-OCH_3	A	44.9 ± 17.1	643 ± 91	12.8 ± 3.6	14
12[a]	6-OCH_3	A	0.35 ± 0.04	238 ± 28	8.71 ± 0.21	680
13	7-OCH_3	A	115 ± 34	672 ± 173	4.12 ± 0.62	6
14[b]	5-OCH_3	B	1.57 ± 0.15	9.24 ± 1.37	4.51 ± 0.39	6
15	6-OCH_3	B	3.16 ± 0.52	9.02 ± 2.92	7.06 ± 2.44	3
16	7-OCH_3	B	0.90 ± 0.21	8.27 ± 2.21	42.4 ± 7.0	9
17	6-OH	A	340 ± 80	600 ± 150	352 ± 85	2
18	7-OH	A	733 ± 116	412 ± 95	30.0 ± 4.0	0.6
19	6-OH	B	6.78 ± 2.42	26.4 ± 5.3	21.3 ± 10.6	4
20	7-OH	B	5.48 ± 2.17	11.8 ± 3.0	55.0 ± 8.2	2
1, (+)-pentazocine			3.24 ± 0.38			
4, DTG				30.8 ± 2.4		
(±)-ifenprodil					19.8 ± 1.2	

[a] Data already reported (ref 18). [b] Data already reported (ref 33).

Table 3. Binding Affinities and Selectivities



compd	n	R	$K_i \pm$ SEM, (nM) σ_1	$K_i \pm$ SEM, (nM) σ_2	$K_i \pm$ SEM, (nM) $\Delta_8-\Delta_7$ SI	K_i ratio σ_2/σ_1
26	3	A	2020 ± 380	4480 ± 250	345 ± 163	2
27	4	A	175 ± 27	520 ± 107	270[a]	3
28	5	A	138 ± 36	117 ± 3	24[a]	0.8
29	3	B	3450 ± 1660	12.6 ± 3.2	79.9 ± 9.1	0.004

[a] Result from one experiment.

affinity (K_i = 44.9 and 115 nM, respectively) compared to lead compound **12**. The hydroxy derivatives **17** and **18** showed a further dramatic decrease in σ_1 receptor affinity (K_i = 340 and 733 nM, respectively) in comparison to the corresponding counterparts **12** and **13**. As expected, all 3,3-dimethylpiperidine derivatives displayed poor affinity values toward σ_2 receptor (K_i = 238−672 nM). As for the SI site, within the 3,3-dimethylpiperidine series, the methoxy compounds **11** and **13** did not show significant changes in the affinity (K_i = 12.8 and 4.12, respectively) when compared to the lead compound **12**. The hydroxyl derivatives belonging to the 3,3-dimethylpiperidine series showed a decreased binding at the SI site, with compound **18** displaying a moderate affinity (K_i = 30.0).

All cyclohexylpiperazine derivatives **14**−**16**, **19**, and **20** showed high σ_1 and σ_2 receptor affinity values, confirming the hypothesis of the dual mode of binding of the piperazines at the σ_1 receptor.[17] The change in the position of the methoxy group (compound **14**−**16**) led to similar high σ_1 receptor affinity (K_i = 0.90−3.16 nM). Moreover, the hydroxyl derivatives **19** and **20** displayed slightly lower σ_1 receptor affinities (K_i = 6.78 and 5.48 nM, respectively), compared to the corresponding methoxy derivatives **15** and **16**.

As for the σ_2 receptor, the cyclohexylpiperazine derivatives **14**−**16** displayed quite similar high affinity values (K_i = 8.27−9.24 nM), showing how the position of the methoxy group on the naphthalene nucleus was not significant in terms of binding at both σ_1 and σ_2 receptor subtypes. The presence of the hydroxyl group in the cyclohexylpiperazine derivatives (compounds **19** and **20**) resulted in slightly lower affinities in comparison to the corresponding methoxy derivatives, as happens for σ_1 receptor affinity. Moderately significant differences in the affinity toward the SI site were found within the cyclohexylpiperazine series, where the methoxy derivatives **14** and **15** reached considerable K_i values (4.51 and 7.06 nM, respectively).

The carbazole derivatives bearing the 3,3-dimethylpiperidine nucleus (**26**−**28**) displayed both moderate σ_1 receptor affinity (K_i = 138−2020 nM) and σ_2 receptor affinity (K_i = 117−4480 nM), with compound **26** showing the worst affinity values (Table 3). As for the binding at the SI site, compounds **26**−**28** showed the same trend (K_i = 24−345 nM), with compound **26** having the poorest value again. For all the three receptor subtypes, a linear correlation was observed between the affinity and the length of the chain, with the five-methylene compound **28** showing the best affinities. The σ_1 receptor affinity was poor (K_i = 3450 nM) for the cyclohexylpiperazine **29**, but noteworthy was its σ_2 receptor affinity (K_i = 12.6 nM), resulting in a good selectivity relative to the σ_1 receptor subtype (274-fold).

σ Receptor Saturation Binding Assay with Fluorescent Compound 19. σ_1 And σ_2 receptor saturation analysis have been carried out with compound **19** as a fluorescent probe. Unfortunately, σ_1 receptor saturation analysis failed because a low specific binding was found for compound **19**. In guinea pig whole brain membranes, compound **19** could likely bind other sites, resulting in high nonspecific binding. Another saturation experiment was performed in rat C6 glioma cells, a tumor cell line overexpressing σ_1 receptors,[45] and led to the same result.

By contrast, as depicted in Figure 2A, compound **19** displayed a good saturation curve in σ_2 receptor saturation binding analysis, where K_d (21.3 nM) and B_{max} (0.47 pmol/mg of protein) were determined. Moreover, as shown in Figure 2B, the K_d value was confirmed by Scatchard analysis so that compound **19** demonstrated binding only to the σ_2 receptor subtype in such experiment. Comparing saturation analysis and competition binding results, it can be observed that compound **19** displayed K_d value consistent with K_i value (26.4 nM). It could be pointed out that K_d is a direct measurement of σ_2

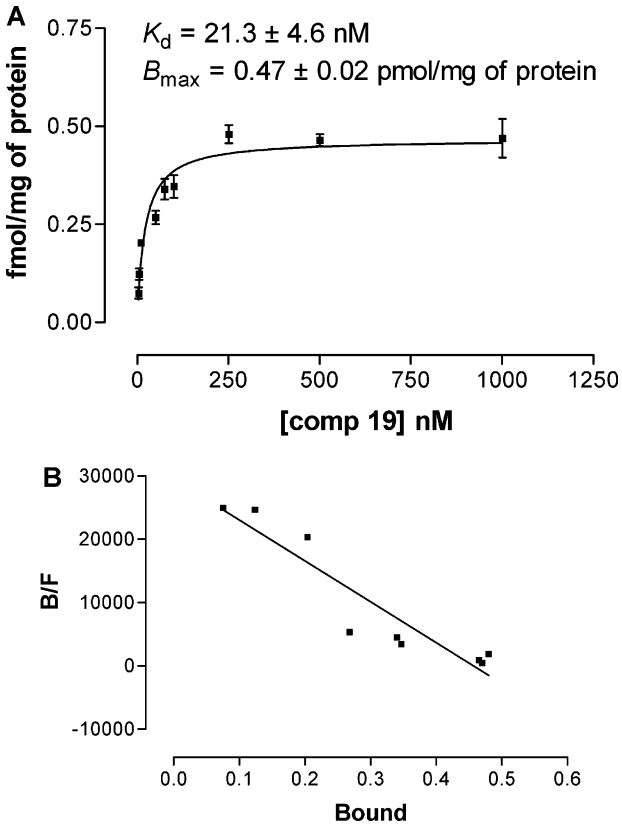


Figure 2. Binding saturation analysis (A) and corresponding Scatchard plot (B) at σ_2 receptor using compound **19** as a probe.

receptor binding, whereas K_i is an indirect measurement by [^{3}H]-DTG competition for the same receptor binding site.

Conclusions

The aim of the present study was to design fluorescent ligands having a fluorescent moiety as intrinsic structural requirement. Moreover, the fluorescent moieties 2-naphthol and 9*H*-carbazole presented structural properties for obtaining ligands with high affinity toward σ_1 and σ_2 receptors and SI site. Among all the examined compounds, the 3,3-dimethylpiperidine derivatives confirmed a good affinity toward σ_1 receptor but showed a moderate selectivity, so that compound **12** still remained the best σ_1 ligand. The cyclohexylpiperazine derivatives showed both σ_1 and σ_2 receptor affinity but no selectivity, except for compound **29**. Compared to the methoxy derivatives, better fluorescent phenolic compounds unfortunately lost σ and SI affinities or at least did not improve them. Among σ receptor ligands bearing the 2-naphthol nucleus, the cyclohexylpiperazine derivative **19** displayed interesting fluorescent properties. In fact, the fluorescent emission of compound **19** was both in the visible field and had good intensity emission. The saturation analysis at σ_2 receptors in rat liver, using compound **19** as a probe, demonstrated that such compound could be employed in "green" competition binding assays instead of a radioligand. Starting from these preliminary results, new compounds having both better fluorescent properties and subnanomolar σ receptor affinity could be designed in the future.

Experimental Section

Chemical Methods. Column chromatography was performed with 1:30 ICN silica gel 60 Å (63−200 or 15−40 μm) as the stationary phase. Melting points were determined in open capillaries on a Gallenkamp electrothermal apparatus. Elemental analyses of hydrochloride salts (C, H, N) were performed on a Eurovector Euro EA 3000 analyzer; the analytical results were within ±0.4% of the theoretical values for the formula given, unless otherwise stated. ^{1}H NMR spectra were recorded at 300 MHz on a Mercury Varian spectrometer using $CDCl_3$ as solvent unless otherwise reported. The chemical shift values were reported in ppm (δ). Recording of mass spectra was done on an Agilent 6890−5973 MSD gas chromatograph/mass spectrometer; only significant *m*/*z* peaks, with their percentage of relative intensity in parentheses, are reported. The fluorescence spectra were measured using a Perkin-Elmer LS55 luminescence spectrofluorimeter. Chemicals were from Aldrich and Across and were used without any further purification.

General Procedure To Obtain Final Compounds 11,13,15−20 and 26−29. In a typical reaction, a representative intermediate (1.0 mmol) among 1-(3-bromopropyl)naphthalenes **10a**−**c** or 9-(ω-chloroalkyl)carbazoles **24** and **25** or mesylalkyl derivative **23** was stirred and refluxed overnight with cyclohexylpiperazine (0.20 g, 1.2 mmol) or 3,3-dimethylpiperidine (0.14 g, 1.2 mmol) and Na_2CO_3 (0.10 g, 1.2 mmol) in CH_3CN (10 mL). The mixture was worked up as already reported.[33] Purification by column chromatography with CH_2Cl_2/MeOH (95:5) as eluent, unless otherwise indicated, afforded the title final compounds.

3,3-Dimethyl-1-[3-(5-methoxynaphthalen-1-yl)propyl]piperidine (11) was obtained as a yellow oil in 81% yield: ^{1}H NMR δ 0.96 [s, 6H, $C(CH_3)_2$], 1.22−1.28 [m, 2H, $CH_2CH_2C(CH_3)_2$], 1.50−1.72 [m, 6H, $NCH_2C(CH_3)_2CH_2CH_2$, $ArCH_2CH_2$], 2.20−2.43 (m, 4H, $CH_2NCH_2CH_2$), 3.08 (t, 2H, J = 7.7 Hz, $ArCH_2$), 3.93 (s, 3H, OCH_3), 6.80−8.18 (m, 6H, aromatic); GC−MS *m*/*z* 312 (M^+ + 1, 6), 311 (M^+, 23), 126 (100). Anal. ($C_{21}H_{29}NO\cdot HCl\cdot{}^3/_4H_2O$) C, H, N.

3,3-Dimethyl-1-[3-(7-methoxynaphthalen-1-yl)propyl]piperidine (13) was obtained as a yellow oil in 61% yield: ^{1}H NMR δ 0.88 [s, 6H, $C(CH_3)_2$], 1.19−1.28 [m, 2H, $CH_2CH_2C(CH_3)_2$], 1.46−1.68 [m, 2H, $CH_2CH_2C(CH_3)_2$], 1.88−2.12 [m, 4H, $ArCH_2CH_2$, $NCH_2C(CH_3)_2$], 2.28−2.45 (m, 4H, $CH_2NCH_2CH_2$), 3.05 (t, 2H, J = 7.6 Hz, $ArCH_2$), 3.95 (s, 3H, OCH_3), 7.12−7.80 (m, 6H, aromatic); GC−MS *m*/*z* 312 (M^+ + 1, 7), 311 (M^+, 29), 126 (100). ($C_{21}H_{29}NO\cdot HCl\cdot{}^1/_2H_2O$) C, H, N.

1-Cyclohexyl-4-[3-(6-methoxynaphthalen-1-yl)propyl]piperazine (15) was obtained as a brown oil in 61% yield: ^{1}H NMR δ 1.05−1.32 (m, 5H, cyclohexyl 5 ax *CH*H), 1.58−2.00 (m, 7H, cyclohexyl 5 eq *CH*H and $ArCH_2CH_2$), 2.22−2.77 (m, 11H, CHN, CH_2N and piperazine), 3.04 (t, 2H, J = 7.8 Hz, $ArCH_2$), 3.95 (s, 3H, OCH_3), 7.10−7.98 (m, 6H, aromatic); GC−MS *m*/*z* 368 (M^+ + 2, 4), 367 (M^+ + 1, 27), 366 (M^+, 100), 195 (87), 181 (64), 171 (27). Anal. ($C_{24}H_{34}N_2O\cdot 2HCl$) C, H, N.

1-Cyclohexyl-4-[3-(7-methoxynaphthalen-1-yl)propyl]piperazine (16) was obtained as an oil with 75% yield: ^{1}H NMR δ 1.00−1.34 (m, 5H, cyclohexyl 5 ax *CH*H), 1.58−2.02 (m, 7H, cyclohexyl 5 eq *CH*H and $ArCH_2CH_2$), 2.20−2.71 (m, 11H, CHN, CH_2N and piperazine], 3.03 (t, 2H, J = 7.7 Hz, $ArCH_2$), 3.97 (s, 3H, OCH_3), 7.10−7.78 (m, 6H, aromatic); GC−MS *m*/*z* 368 (M^+ + 2, 3), 367 (M^+ + 1, 24), 366 (M^+, 93), 323 (22), 195 (100), 181 (75), 171 (44). Anal. ($C_{24}H_{34}N_2O\cdot 2HCl\cdot{}^1/_4H_2O$) C, H, N.

3,3-Dimethyl-1-[3-(9*H*-carbazol-9-yl)propyl]piperidine (26) was obtained as a yellow solid with 95% yield: ^{1}H NMR δ 1.05 (s, 6H, $2CH_3$), 1.22−1.31 [m, 2H, $CH_2CH_2C(CH_3)_2$], 1.60−1.72 [m, 2H, $CH_2CH_2C(CH_3)_2$], 1.95−2.10 [m, 4H, $ArCH_2CH_2$, $NCH_2C(CH_3)_2$], 2.18−2.35 (m, 4H, $CH_2NCH_2CH_2$), 4.41 (t, 2H, J = 6.7 Hz, $ArCH_2$), 7.19−8.17 (m, 8H, aromatic); GC−MS *m*/*z* 322 (M^+ + 2, 2), 321 (M^+ + 1, 15), 320 (M^+, 52), 180 (45), 126 (100). Anal. ($C_{22}H_{28}N_2\cdot HCl$) C, H, N.

3,3-Dimethyl-1-[4-(9*H*-carbazol-9-yl)butyl]piperidine (27) was obtained as a yellow solid in 71% yield after column chromatography with CH_2Cl_2/MeOH (9:1) as eluent; ^{1}H NMR δ 0.91 (s, 6H, $2CH_3$), 1.16−1.24 [m, 2H, $CH_2CH_2C(CH_3)_2$], 1.50−1.68 [m, 4H, $Ar(CH_2)_2CH_2$, $CH_2CH_2C(CH_3)_2$], 1.84−2.03 [m, 4H, $ArCH_2CH_2$, $NCH_2C(CH_3)_2$], 2.18−2.36 (m, 4H, $CH_2NCH_2CH_2$), 4.33 (t, 2H, J = 7.3 Hz, $ArCH_2$), 7.18−8.14 (m, 8H, aromatic); GC−MS *m*/*z* 336 (M^+ + 2, 1), 335 (M^+ + 1, 7), 334 (M^+, 26), 180 (45), 126 (100). Anal. ($C_{23}H_{30}N_2\cdot HCl\cdot{}^1/_4H_2O$) C, H, N.

3,3-Dimethyl-1-[5-(9*H*-carbazol-9-yl)pentyl]piperidine (28) was obtained as a yellow oil in 34% yield: ^{1}H NMR δ 0.91 (s, 6H, $2CH_3$), 1.14−1.28 [m, 2H, $CH_2CH_2C(CH_3)_2$], 1.30−1.44 [m, 2H, $Ar(CH_2)_2CH_2$], 1.45−1.71 [m, 4H, $Ar(CH_2)_3CH_2$, $CH_2CH_2C(CH_3)_2$], 1.72−1.95 [m, 4H, $ArCH_2CH_2$, $NCH_2C(CH_3)_2$], 2.18−2.41 (m, 4H, $CH_2NCH_2CH_2$), 4.30 (t, 2H, J = 7.0 Hz, $ArCH_2$), 7.18−8.12 (m, 8H, aromatic); GC−MS *m*/*z* 350 (M^+ + 2, 1), 349 (M^+ + 1, 6), 348 (M^+, 26), 180 (28), 126 (100). Anal. ($C_{24}H_{32}N_2 \cdot HCl \cdot {}^1/_4H_2O$) C, H, N.

1-Cyclohexyl-4-[3-(9*H*-carbazol-9-yl)propyl]piperazine (29) was obtained as a yellow solid in 82% yield: ^{1}H NMR δ 1.05−1.37 (m, 5H, cyclohexyl 5 ax *CH*H), 1.58−2.08 (m, 7H, cyclohexyl 5 eq *CH*H and $ArCH_2CH_2$), 2.20−2.72 (m, 11H, CHN, CH_2N and piperazine), 4.39 (t, 2H, J = 6.7 Hz, $ArCH_2$), 7.18−8.12 (m, 8H, aromatic); GC−MS *m*/*z*: 377 (M^+ + 2, 3), 376 (M^+ + 1, 26), 375 (M^+, 99), 332 (37), 195 (84), 180 (100). Anal. ($C_{25}H_{33}N_3 \cdot 2HCl \cdot {}^1/_4H_2O$) C, H, N.

General Procedure To Obtain Final Compounds (17−20). A solution of BBr_3 (0.12 mL, 1.3 mmol) in anhydrous CH_2Cl_2 (10 mL) was added in a dropwise manner to a solution of one appropriate compound among **12**, **13**, **15**, and **16** (1.35 mmol) in the same solvent (10 mL) cooled at −78 °C under N_2 atmosphere. The mixture was stirred overnight and allowed to reach room temperature. After cooling, the reaction was quenched with H_2O and then K_2CO_3 saturated solution was added. The resulting mixture was extracted with CH_2Cl_2 (3 × 5 mL). The organic layers were collected, dried (Na_2SO_4), and then concentrated to dryness to afford the crude residue as a solid compound. Purification by crystallization from MeOH/Et_2O, unless otherwise indicated, gave the final compound.

3,3-Dimethyl-1-[3-(6-hydroxynaphthalen-1-yl)propyl]piperidine (17). Purification by crystallization from MeOH/CH_2Cl_2 afforded the target compound as white needles in 30% yield: mp 216−218 °C; ^{1}H NMR (DMSO-d_6) δ 0.92 (s, 6H, $2CH_3$), 1.12−1.24 [m, 2H, $CH_2CH_2C(CH_3)_2$], 1.46−1.57 [m, 2H, $CH_2CH_2C(CH_3)_2$], 1.66−1.78 (m, 2H, $ArCH_2CH_2$), 1.92−2.05 [m, 2H, $NCH_2C(CH_3)_2$], 2.18−2.28 (m, 4H, $CH_2NCH_2CH_2$), 2.96 (t, 2H, J = 7.5 Hz, $ArCH_2$), 7.05−7.98 (m, 6H, aromatic), 9.68 (s, 1H, OH, D_2O exchanged); GC−MS *m*/*z* 298 (M^+ + 1, 2), 297 (M^+, 10), 126 (100). Anal. ($C_{20}H_{27}NO \cdot HCl \cdot {}^3/_4H_2O$) C, H, N; H: calculated 8.56, found 8.07.

3,3-Dimethyl-1-[3-(7-hydroxynaphthalen-1-yl)propyl]piperidine (18) was obtained as light brown needles in 58% yield: mp 210−212 °C; ^{1}H NMR (DMSO-d_6) δ 0.90 (s, 6H, $2CH_3$), 1.12−1.24 [m, 2H, $CH_2CH_2C(CH_3)_2$], 1.47−1.58 [m, 2H, $CH_2CH_2C(CH_3)_2$], 1.70−1.84 (m, 2H, $ArCH_2CH_2$), 1.90−2.08 [m, 2H, $NCH_2C(CH_3)_2$], 2.18−2.40 (m, 4H, $CH_2NCH_2CH_2$), 2.90 (t, 2H, J = 7.5 Hz, $ArCH_2$), 7.04−7.75 (m, 6H, aromatic), 9.67 (s, 1H, OH, D_2O exchanged); GC−MS *m*/*z* 298 (M^+ + 1, 2), 297 (M^+, 12), 126 (100). Anal. ($C_{20}H_{27}NO \cdot HCl \cdot {}^3/_4H_2O$) C, H, N.

1-Cyclohexyl-4-[3-(6-hydroxynaphthalen-1-yl)propyl]piperazine (19) was obtained as white needles in 30% yield: mp 165−167 °C; ^{1}H NMR (DMSO-d_6) δ 0.90−1.22 (m, 5H, cyclohexyl 5 ax *CH*H), 1.42−1.95 (m, 7H, cyclohexyl 5 eq *CH*H and $ArCH_2CH_2$), 2.20−2.98 (m, 11H, CHN, CH_2N and piperazine), 2.96 (t, 2H, J = 7.3 Hz, $ArCH_2$), 7.05−7.85 (m, 6H, aromatic), 9.66 (br s, 1H, OH, D_2O exchanged); GC−MS *m*/*z* 354 (M^+ + 2, 2), 353 (M^+ + 1, 13), 352 (M^+, 52), 309 (24), 195 (100), 181 (74), 157 (42), 111 (31). Anal. ($C_{23}H_{32}N_2O \cdot 2HCl \cdot {}^1/_2H_2O$) C, H, N.

1-Cyclohexyl-4-[3-(7-hydroxynaphthalen-1-yl)propyl]piperazine (20) was obtained as brown needles in 40% yield: mp 283 °C (dec); ^{1}H NMR (DMSO-d_6) δ 0.95−1.25 (m, 5H, cyclohexyl 5 ax *CH*H), 1.48−1.85 (m, 7H, cyclohexyl 5 eq *CH*H and $ArCH_2CH_2$), 2.05−2.55 (m, 11H, CHN, CH_2N and piperazine), 2.88 (t, 2H, J = 7.5 Hz, $ArCH_2$), 7.00−7.78 (m, 6H, aromatic), 9.70 (br s, 1H, OH, D_2O exchanged); GC−MS *m*/*z* 354 (M^+ + 2, 2), 353 (M^+ + 1, 18), 352 (M^+, 74), 309 (22), 195 (100), 181 (85), 157 (51), 111 (32). Anal. ($C_{23}H_{32}N_2O \cdot 2HCl \cdot {}^5/_4H_2O$) C, H, N; H: calculated 8.21, found 7.61.

Pharmacological Methods. Radioligand Competition Binding Assays. All the procedures for the binding assays were previously described.[32] σ_1 And σ_2 receptor binding was carried out according to Matsumoto et al.[46] and $\Delta_8-\Delta_7$ SI according to the method of Moebius et al.[47] The radioligands [^{3}H]DTG (30 Ci/mmol) and (+)-[^{3}H]pentazocine (34 Ci/mmol) were purchased from Perkin-Elmer Life Sciences (Zavantem, Belgium). (±)-[^{3}H]Emopamil (83 Ci/mmol) was purchased from American Radiolabeled Chemicals Inc. (St. Louis, MO). (+)-Pentazocine was obtained from Sigma-Aldrich-RBI s.r.l. (Milan, Italy). DTG and (±)-ifenprodil were purchased from Tocris Cookson Ltd., UK. Male Dunkin guinea pigs and Wistar Hannover rats (250−300 g) were from Harlan, Italy.

σ_2 Receptor Saturation Binding Assay. The saturation binding experiment was carried out as described by Colabufo et al. with minor modifications.[23] In a GF/C Millipore 96-well plate, each well in a final volume of 250 μL received 15 μL of incubation buffer (50 mM Tris dressed with 1 μM (+)-pentazocine to mask σ_1 receptors, pH 8.0), 200 μg of rat liver membranes suspended in 210 μL of incubation buffer, and 25 μL compound **19** at a range of concentrations from 1 μM to 1 nM. The nonspecific binding was determined in the presence of 10 μM DTG. The plate was equilibrated for 120 min at 25 °C. The incubation buffer was removed by filtering and in each well was added 250 μL of 2 N NaOH. The obtained solutions were then transferred into a 96-well plate suitable for fluorescence analyses. For each sample, the intensity fluorescence emission at λ = 416 nm was evaluated by exciting at λ = 240 nm. The emission fluorescence calibration curve of compound **19** was carried out in 2 N NaOH.

Supporting Information Available: Elemental analyses of the end products, description of the preparation of intermediate compounds **21**−**23**, and ^{1}H NMR and GC−MS data for the products **10c** and **21**−**25**. This material is available free of charge via the Internet at http://pubs.acs.org.

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