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Article

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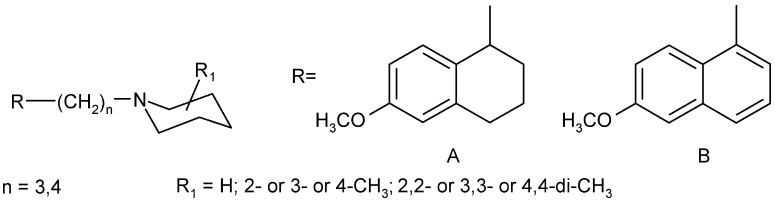

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Methyl Substitution on the Piperidine Ring of *N*-[ω-(6-Methoxynaphthalen-1-yl)alkyl] Derivatives as a Probe for Selective Binding and Activity at the σ_1 Receptor

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The *N*-(6-methoxy-1,2,3,4-tetrahydronaphthalen-1-yl)propyl and *N*-(6-methoxynaphthalen-1-yl)propyl derivatives as well as their upper homologous butyl derivatives of various methylpiperidines were prepared. The piperidine moiety bearing monomethyl or geminal dimethyl groups was employed as a probe to explore σ-subtype affinities and selectivities by radioligand binding assays at σ_1 and σ_2 receptors and the Δ_8-Δ_7 sterol isomerase (SI) site. 4-Methyl derivative **31** was the most potent σ_1 ligand (K_i = 0.030 nM) with a good selectivity profile (597-fold and 268-fold relative to σ_2 receptor and SI site, respectively), whereas 3,3-dimethyl derivative **26** (K_i = 0.35 nM) was the most selective (680-fold) relative to the σ_2 receptor. Both compounds can be proposed as tools for PET experiments. Furthermore, the naphthalene compounds **26**, **28**, **31**, and **33** demonstrated antiproliferative activity in rat C6 glioma cells (EC_{50} = 15.0 μM for **33**), revealing a putative σ_1 antagonist activity and opening a useful perspective in tumor research and therapy.

Introduction

Presently, sigma (σ) receptors are known to be intracellular binding sites of brain and peripheral organs as well as of endocrine, immune, and reproductive tissues.[1−3] The σ receptor family includes σ_1 and σ_2 subtypes, which have been identified on the basis of their pharmacological profile, function, and molecular size.[4] Cloning of σ_1 receptor revealed a 223-amino acid protein, belonging to a unique family and different from any other known protein.[5] The σ_1 receptor was supposed to be a mammalian sterol isomerase (SI) based on its presence in tissues where steroids biosynthesis takes place and on 30% identity shared with a yeast SI.[6] Nevertheless, σ_1 receptor neither proved to have any isomerase activity nor to share any structural homology with the cholesterol biosynthesis Δ_8-Δ_7 isomerase, which is the functional mammalian counterpart of yeast SI.[3] The σ_1 receptor mainly plays modulatory functions on dopamine, acetylcoline, NMDA, and opioid receptors, whereas the σ_2 receptor is involved in regulation of cell proliferation and apoptosis through the control of intracellular Ca^{2+} storage and depletion.[7] A mediator role in cell signaling, particularly through cell Ca^{2+} mobility, has also been suggested for the σ_1 receptor,[8] supported by its localization in membranes of endoplasmic reticulum and organelles and by its supposed one or two transmembrane domains.[3] Both σ receptor subtypes are overexpressed in many tumor cell lines and represent attractive targets for diagnostic imaging of tumors.[9] In this respect, σ receptor ligands can play an important role in neurology and oncology[10−12] to develop both drugs and radiolabeled tracers as new PET[13−15] and SPECT agents.[16]

Although no specific σ agent has reached the market so far, several σ_1 ligands have been researched in clinical trials.[17] No endogenous σ_1 ligand has certainly been recognized and σ_1 agents are considered agonists when they behave as prototypical benzomorphan σ_1 ligands, such as (+)-pentazocine (**1**; Chart 1). Therefore, purportedly σ_1 receptor agonists are thought to be potentially useful for the treatment of depressive and mnemonic disorders, anxiety, and Alzheimer's disease and for the improvement of cognitive deficits.[3,18,19] 1-(3,4-Dimethoxyphenethyl)-4-(3-phenylpropyl)piperazine (**2**, AGY 94806, former SA 4503) has been studied in phase I trails for the treatment of depression.[20] 2-(4-Morpholino)ethyl-1-phenylcyclohexane-1-carboxylate (**3**, PRE 084) showed improvement in learning deficits in many behavioral studies.[21] Claimed σ_1 receptor antagonists could be employed in the treatment of psychosis and neurodamage and for reduction of the locomotory effects caused by cocaine abuse.[3] At the preclinical level 4-(4-fluorophenyl)-1-[4-(1,2,4-triazol-1-yl)butyl]-1,2,3,6-tetrahydropyridine (**4**, E 5842) proved to be an atypical antipsychotic devoid of EPS symptoms.[22] In functional activity tests, *N,N*-dipropyl-2-[4-methoxy-3-(2-phenylethoxy)phenyl]ethylamine (**5**, NE 100)[23] and poorly selective ($\sigma_1 > \sigma_2$ affinity) D_2-antagonist haloperidol (**6**) are largely used as σ_1-antagonists. One of the most selective σ_1 receptor ligands known, the 2-(1-pyrrolidinyl)ethyl ester of 3,4-dichlorophenylacetic acid (**7**, AC 915),[24] could undergo enzymatic ester hydrolysis and not be suitable for animal tissues assays and in vivo assays. Some spiropiperidines such as 1′-benzyl-3-methoxy-3,4-dihydrospiro[[2]benzopyran-1,4′-piperidine] (**8**) demonstrated nanomolar σ_1 receptor affinity and high selectivity relative to σ_2 receptor.[25] To better define structural requirements for σ_1 binding, a significant contribution derived from structure−affinity rela-

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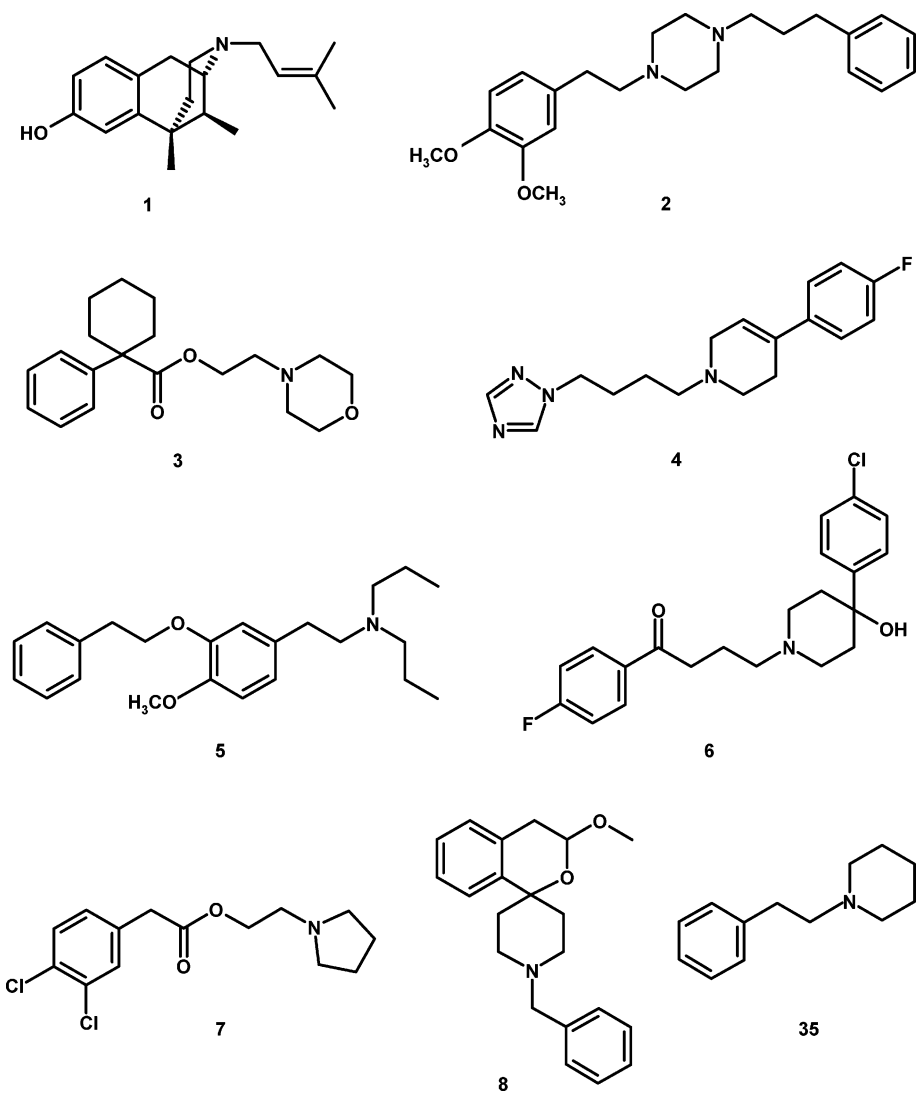
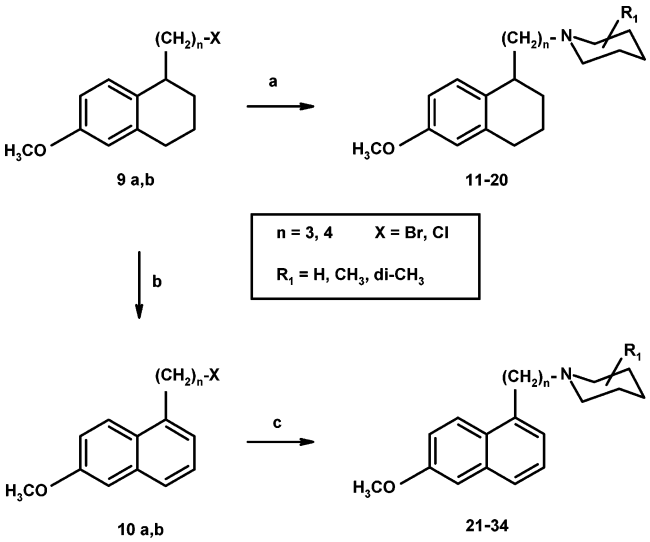
Chart 1



tionship (SAfiR) studies on several series of arylalkylamine analogues.[26,27] The results provided a σ_1 pharmacophore model, where the amine N-atom represented an important element for σ_1 receptor binding.[28] Therefore, the surroundings of the N-atom could be expected to be crucial for receptor binding. Moreover, the replacement of some amino acids by their analogues in the transmembrane domain provided evidence that Ser99, Tyr103, Leu105, and Leu106 played a critical role in ligand binding at the σ_1 receptor.[29] Much more recently, a five-point pharmacophore model was derived by molecular modeling studies on the basis of the conformational and electrostatic properties of some σ_1 receptor ligands.[30] Although many high-affinity σ_1 ligands have been developed, structural requirements for σ_1 activity still have to be defined.

In the last years we have been dealing with the synthesis and radioligand binding evaluation of *N*-(ω-nucleoalkyl)piperidines[31] and -piperazines,[32] and among them, several 3,3-dimethylpiperidine derivatives displayed high σ_1 affinity and sometime certain selectivity relative to σ_2 receptor, but not to Δ_8-Δ_7 SI.[33] Furthermore, 4-methylpiperidine too has been reported to serve like a suitable structural moiety for high-affinity σ_1 ligands, as we recently proved.[34] Indeed, it has also been stated that simple changes in the methyl substitution pattern on the piperidine ring gave rise to major changes in σ_1 receptor affinity and selectivity.[35] On the basis of these results, we extended our SAfiR investigation on analogous compounds bearing one methyl group or two geminal methyl groups in every possible position on the piperidine ring. Among the best tetralin derivatives of 3,3-dimethylpiperidine, we focused our attention on 6-methoxyl derivatives, because they shared the 6-oxytetralin framework with (+)-pentazocine. Moreover, the presence of the methoxyl group assured easy ^{11}C labeling for PET analysis. Thus, the 6-methoxytetralin moiety was linked to the piperidine N-atom by a three- or four-methylene chain as in our lead compounds **14** and **19**, respectively. For the tetralin class, only derivatives with a symmetrical methylpiperidine were prepared and tested as racemic mixtures, as the piperidine substitution would introduce a second stereogenic center besides the C-1. Most of the compounds were aromatized to the corresponding naphthyl derivatives in order to remove the C-1 stereogenic center, and the chiral 2-methyl- and 3-methylpiperidine derivatives were prepared as couples of pure enantiomers. Furthermore, the more planar and electron-rich naphthalene moiety was expected to enhance someway the selectivity, in particular, relative to SI. Some of these high-affinity σ_1 ligands were tested in rat C6 glioma tumor

Scheme 1[a]



[a] (a) Piperidine or 2,2-dimethylpiperidine or 3,3-dimethylpiperidine or 4-methylpiperidine or 4,4-dimethylpiperidine; (b) DDQ; (c) the same piperidines as step a or (−)-(*R*)- and (+)-(*S*)-2-methylpiperidine or (−)-(*R*)- and (+)-(*S*)-3-methylpiperidine.

cell line to define their intrinsic activity. In this assay, σ_1 antagonists and σ_2 agonists had proven to exert antiproliferative and cytotoxic effects, whereas σ_1 agonists did not exert such activity. [36]

Chemistry

The synthesis of the final compounds **11**−**34** is depicted in the Scheme 1. Compounds **14** and **19** have already been reported.[33] The haloalkyl derivatives **9a,b** were prepared starting from 6-methoxy-1-tetralone through the appropriate Grignard's reagent and following a previously reported synthetic route.[37,31] The same intermediates **9a,b** were subsequently aromatized by DDQ (2,3-dichloro-5,6-dicyano-1,4-benzoquinone) to the corresponding haloalkylnaphthalenes **10a**,**b**.[38] Compounds **11**−**34** were obtained by alkylating the appropriate piperidines with haloalkyl derivatives **9a,b** and **10a,b**. 2,2-Dimethylpiperidine,[39] 3,3-dimethylpiperidine,[33] and 4,4-dimethylpiperidine[40] were obtained following the literature procedures. Enantiomeric resolution of commercially available 3-methylpiperidine and 2-methylpiperidine was achieved according to the literature.[41,42] The unsubstituted piperidine and 4-methylpiperidine were purchased. All 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.[43]

Biology

Receptor Binding Studies. All the target compounds **11**−**34**, as hydrochloride salts, were evaluated for in vitro affinity at σ_1 and σ_2 receptors and at the Δ_8-Δ_7 SI site by radioreceptor binding assays. Compounds **14** and **19** were tested in a previous work.[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) sterol Δ_8-Δ_7 isomerase 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 (77−91%), (b) DTG (86−96%), (c) (±)-ifenprodil [2-(4-benzylpiperidino)-1-(4-hydroxyphenyl)-1-propanol] (66−87%). 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 three 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 the Prism ver 3.0, GraphPad software.[44]

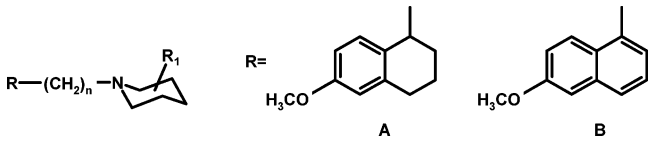
Table 1. Physical Properties

compd	formula[a]	% yield	mp, °C[b]	ClogP[c]
11	$C_{19}H_{29}NO{\cdot}HCl{\cdot}^1/_2H_2O$	83	154−156	5.39
12	$C_{20}H_{31}NO{\cdot}HCl$	83	192−194	5.91
13	$C_{21}H_{33}NO{\cdot}HCl{\cdot}^1/_2H_2O$	70	153−154	6.43
14[d]				6.43
15	$C_{21}H_{33}NO{\cdot}HCl{\cdot}^1/_2H_2O$	75	206−208	6.43
16	$C_{20}H_{31}NO{\cdot}HCl$	80	160−162	5.92
17	$C_{21}H_{33}NO{\cdot}HCl$	80	164−166	6.44
18	$C_{22}H_{35}NO{\cdot}HCl{\cdot}^1/_2H_2O$	20	154−156	6.95
19[d]				6.95
20	$C_{22}H_{35}NO{\cdot}HCl{\cdot}^1/_2H_2O$	70	175−177	6.95
21	$C_{19}H_{25}NO{\cdot}HCl$	80	170−172	4.97
(−)-(*R*)-**22**	$C_{20}H_{27}NO{\cdot}HCl$	50	175−177	5.49
(+)-(*S*)-**22**	$C_{20}H_{27}NO{\cdot}HCl$	50	178−180	5.49
(−)-(*R*)-**23**	$C_{20}H_{27}NO{\cdot}HCl$	60	183−185	5.49
(+)-(*S*)-**23**	$C_{20}H_{27}NO{\cdot}HCl$	60	180−182	5.49
24	$C_{20}H_{27}NO{\cdot}HCl{\cdot}^2/_5H_2O$	80	169−171	5.49
25	$C_{21}H_{29}NO{\cdot}HCl{\cdot}^1/_2H_2O$	68	226−228	6.01
26	$C_{21}H_{29}NO{\cdot}HCl{\cdot}^1/_4H_2O$	80	206−208	6.01
27	$C_{21}H_{29}NO{\cdot}HCl{\cdot}^1/_4H_2O$	80	165−167	6.01
28	$C_{20}H_{27}NO{\cdot}HCl$	75	175−177	5.50
(−)-(*R*)-**29**	$C_{21}H_{29}NO{\cdot}HCl{\cdot}^1/_2H_2O$	45	153−155	6.02
(+)-(*S*)-**29**	$C_{21}H_{29}NO{\cdot}HCl$	45	150−152	6.02
(−)-(*R*)-**30**	$C_{21}H_{29}NO{\cdot}HCl$	50	150−152	6.02
(+)-(*S*)-**30**	$C_{21}H_{29}NO{\cdot}HCl$	50	154−156	6.02
31	$C_{21}H_{29}NO{\cdot}HCl$	75	157−159	6.02
32	$C_{22}H_{31}NO{\cdot}HCl{\cdot}^1/_2H_2O$	28	199−201	6.54
33	$C_{22}H_{31}NO{\cdot}HCl$	65	157−159	6.54
34	$C_{22}H_{31}NO{\cdot}HCl{\cdot}^1/_2H_2O$	70	165−167	6.54

[a] Elemental analyses for C, H, N were within ±0.4% of the theoretical values for the formulas given. [b] Recrystallized from MeOH/Et_2O. [c] Referred to the corresponding free bases. [d] See ref 33.

Antiproliferative Assay. To define agonist or antagonist activity, a functional biochemical assay on rat C6 glioma cells was carried out. Among the σ_1 ligands with highest selectivity relative to σ_2 receptor, some naphthalene compounds were chosen to be tested along with their tetralin counterparts. Furthermore, the following reference compounds were tested: σ_1 agonist **1**, mixed σ_1/σ_2 agonist DTG, σ_1 antagonist **5**, σ_1 ligand **7**, and σ_2 antagonist *N*-(2-phenylethyl)piperidine (**35**, AC 927).[45,46] All selected compounds were tested for evaluating σ_1-mediated antiproliferative effect in cells where σ_2 receptors were masked by 100 μM selective σ_2 antagonist **35**. Under the assay conditions, the σ_1 activity component for DTG was measured. The activity of compound **35** was determined in the absence of any masking agent. Moreover, saturation analysis with (±)-[^{3}H]emopamil proved the absence of the Δ_8–Δ_7 SI site

Table 2. Binding Affinities and Selectivities



				$K_i \pm$ SEM (nM)			K_i ratio	
compd	R	*n*	R_1	σ_1	σ_2	Δ_8-Δ_7 SI	σ_2/σ_1	SI/σ_1
11	A	3	H	1.20 ± 0.44	26.2 ± 5.8	17.9 ± 5.0	22	15
12	A	3	4-CH_3	1.78 ± 0.33	31.4 ± 9.4	5.34 ± 1.88	18	3
13	A	3	2,2-di-CH_3	178 ± 35	115 ± 2	16.4 ± 2.3	0.6	0.1
14[a]	A	3	3,3-di-CH_3	2.36 ± 0.44	172 ± 28	0.57 ± 0.02	73	0.2
15	A	3	4,4-di-CH_3	1.18 ± 0.12	31.4 ± 3.1	5.39 ± 1.92	27	3
16	A	4	H	1.01 ± 0.41	48.7 ± 9.2	3.57 ± 0.85	48	3.5
17	A	4	4-CH_3	0.42 ± 0.04	36.3 ± 5.2	5.55 ± 0.15	86	13
18	A	4	2,2-di-CH_3	6.16 ± 1.59	29.5 ± 6.4	8.97 ± 2.04	4.8	1.4
19[a]	A	4	3,3-di-CH_3	2.12 ± 0.30	247 ± 52	0.67 ± 0.19	117	0.3
20	A	4	4,4-di-CH_3	0.30 ± 0.08	17.5 ± 4.1	4.11 ± 1.92	58	14
21	B	3	H	7.80 ± 2.90	175 ± 24	37.6 ± 9.2	22	4.8
(−)-(*R*)-**22**	B	3	2-CH_3	1.83 ± 0.64	86.2 ± 2.3	11.1 ± 1.9	47	6.1
(+)-(*S*)-**22**	B	3	2-CH_3	7.05 ± 1.68	104 ± 11	46.5 ± 2.3	15	6.6
(−)-(*R*)-**23**	B	3	3-CH_3	1.35 ± 0.42	60.2 ± 1.5	19.3 ± 6.1	45	14
(+)-(*S*)-**23**	B	3	3-CH_3	3.32 ± 0.62	59.3 ± 7.1	3.41 ± 0.85	18	1
24	B	3	4-CH_3	1.50 ± 0.43	38.9 ± 2.8	19.5 ± 0.7	26	13
25	B	3	2,2-di-CH_3	1060 ± 160	94.0 ± 3.4	14.9 ± 3.0	0.09	0.014
26	B	3	3,3-di-CH_3	0.35 ± 0.04	238 ± 28	8.71 ± 0.21	680	25
27	B	3	4,4-di-CH_3	1.47 ± 0.45	26.3 ± 5.5	9.04 ± 3.59	18	6.1
28	B	4	H	1.14 ± 0.04	151 ± 37	19.5 ± 1.6	132	17
(−)-(*R*)-**29**	B	4	2-CH_3	1.43 ± 0.51	49.2 ± 11.0	6.44 ± 1.70	34	4.5
(+)-(*S*)-**29**	B	4	2-CH_3	0.50 ± 0.15	53.8 ± 7.4	7.20 ± 2.05	108	14
(−)-(*R*)-**30**	B	4	3-CH_3	0.24 ± 0.02	64.0 ± 14.5	14.6 ± 0.4	266	61
(+)-(*S*)-**30**	B	4	3-CH_3	0.66 ± 0.24	32.7 ± 10.1	2.81 ± 0.57	50	4.3
31	B	4	4-CH_3	0.030 ± 0.013	17.9 ± 5.3	8.04 ± 1.34	597	268
32	B	4	2,2-di-CH_3	22.2 ± 1.9	28.6 ± 4.8	11.7 ± 2.0	1.3	0.5
33	B	4	3,3-di-CH_3	0.36 ± 0.12	67.4 ± 10.1	1.89 ± 0.33	187	5.3
34	B	4	4,4-di-CH_3	2.25 ± 0.16	17.9 ± 2.0	1.82 ± 0.65	8	0.8
7·HCl[b]				2.51 ± 0.68	>10^4	>10^4		
1				2.80 ± 0.29		>10^4		
5[a]				1.03 ± 0.14	212 ± 24	14.6 ± 4.1		
DTG					32.3 ± 2.4			
(±)-ifenprodil						4.70 ± 0.97		

[a] Formerly published data (ref 33). [b] Data already reported (ref 34).

in such a tumor cell line. The EC_{50} values were obtained from a nonlinear iterative curve fitting by Prism ver 3.0, GraphPad software.

Results and Discussion

Radioligand Binding and σ_1 SAfiR. The results of radioligand binding experiments for the examined compounds are listed in Table 2. Taken together, the affinity values recorded were not greatly different from those of their analogues previously studied.[33] However, some interesting exceptions can be noted. As regards σ_1 receptor affinity, compound **31** displayed the lowest K_i value (0.030 nM), whereas compound **25** was the worst σ_1 receptor ligand among all our piperidines (K_i = 1060 nM). Subnanomolar σ_1 affinities (K_is 0.24−0.66 nM) were also reached by tetralin compounds **17** and **20** and by naphthalene derivatives **26**, (+)-(*S*)-**29**, (−)-(*R*)-**30** and (+)-(*S*)-**30**, and **33**. These results represented an improvement in σ_1 receptor affinity and selectivity, compared to the tetralin derivatives of 3,3-dimethylpiperidine. Indeed, compounds **26** and **31** were found to be highly selective σ_1 receptor ligands (680- and 597-fold, respectively) relative to the σ_2 receptor and compound **31** also relative to the SI site (268-fold). As for σ_2 receptor affinities, the K_i values (17.5−247 nM) were generally comparable to those of our previous 3,3-dimethylpiperidines[33] and worse than those of our *N*-cyclohexylpiperazine analogues.[32] Moreover, all these new compounds displayed lower affinity toward the SI site compared to the mixed σ_1/SI high-affinity ligands **14** and **19** previously prepared.

All the compounds assayed had fairly high values of the calculated logarithm of the partition coefficient (ClogP), ranging from 4.97 to 6.95 (Table 1). ClogP values for most of the high-affinity σ_1 ligands herein reported (K_is 0.24−3.32 nM) fell at 6 ± 0.5. No correlation between σ_1 affinity and ClogP value was observed either for all the compounds or within the four series taken separately: the *N*-(6-methoxy-1,2,3,4-tetrahydronaphthlen-1-yl)propyl and *N*-(6-methoxynaphthalen-1-yl)propyl derivatives as well as their upper homologous butyl derivatives. Singularly, the highest affinity σ_1 ligand **31** and the lowest affinity **25** presented nearly the same ClogP value (6.02 and 6.01, respectively).

Chiral 6-methoxy-1,2,3,4-tetrahydronaphthalene derivatives **11**−**20** have been tested as racemic mixture. The piperidine moiety in such compounds did not present any stereogenic center. In the three-methylene chain series (compounds **11**−**15**), the high σ_1 affinity (K_is 1.18−2.36 nM) was almost unaffected by the position of dimethyl substitution. Only 2,2-dimethyl derivative **13** showed a moderate affinity (K_i = 178 nM) and no

selectivity. If the compounds of this series were compared to their upper homologues **16**−**20**, a little σ_1 affinity enhancement was observed for 4-methyl derivative **17** (K_i = 0.42 nM) and 4,4-dimethyl derivative **20** (K_i = 0.30 nM), whereas a 30-fold enhancement occurred for 2,2-dimethyl derivative **18** (K_i = 6.16 nM) compared to compound **13**. This may be due to the steric hindrance of two methyl groups, which are not tolerated when a propylene chain links the tetralin moiety to 2,2-dimethylpiperidine. Therefore, the 4-methyl or 4,4-dimethyl substitution on the piperidine ring results in the highest σ_1 affinity for the 6-methoxy-1,2,3,4-tetrahydronaphthalenes bearing a four-methylene chain. Unfortunately, due to the fairly good σ_2 affinity, none of these compounds showed higher selectivity relative to σ_2 receptor, when compared to the lead compound **19**.

Poorly significant differences in σ_1 affinity were observed for the series of 6-methoxynaphthalene analogues with a three-methylene chain (compounds **21**−**27**) compared to compounds **11**−**15**. Nevertheless, the results for compounds **25** and **26** stressed the importance of dimethyl substitution on the piperidine ring in this series. In fact, while the 3,3-dimethylpiperidine derivative **26** reached a high σ_1 affinity (K_i = 0.35 nM) and the best selectivity (680-fold) relative to the σ_2 receptor, the 2,2-dimethylpiperidine derivative **25** gave the worst σ_1 affinity result (K_i = 1060 nM), reversing the σ-subtype selectivity ratio. The σ_1 affinities of the remaining compounds of this series fell in the nanomolar range, without remarkable selectivities. The monomethyl derivatives (−)-(*R*)-**22** and (+)-(*S*)-**22** as well as (−)-(*R*)-**23** and (+)-(*S*)-**23** displayed comparable σ_1 affinities and moderate selectivities. Therefore, the chirality played a minimal role, possibly due to the easy interchangeable conformations of the piperidine ring. Moreover, a single methyl group in either 2- or 3-position was ineffective in enhancing or hindering the receptor interactions.

The series of (6-methoxynaphthalen-1-yl)butyl derivatives **28**−**34** presented several high-affinity σ_1 ligands. The highest affinity enhancement was shown by 4-methyl derivative **31** (K_i = 0.030 nM) and 3-methyl derivatives (−)-(*R*)-**30** and (+)-(*S*)-**30** (K_i = 0.24 nM and 0.66 nM, respectively). Also compounds (+)-(*S*)-**29** and **32** gained in σ_1 affinity compared to their respectively lower homologues (+)-(*S*)-**22** and **25**. Compound **33** saved the same σ_1 affinity of its lower homologue **26**, even if its selectivities relative to the σ_2 receptor and SI site were sensibly lower. Also the couple of enantiomers (−)-(*R*)-**29** and (+)-(*S*)-**29** as well as the couple (−)-(*R*)-**30** and (+)-(*S*)-**30** did not present sensible differences in affinities. However, each enantiomer generated a higher affinity profile than the respective homologous counterpart (compounds **22**, **23**).

Functional Assays and σ_1 SAR. The results expressed as EC_{50} values were reported in Table 3. When only a moderate cell growth inhibition was observed at high compound concentrations, the corresponding percentage values at 50 μM were reported. As depicted in Figure 1A, reference compounds **1**, **7**, and DTG were unable to induce an antiproliferative effect. Also the masking agent **35** gave the same result when tested alone, proving to not interfere in the test. By contrast, σ_1 antagonist **5** induced a potent cell proliferation inhibition (EC_{50} = 10.7 μM) that was dose-dependently reverted by σ_1 agonist **1** (Figure 1B). These findings were consistent with previously reported data.[36] Among the best new σ_1 ligands, some naphthalene derivatives **26**, **28**, **31**, and **33** were tested in the antiproliferative assay. To study the effect of the tetralin and naphthalene moiety on the σ_1 receptor activity, the tetralin counterparts **14**, **16**, **17**, and **19** were also selected to be tested. Tetralin derivatives **14**, **16**, **17**, and **19** behaved like the σ_1 agonist **1** (Figure 1A), whereas naphthalene derivatives **26**, **28**, **31**, and **33** displayed antiproliferative activity like σ_1 antagonist **5** (Figure 1B, white bars). The best results were observed for compounds **28**, **31**, and **33** (EC_{50} ranging between 15.0 μM and 25.5 μM), while a moderate potency was displayed by compound **26** (EC_{50} = 40.2 μM). Moreover, 20 μM compound **1** reverted 20−25% of the antiproliferative

Table 3. Antiproliferative Effect Measured as Inhibition of Rat C6 Glioma Cell Proliferation[a]

compd	EC_{50} ± SEM,[b] μM	compd	EC_{50} ± SEM,[b] μM
1	(35%)[c]	**17**	(53%)[c]
5	10.7 ± 0.5	**19**	(31%)[c]
7	(25%)[c]	**26**	40.2 ± 3.5
DTG	(20%)[c]	**28**	19.4 ± 2.5
35	(5%)[c]	**31**	25.5 ± 2.3
14	(47%)[c]	**33**	15.0 ± 1.2
16	(47%)[c]		

[a] All compounds, except **35**, were tested in the presence of 100 μM compound **35** as masking agent. The Δ_8-Δ_7 SI site was not found in this cell line. [b] Mean of $n \geq 3$ separate experiments. [c] EC_{50} not calculated; percentage inhibition at 50 μM given in parentheses.

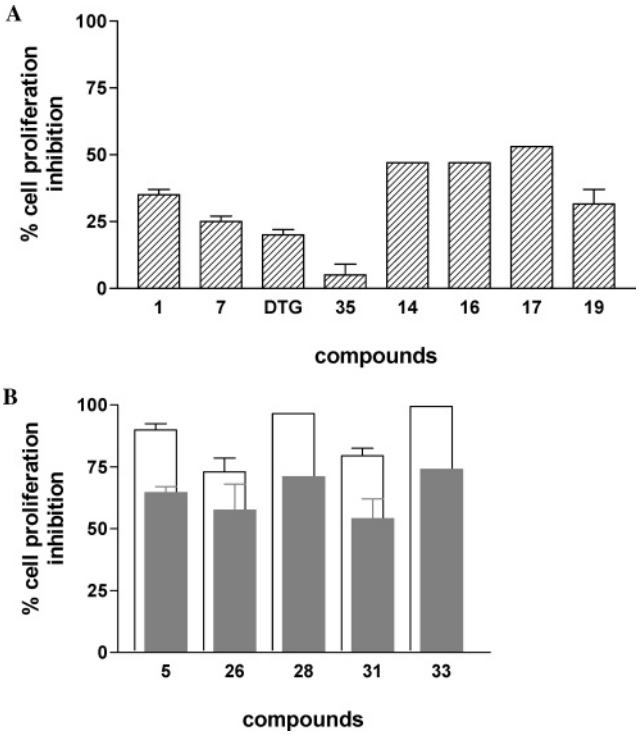


Figure 1. Antiproliferative effects in rat C6 glioma cell line for 50 μM inactive compounds (A) and 30 μM active compounds (B) in the absence (white bars) and in the presence of 20 μM compound **1** (gray bars). $P < 0.001$. One-way ANOVA analysis of variance was used to estimate the significance of difference. Data are means ± SEM of three experiments performed in duplicate. $P < 0.05$ was considered statistically significant.

effect of compounds **5**, **26**, **28**, **31**, and **33** at their EC_{50}. The corresponding results at their 30 μM concentration are matched in Figure 1B (gray bars). Therefore, σ_1 agonist activity can be claimed for tetralin derivatives, while σ_1 antagonist activity is found for the naphthalene derivatives assayed. Despite the few examples reported, it is possible to notice how the replacement of the tetralin with the naphthalene moiety switched the σ_1 ligand activity from agonist to antagonist. Finally, the activities seemed to be not strictly related to the ClogP values of the examined compounds.

Conclusions

Several compounds with nanomolar and subnanomolar affinity values were found in this class of piperidines. Generally, no great differences in affinities were observed when the methyl position changed. However, 2,2-dimethylpiperidine derivatives displayed the lowest σ_1 affinity in all series examined, particularly in the naphthalenepropyl series, giving evidence to the importance of an unshielded piperidine N-atom for the binding at the σ_1 receptor. In this series the three-methylene chain did not properly allow the receptor binding, due to a low conformational freedom. 3,3-Dimethylpiperidine derivatives **14**, **19**, and **26** displayed the best selectivity values within each series they belonged to. 3,3-Dimethylpiperidine derivative **33** also displayed high selectivity relative to σ_2 receptor, even if (*R*)-3-methyl and 4-methyl substitution (compounds **30** and **31**, respectively) generated the highest selectivities in the naphthalenebutyl series. The aromatization to naphthalene derivatives resulted in an increase of σ_1 affinity, accompanied by a slight increase in σ_2 affinity and lowering in SI site affinity. Particularly, naphthalene derivatives **26**, (−)-(*R*)-**30**, and **31** were high-affinity σ_1 ligands, with reduced affinity for the SI site. 4-Methyl group was the most effective substituent in increasing σ_1 affinity and selectivities, when an intermediate butyl chain linked 6-methoxynaphthalene to piperidine moiety. Therefore, the best selectivity profile was shown by the potent σ_1 ligand **31**. Naphthalene compounds **26** and **31** can be proposed as suitable tools for PET experiments.

Furthermore, these compounds along with their analogues **28** and **33** presented a clear antiproliferative activity in rat C6 glioma cells, comparable to that of σ_1 receptor antagonist **5**. This putative σ_1 receptor antagonist activity switched to putative agonist activity for their tetralin counterparts. On the other hand, methyl substitution on the piperidine became indeterminant for antagonist activity. As a little change in the structure turned the activity, an enzyme-like interaction with regulatory function was suggested. Significantly, the changes at the tetralin nucleus, which can mime the A and B rings of a steroid structure, result in important requirements for SI substrates. As the Δ_8-Δ_7 SI site was not found in the rat C6 cell line, a different mechanism than its inhibition has to be supposed for the antiproliferative effects of σ_1 antagonists. In conclusion, these claimed σ_1 antagonist agents open a useful perspective in tumor research and therapy.

Experimental Section

Chemical Methods. Column chromatography was performed with 1:30 ICN silica gel 60 Å (63−200 μm) as the stationary phase. Melting points were determined in open capillaries on a Gallenkamp electrothermal apparatus. Elemental analyses (C, H, N) were performed on an Eurovector Euro EA 3000 analyzer; the analytical results were within ±0.4% of the theoretical values for the formula given. ^{1}H NMR spectra were recorded at 300 MHz on a Mercury Varian spectrometer with $CDCl_3$ as solvent and, where indicated, on a Varian EM-390 at 90 MHz (TMS as internal standard). All values are reported in ppm (δ). The attribution of ^{1}H NMR signals of some final amines was done according to ^{1}H NMR NOESY and COSY given by the corresponding 2-, 3- and 4-methylpiperidines. 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. Optical rotations were measured on the hydrochloride salts with a Perkin-Elmer 341 polarimeter at room temperature (20 °C); concentrations are expressed in grams/100 milliliters. All spectra were in accordance with the assigned structures. Chemicals were from Aldrich or Across and were used without further purification.

Aromatization to 1-(ω-Haloalkyl)naphthalenes (10a,b). General Procedure. This reaction was carried out on the corresponding 1-(ω-haloalkyl)tetralins **9a,b** as previously described.[32] Crude products were purified by column chromatography (petroleum ether/CH_2Cl_2 8:2 as eluent) to yield colorless oils in 65% and 45% yield for **10a** and **10b**, respectively.

General Procedure To Obtain Final Amine Compounds (11−34). In a typical reaction, 1.0 mmol of the intermediate 1-(ω-haloalkyl)-6-methoxy-1,2,3,4-tetrahydronaphthalenes **9a,b** or 1-(ω-haloalkyl)-6-methoxynaphthalenes **10a,b** was stirred and refluxed overnight in CH_3CN with the appropriate piperidine (1.2 mmol) and Na_2CO_3. The workup was carried out as previously described.[32] The crude residue was purified by column chromatography (CH_2Cl_2/MeOH 95:5 as eluent), affording final compound as colorless or pale yellow oil. The corresponding yields were reported in Table 1.

1-[3-(6-Methoxy-1,2,3,4-tetrahydronaphthalen-1-yl)-propyl]piperidine (11): ^{1}H NMR δ 1.39−1.98 [m, 14H, $NCH_2(CH_2)_3$ and $(CH_2)_2CH(CH_2)_2$], 2.25−2.48 [m, 6H, $CH_2N(CH_2)_2$], 2.68−2.79 (m, 3H, benzyl CH and CH_2), 3.78 (s, 3H, OCH_3), 6.58−7.15 (m, 3H, aromatic); GC−MS *m/z* 288 (M^+ + 1, 4), 287 (M^+, 19), 98 (100), 85 (29). Anal. ($C_{19}H_{29}NO\cdot HCl\cdot {}^1/_2H_2O$) C, H, N.

4-Methyl-1-[3-(6-methoxy-1,2,3,4-tetrahydronaphthalen-1-yl)propyl]piperidine (12): ^{1}H NMR δ 0.91 (d, 3H, *J* = 6 Hz, $CHCH_3$), 1.18−1.40 [m, 3H, $CH_3CH(CHH)_2$], 1.45−1.69 [m, 8H, $(CH_2)_2CH(CH_2)_2$], 1.74−1.98 [m, 4H, $(CH_2)_2CH_2N$ and $CH_3CH(CHH)_2$], 2.22−2.38 [m, 2H, $N(CHH)_2$], 2.62−2.75 (m, 3H, benzyl CH and CH_2), 2.85−2.98 [m, 2H, $N(CHH)_2$], 3.78 (s, 3H, OCH_3), 6.55−7.08 (m, 3H, aromatic); GC−MS *m/z* 302 (M^+ + 1, 15), 301 (M^+, 56), 112 (100), 99 (53). Anal. ($C_{20}H_{31}NO\cdot HCl$) C, H, N.

2,2-Dimethyl-1-[3-(6-methoxy-1,2,3,4-tetrahydronaphthalen-1-yl)propyl]piperidine (13): ^{1}H NMR δ 1.05 [s, 6H, $C(CH_3)_2$], 1.35−1.90 [m, 14H, $C(CH_2)_3$ and $(CH_2)_2CH(CH_2)_2$], 2.22−2.58 (m, 4H, CH_2NCH_2), 2.62−2.78 (m, 3H, benzyl CH and CH_2), 3.78 (s, 3H, OCH_3), 6.58−7.15 (m, 3H, aromatic); GC−MS *m/z* 316 (M^+ + 1, 6), 315 (M^+, 27), 300 (100), 126 (65). Anal. ($C_{21}H_{33}NO\cdot HCl\cdot {}^1/_2H_2O$) C, H, N.

4,4-Dimethyl-1-[3-(6-methoxy-1,2,3,4-tetrahydronaphthalen-1-yl)propyl]piperidine (15): ^{1}H NMR δ 0.93 [s, 6H, $C(CH_3)_2$], 1.35−1.48 (m, 4H, CH_2CCH_2), 1.50−1.88 [m, 8H, $(CH_2)_2CH(CH_2)_2$], 2.25−2.50 [m, 6H, $CH_2N(CH_2)_2$], 2.62−2.78 (m, 3H, benzyl CH and CH_2), 3.75 (s, 3H, OCH_3), 6.55−7.15 (m, 3H, aromatic); GC−MS *m/z* 316 (M^+ + 1, 7), 315 (M^+, 29), 126 (100), 113 (30). Anal. ($C_{21}H_{33}NO\cdot HCl\cdot {}^1/_2H_2O$) C, H, N.

1-[4-(6-Methoxy-1,2,3,4-tetrahydronaphthalen-1-yl)-butyl]piperidine (16): ^{1}H NMR δ 1.25−1.88 [m, 16H, $NCH_2(CH_2)_3$ and $(CH_2)_2CH(CH_2)_3$], 2.30 [t, 2H, *J* = 7.8 Hz, $(CH_2)_3CH_2N$], 2.33−2.38 [m, 4H, $N(CH_2)_2$], 2.71−2.80 (m, 3H, benzyl CH and CH_2), 3.78 (s, 3H, OCH_3), 6.58−7.15 (m, 3H, aromatic); GC−MS *m/z* 302 (M^+ + 1, 10), 301 (M^+, 39), 98 (100). Anal. ($C_{20}H_{31}NO\cdot HCl$) C, H, N.

1-[3-(6-Methoxynaphthalen-1-yl)propyl]piperidine (21): ^{1}H NMR δ 1.35−1.78 [m, 8H, $NCH_2(CH_2)_3$ and $ArCH_2CH_2$], 2.37−2.46 [m, 6H, $CH_2N(CH_2)_2$], 3.03 (t, 2H, J = 7.8 Hz, benzyl CH_2), 3.95 (s, 3H, OCH_3), 7.13−8.01 (m, 6H, aromatic); GC−MS *m/z* 284 (M^+ + 1, 3), 283 (M^+, 15), 98 (100). Anal. ($C_{19}H_{25}NO\cdot HCl$) C, H, N.

(−)-(*R*)-2-Methyl-1-[3-(6-methoxynaphthalen-1-yl)propyl]piperidine [(−)-(*R*)-22]: $[\alpha]_D = -16°$ (c = 0.9%, MeOH); ^{1}H NMR δ 1.10 (d, 3H, J = 6.3 Hz, $CHCH_3$), 1.50−1.75 [m, 6H, $CH(CH_2)_3$], 1.84−1.95 (m, 2H, $ArCH_2CH_2$), 2.11−2.38 [m, 2H, $Ar(CH_2)_2CH_2$], 2.45−2.55 (m, 1H, CH_3CH) 2.78−3.06 (m, 4H, NCH_2 and benzyl CH_2), 3.92 (s, 3H, OCH_3), 7.14−7.96 (m, 6H, aromatic); GC−MS *m/z* 298 (M^+ + 1, 4), 297 (M^+, 20), 282 (42), 112 (100). Anal. ($C_{20}H_{27}NO\cdot HCl$) C, H, N.

(+)-(*S*)-2-Methyl-1-[3-(6-methoxynaphthalen-1-yl)propyl]piperidine [(+)-(*S*)-22]: $[\alpha]_D = +17°$ (c = 0.65%, MeOH); GC−MS *m/z* 298 (M^+ + 1, 9), 297 (M^+, 38), 282 (70), 112 (100). Anal. ($C_{20}H_{27}NO\cdot HCl$) C, H, N.

(−)-(*R*)-3-Methyl-1-[3-(6-methoxynaphthalen-1-yl)propyl]piperidine [(−)-(*R*)-23]: $[\alpha]_D = -6.7°$ (c = 0.75%, MeOH); ^{1}H NMR δ 0.85−0.92 (m, 4H, $CHCH_3$), 1.49−2.01 [m, 8H, $CH(CH_2)_2$ and $ArCH_2(CH_2)_2$], 2.36−2.54 [m, 2H, $N(CHH)_2$], 2.78−2.95 [m, 2H, $N(CHH)_2$], 3.04 (t, 2H, J = 7.5 Hz, benzyl CH_2), 3.92 (s, 3H, OCH_3), 7.14−8.02 (m, 6H, aromatic); GC−MS *m/z* 298 (M^+ + 1, 5), 297 (M^+, 21), 112 (100). Anal. ($C_{20}H_{27}NO\cdot HCl$) C, H, N.

(+)-(*S*)-3-Methyl-1-[3-(6-methoxynaphthalen-1-yl)propyl]piperidine [(+)-(*S*)-23]: $[\alpha]_D = +6.2°$ (c = 1%, MeOH). Anal. ($C_{20}H_{27}NO\cdot HCl$) C, H, N.

4-Methyl-1-[3-(6-methoxynaphthalen-1-yl)propyl]piperidine (24): ^{1}H NMR δ 0.95 (d, 3H, J = 6 Hz, $CHCH_3$), 1.21−1.40 [m, 3H, $CH_3CH(CHH)_2$], 1.58−1.68 [m, 2H, $CH_3CH(CHH)_2$], 1.90−2.01 [m, 4H, $ArCH_2(CH_2)_2$], 2.42−2.50 [m, 2H, $N(CHH)_2$], 2.90−2.98 [m, 2H, $N(CHH)_2$], 3.05 (t, 2H, J = 7.5 Hz, benzyl CH_2), 3.95 (s, 3H, OCH_3), 7.18−8.01 (m, 6H, aromatic); GC−MS *m/z* 298 (M^+ + 1, 3), 297 (M^+, 15), 112 (100). Anal. ($C_{20}H_{27}NO\cdot HCl\cdot {}^2/_5H_2O$) C, H, N.

2,2-Dimethyl-1-[3-(6-methoxynaphthalen-1-yl)propyl]-piperidine (25): ^{1}H NMR δ 1.10 [s, 6H, $C(CH_3)_2$], 1.38−1.68 [m, 6H, $C(CH_2)_3$], 1.78−1.88 (m, 2H, $ArCH_2CH_2$), 2.38−2.60 (m, 4H, CH_2NCH_2), 3.05 (t, 2H, J = 7.5 Hz, benzyl CH_2), 3.92 (s, 3H, OCH_3), 7.10−8.02 (m, 6H, aromatic); GC−MS *m/z* 312 (M^+ + 1, 4), 311 (M^+, 17), 296 (100), 126 (45). Anal. ($C_{21}H_{29}NO\cdot HCl\cdot {}^1/_2H_2O$) C, H, N.

3,3-Dimethyl-1-[3-(6-methoxynaphthalen-1-yl)propyl]-piperidine (26): ^{1}H NMR δ 0.95 [s, 6H, $C(CH_3)_2$], 1.19−1.23 (m, 2H, CCH_2CH_2), 1.57−1.65 (m, 2H, CCH_2CH_2), 1.83−1.92 (m, 2H, $ArCH_2CH_2$), 2.03 (br s, 2H, NCH_2C), 2.32−2.40 (m, 4H, CH_2NCH_2), 3.05 (t, 2H, J = 7.8 Hz, benzyl CH_2), 3.93 (s, 3H, OCH_3), 7.12−8.01 (m, 6H, aromatic); GC−MS *m/z* 312 (M^+ + 1, 4), 311 (M^+, 16), 126 (100). Anal. ($C_{21}H_{29}NO\cdot HCl\cdot {}^1/_4H_2O$) C, H, N.

4,4-Dimethyl-1-[3-(6-methoxynaphthalen-1-yl)propyl]-piperidine (27): ^{1}H NMR δ 0.95 [s, 6H, $C(CH_3)_2$], 1.38−1.44 (m, 4H, CH_2CCH_2), 1.80−2.00 (m, 2H, $ArCH_2CH_2$), 2.35−2.50 [m, 6H, $CH_2N(CH_2)_2$], 3.05 (t, 2H, J = 7.5 Hz, benzyl CH_2), 3.92 (s, 3H, OCH_3), 7.13−7.98 (m, 6H, aromatic); GC−MS *m/z* 312 (M^+ + 1, 7), 311 (M^+, 27), 126 (100). Anal. ($C_{21}H_{29}NO\cdot HCl\cdot {}^1/_4H_2O$) C, H, N.

1-[4-(6-Methoxynaphthalen-1-yl)butyl]piperidine (28): ^{1}H NMR δ 1.38−1.80 [m, 10H, $NCH_2(CH_2)_3$ and $ArCH_2(CH_2)_2$], 2.31−2.42 [m, 6H, $CH_2N(CH_2)_2$], 3.03 (t, 2H, J = 7.5 Hz, benzyl CH_2), 3.96 (s, 3H, OCH_3), 7.14−8.02 (m, 6H, aromatic); GC−MS *m/z* 297 (M^+, 26), 98 (100). Anal. ($C_{20}H_{27}NO\cdot HCl$) C, H, N.

Biological Methods. Radioligand Binding Assays. All the procedures for the binding assays were previously described.[33] σ_1 And σ_2 receptor binding was carried out according to Matsumoto et al.[47] and Δ_8-Δ_7 SI according to Moebius et al.[48] 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.

Cell Culture. The rat C6 glioma cells were a gift from Prof. Alberto Corsini (Department of Pharmacological Sciences, University of Milan, Milan, Italy) and were grown in MEM with 10% heat-inactivated fetal calf serum, 5% heat inactivated donor horse serum, 100 U/mL penicillin, 100 μg/mL streptomycin, and 2 mM L-glutamine in a humidified atmosphere 5% CO_2 at 37 °C. Eagle's minimum essential medium (MEM), Dulbecco's modified Eagle's medium (DMEM), trypsin−EDTA, penicillin (10 000 U/mL), streptomycin (10 mg/mL), nonessential amino acid solution (100×), L-glutamine solution (100×), sodium pyruvate solution (100 mM), fetal calf serum, and donor horse serum were purchased from Celbio s.r.l. Disposable culture flasks and Petri dishes were from Corning, Glassworks (Corning, NY).

Antiproliferative Assay. The antiproliferative effect due to σ_1 receptor activity was evaluated as previously described[36] using the MTT assay as reported in the literature.[49] The glioma cells were seeded to 96-well plates in the absence and presence of known concentrations of test compound and in the presence of 100 μM compound **35** to mask σ_2 receptors for 48 h. The medium was removed and replaced with 1 mg/mL of sterilized MTT solution freshly prepared. The plates containing MTT solution were wrapped in aluminum foil and placed in a 5% CO_2 incubator for 1 h at 37 °C. The MTT solution was removed and 100 μL of DMSO was added to each well to dissolve the blue formazan crystals. The optical density was measured at 570 and 650 nm wavelengths using an ELISA spectrophotometer (Spectra Shell). The number of the dead cells was evaluated with Tripan Blue reagent. Assays were performed in duplicate. The compounds **5**, **7**, and **35**, all as hydrochloride salts, were synthesized in our laboratory according to the reported preparative methods.

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Supporting Information Available: Elemental analyses of the end products, ^{1}H NMR and GC−MS data for the products **10a,b**, **17**, **18**, **20**, **29**−**34**, and $[\alpha]_D$ data for the enantiomeric couples **29** and **30**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Su, T.-P. Sigma receptors in the central nervous system and the periphery. In *The sigma receptors*; Itzhak, Y., Ed.; Academic Press: London, 1994; pp 21−44.
(2) Wolfe, S. A. Jr; De Souza, E. B. Role of sigma binding sites in the modulation of endocrine and immune functions. In *The sigma receptors*; Itzhak, Y., Ed.; Academic Press: London, 1994; pp 287−317.
(3) Guitart, X.; Codony, X.; Monroy, X. Sigma receptors: Biology and therapeutic potential. *Psychopharmacology* **2004**, *174*, 301−319.
(4) Quirion, R.; Bowen, W. D.; Itzhak, Y.; Junien, J. L.; Musacchio, J. M.; Rothman, R. B., Su T.-P.; Tam, S. W.; Taylor, D. P. A proposal for the classification of sigma binding sites. *Trends Pharmacol. Sci.* **1992**, *13*, 85−86.
(5) Kekuda, R.; Prasad, P. D.; Fei, Y.-J.; Leibach, F. H.; Ganapathy, V. Cloning and functional expression of the human type 1 sigma receptor (hSigmaR1). *Biochem. Biophys. Res. Commun.* **1996**, *229*, 553−558.
(6) Moebius, F. F.; Striessnig, J.; Glossmann, H. The mysteries of sigma receptors: New family members reveal a role in cholesterol synthesis. *Trends Pharmacol. Sci.* **1997**, *18*, 67−70.
(7) Bowen, W. D. Sigma receptors: Recent advances and new clinical potentials. *Pharm. Acta Helv.* **2000**, *74*, 211−218.
(8) Hayashi, T.; Su, T. P. Regulation ankyrin dynamics; roles of sigma-1 receptors. *PNAS* **2001**, *98*, 491−496.

(9) Vilner, B. J.; John, C. S.; Bowen, W. D. Sigma-1 and sigma-2 receptors are expressed in a wide variety of human and rodent tumor cell lines. *Cancer Res*. **1995**, *55*, 408−413.
(10) Caveliers, V.; Everaert, H.; Lahoutte, T.; Dierickx, L. O.; John, C. S.; Bossuyt, A. Labelled sigma receptor ligands: Can their role in neurology and oncology be extended? *Eur*. *J*. *Nucl*. *Med*. **2001**, *28*, 133−135.
(11) Spruce, B. A.; Campbell, L. A.; McTavish, N.; Copper, M. A.; Appleyard, M. V.; O′Neill, M.; Howie, J.; Samson, J.; Watt, S.; Murray, K.; McLean, D.; Leslie, N. R.; Safrany, S. T.; Ferguson, M. J.; Peters, J. A.; Prescott, A. R.; Box, G.; Hayes, A.; Nutley, B.; Raynaud, F.; Downes, P.; Lambert, J. J.; Thompson, A. M.; Eccles, S. Small molecule antagonists of the σ_1 receptors cause selective release of the death program in tumor and self-reliant cells and inhibit tumor growth *in vitro* and *in vivo*. *Cancer Res*. **2004**, *64*, 4875−4886.
(12) Crawford, K. W., Bowen, W. D. Sigma-2 receptor agonists activate a novel apoptotic pathway and potentiate antineoplastic drugs in breast tumor cell lines. *Cancer Res*. **2002**, *62*, 313−322.
(13) Kawamura, K.; Ishiwata, K.; Tajima, H.; Ishii, S. I.; Matsuno, K.; Homma, Y.; Senda, M. In vivo evaluation of [^{11}C]SA4503 as a PET ligand for mapping CNS sigma$_1$ receptors. *Nucl. Med. Biol.*, **2000**, *27*, 255−261.
(14) Elsinga, P. H.; Kobayashi, T.; Tsukada, H.; Vaalburg, W.; Ishiwata, K. Synthesis and evaluation of [^{18}F]fluoroethyl SA4503 as a PET ligand for the sigma receptor. *Synapse* **2002**, *43*, 259−267.
(15) Kassoiu, M.; Dannals, R. F.; Liu, X.; Wong, D. F.; Ravert, H. T.; Scheffel, U. A. Synthesis and in vivo evaluation of a new PET radioligand for studying sigma-2 receptors. *Bioorg*. *Med*. *Chem*. **2005**, *13*, 3623−3626.
(16) Friebe, M.; Mahmood, A.; Bolzati, C.; Drews, A.; Johannsen, B.; Eisenhut, M.; Kraemer, D.; Davison, A.; Jones, A. G. [^{99m}Tc]Oxotecnetium(V) complexes of amine-amide-dithiol chelates with dialkylaminoalkyl substituents as potential diagnostic probes for malignant melanoma. *J*. *Med*. *Chem*. **2001**, *44*, 3132−3140.
(17) Hayashi, T.; Su, T. P. σ-1 Receptor ligands: Potential in the treatment of neuropsychiatric disorders. *CNS Drugs* **2004**, *18*(5), 269−284.
(18) Su, T. P.; Hayashi, T. Understanding the molecular mechanism of sigma-1 receptors: Towards a hypothesis that sigma-1 receptors are intracellular amplifiers for signal transduction. *Curr*. *Med*. *Chem*. **2003**, *10*, 2073−2080.
(19) Maurice, T. Improving Alzheimer's disease-related cognitive deficits with σ_1 receptor agonists. *Drug News Perspect*. **2002**, *15*, 617−625.
(20) SA-4503 (AGY-94806). *Drugs Future* **2004**, *29*, 970.
(21) Maurice, T. Beneficial effect of the σ_1 receptor agonist PRE-084 against the spatial learning deficit in aged rats. *Eur*. *J*. *Pharmacol*. **2001**, *431*, 223−227.
(22) Ovalle, S.; Andreu, F.; Perez, M. P.; Zamanillo, D.; Guitart, X. Effect of the novel sigma$_1$ receptor ligand and putative atypical anthipsychotic E−5842 on BDNF mRNA expression in the rat brain. *Neuroreport* **2002**, *13*, 2345−2348.
(23) Nakazato, A.; Otha, K.; Sekiguchi, Y.; Okuyama, S.; Chaki, S.; Kawashima, Y.; Hatayama, K. Design, synthesis, structure−activity relationships, and biological characterization of novel arylalkoxyphenylalkylamine σ ligands as potential antipsychotic drugs. *J*. *Med*. *Chem*. **1999**, *42*, 1076−1087.
(24) Maeda, D. Y.; Williams, W.; Bowen, W. D.; Coop, A. A sigma-1 receptor selective analogue of BD 1008. A potential substitute for (+)-opioids in sigma receptor binding assays. *Bioorg*. *Med*. *Chem*. *Lett*. **2000**, *10*, 17−18.
(25) Maier, C. A.; Wunsch, B. Novel spiropiperidines as higly potent and subtype selective σ-receptor ligands. part 1. *J*. *Med*. *Chem*. **2002**, *45*, 438−448.
(26) Glennon, R. A.; Ablordeppey, S. Y.; Ismaiel, A. M.; El-Ashmawy, M. B.; Fischer, J. B.; Burke Howie, K. Structural features important for σ_1 receptor binding. *J*. *Med*. *Chem*. **1994**, *37*, 1214−1219.
(27) Ablordeppey, S. Y.; Fischer, J. B.; Law, H.; Glennon, R. A. Probing the proposed phenyl-A region of the sigma-1 receptor. *Bioorg*. *Med*. *Chem*. **2002**, *10*, 2759−2765.
(28) Ablordeppey, S. Y.; Fischer, J. B.; Glennon, R. A. Is a nitrogen atom an important pharmacophoric element in sigma ligand binding? *Bioorg*. *Med*. *Chem*. **2000**, *8*, 2105−2111.
(29) Yamamoto, H.; Miura, R.; Yamamoto, T.; Shinohara, K.; Watanabe, M.; Okuyama, S.; Nakazato, A.; Nukada, T. Amino acid residues in the transmembrane domain of the type 1 sigma receptor critical for ligand binding. *FEBS Lett*. **1999**, *445*, 19−22.
(30) Gund, T. M.; Floyd, J.; Jung, D. Molecular modeling of σ1 receptor ligands: A model of binding conformational and electrostatic considerations. *J*. *Mol*. *Graph*. *Model*. **2004**, *22*, 221−230.
(31) Berardi, F.; Santoro, S.; Perrone, R.; Tortorella, V.; Govoni, S.; Lucchi, L. *N*-[ω-(tetralin-1-yl)alkyl] derivatives of 3,3-dimethylpiperidine are highly potent and selective σ_1 or σ_2 ligands. *J*. *Med*. *Chem*. **1998**, *41*, 3940−3947.
(32) Berardi, F.; Ferorelli, S.; Abate, C.; Colabufo, N. A.; Contino, M.; Perrone, R.; Tortorella, V. 4-(Tetralin-1-yl)- and 4-(naphthalen-1-yl)alkyl derivatives of 1-cyclohexylpiperazine as σ receptor ligands with agonist σ_2 activity. *J*. *Med*. *Chem*. **2004**, *47*, 2308−2317.
(33) Berardi, F.; Ferorelli, S.; Colabufo, N. A.; Leopoldo, M.; Perrone, R.; Tortorella, V. A multireceptorial binding reinvestigation on a an extended class of σ ligands: *N*-[ω-(indan-1-yl and tetralin-1-yl)alkyl] derivatives of 3,3-dimethylpiperidine reveal high affinities towards σ_1 and EBP sites. *Bioorg*. *Med*. *Chem*. **2001**, *9*, 1325−1335.
(34) Berardi, F.; Loiodice, F.; Fracchiolla, G.; Colabufo, N. A.; Perrone, R.; Tortorella, V. Synthesis of chiral 1-[ω-(4-chlorophenoxy)-alkyl]-4-methylpiperidines and their biological evaluation at σ_1, σ_2, and sterol Δ_8-Δ_7 isomerase sites. *J*. *Med*. *Chem*. **2003**, *46*, 2117−2124.
(35) Maeda, D. Y.; Williams, W.; Kim, W. E.; Tatcher, L. N.; Bowen, W. D.; Coop, A. *N*-Arylpiperidines as high affinity sigma-1 and sigma-2 receptor ligands: Phenylpropylamines as potential leads for selective sigma-2 agents. *Bioorg*. *Med*. *Chem*. **2002**, *12*, 497−500.
(36) Colabufo, N. A.; Berardi, F.; Contino, M.; Niso, M.; Abate, C.; Perrone, R.; Tortorella, V. Antiproliferative and cytotoxic effects of some σ_2 agonists and σ_1 antagonists in tumour cell lines. *Naunyn-Schmiedeberg's Arch*. *Pharmacol*. **2004**, *370*, 106−113.
(37) Perrone, R.; Berardi, F.; Colabufo, N. A.; Leopoldo, M.; Tortorella, V.; Fornaretto, M. G.; Caccia, C.; McArthur R. A. Structure−activity relationship studies on the 5-HT$_{1A}$ receptor. Affinity studies of 1-phenyl-4-[ω-(α- or β-tetralinyl)alkyl]piperazines. **4.** *J*. *Med*. *Chem*. **1996**, *39*, 4928−4934.
(38) Teran, C.; Miranda, R.; Santana, L.; Teijeira, M.; Uriarte, E. Synthesis of linear and angular benzofurocoumarins. *Synthesis* **1997**, 1384−1386.
(39) Castro, P. J. L.; Carling W. R.; Chambers, M. S.; Fletcher, S. R.; Hobbs, S. C.; Matassa, V. G.; Moore, K. W.; Showell, G. A.; Russel, M. G. N. Benzodiazepine derivatives. Merck Sharp and Dohme Ltd. PCT Int. Appl. WO 94 03,437, 1994; GB Appl. 92/16,123, 1992; *Chem*. *Abstr*. **1994**, *121*, 35639j.
(40) Hayashi, T.; Sawamura, M.; Ito, Y. Asymmetric synthesis catalyzed by chiral ferrocenylphosphine-transition metal complexes. 10 Gold(I)-catalyzed asymmetric aldol reaction of isocyanoacetate. *Tetrahedron* **1992**, *48*, 1999−2012.
(41) Marawaha, J.; Palmer, M.; Hoffer, B.; Freedman, R.; Rice, K. C.; Paul, S.; Skolnick, P. Differential electrophysiological and behavioural responses to optically active derivatives of phencyclidine. *Naunyn-Schmiedeberg's Arch*. *Pharmacol*. **1981**, *315*, 203−209.
(42) Adamo, M. F. A.; Aggarwal, V. K.; Sage, M. A. An improved resolution of 2-methylpiperidine and its use in the synthesis of homochiral *trans*-2,6-dialkyl piperidines. *Synth*. *Commun*. **1999**, 1747−1756.
(43) ClogP version 4.0 for Windows, BioByte Corp., Claremont, CA.
(44) Prism ver 3.0 (version for Windows), GraphPad Software Inc., San Diego, CA.
(45) Ablordeppey, S. Y.; Fischer, J. B.; Burke Howie, K. J.; Glennon, R. A. Design synthesis and binding of sigma receptor ligands derived from butaclamol. *Med*. *Chem*. *Res*. **1992**, *2*, 368−375.
(46) Crawford, K. W.; Coop, A.; Bowen, W. D. σ_2 Receptors regulate changes in sphingolipid levels in breast tumor cells. *Eur. J. Pharmacol.* **2002**, *443*, 207−209.
(47) Matsumoto, R. R.; Bowen, W. D.; Tom, M. A.; Nhi Vo, V.; Truong, D. D.; de Costa, B. R. Characterization of two novel σ receptor ligands: Antidystonic effects in rats suggest σ receptor antagonism. *Eur. J. Pharmacol.* **1995**, *280*, 301−310.
(48) Moebius, F. F.; Hanner, M.; Knaus, H. G.; Weber, F.; Striessnig, J.; Glossmann, H. Purification and amino-terminal sequencing of the high affinity phenylalkylamine Ca^{2+} antagonist binding protein from guinea pig liver endoplasmic reticulum. *J. Biol. Chem.* **1994**, *269*, 29314−29320.
(49) Mossman, T. Rapid colorimetric assay for cellular growth and survival. *J*. *Immunol*. *Methods* **1983**, *65*, 55−63.

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