1022 J. Med. Chem. 2011, 54, 1022–1032
DOI: 10.1021/jm1013133

Journal of Medicinal Chemistry Article

Analogues of σ Receptor Ligand 1-Cyclohexyl-4-[3-(5-methoxy-1,2,3,4-tetrahydronaphthalen-1-yl)-propyl]piperazine (PB28) with Added Polar Functionality and Reduced Lipophilicity for Potential Use as Positron Emission Tomography Radiotracers

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Received October 13, 2010

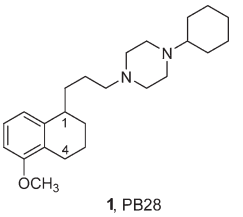
1-Cyclohexyl-4-[3-(5-methoxy-1,2,3,4-tetrahydronaphthalen-1-yl)propyl]piperazine **1** (PB28) represents an excellent lead candidate for therapeutic and/or diagnostic applications in oncology. However, because its utility is limited by its relatively high degree of lipophilicity, novel analogues of **1** with reduced lipophilic character were designed by substituting methylene groups with more polar functional groups in the propylene linker and at the tetralin C4 position. For the chiral analogues, separate enantiomers exhibited substantial and roughly equal affinities within a given receptor subtype, with the greatest difference observed for compound **9** at σ_1 (7.5-fold; (−)-(*S*)-**9** $K_i = 94.6$ nM, (+)-(*R*)-**9** $K_i = 12.6$ nM). Compound (−)-(*S*)-**9** was also found to be the most σ_2-selective agent (σ_2 $K_i = 5.92$ nM), to possess a lipophilicity consistent with entry into tumor cells (log $D_{7.4}$ = 2.38), and to show minimal antiproliferative activity. However, (−)-(*S*)-**9** exhibited moderate activity (EC_{50} = 8.1 μM) at the P-gp efflux pump.

Introduction

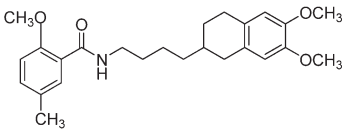
Three decades after their contradictory discovery,[1] the two subtypes of sigma (σ) receptors, namely, σ_1 and σ_2, are receiving much interest despite their still unclear mechanism of action. The isolated and cloned σ_1 subtype[2] has been recently classified as a receptor chaperone at the endoplasmic reticulum (ER[a]) membrane that regulates ER−mitochondrial Ca^{2+} signaling and cell survival.[3] A role in lipid compartmentalization in the ER[4] and in the binding of cholesterol with subsequent remodeling of lipid rafts has also been suggested for this receptor subtype.[5] σ_1 Receptor ligands display neuroprotective and neuroregulative functions and are under evaluation for the treatment of a number of neurological disorders[6] such as depression,[7] schizophrenia,[8] and Alzheimer's and Parkinson's diseases[9−11] and for drug abuse (e.g., cocaine).[12]

The lesser-known σ_2 subtype has yet to be cloned. Recently, an attempt to characterize the σ_2 receptor using a derivative of the high affinity σ_2 ligand 1-cyclohexyl-4-[3-(5-methoxy-1,2,3,4-tetrahydronaphthalen-1-yl)-*n*-propyl]piperazine **1** (PB28; Chart 1) led to the isolation of histone proteins,[13] and in particular, the histone H2A−H2B dimer seemed to be involved.[14]

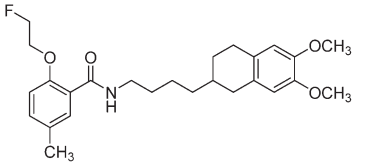
Chart 1. σ_2 Receptor Ligands Assayed in PET Analyses



1, PB28



2, RHM-1



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On the other hand, σ_2 receptor localization followed by fluorescence microscopy with fluorescent σ_2 subtype ligands detected σ_2 receptors in several organelles except the nucleus; this result was in disagreement with the histone hypothesis.[15] However, several high affinity[16] and a few σ_2-selective ligands[17,18] are making new acquisitions possible and interest in these proteins is increasing. σ_2 Receptors are overexpressed in a wide variety of human tumor cell lines in which σ_2 receptor agonists exert antiproliferative actions through different apoptotic pathways. Such evidence endows σ_2 receptor ligands with great diagnostic and therapeutic values.[18−20] In fact, several σ_2 receptor radioligands have been developed as positron emission

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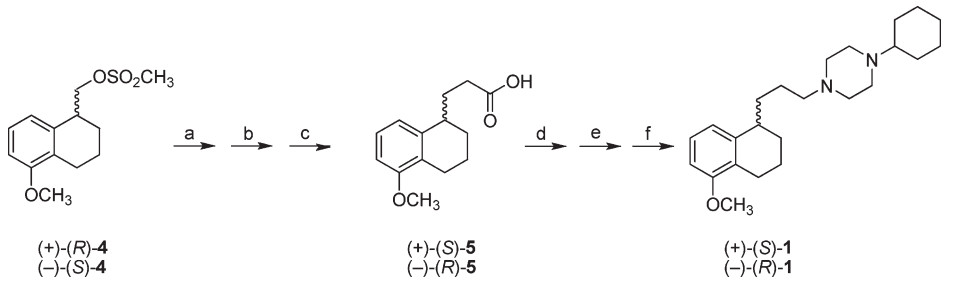
[a] Abbreviations: calcein-AM, calcein acetoxymethyl ester; CoMFA, comparative molecular field analysis; CNS, central nervous system; DMEM, Dulbecco's modified Eagle's medium; ER, endoplasmic reticulum; HSI, human sterol isomerase; MDCK, Madin−Darby canine kidney cells; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; PBS, phosphate buffered saline; PC3, prostate cancer cells; PET, positron emission tomography; P-gp, P-glycoprotein; RPMI, Roswell Park Memorial Institute; SAfiR, structure−affinity relationship; SK-N-SH, human neuroblastoma cells; SPECT, single photon emission computed tomography.

 Published on Web 01/13/2011

Scheme 1. Synthesis of Compound **1** Enantiomers[a]



[a] Reagents: (a) NaH, $CH_2(COOC_2H_5)_2$; (b) 10% KOH; (c) Δ; (d) $LiAlH_4$; (e) CH_3SO_2Cl; (f) 1-cyclohexylpiperazine.

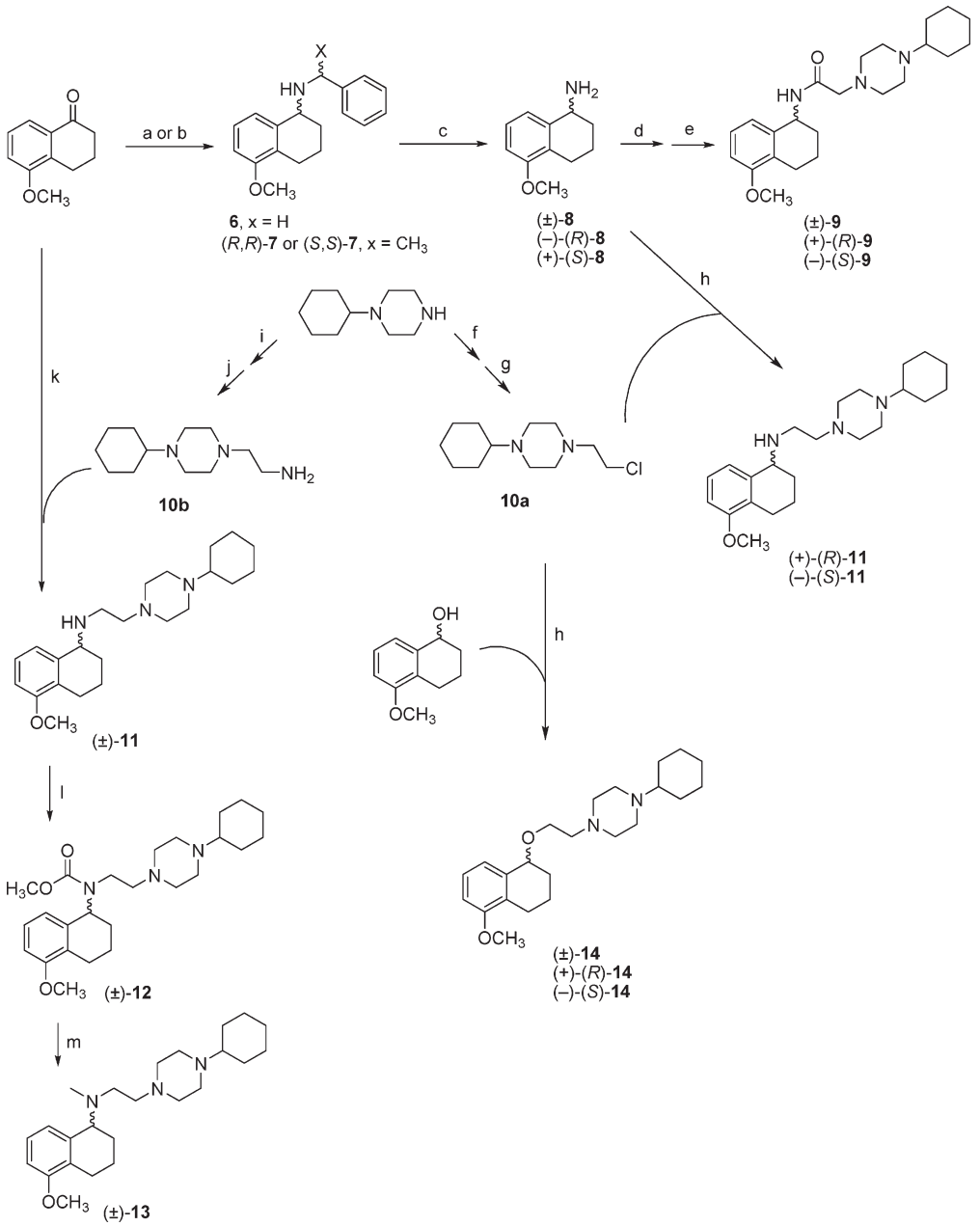
tomography (PET) or single photon emission computed tomography (SPECT) agents[21] with the aim of visualizing and early detecting tumors overexpressing σ_2 receptors. Tumor proliferating cells display a σ_2 receptor density about 10-fold higher than the corresponding quiescent cells, so that σ_2 receptor radiotracers have the potential to characterize the proliferative status of tumors and to be a determining factor in the tumor treatment to be established.[22] σ_2 Receptor high affinity flexible benzamides such as 5-methyl-2-[^{11}C]-methoxy-*N*-[4-(6,7-dimethoxy-3,4-dihydro-1*H*-isoquinolin-2-yl)butyl]benzamide **2** (RHM-1)[23] provided a clear image of murine breast tumors, and the corresponding fluoroethoxy derivative [^{18}F]-radiolabeled *N*-[4-(6,7-dimethoxy-3,4-dihydro-1*H*-isoquinolin-2-yl)-butyl]-2-(2-fluoroethoxy)-5-methylbenzamide **3** is currently under clinical study for imaging solid tumors with PET.[22] Compound **1** is one of the highest affinity σ_2 receptor ligands known.[16,24] It was [^{11}C]-radiolabeled at the methoxy group, and its in vivo evaluation in mice clearly showed a high degree of nonspecific binding in the brain.[25] A possible cause of the nonspecific binding could be a nonoptimal lipophilicity of compound **1** for use as a central nervous system (CNS) radiotracer. Lipophilicity is a determining factor also for the imaging of peripheral tumors. In fact, radiotracers with similar affinities at σ_2 receptors display different tumor uptake depending on their log *P*, suggesting that both the receptor affinity and lipophilicity must be taken into account for the design of receptor-based tumor imaging agents.[22] On the basis of these considerations, we designed a series of compound **1** analogues with a reduced lipophilicity with the aim of obtaining σ_2 receptor-overexpressing tumor imaging agents. A polar functional group was introduced either in the propylene linker or in the tetralin nucleus. In the latter case, an oxygen atom substituting for the tetralin C4 position served to change the tetralin ring system into a chromane one. When the substitution was made in the propylene chain, methylene groups within the chain adjacent to the tetralin C1 position were replaced by an amine, amide or ether group. For each of these analogues, the number of atoms in the chain linking the piperazine ring to the fused bicyclic moiety was kept constant (three, to mimic the high-affinity analogue **1**). Separate enantiomers were synthesized for the compounds where applicable. An enantioselective synthesis was adopted for the resynthesis of the two enantiomers of compound **1**. The corresponding compound **1** derivatives in which naphthalene substitutes for tetralin were also synthesized to determine if a convenient removal of the chirality at the C1 position would result in analogues that retain a high affinity, as suggested by a previously described structure−affinity relationship (SAfiR) study and comparative molecular field analysis (CoMFA) model.[26] The experimental log *P* of the newly synthesized compounds was measured in order to determine its impact on the ability of these compounds to enter tumors. Another property that we considered important to measure was the potential interaction of these newly synthesized molecules with P-glycoprotein (P-gp), one of the main drug efflux pumps overexpressed in a number of chemoresistant tumors. Previous studies demonstrated that compound **1** interacts with P-gp, inhibiting its activity.[20] Therefore the evaluation of the affinity at P-gp of the compound **1** analogues also became important in order to understand whether they may be reliable σ_2 markers in tumors overexpressing P-gp. In addition, given the antiproliferative activity of compound **1**, the antiproliferative activity of the newly synthesized compounds was also evaluated in order to determine if their administration for diagnostic purposes could be accompanied by undesirable strong antiproliferative side effects.

Results and Discussion

Chemistry. The synthesis of target compounds reported herein is depicted in Schemes 1−4. The synthesis of compound **1** enantiomers ((−)-(*R*)- and (+)-(*S*)-**1**), depicted in Scheme 1, was not achieved through fractional crystallization of the diastereomeric salts as reported earlier,[24] but through an enantioselective synthesis, whose pathway has been partially reported.[27] Briefly, key mesylate intermediates (+)-(*R*)- and (−)-(*S*)-**4**[28] were reacted with diethylmalonate, and after alkaline hydrolysis the obtained diacids were decarboxylated, leading to the key acids (+)-(*S*)- and (−)-(*R*)-**5** previously obtained by the fractional crystallization pathway.[24] Their reduction to alcohols with $LiAlH_4$ followed by mesylation led to the corresponding mesyl derivatives that, as previously reported,[24] underwent substitution with cyclohexylpiperazine to afford target compounds (+)-(*S*)- and (−)-(*R*)-**1** with an enantiomeric excess (ee) > 99%.

The syntheses of target compounds (±)-**9**, (±)-**11**, and (±)-**14** along with their corresponding (+)-(*R*)- and (−)-(*S*)-enantiomers together with the synthesis of racemate (±)-**13** are depicted in Scheme 2. 5-Methoxy-1,2,3,4-tetrahydronaphthalen-1-one underwent reductive amination according to a pathway reported for analogue ketones,[29] with either benzylamine[30] or (+)-(*R*)- or (−)-(*S*)-1-phenylethylamine, yielding the corresponding benzyl derivatives **6** and (*R*,*R*)- and (*S*,*S*)-**7** diastereomers, which were obtained with a diastereoselection >95%. Hydrogenation of these benzyl derivatives gave the known tetrahydronaphthalenamine intermediates (±)-, (−)-(*R*)-, and (+)-(*S*)-**8** respectively.[31] Reaction of these amines with bromoacetyl chloride afforded the corresponding bromoacetyl derivatives,[31] which were reacted with 1-cyclohexylpiperazine to yield target compounds (±)-, (+)-(*R*)-, and (−)-(*S*)-**9**. Intermediate **10a**, obtained by alkylation of 1-cyclohexylpiperazine with chloroethanol followed by reaction with $SOCl_2$, was used to

Scheme 2. Synthesis of Tethahydronaphthalene Derivatives[a]

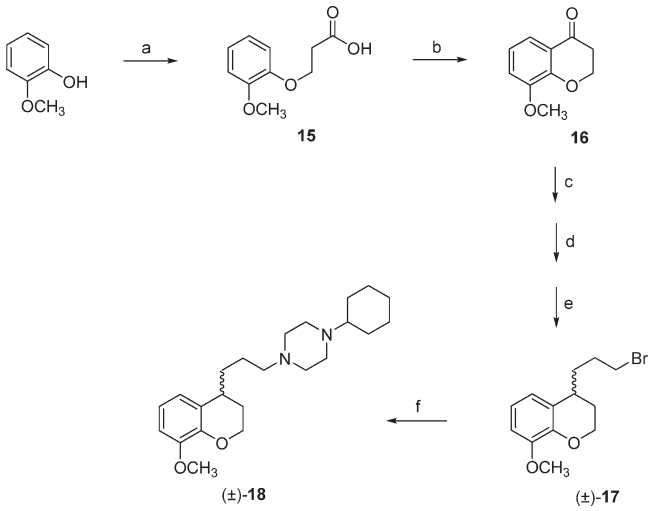


[a] Reagents: (a) benzylamine, $NaBH_4$; (b) (+)-(*R*)-1-phenylethylamine or (−)-(*S*)-1-phenylethylamine, $NaBH_4$; (c) H_2, Pd−C; (d) bromoacetyl chloride; (e) 1-cyclohexylpiperazine; (f) chloroethanol; (g) $SOCl_2$; (h) NaH; (i) $BrCH_2CN$; (j) $BH_3 \cdot DMS$; (k) $NaBH_4$; (l) methylchloroformate; (m) $LiAlH_4$.

alkylate (−)-(*R*)- or (+)-(*S*)-**8**, leading respectively to the target amines (+)-(*R*)- and (−)-(*S*)-**11**. Intermediate amine **10b**, which was obtained by alkylation of 1-cyclohexylpiperazine with $BrCH_2CN$ followed by reduction of the nitrile group to amine with $BH_3 \cdot DMS$, was used for the reductive amination of 5-methoxy-1,2,3,4-tetrahydronaphthalen-1-one, which yielded racemic target amine (±)-**11**. Compound (±)-**11** was reacted with methyl chloroformate to afford the carbamate (±)-**12**, which was subsequently reduced by $LiAlH_4$ to produce target compound (±)-**13**. The synthesis of target compound (±)-**14** and its corresponding enantiomers (+)-(*R*)- and (−)-(*S*)-**14** was achieved by alkylation of the racemic 5-methoxy-1,2,3,4-tetrahydronaphthalen-1-ol[24] and of its (−)-(*R*)- and (+)-(*S*)-enantiomers[32] with intermediate halide **10a**, using NaH as the base. The previously known alcohols (−)-(*R*)- and (+)-(*S*)-5-methoxy-1,2,3,4-tetrahydronaphthalen-1-ol were given by enantioselective reduction of 5-methoxy-1,2,3,4-tetrahydronaphthalen-1-one through the use of chiral Noyori's catalysts,[33] and respectively RuCl-[(1*R*,2*R*)-*p*-$TsNCH(C_6H_5)CH(C_6H_5)NH_2$](*p*-cymene) and RuCl[(1*S*,2*S*)-*p*-$TsNCH(C_6H_5)CH(C_6H_5)NH_2$]($\eta^6$-mesitylene) were used.

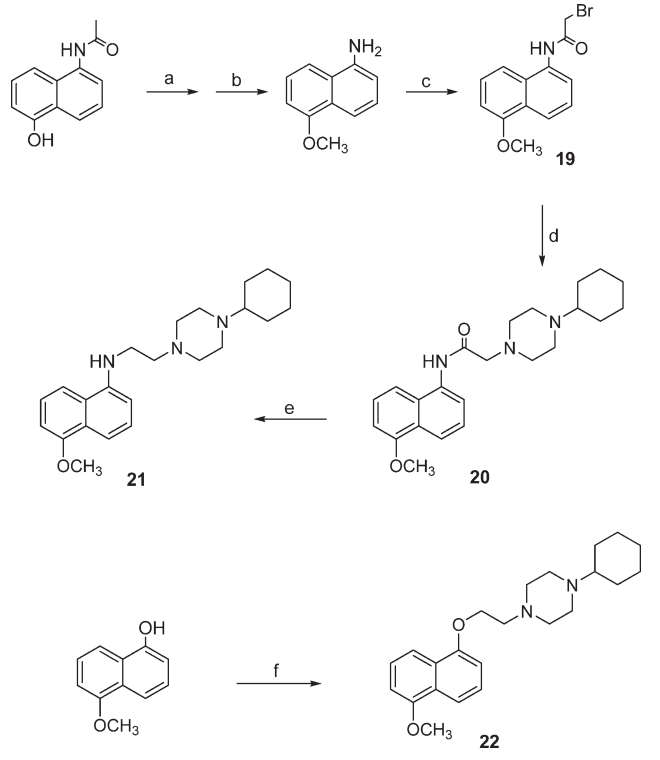
The synthesis of the target compound **18** is depicted in Scheme 3. According to a procedure reported for differently substituted chroman-4-one derivatives,[34] alkylation of guaiacol with 3-bromopropionic acid in the presence of NaH afforded intermediate 3-(2-methoxyphenoxy)propanoic acid **15**. Cyclization of compound **15** using polyphosphoric acid provided known key intermediate 8-methoxy-2,3-dihydro-4*H*-chromen-4-one **16**. Reaction of ketone **16** with

Scheme 3. Synthesis of Chromane Analogue[a]



[a] Reagents: (a) 3-bromopropionic acid, NaH; (b) polyphosphoric acid; (c) cyclopropylmagnesium bromide; (d) HBr/CH_3COOH; (e) H_2, Pd−C; (f) 1-cyclohexylpiperazine.

Scheme 4. Synthesis of Naphthalene Analogues[a]

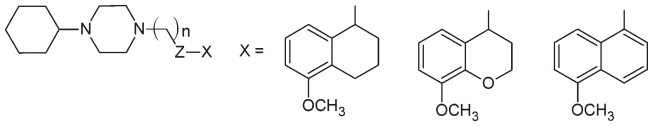


[a] Reagents: (a) MeI; (b) HCl; (c) bromoacetyl chloride; (d) 1-cyclohexylpiperazine (e) $LiAlH_4$; (f) **10a**.

cyclopropylmagnesium bromide prepared in situ, followed by dehydration and reduction of the formed double bond, produced the bromopropyl intermediate (±)-**17** as reported for analogous ketones.[35] Nucleophilic substitution of this bromide (±)-**17** with 1-cyclohexylpiperazine generated target piperazine compound (±)-**18**.

Synthesis of the naphthalene-containing target compounds **20**−**22** is reported in Scheme 4. 5-Methoxynaphthalen-1-amine was obtained by methylation with CH_3I of commercially available *N*-(5-hydroxynaphthalen-1-yl)acetamide[36] followed by acid hydrolysis. Reaction of the obtained amine with bromoacetyl chloride in the presence of $NaHCO_3$ yielded bromoacetamide **19**, which underwent alkylation with 1-cyclohexylpiperazine affording the target compound **20**. Compound **20** was reduced with $LiAlH_4$ to produce target compound **21**. Alkylation of phenol derivative[37] with intermediate chloride **10a** in the presence of K_2CO_3 afforded target compound **22**.

Table 1. Binding Data of Compound **1** (PB28) and Related Analogues at σ Receptors



				$K_i \pm$ SEM (nM)[a]	
compd	X	Z	*n*	σ_1	σ_2
(±)-**1**[b]	A	$-CH_2-$	2	0.38 ± 0.10	0.68 ± 0.20
(−)-(*R*)-**1**[b]	A	$-CH_2-$	2	0.63 ± 0.31	0.49 ± 0.18
(+)-(*S*)-**1**[b]	A	$-CH_2-$	2	0.13 ± 0.03	1.18 ± 0.25
(±)-**9**	A	−CONH−	1	12.6 ± 4.1	13.3 ± 2.9
(+)-(*R*)-**9**	A	−CONH−	1	20.4 ± 7.6	10.1 ± 2.54
(−)-(*S*)-**9**	A	−CONH−	1	94.6 ± 12.6	5.92 ± 0.52
(±)-**11**	A	−NH−	2	23.9 ± 6.2	8.49 ± 1.81
(+)-(*R*)-**11**	A	−NH−	2	13.6 ± 2.7	14.4 ± 1.5
(−)-(*S*)-**11**	A	−NH−	2	23.3 ± 12.8	8.52 ± 0.71
(±)-**13**	A	$-N(CH_3)-$	2	4.17 ± 1.53	15.3 ± 0.7
(±)-**14**	A	−O−	2	3.90 ± 1.28	9.03 ± 4.4
(+)-(*R*)-**14**	A	−O−	2	2.17 ± 0.18	11.1 ± 1.2
(−)-(*S*)-**14**	A	−O−	2	3.24 ± 1.13	4.71 ± 1.48
(±)-**18**	B	$-CH_2-$	2	12.5 ± 2.8	9.72 ± 1.68
20	C	−CONH−	1	42.6 ± 15.7	23.2 ± 5.8
21	C	−NH−	2	5.44 ± 1.64	18.5 ± 5.3
22	C	−O−	2	65%[c]	2.40 ± 0.35
(+)-pentazocine				3.0 ± 0.21	
DTG					25.7 ± 1.41

[a] The stability of each enantiomer was evaluated in the biological experiment conditions. The ee was left unchanged. [b] From ref 16. [c] [^{3}H]-(+)-Pentazocine binding inhibition percentage at 10^{-12} M of the compound. Values are the means of n ≥ 3 separate experiments.

Radioligand Binding and σ_1 and σ_2 Receptor Affinities. Results from binding assays are expressed as inhibition constants (K_i values) in Table 1. Each of the newly synthesized compounds displays nanomolar affinities at both σ subtypes as expected from the previously determined structural requirements for both receptor subtypes,[26,38] However, none of the compounds displays subnanomolar affinity as compound **1** and its enantiomers do, so that the three-methylene chain together with the tetralin ring system appears to be optimal in this series of compounds, thus suggesting a strong hydrophobic interaction of this portion of the molecule at the σ receptors. Despite the fact that affinity at the σ subtypes was reduced, one compound ((−)-(*S*)-**9**) reached an appreciable σ_2 versus σ_1 receptor selectivity (16-fold), which was missing in the lead **1** and its enantiomers. Racemic target compounds (±)-**1**, (±)-**9**, (±)-**11**, (±)-**14** and their corresponding single enantiomers showed a very similar affinity (the largest difference, 7.5-fold, occurs between (±)-**9** and (−)-(*S*)-**9**) so that the interaction at the σ_1 or σ_2 receptor generally appeared not to be enantioselective, at least in this series of compounds in which the stereogenic center is not next to the nitrogen atoms of the piperazine ring. A molecular modeling study where both

enantiomers of compound **1** were docked at the H2A−H2B histone dimer provided a possible explanation for the lack of enantioselectivity,[14] as did a SAfiR study/CoMFA model derived from a set of structurally related σ_2 receptor ligands.[26]

The K_i values at the σ_1 subtype for the present series of compound **1** analogues ranged from 2.17 nM to 94.6 nM (compounds (+)-(*R*)-**14** and (−)-(*S*)-**9** respectively). The oxyethylene linker led to the highest affinities for the racemate and its individual enantiomers (K_i = 3.90 nM, 2.17 nM, and 3.24 nM for compounds (±)-, (+)-(*R*)-, and (−)-(*S*)-**14** respectively) within this series. The amine derivatives (±)-, (+)-(*R*)-, and (−)-(*S*)-**11** displayed higher K_i values (K_i = 23.9 nM, 13.6 nM, and 23.3 nM respectively) and similar lack of enantioselectivity at the receptor. A somewhat different result was found for the corresponding amide derivatives in which the racemate and dextrorotatory enantiomer showed similar binding affinities ((±)-**9**, K_i = 12.6 nM; (+)-(*R*)-**9**, K_i = 20.4 nM), whereas the levorotatory enantiomer displayed significantly lower affinity ((−)-(*S*)-**9**, K_i = 94.6 nM). Thus, amide **9** represents the only clear example of enantioselection at the σ_1 site within this series of molecules. Racemic methylamine (±)-**13** displayed an affinity (K_i = 4.17 nM) comparable to the oxyethylene-bearing compounds showing that methylation of the amine at the tetralin C1 position improved σ_1 receptor affinity of about 5-fold (compare (±)-**13** with (±)-**11**). Results from the substitution of the tetralin ring system with a chromane one (**18**) were in accordance with all the other compounds wherein polar functionality was incorporated into the linker. For the naphthalene derivatives, an appreciable affinity was shown by the amine **21** (K_i = 5.44 nM); the affinity displayed by the corresponding amide **20** (K_i = 42.6 nM) was found to be an order of magnitude lower. The oxyethylene derivative **22** demonstrated noncompetitive binding with (+)-[^{3}H]-pentazocine at the σ_1 receptor, and the percentage of binding inhibition of the radioligand is reported in Table 1.

The K_i values at the σ_2 subtype for the newly synthesized compounds ranged from 2.40 nM to 23.2 nM (compounds **22** and **20** respectively) with most of the compounds having comparable affinities ($K_i \sim 10$ nM) independent of the substitution of the methylene group with an ether, amine, methylamine or amide group. No appreciable enantioselectivity was detected for the enantiomeric pairs at the σ_2 receptor. However, given its low affinity at the σ_1 subtype and a remarkable affinity at the σ_2 receptor (in line with the other compounds of the series), compound (−)-(*S*)-**9** emerged as the most σ_2-selective ligand (16-fold). Within the naphthalene derivatives, **22** displayed the best σ_2 receptor affinity of the overall series (K_i = 2.40 nM). The amine and amide-bearing naphthalene compounds (**20** and **21**) had an affinity 1 order of magnitude lower than **22**; this behavior did not reflect what happened in the tetralin series where compounds bearing an ether, amine or amide group in the linker had substantially the same affinity at the σ_2 receptor ((±)-**9**, (±)-**11**, (±)-**14**, and their enantiomers).

Evaluation of Physicochemical Properties. The pivotal role of PET tracer lipophilicity is well-recognized and it has been reviewed in depth by Waterhouse.[39] Lipophilicity can be measured in various theoretical and experimental ways. The most common experimental lipophilicity measurement involves partitioning of a compound between octanol and aqueous phases (log *P*). When lipophilicity is expressed as log *P* (partitioning of the neutral molecule species) or log $D_{7.4}$ (partitioning of all species present in solution at a given pH and therefore accounting for solubility effects associated with ionization), compounds that seem most effective for imaging have log *P* or log $D_{7.4}$ < 3.0. The ionization constants (pK_a), log *P* and log $D_{7.4}$ of the target compounds were experimentally determined by potentiometric titrations (Table 2). As far as the ionization constants are concerned, it can be noted that the structural modifications performed did not affect the basicity of the piperazine nitrogen bearing the cyclohexyl ring (pK_{a1}). In fact, the pK_{a1} values for all compounds ranged between 8.21 and 8.94. As expected, the basicity of the nitrogen linked to the alkyl chain (pK_{a2}) was strongly dependent on the nature of the functional group inserted in the alkyl chain. In particular, the introduction of the amide group caused a strong reduction of pK_{a2} values for compounds **9** and **20** with a more pronounced effect for the naphthalene derivative (pK_{a2} = 4.88 for **1** and pK_{a2} = 2.80 and 2.08 for **9** and **20**, respectively). The introduction of polar functionality (the Z group in Table 1) in the molecule has different effects depending on its position and the nature. Replacement of the methylene group attached to the tetralin nucleus with an amine function (compound **11**) caused a decrease of the pK_{a2} value of the piperazine nitrogen linked to the alkyl chain (pK_{a2} = 3.50) as compared to **1**. The presence of the electron-donating methyl on the nitrogen linked to the tetralin nucleus (compound **13**) produced a slight increase of the basicity of this same nitrogen (pK_{a3} = 7.98 for **11** and 8.15 for **13**) and a decrease of the pK_a of the piperazine nitrogen that bears the alkyl chain (pK_{a2} = 1.06). The introduction of a nitrogen or oxygen atom in the naphthalene derivatives **21** and **22** caused a decrease of 1.3 to 1.4 log units in the pK_a value (pK_{a2} = 3.55 and 3.50, respectively) compared to **1**. Finally, the introduction of an oxygen atom in the tetralin nucleus (compound **18**) did not influence the basicity of the piperazine nitrogen linked to the alkyl chain (pK_{a2}), presumably because of the greater separation of the chromane oxygen and the piperazine nitrogen.

Table 2. Physicochemical Properties of Compound **1** (PB28) and Related Analogues

compd	pK_{a1}	pK_{a2}	pK_{a3}	log *P*	log $D_{7.4}$
(±)-**1**	8.47	4.88		5.08	3.99
(±)-**9**	8.90	2.80		3.69	2.38
(±)-**11**	8.90	3.50	7.98	3.05	0.89
(±)-**13**	8.94	1.06	8.15	4.83	1.83
(±)-**14**[a]	nd[b]	nd	nd	nd	nd
(±)-**18**	8.71	4.81		nd	nd
20	8.54	2.08		3.44	2.18
21	8.58	3.55		4.08	2.88
22	8.21	3.50		3.94	2.88

[a] Data for compound **14** could not be determined. [b] Not determined.

As far as the lipophilicity is concerned, our study started with the evaluation of the lipophilic properties of compound **1**, which showed a value (log *P* = 5.08) that is abundantly out of the range that was considered optimal for a low degree of nonspecific binding as well as for a high tumor uptake as demonstrated for compound [^{11}C]-**2**.[22] Compound **11** showed the most pronounced decrease in log *P* (3.05; approximately 2 log units) as well as the lowest log $D_{7.4}$ value (0.89) due to the presence of two protonated nitrogens at pH 7.4. The introduction of an *N*-methyl group in derivative **13** (log *P* = 4.83) increased lipophilicity as compared to **11** (log *P* = 3.05). Compound **13** also showed a strong decrease of log $D_{7.4}$ (1.83), again because of the presence of two basic nitrogens. The introduction of the amide function in the

Table 3. Biological Activity of Compound **1** (PB28) and Related Analogues

	$EC_{50} \pm SEM$ (μM)	
compd	P-gp[a]	SK-N-SH[b]
(±)-**1**	3.0[c]	9.04 ± 0.2
(−)-(*R*)-**1**	4.4 ± 0.3	9.07 ± 0.3
(+)-(*S*)-**1**	3.8 ± 0.5	9.37 ± 0.1
(±)-**9**	8.8 ± 1.2	(68%)[d]
(+)-(*R*)-**9**	6.6 ± 0.8	(100%)[d]
(−)-(*S*)-**9**	8.1 ± 0.6	(100%)[d]
(±)-**11**	10 ± 1.1	(48%)[d]
(+)-(*R*)-**11**	9.8 ± 1.0	(31%)[d]
(−)-(*S*)-**11**	3.2 ± 0.3	(33%)[d]
(±)-**13**	8.8 ± 0.5	(82%)[d]
(±)-14	2.4 ± 0.7	(72%)[d]
(+)-(*R*)-**14**	5.3 ± 0.2	(91%)[d]
(−)-(*S*)-**14**	1.7 ± 0.1	(93%)[d]
(±)-**18**	5.2 ± 0.4	(100%)[d]
20	9.1 ± 0.9	(75%)[d]
21	3.6 ± 0.2	44.0 ± 1.2
22	3.4 ± 0.5	41.6 ± 2.0

[a] Transport inhibition in MDCK-MDR1 cells with calcein-AM (2.5 μM) as probe. [b] Antiproliferative effect measured in human SK-N-SH neuroblastoma cell line. [c] From ref 48. [d] EC_{50} not calculated; percentage of cell vitality at 100 μM given in parentheses. Values are the means of n ≥ 2 separate experiments.

alkyl chain led to a 1.5 log unit decrease in log *P* values for both **9** (log *P* = 3.69) and naphthalene analogue **20** (log *P* = 3.44). Finally, the introduction of a nitrogen or oxygen atom in the alkyl chain of the naphthalene derivatives **21** and **22** led to a decrease of about 1 log unit as compared to **1** (log *P* = 4.08 and 3.94, respectively). All in all, the results clearly indicated that the structural modifications led to a substantial decrease in lipophilicity of the newly prepared compounds. Moreover, compounds **9** and **20** showed log $D_{7.4}$ values within the optimal range for a useful PET radiotracer (log $D_{7.4}$ = 2.38 and 2.18, respectively).

Functional Assays and SAR

Calcein-AM Experiment. Activity at the P-gp efflux pump, expressed as EC_{50} in Table 3, was determined for each of the target compounds. The MDCK-MDR1 cells used in the assay overexpress the P-gp transporter, so that the measured biological effect is ascribed to the inhibition of this pump. All the compounds of this series display activity at P-gp with EC_{50} values in a relatively small range from 1.7 μM to 10 μM (compounds (−)-(*S*)-**14** and (±)-**11**, respectively). As was typical of this series of compounds, enantiomers (−)-(*R*)- and (+)-(*S*)-**1** displayed comparable inhibitory activity in the P-gp assay (EC_{50} = 4.4 μM and 3.8 μM, respectively); these values were also close to the activity of their racemate (±)-**1**, revealing that P-gp also does not discriminate based on absolute configuration. The same behavior was shown by the other couples of enantiomers, with just a small difference in the P-gp activity of the two enantiomers for compounds **14** and **11**. The most selective σ_2 receptor ligand of this series, (−)-(*S*)-**9**, displayed a moderate P-gp activity (EC_{50} = 8.1 μM). Therefore, the use of its corresponding radioligand for PET imaging of σ_2 receptors in tumor diagnosis may be accompanied by the drawback of the contemporaneous imaging of the overexpression of P-gp which occurs in some resistant tumors. However, once the effectiveness of the radiolabeled (−)-(*S*)-**9** is ascertained by in vivo assays, such a drawback may be overcome through a coadministration of the radioligand with a cold P-gp inhibitor. With such an approach the imaging of σ_2 receptors may be distinguished from the imaging of P-gp, adding new pieces information about the nature of the tumor.

Antiproliferative Activity in SK-N-SH Neuroblastoma Cells. The antiproliferative activity values of the compound **1** analogues are expressed as EC_{50} in Table 3. As previously reported, the SK-N-SH human neuroblastoma cell line proved to be a good model for the evaluation of the σ_2 receptor-mediated antiproliferative activity since σ_1 receptors were present in a low-affinity state.[40] Compound **1**, whose antiproliferative activity in SK-N-SH cell line had previously been determined,[40,41] was used as the reference compound. However, the activity of the two individual enantiomers of compound **1** has not previously been assayed before in cancer cells. The results presented here show that the enantiomers of **1** are approximately equipotent, each one having about the same EC_{50} value as the racemate ((±)-, (−)-(*R*)-, and (+)-(*S*)-**1**; EC_{50} = 9.04, 9.07, and 9.37). The same configuration-independent potency profile in antiproliferative effect was also observed in another kind of functional assay: the inhibition of the electrically evoked twitch in guinea pig bladder.[24] Compounds (−)-(*R*)- and (+)-(*S*)-**1** displayed all σ_2 receptor-mediated agonist activity, showing an antiproliferative effect independent of the absolute configuration, as also seen in the SK-N-SH assay reported here. Among the newly synthesized compounds, only naphthalene derivatives **21** and **22** displayed a moderate σ_2 receptor mediated agonist activity (EC_{50} = 44.0 μM and 41.6 μM, respectively). For all of the other compounds the EC_{50} could not be recorded since administration of compounds at 100 μM left the percentage of cell vitality very high (ranging from 31% to 100%) with compounds (+)-(*R*)- and (−)-(*S*)-**9**, and (±)-**18** not causing any antiproliferative effect at all (100% living cells). An interesting observation worthy of further investigation is the evidence that when a methylene group in the propylene chain adjacent to the tetralin C1 position or in the tetralin nucleus at the C4 position of compound **1** is substituted by a more hydrophilic function, the σ_2 receptor-mediated activity is reverted (antagonist) or drastically reduced. However, such results further suggest a diagnostic, rather than a therapeutic, role for these newly synthesized molecules given the lack of antiproliferative/cytotoxic effect.

Conclusions

All of the compounds reported herein are σ receptor ligands with nanomolar affinity at both subtypes. However, the subnanomolar affinities associated with the propylene chain lead compound (±)-**1** and its enantiomers were not obtained in the newly reported molecules designed with increased polar functionality. No substantial discrimination between enantiomers for the enantiomeric couples synthesized was observed by the σ receptors with one possible exception: (−)-(*S*)-**9** displayed a 7.5-fold lower affinity at the σ_1 receptor (σ_1 K_i = 94.6 nM) than its (+)-(*R*)-counterpart (σ_1 K_i = 12.6 nM). This compound also emerged as the most σ_2 receptor-selective agent with a remarkable σ_2 receptor affinity (σ_2 K_i = 5.92 nM) within this series. Such properties, together with an appropriate lipophilicity (log $D_{7.4}$ = 2.38), and the lack of antiproliferative activity in SK-N-SH cells, suggest further studies for the evaluation of compound (−)-(*S*)-**9** as a PET tracer for the imaging of σ_2 receptor-overexpressing cancers. The micromolar activity of (−)-(*S*)-**9** at the P-gp pump, which may represent a limitation for its use as σ_2 receptor PET agent when tumors overexpress also P-gp, may be overcome by a

coadministration with a P-gp inhibitor. The results coming from the PET analysis without and with the P-gp cold inhibitor may add new important pieces of information about the nature of the tumor (e.g., its proliferative status from σ_2 receptor content, and its resistance to therapies from P-gp content) for the therapy to be established.

Experimental Section

Chemistry. Both column chromatography and flash column chromatography were performed with 60 Å pore size silica gel as the stationary phase (1:30 w/w, 63−200 μm particle size, from ICN and 1:15 w/w, 15−40 μm particle size, from Merck respectively). Melting points were determined in open capillaries on a Gallenkamp electrothermal apparatus. Purity of tested compounds was established by combustion analysis, confirming a purity ≥95%. 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. ^{1}H NMR spectra were recorded on a Mercury Varian 300 MHz using $CDCl_3$ as solvent. The following data were reported: chemical shift (δ) in ppm, multiplicity (s = singlet, d = doublet, t = triplet, m = multiplet), integration and coupling constant(s) in hertz. Recording of mass spectra was done on an Agilent 6890-5973 MSD gas chromatograph/mass spectrometer and on an Agilent 1100 series LC-MSD trap system VL mass spectrometer; only significant *m*/*z* peaks, with their percentage of relative intensity in parentheses, are reported. Optical rotations were measured with a Perkin-Elmer 341 at room temperature (20 °C); concentrations are expressed as grams/100 mL. Chemicals were from Aldrich and Acros, and were used without any further purification.

(±)-, (+)-(*R*)-, and (−)-(*S*)-*N*-[(5-Methoxy-1,2,3,4-tetrahydronaphthalen)-1-yl]-2-(4-cyclohexylpiperazine-1-yl)acetamide [(±)-9, (+)-(*R*)-9, (−)-(*S*)-9]. A mixture of 1-cyclohexylpiperazine (0.20 g, 1.2 mmol), $NaHCO_3$ (0.11 g, 1.4 mmol) and one among (±)- (+)-(*R*)- or (−)-(*S*)-*N*-(5-methoxy-1,2,3,4-tetrahydronaphthalen-1-yl)bromoacetamide intermediates (0.30 g, 1.0 mmol) in CH_3CN (20 mL) was stirred under reflux overnight. After cooling, the mixture was evaporated under reduced pressure. The crude was taken up with H_2O (15 mL) and extracted with CH_2Cl_2 (3 × 10 mL). The organic phases collected were dried (Na_2SO_4) and evaporated under reduced pressure to give a foaming solid. After purification by column chromatography using CH_2Cl_2/MeOH (9:1) as eluent the target compound was obtained as a white foaming solid in 80% yield.

(±)-**9**: ^{1}H NMR δ 1.00−1.35 (m, 5H, cyclohexyl), 1.55−2.05 (m, 9H, cyclohexyl, Ar$CH_2CH_2CH_2$), 2.20−2.40 (m, 1H, cyclohexyl CHN), 2.55−2.78 (m, 10H, piperazine, benzyl CH_2), 3.00−3.08 (m, 2H, CH_2CO), 3.80 (s, 3H, OCH_3), 5.05−5.15 (m, 1H, CHNH), 6.65−7.20 (m, 3H, aromatic), 7.35 (br s, 1H, NH, D_2O exchanged); GC−MS *m*/*z* 385 (M^+, 9), 181 (100); LC−MS (ESI^+) *m*/*z* 386 $[M + H]^+$. Anal. ($C_{23}H_{35}N_3O_2 \cdot 2HCl$) C, H, N.

(+)-(*R*)-**9**: ^{1}H NMR, GC−MS and LC−MS data are the same reported for racemic compound. $[\alpha]_D$ = +48.5° (*c* = 0.75, MeOH). Anal. ($C_{23}H_{35}N_3O_2 \cdot 2HCl \cdot {}^3/_4H_2O$) C, H, N.

(−)-(*S*)-**9** ^{1}H NMR, GC−MS and LC−MS data are the same reported for racemic compound. $[\alpha]_D$ = −51.6° (*c* = 0.68, MeOH). Anal. ($C_{23}H_{35}N_3O_2 \cdot 2HCl \cdot H_2O$) C, H, N.

HPLC analyses on a Daicel Chiralcel OD (*n*-hexane/*i*-propylamine/diethylamine, 9:1:0.1, flow rate 0.5 mL/min, λ = 280 nm) on both the enantiomers displayed ee >99%.

(+)-(*R*)- and (−)-(*S*)-4-Cyclohexyl-1-[*N*-(5-methoxy-1,2,3,4-tetrahydronaphthalen-1-yl)-2-aminoethyl]piperazine [(+)-(*R*)-11, (−)-(*S*)-11]. A solution of 1-cyclohexylpiperazine (1.0 g, 5.9 mmol), 2-chloroethanol (0.47 mL, 7.1 mmol) and Et_3N (1.0 mL, 7.2 mmol) in CH_3CN (30 mL) was stirred under reflux overnight. Then the solvent was removed by evaporation under reduced pressure and the crude taken up with H_2O (20 mL) and extracted with CH_2Cl_2 (3× 20 mL). The collected organic phases were dried (Na_2SO_4) and evaporated to give a dense oil. Purification of the crude was achieved preparing the hydrochloride salt, which was recrystallized from MeOH, affording 2-(4-cyclohexylpiperazine-1-yl)ethanol dihydrochloride in 80% yield: LC−MS (ESI^+) *m*/*z* 213 $[M - HCl_2]^+$. These last crystals (1.0 g, 3.5 mmol) and $SOCl_2$ (0.51 mL, 7.0 mmol) in $CHCl_3$ (20 mL) were stirred under reflux overnight. After cooling, the white solid was filtered and washed with $CHCl_3$ yielding intermediate **10a** in 75% yield: LC−MS (ESI^+) *m*/*z* 231 $[M - HCl_2]^+$. This last intermediate was made free base (1.6 g, 6.1 mmol) and added to a mixture of Na_2CO_3 (0.70 g, 6.6 mmol) and amine (−)-(*R*)- or (+)-(*S*)-**8** (1.1 g, 6.2 mmol) in CH_3CN (20 mL). The mixture was stirred under reflux overnight. After cooling, more **10a** was added (0.60 g, 2.3 mmol) and the mixture was refluxed again for an additional 6 h. After cooling, the reaction mixture was evaporated under reduced pressure and the crude obtained was taken up with H_2O (15 mL) and extracted with CH_2Cl_2 (3 × 15 mL). The organic phases collected were dried (Na_2SO_4) and evaporated under reduced pressure to give a crude yellow oil. After purification by flash column chromatography using CH_2Cl_2/MeOH (from 9:1 to 8:2) as eluent the target compound was obtained as clear yellow oil in 40% yield.

(+)-(*R*)-**11**: ^{1}H NMR, GC−MS and LC−MS data are the same reported below for racemic compound. $[\alpha]_D$ = +5.03° (*c* = 0.62, MeOH). Anal. ($C_{23}H_{37}N_3O \cdot 3HCl \cdot H_2O$) C, H, N.

(−)-(*S*)-**11**: ^{1}H NMR, GC−MS and LC−MS data are the same reported for racemic compound. $[\alpha]_D$ = −5.8° (*c* = 1, MeOH). Anal. ($C_{23}H_{37}N_3O \cdot 3HCl \cdot 1.5H_2O$) C, H, N.

HPLC analyses on a Daicel Chiralcel OD (*n*-hexane/*i*-propylamine/diethylamine, 99:1:0.1, flow rate 0.4 mL/min, λ = 280 nm) on both the enantiomers displayed ee >99%.

(±)-4-Cyclohexyl-1-[*N*-(5-methoxy-1,2,3,4-tetrahydronaphthalen-1-yl)-2-aminoethyl]piperazine [(±)-11]. A solution of 1-cyclohexylpiperazine (2.50 g, 14.8 mmol), chloroacetonitrile (1.12 mL, 17.8 mmol), and Et_3N (4.2 mL, 29.7 mmol) was stirred at room temperature overnight. Water was added to the reaction and extracted with CH_2Cl_2 (3×20 mL). The organic phases collected were dried (Na_2SO_4) and evaporated under reduced pressure to give (4-cyclohexylpiperazin-1-yl)acetonitrile intermediate as a colorless oil in 90% yield: GC−MS *m*/*z* 208 (M^+ + 1, 36), 207 (M^+, 36), 164 (100). To a solution of (4-cyclohexylpiperazin-1-yl)acetonitrile (2.76 g, 13.3 mmol) in dry THF (50 mL) cooled to 0 °C and kept under a stream of N_2, $BH_3 \cdot S(CH_3)_2$ (4.35 mL, ∼10 M as BH_3) was added in a dropwise manner. The mixture was refluxed for 4 h, and after cooling to 0 °C, MeOH was added to quench the reaction until foaming ceased. HCl (3 N) (50 mL) was added to the mixture, and the reaction was refluxed for 1 h. Organic solvents were then evaporated, and the aqueous solution was turned basic by the addition of NaOH (5 N) (40 mL). The basic solution was extracted with AcOEt (4 × 30 mL), and the organic phases collected were dried (Na_2SO_4) and evaporated under reduced pressure to give 2-(4-cyclohexylpiperazin-1-yl)ethanamine **10b** as a colorless oil with 85% yield: GC−MS *m*/*z* 211 (M^+, 6), 181 (100). Without any further purification, the compound was used for the next step. A solution of 5-methoxy-1,2,3,4-tetrahydronaphthalen-1-one (0.92 g, 5.2 mmol), **10b** and CF_3COOH (0.05 mL) in toluene (20 mL) was refluxed overnight with azeotropic removal of H_2O. Toluene was evaporated until 4 mL was left, and such solution was cooled and added in a dropwise manner to a suspension of $NaBH_4$ (0.30 g, 7.9 mmol) in EtOH (4 mL) cooled at 0 °C. The resulting mixture was kept at room temperature under stirring for 4 h and then H_2O (5 mL) was added. The solvent was evaporated under reduced pressure, and the crude obtained was taken up with H_2O (15 mL) and extracted with AcOEt (4×15 mL). The organic phases collected were dried (Na_2SO_4) and evaporated under reduced pressure to give a yellow oil. After purification by flash column chromatography using CH_2Cl_2/MeOH (from 9:1 to 8:2) as eluent the target compound was obtained as a clear yellow oil in a 50% yield: ^{1}H NMR δ

1.05−1.35 (m, 5H, cyclohexyl), 1.58−1.98 (m, 9H, cyclohexyl, $ArCH_2CH_2CH_2$), 2.18−2.30 (m, 1H, CHN), 2.38−2.98 (m, 14H, CH_2N, CH_2NH, benzyl and piperazine CH_2), 3.68−3.78 (m, 1H, CHNH), 3.80 (s, 3H, OCH_3), 6.65−7.20 (m, 3H, aromatic); GC−MS *m*/*z* 371 (M^+, 1), 181 (100); LC−MS (ESI^+) *m*/*z* 372 $[M + H]^+$; LC−MS−MS 372: 212, 161. Anal. ($C_{23}H_{37}N_3O \cdot 3HCl \cdot H_2O$) C, H, N.

(±)-4-Cyclohexyl-1-[*N*-(5-methoxy-1,2,3,4-tetrahydronaphthalen-1-yl)-*N*-methyl-2-aminoethyl]piperazine [(±)-13]. To a solution of (±)-**11** (1.54 mmol, 0.57 g) in anhydrous DMF (5 mL) kept at 0 °C and under a stream of N_2, methyl chloroformate (2.20 mmol, 0.17 mL) and pyridine (2.96 mmol, 0.24 mL) were added in a dropwise manner. The solution was stirred at room temperature for 2 h. After evaporating the solvent under reduced pressure, the crude was taken up with H_2O (15 mL) and extracted with AcOEt (3 × 15 mL), yielding a yellow gummy crude, which was purified by column chromatography using CH_2Cl_2/MeOH (95:5) as eluent. The carbamate intermediate compound **12** was obtained in a 50% yield as a clear yellow gummy solid: ^{1}H NMR δ 1.05−2.10 (m, 14H, cyclohexyl CH_2, $ArCH_2CH_2CH_2$), 2.20−2.30 (m, 1H, cyclohexyl NCH), 2.40−3.05 (m, 12H, CH_2N and benzyl and piperazine CH_2), 3.12−3.18 (m, 2H, $NCOCH_2$), 3.70−3.78 (s + s, 3H, CH_3OCO), 3.80 (s, 3H, OCH_3), 6.62−7.15 (m, 3H, aromatic); GC−MS *m*/*z* 371 (M^+, 7.5), 181 (100). A solution of the intermediate carbamate **12** in dry THF (5 mL) was added in a dropwise manner to a suspension of $LiAlH_4$ (2.90 mmol, 0.11 g) in dry THF (10 mL) kept at 0 °C and under a stream of N_2. The mixture was refluxed for 3 h, and after cooling down, it was quenched with H_2O (5 mL) and extracted with Et_2O (3×15 mL). The organic phases collected were washed with brine, dried (Na_2SO_4) and evaporated under reduced pressure to produce the target compound as colorless oil in 85% yield: ^{1}H NMR δ 1.05−1.38 (m, 5H, cyclohexyl), 1.45−2.05 (m, 9H, cyclohexyl, $ArCH_2CH_2CH_2$), 2.22 (s, 3H, NCH_3), 2.38−2.80 (m, 15H, cyclohexyl CHN, $CH_3NCH_2CH_2N$, benzyl and piperazine CH_2), 3.80 (s, 3H, OCH_3), 3.82−3.84 (m, 1H, $CHNCH_3$), 6.62−7.30 (m, 3H, aromatic); GC−MS *m*/*z* 385 (M^+, 1), 181 (47), 161 (100). Anal. ($C_{24}H_{39}N_3O \cdot 3HCl \cdot 2H_2O$) C, H, N.

(±)-, (+)-(*R*)-, and (−)-(*S*)-1-Cyclohexyl-4-[2-(5-methoxy-1,2,3,4-tetrahydronaphthalen-1-yloxy)ethyl]piperazine [(±)-14, (+)-(*R*)-14, (−)-(*S*)-14]. To a suspension of NaH (3.12 mmol, 0.075 g) in dry THF (10 mL) at 0 °C, a solution of 5-methoxy-1,2,3,4-tetrahydronaphthalen-1-ol or its (*R*)-(−)- and (*S*)-(+)-enantiomers (2.60 mmol, 0.46 g) in the same solvent (10 mL) was added in a dropwise manner. The mixture was warmed to room temperature, and after 15-crown-5 ether (0.005 mL) was added, it was kept under stirring for 10 min. After cooling to 0 °C, a solution of **10a** (2.60 mmol, 0.6 g) in dry THF (10 mL) was added in a dropwise manner to the mixture, which was then refluxed under stirring overnight. After cooling down, H_2O (20 mL) was added and the mixture was extracted with AcOEt (3 × 15 mL). The collected organic phases were dried (Na_2SO_4) and evaporated under reduced pressure to produce the crude as orange oil. After purification by flash column chromatography using petroleum ether/AcOEt (1:1) and gradually using only AcOEt, the target compound was obtained as a yellow gummy oil in 30% yield: ^{1}H NMR δ 1.05−1.30 (m, 5H, cyclohexyl), 1.58−2.00 (m, 9H, cyclohexyl and $ArCH_2CH_2CH_2$), 2.15−2.25 (m, 1H, NCH), 2.45−2.80 (m, 12H, piperazine, CH_2N and benzyl CH_2), 3.58−3.75 (m, 2H, CH_2O), 3.80 (s, 3H, OCH_3), 4.42 (t, 1H, *J* = 4.7 Hz, CHO), 6.75−7.18 (m, 3H, aromatic). Attempts to obtain the hydrochloride salt failed since a decomposition of the target compound occurred. Thus, maleate salt was obtained in the usual way and recrystallization was made from MeOH. LC−MS [M + H$^+$]: 373. LC−MS−MS 373: 213, 161. Anal. ($C_{23}H_{36}N_2O_2 \cdot 2C_4H_4O_4 \cdot H_2O$) C, H, N.

(+)-(*R*)-**14**: ^{1}H NMR and LC−MS data are the same reported for racemic compound. $[\alpha]_D = +5.0°$ (*c* = 0.6, $CHCl_3$) calculated on the maleate salt. Anal. ($C_{23}H_{36}N_2O_2 \cdot 2C_4H_4O_4$) C, H, N.

(−)-(*S*)-**14** ^{1}H NMR and LC−MS data are the same reported for racemic compound. $[\alpha]_D = -5.3°$ (*c* = 0.6, $CHCl_3$) calculated on the maleate salt. Anal. ($C_{23}H_{36}N_2O_2 \cdot 2C_4H_4O_4$) C, H, N.

HPLC analyses on a Daicel Chiralcel OD (*n*-hexane/*i*-propylamine/diethylamine, 97:3:0.1, flow rate 0.8 mL/min, λ = 270 nm) on both the enantiomers displayed ee > 99%.

(±)-1-Cyclohexyl-4-[3-(8-methoxy-3,4-dihydro-2*H*-chromen-4-yl)propyl]piperazine [(±)-18]. A mixture of 1-cyclohexylpiperazine (0.20 g, 1.2 mmol), Na_2CO_3 (0.20 g, 1.4 mmol) and intermediate (±)-**17** in CH_3CN (20 mL) was stirred under reflux overnight. After cooling the mixture was evaporated under reduced pressure. The crude was taken up with H_2O (15 mL) and extracted with CH_2Cl_2 (3 × 10 mL). The organic phases collected were dried (Na_2SO_4) and evaporated under reduced pressure to give a dark brown oil. After purification by column chromatography using CH_2Cl_2/MeOH (98:2) as eluent the target compound was obtained as brown oil in 80% yield: ^{1}H NMR δ 1.05−1.30 (m, 6H, cyclohexyl), 1.48−1.78 (m, 4H, cyclohexyl), 1.79−1.98 (m, 5H, $CHCH_2CH_2$, CHN), 2.00−2.12 (m, 2H, OCH_2CH_2), 2.27−2.84 (m, 11H, piperazine, CH_2N, and benzyl CH), 3.85 (s, 3H, OCH_3), 4.21−4.30 (m, 2H, OCH_2), 6.65−6.82 (m, 3H, aromatic); GC/MS *m*/*z* 373 ($M^+ + 1$, 6), 372 (M^+, 28), 181 (100); LC−MS (ESI^+) *m*/*z* 373 $[M + H]^+$. Anal. ($C_{23}H_{36}N_2O_2 \cdot 2HCl \cdot 1/2H_2O$) C, H, N.

***N*-(5-Methoxynaphthalen-1-yl)-2-(4-cyclohexylpiperazin-1-yl)-acetamide (20).** To a solution of intermediate **19** (0.78 mmol, 0.23 g) and 1-cyclohexylpiperazine (0.95 mmol, 0.16 g) in CH_3CN (15 mL), K_2CO_3 (0.13 g, 0.95 mmol) was added, and the mixture was refluxed under stirring overnight. The solvent was removed under reduced pressure, and the residue was taken up with H_2O (15 mL) and extracted with CH_2Cl_2 (3 × 10 mL). The organic phases were collected and dried (Na_2SO_4), and the crude oil was purified by column chromatography using CH_2Cl_2/MeOH (95:5) as eluent to give the target compound as brown oil in 85% yield: ^{1}H NMR δ 1.10−1.40 (m, 5H, cyclohexyl), 1.42−2.15 (m, 6H, cyclohexyl), 2.80−3.05 (m, 8H, piperazine), 3.35 (s, 2H, $COCH_2N$), 4.00 (s, 3H, OCH_3), 6.80−8.30 (m, 6H, aromatic), 9.60−9.80 (br s; NH); GC/MS *m*/*z* 382 ($M^+ + 1$, 2), 381 (M^+, 9), 181 (100); LC−MS (ESI^+) *m*/*z* 382 $[M + H]^+$; LC−MS−MS 382: 181. Anal. ($C_{23}H_{31}N_3O_2 \cdot 2HCl \cdot {}^5/_4H_2O$) C, H, N.

4-Cyclohexyl-1-[*N*-(5-methoxynaphthalen-1-yl)-2-aminoethyl]-piperazine (21). To a solution of target compound **20** (0.28 g, 0.73 mmol) in dry THF (10 mL), $BH_3 \cdot THF$ 1 M (3.6 mL, 3.6 mmol) was added in a dropwise manner. The mixture was stirred under reflux for 3.5 h and then cooled, and then MeOH (5 mL) and *i*-propanol saturated with HCl were added to the mixture. The mixture was brought to reflux and, after 30 min, cooled and the solvent evaporated under reduced pressure. The solid obtained was treated with a saturated solution of Na_2CO_3 and extracted with CH_2Cl_2 (3 × 10 mL). The organic phases were collected and dried (Na_2SO_4), and the crude oil was purified by column chromatography using CH_2Cl_2/MeOH (85:15) as eluent to give the target compound as yellow oil in 78% yield: ^{1}H NMR δ 1.05−1.35 (m, 5H, cyclohexyl), 1.58−2.05 (m, 5H, cyclohexyl), 2.25−2.50 (m, 1H, cyclohexyl CHN), 2.58−2.78 (m, 8H, piperazine), 2.80 (t, 2H, *J* = 5.7 Hz, CH_2N), 3.25−3.35 (m, 2H, CH_2NH), 4.00 (s, 3H, OCH_3), 5.18 (br s, 1H, NH D_2O exchanged), 6.58−7.65 (m, 6H, aromatic); GC/MS *m*/*z* 367 (M^+, 4), 181 (100); LC−MS (ESI^+) *m*/*z* 368 $[M + H]^+$. Anal. ($C_{23}H_{33}N_3O \cdot 2HCl \cdot 1/4H_2O$) C, H, N.

4Cyclohexyl-1-[2-[(5-methoxynaphthalen-1-yl)oxy]ethyl]piperazine (22). A mixture of 5-methoxynaphthalen-1-ol (4.79 mmol, 0.83 g), intermediate **10a** (5.75 mmol, 1.32 g) and K_2CO_3 (5.75 mmol, 0.79 g) in acetone (35 mL) was stirred under reflux overnight. The solvent was then evaporated under reduced pressure, and the residue was taken up with H_2O (15 mL) and extracted with AcOEt (3 × 15 mL). The organic phases collected were dried (Na_2SO_4) and evaporated under reduced pressure to afford a crude foaming oil, which was purified by

column chromatography using CH_2Cl_2/MeOH (95:5) as eluent to give the target compound as a brown oil in 45% yield: ^{1}H NMR δ 1.05−1.35 (m, 5H, cyclohexyl), 1.58−2.05 (m, 5H, cyclohexyl), 2.35−2.45 (m, 1H, CHN), 2.65−2.85 (m, 8H, piperazine), 2.98 (t, 2H, J = 5.6 Hz, CH_2N), 4.00 (s, 3H, OCH_3), 4.27 (t, 2H, J = 5.6 Hz, CH_2O), 6.80−7.90 (m, 6H, aromatic); GC/MS m/z 368 (M^+, 2), 195 (100), 181 (65); LC−MS (ESI^+) m/z 369 $[M + H]^+$; LC−MS−MS 369: 195. Anal. ($C_{23}H_{32}N_2O_2 \cdot 2HCl \cdot 1/4H_2O$) C, H, N.

Lipophilicity Data. Lipophilicity data of target compounds **1**, **9**, **11**, **13**, and **20**−**22** were obtained by the pH metric technique using a GLpK_a apparatus (Sirius Analytical Instruments Ltd., Forrest Row, East Sussex, United Kingdom) as previously described.[42] The low aqueous solubility of the investigated compounds required pK_a measurements to be performed in the presence of methanol as cosolvent. Three separate 20 mL semiaqueous solutions of approximately 5×10^{-5} M, in 20−45% w/w of MeOH, were initially acidified with 0.5 M HCl to pH 2.5. The solutions were then titrated with 0.5 M KOH to pH 11. The initial estimates of the p$_s K_a$ values, which are the apparent ionization constants in the mixed solvent, were obtained by *Bjerrum* plots. These values were then refined by a weighted nonlinear least-squares procedure (Refinement Pro 1.0 software) to create a multiset, where the refined values were extrapolated to zero cosolvent concentration using the Yasuda−Shedlovsky equation.[43] To obtain log P data, at least four separate titrations were performed on each compound, on approximately 5×10^{-5} M, using various of *n*-octanol/water ratios (from 0.005 to 1). The biphasic solutions were initially acidified to pH 2.5 with 0.5 M HCl and then titrated with 0.5 M KOH to pH 11. The obtained data were refined as described above. The log P values were obtained by the multiset approach, as described elsewhere.[42] All titrations were carried out at 25 ± 0.1 °C under an inert nitrogen gas atmosphere to exclude CO_2.

Radioligand Binding Assays. The novel target compounds (±)-, (+)-(*R*)-, and (−)-(*S*)-**9**, (±)-, (+)-(*R*)-, and (−)-(*S*)-**11**, (±)-**13**, (±)-**18** and **20**−**22** were assayed as hydrochloride salts. (±)-, (+)-(*R*)-, and (−)-(*S*)-**14** were obtained as a maleate salts. All compounds were evaluated for in vitro affinity at σ_1 and σ_2 receptors by radioligand binding assays. The specific radioligands and tissue sources were, respectively, (a) σ_1 receptor, (+)-[^{3}H]-pentazocine ((+)-[2*S*-(2*R*,6*R*,11*R*)]-1,2,3,4,5,6-hexahydro-6,11-dimethyl-3-(3-methyl-2-butenyl)-2,6-methano-3-benzazocine-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. The specific binding was respectively defined in the presence of (a) 10 μM (+)-pentazocine; (b) 10 μM DTG. The following compounds were used to define the specific binding reported in parentheses: (a) (+)-pentazocine (75−84%), (b) DTG (82−93%). Concentrations required to inhibit 50% of radioligand specific binding (IC_{50}) were determined using six to nine different concentrations of the drug studied in at least three experiments with samples in duplicate. Scatchard parameters (K_d and B_{max}) and apparent inhibition constant (K_i) values were determined by nonlinear curve fitting, using the Prism v. 3.0 (GraphPad Software).[44]

All the procedures for the binding assays have been previously described. σ_1 And σ_2 receptor binding were carried out according to Matsumoto et al.[45] HSI binding was carried out according to Moebius et al.[46] [^{3}H]-DTG (30 Ci/mmol) and (+)-[^{3}H]-pentazocine (34 Ci/mmol) were purchased from PerkinElmer Life Sciences (Zavantem, Belgium). The radioligand (±)-[^{3}H]-emopamil (83 Ci/mmol) was purchased from American Radiolabeled Chemicals, Inc. (St. Louis, MO). DTG and (±)-ifenprodil were purchased from Tocris Cookson Ltd., U.K. (+)-Pentazocine was obtained from Sigma-Aldrich-RBI srl (Milan, Italy). Male Dunkin guinea pigs and Wistar Hannover rats (250−300 g) were from Harlan, Italy.

Cell Culture. MDCK-MDR1 cell line was a gift from Prof. P. Borst, NKI-AVL Institute, Amsterdam, Netherlands. MDCK-MDR1 cells were grown in DMEM high glucose supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin, in a humidified incubator at 37 °C with a 5% CO_2 atmosphere. The human SK-N-SH neuroblastoma cell lines were obtained from Interlab Cell Line Collection (ICLC, Genoa). SK-N-SH cells were routinely cultured in RPMI 1640 supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin, 1 mM sodium pyruvate, and 1% nonessential amino acids in a humidified incubator at 37 °C with a 5% CO_2 atmosphere. Cell culture reagents were purchased from Celbio srl (Milano, Italy). CulturePlate 96-well plates were purchased from PerkinElmer Life Science; calcein-AM and MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) were obtained from Sigma-Aldrich (Milan, Italy).

Calcein-AM Experiment. The P-gp inhibiting activity of (±)-, (−)-(*R*)-, and (+)-(*S*)-**1** and of the newly synthesized compounds (±)-**9**, (±)-**11**, and (±)-**14**, along with their corresponding (+)-(*R*)- and (−)-(*S*)-enantiomers, together with compounds (±)-**13**, (±)-**18**, and **20**−**22** was determined by fluorescence measurement using calcein-AM fluorescent probes in an MDCK-MDR1 cell line according to the experiment previously described by Feng et al. with minor modifications.[47] Each cell line (50 000 cells/well) was seeded into black CulturePlate 96-well plates with 100 μL of medium and allowed to become confluent overnight. Test compounds were dissolved in 100 μL of culture medium and were added to the cell monolayers. The plates were then incubated at 37 °C for 30 min. Calcein-AM was added in 100 μL of phosphate-buffered saline (PBS) to yield a final concentration of 2.5 μM, and plate incubation was continued for 30 min. Each well was washed three times with ice-cold PBS. Saline buffer was added to each well, and the plates were read with a Victor3 fluorometer (PerkinElmer) at excitation and emission wavelengths of 485 and 535 nm, respectively. Under these experimental conditions, calcein cell accumulation in the absence and presence of tested compounds was evaluated, and basal-level fluorescence was estimated by untreated cell fluorescence. In treated wells, the increase in fluorescence was measured relative to the basal level. EC_{50} values were determined by fitting the percent fluorescence increase percentage versus log [dose]. The EC_{50} values were obtained from nonlinear iterative curve fitting by Prism v.3.0, GraphPad software.[44]

Antiproliferative Assay. The functional biochemical assays were performed on human SK-N-SH neuroblastoma cell line, where the expression of σ_2 receptor had been previously reported.[40] Reference compound **1** and all of the σ receptor ligands reported herein were evaluated for their possible σ_2-mediated antiproliferative effect. Determination of cell growth was performed using the MTT assay at 24 and 48 h (EC_{50} values determined at 48 h). On day 1, 30 000 cells/well were seeded into 96-well plates in a volume of 100 μL. On day 2, the various drug concentrations (0.1 μM−100 μM) were added. In all the experiments, the various drug solvents (ethanol, DMSO) were added in each control to evaluate a possible solvent cytotoxicity. After the established incubation time with drugs, MTT (0.5 mg/mL) was added to each well, and after 3 h incubation at 37 °C, the supernatant was removed. The formazan crystals were solubilized using 100 μL of DMSO, and the absorbance values at 570 and 630 nm were determined on the microplate reader Victor 3 from PerkinElmer Life Sciences. The EC_{50} values were obtained from nonlinear iterative curve fitting by Prism, version 3.0 (GraphPad Software).[44]

Supporting Information Available: Elemental analyses of the novel end products; formulas, melting points of hydrochloride and maleate salts, description of the preparation and spectroscopy data for the intermediate compounds (−)-(*R*)- and (+)-(*S*)-**5**, **6**,

(*R*,*R*)- and (*S*,*S*)-**7**, **16**, **17** and **19**. This material is available free of charge via the Internet at http://pubs.acs.org.

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