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Thiophene Bioisosteres of Spirocyclic σ Receptor Ligands: Relationships between Substitution Pattern and σ Receptor Affinity

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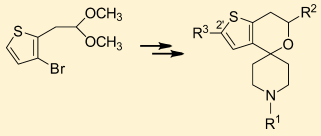
Supporting Information

ABSTRACT: On the basis of the 6′,7′-dihydrospiro-[piperidine-4,4′-thieno[3,2-*c*]pyran] framework, a series of more than 30 σ ligands with versatile substituents in 1-, 2′-, and 6′-position has been synthesized and pharmacologically evaluated in order to find novel structure−affinity relationships. It was found that a cyclohexylmethyl residue at the piperidine *N*-atom instead of a benzyl moiety led to increased σ_2 affinity and therefore to decreased σ_1/σ_2 selectivity. Small substituents (e.g., OH, OCH_3, CN, CH_2OH) in 6′-position adjacent to the O-atom were well tolerated by the σ_1 receptor. Removal of the substituent in 6′-position resulted in very potent but unselective σ ligands (**13**). A broad range of substituents with various lipophilic and *H*-bond forming properties was introduced in 2′-position adjacent to the *S*-atom without loss of σ_1 affinity. However, very polar and basic substituents in both 2′- and 6′-position decreased the σ_1 affinity considerably. It is postulated that the electron density of the thiophene moiety has a big impact on the σ_1 affinity.



R^1	R^2	R^3	σ_1	σ_1/σ_2
$CH_2C_6H_{11}$	H	H	0.66 nM	5
Bn	CN	H	1.4 nM	559
Bn	OCH_3	CN	1.1 nM	>900

INTRODUCTION

The observation of atypical pharmacological effects of typical opioid receptor ligands of the benzomorphan type led to the subclassification of opioid receptors into μ-, κ-, and σ-opioid receptors.[1] However, this classification was disproved because the opioid antagonist naloxone was not able to antagonize σ ligand mediated effects.[2] Later, it was postulated that the σ receptor and the phencyclidine (PCP) binding site of the NMDA receptor are identical.[3] This hypothesis was also disproved by the receptor binding profile of the antipsychotic agent haloperidol showing high affinity toward σ receptors but no affinity toward the PCP binding site of the NMDA receptor.[4] Today, σ receptors are well established as a nonopioid, nonphencyclidine, and haloperidol-sensitive receptor family with a characteristic binding profile and widespread distribution in the central nervous system (CNS) as well as in endocrine, immune, and some peripheral tissues (e.g., lung, kidney, heart, liver).[5,6]

Depending on their different interactions with dextrorotatory benzomorphans, the class of σ receptors was subdivided into two subtypes, which were termed σ_1 and σ_2 receptor. About 15 years ago, the σ_1 receptor was cloned from various species and tissues including guinea pig liver,[7] rat brain,[8] and human placental choriocarcinoma cell lines.[9] These cloned σ_1 receptors are more than 92% identical and more than 95% similar on the level of amino acid sequence. The rat gene encodes for a protein of 223 amino acids with a molecular weight of 25.3 kDa. With the exception of the yeast enzyme sterol-Δ^8/Δ^7-isomerase showing a 30% homology to the σ_1 receptor protein, mammalian receptors or even other known proteins do not reveal any homology with the cloned σ_1 receptor. An X-ray crystal structure of the membrane bound σ_1 receptor is not yet available. However, Aydar et al. postulated a σ_1 receptor model consisting of two transmembrane domains and both the amino and carboxy termini located intracellularly.[10] Very recently Pricl et al. reported on a calculated 3D model of the σ_1 receptor protein analyzing the ligand binding site on the level of amino acids.[11]

The σ_2 receptor is less characterized than the σ_1 receptor. A very recent report showed the identity of the σ_2 receptor and the progesterone receptor membrane component 1 (pgrmc1), which has been cloned in 1996. This protein is comprised of 194 amino acids and has a molecular weight of 21.67 kDa.[12,13]

The intracellular signal transduction pathway after activation of σ_1 receptors is not yet completely elucidated. It has been shown that σ_1 receptors play a crucial role in the regulation of a variety of ion channels including K^+-channels[14,15] and Ca^{2+}-channels.[16,17] Moreover, σ_1 receptors are involved in the modulation of various neurotransmitter systems. In particular the glutamatergic,[18] dopaminergic,[19] and cholinergic[20] neurotransmission is influenced by σ_1 receptors.

Because σ_1 receptors play a crucial role in several neurological processes, their corresponding ligands represent promising drug candidates for the treatment of various neurological and psychiatric disorders. The potential of σ_1 ligands for the treatment of anxiety, depression, memory disorders, Alzheimer's disease as well as alcohol and cocaine abuse has been shown in animal models.[21−24] Moreover, σ_1 receptors are involved in the perception of pain as an endogenous antiopioid system. Whereas σ_1 agonists (e.g., (+)-pentazocine) reduce μ-, κ-, and δ-opioid receptor mediated

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analgesia, σ_1 antagonists (e.g., haloperidol) enhance opioid induced antinociception.[25] The potential of σ_1 antagonists for the treatment of neuropathic pain has been shown by the successful phase II clinical trial with a pyrazole-based σ_1 antagonist.[26] The high density of σ_1 (and σ_2 receptors) in some human tumor cell lines (e.g., lung, prostate, breast cancer cells) has stimulated the development of novel σ ligand-based antitumor drugs and tumor diagnostics.[27,28]

In Figure 1, structurally diverse drugs in clinical use for different indications binding with high affinity toward σ_1

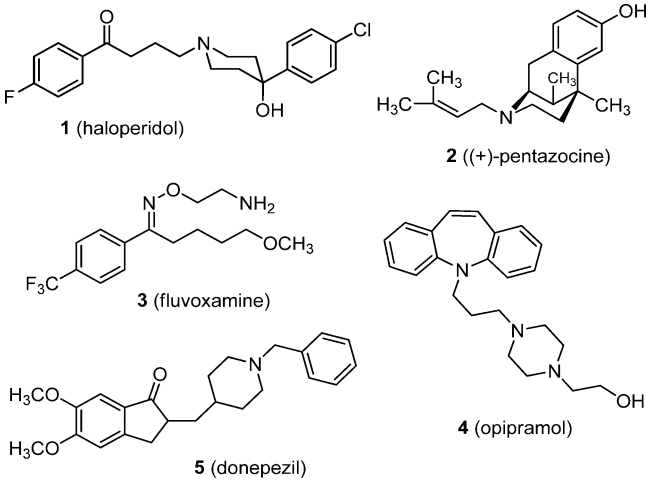


Figure 1. Clinically used drugs with additional σ_1 receptor affinity.

receptors are summarized. In addition to dopamine D_2 receptor antagonistic effects, the prototypical butyrophenone antipsychotic haloperidol (**1**, K_i (σ_1) = 3.9 nM) has potent σ_1 receptor antagonistic activity. Whereas racemic pentazocine ((±)-**2**) is used as strong analgesic, its dextrorotatory enantiomer (+)-**2** is a potent σ_1 receptor agonist (K_i (σ_1) = 4.2 nM). Both antidepressants, the selective serotonin reuptake inhibitor fluvoxamine (**3**, K_i (σ_1) = 36 nM)[29] and the tricyclic antidepressant opipramol (**4**),[30] possess high affinity toward σ_1 receptors. The neuroprotective effects of the acetylcholine esterase inhibitor donepezil (**5**), which is clinically used for the treatment of Alzheimer's disease, are supported by its σ_1 receptor antagonistic activity.[31]

Our interest has been focused on the development of novel σ_1 receptor ligands with reduced conformational flexibility giving information on the complementary surface of the σ_1 receptor binding site.[32,33] The spirocyclic benzofurans **6** and benzopyrans **7** represent very potent σ_1 receptor antagonists showing high selectivity over the σ_2 subtype.[34,35] (Figure 2) Recently, we have reported the synthesis and biological activity of the corresponding thiophene bioisosteres **8** exceeding the σ_1 receptor affinity of the parent benzene derivatives **6** and **7**.[36] Because of the characteristic chemical features of the thiophene moiety, the spirocyclic thienopyrans **8** represent an optimal starting point for broad variations of the substituents of the spirocyclic system. Herein we report on the synthesis and pharmacological evaluation of spirocyclic thienopyrans **9** being modified in the 1-, 2′- and 6′-position. Various polar substituents allowing the formation of *H*-bonds and lipophilic substituents increasing hydrophobic interactions with the receptor protein are envisaged to study ligand receptor interactions.

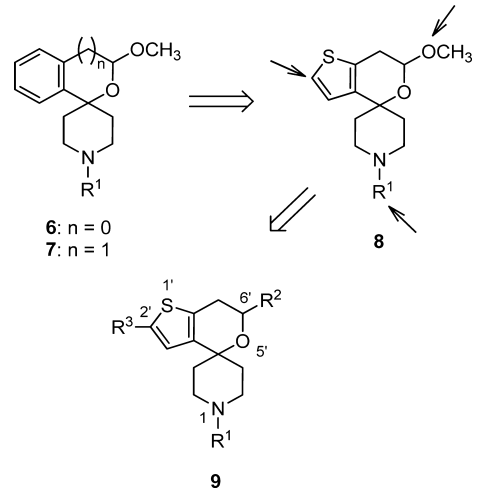
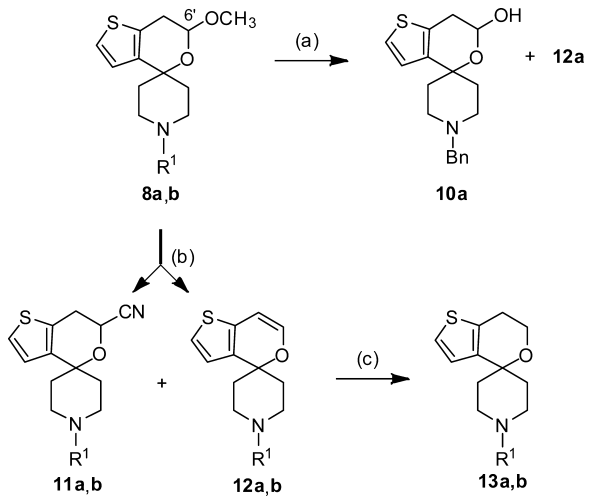


Figure 2. Development of spirocyclic σ_1 receptor ligands **9** with variations in the 1-, 2′-, and 6′-position.

CHEMISTRY

For the synthesis of spirocyclic thiophene based σ_1 ligands with various substituents in the 6′-position, the lactol (intramolecular hemiacetal) **10a** and the nitriles **11** were selected as building blocks (Scheme 1). The lactol **10a** was prepared by hydrolysis of the methyl acetal **8a** with diluted HCl. Although the elimination product **12a** was formed as side product, the desired lactol **10a** was isolated in 64% yield. Treatment of the methyl acetals **8a** and **8b** with an excess of trimethylsilyl cyanide (TMSCN) and 1.6 equiv of $BF_3{\cdot}Et_2O$[37] at −20 °C provided the nitriles **11a** and **11b** in 79% and 43% yields,

Scheme 1[a]



8, 11-13	R^1
a	Bn
b	$CH_2C_6H_{11}$

[a]Reagents and reaction conditions: (a) 2 M HCl, CH_3CN, reflux, 1 h, 64% **10a**, 15−20% **12a**; (b) TMSCN, $BF_3.Et_2O$, CH_2Cl_2, −20 °C, 30 min, 79% **11a**, 18% **12a**, 43% **11b**, 30%, **12b**;(c) H_2, Pd/C, CH_3OH, rt, 6−14 h, 85% **13a**, 64% **13b**.

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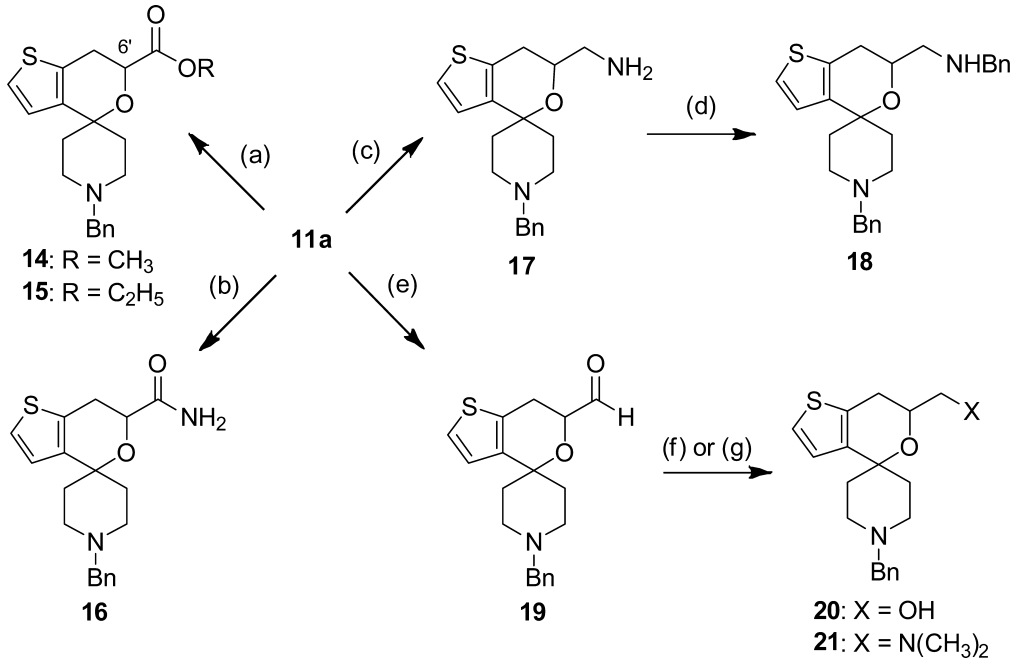
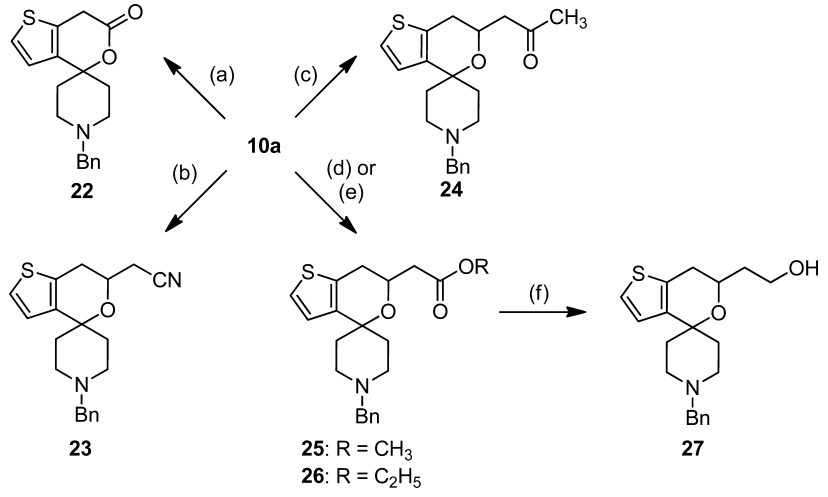
Scheme 2[a]



[a]Reagents and reaction conditions: (a) MeOH or EtOH, H_2SO_4 conc, reflux, 18−40 h, 56% **14**, 71% **15**; (b) $TiCl_4$, AcOH, 0 °C, 15 min, then H_2O, rt, 2 h, 60%; (c) $LiAlH_4$, THF, −10 °C, 1 h, 57 b%; (d) PhCH=O, $NaBH(OAc)_3$, CH_2Cl_2, rt, 1 h, 25%; (e) DIBAL, toluene, −78 °C, 45 min, 37%; (f) $NaBH_4$, MeOH, rt, 1 h, 70%; (g) $HN(CH_3)_2$, $NaBH(OAc)_3$, CH_2Cl_2, rt, 2 h, 46%.

Scheme 3[a]



[a]Reagents and reaction conditions: (a) Pr_4NRuO_4 (5 mol %), NMMO (5 equiv), CH_2Cl_2/CH_3CN, molecular sieves 4 Å, rt, 1.5 h, 66%; (b) $(Ph)_3P{=}CHCN$, Cs_2CO_3, THF, reflux, 3.5 h, 94%; (c) $(Ph)_3P{=}CH(C{=}O)CH_3$, Cs_2CO_3, THF, reflux, 12 h, 67%; (d) $(Ph)_3P{=}CHCO_2CH_3$, Cs_2CO_3, THF, reflux, 6 h, 70%; (e) $(Ph)_3P{=}CHCO_2C_2H_5$, KO^{t}Bu, THF, reflux, 10.5 h, 47%; (f) **26**, $LiAlH_4$, THF, −20 °C, 30 min, 80%.

respectively. Higher temperature or lower amounts of TMSCN led to reduced yields of the nitriles **11** and increased formation of the elimination products **12**. Nevertheless, the elimination products **12a** and **12b** were converted into the hydrogenated thienopyrans **13a** and **13b** upon treatment with H_2 and Pd/C.

The nitrile **11a** was employed for the introduction of substituents with one C-atom in 6′-position (Scheme 2). At first, the nitrile **11a** was converted into the esters **14** and **15** upon heating with concentrated H_2SO_4 in methanol or ethanol. The primary amide **16** was obtained by reaction of the nitrile **11a** with $TiCl_4$ (5 equiv) at 0 °C and subsequent hydrolysis.[38] $LiAlH_4$ reduction of the nitrile **11a**[39] led to the primary amine **17**, which was monobenzylated with benzaldehyde and $NaBH(OAc)_3$[40] to form the benzylamine **18**. The aldehyde **19** was prepared by reduction of the nitrile **11a** with diisobutylaluminum hydride (DIBAL). Reduction of the aldehyde **19** with $NaBH_4$ yielded the primary alcohol **20**, which can be regarded as homologue of the hemiacetal **10a** and constitutional isomer of the methyl acetal **8a**. Reductive amination[40] of the aldehyde **19** with dimethylamine and $NaBH(OAc)_3$ led to the tertiary amine **21**.

The lactol **10a** served as starting material for the synthesis of lactone **22** and derivatives **23**−**27** with a side chain of two carbon atoms (Scheme 3). Oxidation of the lactol **10a** was

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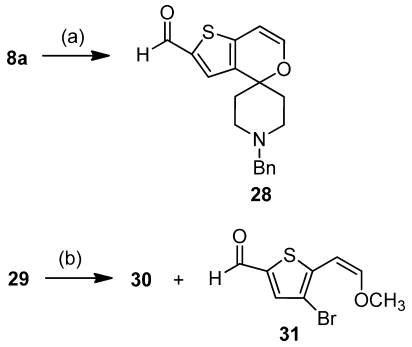
performed with Pr_4NRuO_4 (TPAP, 5 mol %) and an excess of *N*-methylmorpholine-*N*-oxide (NMMO, 1.5 equiv)[41] to afford the lactone **22** in 66% yield. The lactol substructure of **10a** has a tetrahedral geometry and can serve as an *H*-bond donor and acceptor, whereas the carbonyl moiety of **22** is planar and can only form an *H*-bond with another *H*-bond donating moiety.

For the introduction of C_2-side chains in 6′-position the lactol **10a** was reacted with phosphorylides of the type $Ph_3P{=}$CH-A (A = acceptor group). In this tandem reaction,[42] the hemiacetal **10a** was opened to form an hydroxyaldehyde, which reacted with the Wittig reagents to give an α,β-unsaturated ketone, nitrile or ester. The intramolecular conjugate addition of the tertiary alcohol at the electron deficient double bond was supported by the base Cs_2CO_3, providing the benzofuran derivatives **23**–**25** in 67–94% yields. The yield of the ethyl ester **26** did not exceed 44%, although KO^{t}Bu was used for the cyclization step. Reduction of the ethyl ester **26** with $LiAlH_4$ led to the hydroxyethyl derivative **27**, representing the next homologue in the series lactol **10a** and hydroxymethyl derivative **20**.

To introduce different substituents in 2′-position of the spirocyclic system, it was planned to modify the thienopyran **8a** after lithiation in α-position. However, deprotonation with LDA or *n*-BuLi and reaction with 1-formylpiperidine led predominantly to base catalyzed elimination of methanol, resulting in the aldehyde **28** as main product (Scheme 4).

Scheme 4[a]



[a]Reagents and reaction conditions: (a) *n*-BuLi, THF, 0 °C, 3 min, then 1-formylpiperidine, 0 °C, 1 h, 21%; (b) LDA, THF, 0 °C, 30 min, then 1-formylpiperidine, 0 °C, 30 min, 35% **30**, 20% **31**.

Therefore the substitution was performed at an earlier step of the synthesis sequence. The brominated thienylacetaldeyhde acetal **29**, which is the starting compound of the spirocyclic thienopyrans **8**,[36] was deprotonated with LDA at 0 °C and the thienyllithium intermediate was trapped with 1-formylpiperidine. This reaction led to the aldehydes **30** and **31** in 35% and 20% yields, respectively. Again the base catalyzed the elimination to produce the enol ether **31**. However, optimization of the reaction conditions (reduction of the temperature to −45 °C, increase of the amount of LDA to 1.8 equiv, reduction of the reaction time to 30 min after addition of 1-formylpiperidine) led to the aldehyde **30** in 93% yield without formation of the enol ether **31**. The aldehyde **30** was protected as dimethyl acetal **33** for further transformations (Scheme 5).

The introduction of an electron withdrawing chlorine atom in α-position was achieved by an electrophilic aromatic substitution of **29** with *N*-chlorosuccinimide (NCS) (Scheme 5). Furthermore, the sterically demanding benzyl residue should be introduced into the thiophene moiety, which increases the electron density in the thiophene ring and the lipophilic interactions with the receptor protein but is not able to form any *H*-bonds. The direct benzylation of **29** with benzyl bromide after deprotonation with LDA provided the benzylated derivative **35** in only 24% yield. Therefore the hydroxybenzyl derivative **32** was prepared by addition of phenylmagnesium bromide to the aldehyde **30** (87%) or by addition of benzaldehyde to the lithiated thiophene derivative **29** (92%). Finally, the hydroxy group of **32** was removed reductively with butylsilane ($BuSiH_3$) in the presence of tris(pentafluorophenyl)borane ($B(C_6F_5)_3$)[43] to obtain the benzylated product **35** in 58% yield.

Next, the spirocyclic piperidines **39**–**41** were obtained by performing a halogen-metal exchange of the brominated thiophene derivatives **33**–**35** with *n*-BuLi and subsequent trapping of the thienyllithium intermediates with 1-benzylpiperidin-4-one. Finally, intramolecular transacetalization of **36**–**38** was catalyzed by *p*-toluenesulfonic acid to afford the spirocyclic piperidines **39**–**41** (Scheme 5).

The thiophenecarbaldehyde acetal **39** was exploited for the introduction of additional substituents in 2′-position. Treatment of **39** with 1 M HCl at room temperature led exclusively to hydrolysis of the dimethyl acetal, providing the aldehyde **42** in 96% yield (Scheme 6). The high chemoselectivity of this transformation is explained by the higher reactivity of the aromatic dimethyl acetal compared with the reactivity of the aliphatic acetal within the pyran moiety.

Treatment of the aldehyde **42** with $LiAlH_4$ gave the primary alcohol **43** in 57% yield. Reductive amination with dimethylamine and $NaBH(OAc)_3$ converted the aldehyde **42** into the tertiary amine **44**. Nucleophilic addition of the Grignard reagent phenylmagnesium bromide to the aldehyde **42** produced the hydroxybenzyl derivative **45**, which was oxidized with TPAP and NMMO to afford the ketone **46**. The oxime **47** was obtained by condensation of the aldehyde **42** with hydroxylamine. Dehydration of the oxime **47** with phthalic anhydride[44] led to the nitrile **48**, which was transformed into the methyl ester **49** upon heating with methanol and concentrated H_2SO_4.

RECEPTOR BINDING STUDIES

The σ_1 and σ_2 receptor affinities of the spirocyclic thiophenes of the general formula **9** were determined in competition experiments with radioligands. The highly σ_1 selective radioligand [^{3}H]-(+)-pentazocine (compare compound **2** in Figure 1) and homogenates of guinea pig brains were used in the σ_1 assay. The nonspecific binding was recorded with a large excess of nonradiolabeled (+)-pentazocine. In the σ_2 assay membrane preparations of rat liver served as source for σ_2 receptors. The nonselective radioligand [^{3}H]-di-*o*-tolylguanidine was employed in the σ_2 assay because a σ_2 selective radioligand is not commercially available. To mask the σ_1 receptors, an excess of nontritiated (+)-pentazocine (500 nM) was added to the assay solution. A high concentration of nontritiated di-*o*-tolylguanidine was used to determine the nonspecific binding. Generally the IC_{50}-values of all ligands were recorded with six different test compound concentrations ranging from 10 μM to 0.1 nM.[34,35,45,46] In the case of very potent ligands (e.g., **8a**, **11a**), a further dilution step (0.01 nM) was necessary to improve the accuracy of the nonlinear regression analysis. The

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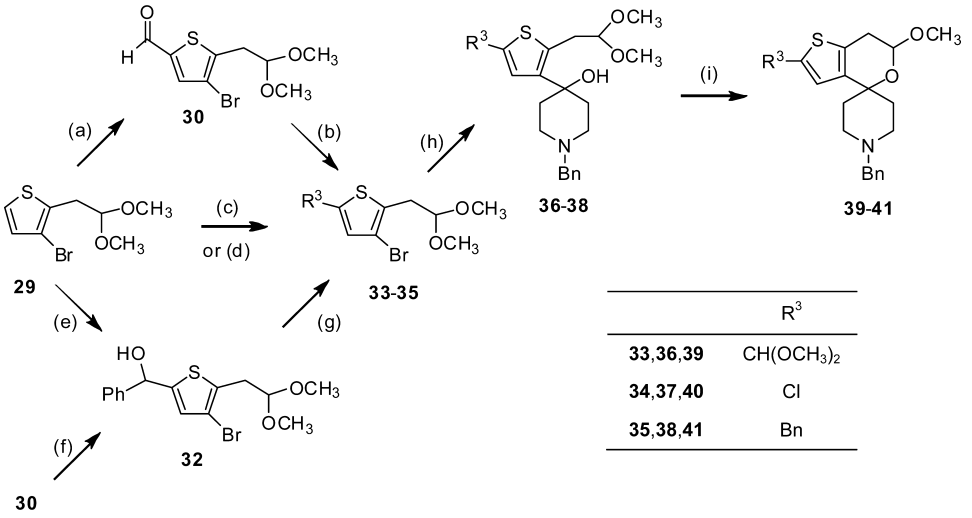
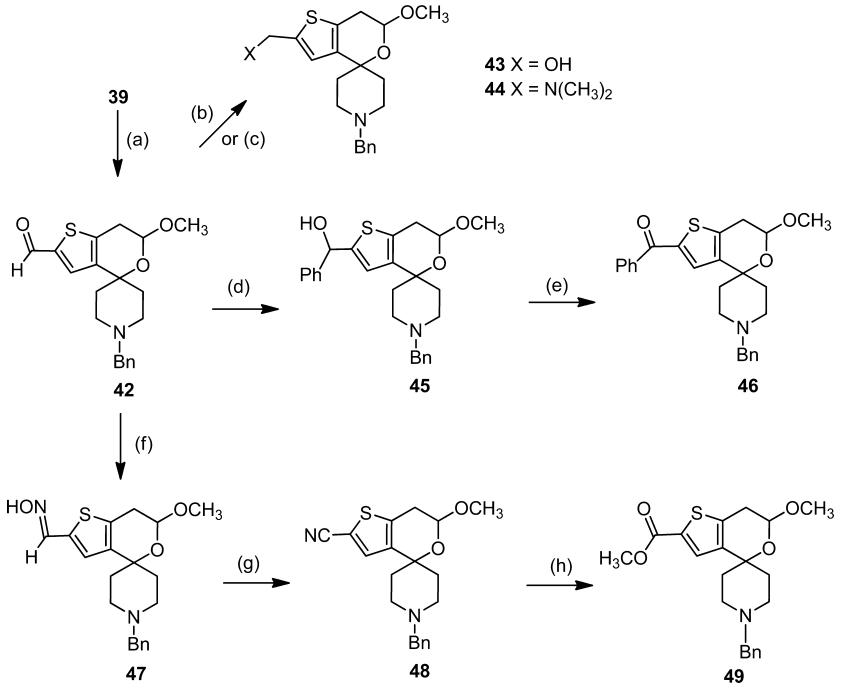
Scheme 5[a]



	R^3
33,36,39	$CH(OCH_3)_2$
34,37,40	Cl
35,38,41	Bn

[a]Reagents and reaction conditions: (a) LDA, THF, −40 °C, 10 min, then *N*-formylpiperidine, −40 °C, 30 min, 93%; (b) CH_3OH, $HC(OCH_3)_3$, *p*-$TolSO_3H$, 65 °C, 3 h, 92%; (c) NCS, CH_2Cl_2/CH_3OH 1:1, rt, 3 h, 60%; (d) LDA, THF, −45 °C, 10 min, then benzyl bromide, −40 °C, 30 min, 24%; (e) LDA, THF, −40 °C, 10 min, then benzaldehyde, −40 °C, 30 min, 92%; (f) PhMgBr, THF, 0 °C, 10 min, 87%; (g) $BuSiH_3$, $B(C_6F_5)_3$, CH_2Cl_2, rt, 8 h, 58%; (h) *n*-BuLi, THF, −78 °C, 15 min, then 1-benzylpiperidin-4-one, −78 °C, 1−2 h, rt, 1 h; (i) CH_3OH, $HC(OCH_3)_3$, *p*-$TolSO_3H$, rt, 1−3 H, 32% **39**, 33% **40**, 26% **41**.

Scheme 6[a]



[a]Reagents and reaction conditions: (a) 1 M HCl, CH_3CN, rt, 15 min, 96%; (b) $LiAlH_4$, THF, −40 °C, 30 min, 57%; (c) $HN(CH_3)_2$, $NaBH(OAc)_3$, CH_2Cl_2, rt, 1.5 h, 77%; (d) PhMgBr, THF, 0 °C, 15 min, 98%; (e) Pr_4NRuO_4 (5 mol %), NMMO (5 equiv), CH_2Cl_2, molecular sieves 4 Å, rt, 30 min, 72%; (f) H_2NOH HCl, pyridine, 60 °C, 30 min, 70%; (g) phthalic anhydride, pyridine, 90 °C. 1 h, 67%; (i) CH_3OH, H_2SO_4 conc, reflux, 22 h, 60%.

IC_{50}-values were transformed into K_i-values by the equation of Cheng and Prusoff.[47]

To investigate the receptor selectivity, the affinity of all spirocyclic thiophenes toward the phencyclidine binding site of the NMDA receptor was recorded with pig brain cortex preparations and the radioligand [^{3}H]-(+)-MK-801.[48] Moreover, the affinities of selected compounds toward the human σ_1 receptor, the 5-HT_6, 5-HT_7, and α_{2A} receptor were determined in receptor binding studies.

DISCUSSION

The σ receptor binding data of the synthesized spirocyclic thiophene derivatives are summarized in Tables 1−3. Table 1 shows that the σ_1 affinities of the cyclohexylmethyl derivatives

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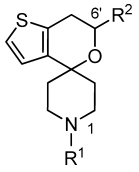
Table 1. Affinities of the Spiro[piperidine-4,4′-thieno[3,2-*c*]pyrans] with Different Substituents in Position 1 and 6′ towards σ_1 and σ_2 Receptors



			$K_i \pm$ SEM (nM) (n = 3)[a]		
compd	R^1	R^2	σ_1	σ_2	σ_1/σ_2 selectivity
6a[34]	Bn[b]		1.1 ± 0.22	1280 ± 137	1160
7a[34]	Bn[b]		1.3 ± 0.18	3500 ± 352	2700
8a[36]	Bn	OCH_3	0.32 ± 0.1	1260	3940
8b	$CH_2C_6H_{11}$	OCH_3	0.29 ± 0.1	25 ± 8.0	86
10a	Bn	OH	3.6 ± 0.7	391	109
11a	Bn	CN	1.4 ± 0.2	783	560
11b	$CH_2C_6H_{11}$	CN	0.46 ± 0.04	26 ± 4.0	57
12a	Bn	$C^{6'}=C^{7'}$	2.0 ± 0.5	61 ± 18	31
12b	$CH_2C_6H_{11}$	$C^{6'}=C^{7'}$	1.7 ± 0.1	45 ± 12	26
13a	Bn	H	0.31 ± 0.06	13 ± 2.5	42
13b	$CH_2C_6H_{11}$	H	0.66 ± 0.16	3.3 ± 0.3	5
haloperidol			3.9 ± 1.5	78 ± 2.0	20
di-*o*-tolylguanidine			61 ± 18	42 ± 15	0.7
(+)-pentazocine			4.2 ± 1.1		

[a]Triplicates were recorded only for high affinity compounds. The affinity of compounds, which did not reduce significantly the radioligand binding in the first assay, were recorded only once. [b]The structures of **6a** and **7a** are shown in Figure 2.

(**b**-series) are comparable with the σ_1 affinities of the corresponding benzyl derivatives (**a**-series). In both series of compounds, the cyano moiety can be regarded as bioisosteric replacement of the methoxy group because the corresponding K_i-values of **8** and **11** are very similar (e.g., **8b**, K_i = 0.30 nM; **11b**, K_i = 0.46 nM). In addition to the methoxy derivatives **8** and the nitriles **11** the unsubstituted compounds **12** and **13** reveal low nanomolar and even subnanomolar σ_1 affinities.

Taking the σ_2 affinity into account, two general rules were derived from this series of compounds. Replacement of the *N*-benzyl moiety with the *N*-cyclohexylmethyl residue led to a considerable increase of the σ_2 affinity and thus to a reduced σ_1/σ_2-selectivity (e.g., **11a**, σ_1/σ_2 = 560; **11b**, σ_1/σ_2 = 57). Second, an increased σ_2-affinity and thus reduced σ_1/σ_2-selectivity was observed upon removal of the substituent in 6′-position of the spirocyclic system (e.g., **8a**, σ_1/σ_2 3940; **12a**, σ_1/σ_2 = 31; **13a**, σ_1/σ_2 = 42). Therefore, in the next series of compounds, the *N*-benzyl moiety was retained and the substituents in 6′-position were varied. (Table 2)

In Table 2, the affinity data of spirocyclic thiophene derivatives with various substituents in 6′-position are summarized. Small substituents in 6′-position like a methoxy group of **8a**, a hydroxy group of **10a**, and a cyano group of **11a** gave very potent σ_1 ligands. However, a carbonyl group (**22**) was not tolerated by the σ_1 receptor protein. Increasing of the chain length of the homologous alcohols **10a**, **20**, and **27** led to continuous decrease of σ_1 affinity, indicating that the size of the substituent tolerated by the σ_1 receptor is limited. Comparing the σ_1 affinities of the homologous nitriles **11a** and **23** showed the same tendency of 50-fold reduced σ_1 affinity by extension of the side chain. Esters **14** and **15** bearing the carbonyl moiety directly at the spirocyclic system revealed lower σ_1 affinities than the corresponding homologous **24**–**26** with an additional CH_2-moiety between the spirocyclic system and the carbonyl moiety. Very polar (e.g., primary amide in **16**) and basic substituents (e.g., primary amine in **17**, benzylamine in **18**, dimethylamine in **21**) were less tolerated by the σ_1 receptor protein.

It is remarkable that the compounds with substituents in 6′-position generally show low affinity to the σ_2 receptor, indicating high σ_1/σ_2 selectivity.

To expand the structure–affinity relationships of these types of spirocyclic compounds, substituents with various *H*-bond forming and lipophilic properties were introduced in 2′-position adjacent to the *S*-atom. For this purpose the benzyl substituent at the piperidine *N*-atom and the methoxy group in 6′-position were selected because these structural elements led to the highest σ_1 affinity and σ_1/σ_2 selectivity in this class of compounds (compare compound **8a**). The σ affinities of the compounds are depicted in Table 3.

It can be clearly seen that the σ_1 receptor accepts a broad variety of substituents at the 2′-position of the spirocyclic system. These substituents include the lipophilic benzyl, α-hydroxybenzyl, and benzoyl moieties of **41** (K_i = 3.3 nM), **45** (K_i = 3.6 nM), and **46** (K_i = 3.7 nM), which cannot form any *H*-bonds (**41**), react as *H*-bond donor and acceptor (**45**), or only as *H*-bond acceptor (**46**). Similar high σ_1 receptor affinities were determined for the acetal **39**, the chloro derivative **40**, the alcohol **43**, and the nitrile **48**. Low σ_1 affinities for compounds bearing very polar or basic substituents in 2′-position (e.g., oxime **47**, tertiary amine **44**) were observed to be following the same trend as the compounds with polar and basic substituents in the 6′-position.

As discussed before, the *N*-benzyl moiety and the 6′-methoxy group are responsible for the preferred interaction of the ligands with the σ_1 receptor. Table 3 clearly shows that additional substituents in 2′-position do not change this situation. The σ_2 affinity of all 2′-substituted compounds is very low, leading to high σ_1/σ_2 selectivity of these compounds.

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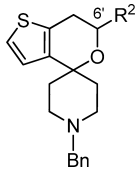
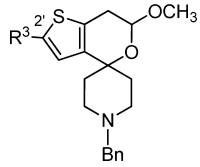
Table 2. Affinities of the Spiro[piperidine-4,4′-thieno[3,2-*c*]pyrans] with Different Substituents in Position 6′ towards σ_1 and σ_2 Receptors



		$K_i \pm$ SEM (nM) (n = 3)[a]		
compd	R^2	σ_1	σ_2	σ_1/σ_2 selectivity
8a[36]	OCH_3	0.32 ± 0.10	1260	3940
10a	OH	3.6 ± 0.7	391	109
11a	CN	1.4 ± 0.20	783	559
14	CO_2CH_3	29 ± 3.2	>1000	>34
15	$CO_2C_2H_5$	144	>1000	>7.0
16	$CONH_2$	350	>1000	>2.8
17	CH_2NH_2	98	>1000	>10
18	CH_2NHBn	196 ± 24	621	3.1
20	CH_2OH	5.4 ± 1.5	>1000	>185
21	$CH_2N(CH_3)_2$	63	>1000	>16
22	=O	296	>1000	>3
23	CH_2CN	64 ± 16	>1000	>15
24	$CH_2C(=O)CH_3$	9.4 ± 4.9	>1000	>106
25	$CH_2CO_2CH_3$	12 ± 0.7	>1000	>83
26	$CH_2CO_2C_2H_5$	20 ± 2.8	>1000	>50
27	CH_2CH_2OH	27 ± 4.3	>1000	>37
haloperidol		3.9 ± 1.5	78 ± 2.0	20
di-*o*-tolylguanidine		61 ± 18	42 ± 15	0.7
(+)-pentazocine		4.2 ± 1.1		

[a]Triplicates were recorded only for high affinity compounds. The affinity of compounds, which did not reduce significantly the radioligand binding in the first assay, were recorded only once.

Table 3. Affinities of the Spiro[piperidine-4,4′-thieno[3,2-*c*]pyrans] with Different Substituents in Position 2′ towards σ_1 and σ_2 Receptors



		$K_i \pm$ SEM (nM) (n = 3)[a]		
compd	R^3	σ_1	σ_2	σ_1/σ_2 selectivity
8a[36]	H	0.32 ± 0.10	1260	3940
39	$CH(OCH_3)_2$	2.3 ± 0.4	>1000	>430
40	Cl	1.3 ± 0.1	>1000	>770
41	$CH_2C_6H_5$	3.3 ± 0.7	411	125
43	CH_2OH	5.8 ± 1.2	>1000	>170
44	$CH_2N(CH_3)_2$	17 ± 0.6	898	53
45	$CH(OH)C_6H_5$	3.6 ± 1.5	882 ± 17	245
46	$(C=O)C_6H_5$	3.7 ± 1.4	427 ± 163	115
47	CH=NOH	39 ± 2.7	>1000	>25
48	CN	1.1 ± 0.1	>1000	>900
49	CO_2CH_3	11 ± 1.3	>1000	>90
haloperidol		3.9 ± 1.5	78 ± 2.0	20
di-*o*-tolylguanidine		61 ± 18	42 ± 15	0.7
(+)-pentazocine		4.2 ± 1.1		

[a]Triplicates were recorded only for high affinity compounds. The affinity of compounds, which did not reduce significantly the radioligand binding in the first assay, were recorded only once.

Next, the affinity of the nitrile **11a** and the unsubstituted compound **13a** at the human σ_1 receptor was recorded in addition to their affinities toward the guinea pig brain σ_1 receptor. The resulting K_i-values of 4.5 ± 0.5 nM (**11a**) and 2.1 ± 0.4 nM (**13a**) are in good accordance with the K_i-values obtained in the guinea pig brain assay (see Table 1).

Because of the historical correlation of the σ receptor and the phencyclidine binding site of the NMDA receptor (see Introduction) and, moreover, the interaction of these receptors with similar compounds,[49,50] the NMDA receptor affinity of all synthesized spirocyclic compounds was also recorded. Up to a test compound concentration of 1 μM, significant interactions with the NMDA receptor were not observed, indicating high selectivity of the spirocyclic thiophenes over this receptor. Additionally, the nitrile **11a** and the unsubstituted compound **13a** did not interact with the 5-HT_6, 5-HT_7, and α_{2A} receptors.

Replacement of the benzene moiety of the benzopyran **7a** with the thiophene moiety (**8a**) led to a 4-fold increase of the σ_1 receptor affinity. Introduction of a chloro (**40**) or cyano group (**48**) in 2′-position adjacent to the *S*-atom resulted in σ_1 affinities, which are very similar to the σ_1 affinities of the benzene derivative **7a**. As the introduction of a chloro or cyano substituent into the thiophene system decreases the electron density of the thiophene ring, it can be assumed that the electron density of the aromatic system has a significant impact on the interaction with the σ_1 receptor. Compound **8a** with the unsubstituted electron rich thiophene ring has a σ_1 receptor affinity close to the optimum. The reduced negative electrostatic potential (red color) of the thiophene ring of **40** and **48** compared with **8a** is displayed in Figure 3.

CONCLUSION

A flexible synthetic approach allows the introduction of versatile substituents in various positions of the spirocyclic system **9**. The large series of compounds represents the basis for the establishment of structure−affinity relationships. To achieve high σ_1 affinity and high selectivity against the σ_2 subtype, a benzyl moiety should be attached to the piperidine *N*-atom and a small substituent (e.g., OH, CN, CH_2OH) should be introduced into the6′-position. Whereas compounds without a substituent in the 6′-position represent potent but unselective σ ligands, compounds with larger substituents in the 6′-position show reduced σ_1 affinity. The σ_1 receptor tolerates well substituents in the 2′-position with different lipophilic and *H*-bond forming properties. However, it does not accept very polar and basic substituents in the 2′- and 6′-positions. The similar K_i-values obtained for **11a** and **13a** in σ_1 assays using guinea pig and human σ_1 receptors show the good correlation between guinea pig brain and human σ_1 receptors.

EXPERIMENTAL SECTION

Chemistry, General. Unless otherwise noted, moisture sensitive reactions were conducted under dry nitrogen. Flash chromatography (fc): silica gel 60, 40−64 μm (Merck). Parentheses include: diameter of the column, eluent, R_f value. ^{1}H NMR (400 MHz): Mercury-400BB spectrometer (Varian); δ in ppm related to tetramethylsilane; 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. Elemental analysis: CHN-Rapid Analysator (Fons-Heraeus). The purity of all test compounds was determined by HPLC analysis (purity >95%).

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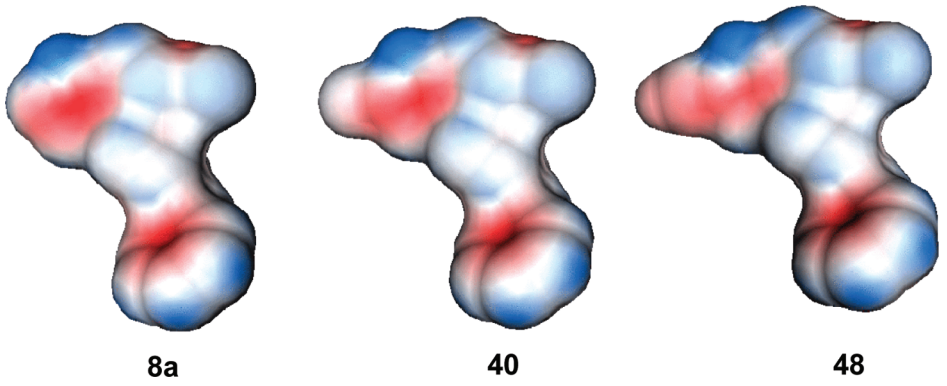

Figure 3. 3D electrostatic contour plots (Connolly Analytic) calculated with the molecular modeling software MOE (Molecular Operating Environment) for the AM1 minimized (*R*)-enantiomers of σ_1 ligands **8a**, **40**, and **48**. Blue regions of the surface indicate a positive, red regions a negative electrostatic potential.

Additionally elemental analyses were performed for key compounds; all values are within ±0.4%.

*1-Benzyl-6′,7′-dihydrospiro[piperidine-4,4′-thieno[3,2-c]pyran]-6′-ol (**10a**).* HCl (2 M, 1 mL) was added to a solution of **8a** (74 mg, 0.22 mmol) in CH_3CN (4 mL). The mixture was heated to reflux for 1 h. After addition of 2 M NaOH (pH > 9), the mixture was extracted with Et_2O (3 × 5 mL). The organic layer was dried (Na_2SO_4), filtered, and concentrated in vacuo, and the residue was purified by recrystallization with CH_3CN. Colorless solid, mp 184 °C, yield 45 mg (64%), $C_{18}H_{21}NO_2S$ (315.2). ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.76 (dd, *J* = 13.6/2.3 Hz, 1 H, $N(CH_2CH_2)_2$), 1.80−1.89 (m, 2 H, $N(CH_2CH_2)_2$), 2.04 (td, *J* = 13.2/4.4 Hz, 1 H, $N(CH_2CH_2)_2$), 2.37 (td, *J* = 11.7/4.5 Hz, 1 H, $N(CH_2CH_2)_2$), 2.43 (td, *J* = 11.7/2.5 Hz, 1 H, $N(CH_2CH_2)_2$), 2.62−2.72 (m, 2 H, $N(CH_2CH_2)_2$), 2.74 (dd, *J* = 15.5/7.3 Hz, 1 H, $ThCH_2CH$), 2.91 (s, 1 H, *OH*), 3.00 (dd, *J* = 15.5/3.1 Hz, 1 H, $ThCH_2CH$), 3.48 (d, *J* = 14.2 Hz, 1 H, NCH_2Ph), 3.51 (d, *J* = 14.2 Hz, 1 H, NCH_2Ph), 5.25 (dd, *J* = 7.3/3.1 Hz, 1 H, $ThCH_2CH$), 6.74 (d, *J* = 5.2 Hz, 1 H, 3′-*H*-Th), 7.04 (d, *J* = 5.2 Hz, 1 H, 2′-*H*-Th), 7.16−7.29 (m, 5 H, Ph-*H*).

*1-Benzyl-6′,7′-dihydrospiro[piperidine-4,4′-thieno[3,2-c]pyran]-6′-carbonitrile (**11a**) and 1-Benzylspiro[piperidine-4,4′-thieno[3,2-c]pyran] (**12a**).* Trimethylsilyl cyanide (TMSCN, 0.88 mL, 7 mmol) and $BF_3{\cdot}Et_2O$ (0.16 mL, 1.6 mmol) were added to a cooled solution (−20 °C) of **8a** (290 mg, 0.88 mmol) in CH_2Cl_2 (3.5 mL). At first, the reaction mixture was stirred for 30 min at −20 °C, then for 15 min at 0 °C. Then MeOH (3 mL) and 2 M NaOH (3 mL) were added (pH > 9) and the mixture was extracted with CH_2Cl_2 (3 × 5 mL). The organic layer was dried (Na_2SO_4), filtered, concentrated in vacuo, and the residue was purified by fc (2 cm, cyclohexane:EtOAc, 7:3, R_f (**11a**) = 0.34), R_f (**12a**) = 0.42).

11a: Colorless solid, mp 123 °C, yield 228 mg (79%), $C_{19}H_{20}N_2OS$ (324.4). ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.77 (dd, *J* = 13.8/2.7 Hz, 1 H, $N(CH_2CH_2)_2$), 1.80−1.89 (m, 2 H, $N(CH_2CH_2)_2$), 2.03 (td, *J* = 13.2/4.5 Hz, 1 H, $N(CH_2CH_2)_2$), 2.33 (td, *J* = 11.8/3.1 Hz, 1 H, $N(CH_2CH_2)_2$), 2.43 (td, *J* = 12.6/2.7 Hz, 1 H, $N(CH_2CH_2)_2$), 2.64−2.75 (m, 2 H, $N(CH_2CH_2)_2$), 3.04 (dd, *J* = 15.6/3.9 Hz, 1 H, $ThCH_2CH$), 3.16 (dd, J = 15.6/9.0 Hz, 1 H, $ThCH_2CH$), 3.48 (d, *J* = 13.0 Hz, 1 H, NCH_2Ph), 3.53 (d, *J* = 13.0 Hz, 1 H, NCH_2Ph), 4.65 (dd, *J* = 9.0/3.3 Hz, 1 H, $ThCH_2CH$), 6.75 (d, *J* = 5.1 Hz, 1 H, 3′-*H*-Th), 7.09 (d, *J* = 5.1 Hz, 1 H, 2′-*H*-Th), 7.19−7.31 (m, 5 H, Ph-*H*). Anal. ($C_{19}H_{20}N_2OS$) C, H, N.

12a: Colorless solid, mp 71 °C, yield 47 mg (18%), $C_{18}H_{19}NOS$ (297.4). ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.84 (td, *J* = 13.4/4.6 Hz, 2 H, $N(CH_2CH_2)_2$), 2.16 (dd, *J* = 14.3/2.1 Hz, 2 H, $N(CH_2CH_2)_2$), 2.37 (td, *J* = 12.5/2.4 Hz, 2 H, $N(CH_2CH_2)_2$), 2.68 (m, 2 H, $N(CH_2CH_2)_2$), 3.50 (s, 2 H, N-CH_2-Ph), 5.71 (d, *J* = 5.9 Hz, 1 H, Th-CH=C*H*), 6.38 (d, *J* = 5.9 Hz, 1 H, Th-C*H*=CH), 6.71 (d, *J* = 5.1 Hz, 1 H, 3′-*H*-Th), 6.97 (d, *J* = 5.1 Hz, 1 H, 2′-*H*-Th), 7.19−7.31 (m, 5 H, Ph-*H*).

*1-Benzyl-6′,7′-dihydrospiro[piperidine-4,4′-thieno[3,2-c]pyran] (**13a**).* Pd/C (50 mg, 10% (m/m)) was added to a solution of **12a** (200 mg, 0.67 mmol) in CH_3OH (20 mL). The mixture was shaken under H_2 pressure (4.5 bar) for 14 h at rt. After filtration, the solvent was evaporated in vacuo. The residue was purified by fc (2 cm, cyclohexane:EtOAc, 7:3, R_f = 0.2). Colorless oil, yield 172 mg (85%), $C_{18}H_{21}NOS$ (299.4). ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.77 (dd, *J* = 14.4/2.3 Hz, 2 H, $N(CH_2CH_2)_2$), 1.91 (td, *J* = 13.4/4.5 Hz, 2 H, $N(CH_2CH_2)_2$), 2.33 (td, *J* = 12.6/2.5 Hz, 2 H, $N(CH_2CH_2)_2$), 2.64−2.67 (m, 2 H, $N(CH_2CH_2)_2$), 2.75 (t, *J* = 5.4 Hz, 2 H, $ThCH_2CH_2$), 3.49 (s, 2 H, NCH_2Ph), 3.85 (t, *J* = 5.4 Hz, 2 H, $ThCH_2CH_2$), 6.74 (d, *J* = 5.5 Hz, 1 H, 3′-*H*-Th), 6.98 (d, *J* = 5.5 Hz, 1 H, 2′-*H*-Th), 7.19−7.30 (m, 5 H, Ph-*H*).

*1-(Cyclohexylmethyl)-6′,7′-dihydrospiro[piperidine-4,4′-thieno-[3,2-c]pyran] (**13b**).* Pd/C (15 mg, 10% (m/m)) was added to a solution of **12b** (60 mg, 0.2 mmol) in CH_3OH (8 mL). The mixture was shaken under H_2 pressure (4.5 bar) for 6 h at rt. After filtration, the solvent was evaporated in vacuo. The residue was purified by fc (0.7 cm, cyclohexane:EtOAc, 9:1, R_f = 0.2). Colorless solid, mp 77 °C, yield 31 mg (51%), $C_{18}H_{27}NOS$ (305.5). ^{1}H NMR ($CDCl_3$): δ (ppm) = 0.83−0.95 (m, 2 H, cHex-*H*), 1.13−1.30 (m, 3 H, cHex-*H*), 1.45−1.55 (m, 1 H, NCH_2-C*H*$(CH_2)_5$), 1.63−1.74 (m, 3 H, cHex-*H*), 1.75−1.83 (m, 2 H, cHex-*H*), 1.83 (dd, *J* = 14.4/2.8 Hz, 2 H, $N(CH_2CH_2)_2$), 1.96 (td, *J* = 13.6/4.5 Hz, 2 H, $N(CH_2CH_2)_2$), 2.17 (d, *J* = 7.2 Hz, 2 H, NCH_2cHex), 2.27 (td, *J* = 12.8/2.8 Hz, 2 H, $N(CH_2CH_2)_2$), 2.65−2.71 (m, 2 H, $N(CH_2CH_2)_2$), 2.82 (t, *J* = 5.6 Hz,2 H, $ThCH_2CH_2$), 3.92 (t, *J* = 5.6 Hz, 2 H, $ThCH_2CH_2$), 6.81 (d, *J* = 5.2 Hz, 1 H, 3′-*H* -Th), 7.06 (d, J = 5.2 Hz, 1 H, 2′-*H*-TH). Anal. ($C_{18}H_{27}NOS$) C, H, N.

*(1-Benzyl-6′,7′-dihyrospiro[piperidine-4,4′-thieno[3,2-c]pyran]-6′-yl)methanol (**20**).* $NaBH_4$ (20 mg, 0.52 mmol) was added to a solution of **19** (85 mg, 0.26 mmol) in CH_3OH (3 mL) at 0 °C. After 15 min stirring at 0 °C, the mixture was stirred at rt for 45 min. Then, brine was added and the mixture was extracted with CH_2Cl_2 (3 × 5 mL). The organic layer was dried (Na_2SO_4), filtered, and concentrated in vacuo. The residue was purified by fc (1 cm, cyclohexane:EtOAc, 7:3, R_f = 0.08). Colorless solid, mp 108 °C, yield 60 mg (70%), $C_{19}H_{23}NO_2S$ (329.5). ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.69 (dd, *J* = 13.6/2.8 Hz, 1 H, $N(CH_2CH_2)_2$), 1.83 (td, *J* = 12.8/4.4 Hz, 1 H, $N(CH_2CH_2)_2$), 2.02 (dd, *J* = 13.6/2.8 Hz, 1 H, $N(CH_2CH_2)_2$), 2.15 (td, *J* = 13.2/4.8 Hz, 1 H, $N(CH_2CH_2)_2$), 2.30 (td, *J* = 12.8/2.4 Hz, 1 H, $N(CH_2CH_2)_2$), 2.44 (td, *J* = 12.0/2.4 Hz, 1 H, $N(CH_2CH_2)_2$), 2.64 (dd, *J* = 16.0/4.0 Hz, 1 H, $ThCH_2$), 2.67−2.78 (m, 3 H ($N(CH_2CH_2)_2$) ($ThCH_2$)), 3.55 (s, 2 H, NCH_2Ph), 3.70 (dd, *J* = 11.0/7.3 Hz, 1 H, $ThCH_2CHCH_2$), 3.78−3.84 (m, 1 H, $ThCH_2CHCH_2$), 3.90−3.97 (m, 1 H, $ThCH_2CHCH_2$), 6.81 (d, *J* = 5.2 Hz, 1 H, 3′-*H*-Th), 7.08 (d, *J* = 5.2 Hz, 1 H, 2′-*H*-Th), 7.23−7.34 (m, 5 H, Ph-*H*). A signal for the O−H proton is not seen.

*1-(1-Benzyl-6′,7′-dihydrospiro[piperidine-4,4′-thieno[3,2-c]-pyran]-6′-yl)acetone (**24**).* Acetylmethylenetriphenylphosphorane (91 mg, 0.28 mmol) and Cs_2CO_3 (67 mg, 0.20 mmol) were added to a solution of **10a** (60 mg, 0.19 mmol) in THF (4 mL). The mixture was stirred under reflux for 12 h. Then H_2O (10 mL) was added and the

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mixture was extracted with CH_2Cl_2 (3 × 5 mL). The organic layer was dried (Na_2SO_4), filtered, and concentrated in vacuo. The residue was purified by fc (1 cm, ccyclohexane:EtOAc, 7:3, R_f = 0.25). Colorless oil, yield 45 mg (67%), $C_{21}H_{25}NO_2S$ (355.5). ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.58 (dd, J = 14.2/5.2 Hz, 1 H, $N(CH_2CH_2)_2$), 1.73 (td, J = 12.9/4.4 Hz, 1 H, $N(CH_2CH_2)_2$), 1.94 (dd, J = 14.2/5.2 Hz, 1 H, $N(CH_2CH_2)_2$), 2.04 (td, J = 13.2/4.2 Hz, 1 H, $N(CH_2CH_2)_2$), 2.17 (td, J = 11.8/2.4 Hz, 1 H, $N(CH_2CH_2)_2$), 2.18 (s, 3 H, CH_3), 2.30 (td, J = 11.8/2.4 Hz, 1 H, $N(CH_2CH_2)_2$), 2.49 (dd, J = 15.0/4.0 Hz, 1 H, $ThCH_2CHCH_2$), 2.55 (dd, J = 15.6/10.2 Hz, 1 H, $ThCH_2CHCH_2$), 2.58−2.67 (m, 2 H, $N(CH_2CH_2)_2$), 2.70 (dd, J = 15.6/3.1 Hz, 1 H, $ThCH_2CHCH_2$) 2.82 (dd, J = 15.0/8.5 Hz, 1 H, $ThCH_2CHCH_2$), 3.45 (d, J = 13.0 Hz, 1 H, NCH_2Ph), 3.48 (d, J = 13.0 Hz, 1 H, NCH_2Ph), 4.18 (m, 1 H, $ThCH_2CHCH_2$), 6.72 (d, J = 5.2 Hz, 1 H, 3′-*H*-Th), 6.99 (d, J = 5.2 Hz, 1 H, 2′-*H*-Th), 7.17−7.24 (m, 5 H, Ph-*H*).

*(1-Benzyl-6′-methoxy-6′,7′-dihydrospiro[piperidine-4,4′-thieno-[3.2-c]pyran]-2′-carb-aldehyde Dimethyl Acetal (**39**).* Trimethyl orthoformate (2 mL) and *p*-toluenesulfonic acid monohydrate (260 mg, 1.37 mmol) were added to a solution of **36** (460 mg, unpurified) in MeOH (15 mL). The mixture was stirred for 45 min at rt. After addition of 2 M NaOH (5 mL), the mixture was extracted with CH_2Cl_2 (3 × 5 mL). The organic layer was dried (Na_2SO_4), filtered, and concentrated in vacuo. The residue was purified by fc (3 cm, cyclohexane:EtOAc, 7:3, R_f = 0.23 (cyclohexane:EtOAc, 1:1)). Colorless solid, mp 71 °C, yield 287 mg (32% related to **33** over two steps), $C_{22}H_{29}NO_4S$ (403.5). ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.81 (dd, J = 13.6/2.8 Hz, 1 H, $N(CH_2CH_2)_2$), 1.85 (td, J = 12.0/4.0 Hz, 1 H, $N(CH_2CH_2)_2$), 1.94 (dd, J = 13.6/2.8 Hz, 1 H, $N(CH_2CH_2)_2$), 2.08 (td, J = 13.6/4.8 Hz, 1 H, $N(CH_2CH_2)_2$), 2.42 (td, J = 12.0/3.2 Hz, 1 H, $N(CH_2CH_2)_2$), 2.51 (td, J = 12.0/2.8 Hz, 1 H, $N(CH_2CH_2)_2$), 2.72−2.79 (m, 2 H, $N(CH_2CH_2)_2$), 2.74 (dd, J = 15.6/7.2 Hz, 1 H, $ThCH_2CH$), 2.94 (dd, J = 15.6/3.6 Hz, 1 H, $ThCH_2CH$), 3.35 (s, 6 H, $CH(OCH_3)_2$), 3.55 (s, 3 H, $ThCH_2CHOCH_3$), 3.57 (d, J = 16.0 Hz, 1 H, NCH_2Ph), 3.59 (d, J = 16.0 Hz, 1 H, NCH_2Ph), 4.86 (dd, J = 7.2/3.6 Hz, 1 H, $ThCH_2CH$), 5.51 (s, 1 H, $ThCH$), 6.79 (s, 1 H, 3′-*H*-Th), 7.19−7.32 (m, 5 H, Ph-*H*).

*1-Benzyl-2′-chloro-6′-methoxy-6′,7′-dihydrospiro[piperidine-4,4′-thieno[3.2-c]-pyran] (**40**).* Trimethyl orthoformate (0.5 mL) and *p*-toluenesulfonic acid monohydrate (120 mg, 0.63 mmol) were added to a solution of **37** (120 mg, unpurified) in MeOH (6 mL). The mixture was stirred for 3 h at rt. After addition of 2 M NaOH (5 mL), the mixture was extracted with CH_2Cl_2 (3 × 5 mL). 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, R_f = 0.36). Colorless solid, mp 117 °C, yield 77 mg (33% related to **34** over two steps), $C_{19}H_{22}ClNO_2S$ (363.6). ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.81 (dd, J = 13.2/2.8 Hz, 1 H, $N(CH_2CH_2)_2$), 1.83 (td, J = 13.6/4.4 Hz, 1 H, $N(CH_2CH_2)_2$), 1.93 (dd, J = 14.0/2.8 Hz, 1 H, $N(CH_2CH_2)_2$), 2.01 (td, J = 13.6/4.4 Hz, 1 H, $N(CH_2CH_2)_2$), 2.41 (td, J = 11.6/2.8 Hz, 1 H, $N(CH_2CH_2)_2$), 2.50 (td, J = 11.6/2.8 Hz, 1 H, $N(CH_2CH_2)_2$), 2.72−2.79 (m, 2 H, $N(CH_2CH_2)_2$), 2.74 (dd, J = 15.6/7.2 Hz, 1 H, $ThCH_2CH$), 2.86 (dd, J = 15.6/3.2 Hz, 1 H, $ThCH_2CH$), 3.54 (s, 3 H, OCH_3), 3.54 (d, J = 12.8 Hz, 1 H, NCH_2Ph), 3.58 (d, J = 12.8 Hz, 1 H, NCH_2Ph), 4.86 (dd, J = 7.2/3.2 Hz, 1 H, $ThCH_2CH$), 6.62 (s, 1 H, 3′-*H* -Th), 7.20−7.35 (m, 5 H, Ph-*H*).

*1-Benzyl-6′-methoxy-6′,7′-dihydrospiro[piperidine-4,4′-thieno-[3,2-c]pyran]-2′-carbo-nitrile (**48**).* Hydroxylamine hydrochloride (50 mg, 0.72 mmol) was added to a solution of **42** (150 mg, 0.42 mmol) in pyridine (4 mL). After stirring the mixture for 30 min at 60 °C, phthalic acid anhydride (240 mg, 1.6 mmol) was added and the mixture stirring was continued for 60 min at 90 °C. Then brine was added, and the mixture was extracted with CH_2Cl_2 (3 × 5 mL). The organic layer was dried (Na_2SO_4), filtered, and concentrated in vacuo. The residue was purified by fc (3 cm, cyclohexane:EtOAc; 4:6, R_f = 0.37). Colorless solid, mp 154 °C, yield 68 mg (46%), $C_{20}H_{22}N_2O_2S$ (354.4). ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.80−1.88 (m, 2 H, $N(CH_2CH_2)_2$), 1.92 (dd, J = 14.0/2.8 Hz, 1 H, $N(CH_2CH_2)_2$), 2.03 (td, J = 12.8/4.8 Hz, 1 H, $N(CH_2CH_2)_2$), 2.42 (td, J = 11.6/2.8 Hz, 1 H, $N(CH_2CH_2)_2$), 2.51 (td, J = 11.8/2.8 Hz, 1 H, $N(CH_2CH_2)_2$), 2.74−2.83 (m, 2 H, $N(CH_2CH_2)_2$), 2.87 (dd, J = 16.4/6.4 Hz, 1 H, $ThCH_2CH$), 3.10 (dd, J = 16.4/3.6 Hz, 1 H, $ThCH_2CH$), 3.56 (s, 3 H, OCH_3), 3.57 (d, J = 13.2 Hz, 1 H, NCH_2Ph), 3.59 (d, J = 13.2 Hz, 1 H, NCH_2Ph), 4.92 (dd, J = 6.4/3.6 Hz, 1 H, $ThCH_2CH$), 7.33 (s, 1 H, 3′-*H*-Th), 7.20−7.38 (m, 5 H, Ph-*H*). Anal. ($C_{20}H_{22}N_2O_2S$) C, H, N.

Receptor Binding Studies. *Materials and General Procedures.* guinea pig brains and rat livers were commercially available (Harlan-Winkelmann, Germany). Homogenizer: Elvehjem potter (B. Braun Biotech International). Centrifuge: high-speed cooling centrifuge model Sorvall RC-5C plus (Thermo Finnigan). Filter: Printed Filtermat Type A (Perkin-Elmer), presoaked in 0.5% aqueous polyethylenimine for 2 h at rt 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 radioactivity bound to the filter was measured using a MicroBeta Trilux scintillation analyzer (Perkin-Elmer). The overall counting efficiency was 20%.

Membrane Preparation for the σ_1 Sssay.[34,46] 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[51] using bovine serum albumin as standard, and subsequently the preparation was frozen (−80 °C) in 1.5 mL portions containing about 1.5 mg protein/mL.

Performing of the σ_1 Assay.[31−34,46] The test was performed with the radioligand [^{3}H]-(+)-pentazocine (22 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 120 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 put on the filtermat and melted at 95 °C. After 5 min, the solid scintillator was allowed to solidify at rt. 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.[52]

Data Analysis. Usually, all experiments were carried out in triplicates using standard 96-well-multiplates (Diagonal). The IC_{50}-values were determined in competition experiments with at least 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.[47] The K_i-values of highly affine compounds are given as mean values ± SEM from three independent experiments.

Experimental details for the σ_2 assay: see refs 34,46.

Experimental details for the NMDA assay: see ref 48.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Physical and spectroscopic data of all new compounds. Purity data. General chemistry methods. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

PCP, 1-(1-phenylcyclohexyl)piperidine (phencyclidine); NMDA, *N*-methyl-D-aspartate; CNS, central nervous system; TMSCN, trimethylsilyl cyanide; DIBAL, diisobutylaluminum hydride; TPAP, tetrapropylammonium perruthenate; NMMO, *N*-methylmorpholine-*N*-oxide; LDA, lithium diisopropylamide; NCS, *N*-chlorosuccinimide; THF, tetrahydrofuran

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