Thiophene Bioisosteres of Spirocyclic σ Receptor Ligands. 1. N-Substituted Spiro[piperidine-4,4′-thieno[3,2-*c*]pyrans]

Christoph Oberdorf,[†] Dirk Schepmann,[†] Jose Miguel Vela,[‡] Jose Luis Diaz,[‡] Jörg Holenz,[‡] and Bernhard Wünsch*[,†]

Institut für Pharmazeutische and Medizinische Chemie der Universität Münster, Hittorfstrasse 58-62, D-48149 Münster, Germany, and Esteve, Av. Mare de Deu de Montserrat 221, 08041 Barcelona, Spain

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Herein, the synthesis and pharmacological evaluation of thiophene bioisosteres of the highly potent spirocyclic benzopyran **1** are detailed. The synthesis of 1-benzyl-6′-methoxy-6′,7′-dihydrospiro[piperidine-4,4′-thieno[3.2-*c*]pyran] (**2a**) was performed starting with 3-bromothiophene (**3**). After introduction of the acetaldehyde substructure (**7**), halogen metal exchange, addition of 1-benzylpiperidin-4-one, and cyclization led to the spirocyclic thienopyran **2a**. The removal of the benzyl group afforded the secondary amine **2f**, which was substituted with various residues. With respect to σ_1 affinity the *N*-benzyl derivative **2a**, the *N*-cyclohexylmethyl derivative **2d**, and the *N*-*p*-fluorobenzyl derivative **2i** represent the most potent compounds of this series binding with K_i values of 0.32, 0.29, and 0.62 nM, respectively. Electronic properties of the substituents have only little impact on σ_1 affinity. The most potent σ_1 ligands display high selectivity against σ_2, 5-HT$_{1A}$, 5-HT$_6$, 5-HT$_7$, α_{1A}, α_2, and NMDA receptors. The activity of **2a** in the mouse capsaicin assay seems to indicate σ_1 antagonistic activity.

Introduction

About 30 years ago, the σ receptor was first discovered and classified as an opioid receptor, because typical opioid receptor ligands of the benzomorphan type showed atypical pharmacological effects.[1] However, this classification was abolished when further investigations revealed that σ ligand mediated effects were not antagonized by the opioid antagonist naloxone.[2] The assumption that the σ receptor and the phencyclidine (PCP[a]) binding site of the NMDA receptor are identical was also disproved because the antipsychotic agent haloperidol binds with high affinity to σ receptors but has no affinity to the PCP binding site.[3] Today, σ receptors are well established as a non-opioid, non-phencyclidine, and haloperidol-sensitive receptor family with its own binding profile and a characteristic distribution in the central nervous system (CNS) as well as in endocrine, immune, and some peripheral tissues, like kidney, liver, lung, and heart.[4,5]

The class of σ receptors comprises two subtypes, termed σ_1 and σ_2 receptor. The two subtypes have been pharmacologically characterized. Haloperidol and di-*o*-tolylguanidine bind with high affinity to σ_1 as well as to σ_2 receptors, whereas (+)-benzomorphans, e.g., (+)-pentazocine, displays high affinity and selectivity for the σ_1 subtype. The σ_1 receptor was cloned from various tissues including guinea pig liver[6] and rat brain.[7] Recently, the σ_1 receptor was also cloned from human placental choriocarcinoma cell lines.[8] The similarity of the amino acid sequence of the σ_1 receptors cloned from various species is greater than 95%, and the σ_1 receptors are more than 92% identical. Surprisingly, there is no significant homology between the σ_1 receptor protein and any other known mammalian receptors or even proteins. However, the yeast enzyme ergosterol-Δ^8/Δ^7-isomerase shows a ~30% homology to the σ_1 receptor. The rat brain σ_1 gene encodes for a protein consisting of 223 amino acids with a molecular weight of 23 kDa. The membrane localized σ_1 receptor has not been crystallized yet, but in 2002, Aydar et al. postulated a σ_1 receptor model with two transmembrane regions and both the C-and N-termini localized intracellularly.[9] In 2003, σ_1 knockout mice were generated, which are viable and fertile but show a significant decrease in motility.[10]

The σ_2 receptor has not been cloned yet. The molecular weight was estimated to be about 21.5 kDa.[11] Endogenous ligands for the σ_1 and σ_2 receptor have not been identified so far. However, it has been shown that neuro(active)steroids, e.g., pregnenolone, progesterone, and dehydroepiandrosterone (DHEA), interact with moderate affinity with the σ_1 receptor, and therefore, they are discussed to be its endogenous ligands.[12]

Although the intracellular signal transduction pathway is not yet elucidated, σ_1 receptors are involved in the modulation of various systems including the glutamatergic,[13] dopaminergic,[14] and cholinergic[15] neurotransmission. Additionally, the influence on the regulation and activity of a variety of ion channels including K^+-channels[16,17] and Ca^{2+}-channels[18,19] is an important feature of σ_1 receptors.

The σ_1 receptors play a role in several physiological and pathophysiological processes. In particular σ_1 antagonists can be used for the treatment of psychosis, since they do not lead to the extrapyramidal motoric side effects of typical D_2 receptor antagonists.[20] Several neuroleptic drugs like haloperidol bind not only at dopamine D_2 receptors but also at σ receptors.[21,22] Furthermore, σ_1 ligands represent a new principle for the treatment of neuropathic pain,[23,24] depressions,[25,26] cocaine abuse,[27] and epilepsy.[28] Neuroprotective and antiamnesic properties of some σ_1 ligands have been explored for the treatment of diseases associated with the loss of cognitive functions, e.g., Alzheimer's disease and Parkinson's disease.[29,30] Because of the high expression of σ_1 and σ_2 receptors in some human tumor cell lines, radiolabeled σ_1 and σ_2 ligands could be applied as selective tumor imaging agents for the diagnosis of tumors and their metastases.[31,32]

* To whom correspondence should be addressed. Phone: +49-251-8333311. Fax: +49-251-8332144. E-mail: wuensch@uni-muenster.de.
[†] Universität Münster.
[‡] Esteve.

[a] Abbreviations: CNS, central nervous system; DHEA, dehydroepiandrosterone; 5-HT, 5-hydroxytryptamine; NMDA, *N*-methyl-D-aspartate; PCP, phencyclidine.

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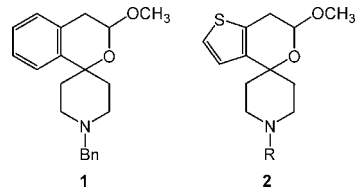


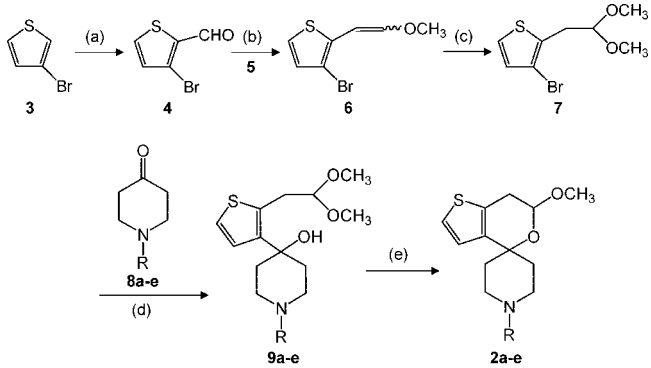
Figure 1. Comparison of spirocyclic benzopyran σ_1 receptor ligand **1** with the bioisosteric thienopyran derivatives **2**.

The structures of σ_1 receptor ligands are quite diverse. In order to gain more information about the σ_1 binding site, it is necessary to develop conformationally restricted ligands with high σ_1 affinity and high selectivity against the σ_2 receptor. In 2002, the synthesis of the spirocyclic benzopyran **1** (Figure 1) with a σ_1 affinity of 1.3 nM was reported. Moreover, this compound displays high selectivity against the σ_2 subtype.[33,34] Herein, we report on the synthesis of thiophene bioisosteres of **1** with the general structure **2**. Thiophene is a common heterocycle for the bioisosteric benzene replacement in drug development.[35] The size of the thiophene heterocycle is similar to the size of benzene, but the electronic properties and in particular the electron density are quite different. The relationship between the N-substituent of spirocyclic thienopyran derivatives **2** and their σ_1 and σ_2 affinity is detailed.

Chemistry

The synthesis of σ ligands with the general structure **2** started with 3-bromothiophene (**3**) (Scheme 1). Regioselective lithiation at position 2 of the thiophene ring led to a thienyllithium intermediate, which was trapped with 1-formylpiperidine to form 3-bromothiophene-2-carbaldehyde (**4**) in 79% yield. The aldehyde **4** reacted with the P-ylide, which had been formed by deprotonation of the phosphonium salt $Ph_3P^+CH_2OCH_3Cl^-$ (**5**) with KO^{t}Bu, to afford the enol ether **6** as a mixture of (*E*)- and (*Z*)-isomers (ratio 1:2). In the next step methanol was added to the double bond of **6** in the presence of catalytic amounts of

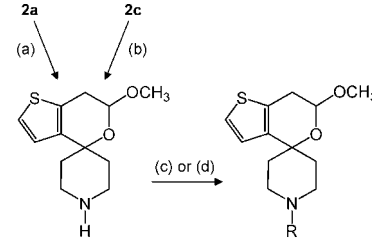
Scheme 1. Synthesis of Spirocyclic σ Receptor Ligands with Thienopyran Substructure[a]



2, 8, 9	a	b	c	d	e
R	$CH_2C_6H_5$	$(CH_2)_2C_6H_5$	$CO_2C_2H_5$	$CH_2C_6H_{11}$	$(CH_2)_2CH(CH_3)_2$

[a] Reagents and conditions: (a) LDA, THF, 0 °C, 0.5 h, then 1-formylpiperidine, THF, 0 °C, 2 h, yield 79%; (b) $Ph_3P^+CH_2OCH_3Cl^-$ (**5**), KO^{t}Bu, THF, −10 °C, 2 h, yield 83%; (c) *p*-TosOH·H_2O, $CH(OCH_3)_3$, MeOH, reflux, 24 h, yield 93%; (d) *n*-BuLi, THF, −78 °C, 15 min, then **8a**−**e**, THF, −78 °C, 1−2 h, yield **9a** 68%, yield **9b** 8%, yield **9c** nd*, yield **9d** 46%, yield **9e** nd*; (e) *p*-TosOH·H_2O, $CH(OCH_3)_3$, MeOH, room temp, 1−3 h, yield **2a** 75%, yield **2b** 44%, yield **2c** 53%,* yield **2d**: 68%, yield **2e**: 16%*. (*) The hydroxy acetals **9c** and **9e** were directly cyclized to give the thienopyrans **2c** and **2e**. Therefore, the yields of **2c** and **2e** are calculated starting from the bromo acetal **7** (two steps).

Scheme 2. N-Deprotection (a) and (b) and Alkylation (c) and (d)[a]



2 R	2 R	2 R
g $(CH_2)_3C_6H_5$	**j** $CH_2C_6H_4$-*p*-F	**m** C_4H_9
h $(CH_2)_4C_6H_5$	**k** CH_2-2-Th	**n** C_5H_{11}
i $CH_2C_6H_4$-*p*-OCH_3	**l** $CH_2CH{=}C(CH_3)_2$	**o** C_8H_{17}

[a] Reagents and conditions: (a) $ClCO_2CHClCH_3$, THF, −78 °C, 20 min, then MeOH, reflux, 40 min, yield 65%; (b) 2 M NaOH, dioxane/H_2O, 100 °C, 5 h, yield 54%; (c) **2g**,**h**,**j**: R-Cl, CH_3CN, K_2CO_3, reflux, yield **2g** 38%, yield **2h** 34%, yield **2j** 48%; **2d**,**l**,**m**,**o** R-Br, CH_3CN, K_2CO_3, reflux, yield **2d** 95%, yield **2l** 21%, yield **2m** 65%, **2o** 87%. (d) **2e**,**i**,**k**,**n:** **2e** isovaleraldehyde, **2i** *p*-methoxybenzaldehyde, **2k** thiophene-2-carbaldehyde; **2n** valeraldehyde, $NaBH(OAc)_3$, CH_2Cl_2, room temp, 2−4 h, yield, **2e** 83%, yield, **2i** 92%, yield; **2k** 82%, yield; **2n** 88%.

p-toluenesulfonic acid (10 mol%) to form the desired dimethyl acetal **7** in 93% yield. The halogen−metal exchange of the bromo acetal **7** with *n*-butyllihtium at −78 °C led to a thienyllithium intermediate, which was trapped with the 1-substituted piperidin-4-ones **8a** and **8b**. The intramolecular transacetalization of the resulting hydroxy acetals **9a** and **9b** was carried out with catalytic amounts of *p*-toluenesulfonic acid at room temperature to provide the spirocyclic thienopyrans **2a** and **2b** in 75% and 44% yield, respectively.

In order to get access to further N-substituted spiro[piperidin-4,4′-thieno[3.2-*c*]pyrans], the benzyl group of **2a** should be split off to obtain the secondary amine **2f** (Scheme 2). Attempts to remove the *N*-benzyl moiety of **2a** via transfer hydrogenolysis under reflux conditions with ammonium formate and Pd/C as catalyst[36] failed, even with an excess of the catalyst and very long reaction times of 3−4 days. Hydrogenolysis with hydrogen at room temperature in the presence of Pd/C also did not provide the secondary amine **2f**. An explanation for this phenomenon might be poisoning of the catalyst Pd/C by the thiophene sulfur. As solution of this problem, a catalyst independent debenzylation method seemed to be reasonable for synthesizing the secondary amine **2f**. α-Chloroethyl chloroformate is a reagent, which is able to remove N-substituents without any catalysts. Benzyl protected tertiary amines react with α-chloroethyl chloroformate under cleavage of the benzyl group to form an unstable α-chloroethyl carbamate that can easily be hydrolyzed.[37] This deprotection method provided the secondary amine **2f** in 65% yield (Scheme 2).

Alternatively, the secondary amine **2f** was synthesized by reaction of the bromo acetal **7** with the ethoxycarbonyl protected piperidone **8c** instead of the benzyl protected piperidone **8a**. Subsequent cyclization of the hydroxy acetal **9c** and hydrolysis of the ethyl carbamate **2c** upon heating with an excess of 2 M NaOH under reflux gave the secondary amine **2f** in 54% yield. However, the debenzylation of **2a** with α-chloroethyl chloroformate gave slightly higher yields than the hydrolysis of **2c** and was also advantageous because of the milder reaction conditions and shorter reaction times (1 h instead of 5 h).

The secondary amine **2f** was transformed either by alkylation with alkyl halides or by reductive alkylation with aldehydes and $NaBH(OAc)_3$[38] into the substituted spirocycles **2d**,**e**,**g**−**o**

Table 1. Affinities of the Spiro[piperidine-4,4′-thieno[3,2-*c*]pyrans] toward σ_1 and σ_2 Receptors

		$K_i \pm$ SEM (nM)[a]		
compd	R	σ1 ([^{3}H]-(+)-pentazocine)	σ_2 ([^{3}H]di-*o*-tolylguanidine)	σ_1/σ_2 selectivity
1[33]		1.3 ± 0.2	3500 ± 350	2708
2a	$CH_2C_6H_5$	0.32 ± 0.1	1260 ($n = 1$)	3940
2b	$(CH_2)_2C_6H_5$	6.0 ± 3.0	191 ($n = 1$)	32
2d	$CH_2C_6H_{11}$	0.29 ± 0.1 *(n = 4)*	25 ± 8.0	86
2e	$(CH_2)_2CH(CH_3)_2$	1.0 ± 0.3	285 ($n = 1$)	294
2f	H	602 ($n = 1$)	17%[b]	
2g	$(CH_2)_3C_6H_5$	2.4 ± 1.0	203 ($n = 1$)	90
2h	$(CH_2)_4C_6H_5$	7.7 ± 1.2	99 ± 13	13
2i	$CH_2C_6H_4$-*p*-OCH_3	2.2 ± 0.6	2970 ($n = 1$)	1325
2j	$CH_2C_6H_4$-*p*-F	0.62 ± 0.3	425 ($n = 1$)	685
2k	CH_2-2-Th	2.0 ± 0.4	1040 ($n = 1$)	515
2l	$CH_2CHC(CH_3)_2$	1.0 ± 0.2	162 ± 65	169
2m	C_4H_9	4.3 ± 0.3	246 ($n = 1$)	57
2n	C_5H_{11}	1.6 ± 0.3	460 ($n = 1$)	280
2o	C_8H_{17}	5.1 ± 1.7 ($n = 4$)	1200 ($n = 1$)	238
haloperidol		3.9 ± 1.5 ($n = 5$)	78 ± 2.0	21
di-*o*-tolylguanidine		61 ± 18 ($n = 5$)	42 ± 15 ($n = 7$)	1.4

[a] The K_i values were determined in three independent experiments ($n = 3$) unless otherwise noted. [b] Percent inhibition at a concentration of 1 μM test compound.

(Scheme 2). Because of the extraordinarily high affinity of the *N*-benzyl substituted compound **2a** (Table 1), the homologous arylalkyl derivatives **2b**, **2g**, and **2h** were synthesized and the secondary amine **2f** was substituted with benzyl bioisosteric groups, e.g., thienylmethyl (**2k**) and cyclohexylmethyl (**2d**) residues. Furthermore, spirocyclic pyran derivatives with electron rich (**2i**) and electron poor (**2j**) benzyl moieties were generated. To explore the influence of alkyl residues on the σ receptor affinity, the secondary amine **2f** was substituted with linear (**2m**, **2n**, **2o**) and branched alkyl moieties (**2e**, **2l**).

In order to shorten the synthesis, the highly potent σ_1 ligands **2d** and **2e** were also synthesized by addition of the appropriate N-substituted piperidones **8d** and **8e** to the bromo acetal **7** after treatment of **7** with *n*-BuLi. The piperidones **8d** and **8e** were prepared by alkylation of piperidin-4-one hydrochloride with cyclohexylmethyl bromide and isopentyl bromide, respectively.[39] Transacetalization of the formed hydroxy acetals **9d** and **9e** led to the spirocyclic pyran derivatives **2d** and **2e**.

σ Receptor Binding Studies

The σ_1 and σ_2 receptor affinities of the spirocyclic compounds **2a**,**b**,**d**−**o** were determined in competition experiments with radioligands. In the σ_1 assay membrane preparations of guinea pig brains were used as receptor material and [^{3}H]-(+)-pentazocine was used as radioligand. The nonspecific binding was determined in the presence of a large excess of nontritiated (+)-pentazocine. Homogenates of rat liver served as source for σ_2 receptors in the σ_2 assay. Since a σ_2 selective radioligand is not commercially available, the nonselective radioligand [^{3}H]-di-*o*-tolylguanidine was employed in the presence of an excess of nonradiolabeled (+)-pentazocine (500 nM) for selective masking of σ_1 receptors. An excess of nontritiated di-*o*-tolylguanidine was used for determination of the nonspecific binding. For highly potent ligands, the affinities were determined with six different test compound concentrations from 10 μM to 0.1 nM. When the screening with high test compound concentrations indicated low affinity, only the inhibition of the radioligand binding (in %) at a test compound concentration of 1 μM is listed.[34,40] The K_i values were calculated according to the equation of Cheng and Prusoff.[41] The σ receptor binding data of the spirocyclic thiophene derivatives **2** are summarized in Table 1.

Discussion

The bioisosteric replacement of the benzene ring of the spirocyclic benzopyran **1** for a thiophene ring results in a 4-fold increased σ_1 affinity of 0.32 nM for the spirocyclic derivative **2a** and, furthermore, an improved σ_1/σ_2 selectivity (3940-fold). As already mentioned, the size of the thiophene ring is similar to the size of the benzene ring, but the electronic properties are quite different. The replacement of the benzopyran substructure for the thienopyran substructure leads to a higher electron density in the aromatic part of **2a** (see Figure 2). Furthermore, the thiophene ring represents a better H-bond acceptor than the benzene ring. Altogether, these effects might be the reason for the increased σ_1 receptor affinity.

The unsubstituted secondary amine **2f** showed only low affinity for both σ receptor subtypes. According to the pharmacophore model of Glennon et al., two hydrophobic residues in defined distances to the basic amine are required for high σ_1 affinity.[42,43] In compound **2f** the second hydrophobic residue is missing, which explains the loss of affinity. Nevertheless, the thiophene derivative **2f** binds with considerably higher affinity for σ_1 receptors ($K_i = 602$ nM) than the corresponding secondary amine with spirocyclic benzopyran structure ($K_i =$ 19 800 nM).[33]

Replacement of the *N*-benzyl moiety of **2a** by the electron rich bioisosteric thienylmethyl (**2k**) and the *p*-methoxybenzyl residues (**2i**) resulted in compounds with high affinity for the σ_1 receptor ($K_i = 2.0$ and 2.2 nM) and a selectivity against the σ_2 receptor (see Table 1). Compared with **2a**, the electron poor *p*-fluorobenzyl derivative **2j** shows nearly the same subnanomolar σ_1 receptor affinity but an increased σ_2 affinity and thus a reduced selectivity (**2a**, $K_i(\sigma_2) = 1260$ nM; **2j**, $K_i(\sigma_2) = 425$ nM). In the case of aromatic substituents, the electronic properties of the aromatic system seem to influence the σ_2 affinity but have only minor effects toward the σ_1 affinity.

The cyclohexylmethyl moiety of the spirocyclic thienopyran **2d** represents a further bioisosteric replacement of the benzyl residue, which is nonaromatic and because of its chair conformation somewhat more voluminous. The σ_1 affinity of **2d** is extraordinarily high ($K_i = 0.29$ nM). However, **2d** displays also

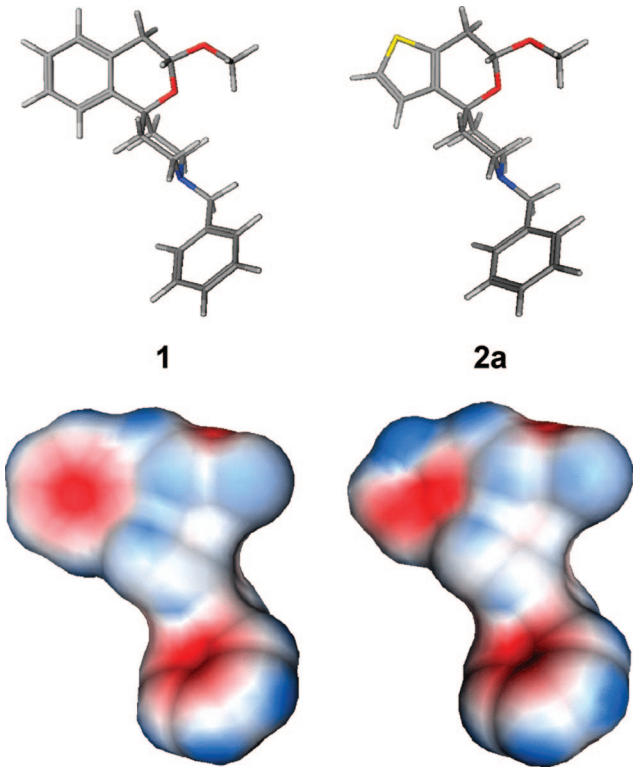


Figure 2. 3D electrostatic contour plots (Connolly analytic) calculated with the molecular modeling program MOE (Molecular Operating Environment) of the AM1 minimized (*R*)-enantiomers of σ_1 ligands **1** and **2a**. Blue regions of the surface indicate a positive electrostatic potential, and red regions indicate a negative electrostatic potential.

rather high σ_2 receptor affinity (K_i = 25 nM) and thus represents an unselective σ ligand in contrast to the bioisosteric *N*-benzyl derivative **2a**. The high affinity of **2d** indicates that an aromatic system attached to the basic nitrogen is not essential for high σ_1 affinity. In order to investigate the effect of nonaromatic moieties on the σ receptor affinities, compounds **2e**,**l**−**o** with linear and branched alkyl substituents were examined. Very low K_i values indicating high σ_1 affinity were determined for the pentyl (**2n**, K_i = 1.6 nM), isopentyl (**2e**, K_i = 1.0 nM), and isopentenyl derivatives (**2l**, K_i = 1.0 nM). The σ_1 affinities of the *N*-butyl and *N*-octyl derivatives **2m** and **2o** are slightly lower. All *N*-alkyl substituted thienopyrans display a considerably reduced σ_1/σ_2 selectivity compared to the *N*-benzyl compound **2a**.

In order to explore the influence of the distance between the aromatic system and the basic amine on the σ_1 and σ_2 receptor affinity and selectivity, the homologous *N*-arylalkyl derivatives **2b**, **2g**, and **2h** were synthesized. The *N*-phenylethyl and *N*-phenylbutyl derivatives **2b** and **2h** show a 20- to 25-fold reduced σ_1 affinity compared to **2a**. Surprisingly, the phenylpropyl derivative **2g** possesses a higher σ_1 affinity (K_i = 2.4 nM) than **2b** and **2h**. The reduced but still high σ_1 affinity of the three homologous arylalkyl derivatives **2b**, **2g**, **2h** demonstrates the tolerance of the σ_1 binding site for bulky and highly flexible moieties at the basic amino group. In contrast to the slightly decreased σ_1 affinity of the arylalkyl compounds **2b**, **2g**, and **2h** the σ_2 affinity increases with further elongation of the nitrogen−phenyl distance up to the phenylbutyl derivative **2h** with a K_i value of 99 nM. Thus, the phenylbutyl derivative **2h** shows the lowest σ_1/σ_2-selectivity (factor 13) of all investigated compounds.

Receptor Selectivity. In order to get information about the selectivity of the spirocyclic thiophene derivatives **2**, the selectivity toward the phencyclidine binding site of the NMDA receptor was investigated in receptor binding studies using MK-801 as radioligand.[40] At a concentration of 10 μM, the spirocyclic compounds **2** did not significantly bind to the NMDA receptor. Furthermore, the affinity of the highly potent σ_1 ligands toward the 5-HT_{1A} (human), 5-HT_6 (human), 5-HT_7 (human), α_1 (rat) and α_{2A} (human) receptors, and the 5-HT transporter was determined. All compounds exhibit a negligible affinity for the investigated receptor and transporter systems, indicating high selectivity for the σ_1 receptor over these receptors and transporters.

In Vivo Activity. It has been shown that σ_1 antagonists are able to reduce pain responses in the capsaicin assay.[23,24] Therefore, σ_1 antagonists are interesting compounds for the treatment of neuropathic pain. The thiophene derivative **2a** was investigated in the capsaicin assay in rats and showed at a dose of 32 mg/kg body weight (ip) a low analgesic effect of 10%. This activity indicates the spirocyclic compound **2a** could be a σ_1 antagonist.

Conclusion

The presented data indicate that the benzene−thiophene exchange leads to highly potent σ_1 receptor ligands. The thiophene derivative **2a** even exceeds the σ_1 affinity of **1** with benzopyran substructure. A similar increase of σ_1 affinity could be observed for the secondary amine **2f** compared to the NH-benzopyran derivative. An aromatic residue at the basic amine is not necessary for a high σ_1 affinity. Alkyl moieties but even better cycloalkyl moieties led to extraordinarily high σ_1 affinity but a reduced selectivity toward σ_2 receptors. The thiophene derivatives show high selectivity for the σ_1 receptor over NMDA, 5-HT_{1A}, 5-HT_6, 5-HT_7, α_{1A}, and α_2 receptors and the 5-HT transporter. Most probably, the synthesized compounds with thienopyran substructure act as antagonists at the σ_1 receptor, since **2a** reduces pain response in the capsaicin assay.

Experimental Section

Chemistry. General. For glash chromatography (fc), silica gel 60, 40−64 μm (Merck), was used. Parameters in parentheses include diameter of the column, eluent, R_f value. For ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz), a mercury 400BB spectrometer (Varian) was used. The reported δ in ppm is referenced to tetramethylsilane, and coupling constants are given with 0.5 Hz resolution. The assignments of ^{13}C and ^{1}H NMR signals were supported by 2D NMR techniques.

(*E*/*Z*)-3-Bromo-2-(2-methoxyvinyl)thiophene (6). Under N_2, the Wittig reagent **5** (13.1 g, 38.3 mmol) was suspended in THF (100 mL) at −10 °C and a solution of KO^{t}Bu (1 M in THF, 38.3 mL, 38.3 mmol) was added. After 5 min **4** (5.64 g, 29.5 mmol) was added and the mixture was stirred for 2 h at −10 °C. Then H_2O (80 mL) was added, and the mixture was extracted with Et_2O (3×). The organic layer was dried (Na_2SO_4), filtered, and concentrated in vacuo. The precipitate of triphenylphosphane oxide was washed with a mixture of cyclohexane/EtOAc, 1:1, and the organic layer was again concentrated in vacuo. The residue was purified by distillation. Colorless liquid, bp 72 °C (5.6×10^{-2} mbar), yield 5.39 g (83%). C_7H_7BrOS (219.1). ^{1}H NMR ($CDCl_3$): δ 3.64 (s, 1 H, **6**-(*E*) OC*H*$_3$), 3.77 (s, 2 H, **6**-(*Z*) OC*H*$_3$), 5.70 (dd, J = 6.6/1.9 Hz, 0.67 H, **6**-(*Z*) ThC*H*═CH), 5.89 (d, J = 12.9 Hz, 0.33 H, **6**-(*E*) ThC*H*═CH), 6.18 (d, J = 6.6 Hz, 0.67 H, **6**-(*Z*) ThCH═C*H*), 6.82 (d, J = 5.4 Hz, 0.33 H, **6**-(*E*) 4-*H*-Th), 6.85 (d, J = 5.4 Hz, 0.67 H, **6**-(*Z*) 4-*H*-Th), 6.89 (d, J = 5.4 Hz, 0.33 H, **6**-(*E*) 5-*H*-Th), 6.97 (d, J = 12.9 Hz, 0.33 H, **6**-(*E*) ThCH═C *H*), 7.09 (dd, J = 5.4/0.9 Hz, 0.67 H, **6**-(*Z*) 5-*H*-Th).

2-(3-Bromothiophen-2-yl)acetaldehyde Dimethyl Acetal (7). The enol ether **6** (5.39 g, 24.6 mmol), *p*-toluenesulfonic acid monohydrate (470 mg, 2.46 mmol), and trimethyl orthoformate (5 mL) were dissolved in MeOH. The solution was heated to reflux for 24 h.

After neutralization with NaOH (2 M, 5 mL), H_2O was added and the mixture was extracted with CH_2Cl_2 (3×). The organic layer was dried (Na_2SO_4) and concentrated in vacuo, and the residue was purified by distillation. Colorless liquid, bp 78 °C (5.6×10^{-2} mbar), yield 5.73 g (93%). $C_8H_{11}BrO_2S$ (251.1). ^{1}H NMR ($CDCl_3$): δ 3.03 (d, J = 5.6 Hz, 2 H, ThCH_2CH), 3.31 (s, 6 H, CH(OCH_3)$_2$), 4.47 (t, J = 5.6 Hz, 1 H, ThCH$_2$CH), 6.84 (d, J = 5.4 Hz, 1 H, 4-*H*-Th), 7.15 (d, J = 5.4 Hz, 1 H, 5-*H*-Th).

2-[3-(1-Benzyl-4-hydroxypiperidin-4-yl)-2-thienyl]acetaldehyde Dimethyl Acetal (9a). A solution of *n*-BuLi (1.35 M in *n*-hexane, 8.3 mL, 11.3 mmol) was added dropwise over 2−3 min to a cooled (−78 °C) solution of dimethyl acetal **7** (2.18 g, 8.7 mmol) in THF (100 mL). After the mixture was stirred for 15 min, a solution of **8a** (1.81 g, 9.57 mmol) in THF (1.5 mL) was added at −78 °C, and the mixture was stirred for 1 h at −78 °C and for 2 h at room temperature. After addition of H_2O (20 mL) and a solution of $NaHSO_3$ (10 mL, 10%) the mixture was extracted with CH_2Cl_2 (3×). The organic layer was dried (Na_2SO_4), filtered, and concentrated in vacuo. The residue was purified by fc (∅ = 5 cm, cyclohexane/EtOAc, 7:3, R_f = 0.28). Pale-yellow oil, yield 2.14 g (68%). $C_{20}H_{27}NO_3S$ (361.5). ^{1}H NMR ($CDCl_3$): δ 1.67 (dd, J = 14.0/2.5 Hz, 2 H, N(CH$_2$CH_2)$_2$), 2.04 (td, J = 13.2/4.5 Hz, 2 H, N(CH$_2$CH_2)$_2$), 2.45 (td, J = 12.3/2.1 Hz, 2 H, N(CH_2CH$_2$)$_2$), 2.62−2.71 (m, 2 H, N(CH_2CH$_2$)$_2$), 3.30 (s, 6 H, CH(OCH_3)$_2$, 3.38 (d, J = 5.4 Hz, 2 H, ThCH_2CH), 3.50 (s, 2 H, NCH_2Ph), 3.78 (s, 1 H, OH), 4.44 (t, J = 5.4 Hz, 1 H, ThCH$_2$CH), 6.83 (d, J = 5.4 Hz, 1 H, 4-*H*-Th), 6.99 (d, J = 5.4 Hz, 1 H, 5-*H*-Th), 7.12−7.23 (m, 5 H, Ph-*H*).

2-{3-[1-(Cyclohexylmethyl)-4-hydroxypiperidin-4-yl]-2-thienyl}acetaldehyde Dimethyl Acetal (9d). A solution of *n*-BuLi (1.5 M in *n*-hexane, 5.2 mL, 7.0 mmol) was added dropwise over 2−3 min to a cooled (−78 °C) solution of dimethyl acetal **7** (1.18 g, 4.7 mmol) in THF (100 mL). After the mixture was stirred for 15 min, a solution of **8d** (1.0 g, 5.2 mmol) in THF (2 mL) was added at −78 °C and the mixture was stirred for 1.5 h at −78 °C and for 2 h at room temperature. After addition of H_2O (20 mL) the mixture was extracted with CH_2Cl_2 (3×). The organic layer was dried (Na_2SO_4), filtered and concentrated in vacuo. The residue was purified by fc (∅ = 4 cm, cyclohexane/EtOAc, 1:1, R_f = 0.21). Colorless oil, yield 0.80 g (46%). $C_{20}H_{33}NO_3S$ (367.5). ^{1}H NMR ($CDCl_3$): δ 0.77−0.87 (m, 2 H, cHex-*H*), 1.07−1.20 (m, 3 H, cHex-*H*), 1.40−1.48 (m, 1 H, cHex-*H*), 1.55−1.75 (m, 5 H, cHex-*H*), 1.66 (dd, J = 13.7/2.6 Hz, 2 H, N(CH$_2$CH_2)$_2$), 2.03 (td, J = 12.6/3.3 Hz, 2 H, N(CH$_2$CH_2)$_2$), 2.11 (d, J = 7.4 Hz, 2 H, NCH$_2$cHex), 2.31 (t, J = 11.7 Hz, 2 H, N(CH_2CH$_2$)$_2$), 2.59−2.65 (m, 2 H, N(CH_2CH$_2$)$_2$), 3.29 (s, 6 H, (OCH_3)$_2$), 3.87 (d, J = 4.2 Hz, 2 H, ThCH_2CH), 3.69 (s, 1 H, OH), 4.44 (t, J = 4.2 Hz, 1 H, ThCH$_2$CH), 6.83 (d, J = 5.2 Hz, 1 H, 4-*H*-Th), 7.00 (d, J = 5.2 Hz, 1 H, 5-*H*-Th).

General Procedure A for the Cyclization of 9a−e to give 2a−e (Table 2). The corresponding hydroxy acetal **9a−e** was dissolved in MeOH. Trimethyl orthoformate and 1.2 equiv of *p*-toluenesulfonic acid monohydrate were added, and the solution was stirred at room temperature for 1−24 h). Then NaOH (2 M, 5−10 mL) and H_2O (10−20 mL) were added and the mixture was extracted with CH_2Cl_2 (3×). The organic layer was dried (Na_2SO_4), filtered, and concentrated in vacuo. The residue was purified by fc.

Table 2. Cyclization of **9a−e**: Reagents and Conditions

product	hydroxyacetal, amount (g (mmol))	MeOH (mL)	TMOF[a] (mL)	t (h)	fc ∅[b] (cm)
2a	**9a**, 0.29 (0.81)	15	2	24	2
2b	**9b**, 0.055 (0.14)	5	1	24	1
2c	**9c**, 1.49[c]	50	5	1	3
2d	**9d**, 0.80 (2.2)	30	3	3	2.5
2e	**9e**, 1.20[c]	40	4	2	4

[a] Trimethyl orthoformate. [b] fc solvent: cyclohexane/EtOAc, 7:3. [c] Amount that includes a small amount of ketone **8c** and **8e**.

1-Benzyl-6′-methoxy-6′,7′-dihydrospiro[piperidine-4,4′-thieno[3.2-*c*]pyran] (2a). Synthesis was according to general procedure A. Colorless solid, mp 78 °C, yield 199 mg (75%), R_f = 0.21 (cyclohexane/EtOAc, 7:3). Anal. ($C_{19}H_{23}NO_2S$) C, H, N. ^{1}H NMR ($CDCl_3$): δ 1.76 (dd, J = 13.5/2.1 Hz, 1 H, N(CH$_2$CH_2)$_2$), 1.80−1.93 (m, 2 H, N(CH$_2$CH_2)$_2$), 2.04 (td, J = 13.4/4.5 Hz, 1 H, N(CH$_2$CH_2)$_2$), 2.37 (td, J = 11.6/3.7 Hz, 1 H, N(CH_2CH$_2$)$_2$), 2.45 (td, J = 12.4/2.6 Hz, 1 H, N(CH_2CH$_2$)$_2$), 2.65−2.73 (m, 2 H, N(CH_2CH$_2$)$_2$), 2.77 (dd, J = 15.6/7.5 Hz, 1 H, ThCH_2CH), 2.92 (dd, J = 15.6/3.3 Hz, 1 H, ThCH_2CH), 3.48 (d, J = 13.0 Hz, 1 H, NCH_2Ph), 3.49 (s, 3 H, OCH_3), 3.54 (d, J = 13.0 Hz, 1 H, NCH_2Ph), 4.81 (dd, J = 7.2/3.3 Hz, 1 H, ThCH$_2$CH), 6.73 (d, J = 5.4 Hz, 1 H, 3′-*H*-Th), 7.02 (d, J = 5.4 Hz, 1 H, 2′-*H*-Th), 7.15−7.31 (m, 5 H, Ph-*H*). ^{13}C NMR ($CDCl_3$): δ 31.7 (1 C, Th*C*H$_2$CH), 35.7 (1 C, N(CH$_2$*C*H$_2$)$_2$), 38.8 (1 C, N(CH$_2$*C*H$_2$)$_2$), 49.4 (1 C, N(*C*H$_2$CH$_2$)$_2$), 49.5 (1 C, N(*C*H$_2$CH$_2$)$_2$), 56.8 (1 C, O*C*H$_3$), 63.7 (1 C, N*C*H$_2$Ph), 74.7 (1 C, Th*C*O), 96.9 (1 C, ThCH$_2$*C*H), 123.4 (1 C, *C*-2′-Th), 124.0 (1 C, *C*-3′-Th), 127.2 (1 C, para-*C*-Ph), 128.4 (2 C, meta-*C*-Ph), 129.5 (2 C, ortho-*C*-Ph), 130.7 (1 C, *C*-7a′-Th), 138.7 (1 C, Ph-*C*), 140.8 (1 C, *C*-3a′-Th). (329.5).

1-(Cyclohexylmethyl)-6′-methoxy-6′,7′-dihydrospiro[piperidine-4,4′-thieno[3.2-*c*]pyran] (2d). Synthesis was according to general procedure A. Colorless solid, mp 98 °C, yield 490 mg (68%), R_f = 0.45 (cyclohexane/EtOAc, 7:3). Anal. ($C_{19}H_{29}NO_2S$) C, H, N. ^{1}H NMR ($CDCl_3$): δ 0.82−0.87 (m, 2 H, cHex-*H*), 1.12−1.20 (m, 2 H, cHex-*H*), 1.41−1.51 (m, 1 H, cHex-*H*), 1.56−1.86 (m, 9 H N(CH$_2$CH_2)$_2$ and cHex-*H*), 2.05 (td, J = 13.2/4.5 Hz, 1 H, N(CH$_2$CH_2)$_2$), 2.12 (d, J = 7.0 Hz, 2 H, CH_2cHex), 2.27 (td, J = 12.1/3.3 Hz, 1 H, N(CH_2CH$_2$)$_2$), 2.34 (td, J = 12.5/2.3 Hz, 1 H, N(CH_2CH$_2$)$_2$), 2.64−2.70 (m, 2 H, N(CH_2CH$_2$)$_2$), 2.78 (dd, J = 16.0/7.1 Hz, 1 H, ThCH_2CH), 2.92 (dd, J = 15.6/3.1 Hz, 1 H, ThCH_2CH), 3.51 (s, 3 H, OCH_3), 4.81 (dd, J = 7.4/3.1 Hz, 1 H, ThCH$_2$CH), 6.72 (d, J = 5.1 Hz, 1 H, 3′-*H*-Th), 7.02 (d, J = 5.5 Hz, 1 H, 2′-*H*-Th). The synthesis of **2d** was also performed according to general procedure B using cyclohexylmethyl bromide (see Table 3). Colorless solid, mp 98 °C, yield 118 mg (95%).

6′-Methoxy-6′,7′-dihydrospiro[piperidine-4,4′-thieno[3,2-*c*]pyran] (2f). Method 1. To a solution of carbamate **2c** (0.92 g, 2.96 mmol) in dioxane/H_2O, 1:1 (50 mL), NaOH (2 M, 100 mL, 70 equiv) was added, and the mixture was heated to reflux for 5 h. After the mixture was cooled to room temperature, the aqueous solution was extracted with Et_2O (3×). The organic layer was dried (Na_2SO_4), filtered, and concentrated in vacuo. The residue was purified by fc (∅ = 3 cm, MeOH/NH_3, 98:2, R_f = 0.25). Colorless solid, mp 103 °C, yield 0.385 g (54%).

Method 2. The *N*-benzyl protected compound **2a** (100 mg, 0.31 mmol) was dissolved in THF (8 mL), and α-chloroethyl chloroformate (45 μL, 0.40 mmol) was added slowly at −78 °C. After stirring for 20 min, the mixture was warmed up to room temperature and THF was removed in vacuo. The residue was dissolved in MeOH (10 mL), and the solution was heated to reflux for 40 min. Then MeOH was evaporated in vacuo and the residue was purified by fc (∅ = 0.7 cm, EtOAc/MeOH/NH_3, 90:10:2, R_f = 0.25 (MeOH/NH_3, 98:2)). Colorless solid, mp 103 °C, yield 48 mg (65%). $C_{12}H_{17}NO_2S$ (239.3). ^{1}H NMR ($CDCl_3$): δ 1.68 (td, J = 14.0/4.7 Hz, 1 H, N(CH$_2$CH_2)$_2$), 1.76 (dd, J = 14.0/2.7 Hz, 1 H, N(CH$_2$CH_2)$_2$), 1.84−1.96 (m, 2 H, N(CH$_2$CH_2)$_2$), 2.78 (dd, J = 15.6/7.5 Hz, 1 H, ThCH_2CH), 2.84−2.88 (m, 1 H, N(CH_2CH$_2$)$_2$), 2.88−2.93 (m, 1 H, N(CH_2CH$_2$)$_2$), 2.92 (dd, J = 15.6/3.3 Hz, 1 H,

Table 3. Alkylation of **2f**: Reagents and Conditions

product	amount of **2f** (mg)	CH_3CN (mL)	alkyl halide, amount (mg (mmol))	t (h)	fc ∅[a] (cm)
2d	90 (0.37)	8	$BrCH_2C_6H_{11}$, 80 (0.45)	24	1.5
2g	120 (0.5)	15	$Cl(CH_2)_3C_6H_5$, 119 (0.6)	16	2.0
2h	89 (0.36)	10	$Cl(CH_2)_4C_6H_5$, 76 (0.45)	24	1.5
2j	90 (0.37)	8	$ClCH_2C_6H_4$-*p*-F, 65 (0.6)	20	2.0
2l	59 (0.24)	5	$BrCH_2CHC(CH_3)_2$, 45 (0.3)	20	1.0
2m	90 (0.37)	8	BrC_4H_9, 79 (0.57)	21	1.0
2o	180 (0.75)	20	BrC_8H_{17}, 174 (0.9)	16	2.0

[a] fc solvent: cyclohexane/EtOAc, 7:3.

ThCH_2CH), 3.04 (td, J = 12.3/2.7 Hz, 1 H, N(CH_2CH_2)$_2$), 3.15 (td, J = 12.3/3.0 Hz, 1 H, N(CH_2CH_2)$_2$), 3.52 (s, 3 H, OCH_3), 4.83 (dd, J = 7.2/3.3 Hz, 1 H, ThCH_2C*H*), 6.72 (d, J = 5.1 Hz, 1 H, 3′-*H*-Th), 7.03 (d, J = 5.1 Hz, 1 H, 2′-*H*-Th). A signal for the N−H proton is not seen.

Genaral Procedure B for the Alkylation of 2f with Alkyl Halides (Table 3). The secondary amine **2f** was dissolved in CH_3CN, and the alkyl halide and 6 equiv of K_2CO_3 were added. The mixture was heated to reflux for 16−24 h. K_2CO_3 was removed by filtration, and the solution was transferred into a separation funnel. After addition of brine (10−20 mL), the aqueous solution was extracted with Et_2O (3×). The organic layer was dried (Na_2SO_4), filtered, and concentrated in vacuo. The residue was purified by fc.

General Procedure C for the Reductive Alkylation of 2f (Table 4). The secondary amine **2f** was dissolved in CH_2Cl_2, and the corresponding aldehyde and $NaBH(OAc)_3$ were added. After the mixture was stirred for 2−4 h at room temperature, saturated saturated solution of $NaHCO_3$ (10 mL) was added and the aqueous layer was extracted with CH_2Cl_2 (3×). The organic layer was dried (Na_2SO_4), filtered, and concentrated in vacuo. The residue was purified by fc (∅ = 2 cm, cyclohexane/EtOAc, 7:3).

Table 4. Reductive Alkylation of **2f**: Reagents and Conditions

product	amount of **2f** (mg (mmol))	aldehyde, amount (mg (mmol))	amount of $NaBH(OAc)_3$ (mg (mmol))	t (h)
2e	58 (0.24)	O=CHCH_2CH(CH_3)$_2$, 22 (0.25)	80 (0.38)	4
2i	70 (0.29)	O=CHC$_6$H$_4$-*p*-OCH_3, 45 (0.33)	75 (0.35)	2
2k	58 (0.24)	O=CH-2-Th, 31 (0.28)	80 (0.38)	
2n	62 (0.26)	O=CH(CH_2)$_3$CH_3, 24 (0.28)	80 (0.38)	2

Receptor Binding Studies. Materials and General Procedures. Guinea pig brains and rat livers were commercially available (Harlan-Winkelmann, Germany). The homogenizer was an Elvehjem Potter (B. Braun Biotech International). The centrifuge was a high-speed cooling model Sorvall RC-5C Plus (Thermo Finnigan). The filter was printed filtermat type B (Perkin-Elmer) presoaked in 0.5% aqueous polyethylenimine for 2 h at room temperature before use. The filtration was carried out with a MicroBeta FilterMate-96 Harvester (Perkin-Elmer). The scintillation analysis was performed using Meltilex (type A) solid scintillator (Perkin-Elmer). The solid scintillator was melted on the filtermat at a temperature of 95 °C for 5 min. After solidification of the scintillator at room temperature, the scintillation was measured using a MicroBeta Trilux scintillation analyzer (Perkin-Elmer). The counting efficiency was 20%.

Membrane Preparation for the σ_1 Assay.[33,40] Five guinea pig brains were homogenized with the potter (500−800 rpm, 10 up-and-down strokes) in 6 volumes of cold 0.32 M sucrose. The suspension was centrifuged at 1200*g* for 10 min at 4 °C. The supernatant was separated and centrifuged at 23500*g* for 20 min at 4 °C. The pellet was resuspended in 5−6 volumes of buffer (50 mM Tris, pH 7.4) and centrifuged again at 23500*g* (20 min, 4 °C). This procedure was repeated twice. The final pellet was resuspended in 5−6 volumes of buffer, the protein concentration was determined according to the method of Bradford[44] using bovine serum albumin as standard, and subsequently the preparation was frozen (−80 °C) in 1.5 mL portions containing about 1.5 mg of protein/mL.

Performing of the σ_1 Assay.[33,40] The test was performed with the radioligand [^{3}H]-(+)-pentazocine (42.5 Ci/mmol, Perkin-Elmer). The thawed membrane preparation (about 75 μg of the protein) was incubated with various concentrations of test compounds, 2 nM [^{3}H]-(+)-pentazocine, and buffer (50 mM Tris, pH 7.4) in a total volume of 200 μL for 180 min at 37 °C. The incubation was terminated by rapid filtration through the presoaked filtermats by using the cell harvester. After washing each well five times with 300 μL of water, the filtermats were dried at 95 °C. Subsequently, the solid scintillator was placed on the filtermat and melted at 95 °C. After 5 min, the solid scintillator was allowed to solidify at room temperature. The bound radioactivity trapped on the filters was counted in the scintillation analyzer. The nonspecific binding was determined with 10 μM unlabeled (+)-pentazocine. The K_d value of the radioligand [^{3}H]-(+)-pentazocine is 2.9 nM.[45]

Membrane Preparation for the σ_2 Assay.[33,40] Two rat livers were cut into small pieces and homogenized with a potter (500−800 rpm, 10 up-and-down strokes) in 6 volumes of cold 0.32 M sucrose. The suspension was centrifuged at 1200*g* for 10 min at 4 °C. The supernatant was separated and centrifuged at 31000*g* for 20 min at 4 °C. The pellet was resuspended in buffer (50 mM Tris, pH 8.0) and incubated at room temperature for 30 min. After the incubation, the suspension was centrifuged again at 31000*g* for 20 min at 4 °C. The final pellet was resuspended in buffer, the protein concentration was determined according to the method of Bradford[44] using bovine serum albumin as standard, and subsequently the preparation was frozen (−80 °C) in 1.5 mL portions containing about 2 mg of protein/mL.

Performing of the σ_2-Assay.[33,40] The test was performed with the radioligand [^{3}H]-di-*o*-tolylguanidine (50 Ci/mmol, ARC). The thawed membrane preparation (about 100 μg of the protein) was incubated with various concentrations of test compounds, 3 nM [^{3}H]-di-*o*-tolylguanidine, 500 nM (+)-pentazocine, and buffer (50 mM Tris, pH 8.0) in a total volume of 200 μL for 180 min at room temperature. The incubation was terminated by rapid filtration through the presoaked filtermats using a cell harvester. After each well was washed five times with 300 μL of water, the filtermats were dried at 95 °C. Subsequently, the solid scintillator was placed on the filtermat and melted at 95 °C. After 5 min, the solid scintillator was allowed to solidify at room temperature. The bound radioactivity trapped on the filters was counted in the scintillation analyzer. The nonspecific binding was determined with 10 μM unlabeled ditolylguanidine. The K_d value of the radioligand [^{3}H]ditolylguanidine is 17.9 nM.[46]

NMDA Assay. The preparation of the receptor material and the assay were performed according to ref 40.

Data Analysis. All experiments were carried out in triplicate using standard 96-well multiplates (Diagonal). The IC_{50} values were determined in competition experiments with six concentrations of the test compounds and were calculated with the program GraphPad Prism 3.0 (GraphPad Software) by nonlinear regression analysis. The K_i values were calculated according to Cheng and Prusoff.[41] The K_i values are given as mean values +/−SEM from three independent experiments.

Supporting Information Available: Physical and spectroscopic data of all new compounds, purity data, and general chemistry methods. This material is available free of charge via the Internet at http://pubs.acs.org.

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