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PAPER

Exploitation of an additional hydrophobic pocket of σ_1 receptors: Late-stage diverse modifications of spirocyclic thiophenes by C–H bond functionalization

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The hypothesis that the σ_1 receptor will tolerate an additional aryl moiety in position 1 of the spirocyclic system was based on spirocyclic pyrazole derivatives, pharmacophore models of σ_1 receptor ligands and DFT calculations. The strategy of introducing the aryl residue at the final step of the synthesis allowed the preparation of a large set of diverse ligands for the exploitation of the hydrophobic pocket of the σ_1 receptor protein. The catalyst system $PdCl_2$/2,2′-bipyridyl/Ag_2CO_3 is able to introduce various aryl groups onto the α-positions of spirocyclic thiophene derivatives **5** and **6** to afford the target aryl-appended spirocyclic thiophenes **3** and **4**. Although the σ_1 affinity of the 1-phenyl substituted spirocyclic thiophenes **3a** and **4a** is slightly reduced compared with the σ_1 affinity of the non-arylated compounds **5** and **6**, both compounds represent very potent σ_1 receptor ligands (**3a**: K_i = 4.5 nM; **4a**: K_i = 1.0 nM). This result indicates that an aryl moiety in position 1 is well tolerated by the σ_1 receptor protein. The substitution pattern of the additional phenyl moiety has only weak effects on the σ_1 affinity. Even ligands **3f** and **4h** with extended naphthyl residue show high σ_1 affinity. However, decrease of σ_1 affinity by extension of the π-system to a biphenylyl substituent (**4j**: K_i = 30 nM) indicates that the biphenylyl residue is too large for the primary hydrophobic binding pocket of the σ_1 receptor.

1. Introduction

σ Receptors are well established as a non-opioid, non-phencyclidine and haloperidol sensitive receptor family with an unique binding profile and a characteristic distribution in the central nervous system as well as in endocrine, immune and some peripheral tissues like kidney, liver, lung and heart. At least two σ receptor subtypes have been identified thus far, and are termed σ_1 and σ_2 receptor.[1–3]

Modulation of σ_1 receptor activity offers some potential for the treatment of acute and chronic neurological disorders, like schizophrenia, depression, Alzheimer's and Parkinson's disease, (neuropathic) pain as well as alcohol and cocaine abuse.[4–7] Furthermore, some human tumor cell lines are able to express a large number of σ_1 and/or σ_2 receptors. Consequently σ_1 (and σ_2) ligands may be used for diagnosis and therapy of cancer.[8,9]

Despite enormous efforts of research, the signal transduction pathway after activation of σ_1 receptors is not completely understood so far and the pharmacological effects cannot be correlated to a distinct biochemical mechanism. Nevertheless it has been shown that σ_1 receptors are involved in the modulation of various neurotransmitter systems, including the glutamatergic, dopaminergic and cholinergic neurotransmitter systems.[10] Additionally, a variety of ion channels (*e.g.* K^+-, Na^+- and Ca^{2+} channels) is regulated by σ_1 receptors.[11–14]

We aim to develop novel compounds with high σ_1 receptor affinity and high selectivity over the σ_2 subtype as well as some other relevant receptors in the central nervous system. Recently we have shown that the substituent in position 1 of spirocyclic pyrazoles **1** has a considerable influence on the σ_1 affinity. The spirocyclic pyrazole **1b** bearing a phenyl moiety in position 1 (K_i = 1.5 nM) is almost 15-fold more active than the corresponding methyl derivative **1a** (K_i = 21 nM). (Fig. 1) We assume that the phenyl substituent of **1b** is able to occupy an additional hydrophobic pocket of the σ_1 receptor protein.[15]

Bioisosteric replacement of the pyrazole ring by a thiophene ring led to the very potent σ_1 ligand **2** (K_i = 0.32 nM).[16] The combination of the high affinity thiophene substructure with an additional aryl substituent as shown for the pyrazole **1b** should lead to a new compound class of σ_1 ligands with promising activity and selectivity profile. For this purpose regioisomeric thiophene derivatives **3** and **4** with various aryl substituents in position 1 were designed. Aryl substituents with different electronic and steric properties should allow exploring the character and the size of the complementary hydrophobic binding pocket of the σ_1 receptor.

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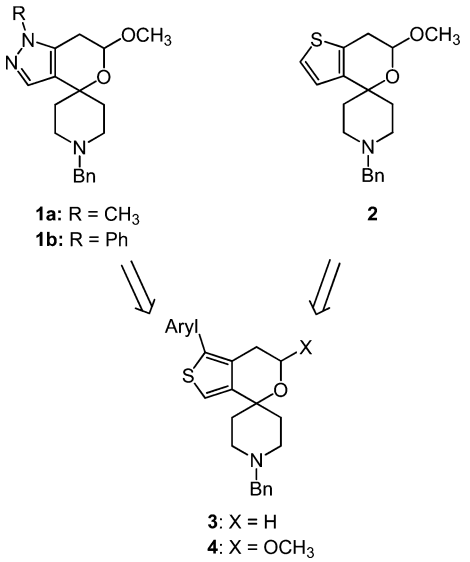


Fig. 1 Design of thiophene-based σ_1 receptor ligands with additional aryl substituent in position 1.

2. Pharmacophore models of σ_1 ligands and DFT calculations of preferred conformations

Various pharmacophore models for σ_1 receptor ligands have been reported in literature.[17,18] The common features of these models are a basic amino group and at least two hydrophobic regions in defined distances to the basic amino group. According to the model of *Glennon* the distances are defined as 2.5–3.9 Å and 6–10 Å, respectively.[19,20] The 3D computer-based σ_1 pharmacophore model of *Langer et al.* postulates four hydrophobic groups and a positive ionizable feature in definite spatial arrangements and distances (Fig. 2, top).[21] The postulated distances between the basic amine and three hydrophobic groups are calculated as 4.1 Å, 6.3 Å and 9.8 Å and are in good accordance with the distances in the *Glennon* model. In the model of *Zampieri et al.* five pharmacophoric features are described: one basic amine, two hydrophobic aromatic groups, one hydrophobic group and an additional H-bond acceptor.[22] The corresponding distances of 3.58 Å as well as 7.01 Å and 8.50 Å correlate nicely with the distances postulated in the *Glennon* and *Langer* models.

In order to define the corresponding distances within the designed spirocyclic thiophene derivative **4a**, a conformational analysis using molecular dynamics simulation was performed (PCMODEL, option "dynam" (leapfrog Verlet algorithm)).[23] The geometry optimization led to two main conformations, one with the thienyl moiety in the axial orientation with respect to the central piperidine ring, and the second one with the thienyl ring in the equatorial position. The calculated energies of both conformations are almost identical. Consequently both conformers were taken into account to calculate the intramolecular distances. Furthermore, conformers with an axially arranged benzyl moiety at the basic piperidine nitrogen atom were also found to be within an acceptable energy range and therefore were also considered.

To gain further insights into the structure and energy differences of various conformations of compound **4a**, theoretical calculations were performed on several conformers of **4a**. (Fig. 2) For geometry optimization the DFT method B3LYP/6-31G(d)[24,25] was used. For the determination of the relative energies the SCS-MP2-method of *Grimme*[26] using the 6-311+G(d,p) basis set was applied (SCS-MP2/6-311+G(d,p)//B3LYP/6-31G(d) using the GAUSSIAN 09[27] package of programs. The relative energies discussed in the following section include DFT zero point correction.

Among the various conformations and configurations studied, isomer **4a(I)** with the thiophene moiety in the axial position and the benzyl group in the equatorial orientation of the piperidine ring in a chair conformation was found to be the lowest-energy conformer. Isomer **4a(II)** bearing both the benzyl group and the thiophene moiety in equatorial orientation is calculated to be slightly higher in energy by 0.88 kcal mol^{-1}, whereas the isomers **4a(III)** and **4a(IV)** with the benzyl group in axial orientation at N1 are significantly higher in energy by 2.55 and 2.60 kcal mol^{-1}, respectively. **4a(III)** bears the thiophene substructure (C4) in equatorial orientation, whereas **4a(IV)** has the thiophene unit (C4) in axial orientation of the piperidine ring. As expected, isomers featuring twist-piperidine conformations are considerably higher in energy.

As described before, Langer *et al.* generated a 3D computer model based on the most potent σ_1 ligands known at the time. The Langer model was used as a platform to design the structure of novel σ_1 ligands. The hydrophobic features of the model are represented by the thiophene system, the phenyl residue and the *N*-benzyl moiety of **4a**. The central basic amine is consistent with the positive ionizable feature in the model. (Fig. 2)

In order to apply the model proposed by Langer for comparison of **4a** with literature data, the distances between the piperidine nitrogen atom to the center of the phenyl ring of the benzyl moiety, to the center of the thiophene ring and to the center of the phenyl ring adjacent to the thiophene were determined.

The distance 1 (*N*-Benzyl) is slightly larger in case of equatorial orientation of the benzyl group (3.765–3.766 Å) than in the case of axial orientation (3.711–3.759 Å). The distances 2 and 3 are also greater in the case of equatorial orientation of the thiophene ring (distance 2/*N*-thiophene: 5.522–5.592 Å, distance 3/*N*-Phenyl: 9.450–9.502 Å) than in case of axial orientation (distance 2/*N*-thiophene: 4.946–4.947 Å, distance 3/*N*-Phenyl: 9.083–9.093 Å). As an example, the energetically most favorable conformer **4a(I)** with axial orientation of the thiophene moiety and equatorial orientation of the *N*-benzyl group is mapped onto the model. The calculated distances indicate that **4a(I)** is in good accordance with the pharmacophore model of Langer since distance 1 (*N*-Benzyl: 3.765 Å), distance 2 (*N*-Thiophene: 4.946 Å) and distance 3 (*N*-Phenyl: 9.083 Å) agree well with the postulated distances in the model.

3. Chemistry

In order to synthesize spirocyclic thiophenes **3** and **4** with a large variety of aryl substituents in position 1, the building blocks **5** and **6** should be prepared and arylated regioselectively in α-position (Fig. 3). The direct introduction of an aryl moiety in the last step of synthesis would allow the generation of a diverse set of aryl substituted spirocyclic σ_1 receptor ligands.

The synthesis of novel spirocyclic thiophene derivatives **3** and **4** started with commercially available 3,4-dibromothiophene (**7**). (Scheme 1) Halogen-metal exchange with n-BuLi and subsequent trapping of the resulting thienyllithium intermediate with DMF

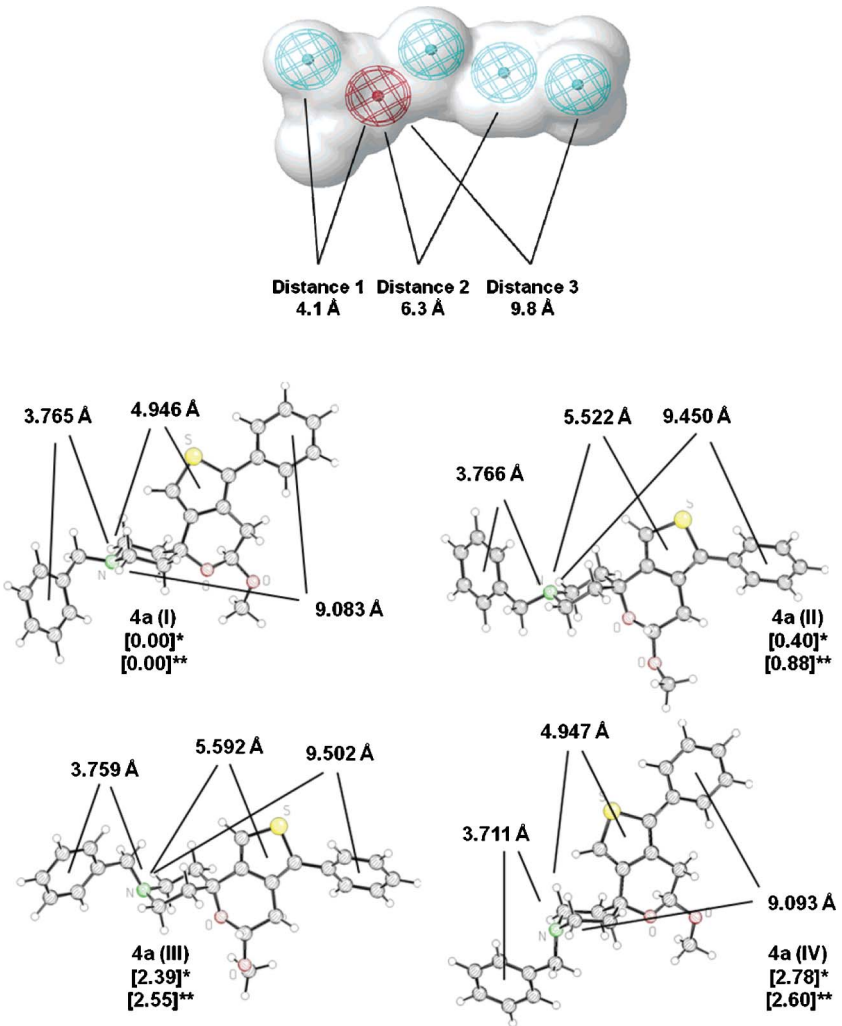


Fig. 2 Top: Pharmacophore model according to Langer *et al.*[21] Bottom: Calculated distances of pharmacophoric elements and relative energies of four energetically favored conformers of spirocyclic thiophene **4a** (SCS-MP2/6-311+G(d,p)//B3LYP/6-31G(d), energies in [kcal mol^{-1}]). **4a(I)**: thiophene moiety in axial and benzyl group in equatorial orientation; **4a(II)**: both thiophene moiety and benzyl group in equatorial orientation; **4a(III)**: thiophene moiety in equatorial and benzyl group in axial orientation; **4a(IV)**: both thiophene moiety and benzyl group in axial orientation. * B3LYP/6-31G(d)+ZPE [kcal mol^{-1}]. ** SCS-MP2/6-311G(d,p)//B3LYP/6-31G(d)+ZPE [kcal mol^{-1}].

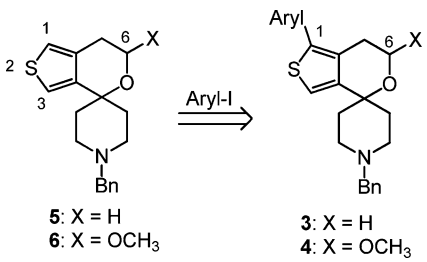
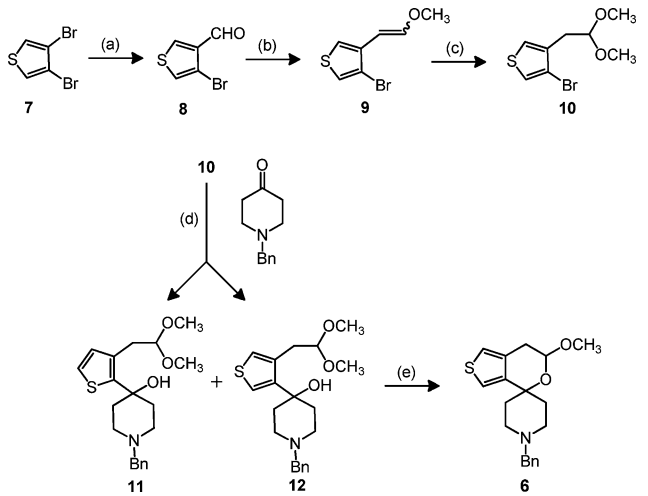


Fig. 3 Synthesis strategy for thiophene-based σ_1 ligands with additional aryl moiety in position 1.

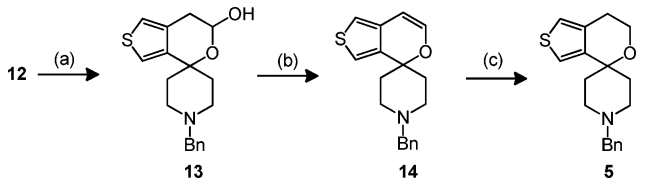
afforded thiophenecarbaldehyde **8** in 84% yield.[28] Homologation of the resulting aldehyde **8** with $Ph_3PCH_2OCH_3^+$ Cl^- and KO^{t}Bu[16,29] led to a mixture of (*E*)- and (*Z*)-configured enol ethers **9**, which was then converted into the dimethyl acetal **10** upon reaction with methanol.

A second halogen-metal exchange with *n*-BuLi at −78 °C in THF followed by addition to 1-benzylpiperidin-4-one afforded regioisomeric hydroxy acetals **11** and **12**. The formation of undesired regioisomer **11** could be explained by intermolecular deprotonation of the thienyllithium intermediate to generate an anion at the thermodynamically more sTable 2-position. The ratio of regioisomers was considerably influenced by the reaction temperature. At −78 °C the ratio of **11**/**12** was 20 : 80, whereas increasing the reaction temperature to −50 °C led to a decreased ratio of 40 : 60. Finally at −100 °C the ratio of **11**/**12** remained at 20 : 80, but the experimental procedure was less convenient. After isolation by flash chromatography, hydroxy acetal **12** was cyclized with *p*-toluenesulfonic acid in methanol to provide the spirocyclic piperidine **6** in 43% yield over two reaction steps.

Cyclic hemiacetal **13** was obtained by cyclization of hydroxy acetal **12** with diluted HCl. (Scheme 2) Treatment of **13** with methanesulfonyl chloride and triethylamine in CH_2Cl_2 afforded the enol ether **14** in 51% yield. Subsequently, **14** was reduced with H_2 in the presence of Pd/C to provide the spirocyclic thienopyran **5** in 42% yield.



Scheme 1 Synthesis of the spirocyclic thiophene derivative **6**. *Reagents and conditions*: (a) *n*-BuLi, Et_2O, −78 °C, 15 min, then DMF, −78 °C, 3 h, 84%. (b) $Ph_3PCH_2OCH_3^+$ Cl^-, KO^{t}Bu, Et_2O, −20 °C, 2 h, 90%. (c) CH_3OH, *p*-TosOH, 65 °C, 18 h, 90%. (d) *n*-BuLi, THF, −78 °C, 15 min, then 1-benzylpiperidin-4-one, −78 °C, 3 h, 53% (**12**), 12% (**11**). (e) *p*-TosOH, CH_3OH, rt, 24 h, 51%.

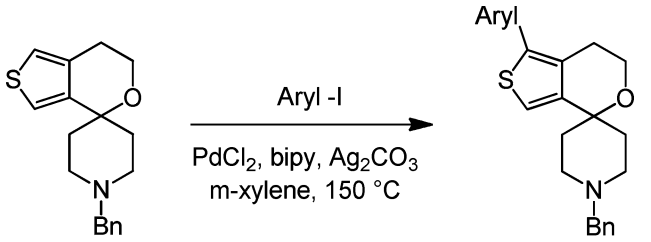


Scheme 2 Synthesis of spirocyclic thiophene derivatives without acetalic substructure. *Reagents and conditions*: (a) HCl, H_2O, THF, rt, 18 h, 76%. (b) $MeSO_2Cl$, NEt_3, CH_2Cl_2, rt, 3 h, 51%. (c) H_2, balloon, Pd/C, CH_3OH, rt, 2 h, 42%.

With the requisite core spirocyclic thiophene structures (**5** and **6**) in hand, we questioned whether we should conduct a lengthy but reliable cross-coupling technology or search for a direct C–H bond functionalization methodology for the introduction of aryl groups onto the thiophene core of **5** and **6**. The Pd-catalyzed cross-coupling reactions of metalated arene/heteroarene and halogenated arene/heteroarene species as exemplified by Suzuki–Miyaura coupling are undoubtedly among the most reliable methods for making biaryls and heterobiaryls,[30] the C–H bond arylation of arenes and heteroarenes holds significant potential for streamlining overall synthetic routes.[31] Indeed, the quest for such methods has been the driving force behind enormous efforts in the synthetic community including our group, culminating in a wealth of useful catalytic systems for reactions assembling arenes through C–H bond functionalization. Thus, we envisaged that direct C–H bond arylation of thiophenes **5** and **6** would allow us to access the target spirocyclic thiophenes **3** and **4** in a step-economical and late-stage-diversifying manner.

The palladium-catalyzed direct C–H bond arylation of thiophenes with haloarenes was first discovered by the group of Ohta in 1990.[32] After this pioneering work, a number of research groups reported various catalysts (Pd,[33] Rh,[34,35] Ir,[36] Cu[37]) promoting the C–H bond arylation of thiophenes with haloarenes. Although these arylation reactions typically occur regioselectively at the α-position of thiophene ring, several unique catalysts promoting β-selective arylation have also been established recently.[38–40] In view of broad substrate scope, high α-regioselectivity, and operational simplicity, we decided to apply one of our catalytic systems, $PdCl_2$/2,2′-bipyridyl/Ag_2CO_3,[38,41] to the arylation of spirocyclic thiophenes **5** and **6**. In particular electron-rich and electron-poor aryl moieties were taken into account, which is of great interest both for synthetic and pharmacological features.

Table 1 Yields after α-arylation of the non-acetalic spirocyclic thiophene **5** with various iodoarenes



Compd.	Aryl	Yield (gpc)[a]	Yield (ptlc)[b]
3a	C_6H_5	54%	35%
3b	*p*-$MeOC_6H_4$	65%	32%
3c	*p*-MeC_6H_4	49%	34%
3d	*p*-$NO_2C_6H_4$	48%	11%
3e	*p*-CNC_6H_4	59%	49%
3f	1-naphthyl	67%	35%

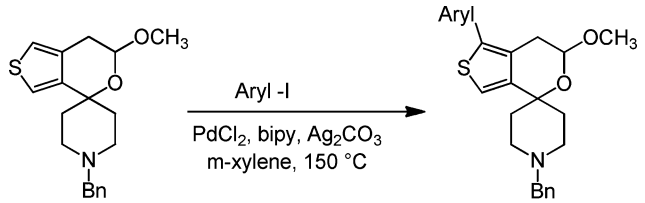
[a] gpc: yields after gel permeation chromatography; [b] ptlc: yields after preparative thin layer chromatography.

At first the spirocyclic system **5** without an alkoxy substituent at the pyran moiety was reacted with iodobenzene, $PdCl_2$, 2,2′-bipyridyl and Ag_2CO_3 in boiling *m*-xylene (150 °C) to provide the phenylated product **3a** in 54% yield (Table 1). Similar yields were obtained when electron-rich iodoarenes (*e.g.* 1-iodo-4-methoxybenzene, → **3b**), electron-deficient iodoarenes (*e.g. p*-iodobenzonitrile, → **3e**), and extended π-systems (*e.g.* 1-iodonaphthalene, → **3f**) were used. These results clearly indicate that the applied reaction conditions are compatible with the tertiary amine of the piperidine ring and the ether moiety of the pyran system.

The α-arylation of spirocyclic thiophene **6** was expected to be more problematic, due to the additional methoxy substituent in position 6 of the pyran ring (acetal). However, treatment of **6** with iodobenzene, $PdCl_2$, 2,2′-bipyridyl and Ag_2CO_3 in *m*-xylene led to the arylated product **4a** in even higher yields (67%) (Table 2). As demonstrated for the unsubstituted spirocyclic system **5**, the acetalic compound **6** reacted with electron-rich and electron-deficient iodoarenes as well as with extended π-systems to produce the arylated compounds **4a–4j** in 27–79% yields. These transformations represent the first examples of the α-arylation of highly functionalized thiophenes using the $PdCl_2$/2,2′-bipyridyl/Ag_2CO_3 method. The tolerance of the acid-labile acetal substructure of **6** is of particular importance.

All compounds were purified by gel permeation chromatography (gpc) to isolate the arylated products. In order to use pure compounds in the receptor binding studies, these arylated products were further purified by preparative thin layer chromatography (ptlc) before testing.

Table 2 Yields after α-arylation of the acetalic spirocyclic thiophene **6** with various iodoarenes



6 **4a-j**

Compd.	Aryl	Yield (gpc)[a]	yield (ptlc)[b]
4a	C_6H_5	67%	48%
4b	*p*-$MeOC_6H_4$	52%	47%
4c	*p*-MeC_6H_4	42%	38%
4d	*p*-$NO_2C_6H_4$	80%	45%
4e	*p*-AcC_6H_4		60%
4f	*p*-CNC_6H_4	50%	40%
4g	*p*-$CF_3C_6H_4$	38%	19%
4h	1-naphthyl	72%	46%
4i	3-pyridyl	27%	18%
4j	*p*-biphenyl	39%	38%

[a] gpc: yields after gel permeation chromatography; [b] ptlc: yields after preparative thin layer chromatography.

4. Receptor Affinity

The σ_1 and σ_2 receptor affinities of the spirocyclic thienopyran derivatives **3** and **4** as well as the corresponding synthetic precursors **5,6,13** and **14** were determined in competition experiments with radioligands. In the σ_1 assay different amounts of the test compound compete with the potent and selective radioligand [^{3}H]-(+)-pentazocine for σ_1 receptors in a membrane preparation of guinea pig brains. Non-specific binding was determined in the presence of a large excess of non-tritiated (+)-pentazocine.[42–45] Membrane preparations of guinea pig brains represent the standard source for σ_1 receptors in the σ_1 assay. The resulting K_i-values are rather reliable, since σ_1 receptors of different species (human, guinea pig, rat) are more than 92% identical and more than 95% similar at the level of amino acid sequence.[46] In the σ_2 assay homogenates of rat liver served as the source for σ_2 receptors and [^{3}H]-di-*o*-tolylguanidine was employed as radioligand. Due to the low σ receptor selectivity of di-*o*-tolylguanidine, an excess amount of non-radiolabeled (+)-pentazocine was added to mask σ_1 receptors. A large excess of non-tritiated di-*o*-tolylguanidine was used for the determination of non-specific binding.[42–45]

In Table 3 the σ_1 and σ_2 receptor affinities of the synthesized compounds are summarized. The σ_1 receptor affinities of the non-arylated regioisomeric thiophenes **5** (K_i = 0.35 nM) and **6** (K_i = 0.22 nM) are in the same range as the σ_1 receptor affinity of the lead compound **2** (K_i = 0.32 nM). Although introduction of a phenyl moiety in position 1 led to a slightly reduced σ_1 affinity, the resulting compounds **3a** (K_i = 4.5 nM) and **4a** (K_i = 1.0 nM) still represent very potent σ_1 ligands. Obviously, the σ_1 receptor tolerates a lipophilic phenyl moiety in position 1 of the thiophene system. In contrast to the pyrazole series of compounds **1**, the σ_1 affinity is not increased by the additional phenyl moiety. A reason might be that the σ_1 affinity of the parent compounds **5** and **6** is already very high.

In both series of compounds introduction of an electron rich or electron poor substituent into the phenyl moiety has only little influence on the σ_1 affinity. In the series of compounds

Table 3 σ_1 and σ_2 receptor affinities of the synthesized spirocyclic thiophenes and reference compounds

			$K_i \pm$ SEM [nM] ($n = 3$)		Selectivity
Compd.	X	Aryl	σ_1	σ_2	σ_1/σ_2
1a	OCH_3	CH_3	21 ± 2.3	> 1 μM	> 47
1b	OCH_3	C_6H_5	1.5 ± 0.08	> 1 μM	> 660
2	OCH_3	H	0.32 ± 0.10	> 1 μM	> 3125
3a	H	C_6H_5	4.5 ± 2.9	> 1 μM	> 222
3b	H	*p*-$MeOC_6H_4$	1.5 ± 0.54	926	617
3c	H	*p*-MeC_6H_4	3.6 ± 0.40	1.6 μM	444
3d	H	*p*-$NO_2C_6H_4$	1.7 ± 0.79	> 1 μM	> 588
3e	H	*p*-CNC_6H_4	3.4 ± 0.90	> 1 μM	> 294
3f	H	1-naphthyl	4.0 ± 1.9	51	13
4a	OCH_3	C_6H_5	1.0 ± 0.40	>1 μM	> 1000
4b	OCH_3	*p*-$MeOC_6H_4$	2.2 ± 0.13	751	341
4c	OCH_3	*p*-MeC_6H_4	2.0 ± 0.81	> 1 μM	> 500
4d	OCH_3	*p*-$NO_2C_6H_4$	1.0 ± 0.16	> 1 μM	> 1000
4e	OCH_3	*p*-AcC_6H_4	1.6 ± 0.86	> 1 μM	> 625
4f	OCH_3	*p*-CNC_6H_4	0.25 ± 0.14	923	3692
4g	OCH_3	*p*-$CF_3C_6H_4$	5.7 ± 2.3	>1 μM	> 175
4h	OCH_3	1-naphthyl	5.0 ± 0.50	2.1 μM	420
4i	OCH_3	3-pyridyl	2.2 ± 0.42	> 1 μM	> 450
4j	OCH_3	*p*-biphenyl	30 ± 18	> 1 μM	> 33
5	H	H	0.35 ± 0.06	230	657
6	OCH_3	H	0.22 ± 0.06	806	3664
13	OH	H	3.2 ± 0.41	266	83
14	$HC^3{=}C^4H$	H	1.9 ± 0.66	84.6 ± 25.4	45
haloperidol			3.9 ± 1.5	78 ± 2.0	20
di-*o*-tolylguanidine			61 ± 8	42 ± 15	0.7

3, the methoxyphenyl derivative **3b** is slightly more potent than the unsubstituted phenyl derivative **3a**, whereas in the methoxy substituted series **4** an opposite tendency is observed. In both series, extension of the π-system to a 1-naphthyl system (**3f,4h**) does not reduce σ_1 affinity significantly. However, extension of the phenyl substituent to a 4-phenylphenyl (biphenylyl) residue led to **4j** with the lowest σ_1 affinity (K_i = 30 nM) within this compound class. The *p*-cyanophenyl substituted derivative **4f** (K_i = 0.25 nM) shows the same σ_1 affinity as the non-arylated parent compound **6** and represents the most potent σ_1 ligand of this series. We assume that the σ_1 receptor protein accepts both electron-rich and electron-poor phenyl moieties in position 1 of the thiophene system. Moreover, the size of the aromatic system in position 1 may be expanded to a naphthyl residue without loss of σ_1 affinity, but not to a biphenylyl residue.

The similar σ_1 affinities of non-arylated and arylated spirocyclic thiophenes are surprising. We assume that there is a hydrophobic pocket within the σ_1 receptor protein which is not addressed by the small non-arylated derivatives. However, an additional aryl moiety can open the hydrophobic pocket and allow further lipophilic interactions. These additional interactions of the aryl moiety do only modulate the ligand - σ_1 receptor interactions, which are mainly determined by the residual spirocyclic framework.

The σ_2 receptor affinities of all investigated compounds are considerably lower than their σ_1 affinities indicating high selectivity against the σ_2 subtype. As a general rule, the methoxy group in position 6 (compound series **4**) leads to reduced σ_2 affinity and thus increased $\sigma_1 : \sigma_2$ selectivity compared with compounds without a substituent in position 6 (compare compounds **4f** ($\sigma_1 : \sigma_2$ = 3692) with **3e** ($\sigma_1 : \sigma_2$ = > 294) and **6** ($\sigma_1 : \sigma_2$ = 3664) with **5** ($\sigma_1 : \sigma_2$ = 657) and **14** ($\sigma_1 : \sigma_2$ = 45)). Introduction of an aryl moiety with an increased π-system in position 1 (1-naphthyl, biphenylyl residue) results in reduced $\sigma_1 : \sigma_2$ selectivity. The naphthyl substituted compound **3f** without a substituent in position 6 shows the lowest $\sigma_1 : \sigma_2$ selectivity (13) of this series of compounds. A polar OH-moiety in position 6 (compound **13**) also favors the interaction with σ_2 receptors.

5. Conclusion

In summary, the regioselective α-arylation of the thiophene derivatives **5** and **6** allows the late-stage introduction of diverse aromatic substituents into the spirocyclic σ_1 ligands without previous activation of the C–H bond. The phenylated thiophenes **3a** and **4a** show a slightly reduced σ_1 receptor affinity compared to the extraordinarily potent, non-arylated spirocyclic thiophenes **5** and **6**. Nevertheless, both phenylated compounds **3a** and **4a** interact in the low nanomolar range with σ_1 receptors (**3a**: K_i = 4.5 nM, **4a**: K_i = 1.0 nM). The substitution pattern of the aryl residue does not considerably influence the σ_1 affinity. Whereas the larger naphthyl residue was also well accepted by the σ_1 receptor protein, the biphenylyl substituted compound **4j** showed reduced σ_1 affinity. With exception of naphthyl derivative **3f** all compounds have very high selectivity against the σ_2 subtype. The most promising compounds of this series are the non-arylated compound **6** (K_i = 0.22 nM) and the *p*-cyanophenyl substituted compound **4f** (K_i = 0.25 nM), possessing subnanomolar σ_1 affinities. Furthermore, molecular modelling investigations including DFT-calculations demonstrated that the spirocyclic thiophene **4a** fits well onto the σ_1 pharmacophore model described by *Langer*.

6. Experimental

6.1. Chemistry

6.1.1. General. Unless otherwise noted, moisture and oxygen sensitive reactions were conducted in dry glassware (Schlenk flask sealed with a rubber septum) under N_2 (dried with phosphorous pentoxide (Granusic® A, Baker)). THF and Et_2O were dried with sodium/benzophenone and were freshly distilled before use. Methanol was dried with magnesium and iodine and was used freshly distilled. DMF was dried with CaH_2, filtered, distilled and stored over molecular sieves 3 Å. The concentration of *n*-BuLi was determined by titration with 1,3-diphenyl-2-propanone *p*-toluenesulfonylhydrazone in THF under N_2-atmosphere.[47] Thin layer chromatography (tlc): Silica gel 60 F254 plates (Merck). Flash chromatography (fc): Silica gel 60, 40–64 μm (Merck); parentheses include: diameter of the column [cm], length of the stationary phase [cm], eluent, fraction size [mL] and retention factor R_f. Gel permeation chromatography (gpc): LC-9204 instrument (JAI) with JAIGEL-1H/JAIGEL-2H columns, eluent $CHCl_3$; preparative thin layer chromatography (prep. tlc): Wako-gel® B5-F silica coated plates (0.75 mm) prepared in the Itami laboratory; IR: IR spectrophotometer 480Plus FT-ATR-IR (Jasco). ^{1}H NMR (400 MHz, 300 MHz, 600 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a Unity Mercury Plus 400 (400 MHz) NMR spectrometer (Varian), JNM-ECA-400 (400 MHz) spectrometer (JEOL), Brucker AV 300 (300 MHz), and Varian Unity Plus 600 (600 MHz) operating at 23 °C. Chemical shifts δ are reported in parts per million (ppm) against the reference compound tetramethylsilane and calculated using the chemical shift of the signal of the residual non-deuterated solvent. Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, dd = doublet of doublets, t = triplet, q = quartet, m = multiplet), coupling constant (Hz) and integration. MS: HRMS (ESI): Finnigan MAT 4200 s, Brucker Daltonics Micro Tof and Waters Micromass Quatro LCZ, peaks are given in *m/z* (% of basis peak). EI, electron impact, MAT GCQ (Thermo-Finnigan); HRMS: JMS-T100TD instrument (DART); HPLC: Merck Hitachi Equipment; UV detector: L-7400; autosampler:L-7200; pump: L-7100; degasser: L-7614; column: LiChrospher® 60 RP-select B (5 μm); LiChroCART® 250-4 mm cartridge; flow rate: 1.000 mL min^{-1}; injection volume: 5.0 μL; detection at λ = 210 nm; solvents: A: water with 0.05% (*v/v*) trifluoroacetic acid; B: acetonitrile with 0.05% (v/v) trifluoroacetic acid: gradient elution: (A%): 0–4 min: 90%, 4–29 min: gradient from 90%,to 0%, 29–31 min: 0%, 31–31.5 min: 0% to 90%, 31.5–40 min: 90%. The purity of test compounds was greater than 95%, which was determined by the given HPLC method.

6.1.2. 4-Bromothiophene-3-carbaldehyde (8)[28]. Under N_2 3,4-dibromothiophene (**7**, 22.2 g, 92 mmol) was dissolved in dry Et_2O (35 mL) and cooled down to −78 °C. After stirring the mixture at −78 °C for 10 min a solution of n-butyllithium in n-hexane (1.6 M, 57.2 mL, 92 mmol) was added dropwise and the mixture was stirred for another 15 min. The resulting aryllithium intermediate was subsequently trapped with freshly distilled DMF

(7.08 mL, 92 mmol) and the mixture was stirred for 3 h at −78 °C. The solution was warmed up to rt, water (40 mL) was added, the organic layer was separated, the aqueous layer was extracted twice with Et_2O, the combined organic layers were dried (K_2CO_3), concentrated *in vacuo* and the residue was purified by high vacuum distillation. Pale yellow oil, bp 41 °C (9×10^{-3} mbar), yield 14.8 g (84%). C_5H_3BrOS (191.0 g mol^{-1}). MS (EI): m/z = 189 [$^{79}BrM^+$ − H], 191 [$^{81}BrM^+$ − H]. IR (neat): ν (cm^{-1}) = 3103 (C–H_{aryl}), 2848 (C–H), 1683 (C═O). ^{1}H NMR ($CDCl_3$): δ (ppm) = 7.37 (d, J = 3.4 Hz, 1H, 5-H-thioph), 8.16 (d, J = 3.5 Hz, 1H, 2-H-thioph), 9.95 (s, 1H, CH═O).

6.1.3. (*E*)- and (*Z*)-3-Bromo-4-(2-methoxyvinyl)thiophene (9). Under N_2 dried $MeOCH_2PPh_3^+Cl^-$ (42.6 g, 120 mmol) was suspended in dry Et_2O (200 mL) and the mixture was cooled down to −20 °C. KO^{t}Bu (13.9 g, 120 mmol) was dissolved in dry THF (77 mL) to give a 1.6 M solution. This solution was added dropwise to the stirred suspension of the Wittig reagent so that the temperature was kept below −10 °C and the mixture was stirred for 30 min after complete addition of KO^{t}Bu. Next the aldehyde **8** (14.8 g, 80 mmol) was dissolved in dry Et_2O (3–5 mL) and the solution was added slowly to the suspension. The mixture was stirred for 2 h at −20 °C and overnight at rt. After addition of water the aqueous layer was extracted twice with Et_2O. The combined organic layers were dried (K_2CO_3), concentrated *in vacuo* and the side product Ph_3PO was removed by multiple recrystallization. The remaining oil was purified by high vacuum distillation. Yellow oil, bp 58–62 °C (1.2×10^{-2} mbar), yield 15.8 g (90%). C_7H_7BrOS (219.1 g mol^{-1}). MS (EI): m/z = 218 [$^{79}BrM^+$], 220 [$^{91}BrM^+$], 175 [$^{79}BrMH^+ - CH(OCH_3)$], 177 [$^{81}BrMH^+ - CH(OCH_3)$]. IR (neat): ν (cm^{-1}) = 3110 (C–H_{aryl}), 2831 (OCH_3), 1639 (C═C), 1216 (C–O), 1098 (C–O). ^{1}H NMR ($CDCl_3$): δ (ppm) = 3.69 (s, 3·0.6H, OCH_3, (*E*)-isomer), 3.80 (s, 3·0.4H, OCH_3, (*Z*)-isomer), 5.44 (d, J = 6.8 Hz, 0.4H, thiophC*H*═CH, (*Z*)-isomer), 5.75 (d, J = 13.0 Hz, 0.6H, thiophC*H*═CH, (*E*)-isomer), 6.24 (d, J = 6.8 Hz, 0.4H, thiophCH═C*H*, (*Z*)-isomer), 6.97 (d, J = 12.9 Hz, 0.6H, thiophCH═C*H*, (*E*)-isomer), 7.01 (d, J = 3.4 Hz, 0.6H, 5-H-thioph, (*E*)-isomer), 7.18 (d, J = 3.5 Hz, 0.4H, 5-H-thioph, (*Z*)-isomer) 7.23 (d, J = 3.4 Hz, 0.6H, 2-H-thioph, (*E*)-isomer), 7.71 (d, J = 3.5 Hz, 0.4H, 2-H-thioph, (*Z*)-isomer). The ratio of (*E*)-**9** and (*Z*)-**9** is 6 : 4.

6.1.4. 2-(4-Bromothiophen-3-yl)acetaldehyde dimethyl acetal (10). Enol ether **9** (360 mg, 1.62 mmol) was dissolved in dry MeOH (30 mL) and catalytic amounts of *p*-toluenesulfonic acid were added. The mixture was stirred overnight under reflux. After cooling down to rt CH_2Cl_2 (100 mL) was added and the solution was alkalized with 2 M NaOH. The aqueous layer was extracted twice with CH_2Cl_2. The combined organic layers were dried (K_2CO_3), filtered and purified by fc (3 cm, 15 cm, cyclohexane : EtOAc = 19 : 1, 20 mL, R_f 0.22). Colorless oil, yield 369 mg (90%). $C_8H_{11}BrO_2S$ (251.1 g mol^{-1}). MS (EI): m/z = 219 [$^{79}BrM^+ - OCH_3$], 221 [$^{81}BrM^+ - OCH_3$], 175 [$^{79}BrM^+ - CH(OCH_3)_2$], 177 [$^{81}BrM^+ - CH(OCH_3)_2$], 140 [$M^+ - Br, - OCH_3$]. IR (neat): ν (cm^{-1}) = 3107 (C–H_{aryl}), 2830 (C–O), 1059 (C_{aryl}–Br). ^{1}H NMR ($CDCl_3$): δ (ppm) = 2.93 (d, J = 5.6 Hz, 2H, thiophCH_2CH), 3.36 (s, 6H, CH(OCH_3)$_2$), 4.61 (t, J = 5.6 Hz, 1H, thiophCH$_2$C*H*), 7.16 (d, J = 3.4 Hz, 1H, 2-H-thioph), 7.24 (d, J = 3.4 Hz, 1H, 5-H-thioph).

6.1.5. 1-Benzyl-4-[3-(2,2-dimethoxyethyl)thiophen-2-yl]piperidin-4-ol (11) and 1-Benzyl-4-[4-(2,2-dimethoxyethyl)thiophen-3-yl]piperidin-4-ol (12). Under N_2 and at −78 °C *n*-BuLi in n-hexane (1.26 M, 2.74 mL, 3.44 mmol) was added slowly to a stirred solution of bromoacetal **10** (0.67 g, 2.65 mmol) in dry THF (15 mL). The mixture was stirred for 15 min at −78 °C. Then 1-benzylpiperidin-4-one (0.57 mL, 3.2 mmol) was added slowly. After stirring at −78 °C for 3 h, the mixture was warmed to rt and water was added. The aqueous layer was separated and extracted twice with CH_2Cl_2. The combined organic layers were dried (K_2CO_3), filtered and the solvent was removed in vacuo. The regioisomeric alcohols were separated by fc (5 cm, 15 cm, cyclohexane : EtOAc = 8 : 2, 20 mL).

11 (R_f 0.35, cyclohexane : EtOAc = 3 : 7): Pale yellow oil, yield 0.11 g (12%). $C_{20}H_{27}NO_3S$ (361.5 g mol^{-1}). MS (EI): m/z = 361 [M^+], 328 [$M^+ - HOCH_3$, − H], 298 [$M^+ - OCH_3 - HOCH_3$], 91 [$^+CH_2Ph$]. IR (neat): ν (cm^{-1}) = 3430 (O–H), 3026 (C–H_{aryl}), 2934 (C–H), 1118 (C–O), 697 (C–H). ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.90–1.97 (m, 2H, N(CH$_2$CH_2)$_2$), 2.07–2.16 (m, 2H, N(CH$_2$CH_2)$_2$), 2.49–2.56 (m, 2H, N(CH_2CH$_2$)$_2$), 2.71–2.80 (m, 2H, N(CH_2CH$_2$)$_2$), 3.22 (d, J = 5.5 Hz, 2H, thiophCH_2CH), 3.35 (s, 6H, CH(OCH_3)$_2$), 3.57 (s, 2H, NCH_2Ph), 3.62 (s, 1H, O*H*), 4.48 (t, J = 5.5 Hz, 1H, thiophCH$_2$C*H*), 6.81 (d, J = 5.1 Hz, 1H, 3-H-thioph), 7.09 (d, J = 5.1 Hz, 1H, 2-H-thioph), 7.29–7.37 (m, 5H, Ph-*H*).

12 (R_f 0.22, cyclohexane : EtOAc = 3 : 7): Pale yellow oil, yield 0.50 g (53%). $C_{20}H_{27}NO_3S$ (361.5 g mol^{-1}). MS (EI): m/z = 361 [M^+], 330 [$M^+ - OCH_3$], 298 [$M^+ - OCH_3 - HOCH_3$], 91 [$^+CH_2Ph$]. IR (neat): ν (cm^{-1}) = 3442 (O–H), 3027 (C–H_{aryl}), 2926 (C–H), 1119 (C–O), 698 (C–H). ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.88–1.95 (m, 2H, N(CH$_2$CH_2)$_2$), 2.06 (td, J = 12.9/4.3 Hz, 2H, N(CH$_2$CH_2)$_2$), 2.51 (td, J = 11.9/2.5 Hz, 2H, N(CH_2CH$_2$)$_2$), 2.70–2.78 (m, 2H, N(CH_2CH$_2$)$_2$), 3.24 (d, J = 5.6 Hz, 2H, thiophCH_2CH), 3.35 (s, 6H, CH(OCH_3)$_2$), 3.51 (s, 1H, O*H*), 3.57 (s, 2H, NCH_2Ph), 4.52 (t, J = 5.6 Hz, 1H, thiophCH$_2$C*H*), 7.07 (m, 2H, 2-*H*-thioph, 5-*H*-thioph), 7.26–7.37 (m, 5H, Ph-*H*).

6.1.6. 1-Benzyl-6′-methoxy-6′,7′-dihydrospiro[piperidine-4,4′-thieno[3,4-*c*]pyran] (6). Hydroxy acetal **12** (421 mg, 1.16 mmol) was dissolved in dry MeOH (25 mL) and *p*-toluenesulfonic acid (251 mg, 1.45 mmol) was added. The solution was stirred for 24 h at rt. Then 2 M NaOH was added until pH 8 and the solution was diluted with water (20 mL). The aqueous layer was separated and extracted twice with CH_2Cl_2. The combined organic layers were dried (K_2CO_3), filtered and the solvent was removed in vacuo. The remaining oil was purified by fc (2.5 cm, 15 cm, cyclohexane : EtOAc = 3 : 2, 20 mL, R_f 0.22). Pale yellow oil, yield 194 mg (51%). $C_{19}H_{23}NO_2S$ (329.5 g mol^{-1}). Purity (HPLC): 98.5%, t_r = 16.8 min. MS (EI): m/z = 329 [M^+], 314 [$M^+ - CH_3$], 298 [$M^+ - OCH_3$], 238 [$M^+ - CH_2Ph$], 91 [$^+CH_2Ph$]. IR (neat): ν (cm^{-1}) = 3026 (C–H_{aryl}), 2921, 2809 (C–H), 1067 (C–O), 698 (C–H). ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.82–1.98 (m, 2H, N(CH$_2$CH_2)$_2$), 2.02–2.16 (m, 2H, N(CH$_2$CH_2)$_2$), 2.44 (td, J = 12.8/2.4 Hz, 1H, N(CH_2CH$_2$)$_2$), 2.55 (td, J = 12.0/2.6 Hz, 1H, N(CH_2CH$_2$)$_2$), 2.72–2.82 (m, 3H, thiophCH_2CH, N(CH_2CH$_2$)$_2$), 2.96 (dd, J = 15.3/3.1 Hz, 1H, thiophCH_2CH), 3.53 (s, 3H, OCH_3), 3.57 (d, J = 13.0 Hz, 1H, NCH_2Ph), 3.61 (d, J = 13.6 Hz, 1H, NCH_2Ph), 4.84 (dd, J = 7.4/3.1 Hz, 1H, thiophCH$_2$C*H*), 6.91 (d, J = 3.0 Hz, 1H, 1-H-thioph), 6.96 (d, J = 3.0 Hz, 1H,

3-*H*-thioph), 7.27–7.39 (m, 5H, Ph-*H*). ^{13}C NMR ($CDCl_3$): δ (ppm) = 32.8 (1C, thiophCH_2CH), 37.7 (1C, N(CH_2CH_2)$_2$), 40.0 (1C, N(CH_2CH_2)$_2$), 49.4 (1C, N(CH_2CH_2)$_2$), 49.4 (1C, N(CH_2CH_2)$_2$), 56.6 (1C, OCH_3), 63.5 (1C, NCH_2Ph), 74.3 (1C, thiophC_{spiro}), 97.4 (1C, thiophCH$_2$*C*H), 118.5 (1C, *C*-1′-thioph), 120.0 (1C, *C*-3′-thioph), 127.3 (Ph-*C*H), 128.5 (Ph-*C*H), 129.6 (Ph-*C*H), 133.5 (1C, C_{quart}), 138.5 (1C, C_{quart}), 143.4 (1C, C_{quart}).

6.1.7. 1-Benzyl-6′,7′-dihydrospiro[piperidine-4,4′-thieno[3,4-*c*]pyran]-6′-ol (13). Hydroxy acetal **12** (1.06 g, 2.94 mmol) was dissolved in THF (5 mL) and hydrochloric acid (1 M, 30 mL, 30 mmol) was added. The mixture was stirred at rt overnight. Subsequently 2 M NaOH was added dropwise until pH = 8, the aqueous layer was separated and extracted twice with CH_2Cl_2. The combined organic layers were dried (K_2CO_3), filtered and the solvent was removed in vacuo. The remaining resin was purified by fc (6 cm, 15 cm, cyclohexane : EtOAc = 3 : 2, 20 mL, R_f 0.18). Colorless solid, mp 146 °C, yield 703.1 mg (76%). $C_{18}H_{21}NO_2S$ (315.4 g mol^{-1}). Purity (HPLC): 97.3%, t_r = 14.0 min. MS (EI): *m*/*z* = 315 [M$^+$], 224 [M$^+$ – CH_2Ph], 91 [$^+CH_2$Ph]. IR (neat): *v* (cm^{-1}) = 3079 (O–H), 2937 (C–H), 2836 (C–H), 1051 (C–O), 698 (C–H). ^{1}H NMR ($CDCl_3$): δ (ppm) = ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.91–2.20 (m, 4H, N($CH_2C\mathit{H}_2$)$_2$), 2.44–2.61 (m, 2H, N($C\mathit{H}_2CH_2$)$_2$), 2.68–2.85 (m, 3H, thioph$C\mathit{H}_2$CH, N($C\mathit{H}_2CH_2$)$_2$), 3.05 (dd, J = 15.3/3.0 Hz, 1H, thioph$C\mathit{H}_2$CH), 3.59 (s_{broad}, 2H, N$C\mathit{H}_2$Ph), 5.30 (dd, J = 7.4/3.0 Hz, 1H, thiophCH$_2$C*H*), 6.94 (d, *J* = 3.0 Hz, 3′-*H*-thioph), 6.99 (d, *J* = 2.9 Hz, 1H, 1′-*H*-Thioph), 7.28–7.41 (m, 5H, Ph-*H*). A signal for the OH-proton is not visible. ^{13}C NMR ($CDCl_3$): δ (ppm) = 34.3 (1C, thioph$C$H$_2$CH), 37.3 (1C, N($CH_2C\mathit{H}_2$)$_2$), 39.9 (1C, N($CH_2C\mathit{H}_2$)$_2$), 49.3 (1C, N(CH$_2$CH$_2$)$_2$), 49.5 (1C, N(CH$_2$CH$_2$)$_2$), 63.4 (1C, NCH$_2$Ph), 74.6 (1C, thiophC_{spiro}), 90.5 (1C, thiophCH$_2$*C*H), 118.7 (1C, *C*-1′-thioph), 120.3 (1C, *C*-3′-thioph), 127.4 (Ph-*C*H), 128.5 (Ph-*C*H), 129.6 (Ph-*C*H), 133.2 (1C, C_{quart}), 142.9 (1C, C_{quart}). One signal for a quaternary carbon atom is not visible.

6.1.8. 1-Benzylspiro[piperidine-4,4′-thieno[3,4-*c*]pyran] (14). Under N_2 the hemiacetal **13** (284 mg, 0.9 mmol) was dissolved in dry CH_2Cl_2 (10 mL) and cooled down to 0 °C. After 10 min NEt_3 (0.32 mL, 2.28 mmol) and $MeSO_2Cl$ (0.12 mL, 1.52 mmol) were added dropwise. The solution was stirred for 2 h at rt and finally heated to reflux for 1 h. After cooling down to rt the mixture was alkalized with saturated $NaHCO_3$ solution. The aqueous layer was separated and extracted twice with CH_2Cl_2. The combined organic layers were dried (K_2CO_3), filtered and the solvent was removed in vacuo. The residue was purified by fc (3 cm, 15 cm, cyclohexane : EtOAc = 7 : 3, 20 mL, R_f 0.34). Colorless solid, mp 85 °C, yield 137 mg (51%). $C_{18}H_{19}NOS$ (297.4 g mol^{-1}). Purity (HPLC): 95.1%, t_r = 17.6 min. MS (EI): *m*/*z* = 297 [M$^+$], 206 [M$^+$ – CH_2Ph], 91 [$^+CH_2$Ph]. R (neat): *v* (cm^{-1}) = 3089 (C–H), 2923 (C–H), 1615 (C=C), 1041 (C–O). ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.93 (td, J = 13.4/4.6 Hz, 2H, N($CH_2C\mathit{H}_2$)$_2$), 2.16 (dd, J = 14.0/2.4 Hz, 2H, N($CH_2C\mathit{H}_2$)$_2$), 2.42 (td, J = 12.0/2.4 Hz, 2H, N($C\mathit{H}_2CH_2$)$_2$), 2.70–2.77 (m, 2H, N($C\mathit{H}_2CH_2$)$_2$), 3.57 (s, 2H, N$C\mathit{H}_2$Ph), 5.80 (d, *J* = 5.9 Hz, 1H, 7-H), 6.44 (d, *J* = 5.9 Hz, 1H, 6-H), 6.77 (d, *J* = 2.7 Hz, 1H, 1-H-thioph), 6.91 (d, *J* = 2.1 Hz, 1H, 3-H-thioph), 7.29–7.38 (m, 5H, Ph-*H*). ^{13}C NMR ($CDCl_3$): δ (ppm) = 35.7 (2C, N(CH_2CH$_2$)$_2$), 49.1 (2C, N(CH$_2$CH$_2$)$_2$), 63.4 (1C, NCH$_2$Ph), 101.0 (1C, thioph$C$H=CH), 115.5 (1C, *C*-3′-thioph), 117.9 (1C, *C*-1′-thioph), 127.3 (1C, Ph-*C*), 128.4 (2C, Ph-*C*), 129.5 (2C, Ph-*C*), 131.2 (1C, C_{quart}), 138.2 (1C, C_{quart}), 139.9 (1C, C_{quart}), 142.4 (1C, thiophCH=*C*H). The solvent signals probably overlap with the quaternary C_{spiro} signal.

6.1.9. 1-Benzyl-6′,7′-dihydrospiro[piperidine-4,4′-thieno[3,4-*c*]-pyran] (5). The enol ether **14** (91.7 mg, 0.31 mmol) was dissolved in dry MeOH (10 mL) and 10% Pd/C (50 mg) was added. The suspension was stirred under H_2 atmosphere (balloon) for 2 h. Afterwards the catalyst was filtered off and the remaining residue was washed with 2 M HCl and with water. The filtrate was alkalized with 2 M NaOH and extracted twice with CH_2Cl_2. The combined organic layers were dried over K_2CO_3, the solvent was removed *in vacuo* and the crude product was purified by fc (2.5 cm, 15 cm, cylcohexane : EtOAc = 7 : 3, 15 mL, R_f 0.25). Colorless oil, yield 38.4 mg (42%). $C_{18}H_{21}NOS$ (299.4 g mol^{-1}). Purity (HPLC): 97.9%, t_r = 17.5 min. MS (EI): *m*/*z* = 299 [M$^+$], 208 [M$^+$ – CH_2Ph], 91 [$^+CH_2$Ph]. IR (neat): *v* (cm^{-1}) = 3028 (C–H_{aryl}), 2922 (C–H), 1080 (C–O), 697 (C–H). ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.88 (dd, J = 14.9/2.4 Hz, 2H, N($CH_2C\mathit{H}_2$)$_2$), 1.96 (td, J = 14.5/4.2 Hz, 2H, N($CH_2C\mathit{H}_2$)$_2$), 2.39 (td, J = 12.1/2.9 Hz, 2H, N($C\mathit{H}_2CH_2$)$_2$), 2.66 (t, 5.2 Hz, 2H, thioph$C\mathit{H}_2CH_2$), 2.72 (d, *J* = 11.6 Hz, 2H, N($C\mathit{H}_2CH_2$)$_2$), 3.56 (s, 2H, N$C\mathit{H}_2$Ph), 3.72 (t, *J* = 5.6 Hz, 2H, thiophCH$_2C\mathit{H}_2$), 6.79 (d, 3.0 Hz, 1H, 3′-*H*-thioph), 6.87 (d, 3.0 Hz, 1H, 1′-*H*-thioph), 7.27–7.38 (m, 5H, Ph-*H*). ^{13}C NMR ($CDCl_3$): δ (ppm) = 27.1 (1C, thioph$C$H$_2$CH$_2$), 37.3 (2C, N($CH_2C\mathit{H}_2$)$_2$), 49.1 (2C, N(CH$_2$CH$_2$)$_2$), 59.2 (1C, thiophCH$_2C$H$_2$), 63.1 (1C, NCH$_2$Ph), 73.6 (1C, thiophC_{spiro}), 118.9 (1C, *C*H-1′-thioph), 119.4 (1C, *C*H-3′-thioph), 127.5 (1C, Ph-*C*H), 128.5 (2C, Ph-*C*H), 129.8 (2C, Ph-*C*H), 134.8 (1C, C_{quart}), 137.2 (1C, C_{quart}), 143.7 (1C, C_{quart}).

6.1.10. General procedure A for the α-arylation of spirocyclic thiophene derivatives 5 and 6. A 20 mL glass vessel was equipped with a magnetic stirring bar and closed by a J. Young® O-ring tap. The flask was flame-dried under vacuo and filled with Ar after cooling to rt. Under a permanent stream of Ar the Pd-catalyst (10 mol %) and Ag_2CO_3 (1 equiv.) were filled into the vessel and suspended in dry *m*-xylene (0.4 mL). This mixture was stirred at 60 °C for 30 min. Finally, a solution of the iodoarene (1.1 equiv.) and a solution of the spirocyclic thiophene **5** or **6** (1 equiv.) in dry *m*-xylene (0.6 mL in total) were added dropwise. The vessel was sealed with the O-ring tap and heated at 150 °C for 12 h in a 8-well reaction block. After cooling the vessel to rt the mixture was filtered through a short silica pad (EtOAc). The filtrate was concentrated *in vacuo* and the crude product was purified by gel permeation chromatography ($CHCl_3$) followed by preparative thin layer chromatography to yield the corresponding arylthiophene in high purity.

6.1.11. 1-Benzyl-1′-phenyl-6′,7′-dihydrospiro[piperidine-4,4′-thieno[3,4-*c*]pyran] (3a). According to General procedure A spirocyclic thiophene **5** (27.7 mg, 0.093 mmol) was reacted with iodobenzene (11.3 µL, 0.10 mmol), Ag_2CO_3 (28 mg, 0.10 mmol) and $PdCl_2$/2,2′-bipyridyl (3.6 mg, 0.01 mmol) in *m*-xylene (1.2 mL). The crude product was purified by $CHCl_3$-gpc and prep. tlc (*h* = 15 cm, hexane : EtOAc = 9 : 1, R_f 0.42). Colorless solid, yield 18.8 mg (54%) after gpc; yield 12.0 mg (35%) after prep. tlc. $C_{24}H_{25}NOS$ (375.5 g mol^{-1}). Exact MS (HRMS): *m*/*z* = calcd. for $C_{24}H_{25}NOS$ [M$^+$] 375.1657, found 375.1643. ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.95–2.06 (m, 4H, N($CH_2C\mathit{H}_2$)$_2$), 2.42 (td, J = 10.8/4.9

Hz, 2H, N(CH_2CH$_2$)$_2$), 2.74 (d, J = 11.5 Hz, 2H, N(CH_2CH$_2$)$_2$), 2.86 (t, J = 5.5 Hz, 2H, thiophCH_2CH$_2$), 3.58 (s, 2H, NCH_2Ph), 3.82 (t, J = 5.5 Hz, 2H, thiophCH$_2$CH_2), 6.97 (s, 1H, 3′-H-thioph), 7.27–7.50 (m, 10H, Ph-H). ^{13}C NMR (CDCl$_3$): δ (ppm) = 27.8 (1C, thiophCH_2CH$_2$), 37.9 (2C, N(CH$_2$CH_2)$_2$), 49.3 (2C, N(CH_2CH$_2$)$_2$), 59.3 (1C, thiophCH$_2$CH_2), 63.6 (1C, NCH_2Ph), 73.9 (1C, thiophC_{spiro}), 118.0 (1C, CH-3′-thioph), 127.3 (Ph-CH), 127.5 (Ph-CH), 128.5 (Ph-CH), 128.6 (Ph-CH), 128.9 (Ph-CH), 129.6 (Ph-CH), 131.1 (1C, C_{quart}), 134.6 (1C, C_{quart}), 137.5 (1C, C_{quart}), 138.6 (1C, C_{quart}), 145.5 (1C, C_{quart}).

6.1.12. 1-Benzyl-1′-(4-methoxyphenyl)-6′,7′-dihydrospiro[piperidine-4,4′-thieno[3,4-*c*]pyran] (3b). According to General procedure A spirocyclic thiophene **5** (28.4 mg, 0.095 mmol) was reacted with *p*-iodoanisole (26.7 mg, 0.11 mmol), Ag_2CO_3 (30.2 mg, 0.11 mmol) and $PdCl_2$/2,2′-bipyridyl (4.1 mg, 0.012 mmol) in *m*-xylene (1.2 mL). The crude product was purified by $CHCl_3$-gpc and prep. tlc (h = 15 cm, hexane : EtOAc = 9 : 1, R_f 0.22). Colorless solid, yield 25.0 mg (65%) after gpc; 12.3 mg (32%) after prep. tlc. $C_{25}H_{27}NO_2S$ (405.6 g mol^{-1}). Exact MS (HRMS): m/z = calcd. for $C_{25}H_{27}NO_2S$ [M$^+$] 405.1762, found 405.1779. ^{1}H NMR (CDCl$_3$): δ (ppm) = 1.96–2.04 (m, 4H, N(CH$_2$CH_2)$_2$), 2.42 (td, J = 11.1/4.4 Hz, 2H, N(CH_2CH$_2$)$_2$), 2.74 (d, J = 11.5 Hz, 2H, N(CH_2CH$_2$)$_2$), 2.81 (t, J = 5.5 Hz, 2H, thiophCH_2CH$_2$), 3.58 (s, 2H, NCH_2Ph), 3.81 (t, J = 5.5 Hz, 2H, thiophCH$_2$CH_2), 3.83 (s, 3H, OCH_3), 6.91 (s, 1H, 3′-H-thioph), 6.93 (d, J = 8.9 Hz, 2H, o-H$_3$CO-Ph-H), 7.27–7.41 (m, 7H, Ph-H, H$_3$CO-Ph-H). ^{13}C NMR (CDCl$_3$): δ (ppm) = 27.7 (1C, thiophCH_2CH$_2$), 37.9 (2C, N(CH$_2$CH_2)$_2$), 49.3 (2C, N(CH_2CH$_2$)$_2$), 55.4 (1C, Ph-OCH_3), 59.3 (1C, thiophCH$_2$CH_2), 63.6 (1C, NCH_2Ph), 73.9 (1C, thiophC_{spiro}), 114.3 (2C, o-H$_3$CO-Ph-CH), 117.1 (1C, CH-3′-thioph), 127.1 (1C, C_{quart}), 127.3 (Ph-CH), 128.5 (Ph-CH), 129.6 (Ph-CH), 129.8 (Ph-CH), 130.3 (1C, C_{quart}), 137.3 (1C, C_{quart}), 145.4 (1C, C_{quart}), 159.2 (1C, C_{quart}). One signal for a quaternary carbon atom is not visible.

6.1.13. 1-Benzyl-1′-(4-methylphenyl)-6′,7′-dihydrospiro[piperidine-4,4′-thieno[3,4-*c*]pyran] (3c). According to General procedure A spirocyclic thiophene **5** (22.9 mg, 0.077 mmol) was reacted with *p*-iodotoluene (23.0 mg, 0.11 mmol), Ag_2CO_3 (23.5 mg, 0.09 mmol) and $PdCl_2$/2,2′-bipyridyl (3.1 mg, 0.01 mmol) in *m*-xylene (1.0 mL). The crude product was purified by $CHCl_3$-gpc and prep. tlc (h = 15 cm, hexane : EtOAc = 12 : 1, NHEt$_2$ 2%, R_f 0.56). Colorless solid, yield 14.6 mg (49%) after gpc; yield 10.0 mg (34%) after prep. tlc. $C_{25}H_{27}NOS$ (389.6 g mol^{-1}). ^{1}H NMR (CDCl$_3$): δ (ppm) = 1.96–2.05 (m, 4H, N(CH$_2$CH_2)$_2$), 2.37 (s, 3H, H_3C-Ph), 2.42 (td, J = 11.5/4.4 Hz, 2H, N(CH_2CH$_2$)$_2$), 2.74 (d, J = 11.5 Hz, 2H, N(CH_2CH$_2$)$_2$), 2.84 (t, J = 5.5 Hz, 2H, thiophCH_2CH$_2$), 3.58 (s, 2H, NCH_2Ph), 3.81 (t, J = 5.5 Hz, 2H, thiophCH$_2$CH_2), 6.94 (s, 1H, 3′-H-thioph), 7.20 (d, J = 7.9 Hz, 2H, o-H$_3$C-Ph-H), 7.27–7.39 (m, 7H, Ph-H, H$_3$C-Ph-H). ^{13}C NMR (CDCl$_3$): δ (ppm) = 21.2 (1C, Ph-CH$_3$), 27.8 (1C, thiophCH_2CH$_2$), 37.9 (2C, N(CH$_2$CH_2)$_2$), 49.4 (2C, N(CH_2CH$_2$)$_2$), 59.3 (1C, thiophCH$_2$CH_2), 63.6 (1C, NCH_2Ph), 74.0 (1C, thiophC_{spiro}), 117.5 (1C, CH-3′-thioph), 127.2 (Ph-CH), 128.5 (Ph-CH), 128.5 (Ph-CH), 129.6 (Ph-CH), 130.7 (1C, C_{quart}), 131.7 (1C, C_{quart}), 137.3 (1C, C_{quart}), 137.6 (1C, C_{quart}), 138.8 (1C, C_{quart}), 145.5 (1C, C_{quart}).

6.1.14. 1-Benzyl-1′-(4-nitrophenyl)-6′,7′-dihydrospiro[piperidine-4,4′-thieno[3,4-*c*]pyran] (3d). According to General procedure A spirocyclic thiophene **5** (27.8 mg, 0.093 mmol) was reacted with *p*-iodonitrobenzene (28.7 mg, 0.12 mmol), Ag_2CO_3 (28.1 mg, 0.10 mmol) and $PdCl_2$/2,2′-bipyridyl (4.0 mg, 0.012 mmol) in *m*-xylene (1.2 mL). The crude product was purified by $CHCl_3$-gpc and prep. tlc (h = 15 cm, hexane : EtOAc = 9 : 1, NHEt$_2$ 2%, R_f 0.60). Pale yellow solid, yield 18.7 mg (48%) after gpc; yield 4.4 mg (11%) after prep. tlc. $C_{24}H_{24}N_2O_3S$ (420.5 g mol^{-1}). Exact MS (HRMS): m/z = calcd. for $C_{24}H_{24}N_2O_3S$ [M$^+$] 420.1508, found 420.1523. ^{1}H NMR (CDCl$_3$): δ (ppm) = 1.95–2.06 (m, 4H, N(CH$_2$CH_2)$_2$), 2.42 (td, J = 10.8/5.6 Hz, 2H, N(CH_2CH$_2$)$_2$), 2.75 (d, J = 10.8 Hz, 2H, N(CH_2CH$_2$)$_2$), 2.88 (t, J = 5.3 Hz, 2H, thiophCH_2CH$_2$), 3.58 (s, 2H, NCH_2Ph), 3.84 (t, J = 5.4 Hz, 2H, thiophCH$_2$CH_2), 7.11 (s, 1H, 3′-H-thioph), 7.27–7.40 (m, 5H, Ph-H), 7.62 (d, J = 7.9 Hz, 2H, m-NO$_2$-Ph-H), 8.26 (d, J = 7.8 Hz, 2H, o-NO$_2$-Ph-H). ^{13}C NMR (CDCl$_3$): δ (ppm) = 28.1 (1C, thiophCH_2CH$_2$), 37.8 (2C, N(CH$_2$CH_2)$_2$), 49.2 (2C, N(CH_2CH$_2$)$_2$), 59.0 (1C, thiophCH$_2$CH_2), 63.5 (1C, NCH_2Ph), 73.9 (1C, thiophC_{spiro}), 120.5 (1C, CH-3′-thioph), 124.3 (Ph-CH), 127.3 (Ph-CH), 128.5 (Ph-CH), 128.7 (Ph-CH), 129.6 (Ph-CH), 133.6 (1C, C_{quart}), 135.1 (1C, C_{quart}), 138.6 (1C, C_{quart}), 141.3 (1C, C_{quart}), 146.4 (1C, C_{quart}), 146.7 (1C, C_{quart}).

6.1.15. 4-(1-Benzyl-6′,7′-dihydrospiro[piperidine-4,4′-thieno-[3,4-*c*]pyran]-1′-yl) benzonitrile (3e). According to General procedure A spirocyclic thiophene **5** (23.9 mg, 0.080 mmol) was reacted with *p*-iodobenzonitrile (21.8 mg, 0.09 mmol), Ag_2CO_3 (23.8 mg, 0.09 mmol) and $PdCl_2$/2,2′-bipyridyl (3.3 mg, 0.01 mmol) in *m*-xylene (1.0 mL). The crude product was purified by $CHCl_3$-gpc and prep. tlc (h = 15 cm, hexane : EtOAc = 9 : 1, NHEt$_2$ 2%, R_f 0.36). Colorless solid, yield 19 mg (59%) after gpc; yield 15.7 mg (49%) after prep. tlc. $C_{25}H_{24}N_2OS$ (400.5 g mol^{-1}). Exact MS (HRMS): m/z = calcd. for $C_{25}H_{24}N_2OS$ [M$^+$] 400.1609, found 400.1606. ^{1}H NMR (CDCl$_3$): δ (ppm) = 1.93–2.04 (m, 4H, N(CH$_2$CH_2)$_2$), 2.42 (td, J = 11.0/4.5 Hz, 2H, N(CH_2CH$_2$)$_2$), 2.74 (d, J = 11.5 Hz, 2H, N(CH_2CH$_2$)$_2$), 2.86 (t, J = 5.5 Hz, 2H, thiophCH_2CH$_2$), 3.58 (s, 2H, NCH_2Ph), 3.83 (t, J = 5.5 Hz, 2H, thiophCH$_2$CH_2), 7.08 (s, 1H, 3′-H-thioph), 7.27–7.41 (m, 5H, Ph-H), 7.56 (d, J = 8.2 Hz, 2H, m-NC-Ph-H), 7.68 (d, J = 8.1 Hz, 2H, o-NC-Ph-H). ^{13}C NMR (CDCl$_3$): δ (ppm) = 28.0 (1C, thiophCH_2CH$_2$), 37.9 (2C, N(CH$_2$CH_2)$_2$), 49.2 (2C, N(CH_2CH$_2$)$_2$), 59.0 (1C, thiophCH$_2$CH_2), 63.6 (1C, NCH_2Ph), 73.9 (1C, thiophC_{spiro}), 110.7 (1C, Ph-C_{quart}-C≡N), 119.0 (1C, C≡N), 120.0 (1C, CH-3′-thioph), 127.3 (Ph-CH), 128.5 (Ph-CH), 128.8 (Ph-CH), 129.6 (Ph-CH), 132.7 (Ph-CH), 133.1 (1C, C_{quart}), 135.4 (1C, C_{quart}), 138.7 (1C, C_{quart}), 139.3 (1C, C_{quart}), 146.2 (1C, C_{quart}).

6.1.16. 1-Benzyl-1′-(naphthalen-1-yl)-6′,7′-dihydrospiro[piperidine-4,4′-thieno [3,4-*c*]pyran] (3f). According to General procedure A spirocyclic thiophene **5** (34.7 mg, 0.11 mmol) was reacted with 1-iodonaphthalene (19 μL, 0.13 mmol), Ag_2CO_3 (34.8 mg, 0.13 mmol) and $PdCl_2$/2,2′-bipyridyl (4.0 mg, 0.01 mmol) in *m*-xylene (1.2 mL). The crude product was purified by $CHCl_3$-gpc and prep. tlc (h = 15 cm, hexane : EtOAc = 11 : 1, NHEt$_2$ 2%, R_f 0.40). Colorless solid, yield 33.4 mg (67%) after gpc; yield 17.4 mg (35%) after prep. tlc. $C_{28}H_{27}NOS$ (425.6 g mol^{-1}). Exact MS (HRMS): m/z = calcd. for $C_{28}H_{27}NOS$ [M$^+$] 425.1813, found 425.1805. ^{1}H NMR (CDCl$_3$): δ (ppm) = 2.01–2.14 (m, 4H, N(CH$_2$CH_2)$_2$), 2.41–2.50 (m, 4H, N(CH_2CH$_2$)$_2$, thiophCH_2CH$_2$), 2.78 (d, J = 11.4 Hz, 2H, N(CH_2CH$_2$)$_2$, 3.60 (s, 2H, NCH_2Ph), 3.79 (t, J = 5.6 Hz, 2H, thiophCH$_2$CH_2), 7.09 (s, 1H, 3′-H-thioph), 7.27–7.31 (m, 1H,

Ar-*H*), 7.32–7.42 (m, 4H, Ar-*H*), 7.43–7.54 (m, 4H, Ar-*H*), 7.76 (d, *J* = 8.2 Hz, 1H, Ar-*H*), 7.82–7.93 (m, 2H, Ar-*H*). ^{13}C NMR ($CDCl_3$): δ (ppm) = 26.8 (1C, thiophCH_2CH_2), 37.9 (2C, N(CH_2CH_2)$_2$), 49.3 (2C, N(CH_2CH_2)$_2$), 59.1 (1C, thiophCH_2CH_2), 63.6 (1C, NCH_2Ph), 73.9 (1C, thiophC_{spiro}), 118.4 (1C, *C*H-3′-thioph), 125.4 (1C, Ar-*C*H), 126.2 (1C, Ar-*C*H), 126.3 (1C, Ar-*C*H), 126.6 (1C, Ar-*C*H), 127.3 (1C, Ar-*C*H), 128.5 (1C, Ar-*C*H), 128.6 (1C, Ar-*C*H), 128.9 (1C, Ar-*C*H), 129.3 (1C, Ar-*C*H), 129.6 (1C, Ar-*C*H), 131.8 (1C, C_{quart}), 132.5 (1C, C_{quart}), 133.2 (1C, C_{quart}), 133.9 (1C, C_{quart}), 134.9 (1C, C_{quart}), 138.7 (1C, C_{quart}), 144.5 (1C, C_{quart}).

6.1.17. 1-Benzyl-6′-methoxy-1′-phenyl-6′,7′-dihydrospiro[piperidine-4,4′-thieno[3,4-*c*]pyran] (4a). According to General procedure A spirocyclic thiophene **6** (87.8 mg, 0.27 mmol) was reacted with iodobenzene (35.2 mg, 0.17 mmol), Ag_2CO_3 (49.3 mg, 0.18 mmol) and $PdCl_2$/2,2′-bipyridyl (5.5 mg, 0.016 mmol) in *m*-xylene (1.5 mL). The crude product was purified by $CHCl_3$-gpc and prep. tlc (*h* = 15 cm, hexane : EtOAc = 3 : 2, R_f 0.4). Pale yellow resin, yield 46.3 mg (67%) after gpc; yield 33.2 mg (48%) after prep.tlc. $C_{25}H_{27}NO_2S$ (405.6 g mol^{-1}). Exact MS (HRMS): *m*/*z* = calcd. for $C_{25}H_{27}NO_2S$ [M$^+$] 405.1762, found 405.1782. ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.91 (td, J = 13.6/4.6 Hz, 1H, N(CH_2CH_2)$_2$), 1.96–2.06 (m, 1H, N(CH_2CH_2)$_2$), 2.06–2.19 (m, 2H, N(CH_2CH_2)$_2$), 2.47 (td, J = 11.8/2.3 Hz, 1H, N(CH_2CH_2)$_2$), 2.58 (td, J = 11.9/2.7 Hz, 1H, N(CH_2CH_2)$_2$), 2.80 (dd, J = 13.5/11.4 Hz, 2H, N(CH_2CH_2)$_2$), 2.88 (dd, J = 15.5/7.4 Hz, 1H, thiophCH_2CH), 3.02 (dd, J = 15.5/3.0 Hz, 1H, thiophCH_2CH), 3.52 (s, 3H, OCH_3), 3.58 (d, *J* = 13.0 Hz, 1H, NCH_2Ph), 3.62 (d, *J* = 13.0 Hz, 1H, NCH_2Ph), 4.80 (dd, J = 7.4/3.1 Hz, 1H, thiophCH_2C*H*), 6.96 (s, 1H, 3′-*H*-thioph), 7.27–7.46 (m, 10H, Ph-*H*). ^{13}C NMR ($CDCl_3$): δ (ppm) = 33.3 (1C, thiophCH_2CH), 37.5 (1C, N(CH_2CH_2)$_2$), 39.9 (1C, N(CH_2CH_2)$_2$), 49.4 (2C, N(CH_2CH_2)$_2$), 56.7 (1C, OCH_3), 63.4 (1C, NCH_2Ph), 74.0 (1C, thiophC_{spiro}), 97.5 (1C, thiophCH_2CH), 117.5 (1C, *C*H-3′-thioph), 127.5 (Ph-*C*H), 128.1 (Ph-*C*H), 128.2 (Ph-*C*H), 128.5 (Ph-*C*H), 128.5 (Ph-*C*H), 128.8 (Ph-*C*H), 129.1 (Ph-C_{quart}), 129.6 (Ph-*C*H), 131.6 (Ph-*C*H), 134.0 (1C, C_{quart}), 138.1 (1C, C_{quart})(Ph-C), 138.4 (1C, C_{quart})(Ph-C), 144.2 (1C, C_{quart}).

6.1.18. 1-Benzyl-6′-methoxy-1′-(4-methoxyphenyl)-6′,7′-dihydrospiro[piperidine-4,4′-thieno[3,4-*c*]pyran] (4b). According to General procedure A spirocyclic thiophene **6** (20.0 mg, 0.061 mmol) was reacted with *p*-iodoanisole (15.9 mg, 0.07 mmol), Ag_2CO_3 (16.9 mg, 0.06 mmol) and $PdCl_2$/2,2′-bipyridyl (2.2 mg, 0.007 mmol) in *m*-xylene (1.0 mL). The crude product was purified by $CHCl_3$-gpc and prep. tlc (*h* = 15 cm, hexane : EtOAc = 3 : 2, NEt_3 2%, R_f 0.36). Colorless solid, yield 13.8 mg (52%) after gpc; 12.5 mg (47%) after prep. tlc. $C_{26}H_{29}NO_3S$ (435.6 g mol^{-1}). Exact MS (HRMS): *m*/*z* = calcd. for $C_{26}H_{29}NO_3S$ [M$^+$] 435.1868, found 435.1877. ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.90 (td, J = 13.5/3.0 Hz, 1H, N(CH_2CH_2)$_2$), 1.96–2.15 (m, 3H, N(CH_2CH_2)$_2$), 2.46 (td, J = 12.1/1.6 Hz, 1H, N(CH_2CH_2)$_2$), 2.57 (td, J = 11.5/1.3 Hz, 1H, N(CH_2CH_2)$_2$), 2.74–2.89 (m, 3H, N(CH_2CH_2)$_2$, thiophCH_2CH), 2.98 (dd, J = 15.4/3.0 Hz, 1H, thiophCH_2CH), 3.52 (s, 3H, OCH_3), 3.58 (d, *J* = 12.5 Hz, 1H, NCH_2Ph), 3.60 (d, *J* = 13.4 Hz, 1H, NCH_2Ph), 3.83 (s, 3H, OCH_3), 4.79 (dd, J = 7.4/3.1 Hz, 1H, thiophCH_2C*H*), 6.90 (s, 1H, 3′-*H*-thioph), 6.92 (d, *J* = 8.7 Hz, 2H, *o*-H_3CO-Ph-*H*), 7.30–7.40 (m, 7H, Ph-*H*). ^{13}C NMR ($CDCl_3$): δ (ppm) = 33.2 (1C, thiophCH_2CH), 37.6 (1C, N(CH_2CH_2)$_2$), 40.1 (1C, N(CH_2CH_2)$_2$), 49.4 (2C, N(CH_2CH_2)$_2$), 55.5 (1C, Ph-OCH_3), 56.6 (1C, OCH_3), 63.5 (1C, NCH_2Ph), 74.2 (1C, thiophC_{spiro}), 97.6 (1C, thiophCH_2CH), 114.4 (Ph-*C*H), 116.7 (1C, *C*H-3′-thioph), 126.8 (Ph-*C*H), 127.3 (1C, C_{quart}), 128.5 (Ph-*C*H), 128.6 (1C, C_{quart}), 129.4 (1C, C_{quart}), 129.6 (Ph-*C*H), 129.9 (Ph-*C*H), 138.0 (1C, C_{quart}), 144.6 (1C, C_{quart}), 159.4 (1C, Ph-C_{quart}-OCH_3).

6.1.19. 1-Benzyl-6′-methoxy-1′-(4-methylphenyl)-6′,7′-dihydrospiro[piperidine-4,4′-thieno[3,4-*c*]pyran] (4c). According to General procedure A spirocyclic thiophene **6** (28.3 mg, 0.086 mmol) was reacted with *p*-iodotoluene (22.5 mg, 0.10 mmol), Ag_2CO_3 (25.5 mg, 0.09 mmol) and $PdCl_2$/2,2′-bipyridyl (3.7 mg, 0.011 mmol) in *m*-xylene (1.2 mL). The crude product was purified by $CHCl_3$-gpc and prep. tlc (*h* = 15 cm, hexane : EtOAc = 4 : 1, NEt_3 2%, R_f 0.62). Colorless solid, yield 15.2 mg (42%) after gpc; 13.7 mg (38%) after prep. tlc. $C_{26}H_{29}NO_2S$ (419.6 g mol^{-1}). Exact MS (HRMS): *m*/*z* = calcd. for $C_{26}H_{29}NO_2S$ [M$^+$] 419.1919, found 419.1912. ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.90 (td, J = 13.3/3.9 Hz, 1H, N(CH_2CH_2)$_2$), 1.99 (dd, J = 13.7/2.7 Hz, 1H, N(CH_2CH_2)$_2$), 2.04–2.15 (m, 2H, N(CH_2CH_2)$_2$), 2.37 (s, 3H, H_3C-Ph), 2.46 (td, J = 12.7/2.3 Hz, 1H, N(CH_2CH_2)$_2$), 2.57 (td, J = 12.1/2.7 Hz, 1H, N(CH_2CH_2)$_2$), 2.74–2.83 (m, 2H, N(CH_2CH_2)$_2$), 2.86 (dd, J = 15.5/7.5 Hz, 1H, thiophCH_2CH), 3.00 (dd, J = 15.5/3.1 Hz, 1H, thiophCH_2CH), 3.52 (s, 3H, OCH_3), 3.58 (d, *J* = 13.0 Hz, 1H, NCH_2Ph), 3.62 (d, *J* = 13.1 Hz, 1H, NCH_2Ph), 4.79 (dd, J = 7.4/3.1 Hz, 1H, thiophCH_2C*H*), 6.93 (s, 1H, 3′-*H*-thioph), 7.19 (d, *J* = 7.9 Hz, 2H, *o*-H_3C-Ph-*H*), 7.27–7.40 (m, 7H, Ph-*H*, H_3C-Ph-*H*). ^{13}C NMR ($CDCl_3$): δ (ppm) = 21.2 (1C, Ph-CH_3), 33.3 (1C, thiophCH_2CH), 37.6 (1C, N(CH_2CH_2)$_2$), 40.1 (1C, N(CH_2CH_2)$_2$), 49.4 (2C, N(CH_2CH_2)$_2$), 56.6 (1C, OCH_3), 63.6 (1C, NCH_2Ph), 74.2 (1C, thiophC_{spiro}), 97.5 (1C, thiophCH_2CH), 117.0 (1C, *C*H-3′-thioph), 127.3 (Ph-*C*H), 128.5 (Ph-*C*H), 128.5 (Ph-*C*H), 128.9 (1C, C_{quart}), 129.6 (Ph-*C*H), 129.6 (Ph-*C*H), 131.3 (1C, C_{quart}), 137.5 (1C, C_{quart}), 138.2 (1C, C_{quart}), 138.6 (1C, C_{quart}), 144.6 (1C, C_{quart}).

6.1.20. 1-Benzyl-6′-methoxy-1′-(4-nitrophenyl)-6′,7′-dihydrospiro-[piperidine-4,4′-thieno [3,4-*c*]pyran] (4d). According to General procedure A spirocyclic thiophene **6** (30.5 mg, 0.093 mmol) was reacted with *p*-iodo-nitrobenzene (26.1 mg, 0.10 mmol), Ag_2CO_3 (26.0 mg, 0.09 mmol) and $PdCl_2$/2,2′-bipyridyl (3.5 mg, 0.01 mmol) in *m*-xylene (1.2 mL). The crude product was purified by $CHCl_3$-gpc and prep. tlc (*h* = 15 cm, hexane : EtOAc = 3 : 2, NEt_3 2%, R_f 0.33). Pale yellow solid, yield 33.3 mg (80%) after gpc; 18.8 mg (45%) after prep. tlc. $C_{25}H_{26}N_2O_4S$ (450.5 g mol^{-1}). Exact MS (HRMS): *m*/*z* = calcd. for $C_{25}H_{26}N_2O_4S$ [M$^+$] 450.1613, found 450.1595. ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.90 (td, J = 13.3/4.0 Hz, 1H, N(CH_2CH_2)$_2$), 1.99 (dd, J = 13.9/2.4 Hz, 1H, N(CH_2CH_2)$_2$), 2.03–2.15 (m, 2H, N(CH_2CH_2)$_2$), 2.46 (td, J = 12.8/2.5 Hz, 1H, N(CH_2CH_2)$_2$), 2.57 (td, J = 11.9/2.2 Hz, 1H, N(CH_2CH_2)$_2$), 2.80 (dd, J = 14.5/12.6 Hz, 2H, N(CH_2CH_2)$_2$), 2.92 (dd, J = 15.7/6.8 Hz, 1H, thiophCH_2CH), 3.03 (dd, J = 15.5/3.1 Hz, 1H, thiophCH_2CH), 3.52 (s, 3H, OCH_3), 3.58 (d, *J* = 13.1 Hz, 1H, NCH_2Ph), 3.62 (d, *J* = 13.1 Hz, 1H, NCH_2Ph), 4.84 (dd, J = 6.7/3.1 Hz, 1H, thiophCH_2C*H*), 7.11 (s, 1H, 3′-*H*-thioph), 7.28–7.40 (m, 5H, Ph-*H*), 7.59 (d, *J* = 8.3 Hz, 2H, *m*-NO_2-Ph-*H*), 8.25 (d, *J* = 8.1 Hz, 2H, *o*-NO_2-Ph-*H*). ^{13}C NMR ($CDCl_3$): δ (ppm) = 33.5 (1C, thiophCH_2CH), 37.8 (1C,N(CH_2CH_2)$_2$), 40.0 (1C, N(CH_2CH_2)$_2$), 49.3 (2C, N(CH_2CH_2)$_2$), 56.7 (1C, OCH_3), 63.5

(1C, NCH$_2$Ph), 74.1 (1C, thiophC$_{spiro}$), 97.2 (1C, thiophCH$_2$CH), 120.0 (1C, CH-3′-thioph), 124.3 (Ph-CH), 127.4 (Ph-CH), 128.5 (Ph-CH), 128.9 (Ph-CH), 129.6 (Ph-CH), 130.5 (1C, C_{quart}), 131.6 (1C, C_{quart}), 135.7 (1C, C_{quart}), 140.9 (1C, C_{quart}), 145.3 (1C, C_{quart}), 146.83 (1C, C_{quart}).

6.1.21. 1-[4-(1-Benzyl-6′-methoxy-6′,7′-dihydrospiro[piperidine-4,4′-thieno[3,4-*c*]pyran]-1′-yl)phenyl]ethanone (4e). According to General procedure A spirocyclic thiophene **6** (21.4 mg, 0.065 mmol) was reacted with *p*-iodo-acetophenone (17.9 mg, 0.07 mmol), Ag_2CO_3 (17.0 mg, 0.06 mmol) and $PdCl_2$/2,2′-bipyridyl (2.1 mg, 0.006 mmol) in *m*-xylene (1.0 mL). The crude product was purified by prep. tlc (*h* = 15 cm, hexane : EtOAc = 3 : 2, NEt_3 2%, R_f 0.52). Colorless solid, yield 17.4 mg (60%) after prep. tlc. $C_{27}H_{29}NO_3S$ (447.6 g mol^{-1}). Exact MS (HRMS): *m*/*z* = calcd. for $C_{27}H_{29}NO_3S$ [M$^+$] 447.1868, found 447.1890. ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.91 (td, J = 13.3/4.1 Hz, 1H, N(CH$_2$CH_2)$_2$), 1.99 (dd, J = 13.8/2.6 Hz, 1H, N(CH$_2$CH_2)$_2$), 2.05–2.16 (m, 2H, N(CH$_2$CH_2)$_2$), 2.47 (td, J = 12.4/2.7 Hz, 1H, N(CH_2CH$_2$)$_2$), 2.53–2.59 (m, 1H, N(CH_2CH$_2$)$_2$), 2.61 (s, 3H, CH_3C═O), 2.74–2.86 (m, 2H, N(CH_2CH$_2$)$_2$), 2.92 (dd, J = 15.6/7.1 Hz, 1H, thiophCH_2CH), 3.03 (dd, J = 15.6/3.1 Hz, 1H, thiophCH_2CH), 3.52 (s, 3H, OCH_3), 3.58 (d, *J* = 13.1 Hz, 1H, NCH_2Ph), 3.62 (d, *J* = 13.1 Hz, 1H, NCH_2Ph), 4.82 (dd, J = 7.1/3.1 Hz, 1H, thiophCH$_2$C*H*), 7.04 (s, 1H, 3′-*H*-thioph), 7.27–7.40 (m, 5H, Ph-*H*), 7.53 (d, *J* = 8.4 Hz, 2H, *m*-CH$_3$C═O-Ph-*H*), 7.98 (d, *J* = 8.3 Hz, 2H, *o*-CH$_3$C═O-Ph-*H*). ^{13}C NMR ($CDCl_3$): δ (ppm) = 26.6 (1C, CH$_3$C═O), 33.5 (1C, thiophCH$_2$CH), 37.8 (1C, N(CH$_2$CH$_2$)$_2$), 40.1 (1C, N(CH$_2$CH$_2$)$_2$), 49.4 (2C, N(CH$_2$CH$_2$)$_2$), 56.6 (1C, OCH$_3$), 63.5 (1C, NCH$_2$Ph), 74.2 (1C, thiophC$_{spiro}$), 97.4 (1C, thiophCH$_2$CH), 118.9 (1C, CH-3′-thioph), 127.4 (Ph-CH), 128.5 (Ph-CH), 129.0 (Ph-CH), 129.6 (Ph-CH), 130.7 (1C, C_{quart}), 135.9 (1C, C_{quart}), 136.9 (1C, C_{quart}), 138.5 (1C, C_{quart}), 139.0 (1C, C_{quart}), 145.1 (1C, C_{quart}), 197.8 (1C, C_{quart}, CH$_3$C═O).

6.1.22. 4-(1-Benzyl-6′-methoxy-6′,7′-dihydrospiro[piperidine-4,4′-thieno[3,4-*c*]pyran]-1′-yl)benzonitrile (4f). According to General procedure A spirocyclic thiophene **6** (33.5 mg, 0.102 mmol) was reacted with *p*-iodobenzonitrile (24.8 mg, 0.11 mmol), Ag_2CO_3 (26.1 mg, 0.09 mmol) and $PdCl_2$/2,2′-bipyridyl (3.1 mg, 0.009 mmol) in *m*-xylene (1.2 mL). The crude product was purified by $CHCl_3$-gpc and prep. tlc (*h* = 15 cm, hexane : EtOAc = 9 : 1, NEt_3 2%, R_f 0.22). Colorless solid, yield 21.7 mg (50%) after gpc; yield 17.4 mg (40%) after prep. tlc. $C_{26}H_{26}N_2O_2S$ (430.6 g mol^{-1}). Exact MS (HRMS): *m*/*z* = calcd. for $C_{26}H_{26}N_2O_2S$ [M$^+$] 430.1715, found 430.1693. ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.84–2.02 (m, 2H, N(CH$_2$CH_2)$_2$), 2.03–2.18 (m, 2H, N(CH$_2$CH_2)$_2$), 2.46 (td, J = 12.6/2.7 Hz, 1H, N(CH_2CH$_2$)$_2$), 2.57 (td, J = 11.9/2.6 Hz, 1H, N(CH_2CH$_2$)$_2$), 2.80 (dd, J = 14.0/11.4 Hz, 2H, N(CH_2CH$_2$)$_2$), 2.89 (dd, J = 15.5/6.9 Hz, 1H, thiophCH_2CH), 3.00 (dd, J = 15.5/3.2 Hz, 1H, thiophCH_2CH), 3.52 (s, 3H, OCH_3), 3.58 (d, *J* = 11.7 Hz, 1H, NCH_2Ph), 3.62 (d, *J* = 13.1 Hz, 1H, NCH_2Ph), 4.82 (dd, J = 6.9/3.2 Hz, 1H, thiophCH$_2$C*H*), 7.07 (s, 1H, 3′-*H*-thioph), 7.27–7.40 (m, 5H, Ph-*H*), 7.53 (d, *J* = 8.6 Hz, 2H, *m*-NC-Ph-*H*), 7.67 (d, *J* = 8.5 Hz, 2H, *o*-NC-Ph-*H*). ^{13}C NMR ($CDCl_3$): δ (ppm) = 33.4 (1C, thiophCH$_2$CH), 37.8 (1C, N(CH$_2$CH$_2$)$_2$), 40.1 (1C, N(CH$_2$CH$_2$)$_2$), 49.3 (2C, N(CH$_2$CH$_2$)$_2$), 56.6 (1C, OCH$_3$), 63.5 (1C, NCH$_2$Ph), 74.1 (1C, thiophC$_{spiro}$), 97.2 (1C, thiophCH$_2$CH), 110.9 (1C, Ph-C_{quart}-C≡N), 119.0 (1C, C≡N), 119.5 (1C, CH-3′-thioph), 127.3 (Ph-CH), 128.5 (Ph-CH), 128.9 (Ph-CH), 129.5 (Ph-CH), 131.2 (1C, C_{quart}), 132.7 (Ph-CH), 136.0 (1C, C_{quart}), 138.5 (1C, C_{quart}), 138.9 (1C, C_{quart}), 145.3 (1C, C_{quart}).

6.1.23. 1-Benzyl-6′-methoxy-1′-[4-(trifluoromethyl)phenyl]-6′,7′-dihydrospiro[piperidine-4,4′-thieno[3,4-*c*]pyran] (4g). According to General procedure A spirocyclic thiophene **6** (20.1 mg, 0.061 mmol) was reacted with *p*-iodo-trifluoromethylbenzene (10 µL, 0.07 mmol), Ag_2CO_3 (18.1 mg, 0.07 mmol) and $PdCl_2$/2,2′-bipyridyl (2.2 mg, 0.007 mmol) in *m*-xylene (1.0 mL). The crude product was purified by $CHCl_3$-gpc and prep. tlc (*h* = 15 cm, hexane : EtOAc = 3 : 2, R_f 0.43). Colorless solid, yield 10.9 mg (38%) after gpc; 5.4 mg (19%) after prep. tlc. $C_{26}H_{26}F_3NO_2S$ (473.6 g mol^{-1}). Exact MS (HRMS): *m*/*z* = calcd. for $C_{26}H_{26}F_3NO_2S$ [M$^+$] 473.1636, found 473.1648. ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.90 (td, J = 13.4/4.2 Hz, 1H, N(CH$_2$CH_2)$_2$), 1.96–2.16 (m, 3H, N(CH$_2$CH_2)$_2$), 2.46 (td, J = 12.6/2.4 Hz, 1H, N(CH_2CH$_2$)$_2$), 2.57 (td, J = 12.2/2.7 Hz, 1H, N(CH_2CH$_2$)$_2$), 2.80 (dd, J = 13.6/11.7 Hz, 2H, N(CH_2CH$_2$)$_2$), 2.89 (dd, J = 15.5/7.1 Hz, 1H, thiophCH_2CH), 3.00 (dd, J = 15.5/3.1 Hz, 1H, thiophCH_2CH), 3.52 (s, 3H, OCH_3), 3.58 (d, *J* = 13.3 Hz, 1H, NCH_2Ph), 3.62 (d, *J* = 12.9 Hz, 1H, NCH_2Ph), 4.82 (dd, J = 7.0/3.2 Hz, 1H, thiophCH$_2$C*H*), 7.03 (s, 1H, 3′-*H*-thioph), 7.27–7.42 (m, 5H, Ph-*H*), 7.54 (d, *J* = 8.1 Hz, 2H, *m*-F$_3$C-Ph-*H*), 7.64 (d, *J* = 8.3 Hz, 2H, *o*-F$_3$C-Ph-*H*). ^{13}C NMR ($CDCl_3$): δ (ppm) = 33.3 (1C, thiophCH$_2$CH), 37.8 (1C, N(CH$_2$CH$_2$)$_2$), 40.1 (1C, N(CH$_2$CH$_2$)$_2$), 49.4 (2C, N(CH$_2$CH$_2$)$_2$), 56.6 (1C, OCH$_3$), 63.6 (1C, NCH$_2$Ph), 74.2 (1C, thiophC$_{spiro}$), 97.3 (1C, thiophCH$_2$CH), 118.8 (1C, CH-3′-thioph), 125.9 (Ph-C-CF$_3$), 126.3 (1C, C_{quart}), 127.4 (Ph-CH), 128.5 (Ph-CH), 128.7 (Ph-CH), 129.2 (1C, C_{quart}), 129.6 (Ph-CH), 130.5 (1C, C_{quart}), 136.4 (1C, C_{quart}), 137.8 (1C, C_{quart}), 145.0 (1C, C_{quart}).

6.1.24. 1-Benzyl-6′-methoxy-1′-(naphthalen-1-yl)-6′,7′-dihydrospiro[piperidine-4,4′-thieno[3,4-*c*]pyran] (4h). According to General procedure A spirocyclic thiophene **6** (35.2 mg, 0.11 mmol) was reacted with 1-iodonaphthalene (17.2 µL, 0.12 mmol), Ag_2CO_3 (26.9 mg, 0.10 mmol) and $PdCl_2$/2,2′-bipyridyl (3.3 mg, 0.01 mmol) in *m*-xylene (1.2 mL). The crude product was purified by $CHCl_3$-gpc and prep. tlc (*h* = 15 cm, hexane : EtOAc = 9 : 1, $NHEt_2$ 2%, R_f 0.28). Colorless solid, yield 35.1 mg (72%) after gpc; 22.3 mg (46%) after prep. tlc. $C_{29}H_{29}NO_2S$ (455.6 g mol^{-1}). Exact MS (HRMS): *m*/*z* = calcd. for $C_{29}H_{29}NO_2S$ [M$^+$] 455.1919, found 455.1938. ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.98 (td, J = 13.3/4.2 Hz, 1H, N(CH$_2$CH_2)$_2$), 2.09 (dd, J = 13.7/2.5 Hz, 1H, N(CH$_2$CH_2)$_2$), 2.12–2.28 (m, 2H, N(CH$_2$CH_2)$_2$), 2.49 (td, J = 13.6/3.0 Hz, 1H, N(CH_2CH$_2$)$_2$), 2.56 (d, *J* = 5.7 Hz, 2H, thiophCH_2CH), 2.57–2.65 (m, 1H, N(CH_2CH$_2$)$_2$), 2.83 (dd, J = 14.0/11.9 Hz, 2H, N(CH_2CH$_2$)$_2$), 3.48 (s, 3H, OCH_3), 3.60 (d, *J* = 13.7 Hz, 1H, NCH_2Ph), 3.64 (d, *J* = 13.8 Hz, 1H, NCH_2Ph), 4.81 (t, *J* = 5.3 Hz, 1H, thiophCH$_2$C*H*), 7.09 (s, 1H, 3′-*H*-thioph), 7.27–7.53 (m, 9H, Ar-*H*), 7.77 (d, *J* = 8.2 Hz, 1H, Ar-*H*), 7.88 (td, J = 7.1/2.2 Hz, 2H, Ar-*H*). ^{13}C NMR ($CDCl_3$): δ (ppm) = 32.5 (1C, thiophCH$_2$CH), 37.8 (1C, N(CH$_2$CH$_2$)$_2$), 40.1 (1C, N(CH$_2$CH$_2$)$_2$), 49.4 (1C, N(CH$_2$CH$_2$)$_2$), 49.5 (1C, N(CH$_2$CH$_2$)$_2$), 56.5 (1C, OCH$_3$), 63.6 (1C, NCH$_2$Ph), 74.3 (1C, thiophC$_{spiro}$), 97.5 (1C, thiophCH$_2$CH), 117.9 (1C, CH-3′-thioph), 125.3 (Ar-CH), 126.2 (Ar-CH), 126.7 (Ar-CH), 127.3 (Ar-CH), 128.4 (1C, C_{quart}), 128.5 (Ar-CH), 128.6 (Ar-CH), 129.0 (Ar-CH), 129.2 (Ar-CH), 129.6 (Ar-CH), 130.1 (1C, C_{quart}), 131.4 (1C, C_{quart}), 131.8

(Ar-*C*H), 132.5 (1C, C_{quart}), 133.9 (Ar-*C*H), 135.5 (1C, C_{quart}), 138.7 (1C, C_{quart}), 143.8 (1C, C_{quart}).

6.1.25. 1-Benzyl-6′-methoxy-1′-(pyridin-3-yl)-6′,7′-dihydrospiro[piperidine-4,4′-thieno [3,4-*c*]pyran] (4i). According to General procedure A spirocyclic thiophene **6** (34 mg, 0.103 mmol) was reacted with 3-iodopyridine (20.4 mg, 0.10 mmol), Ag_2CO_3 (26.8 mg, 0.10 mmol) and $PdCl_2$/2,2′-bipyridyl (3.4 mg, 0.01 mmol) in *m*-xylene (1.2 mL). The crude product was purified by $CHCl_3$-gpc and prep. tlc (h = 15 cm, hexane : EtOAc = 7 : 3, NEt_3 2%, R_f 0.44). Colorless solid, yield 11.4 mg (27%) after gpc; 7.4 mg (18%) after prep. tlc. $C_{24}H_{26}N_2O_2S$ (406.5 g mol^{-1}). Exact MS (HRMS): m/z = calcd. for $C_{24}H_{26}N_2O_2S$ [M$^+$] 406.1715, found 406.1703. ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.83–2.17 (m, 4H, N(CH_2CH_2)$_2$), 2.47 (td, J = 12.1/2.4 Hz, 1H, N(CH_2CH_2)$_2$), 2.57 (td, J = 11.9/2.6 Hz, 1H, N(CH_2CH_2)$_2$), 2.73–2.94 (m, 3H, N(CH_2CH_2)$_2$), thiophCH_2CH), 2.99 (dd, J = 15.4/3.2 Hz, 1H, thiophCH_2CH), 3.51 (s, 3H, OCH_3), 3.57 (d, J = 14.0 Hz, 1H, NCH_2Ph), 3.62 (d, J = 13.5 Hz, 1H, NCH_2Ph), 4.83 (dd, J = 6.8/3.3 Hz, 1H, thiophCH_2CH), 7.05 (s, 1H, 3′-*H*-thioph), 7.28–7.42 (m, 6H, Ph-*H*, 5-*H*-Pyr), 7.73 (d, J = 7.9 Hz, 1H, 4-*H*-Pyr), 8.54 (d, J = 4.8 Hz, 1H, 6-*H*-Pyr), 8.70 (s, 1H, 2-*H*-Pyr). ^{13}C NMR ($CDCl_3$): δ (ppm) = 33.0 (1C, thiophCH_2CH), 37.9 (1C, N(CH_2CH_2)$_2$), 40.1 (1C, N(CH_2CH_2)$_2$), 49.4 (2C, N(CH_2CH_2)$_2$), 56.6 (1C, OCH_3), 63.5 (1C, NCH_2Ph), 74.2 (1C, thiophC_{spiro}), 97.4 (1C, thiophCH_2CH), 118.8 (1C, *C*H-3′-thioph), 123.6 (1C, *C*H-5′-Pyr), 127.4 (Ph-*C*H), 128.5 (Ph-*C*H), 129.6 (Ph-*C*H), 130.4 (1C, C_{quart}), 130.6 (1C, C_{quart}), 134.1 (1C, C_{quart}), 135.7 (1C, *C*H-4′-Pyr), 144. 9 (1C, C_{quart}), 148.7 (1C, *C*H-2′-Pyr), 149.4 (1C, *C*H-6′-Pyr). One signal for a quaternary carbon atom is not visible.

6.1.26. 1-Benzyl-1′-(1,1′-biphenyl-4′-yl)-6′-methoxy-6′,7′-dihydrospiro[piperidine-4,4′-thieno[3,4-*c*]pyran] (4j). According to General procedure A spirocyclic thiophene **6** (31.0 mg, 0.094 mmol) was reacted with 4-iodo-1,1′-biphenyl (32.4 mg, 0.12 mmol), Ag_2CO_3 (33.0 mg, 0.12 mmol) and $PdCl_2$/2,2′-bipyridyl (3.2 mg, 0.01 mmol) in *m*-xylene (1.2 mL). The crude product was purified by $CHCl_3$-gpc and flash chromatography (Ø = 1.5 cm, h = 5 cm, hexane : EtOAc = 4 : 1, 3 mL, R_f 0.22). Pale yellow solid, mp 161.2 °C, yield 17.7 mg (39%) after gpc; yield 17.4 mg (38%) after flash chromatography. $C_{31}H_{31}NO_2S$ (481.6 g mol^{-1}). Purity (HPLC†): 96.7%, t_R = 10.11 min. Exact MS (APCI): m/z = calcd. for $C_{31}H_{32}NO_2S$ [MH$^+$] 482.2148, found 482.2188. ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.91 (td, J = 12.6/4.9 Hz, 1H, N(CH_2CH_2)$_2$), 2.01 (dd, J = 13.7/2.7 Hz, 1H, N(CH_2CH_2)$_2$), 2.07–2.19 (m, 2H, N(CH_2CH_2)$_2$), 2.47 (td, J = 12.6/2.8 Hz, 1H, N(CH_2CH_2)$_2$), 2.58 (td, J = 12.0/2.6 Hz, 1H, N(CH_2CH_2)$_2$), 2.80 (dd, J = 13.4/11.5 Hz, 2H, N(CH_2CH_2)$_2$), 2.93 (dd, J = 15.5/7.4 Hz, 1H, thiophCH_2CH), 3.07 (dd, J = 15.4/3.1 Hz, 1H, thiophCH_2CH), 3.53 (s, 3H, OCH_3), 3.59 (d, J = 12.9 Hz, 1H, NCH_2Ph), 3.62 (d, J = 13.0 Hz, 1H, NCH_2Ph), 4.82 (dd, J = 7.4/3.1 Hz, 1H, thiophCH_2CH), 6.98 (s, 1H, 3′-*H*-thioph), 7.27–7.65 (m, 14H, Ph-*H*). ^{13}C NMR ($CDCl_3$): δ (ppm) = 33.4 (1C, thiophCH_2CH), 37.7 (1C, N(CH_2CH_2)$_2$), 40.2 (1C, N(CH_2CH_2)$_2$), 49.5 (2C, N(CH_2CH_2)$_2$), 56.7 (1C, OCH_3), 63.6 (1C, NCH_2Ph), 74.3 (1C, thiophC_{spiro}), 97.5 (1C, thiophCH_2CH), 117.5 (1C, *C*H-3′-thioph), 127.2 (Ph-*C*H), 127.3 (Ph-*C*H), 127.6 (Ph-*C*H), 127.7 (Ph-*C*H), 128.5 (Ph-*C*H), 129.0 (Ph-*C*H), 129.1 (Ph-*C*H), 129.5 (1C, C_{quart}), 129.5 (Ph-*C*H), 133.2 (1C, C_{quart}), 137.8 (1C, C_{quart}), 138.7 (1C, C_{quart}), 140.4 (1C, C_{quart}), 140.8 (1C, C_{quart}), 144.9 (1C, C_{quart}).†

6.2. Receptor binding studies

6.2.1. 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 solid scintillator was melted on the filtermat at a temperature of 95 °C for 5 min. After solidification of the scintillator at rt, the scintillation was measured using a MicroBeta Trilux scintillation analyzer (Perkin Elmer). The overall counting efficiency was 20%.

6.2.2 Membrane preparation for the σ_1 assay[42–45]. 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 x g for 10 min at 4 °C. The supernatant was separated and centrifuged at 23500 x 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 x 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[48] 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.

6.2.3. Performance of the σ_1 assay[42–45]. 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 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 non-specific binding was determined with 10 µM unlabeled (+)-pentazocine. The K_d-value of the radioligand [^{3}H]-(+)-pentazocine is 2.9 nM.[49]

6.2.4. Membrane preparation for the σ_2 assay[42–45]. 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 x g for 10 min at 4 °C. The supernatant was separated and centrifuged at

† HPLC Agilent Technologies®, quaternary pump; UV detector: Agilent 1200 Series; autosampler: Agilent 1200 Series High Performance autosampler; column: ZORBAX Eclipse Plus C18 2.1 × 150 mm, 3.5 µm; flow rate: 0.40 mL min^{-1}; detection at λ = 254 nm; cut off time: 13 min; solvents: A: H_2O with 5 mM NH_4HCO_2; B: CH_3CN; gradient elution (A%): 0–5 min: gradient from 80% to 0%; 5–13 min: 0%; 13–18 min: 0% to 80%.

31000 x *g* for 20 min at 4 °C. The pellet was resuspended in buffer (50 mM TRIS, pH 8.0) and incubated at rt for 30 min. After the incubation, the suspension was centrifuged again at 31000 x *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[48] using bovine serum albumin as standard, and subsequently the preparation was frozen (–80 °C) in 1.5 mL portions containing about 2 mg protein/mL.

6.2.5. Performance of the σ_2 assay[42–45]. 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 rt. The incubation was terminated by rapid filtration through the presoaked filtermats using a 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 non-specific binding was determined with 10 µM unlabeled ditolylguanidine. The K_d-value of the radioligand [^{3}H]-ditolylguanidine is 17.9 nM.[50]

6.2.6. 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 six concentrations of the test compounds and were calculated with the program GraphPad Prism® 3.0 (GraphPad Software) by non-linear regression analysis. The K_i-values were calculated according to Cheng and Prusoff.[51] The K_i-values are given as mean values ± SEM from three independent experiments.

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References

1 J. M. Walker, W. D. Bowen, F. O. Walker, R. R. Matsumoto, B. De Costa and K. C. Rice, *Pharmacol. Rev.*, 1990, **42**, 355–402.
2 R. Quirion, R. Chicheportiche, P. C. Contreras, K. M. Johnson, D. Lodge, S. W. Tam, J. H. Woods and S. R. Zukin, *Trends Neurosci.*, 1987, **10**, 444–446.
3 R. Quirion, W. D. Bowen, Y. Itzhak, J. L. Junien, J. M. Musachio, R. B. Rothman, T. P. Su, S. W. Tam and D. P. Taylor, *Trends Pharmacol. Sci.*, 1992, **13**, 85–86.
4 T. Hayashi and T. P. Su, *CNS Drugs*, 2004, **18**, 269–284.
5 E. J. Cobos, J. M. Entrena, F. R. Nieto, C. M. Cendan and E. DelPezo, *Curr. Pharmacol.*, 2008, **6**, 344–366.
6 T. Maurice and T. P. Su, *Pharmacol. Ther.*, 2009, **124**, 195–206.
7 M. Ishikawa and K. Hashimoto, *J. Receptor Ligand Channel Res.*, 2010, **3**, 25–36.
8 B. J. Vilner, C. S. John and W. D. Bowen, *Cancer Res.*, 1995, **55**, 408–413.
9 B. Wang, R. Rouzier, C. T. Albarracin, A. Sahin, P. Wagner, Y. Yang, T. L. Smith, F. M. Bernstam, A. C. Marcelo, G. N. Hortobagyi and L. Pusztai, *Breast Cancer Res. Treat.*, 2004, **87**, 205–214.
10 K. Kitaichi, J. G. Chabot, F. F. Moebius, A. Flandorfer, H. Glossmann and R. Quirion, *J. Chem. Neuroanat.*, 2000, **20**, 375–387.
11 P. J. Lupardus, R A. Wilke, E. Aydar, C. P. Palmer, Y. Chen, A. E. Ruoho and M. B. Jackson, *J. Physiol.*, 2000, **526**, 527–539.
12 E. Aydar, C. P. Palmer, V. A. Klyachko and M B. Jackson, *Neuron*, 2002, **34**, 399–410.
13 C. Ela, J. Barg, Z. Vogel, Y. Hasin and Y. Eilam, *J. Pharmacol. Exp. Ther.*, 1994, **269**, 1300–1309.
14 M. Johannessen, S. Ramachandran, L. Riemer, A. Ramos-Serrano, A. E. Ruoho and M. B. Jackson, *Am. J. Physiol.: Cell Physiol.*, 2009, **296**, C1049–C1057.
15 T. Schläger, D. Schepmann, K. Lehmkuhl, J. Holenz, J. M. Vela, H. Buschmann and B. Wünsch, *J. Med. Chem.* submitted.
16 C. Oberdorf, D. Schepmann, J. M. Vela, J. L. Diaz, J. Holenz and B. Wünsch, *J. Med. Chem.*, 2008, **51**, 6531–6537.
17 C. Oberdorf, T. J. Schmidt and B. Wünsch, *Eur. J. Med. Chem.*, 2010, **45**, 3116–3124.
18 E. Rack, R. Fröhlich, D. Schepmann and B. Wünsch, *Bioorg. Med. Chem.*, 2011, **19**, 3141–3151.
19 R. A. Glennon, S. Y. Ablordeppey, A. M. Ismaiel, M. B. El-Ashmawy, J. B. Fischer and K. B. Howie, *J. Med. Chem.*, 1994, **37**, 1214–1219.
20 R. A. Glennon, *Mini-Rev. Med. Chem.*, 2005, **5**, 927–940.
21 C. Laggner, C. Schieferer, B. Fiechtner, G. Poles, R. D. Hoffmann, H. Glossmann, T. Langer and E. F. Moebius, *J. Med. Chem.*, 2005, **48**, 4754–4764.
22 D. Zampieri, M. G. Mamolo, E. Laurini, C. Florio, C. Zanette, M. Fermeglia, P. Posocco, M. S. Paneni, S. Pricl and L. Vio, *J. Med. Chem.*, 2009, **52**, 5380–5393.
23 *PCMODEL*, Serena Software, Version 9.1, 2005, Bloomington, Indiana, USA.
24 D. Becke, *J. Chem. Phys.*, 1993, **98**, 5648.
25 C. Lee, W. Yang and R. G. Parr, *Phys. Rev. B*, 1988, **37**, 785–789.
26 S. J. Grimme, *J. Chem. Phys.*, 2003, **118**, 9095–9102.
27 M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G. A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H. P. Hratchian, A. F. Izmaylov, J. Bloino, G. Zheng, J. L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J. A. Montgomery, Jr., J. E. Peralta, F. Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, K. N. Kudin, V. N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J. M. Millam, M. Klene, J. E. Knox, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. Ochterski, R. L. Martin, K. Morokuma, V. G. Zakrzewski, G. A. Voth, P. Salvador, J. J. Dannenberg, S. Dapprich, A. D. Daniels, O. Farkas, J. B. Foresman, J. V. Ortiz, J. Cioslowski and D. J. Fox, *GAUSSIAN 09 (Revision A.2)*, Gaussian, Inc., Wallingford, CT, 2009.
28 D. W. Hawkins, B. Iddon, D. S. Longthorne and P. J. Rosyk, *J. Chem. Soc., Perkin Trans. 1*, 1994, 2735–2743.
29 B. Wünsch, *Arch. Pharm.*, 1990, **323**, 493–499.
30 J. Hassan, M. Sévignon, C. Gozzi, E. Schulz and M. Lemaire, *Chem. Rev.*, 2002, **102**, 1359.
31 (*a*) D. Alberico, M. E. Scott and M. Lautens, *Chem. Rev.*, 2007, **107**, 174; (*b*) L. Ackermann, R. Vicente and A. R. Kapdti, *Angew. Chem., Int. Ed.*, 2009, **48**, 9792.
32 A. Ohta, Y. Akita, T. Ohkuwa, M. Chiba, R. Fukunaga, A. Miyafuji, T. Nakata, N. Tani and Y. Aoyagi, *Heterocycles*, 1990, **31**, 1951.
33 B. Liégault, D. Lapointe, L. Caron, A. Vlassova and K. Fagnou, *J. Org. Chem.*, 2009, **74**, 1826 and references therein.
34 S. Yanagisawa, T. Sudo, R. Noyori and K. Itami, *J. Am. Chem. Soc.*, 2006, **128**, 11748.
35 S. Yanagisawa, T. Sudo, R. Noyori and K. Itami, *Tetrahedron*, 2008, **64**, 6073.
36 J. Benoît, T. Yamamoto and K. Itami, *Angew. Chem., Int. Ed.*, 2009, **48**, 3644.
37 H.-Q. Do, R. M. K. Khan and O. Daugulis, *J. Am. Chem. Soc.*, 2008, **130**, 15185.
38 S. Yanagisawa, K. Ueda, H. Sekizawa and K. Itami, *J. Am. Chem. Soc.*, 2009, **131**, 14622.
39 K. Ueda, S. Yanagisawa, J. Yamaguchi and K. Itami, *Angew. Chem., Int. Ed.*, 2010, **49**, 8946.
40 S. Kirchberg, S. Tani, K. Ueda, J. Yamaguchi, A. Studer and K. Itami, *Angew. Chem. Int. Ed.*, 2011, **50**, 2387.

41 S. Yanagisawa and K. Itami, *Tetrahedron*, 2011, **67**, 4425.
42 C. A. Maier and B. Wünsch, *J. Med. Chem.*, 2002, **45**, 438–448.
43 C. A. Maier and B. Wünsch, *J. Med. Chem.*, 2002, **45**, 4923–4930.
44 E. Große Maestrup, C. Wiese, D. Schepmann, A. Hiller, S. Fischer, M. Scheunemann, P. Brust and B. Wünsch, *Bioorg. Med. Chem.*, 2009, **17**, 3630–3641.
45 R. Holl, D. Schepmann, R. Fröhlich, R. Grünert, P. J. Bednarski and B. Wünsch, *J. Med. Chem.*, 2009, **52**, 2126–2137.
46 P. D. Prasad, H. W. Li, Y. J. Fei, M. E. Ganapathy, T. Fujita, L. H. Plumley, T. L. Yang-Feng, F. H. Leibach and V. Ganapathy, *J. Neurochem.*, 1998, **70**, 443–451.
47 J. Suffert, *J. Org. Chem.*, 1989, **54**, 509–510.
48 M. M. Bradford, *Anal. Biochem.*, 1976, **72**, 248–254.
49 D. L. DeHaven-Hudkins, L. C. Fleissner and F. Y. Ford-Rice, *Eur. J. Pharmacol., Mol. Pharmacol. Sect.*, 1992, **227**, 371–378.
50 H. Mach, C. R. Smith and S. R. Childers, *Life Sci.*, 1995, **57**, PL57.
51 Y. C. Cheng and W. H. Prusoff, *Biochem. Pharmacol.*, 1973, **22**, 3099–3108.