Novel Spiropiperidines as Highly Potent and Subtype Selective σ-Receptor Ligands. Part 1

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Received July 19, 2001

A series of spiro[[2]benzopyran-1,4′-piperidines] and spiro[[2]benzofuran-1,4′-piperidines] of general structure **10** is prepared, and the affinity for σ_1- and σ_2-receptors is investigated by means of radioligand binding assays. The synthesis of the spiropiperidines **14a** and **23** proceeds from bromine/lithium exchange of the bromoacetals **11** and **21**, addition to piperidin-4-one **12a**, and subsequent cyclization. Systematic variations of the substituent R at the nitrogen atom, the group X in position 3, and the ring size of the oxygen heterocycle are performed. The σ_1- and σ_2-receptor affinities are determined with guinea pig brain and rat liver membrane preparations using [^{3}H]-labeled (+)-pentazocine and ditolylguanidine, respectively. Test results show that a benzyl residue at the piperidine nitrogen atom and a methoxy group in position 3 are advantageous for high σ_1-receptor affinity. In this series the 1′-benzyl-3-methoxy-3,4-dihydrospiro[[2]benzopyran-1,4′-piperidine] (**14a**) and the 1′-benzyl-3-methoxy-3*H*-spiro[[2]-benzofuran-1,4′-piperidine] (**23**) are among the most potent σ_1-ligands interacting in the low nanomolar range with σ_1-receptors (**14a**, K_i = 1.29 nM; **23**, K_i = 1.14 nM). Variation of the nitrogen substituent R from benzyl to H, alkyl, phenyl, or ω-phenylalkyl and the group X from methoxy to hydroxy, carbonyl, or alkyloxy led to reduced σ_1-receptor affinity. In addition to their high σ_1-receptor affinity, the spiropiperidines **14a** and **23** display excellent selectivity toward σ_2-receptors (σ_1/σ_2 = 2708 and 1130) and several other receptor and reuptake systems. Introduction of a polar hydroxy group in position 3 and elongation of the distance between the piperidine nitrogen atom and the phenyl moiety result in ligands with considerable σ_2-receptor affinity and therefore diminished σ_1/σ_2-receptor selectivity. The hemiacetalic 1′-(3-phenylpropyl)-3,4-dihydrospiro[[2]benzopyran-1,4′-piperidin]-3-ol (**15e**) represents the most active σ_2-receptor ligand in this series with a K_i value of 83.1 nM.

Introduction

Originally Martin and co-workers suggested the σ-receptor to be a subtype of the opioid receptors.[1] However, since most of the σ-effects caused by typical σ-ligands are not abolished by the opioid antagonist naloxone, this classification was discarded.[2] Nowadays, σ-receptors are well recognized as a class of haloperidol-sensitive, nonopioid, nonphencyclidine receptors with their own binding profile and characteristic distribution within the central nervous system (CNS) as well as in many tissues outside the CNS (e.g., kidney, liver, lung).[3,4]

σ-Receptors are involved in several physiological and pathophysiological processes. Therefore, ligands interacting with σ-receptors are prone to modulate these (patho)physiological events and influence psychiatric disorders.[3,4] A particular interest has been evoked by the perspective of using σ-agents as atypical antipsychotics, which are devoid of the typical extrapyramidal motoric side effects[5] associated with classical antipsychotic D_2 antagonists. Like haloperidol (**1**), several well-established neuroleptic agents bind not only at dopamine D_2 receptors but also at σ-receptors.[6,7] Furthermore, σ-ligands could be used in the treatment of cocaine abuse,[8] depressions,[9] and epileptic disorders.[10] They also have potential as neuroprotective,[11] antiamnesic,[11] antineoplastic,[12] and tumor imaging agents.[13]

Up to now, at least two distinct σ-receptor subtypes termed σ_1- and σ_2-receptors have been pharmacologically characterized. According to this classification, haloperidol (**1**) and ditolylguanidine (**2**) bind with high affinity at both σ_1- and σ_2-receptors (compare Table 1, entries 9 and 11), whereas (+)-benzomorphans (e.g., (+)-pentazocine (**3**)) display high affinity for σ_1-receptors but low affinity for σ_2-receptors (compare Table 1, entry 12). The stereochemistry of the benzomorphans is important for σ-binding, with σ_1-receptors binding enantioselectively the (+)-enantiomers, whereas (−)-benzomorphans are mixed σ_1- and σ_2-ligands with moderate affinity.[14−16] The existence of a third σ_3-subtype has been intensively discussed.[17] However, recent investigations revealed that there are no significant differences of ligand selectivity and CNS distribution between histamine H_1- and σ_3-receptors.[18]

Meanwhile, the gene coding for the σ_1-receptor of different species and tissues has been cloned. Whereas the σ_1-receptors from human placental cells, guinea pig liver, and rat brain are >92% identical and >95% similar at the level of amino acid sequence, there is no significant homology between the σ_1-receptor and any other known mammalian receptor or even protein. However, a significant homology (~30%) exists between the cloned σ_1-receptor and the yeast sterol-C_8/C_7-isomerase. The role of this observation still remains to be established. The protein, coded by the rat brain σ_1-receptor gene, comprises 223 amino acids (23 kDa) and

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10.1021/jm010992z CCC: $22.00 © 2002 American Chemical Society
Published on Web 12/19/2001

Chart 1

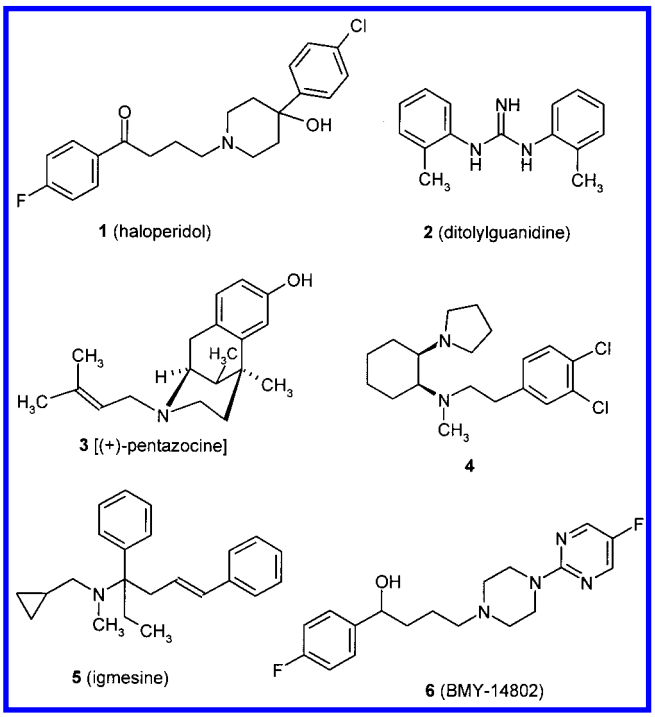
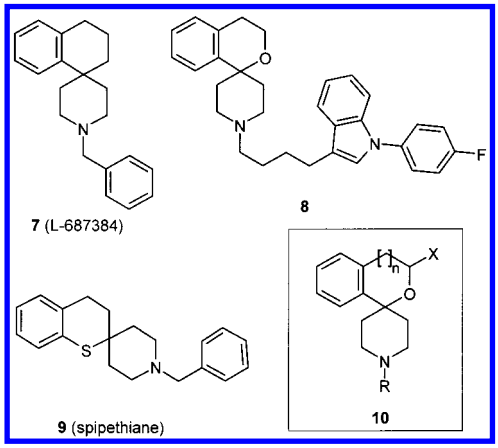


Chart 2



a single putative transmembrane domain. In comparison, the molecular weight of the σ_2-receptor, which has not been cloned yet, is estimated to be about 16−18 kDa.[19,20]

The group of high-affinity σ-ligands contains several structurally unrelated compounds[6] including butyrophenone neuroleptics (e.g., haloperidol, **1**), guanidines (e.g., **2**), dextrorotatory benzomorphans (e.g., (+)-pentazocine, **3**), and cis-configured cyclohexane-1,2-diamines (e.g., **4**).[21,22] The structurally unique cyclopropylmethylamine igmesine (**5**) is in phase II trials for the treatment of depressive disorder.[9] The haloperidol related piperazine derivative BMY-14802 (**6**), which interacts unselectively with σ_1- and σ_2-receptors, has been investigated in clinical trials (phase II) as atypical antipsychotic[23] (see Chart 1 for the structures of compounds **1**−**6**).

Our work dedicated to the development of novel σ-ligands was inspired by the already existing structure−activity relationship studies on the spirocyclic piperidine derivatives **7**−**9** (Chart 2). The tetraline derivative **7** developed by Merck, Sharp, and Dohme revealed an affinity for σ-receptors in the nanomolar range (IC_{50} = 3.8 nM). Thereby, the σ-receptor affinity was determined in a receptor binding assay using [^{3}H]-ditolylguanidine (**2**) as the radioligand, which does not discriminate between σ_1- and σ_2-receptors.[24] In the meantime it was shown that the tetraline derivative **7** possesses significant preference for σ_1-receptors.[25] Some variations of the piperidine nitrogen substituent have been performed, but introduction of heteroatoms or substituents in the aliphatic part of the tetraline substructure is not described.[24]

The spiro[[2]benzopyran-1,4′-piperidine] **8** belongs to the most potent and σ_2-selective ligands described so far (σ_1, IC_{50} = 53 nM; σ_2, IC_{50} = 0.90 nM). Whereas the oxygen heterocycle of **8** has been extensively modified, introduction of substituents into the benzopyran moiety and modifications of the σ_2-pharmacophoric 4-[1-(4-fluorophenyl)indol-3-yl]butyl residue in the benzopyran moiety are not reported.[25] Furthermore, the 1-benzothiopyran derivative **9** with the spiro connection shifted to position 2 represents a very potent and selective ligand for σ_1-receptors (σ_1, K_i = 0.5 nM; σ_2, K_i = 416 nM).[26]

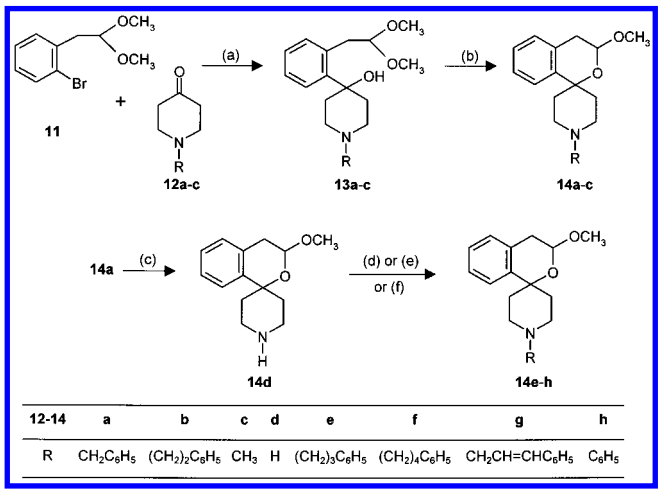
This prompted us to undertake the synthesis of novel spirocyclic σ-ligands with the general structure **10**. In particular, we focused on the systematic variation of the substituent R at the piperidine nitrogen atom, the substituent X in position 3 of the 2-benzopyran ring system, and the ring size of the oxygen heterocycle (n = 0 or 1). The affinities for σ_1- and σ_2-receptors of the synthesized compounds were determined in radioligand binding assays. Relationships between ligand structure and σ_1- and σ_2-receptor affinities and, moreover, σ_1/σ_2-selectivity are discussed.

Chemistry

The synthesis of spiro[[2]benzopyran-1,4′-piperidines] of general formula **10** proceeds from the treatment of 2-(2-bromophenyl)acetaldehyde dimethyl acetal (**11**)[27] with *n*-butyllithium at −78 °C to generate an aryllithium intermediate that was trapped with 1-substituted piperidin-4-ones **12a-c** to afford the hydroxyacetals **13a-c**, respectively. The subsequent cyclization of the hydroxyacetals **13a-c** was catalyzed by *p*-toluenesulfonic acid. The use of methanol as a solvent is crucial in this step to obtain the spiro[[2]benzopyran-1,4′-piperidines] **14a-c** in high yields.

Attempts to cleave the *N*-benzyl substituent of **14a** with hydrogen in the presence of the catalyst Pd/C led to a mixture of the desired secondary amine **14d** and, surprisingly, the methyl derivative **14c**. The identity of the methyl derivative **14c** was unequivocally proven by comparison of the spectroscopic data with an authentic sample prepared by addition of the bromo acetal **11** to 1-methylpiperidin-4-one **12c** and subsequent cyclization.[28] However, using ammonium formate[29] instead of hydrogen led to clean cleavage of the *N*-benzyl substituent of **14a** to provide the secondary amine **14d** in 70% yield (Scheme 1). The secondary amine **14d** reacted with 1-bromo-3-phenylpropane, 1-chloro-4-phenylbutane, and 3-chloro-1-phenylprop-1-ene (cinnamyl chloride) to afford spiro[[2]-benzopyran-1,4′-piperidine] derivatives with *N*-(3-phenylpropyl) **14e**, *N*-(4-phenylbutyl) **14f**, and *N*-cinnamyl **14g** residues, respectively (Scheme 1).

Scheme 1[a]



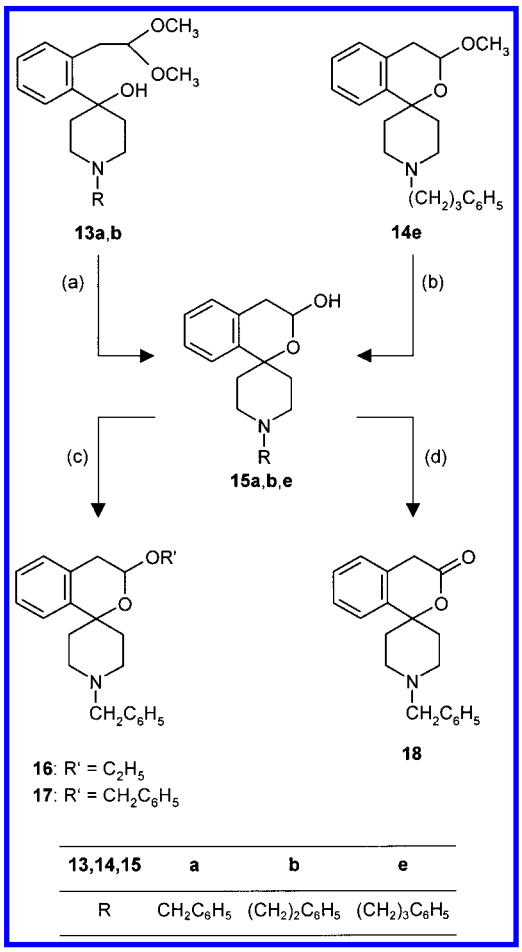
[a] Reagents and reaction conditions: (a) n-BuLi, THF, −78 °C; **13a**, 39%; **13b**, 44%; **13c**, 51%. (b) TsOH, CH_3OH, room temp; **14a**, 86%; **14b**, 92%; **14c**, 75%. (c) Ammonium formate, Pd/C, CH_3OH, 65 °C, 70%. (d) $Ph(CH_2)_3Br$, K_2CO_3, THF, 66 °C; **14e**, 64%. (e) $Ph(CH_2)_4Cl$ or $PhCH{=}CHCH_2Cl$, K_2CO_3, CH_3CN, 82 °C; **14f**, 38%; **14g**, 24%. (f) PhBr, $Pd(OAc)_2$, P^tBu_3, NaO^tBu, *o*-xylene, 130 °C; **14h**, 21%.

The results of the pharmacological tests indicated that a phenyl moiety in the nitrogen substituent is essential for high σ_1- and σ_2-receptor affinities. Therefore, we reasoned that the spiro[[2]benzopyran-1,4′-piperidine] **14h** with the phenyl moiety attached directly to the nitrogen atom should also be included in the study. The synthesis of the *N*-phenyl derivative **14h** succeeded by Pd-catalyzed phenylation of the secondary amine **14d**.[30,31] Thus, the secondary amine **14d** was heated with bromobenzene, P^tBu_3, and NaO^tBu in the presence of 0.25 mol % of the catalyst $Pd(OAc)_2$ to yield the *N*-phenyl derivative **14h**. Thus, the synthesis of the first series of test compounds **14a**−**h** bearing different substituents R at the piperidine nitrogen atom was completed.

Next, we investigated the analogues with modifications on the 2-benzopyran ring system. Treatment of the hydroxy acetals **13a** and **13b** with diluted HCl yielded the lactols (=hemiacetals) **15a** and **15b**, respectively (Scheme 2). The corresponding *N*-(3-phenylpropyl)-substituted 2-benzopyran-3-ol **15e** was obtained by hydrolysis of the methoxy derivative **14e**. Since the *N*-benzyl derivatives showed the most promising σ-receptor affinities (compare Table 1), further modifications were carried out using the *N*-benzyl-substituted lactol **15a**. The homologous ethyl acetal **16** and the benzyl acetal **17** were prepared by acetalization of the lactol **15a** with ethanol and benzyl alcohol, respectively, in the presence of sulfuric acid. Oxidation of the lactol **15a** with a catalytic amount of tetrapropylammonium perruthenate [$(NPr_4)RuO_4$] and an excess of the reoxidant *N*-methylmorpholine *N*-oxide (NMMO)[32] provided the lactone **18**.[33]

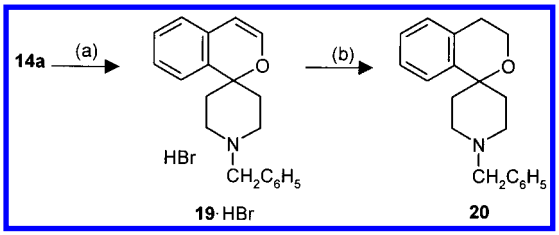
Elimination of methanol succeeded upon heating of the methyl acetal **14a** with *tert*-butyl bromide[34] to yield the dehydro derivative **19**·HBr (Scheme 3). Careful hydrogenation (low H_2 pressure, short reaction time, solvent HOAc) of **19** afforded the *N*-benzyl-substituted spiropiperidine **20**[35] without further substituents in the benzopyran ring system.

Scheme 2[a]



[a] Reagents and reaction conditions: (a) HCl, THF, room temp; **15a**, 69%; **15b**, 64%. (b) HCl, THF, 66 °C; **15e**, 37%. (c) C_2H_5OH or $C_6H_5CH_2OH$, H_2SO_4, room temp; **16**, 81%; **17**, 77%. (d) (NPr_4)-RuO_4, NMMO, molecular sieves 4 Å, CH_2Cl_2, room temp, 43%.

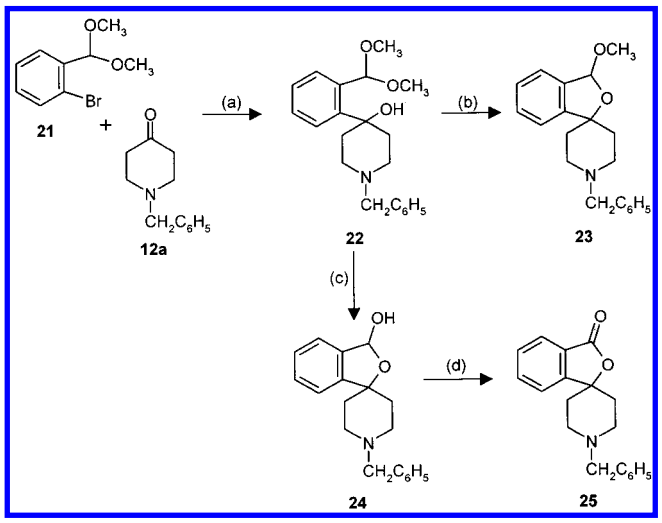
Scheme 3[a]



[a] Reagents and reaction conditions: (a) ^{t}BuBr, $CHCl_3$, 61 °C; **19**·HBr, 24%. (b) H_2, Pd/C, HOAc, room temp, 39%.

Since the five membered indane analogue of **7** (L-693403) and the isobenzofuran analogue of **8** (Lu 28-179) are superior to the parent six-membered spiro compounds **7** and **8** in σ-receptor affinity,[24,25] the five-membered spiro compounds of general structure **10** (n = 0) were also included into the study. Thus, the ring size represents a third structural parameter influencing σ-receptor affinity. Starting with 2-bromobenzaldehyde dimethyl acetal (**21**) halogen/metal exchange with *n*-butyllithium at −95 °C and subsequent reaction with 1-benzylpiperidin-4-one (**12a**) afforded the hydroxy acetal **22** (Scheme 4). Cyclization of the hydroxy acetal **22** with *p*-toluenesulfonic acid in methanol led to the cyclic methyl acetal **23**, whereas hydrolysis with diluted HCl provided the lactol **24**. The lactone **25**[36] was

Scheme 4[a]



[a] Reagents and reaction conditions: (a) n-BuLi, THF, −95 °C. (b) TsOH, CH_3OH, room temp, 36%. (c) HCl, THF, 66 °C, 11%. (d) $(NPr_4)RuO_4$, NMMO, molecular sieves 4 Å, CH_2Cl_2, room temp, 58%.

prepared by oxidation of the lactol **24** with $(NPr_4)RuO_4$ and NMMO.[32]

Receptor Binding Studies

The σ-receptor affinities of the test compounds were determined in competition experiments with radioligands. In the σ_1-assay, homogenates of guinea pig brains were used as the receptor material. The σ_1-selective ligand [^{3}H]-(+)-pentazocine (**3**) was employed as the radioligand, and the nonspecific binding was determined in the presence of a large excess of haloperidol (**1**).[37] Homogenates of rat liver served as source for σ_2-receptors in the σ_2-assay. Since a σ_2-selective radioligand is not available, the nonselective radioligand [^{3}H]-ditolylguanidine (**2**) was employed in the presence of an excess of nonradiolabeled (+)-pentazocine (100 nM) for selective labeling of σ_1-receptors. Performing the σ_2-assay in the presence of an excess of non-tritiated 1,3-di(*o*-tolyl)guanidine led to the nonspecific binding of the radioligand.[38,39]

Results and Discussion

The σ-receptor affinities of 3-methoxy-3,4-dihydrospiro[[2]benzopyran-1,4′-piperidines] **14** with varying piperidine nitrogen substituents R are summarized in Table 1. Ligands with a proton (**14d**, entry 4), a methyl substituent (**14c**, entry 3), or a phenyl residue directly attached to the piperidine nitrogen (**14h**, entry 8) did not interact significantly with σ_1-receptors. However, introduction of a methylene spacer between the nitrogen atom and the phenyl residue led to the very potent σ_1-receptor ligand **14a** (entry 1) showing the highest σ_1-affinity (K_i = 1.29 nM) in this series. Whereas the 3-phenylpropyl derivative **14e** (entry 5) was almost as active as the benzyl derivative **14a**, the 2-phenylethyl (**14b**, entry 2), the 4-phenylbutyl (**14f**, entry 6), and the cinnamyl derivatives (**14g**, entry 7) revealed lower σ_1-receptor affinity.

Generally, the σ_2-receptor affinities of the spiropiperidines **14** were lower than their σ_1-receptor affinities. Thus, the most active σ_1-ligand, the benzyl derivative **14a**, displayed the highest σ_1/σ_2-selectivity (2708-fold, entry 1). However, the σ_2-receptor affinity was increased with elongation of the nitrogen−phenyl distance. In this series the highest σ_2-receptor affinity (311 nM) and, therefore, the lowest σ_1/σ_2-selectivity (30-fold) was found for the 4-phenylbutyl derivative **14f** (entry 6).

The results of the systematic variation of the substituent X in position 3 of the benzopyran scaffold compared with the most active σ_1-ligand **14a** with the benzyl residue at the nitrogen atom (K_i = 1.29 nM) are shown in Table 2. The lactol **15a** with a polar hydroxy group in position 3 (entry 2) displayed a somewhat lower affinity for σ_1-receptors than the methyl acetal **14a**. Enlargement of the acetalic methoxy group led to the ethoxy and benzyloxy derivatives **16** and **17** with reduced σ_1-receptor affinity (entries 5, 6). Oxidation to the lactone **18** was detrimental for the σ_1-receptor affinity (entry 7). A low K_i value (K_i = 1.87 nM) indicating high σ_1-receptor affinity was determined for the corresponding elimination product **19** (entry 8). The most active σ_1-receptor ligand, however, was the unsubstituted benzopyran derivative **20** with a K_i value in the subnanomolar range (K_i = 0.69 nM). Nevertheless, since **20** exhibits a high σ_2-affinity (K_i = 99.7 nM), the σ_1/σ_2-selectivity of **20** was reduced (146-fold, entry 9).

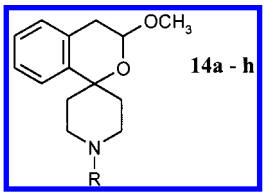
In the hemiacetalic series of ligands **15**, the σ_1-receptor affinity was influenced by the nitrogen substituent in the same way as in the acetalic series **14**. Hence, the benzyl derivative **15a** displayed the highest σ_1-receptor affinity (entry 2), whereas the 2-phenylethyl derivative **15b** was considerably less active (entry 3). Once more, the 3-phenylpropyl derivative **15e** (entry 4) showed higher σ_1-receptor affinity than the 2-phenylethyl derivative **15b** but lower affinity than the benzyl derivative **15a**.

A polar hydroxy group (**15b**, entry 3; **15e**, entry 4) or two protons in position 3 of the benzopyran moiety (**20**, entry 9) were beneficial for σ_2-receptor binding. Among these ligands, the 3-phenylpropyl-substituted lactol **15e** with a trimethylene spacer between the piperidine nitrogen atom and the phenyl residue was the most active σ_2-ligand (K_i = 83.1 nM, entry 4). However, the σ_1-receptor affinity still predominated with a σ_1/σ_2-selectivity factor of 7. The lowest σ_1/σ_2-selectivity factor was found for the 2-phenylethyl derivative **15b** (entry 3) because of its reduced σ_1-receptor affinity.

The σ-receptor affinities of the five-membered 2-benzofuran derivatives **23**−**25** are shown in Table 3. As in the six-membered 2-benzopyran series, the methoxy derivative **23** revealed best interaction with σ_1-receptors (K_i = 1.14 nM, entry 1). Although statistically not significant, the σ_1-receptor affinity of **23** even exceeds the σ_1-receptor affinity of the analogous six-membered acetal **14a**. For comparison, the five-membered analogues of the spirocyclic lead structures **7** and **8** also display higher σ-receptor affinity than their six-membered counterparts.[24,25] In analogy to the six-membered benzopyran derivatives, the lactol **24** (entry 2) and the lactone **25** (entry 3) were less active than the methoxy derivative **23** at σ_1-receptors.

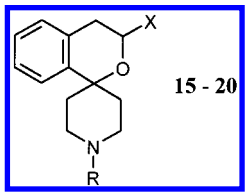
Just like the six-membered benzopyrans, the five-membered benzofurans **23**−**25** revealed selectivity for σ_1-receptors. Again, a polar hydroxy group in position

Table 1. σ-Receptor Affinities of Spiro[[2]benzopyran-1,4′-piperidines] 14**a**−**h** with Various N-Substituents



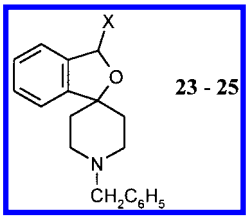
			$K_i \pm$ SEM (3) (nM)		
entry	compd	R	σ_1 ([^{3}H]-(+)-pentazocine)	σ_2 ([^{3}H]-ditolylguanidine)	σ_1/σ_2 selectivity
1	**14a**	$CH_2C_6H_5$	1.29 ± 0.18	3500 ± 352	2708
2	**14b**	$(CH_2)_2C_6H_5$	6.64 ± 1.14	410 ± 58	62
3	**14c**	CH_3	4400 ± 1440	>10000	
4	**14d**	H	19800 ± 1760	>10000	
5	**14e**	$(CH_2)_3C_6H_5$	2.07 ± 0.43	307 ± 99	148
6	**14f**	$(CH_2)_4C_6H_5$	10.2 ± 0.94	311 ± 84	30
7	**14g**	$CH_2CH{=}CHC_6H_5$	13.3 ± 1.55	4000 ± 499	301
8	**14h**	C_6H_5	10100 ± 2280	>10000	
9	haloperidol (**1**)		2.20 ± 0.31	34.2 ± 2.3	16
10	BMY-14802 (**6**)		265 ± 32	391 ± 62	1.5
11	ditolylguanidine (**2**)		164 ± 47	63.9 ± 10.8	0.4
12	(+)-pentazocine (**3**)		3.58 ± 0.20		

Table 2. σ-Receptor Affinities of Spiro[[2]benzopyran-1,4′-piperidines] **15**−**20** with Various Substituents X in Position 3 of the 2-Benzopyran Moiety



				$K_i \pm$ SEM (3) (nM)		
entry	compd	X	R	σ_1 ([^{3}H]-(+)-pentazocine)	σ_2 ([^{3}H]-ditolylguanidine)	σ_1/σ_2 selectivity
1	**14a**	OCH_3	$CH_2C_6H_5$	1.29 ± 0.18	3500 ± 352	2708
2	**15a**	OH	$CH_2C_6H_5$	2.17 ± 0.40	513 ± 72	236
3	**15b**	OH	$(CH_2)_2C_6H_5$	43.7 ± 8.32	107 ± 24	2
4	**15e**	OH	$(CH_2)_3C_6H_5$	12.4 ± 3.2	83.1 ± 16.0	7
5	**16**	OC_2H_5	$CH_2C_6H_5$	5.83 ± 1.48	2960 ± 729	508
6	**17**	$OCH_2C_6H_5$	$CH_2C_6H_5$	95.3 ± 22.3	9890 ± 2200	104
7	**18**	=O	$CH_2C_6H_5$	29.3 ± 8.96	1157 ± 148	39
8	**19**	3,4-=	$CH_2C_6H_5$	1.87 ± 0.27	302 ± 97	162
9	**20**	H	$CH_2C_6H_5$	0.69 ± 0.17	99.7 ± 19.8	146

Table 3. σ-Receptor Affinities of Spiro[[2]benzofuran-1,4′-piperidines] **23**−**25**



			$K_i \pm$ SEM (3) (nM)		
entry	compd	X	σ_1 ([^{3}H]-(+)-pentazocine)	σ_2 ([^{3}H]-ditolylguanidine)	σ_1/σ_2 selectivity
1	**23**	OCH_3	1.14 ± 0.22	1280 ± 137	1130
2	**24**	OH	7.33 ± 0.60	761 ± 13	104
3	**25**	=O	21.3 ± 3.45	1460 ± 108	68

3 (**24**, entry 2) is advantageous for σ_2-binding, but the σ_1-receptor affinity of the lactol **24** still predominates.

The receptor binding profiles of the σ_1-selective spiropiperidines **14a**, **14e**, and **23** were determined by screening these compounds in several receptor and reuptake assays. The results in Table 4 point out that interactions of the spiropiperidines **14a**, **14e**, and **23** with the investigated receptor and reuptake systems are very low. Obviously, the selectivity of the spiropiperidines **14a**, **14e**, and **23** for the σ_1-receptor is very high, usually greater than 1000-fold. However, it should be emphasized that the 3-phenylpropyl derivative **14e** was bound in the submicromolar range at serotonine 5-HT_{2A} receptors (IC_{50} = 0.46 μM, entry 6), at the serotonine reuptake system (IC_{50} = 0.70 μM, entry 13), and the noradrenaline reuptake system (IC_{50} = 0.51 μM, entry 14). Nevertheless, the selectivity of **14e** for σ_1-receptors is still greater than 100-fold with regard to these systems.

Additionally, the μ-opioid and κ-opioid receptor affinities[40] of the spiropiperidines **14a** and **20** were investigated. In the μ-receptor screening with test concentrations of 1 and 10 μM, the test compounds **14a** and **20** displayed low competition with the radioligand [^{3}H]-DAMGO. Obviously, the IC_{50} values are greater than 1 μM. However, the screenings with the same test concentrations pointed to promising κ-receptor affinity.

Table 4. Affinities of the Spiropiperidines **14a**, **14e**, and **23** for Various Receptor and Reuptake Systems

		IC_{50} (μM)		
entry	receptor system (radioligand)	**14a**	**14e**	**23**
1	histamine H_1 (mepyramine)	>10	8.0	>10
2	dopamine D_1 (SCH 23390)	>10	>10	>10
3	NMDA, PCP (MK 801)	>10	>10	>10
4	NMDA, glycine (MDL 105519)	>10	>10	>10
5	serotonine 5-HT_{1A} (5-OH DPAT)	>1	>1	>1
6	serotonine 5-HT_{2A} (ketanserine)	6.6	0.46	>10
7	serotonine 5-HT_3 (GR 65630)	>10	>10	>10
8	noradrenaline α_1 (prazosine)	5.9	1.4	>10
9	noradrenaline α_2 (idazoxane)	3.0	3.1	6.2
10	neurokinin NK_1 (h) ([Sar^9,$Met(O_2)^{11}$]-SP)	>10	>10	>10
11	neurokinin NK_2 (h) ([^{125}I]-NKA)	>10	>10	>10
12	neurokinin NK_3 (h) ([^{125}I]-[$MePhe^7$]-NKB)	>10	>10	>10
13	serotonine reuptake (serotonine)	6.0	0.70	8.0
14	noradrenaline reuptake (noradrenaline)	3.0	0.51	>10
15	dopamine reuptake (dopamine)	5.9	1.6	>10

Therefore, the complete competition curves were recorded using the radioligand [^{3}H]-U-69593. Both test compounds **14a** and **20** revealed submicromolar κ-receptor affinity with K_i values of 418 nM (±37 nM, $n = 2$) and 586 nM (±11 nM, $n = 2$), respectively. We reasoned that the 4-phenylpiperidine substructure of the spiropiperidines **14a** and **20**, which is also present in opioid analgesics (e.g., morphine, pethidine), is responsible for the interaction with κ-opioid receptors.

Conclusion

The presented data indicate that high σ_1-receptor affinity and good σ_1/σ_2-selectivity can be obtained with spiropiperidines **10** bearing a benzyl residue at the piperidine nitrogen atom and a methoxy group in position 3. This result is found in both the six-membered 2-benzopyran series and the five-membered 2-benzofuran series of ligands, the methoxy derivatives **14a** and **23** representing the most active σ_1-ligands, respectively. The σ_1-receptor affinities of **14a** and **23** are only exceeded by that of the unsubstituted spirobenzopyran **20** ($K_i = 0.69$ nM). However, the unsubstituted spirobenzopyran **20** revealed a significantly lower σ_1/σ_2-receptor selectivity (146-fold) in contrast to the σ_1-selective methoxy derivatives **14a** (2708-fold) and **23** (1130-fold).

Experimental Section

1. General. Unless otherwise noted, moisture-sensitive reactions were conducted under dry nitrogen. Tetrahydrofuran (THF) was distilled from sodium/benzophenone ketyl prior to use. Thin layer chromatography (TLC) was conducted with silica gel 60 F_{254} plates (Merck). Flash chromatography (fc)[41] was conducted with silica gel 60, 0.040−0.063 mm (Merck), and the terms in parentheses for fc include the following: diameter of the column [cm], eluent, fraction size [mL], R_f. Melting points were determined with an SMP 2 (Stuart Scientific) apparatus and were uncorrected. Elemental analyses were conducted with an Vario EL (Elementaranalysesysteme GmbH) instrument. The mass spectrometer used was model 5989A (Hewlett-Packard) and models MAT 312, MAT 8200, MAT 44, and TSQ 7000 (Finnigan). In the paragraphs below, EI is defined as electron impact and CI is defined as chemical ionization. IR data were collected from a 1605 FT-IR (Perkin-Elmer) spectrophotometer. The ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) data were collected from a Unity 300 FT NMR spectrometer (Varian), and chemical shifts (δ) are reported in ppm downfield from tetramethylsilane. Coupling constants are given with 0.5 Hz resolution, and the assignments of ^{13}C and of ^{1}H NMR signals were supported by 2D NMR techniques (correlation spectroscopy (COSY)). Compounds **13c** and **14c** have been prepared according to ref 28.

2-[2-(1-Benzyl-4-hydroxypiperidin-4-yl)phenyl]acetaldehyde Dimethyl Acetal (13a). Under an N_2 atmosphere, a 1.6 M solution of *n*-butyllithium in hexane (5.4 mL, 8.4 mmol) was slowly added to a cooled (−78 °C) solution of **11** (1.72 g, 7.0 mmol) in THF (20 mL). The mixture was stirred at −78 °C for 5 min, and then a solution of **12a** (1.4 g, 7.4 mmol) in THF (6 mL) was slowly added. The solution was stirred at −78 °C for 3 h. Then it was allowed to warm to room temperature and stirred for another 2 h. Then water (40 mL) was added, the mixture was extracted with CH_2Cl_2, the organic layer was dried (Na_2SO_4), and the solvent was removed in vacuo to give 1.76 g of crude product. The crude product was purified by fc (5.5 cm, petroleum ether/ethyl acetate 1:2, 50 mL, $R_f = 0.05$) to afford a colorless oil, yield 0.963 g (39%). Anal. ($C_{22}H_{29}NO_3$) H, N; C: calcd, 74.3; found, 73.8. MS (EI): *m/z* 355 [M^+], 340 [$M^+ - CH_3$], 264 [$M^+ - CH_2Ph$], 91 [CH_2Ph^+]. IR (film), ν (cm^{-1}): 3436 (O−H); 2939, 2826 (C−H); 1119, 1067 (C−O); 744, 699 (C−H). ^{1}H NMR ($CDCl_3$): δ (ppm) 1.85 (dd, $J = 13.7/2.4$ Hz, 2 H, $N(CH_2CH_2)_2$), 2.15 (td, $J = 12.4/4.2$ Hz, 2 H, $N(CH_2CH_2)_2$), 2.61 (td, $J = 11.7/2.2$ Hz, 2 H, $N(CH_2CH_2)_2$), 2.78 (br d, $J = 10.7$ Hz, 2 H, $N(CH_2CH_2)_2$), 3.34 (s, 6 H, $CH(OCH_3)_2$), 3.45 (d, $J = 5.9$ Hz, 2 H, $ArCH_2CH$), 3.60 (s, 2 H, NCH_2Ph), 4.01 (s, 1 H, O*H*), 4.54 (t, $J = 5.9$ Hz, 1 H, $CH_2CH(OCH_3)_2$), 7.17−7.44 (m, 9 H, arom).

2-{2-[4-Hydroxy-1-(2-phenylethyl)piperidin-4-yl]-phenyl}acetaldehyde Dimethyl Acetal (13b). Under an N_2 atmosphere a 1.6 M solution of *n*-butyllithium in hexane (0.8 mL, 1.28 mmol) was slowly added to a cooled (−78 °C) solution of **11** (0.273 g, 1.11 mmol) in THF (6 mL). The mixture was stirred at −78 °C for 15 min, then a solution of **12b** (0.266 g, 1.31 mmol) in THF (2 mL) was slowly added and the mixture was stirred at −78 °C for 3.25 h. The mixture was warmed to room temperature and stirred for another 1 h. Then water (9 mL) was added and the mixture was extracted with CH_2Cl_2. The organic layer was dried (Na_2SO_4), and the solvent was removed in vacuo to give 0.424 g of crude product, which was purified by fc (3 cm, ethyl acetate, 20 mL, $R_f = 0.08$) to afford a colorless solid (petroleum ether), mp 117 °C, yield 0.182 g (44%). Anal. ($C_{23}H_{31}NO_3$) C, H, N. MS (EI), *m/z*: 278 [$M^+ - CH_2Ph$]. MS (CI, isobutane), *m/z*: 370 [MH^+], 338 [$M^+ - OCH_3$], 278 [$M^+ - CH_2Ph$]. IR (film), ν (cm^{-1}): 3441 (O−H); 2938, 2828 (C−H); 1114, 1061 (C−O); 756, 697 (C−H). ^{1}H NMR ($CDCl_3$): δ (ppm) 1.90 (dd, $J = 13.7/2.4$ Hz, 2 H, $N(CH_2CH_2)_2$), 2.22 (td, $J = 13.1/3.7$ Hz, 2 H, $N(CH_2CH_2)_2$), 2.71 (m, 4 H, $N(CH_2CH_2)_2$ (2 H), NCH_2CH_2Ph (2 H)), 2.90 (m, 4 H, $N(CH_2CH_2)_2$ (2 H), NCH_2CH_2Ph (2 H)), 3.35 (s, 6 H, $CH(OCH_3)_2$), 3.46 (d, $J = 5.8$ Hz, 2 H, $ArCH_2CH$), 4.06 (s, 1 H, O*H*), 4.55 (t, $J = 5.8$ Hz, 1 H, $ArCH_2CH$), 7.19−7.33 (m, 8 H, arom), 7.38−7.44 (m, 1 H, arom).

1′-Benzyl-3-methoxy-3,4-dihydrospiro[[2]benzopyran-1,4′-piperidine] (14a). A solution of **13a** (0.75 g, 2.1 mmol) and *p*-toluenesulfonic acid monohydrate (0.48 g, 2.5 mmol) in methanol (60 mL) was stirred at room temperature for 4 d. Then a solution of Na_2CO_3 (5% in water, 100 mL) was added. The mixture was extracted with CH_2Cl_2, the organic layer was dried (Na_2SO_4), and the solvent was removed in vacuo. The crude product was purified by fc (3 cm, ethyl acetate, 20 mL, $R_f = 0.38$) to afford a colorless oil **14a**, which slowly solidified on standing, giving white needles (^{i}Pr$_2$O), mp 41 °C, yield 0.587 g (86%). **14a** was converted into the hydrochloride in the usual manner to obtain **14a**·HCl as colorless crystals, mp 182−183 °C. Anal. ($C_{21}H_{26}NO_2Cl$) C, H, N. MS (base, EI), *m/z*: 323 [M^+], 308 [$M^+ - CH_3$], 292 [$M^+ - OCH_3$], 232 [$M^+ - CH_2Ph$], 91 [CH_2Ph^+]. IR (base, film), ν (cm^{-1}): 2938, 2812 (C−H); 1077, 1044 (C−O); 735, 698 (C−H). ^{1}H NMR (base, $CDCl_3$): δ (ppm) 1.86 (d, $J = 13.7$ Hz, 1 H, $N(CH_2CH_2)_2$), 1.96−2.00 (m, 2 H, $N(CH_2CH_2)_2$), 2.22 (td, $J = 12.7/4.4$ Hz, 1 H, $N(CH_2CH_2)_2$), 2.46−2.56 (m, 1 H, $N(CH_2CH_2)_2$), 2.60 (td, $J = 11.7/2.4$ Hz, 1

H, N(CH_2CH$_2$)$_2$), 2.77−2.85 (m, 2 H, N(CH_2CH$_2$)$_2$, 2.89−2.95 (m, 2 H, ArCH_2CH), 3.56 (s, 3 H, OCH_3), 3.62 (s, 2 H, NCH_2-Ph), 4.87 (dd, J = 6.5/4.4 Hz, 1 H, ArCH$_2$CH, 7.09 (d, J = 6.3 Hz, 1 H, 5-H), 7.14−7.43 (m, 8 H, arom). ^{13}C NMR (base, CDCl$_3$): δ (ppm) 35.3 (1 C, ArCH$_2$CH), 36.5 (1 C, N(CH$_2$$CH_2$)$_2$), 39.0 (1 C, N(CH$_2CH_2$)$_2$), 49.3 (2 C, N($CH_2CH_2$)$_2$), 56.1 (1 C, O$CH_3$), 63.4 (1 C, NCH$_2$Ph), 74.9 (1 C, Ar$C$O), 96.2 (1 C, ArCH$_2$$C$H), 124.8 (1 C, C-8), 126.4 (1 C, C-7), 126.6 (1 C, C-6), 126.9 (1 C, C-4″), 128.1 (2 C, C-3″ + C-5″), 129.1 (1 C, C-5), 129.2 (2 C, C-2″ + C-6″), 131.5 (1 C, C-4a), 138.5 (1 C, C-1″), 141.2 (1 C, C-8a).

3-Methoxy-1′-(2-phenylethyl)-3,4-dihydrospiro[[2]-benzopyran-1,4′-piperidine] (14b). A solution of **13b** (55 mg, 0.15 mmol) and *p*-toluenesulfonic acid monohydrate (34 mg, 0.18 mmol) in methanol (10 mL) was stirred at room temperature for 20 h. Then NaOH (15 mg) was added and the solvent was removed under reduced pressure. The residue was purified by fc (2 cm, petroleum ether/ethyl acetate 1:1, 20 mL, R_f = 0.04) to give a colorless oil, yield 46 mg (92%). The base **14b** was converted into the hydrochloride **14b**·HCl in the usual manner to obtain colorless crystals, mp 263−264 °C (dec). Anal. (C$_{22}$H$_{28}$NO$_2$Cl) C, H, N. MS (EI), m/z: 246 [M$^+$ − CH$_2$-Ph]. MS (CI, NH$_3$), m/z: 338 [MH$^+$], 246 [M$^+$ − CH$_2$Ph]. IR (film), ν (cm^{-1}): 2941, 2813 (C−H); 1076, 1043 (C−O); 756, 699 (C−H). ^{1}H NMR (CDCl$_3$): δ (ppm) 1.91 (dd, J = 13.7/2.2 Hz, 1 H, N(CH$_2$CH_2)$_2$), 1.98−2.05 (m, 2 H, N(CH$_2$CH_2)$_2$), 2.23 (td, J = 12.8/4.3 Hz, 1 H, N(CH$_2$CH_2)$_2$), 2.56 (td, J = 11.0/4.9 Hz, 1 H, N(CH_2CH$_2$)$_2$), 2.56−2.74 (m, 3 H, N(CH_2CH$_2$)$_2$ (1 H), NCH_2CH$_2$Ph (2 H)), 2.85−2.98 (m, 6 H, N(CH_2CH$_2$)$_2$ (2 H), NCH$_2$CH_2Ph (2 H), ArCH_2CH (2 H)), 3.58 (s, 3 H, OCH_3), 4.89 (dd, J = 6.7/4.3 Hz, 1 H, ArCH$_2$CH), 7.10 (d, J = 6.4 Hz, 1 H, arom), 7.15−7.34 (m, 8 H, arom).

3-Methoxy-3,4-dihydrospiro[[2]benzopyran-1,4′-piperidine] (14d). Under an N$_2$ atmosphere, ammonium formate (231 mg, 3.65 mmol) was added to a stirred mixture of **14a** (237 mg, 0.73 mmol) and 10% Pd/C (40 mg) in dry methanol (30 mL). This mixture was refluxed for 2.5 h. Then it was filtered through Celite and concentrated in vacuo. The crude product was purified by fc (2 cm, methanol/ammonia 98:2, 12 mL, R_f = 0.09) to yield **14d** as colorless needles (petroleum ether), mp 70−71 °C, yield 120 mg (70%). Anal. (C$_{14}$H$_{19}$NO$_2$) C, H, N. MS (EI), m/z: 233 [M$^+$], 218 [M$^+$ − CH$_3$]. IR (KBr), ν (cm^{-1}): 3561 (N−H); 2941, 2870 (C−H); 1141, 1073 (C−O); 764 (C−H). ^{1}H NMR (CDCl$_3$): δ (ppm) 1.80−1.92 (m, 2 H, N(CH$_2$CH_2)$_2$), 1.99 (dd, J = 14.2/2.4 Hz, 1 H, N(CH$_2$CH_2)$_2$), 2.09 (td, J = 13.2/4.6 Hz, 1 H, N(CH$_2$CH_2)$_2$), 2.30 (br s, 1 H, NH), 2.85−2.95 (m, 2 H, ArCH_2CH), 3.01 (br d, J = 12.7 Hz, 2 H, N(CH_2CH$_2$)$_2$), 3.09−3.33 (m, 2 H, N(CH_2CH$_2$)$_2$), 3.57 (s, 3 H, OCH_3), 4.88 (dd, J = 6.3/3.9 Hz, 1 H, ArCH$_2$CH), 7.09 (d, J = 6.3 Hz, 1 H, arom), 7.14−7.25 (m, 3 H, arom).

3-Methoxy-1′-(3-phenylpropyl)-3,4-dihydrospiro[[2]-benzopyran-1,4′-piperidine] (14e). 1-Chloro-3-phenylpropane (328 mg, 1.65 mmol) and potassium carbonate (1.5 g, 10.9 mmol) were added to a solution of **14d** (320 mg, 1.37 mmol) in THF (40 mL). This mixture was refluxed for 19 h. Then it was filtered and concentrated in vacuo. The crude product was purified by fc (3 cm, petroleum ether/ethyl acetate 1:1, 20 mL, R_f = 0.04) to yield a colorless oil (**14e**, 310 mg, 64%). The base **14e** was converted into the hydrochloride in the usual manner to obtain **14e**·HCl as colorless crystals, mp 196−198 °C. Anal. (C$_{23}$H$_{30}$NO$_2$Cl) C, H, N. MS (base, EI), m/z: 351 [M$^+$], 246 [M$^+$ − CH$_2$CH$_2$Ph]. IR (base, film), ν (cm^{-1}): 2940, 2815 (C−H); 1128, 1076, 1044 (C−O); 754, 700 (C−H). ^{1}H NMR (CDCl$_3$): δ (ppm) 1.84−2.03 (m, 5 H, CH$_2$CH_2CH$_2$Ph (2 H), N(CH$_2$CH_2)$_2$ (3 H)), 2.21 (td, J = 13.1/4.2 Hz, 1 H, N(CH$_2$CH_2)$_2$), 2.40−2.59 (m, 4 H, NCH_2CH$_2$CH$_2$Ph (2 H), N(CH_2CH$_2$)$_2$ (2 H)), 2.69 (t, J = 7.6 Hz, 2 H, NCH$_2$CH$_2$CH_2Ph), 2.80−2.89 (m, 2 H, N(CH_2-CH$_2$)$_2$), 2.91−2.95 (m, 2 H, ArCH_2CH), 3.57 (s, 3 H, OCH_3), 4.88 (dd, J = 6.4/4.0 Hz, 1 H, ArCH$_2$CH), 7.09 (d, J = 6.4 Hz, 1 H, arom), 7.14−7.34 (m, 8 H, arom).

3-Methoxy-1′-(4-phenylbutyl)-3,4-dihydrospiro[[2]-benzopyran-1,4′-piperidine] (14f). 1-Bromo-4-phenylbutane (32 mg, 0.19 mmol) and potassium carbonate (209 mg, 1.5 mmol) were added to a solution of **14d** (44 mg, 0.19 mmol) in acetonitrile (5 mL). This mixture was refluxed for 40 h. Then it was filtered and concentrated in vacuo. The crude product (72 mg) was purified by fc (2 cm, ethyl acetate, 10 mL, R_f = 0.18) to give a colorless oil, yield 26 mg (38%), which solidified on standing, giving a colorless solid, mp 42−44 °C. HRMS for C$_{24}$H$_{31}$NO$_2$: calcd 365.235 479, found 365.235 630 (+0.4 ppm). MS (EI), m/z: 365 [M$^+$], 334 [M$^+$ − OCH$_3$], 246 [M$^+$ − CH$_2$CH$_2$CH$_2$Ph]. IR (film), ν (cm^{-1}): 2935, 2811 (C−H); 1076, 1044 (C−O); 753, 699 (C−H). ^{1}H NMR (CDCl$_3$): δ (ppm) 1.54−1.75 (m, 4 H, NCH$_2$CH_2CH_2CH$_2$Ph), 1.85−2.03 (m, 3 H, N(CH$_2$CH_2)$_2$), 2.21 (td, J = 13.2/4.4 Hz, 1 H, N(CH$_2$CH_2)$_2$), 2.39−2.59 (m, 4 H, NCH_2CH$_2$CH$_2$CH$_2$Ph (2 H), N(CH_2CH$_2$)$_2$ (2 H)), 2.68 (t, J = 7.3 Hz, 2 H, NCH$_2$CH$_2$CH$_2$CH_2Ph), 2.78−2.88 (m, 2 H, N(CH_2CH$_2$)$_2$), 2.91−2.96 (m, 2 H, ArCH_2CH), 3.58 (s, 3 H, OCH_3), 4.88 (dd, J = 6.3/3.9 Hz, 1 H, ArCH$_2$CH), 7.10 (d, J = 6.8 Hz, 1 H, arom), 7.15−7.34 (m, 8 H, arom).

1′-Cinnamyl-3-methoxy-3,4-dihydrospiro[[2]benzopyran-1,4′-piperidine] (14g). 3-Chloro-1-phenylprop-1-ene (cinnamyl chloride, 27 mg, 0.18 mmol) and potassium carbonate (190 mg, 1.4 mmol) were added to a solution of **14d** (40 mg, 0.17 mmol) in acetonitrile (5 mL). This mixture was refluxed for 9 h. Then it was filtered and concentrated in vacuo. The crude product (36 mg) was purified by fc (2 cm, ethyl acetate, 10 mL, R_f = 0.19) to give a colorless oil, yield 14 mg (24%). HRMS for C$_{23}$H$_{27}$NO$_2$: calcd 349.204 179, found 349.204 127 (−0.1 ppm). MS (EI), m/z: 349 [M$^+$], 334 [M$^+$ − CH$_3$], 117 [CH$_2$CHCHPh$^+$]. IR (film), ν (cm^{-1}): 2932, 2813 (C−H); 1671 (C=C (*E*)); 1071, 1044 (C−O); 750, 699 (C−H). ^{1}H NMR (CDCl$_3$): δ (ppm) 1.90 (d, J = 13.7 Hz, 1 H, N(CH$_2$CH_2)$_2$), 1.98−2.06 (m, 2 H, N(CH$_2$CH_2)$_2$), 2.25 (td, J = 12.7/4.4 Hz, 1 H, N(CH$_2$CH_2)$_2$), 2.47−2.68 (m, 2 H, N(CH_2-CH$_2$)$_2$), 2.88−2.98 (m, 4 H, ArCH_2CH, N(CH_2CH$_2$)$_2$), 3.27 (d, J = 6.8 Hz, 2 H, NCH_2CH=CHPh), 3.57 (s, 3 H, OCH_3), 4.88 (dd, J = 6.5/4.2 Hz, 1 H, ArCH$_2$CH), 6.36 (dt, J = 15.6/6.8 Hz, 1 H, (*E*) NCH$_2$CH=CHPh), 6.58 (d, J = 16.1 Hz, 1 H, (*E*) NCH$_2$-CH=CHPh), 7.09 (d, J = 6.8 Hz, 1 H, arom), 7.12−7.43 (m, 8 H, arom).

3-Methoxy-1′-phenyl-3,4-dihydrospiro[[2]benzopyran-1,4′-piperidine] (14h). Under an N$_2$ atmosphere, Pd(OAc)$_2$ (0.1 mg, 0.000 437 mmol, 0.25 mol %) and P^{t}Bu$_3$ (0.35 mg, 0.001 75 mmol, 1 mol %) were added to a suspension of bromobenzene (27.5 mg, 0.175 mmol), **14d** (49.0 mg, 0.21 mmol), and NaO^{t}Bu (28.3 mg, 0.29 mmol) in dry *o*-xylene (3 mL). This mixture was heated at 130 °C for 4 h. After the mixture cooled to room temperature, petroleum ether (3 mL) was added and the mixture was washed with a saturated solution of NaCl (6 mL). The organic layer was dried (MgSO$_4$) and concentrated in vacuo. The residue was purified by fc (1 cm, petroleum ether/ethyl acetate 95:5, 5 mL, R_f = 0.10) to give a colorless oil, yield 11.3 mg (21%), which solidified on standing. HRMS for C$_{20}$H$_{23}$NO$_2$: calcd 309.172 879, found 309.172 914 (+0.1 ppm). MS (EI), m/z: 309 [M$^+$], 294 [M$^+$ − CH$_3$], 77 [Ph$^+$]. IR (film), ν (cm^{-1}): 2940, 2823 (C−H); 1078, 1035 (C−O); 757, 694 (C−H). ^{1}H NMR (CDCl$_3$): δ (ppm) 1.98 (d, J = 13.2 Hz, 1 H, N(CH$_2$CH_2)$_2$), 2.10 (br d, J = 4.9 Hz, 2 H, N(CH$_2$CH_2)$_2$), 2.31 (td, J = 12.7/3.9 Hz, 1 H, N(CH$_2$CH_2)$_2$), 2.89−3.03 (m, 2 H, ArCH_2CH), 3.19−3.40 (m, 2 H, N(CH_2-CH$_2$)$_2$), 3.57 (s, 3 H, OCH_3), 3.62 (br d, J = 11.7 Hz, 2 H, N(CH_2-CH$_2$)$_2$), 4.93 (dd, J = 6.3/3.9 Hz, 1 H, ArCH$_2$CH), 6.88 (t, J = 7.8 Hz, 1 H, arom), 7.04 (d, J = 8.3 Hz, 2 H, arom), 7.09−7.35 (m, 6 H, arom).

1′-Benzyl-3,4-dihydrospiro[[2]benzopyran-1,4′-piperidin]-3-ol (15a). A solution of **13a** (557 mg, 1.57 mmol) in THF (6 mL), HCl (2 M, 8 mL), and water (10 mL) was stirred at room temperature for 11 days. Then a solution of NaOH (2 M) was added to give pH 10 and the mixture was extracted with CH$_2$Cl$_2$. The organic layer was dried (Na$_2$SO$_4$), and the solvent was removed under reduced pressure. The crude product was purified by fc (3 cm, petroleum ether/ethyl acetate 2:1, 20 mL, R_f = 0.02) to yield a colorless solid (petroleum ether/ethyl acetate), mp 163 °C, yield 363 mg (69%). Anal. (C$_{20}$H$_{23}$NO$_2$) C, H, N. MS (EI), m/z: 309 [M$^+$], 232 [M$^+$ − Ph], 218 [M$^+$ − CH$_2$Ph], 91 [CH$_2$Ph$^+$]. IR (KBr), ν (cm^{-1}): 3422 (O−H); 3064, 2942, 2836 (C−H); 1134, 1060, 1020 (C−O); 754,

694 (C−H). ^{1}H NMR ($CDCl_3$): δ (ppm) 1.86 (dd, J = 13.4/2.4 Hz, 1 H, N(CH_2CH_2)$_2$), 1.92−2.02 (m, 2 H, N(CH_2CH_2)$_2$), 2.25 (td, J = 13.0/4.9 Hz, 1 H, N(CH_2CH_2)$_2$), 2.49 (td, J = 10.7/4.9 Hz, 1 H, N(CH_2CH_2)$_2$), 2.58 (td, J = 12.2/2.4 Hz, 1 H, N(CH_2-CH_2)$_2$), 2.71−2.84 (m, 2 H, N(CH_2CH_2)$_2$), 2.88 (dd, J = 15.6/7.3 Hz, 1 H, ArCH_2CH), 3.03 (dd, J = 15.6/2.9 Hz, 1 H, ArCH_2CH), 3.59 (s, 2 H, NCH_2Ph), 5.31 (dd, J = 7.8/2.9 Hz, 1 H, ArCH_2CH), 7.10 (d, J = 6.8 Hz, 1 H, arom), 7.16−7.44 (m, 8 H, arom).

1′-(2-Phenylethyl)-3,4-dihydrospiro[[2]benzopyran-1,4′-piperidin]-3-ol (15b). A solution of **13b** (80 mg, 0.22 mmol) in THF (1 mL), HCl (2 M, 2 mL), and water (2 mL) was stirred for 2.5 days at room temperature. Then diluted NaOH (2 M) was added (pH 10) and the mixture was extracted with CH_2Cl_2. The organic layer was dried (Na_2SO_4), and the solvent was removed under reduced pressure. The crude product was purified by fc (2 cm, petroleum ether/ethyl acetate 1:1, 20 mL, R_f = 0.03) to yield a colorless solid (petroleum ether/ethyl acetate), mp 127 °C, yield 45 mg (64%). Anal. ($C_{21}H_{25}NO_2$) C, H, N. MS (EI), m/z: 232 [M^+ − CH_2Ph]. MS (CI, isobutane), m/z: 324 [MH^+], 232 [M^+ − CH_2Ph]. IR (film), ν (cm^{-1}): 3401 (O−H); 2929, 2827 (C−H); 1117, 1065 (C−O); 757, 700 (C−H). ^{1}H NMR ($CDCl_3$): δ (ppm) 1.91 (br d, J = 13.1 Hz, 1 H, N(CH_2CH_2)$_2$), 2.00−2.07 (m, 2 H, N(CH_2CH_2)$_2$), 2.28 (td, J = 12.9/4.4 Hz, 1 H, N(CH_2CH_2)$_2$), 2.54−2.73 (m, 4 H, N(CH_2-CH_2)$_2$ (2 H), NCH_2CH_2Ph (2 H)), 2.84−2.96 (m, 5 H, N(CH_2-CH_2)$_2$ (2 H), NCH_2CH_2Ph (2 H), ArCH_2CH (1 H)), 3.04 (dd, J = 15.6/3.1 Hz, 1 H, ArCH_2CH), 5.32 (dd, J = 7.6/3.1 Hz, 1 H, ArCH_2CH), 7.11 (d, J = 6.7 Hz, 1 H, arom), 7.16−7.34 (m, 8 H, arom).

1′-(3-Phenylpropyl)-3,4-dihydrospiro[[2]benzopyran-1,4′-piperidin]-3-ol (15e). A solution of **14e** (212 mg, 0.60 mmol) in THF (10 mL) and HCl (2 M, 3 mL) was stirred for 24 days at room temperature and for 4 h at reflux temperature. Then the solution was treated with diluted NaOH (2 M) to give pH 10 and extracted with CH_2Cl_2. The organic layer was dried (Na_2SO_4), and the solvent was removed in vacuo. The crude product was purified by fc (2 cm, petroleum ether/ethyl acetate 1:1, 10 mL, R_f = 0.03) to yield a colorless solid (cyclohexane/ethyl acetate), mp 114−115 °C, yield 75 mg (37%). HRMS for $C_{22}H_{27}NO_2$: calcd 337.204 179, found 337.204 304 (+0.4 ppm). MS (EI), m/z: 337 [M^+], 232 [M^+ − CH_2CH_2Ph]. IR (film), ν (cm^{-1}): 3269 (O−H); 2932 (C−H); 1064, 1017 (C−O); 756, 701 (C−H). ^{1}H NMR ($CDCl_3$): δ (ppm) 1.84−2.00 (m, 5 H, $CH_2CH_2CH_2$Ph (2 H), N(CH_2CH_2)$_2$ (3 H)), 2.23 (td, J = 13.1/4.5 Hz, 1 H, N(CH_2CH_2)$_2$), 2.43−2.59 (m, 4 H, NCH_2CH_2-CH_2Ph (2 H), N(CH_2CH_2)$_2$ (2 H)), 2.67, (t, J = 7.9 Hz, 2 H, N$CH_2CH_2CH_2$Ph), 2.77−2.91 (m, 3 H, N(CH_2CH_2)$_2$ (2 H), ArCH_2CH (1 H)), 3.02 (dd, J = 15.9/3.1 Hz, 1 H, ArCH_2CH), 5.30 (dd, J = 7.6/3.1 Hz, 1 H, ArCH_2CH), 7.10 (d, J = 6.4 Hz, 1 H, arom), 7.16−7.33 (m, 8 H, arom).

1′-Benzyl-3-ethoxy-3,4-dihydrospiro[[2]benzopyran-1,4′-piperidine] (16). A solution of **15a** (50 mg, 0.16 mmol) and one drop of concentrated H_2SO_4 in ethanol (2.3 g) was stirred at room temperature for 3 days. Then solid sodium hydroxide (66 mg) was added. The solvent was removed under reduced pressure, and the residue was purified by fc (1 cm, petroleum ether/ethyl acetate 1:1, 5 mL, R_f = 0.10) to yield a colorless oil, yield 44 mg (81%). The base **16** was converted into the hydrochloride **16**·HCl in the usual manner to obtain colorless crystals, mp 164 °C. Anal. ($C_{22}H_{28}NO_2Cl$) H, N, C: calcd, 70.67; found, 70.13. HRMS (base): calcd 337.204 179, found 337.204 304 (+0.4 ppm). MS (base, EI), m/z: 337 [M^+], 246 [M^+ − CH_2Ph], 91 [CH_2Ph^+]. IR (base, film), ν (cm^{-1}): 2934, 2811 (C−H); 1125, 1041 (C−O); 753, 699 (C−H). ^{1}H NMR ($CDCl_3$): δ (ppm) 1.30 (t, J = 7.0 Hz, 3 H, OCH_2CH_3), 1.89 (dd, J = 13.4/1.6 Hz, 1 H, N(CH_2CH_2)$_2$), 1.97−2.02 (m, 2 H, N(CH_2CH_2)$_2$), 2.21 (td, J = 12.8/4.4 Hz, 1 H, N(CH_2CH_2)$_2$), 2.49 (td, J = 10.6/5.0 Hz, 1 H, N(CH_2CH_2)$_2$), 2.60 (td, J = 11.7/2.6 Hz, 1 H, N(CH_2CH_2)$_2$), 2.78−2.82 (m, 2 H, N(CH_2CH_2)$_2$), 2.93 (d, J = 5.5 Hz, 2 H, ArCH_2CH), 3.52−3.63 (m, 1 H, OCH_2-CH_3), 3.63 (s, 2 H, NCH_2Ph), 3.98−4.10 (m, 1 H, OCH_2CH_3), 4.96 (t, J = 5.5 Hz, 1 H, ArCH_2CH), 7.10 (d, J = 6.6 Hz, 1 H, arom), 7.13−7.42 (m, 8 H, arom).

1′-Benzyl-3-benzyloxy-3,4-dihydrospiro[[2]benzopyran-1,4′-piperidine] (17). One drop of concentrated H_2SO_4 was added to a solution of **15a** (40 mg, 0.129 mmol) in benzyl alcohol (0.5 g), and the mixture was stirred at room temperature for 6 h. Then solid sodium hydroxide (28 mg) was added. This mixture was directly purified by fc (3 cm, petroleum ether/ethyl acetate initially 9:1, then 2:1, 20 mL, R_f = 0.09) to yield a colorless oil, yield 38 mg (77%). HRMS for $C_{27}H_{29}NO_2$: calcd 399.219 829, found 399.219 726 (−0.3 ppm). MS (EI), m/z: 399 [M^+], 308 [M^+ − CH_2Ph], 91 [CH_2Ph^+]. MS (CI, NH_3), m/z: 400 [MH^+]. IR (film), ν (cm^{-1}): 2935, 2812 (C−H); 1093, 1040 (C−O); 738, 698 (C−H). ^{1}H NMR ($CDCl_3$): δ (ppm) 1.85−2.01 (m, 3 H, N(CH_2CH_2)$_2$), 2.21 (td, J = 13.1/4.4 Hz, 1 H, N(CH_2CH_2)$_2$), 2.48−2.56 (m, 1 H, N(CH_2CH_2)$_2$), 2.62 (td, J = 12.8/2.7 Hz, 1 H, N(CH_2CH_2)$_2$), 2.77−2.86 (m, 2 H, N(CH_2CH_2)$_2$), 2.93 (dd, J = 16.2/3.7 Hz, 1 H, ArCH_2CH), 3.01 (ddd, J = 15.6/7.3/0.9 Hz, 1 H, ArCH_2CH), 3.63 (s, 2 H, NCH_2Ph), 4.68 (d, J = 12.2 Hz, 1 H, OCH_2Ph), 4.97 (d, J = 12.2 Hz, 1 H, OCH_2Ph), 5.02 (dd, J = 7.3/3.7 Hz, 1 H, ArCH_2CH), 7.08 (d, J = 6.4 Hz, 1 H, arom), 7.13−7.43 (m, 13 H, arom).

1′-Benzylspiro[[2]benzopyran-1,4′-piperidine]-3(4*H*)-one (18). Under an N_2 atmosphere, solid tetrapropylammonium perruthenate ($(NPr_4)RuO_4$, 3.3 mg, 0.0093 mmol, 5 mol %) was added in one portion to a stirred mixture of **15a** (57 mg, 0.185 mmol), *N*-methylmorpholine *N*-oxide (NMMO, 43 mg, 0.37 mmol), and powdered 4 Å molecular sieves (93 mg) in dry CH_2Cl_2 (3 mL). This mixture was stirred for 1 h at room temperature. Then it was filtered. The filtrate was evaporated under reduced pressure, and the residue was purified by fc (2 cm, petroleum ether/ethyl acetate 2:1, 20 mL, R_f = 0.21) to afford a colorless oil, yield 24 mg (43%), which solidified on standing, giving a colorless solid (^{i}Pr$_2$O), mp 138 °C. Anal. ($C_{20}H_{21}NO_2$) C, H, N. MS (EI), m/z: 307 [M^+], 230 [M^+ − Ph], 216 [M^+ − CH_2Ph], 91 [CH_2Ph^+]. IR (film), ν (cm^{-1}): 2939, 2820 (C−H); 1740 (C=O); 1237, 1105 (C−O); 745, 700 (C−H). ^{1}H NMR ($CDCl_3$): δ (ppm) 1.95 (dd, J = 14.2/2.6 Hz, 2 H, N(CH_2CH_2)$_2$), 2.22 (td, J = 13.0/4.6 Hz, 2 H, N(CH_2CH_2)$_2$), 2.61 (td, J = 12.0/2.1 Hz, 2 H, N(CH_2CH_2)$_2$), 2.82 (br d, J = 11.0 Hz, 2 H, N(CH_2CH_2)$_2$), 3.58 (s, 2 H, NCH_2Ph), 3.75 (d, J = 0.8 Hz, 2 H, ArCH_2CO), 7.11−7.16 (m, 1 H, arom), 7.21−7.38 (m, 8 H, arom).

1′-Benzylspiro[[2]benzopyran-1,4′-piperidine] Hydrobromide (19·HBr). A solution of **14a** (37 mg, 0.11 mmol) and 2-bromo-2-methylpropane (78 mg, 0.55 mmol) in $CHCl_3$ (10 mL) was refluxed for 24 h. Then it was concentrated in vacuo and the residue was recrystallized from ethyl acetate to afford colorless crystals, mp 251−252 °C, yield 10 mg (24%). Anal. ($C_{20}H_{22}NOBr$) C, H, N. MS (base, EI), m/z: 291 [M^+], 214 [M^+ − Ph], 200 [M^+ − CH_2Ph], 91 [CH_2Ph^+]. IR (base, film), ν (cm^{-1}): 2941, 2813 (C−H); 1630 (C=C); 1054 (C−O); 743, 698 (C−H). ^{1}H NMR (base, $CDCl_3$): δ (ppm) 1.99 (td, J = 13.4/4.4 Hz, 2 H, N(CH_2CH_2)$_2$), 2.25 (dt, J = 14.1/2.7 Hz, 2 H, N(CH_2CH_2)$_2$), 2.49 (td, J = 12.0/2.4 Hz, 2 H, N(CH_2CH_2)$_2$), 2.79 (br d, J = 11.0 Hz, 2 H, N(CH_2CH_2)$_2$), 3.60 (s, 2 H, NCH_2Ph), 5.75 (d, J = 5.7 Hz, 1 H, ArCH=CH), 6.50 (d, J = 5.7 Hz, 1 H, ArCH=CH), 6.93−6.96 (m, 1 H, arom), 7.08−7.42 (m, 8 H, arom).

1′-Benzyl-3,4-dihydrospiro[[2]benzopyran-1,4′-piperidine] (20). A mixture of base **19** (27 mg, 0.092 mmol), 10% Pd/C (19 mg), and acetic acid (4 mL) was stirred under a hydrogen atmosphere (0.8 bar) for 1.25 h. Then it was filtered through Celite, and diluted NaOH (2 M, 25 mL) was added. This solution was extracted with CH_2Cl_2, and the organic layer was dried (Na_2SO_4) and evaporated under reduced pressure. The residue was purified by fc (1 cm, petroleum ether/ethyl acetate 3:1, 5 mL R_f = 0.06) to give a colorless oil, yield 11 mg (39%), which solidified on standing, mp 50 °C. HRMS for $C_{20}H_{23}NO$: calcd 293.177 964, found 293.177 874 (−0.3 ppm). MS (EI), m/z: 293 [M^+], 216 [M^+ − Ph], 202 [M^+ − CH_2Ph], 91 [CH_2Ph^+]. IR (film), ν (cm^{-1}): 2929, 2813 (C−H); 1092 (C−O); 737, 697 (C−H). ^{1}H NMR ($CDCl_3$): δ (ppm) 1.88 (dd, J = 14.3/2.4 Hz, 2 H, N(CH_2CH_2)$_2$), 2.05 (td, J = 13.0/4.5 Hz, 2 H, N(CH_2CH_2)$_2$), 2.43 (td, J = 11.9/2.7 Hz, 2 H, N(CH_2CH_2)$_2$), 2.73 (br d, J = 10.7 Hz, 2 H, N(CH_2CH_2)$_2$), 2.82 (t, J = 5.6 Hz,

2 H, ArCH_2CH$_2$), 3.58 (s, 2 H, NCH_2Ph), 3.90 (t, J = 5.6 Hz, 2 H, ArCH$_2$CH_2), 7.05−7.41 (m, 9 H, arom).

1′-Benzyl-3-methoxy-3*H*-spiro[[2]benzofuran-1,4′-piperidine] (23). Under an N_2 atmosphere, a 1.6 M solution of *n*-butyllithium in hexane (8.13 mL, 10 mmol) was slowly added to a cooled (−95 °C) solution of **21** (3.0 g, 13 mmol) in THF (30 mL). The mixture was stirred at −95 °C for 5 min, and then a solution of **12a** (1.89 g, 10 mmol) in THF (10 mL) was slowly added. The solution was stirred at −95 °C for 2 h. Then it was allowed to warm to room temperature and was stirred for another 4 h. Then water (50 mL) was added, the mixture was extracted with CH_2Cl_2, the organic layer was dried (Na_2SO_4), and the solvent was removed in vacuo to give 3.9 g of crude product **22**. The crude product was dissolved in methanol (20 mL), and *p*-toluenesulfonic acid monohydrate (2.8 g, 14.7 mmol) was added. This mixture was stirred for 7 days at room temperature. Then the solution was treated with diluted NaOH (2 M) to give pH 10 and was extracted with CH_2Cl_2. The crude product was purified by fc (8 cm, petroleum ether/ethyl acetate 3:1, 50 mL, R_f = 0.20) to afford a colorless oil (**23**), yield 1.1 g (36%). **23** was converted into the hydrochloride in the usual manner to obtain **23**·HCl as colorless crystals, mp 171−173 °C. Anal. ($C_{20}H_{24}NO_2Cl$) C, H, N. MS (EI), *m*/*z*: 309 [M^+], 294 [$M^+ - CH_3$], 278 [$M^+ - OCH_3$], 218 [$M^+ - CH_2Ph$], 91 [CH_2Ph^+]. IR (base, film), ν (cm^{-1}): 2944, 2813 (C−H); 1085, 1003 (C−O); 747, 699 (C−H). ^{1}H NMR (base, $CDCl_3$): δ (ppm) 1.65 (ddd, J = 13.4/5.5/2.4 Hz, 1 H, N(CH$_2$CH_2)$_2$), 1.82 (ddd, J = 13.4/5.5/2.8 Hz, 1 H, N(CH$_2$CH_2)$_2$), 2.05 (td, J = 12.8/4.0 Hz, 1 H, N(CH$_2$CH_2)$_2$), 2.13 (td, J = 13.0/4.4 Hz, 1 H, N(CH$_2$CH_2)$_2$), 2.52 (br t, J = 11.8 Hz, 2 H, N(CH_2-CH$_2$)$_2$), 2.81−2.95 (m, 2 H, N(CH_2CH$_2$)$_2$), 3.48 (s, 3 H, OCH_3), 3.62 (s, 2 H, NCH_2Ph), 6.09 (s, 1 H, ArC*H*), 7.18−7.22 (m, 1 H, arom), 7.24−7.43 (m, 8 H, arom).

1′-Benzyl-3*H*-spiro[[2]benzofuran-1,4′-piperidin]-3-ol (24). As described for **23** the bromobenzaldehyde acetal **21** (578 mg, 2.5 mmol) was reacted with *n*-butyllithum (1.6 M, 1.72 mL, 2.75 mmol) and **12a** (402 mg, 2.1 mmol) to yield the addition product **22** (510 mg). The crude product **22** was dissolved in THF (2 mL), HCl (2 M, 10 mL), and water (10 mL). This solution was refluxed for 3.5 h. Then the solution was treated with diluted NaOH (2 M) to give pH 10 and was extracted with CH_2Cl_2. The crude product was purified by fc (2 cm, petroleum ether/ethyl acetate 3:2, 10 mL, R_f = 0.08) to afford **24** as a colorless solid (petroleum ether/ethyl acetate), mp 154 °C, yield 68 mg (11%). Anal. ($C_{19}H_{21}NO_2$) C, H, N. MS (EI), *m*/*z*: 295 [M^+], 204 [$M^+ - CH_2Ph$], 91 [CH_2Ph^+]. IR (KBr), ν (cm^{-1}): 3420 (O−H); 2919, 2829 (C−H); 1038, 997 (C−O); 753, 697 (C−H). ^{1}H NMR ($CDCl_3$): δ (ppm) 1.63 (ddd, J = 13.7/5.3/2.9 Hz, 1 H, N(CH$_2$CH_2)$_2$), 1.80 (ddd, J = 13.7/5.4/2.4 Hz, 1 H, N(CH$_2$CH_2)$_2$), 2.02−2.21 (m, 2 H, N(CH$_2$CH_2)$_2$), 2.45−2.60 (m, 2 H, N(CH_2CH$_2$)$_2$), 2.83−2.93 (m, 2 H, N(CH_2CH$_2$)$_2$), 3.62 (s, 2 H, NCH_2Ph), 6.45 (s, 1 H, ArC*H*), 7.22 (dd, J = 6.3/2.0 Hz, 1 H, arom), 7.24−7.45 (m, 8 H, arom).

1′-Benzylspiro[[2]benzofuran-1,4′-piperidin]-3-one (25). Under an N_2 atmosphere, solid tetrapropylammonium perruthenate ((NPr$_4$)RuO$_4$, 3.5 mg, 0.0098 mmol, 5 mol %) was added in one portion to a stirred mixture of **24** (58 mg, 0.196 mmol), *N*-methylmorpholine *N*-oxide (NMMO, 46 mg, 0.39 mmol), and powdered 4 Å molecular sieves (100 mg) in dry CH_2Cl_2 (3 mL). This mixture was stirred for 1.5 h at room temperature. Then it was filtered. The filtrate was evaporated under reduced pressure, and the residue was purified by fc (1 cm, petroleum ether/ethyl acetate 2:1, 5 mL, R_f = 0.06) to afford a colorless oil, yield 33 mg (58%), which solidified on standing. Compound **25** was converted into the hydrochloride in the usual manner to obtain **25**·HCl as colorless crystals, mp 274−275 °C (ref 36, 280−283 °C). MS (EI) for $C_{19}H_{19}NO_2$, *m*/*z*: 293 [M^+], 202 [$M^+ - CH_2Ph$], 91 [CH_2Ph^+]. IR (film), ν (cm^{-1}): 2944, 2813 (C−H); 1762 (C=O); 1072 (C−O); 741, 695 (C−H). ^{1}H NMR (base, $CDCl_3$): δ (ppm) 1.71 (dd, J = 14.3/2.5 Hz, 2 H, N(CH$_2$CH_2)$_2$), 2.23 (td, J = 13.4/4.7 Hz, 2 H, N(CH$_2$CH_2)$_2$), 2.56 (td, J = 12.2/2.7 Hz, 2 H, N(CH_2CH$_2$)$_2$), 2.92 (br d, J = 11.0 Hz, 2 H, N(CH_2CH$_2$)$_2$), 3.62 (s, 2 H, NCH_2Ph), 7.23−7.39 (m, 5 H, arom), 7.42 (dt, J = 7.6/0.9 Hz, 1 H, 7-H), 7.51 (td, J = 7.6/0.9 Hz, 1 H, 5-H), 7.66 (td, J = 7.3/1.2 Hz, 1 H, 6-H), 7.87 (dt, J = 7.3/1.2 Hz, 1 H, 4-H).

2. Receptor Binding Studies. General. Teflon−glass−homogenizer was Potter S (B. Braun Biotech International). The rotor/stator homogenizer was a Ultraturrax T25 basic (Ika Labortechnik). The centrifuge was a high-speed refrigerating centrifuge model J2-HS (Beckman). Whatman glass fiber filters GF/B were presoaked in 0.5% polyethylenimine in water for 2 h at 4 °C before use. Filtration was performed with a Brandel 24-well cell harvester. The scintillation cocktail was Rotiszint eco plus (Roth). The liquid scintillation analyzer was a Tri-Carb 2100 TR (Canberra Packard) with a counting efficiency of 66%. All experiments were carried out in triplicate. IC_{50} values were determined from competition experiments with at least six concentrations of test compounds and were calculated with the curve-fitting program GraphPad Prism 3.0 (GraphPad Software) by nonlinear regression analysis. K_i values were calculated according to Cheng and Prusoff.[42] K_d values for the radioligands were taken from the literature. For compounds with high affinity (low K_i values) mean values ± SEM from at least three independent experiments are given.

3. Investigation of the σ_1-Receptor Affinity.[37] [^{3}H]-(+)-Pentazocine binding to guinea pig brain membrane preparations was performed according to standard radioligand binding assays,[37] which were slightly modified as described below.

Membrane Preparation. Thawed guinea pig brains (Dunkin Hartley, Harlan-Sera-Lab) were homogenized with an ultraturrax (8000 rpm) in 10 volumes of cold 0.32 M sucrose. The homogenate was centrifuged at 1000*g* for 10 min at 4 °C. The supernatant was separated and centrifuged at 22 000*g* for 20 min at 4 °C. The pellet was resuspended in 10 volumes of buffer (50 mM Tris-HCl, pH 7.4) with an ultraturrax (8000 rpm), incubated for 30 min at 25 °C, and centrifuged at 22 000*g* (20 min, 4 °C). The pellet was resuspended in buffer, the protein concentration was determined according to the method of Bradford[43] using bovine serum albumin as standard, and subsequently the preparation was frozen (−83 °C) in 5 mL portions of about 2 mg of protein/mL.

σ_1-Receptor Binding Assay. The test was performed with the radioligand [ring-1,3-^{3}H]-(+)-pentazocine (1036 GBq/mmol; NEN Life Science Products). The thawed membrane preparation (about 150 μg of the protein) was incubated with various concentrations of test compounds, 3 nM [^{3}H]-(+)-pentazocine, and buffer (50 mM Tris-HCl, pH 7.4) in a total volume of 500 μL for 150 min at 37 °C. The incubation was terminated by rapid filtration through presoaked Whatman GF/B filters using a cell harvester. After the sample was washed four times with 2 mL of cold buffer, a total of 3 mL of scintillation cocktail was added to the filters. After at least 8 h, bound radioactivity trapped on the filters was counted in a liquid scintillation analyzer. Nonspecific binding was determined with 10 μM haloperidol.

4. Investigation of the σ_2-Receptor Affinity.[38,39] σ_2-Receptor affinity was determined using rat liver membranes with [^{3}H]-ditolylguanidine in the presence of 100 nM (+)-pentazocine to mask σ_1-binding sites. The assay was performed according to the standard procedure,[38,39] which was slightly modified as described below.

Membrane Preparation. One frozen rat liver (Sprague Dawley, Harlan-Sera-Lab) was allowed to thaw slowly on ice. Then it was homogenized with a potter (800 rpm) in 10 volumes of cold buffer (10 mM Tris-HCl/0.32 M sucrose, pH 7.4). The homogenate was centrifuged at 1000*g* for 10 min at 4 °C. The supernatant was separated and saved on ice. The pellet was resuspended in 30 mL of cold buffer and centrifuged again. Both supernatants were then centrifuged at 31 000*g* for 20 min at 4 °C. The pellet was resuspended in 30 mL of buffer (10 mM Tris-HCl, pH 7.4) by vortexing and gentle potter homogenization. Then it was incubated for 15 min at 25 °C and centrifuged at 31 000*g* (20 min, 4 °C). The pellet was resuspended in buffer, the protein concentration was determined according to the method of Bradford[43] using bovine

serum albumin as standard, and subsequently the preparation was frozen (−83 °C) in 5 mL portions of about 2.5 mg of protein/mL.

***σ_2*-Receptor Binding Assay.** The membrane preparation (about 60 μg of protein) was incubated with 3 nM [^{3}H]-ditolylguanidine (di-[*p*-ring-^{3}H]-1,3-di-*o*-tolylguanidine, 2220 GBq/mmol; American Radiolabeled Chemicals, Inc.) and different concentrations of test compounds in buffer (50 mM Tris-HCl, pH 8.0) in the presence of 100 nM (+)-pentazocine. The total volume was 250 μL. The incubation (120 min, 25 °C) was stopped by addition of 2 mL of ice-cold buffer (10 mM Tris-HCl, pH 8.0) followed by rapid filtration through presoaked Whatman GF/B filters using a cell harvester. After the sample was washed three times with 2 mL of cold buffer, a total of 3 mL of scintillation cocktail was added to the filters. After at least 8 h, bound radioactivity trapped on the filters was counted in a liquid scintillation analyzer. Nonspecific binding was determined with 10 μM nonradiolabeled ditolylguanidine.

Acknowledgment. We thank the Deutsche Forschungsgemeinschaft, the Fonds der Chemischen Industrie, and the Wissenschaftliche Gesellschaft Freiburg for financial support. Thanks are also due to Degussa AG, Janssen Cilag GmbH, and Bristol-Myers Squibb for donation of chemicals and reference compounds. Performance of the receptor screening (Table 4) by Dr. C. A. Seyfried, Department of CNS Research, Merck KGaA, Darmstadt, is gratefully acknowledged.

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JM010992Z