Bioorganic & Medicinal Chemistry 17 (2009) 3630–3641

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Synthesis of spirocyclic σ_1 receptor ligands as potential PET radiotracers, structure–affinity relationships and in vitro metabolic stability

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ARTICLE INFO

Article history:
Received 13 January 2009
Revised 30 March 2009
Accepted 31 March 2009
Available online 5 April 2009

Keywords:
σ_1 Receptor antagonists
Spirocyclic piperidines
Metabolism
^{18}F Radiotracer
Neuroimaging
Structure–affinity-relationships

ABSTRACT

Several 3*H*-spiro[[2]benzofuran-1,4′-piperidines] bearing a *p*-fluorobenzyl residue at the N-atom and various substituents in position 3 of the benzofuran system were synthesized. The crucial reaction steps are the addition of a lithiated benzaldehyde derivative to the *p*-fluorobenzylpiperidone **5** and the $BF_3{\cdot}OEt_2$ catalyzed substitution of the methoxy group of **2a** by various nucleophiles. Structure–affinity relationship studies revealed that compounds with two protons (**2d**), a methoxy group (**2a**), and a cyano group (**2e**) in position 3 possess subnanomolar σ_1 affinity (K_i = 0.18 nM, 0.79 nM, 0.86 nM) and high selectivity against the σ_2 subtype. The metabolites of **2a**, **2d**, and **2e**, which were formed upon incubation with rat liver microsomes, were identified. Additionally, the rate of metabolic degradation of **2a**, **2d**, and **2e** was determined and compared with the degradation rate of the non-fluorinated spirocyclic compound **1**. For the synthesis of the potential PET tracers [^{18}F]**2a** and [^{18}F]**2e** two different radiosynthetic approaches were followed.

1. Introduction

The class of σ receptors contains at least two subtypes, which are termed σ_1 and σ_2 receptor. The σ_1 subtype, showing high affinity for (+)-benzomorphans (e.g., (+)-pentazocine and (+)-SKF-10,047),[1] has a characteristic distribution in the central nervous system (CNS), but is also found in the periphery, for example, in endocrine and immune tissues as well as in some organs like kidney, liver, lung and heart.[2,3]

Studies revealed that ligands interacting with the σ_1 receptor subtype are of particular interest due to their potential for the treatment of acute and chronic neurological disorders, including schizophrenia,[4] depression,[5,6] Alzheimer's disease and Parkinson's disease,[7,8] pain, in particular neuropathic pain,[9,10] as well as alcohol and cocaine abuse.[11,12] A correlation of the σ_1 receptor with Alzheimer's disease has also been shown, since the potent antiamnesic and neuroprotective effects of donepezil against $A\beta_{25-35}$ induced toxicity involve both cholinergic and σ_1 agonistic properties. This dual action might explain the special properties of donepezil compared to other acetylcholinesterase inhibitors.[13]

Since the signal transduction pathway after activation of σ_1 receptors is not completely understood so far, the above-mentioned pharmacological effects cannot be correlated directly to a biochemical mechanism. However, it has been shown that σ_1 receptors are involved in the modulation of various neurotransmitter systems including the glutamatergic,[14] dopaminergic[15] and cholinergic[16] neurotransmission. Additionally, the influence on the regulation and activity of a variety of ion channels including K^+ channels[17,18] and Ca^{2+} channels[19,20] is an important feature of σ_1 receptors.

The main aim of positron emission tomography (PET) with σ_1 receptor binding radiotracers is the quantitative mapping of σ_1 receptors in the living human brain as well as to improve our understanding of the cross-talk between σ_1 receptors and brain neurotransmitter systems. Accordingly, we planned to develop an appropriate radiotracer and to investigate brain σ_1 receptors with PET. For this purpose, a ligand with high σ_1 receptor affinity, high selectivity over the σ_2 subtype and other important CNS receptors (e.g., dopamine, serotonin, noradrenaline and glutamate receptors), sufficient metabolic stability, and a facile approach to a radiotracer is required. This radiotracer is expected to be useful for the investigation of the distribution, function and activity of central σ_1 receptors. In particular, a radiotracer for σ_1 receptors in the brain will contribute to a better understanding of the complex interactions of different receptor systems in the central nervous system and, moreover, may provide a tool for neuroimaging of brain diseases, for example, schizophrenia, Parkinson's disease, Alzheimer's disease and others.

Recently, we have reported on the synthesis and pharmacological evaluation of a new class of spirocyclic piperidines, which bind with high affinity and selectivity to σ_1 receptors.[21,22] In particular

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doi:10.1016/j.bmc.2009.03.060

the spiro[benzofuran-piperidine] **1** represents an extraordinarily potent σ_1 receptor antagonist (K_i = 1.1 nM) with high selectivity against σ_2 receptors (>1000-fold) and more than 60 other receptors, ion channels (including the hERG channel) and transporters.[23] In the capsaicin assay, **1** was able to reduce the neuropathic pain reaction of mice (see Fig. 1).[23]

Starting from the spirocyclic σ_1 receptor antagonist **1** we planned to develop a [^{18}F] labeled PET tracer taking both the pharmacodynamic and pharmacokinetic properties into account. The novel PET tracer should allow the analysis of the distribution and the activity of σ_1 receptors in the complex interactions of various neurotransmitters in the central nervous system. Herein, we wish to report on the synthesis, σ_1 receptor affinity and selectivity, as well as the metabolic stability of *p*-fluorobenzyl substituted spirocyclic piperidines **2**. Moreover, an approach for the radiosynthesis of [^{18}F] labeled compounds is given.

2. Synthesis

At first the *p*-fluorobenzyl derivative **2a** was prepared by alkylation of the secondary amine **7**, which had been obtained upon hydrogenolytic cleavage of the *N*-benzyl derivative **1**.[21] Whereas the reductive alkylation of the secondary amine **7** with *p*-fluorobenzaldehyde and $NaBH(OAc)_3$ led to the *p*-fluorobenzyl derivative in 48% yield, the alkylation of **7** with *p*-fluorobenzyl chloride in the presence of K_2CO_3 gave **2a** in 84% yield (Scheme 1).

In order to shorten the synthesis and avoid the exchange of the *N*-benzyl moiety of **1** against the *p*-fluorobenzyl moiety (two reaction steps) the synthesis of the spirocyclic system was started with 1-(*p*-fluorobenzyl)piperidone (**5**), which was obtained by alkylation of piperidone **4** with *p*-fluorobenzyl chloride. Treatment of the bromoacetal **3** with *n*-BuLi at −85 °C and subsequent trapping of the resulting aryllithium intermediate with the fluorobenzylpiperidone **5** afforded the hydroxy acetal **6**, which was cyclized with *p*-toluenesulfonic acid in methanol to provide the *p*-fluorobenzyl derivative **2a** in 31% yield in only two reaction steps from **3**.

Next, the influence of C-3 substituents of the benzofuran system on the σ_1 and σ_2 receptor affinity was investigated. At first the five membered hemiacetal **2b** was prepared by hydrolysis (1 M HCl in acetonitrile) of the cyclic acetal **2a** (64% yield) or more directly by hydrolysis of the hydroxy acetal **6** (80% yield). Oxidation of the lactol **2b** with tetrapropylammonium perruthenate (TPAP, NPr_4 RuO_4) and *N*-methylmorpholine-N-oxide[24] (NMMO) provided the lactone **2c** in 50% yield.

Furthermore, the cyclic ether **2d** was prepared by reduction of the methyl acetal **2a** with Et_3SiH and $BF_3{\cdot}OEt_2$.[25] TMS-CN (trimehylsilyl cyanide) in the presence of $BF_3{\cdot}OEt_2$[26,27] transformed the cyclic acetal **2a** into the nitrile **2e** and allyltrimethylsilane with $BF_3{\cdot}OEt_2$[26,27] led to the allyl derivative **2f**.

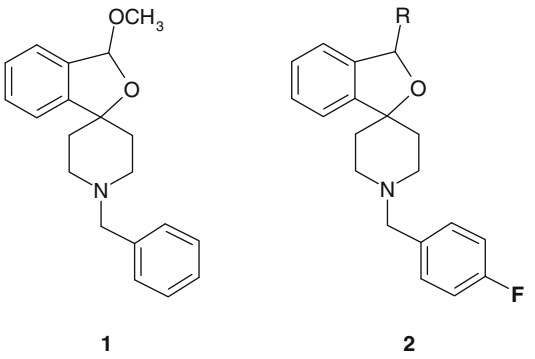


Figure 1. Spirocyclic piperidine **1** with high σ_1 receptor affinity and selectivity and derived fluorinated derivatives **2** designed for PET studies.

3. σ Receptor binding studies

The σ_1 and σ_2 receptor affinities of the spirocyclic compounds **2a–f** were determined in competition experiments with radioligands. In the σ_1 assay membrane preparations of guinea pig brains were used as receptor material and [^{3}H]-(+)-pentazocine as radioligand. The non-specific binding was determined in the presence of a large excess of non-tritiated (+)-pentazocine. Homogenates of rat liver served as source for σ_2 receptors in the σ_2 assay. Since a σ_2 selective radioligand is not commercially available, the non-selective radioligand [^{3}H]-di-*o*-tolylguanidine was employed in the presence of an excess of non-radiolabeled (+)-pentazocine for selective masking of σ_1 receptors. An excess of non-tritiated di-*o*-tolylguanidine was used for determination of the non-specific binding.[21,28]

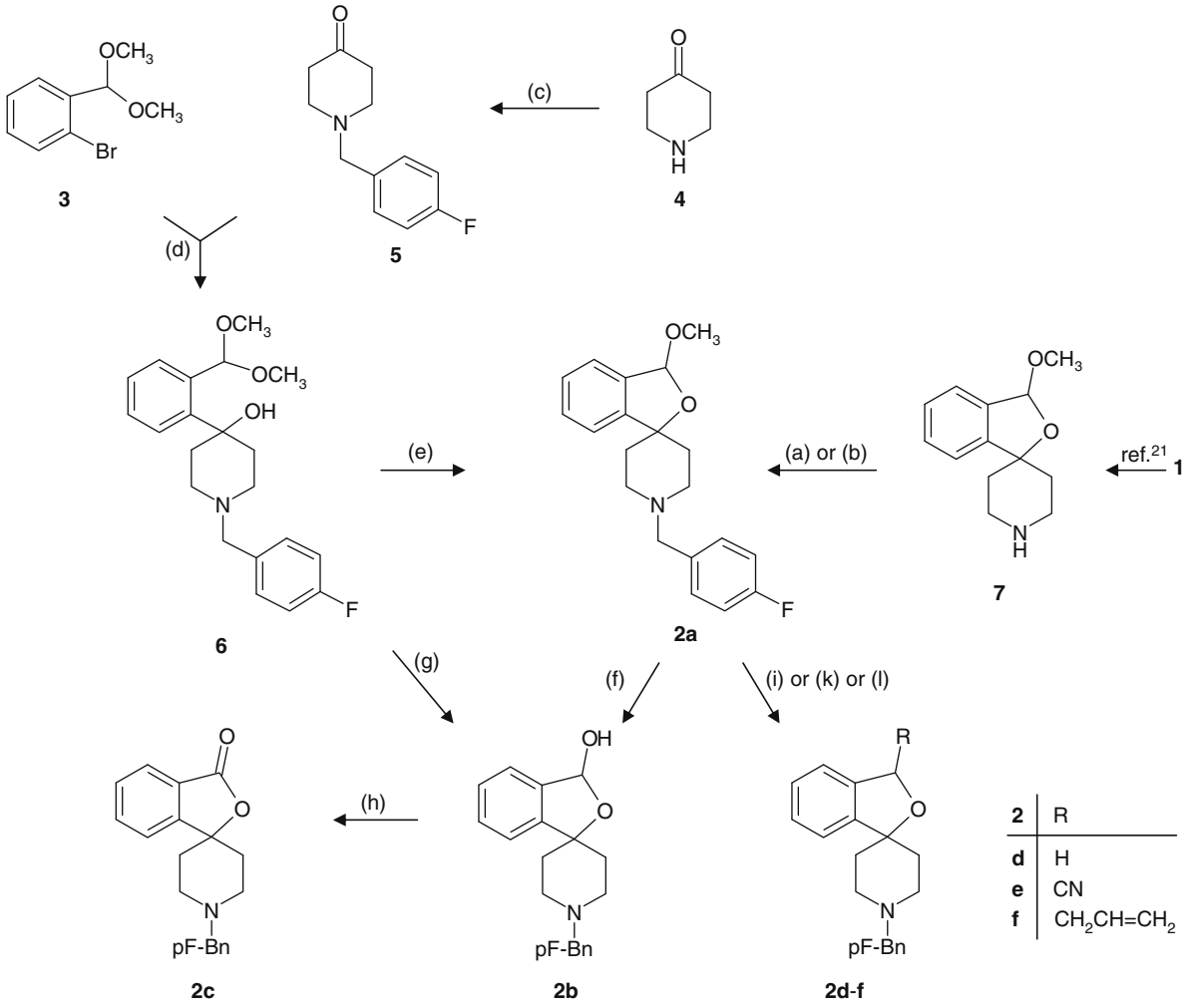
In Table 1 the σ_1 and σ_2 receptor affinities of the fluorinated spirocyclic piperidines are summarized. Comparison of the σ receptor affinities of the spirocyclic piperidines **1** and **2a** shows that introduction of a fluoro substituent in *p*-position of the benzyl moiety led to a slight increase of both σ_1 and σ_2 receptor affinities as well as the σ_1/σ_2 selectivity. A polar hydroxy (**2b**) or carbonyl moiety (**2c**) in position 3 caused a four to 10-fold reduction of the σ_1 receptor affinity. However, removal of any substituent in position 3 led to an extraordinarily potent σ_1 receptor ligand (**2d**, K_i = 0.18 nM) with good selectivity (factor 728) over the σ_2 receptor. The cyano derivative **2e** showed almost the same σ_1 and σ_2 receptor affinities as the methoxy derivative **2a**, which indicates that the cyano group can replace the methoxy group in this class of σ_1 receptor ligands. The high σ_1 receptor affinity and σ_1/σ_2 selectivity of the allyl derivative **2f** (K_i = 1.4 nM) is remarkable, since it indicates that the σ_1 receptor tolerates obviously an allyl substituent in position 3.

In conclusion, the high σ_1 receptor affinity combined with the high σ_1/σ_2 selectivity rendered this class of spirocyclic piperidines **2** with a *p*-fluorobenzyl moiety at the piperidine N-atom attractive for the development of a PET tracer. In particular the spirocyclic piperidines **2a** (R = OCH_3), **2d** (R = H) and **2e** (R = CN) binding in the subnanomolar range at the σ_1 receptor were considered first. Since in a PET study the σ_1 ligands will be administered to animals (rats, mice) the metabolic stability of the potential PET ligands is of central interest.

4. Metabolic stability

The metabolism of the envisaged PET ligands **2a**, **2d** and **2e** labeled with [^{19}F] was investigated with rat liver microsomes. At first the rate of metabolic degradation was determined in comparison to the metabolic degradation of **1**. Several incubations were started at the same time and stopped with a time shift of 10 min each to observe the respective degradation. Directly after termination of the metabolic process haloperidol was added as an internal standard to quantify the remaining parent compound by HPLC analysis. For the exact quantification a matrix calibration was carried out for each spirocyclic σ_1 ligand. Each data point was measured only once.

In Figure 2 the degradation of the three *p*-fluorobenzyl substituted σ_1 ligands **2a**, **2d** and **2e** is compared with the degradation of the benzyl derivative **1**. It is shown that the unsubstituted derivative **2d** was metabolized very fast by rat liver microsomes. After an incubation period of 30 min only 13% of the parent compound **2d** was left. The methyl acetal **2a** with a *p*-fluorobenzyl moiety was slightly more stable than the benzyl derivative **1**. We assume



Scheme 1. Synthesis of *p*-fluorobenzyl (pF-Bn) substituted spirocyclic σ_1 ligands. Reagents and reaction conditions: (a) *p*-fluorobenzaldehyde, $NaBH(OAc)_3$, CH_2Cl_2, 5 h, rt, 48%; (b) *p*-fluorobenzyl chloride, K_2CO_3, CH_3CN, 16 h, reflux, 84%; (c) *p*-fluorobenzyl chloride, K_2CO_3, CH_3CN, H_2O, 2 h, reflux, 71%; (d) *n*-BuLi, THF, -85 °C, 1 h, then 3.5 h, rt; (e) CH_3OH, *p*TosOH, 6 d, rt, 75%; (f) CH_3CN, HCl, 1 h, rt, 64%; (g) THF, HCl, 3.5 h, reflux, 89%; (h) NPr_4RuO_4, NMMO, CH_3CN/CH_2Cl_2, 2 h, rt, 50%; (i) Et_3SiH, $BF_3{\cdot}OEt_2$, CH_2Cl_2, 20 min, -25 °C, then 1 h, 0 °C, 79%; (k) Me_3SiCN, $BF_3{\cdot}OEt_2$, CH_2Cl_2, 20 min, -25 °C, then 1 h, 0 °C, 75%; (l) $allylSiMe_3$, $BF_3{\cdot}OEt_2$, CH_2Cl_2, 20 min, −25 °C, then 6.5 h, 0 °C, then 3.5 h, rt, 62%.

Table 1
Affinities of the lead compound **1** and fluorinated spirocyclic piperidines **2** towards σ_1 and σ_2 receptors

Compound	R	$K_i \pm$ SEM[a] (nM)				σ_1/σ_2 Selectivity
		σ_1 ([^{3}H]-(+)-pentazocine)		σ_2 ([^{3}H]-di-*o*-tolylguanidine)		
1[21]	OCH_3	1.1 ± 0.2		1280 ± 137		1130
2a	OCH_3	0.79 ± 0.05		946	(*n* = 1)	1205
2b	OH	3.3 ± 1.2		414	(*n* = 1)	124
2c	=O	7.7 ± 2.0		1920	(*n* = 1)	249
2d	H	0.18 ± 0.09		131	(*n* = 1)	728
2e	CN	0.86 ± 0.06		268	(*n* = 1)	311
2f	$CH_2CH{=}CH_2$	1.4 ± 0.6		971	(*n* = 1)	684
Haloperidol		3.9 ± 1.5	(*n* = 5)	78 ± 2.0	(*n* = 1)	21
Di-*o*-tolylguanidine		61 ± 18	(*n* = 5)	42 ± 15	(*n* = 7)	1.4

[a] The K_i-values were determined in three independent experiments (*n* = 3) unless otherwise noted.

that the increased metabolic stability of **2a** is due to the occupied *p*-position of the benzyl moiety inhibiting an aromatic hydroxylation at this position. However, the nitrile **2e** represents the most stable compound. Even after an incubation period of 60 min more than 50% of the nitrile **2e** remained unchanged.

In order to learn more about the potential labile positions in the molecules the samples were analyzed using LC–MS techniques in addition to the conventional HPLC with UV detection. The mass detector of the LC–MS system consisted of an ion trap, which allows consecutive fragmentation experiments (MS^n). The performed MS^n experiments combined with spiking of the samples with reference compounds of potential metabolites led to the identification of the respective metabolites of the spirocyclic σ_1 ligands **2a**, **2d** and **2e**.

At first the metabolites obtained after incubation of the most potent spirocyclic compound **2d** (σ_1: K_i = 0.18 nM) with rat liver

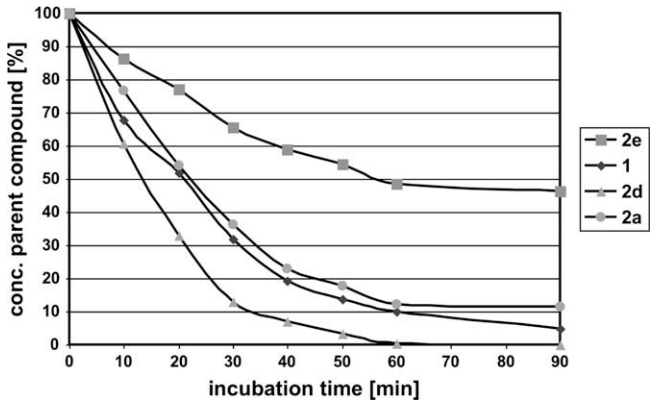


Figure 2. Degradation of the compounds **1**, **2a**, **2d** and **2e** during a period of 90 min; **2e** turned out to be the most stable ligand.

microsomes were investigated. According to LC–MS studies and by comparison with reference compounds three primary metabolites were identified, which had been formed by N-debenzylation (**8**), N-oxidation (**9**) and α-hydroxylation (**2b**). The hemiacetal **2b** was further oxidized to the lactone **2c**. N-Debenzylation of **2b** or α-hydroxylation of **8** led to the hemiacetal **10**, which was further oxidized to give the lactone **11**. Since the major metabolite was the hemiacetal **2b** we assume that the high reactivity of the Ph-CH_2–O substructure in position 3 of **2d** is responsible for the fast metabolic degradation of **2d** (Scheme 2).

The primary metabolites formed upon incubation of the methyl acetal **2a** (σ_1: K_i = 0.79 nM) are the secondary amine **12**, the N-oxide **13**, and the hemiacetal **2b**, resulting from N-debenzylation, N-oxide formation and acetal hydrolysis (or O-demethylation), respectively. Furthermore, oxidation of the hemiacetal **2b** provided the lactone **2c**. The metabolic pattern of the *p*-fluorobenzyl derivative **2a** is very similar to that of the benzyl derivative **1**. [23] The main difference is that the CYP enzymes additionally oxidize the benzyl moiety of **1** to afford an hydroxybenzyl metabolite in considerable amounts. An analogous metabolite could not be formed starting from the *p*-fluorobenzyl derivative **2a**, since the aromatic residue is desactivated by the fluoro atom, which already occupies the *p*-position (see Scheme 3).

Due to the high σ_1 affinity (K_i = 0.86 nM) and σ_1/σ_2 selectivity (factor 311) the nitrile **2e** represents a promising candidate for the development of a PET tracer as well. According to the experiments shown in Figure 2 the metabolic degradation of the nitrile **2e** is rather slow. Careful analysis of the metabolites led to identification of the N-debenzylation product **14** and the lactone **2c** (see Fig. 3). We assume that the lactone **2c** is formed by hydrolytic cleavage of the α-alkoxy nitrile (cyanohydrine ether) to afford the hemiacetal **2b**, which is oxidized by CYP enzymes to end up with the lactone **2c** (see Scheme 4).

Next the metabolism of **2d** and **2e** was investigated using pooled human liver microsomes. The metabolic profiles were very similar to the profiles obtained with rat liver microsomes. However, in case of **2d** the N-oxide **9** and the lactone **2c** were only formed in very small amounts.

In order to learn which CYP enzymes are responsible for the fast degradation the spirocyclic piperidines **2d** and **2e** were incubated with seven recombinant CYP450 isoenzymes (1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 3A4) and the formed metabolites were analyzed.

In case of the nitrile **2e** the lactone **2c** was formed by all isoenzymes, mainly by CYP3A4, CYP2D6, CYP2C9 ad CYP2C8. The secondary amine **14** was produced only by CYP1A2 and CYP3A4 (see Table 2).

In case of **2d** the secondary amine **8** was mainly produced by the isoenzymes CYP3A4, CYP2C19 and CYP1A2. All isoenzymes were involved in the formation of the hemiacetal **2b**, especially

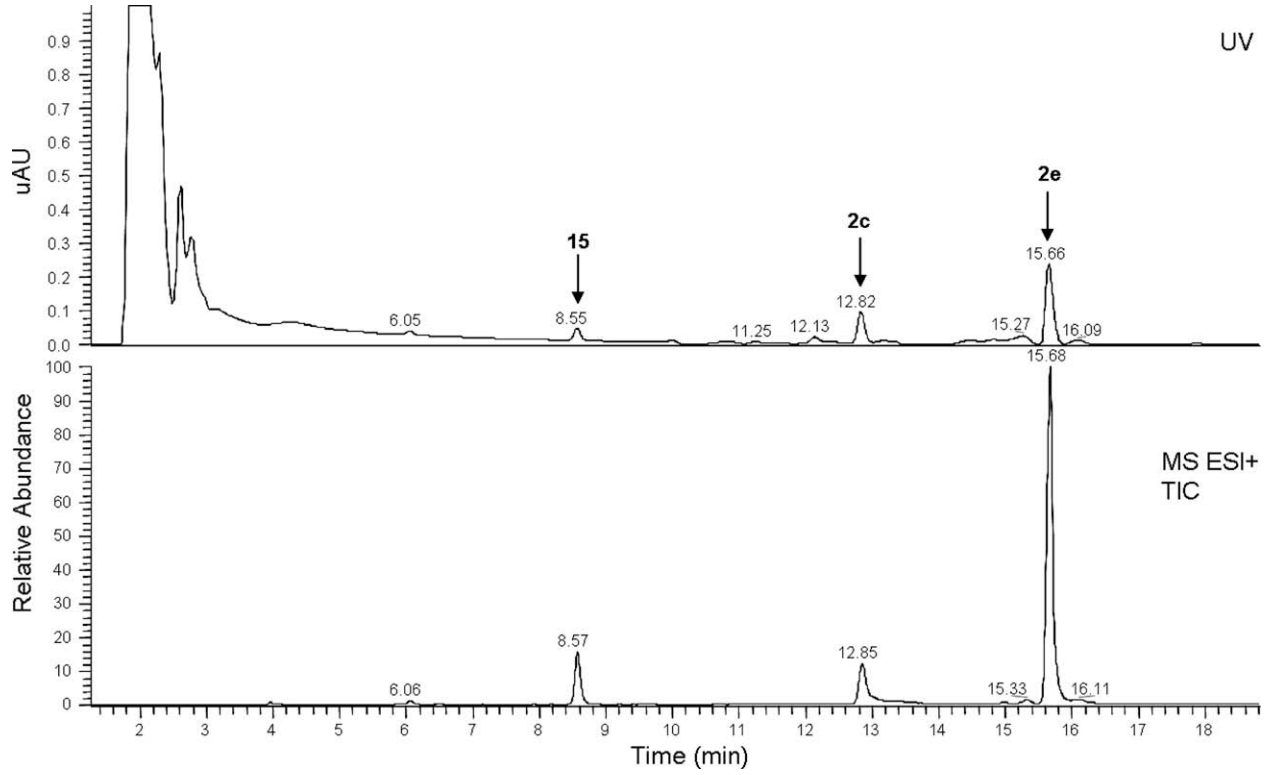
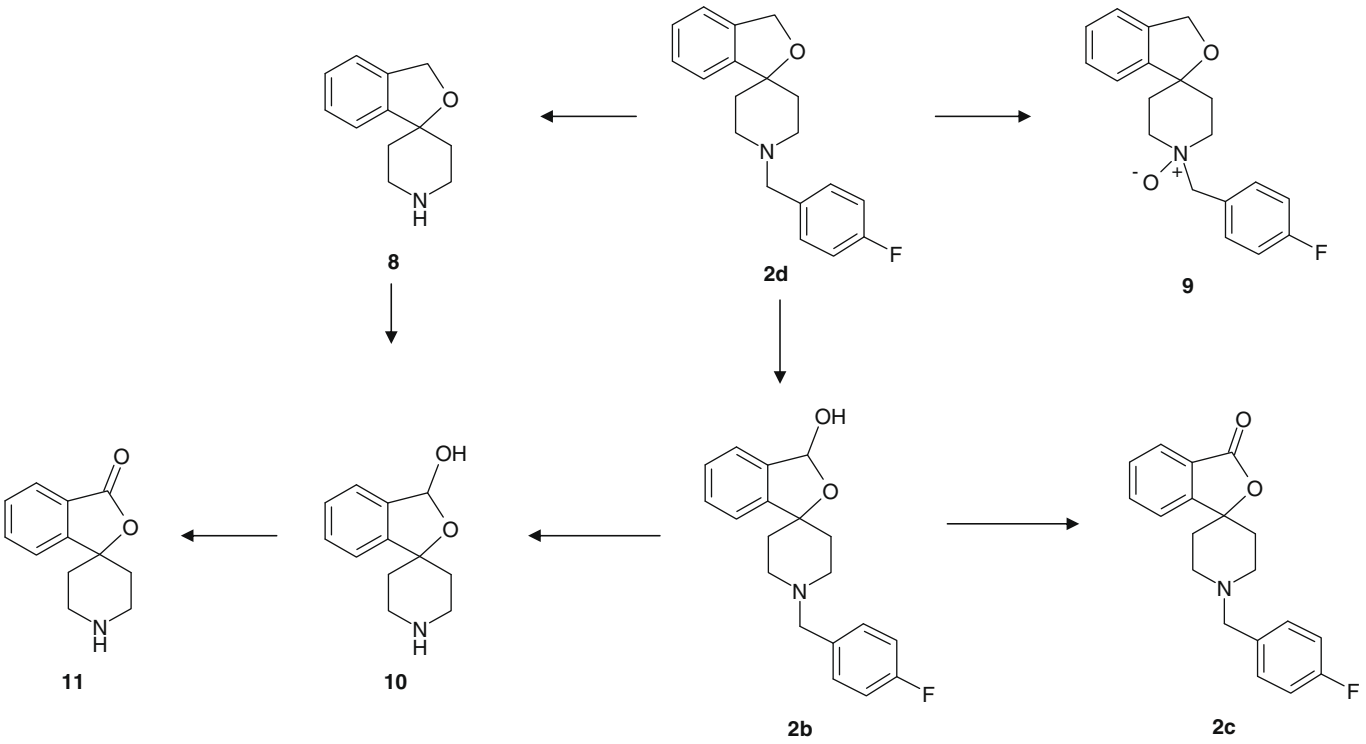
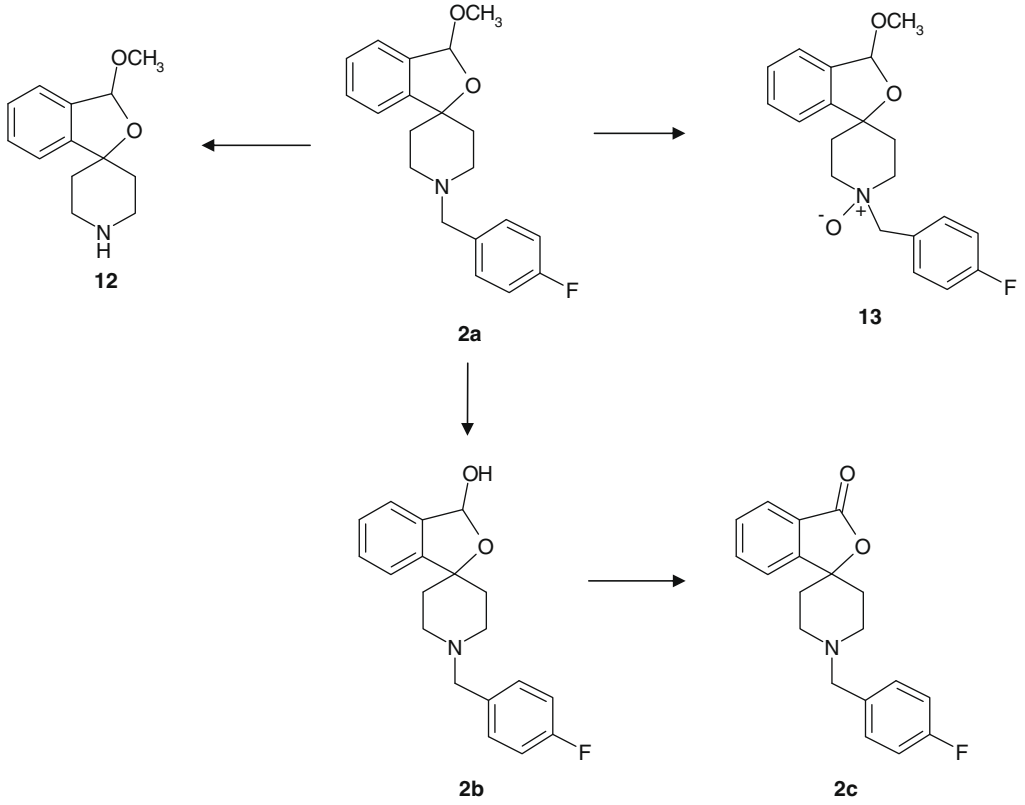


Figure 3. Correlation of HPLC chromatograms with UV (top) and ESI^+-MS detection (bottom): incubation of **2e** with rat liver microsomes; separation on a LiChrospher® RP select B 5 μm (250 × 4 mm) column at a flow rate of 1.0 mL/min, UV detection wavelength: 210 nm.



Scheme 2. Metabolism of the unsubstituted spirocyclic piperidine **2d**.

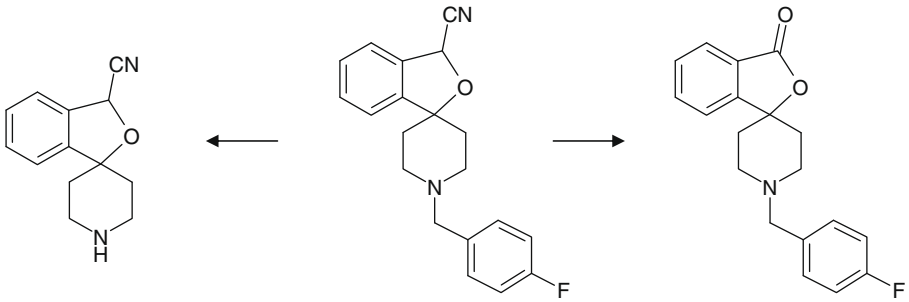


Scheme 3. Metabolism of the methoxy derivative **2a**.

CYP2D6, CYP3A4, CYP2C9 and CYP1A2. The formation of the lactone **2c** could not be observed. The secondary amine of the hemiacetal **10** was generated in very small amounts by CYP3A4 and CYP2C19 (see Table 3).

5. Perspective for a radiochemical approach

With respect to σ_1 receptor affinity and σ_1/σ_2 selectivity the most promising compound of this series is the cyclic ether **2d** with



14 2e 2c

Scheme 4. Metabolism of the nitrile **2e**.

Table 2
Relevance of CYP isoenzymes for the formation of the metabolites of **2e**

	Secondary amine **14**	Lactone **2c**
CYP1A2	+	0
CYP2B6	–	0
CYP2C8	–	+
CYP2C9	–	+
CYP2C19	0	0
CYP2D6	–	+
CYP3A4	+	++

Relevance: ++ (very strong), + (strong), 0 (minor), – (no).

Table 3
Relevance of CYP isoenzymes for the formation of the metabolites of **2d**

	Secondary amine **8**	Secondary amine **10**	Hemiacetal **2b**	Lactone **2c**
CYP1A2	+	–	+	–
CYP2B6	–	–	0	–
CYP2C8	–	–	0	–
CYP2C9	0	–	+	–
CYP2C19	+	0	0	–
CYP2D6	–	–	++	–
CYP3A4	++	0	+	–

Relevance: ++ (very strong), + (strong), 0 (minor), – (no).

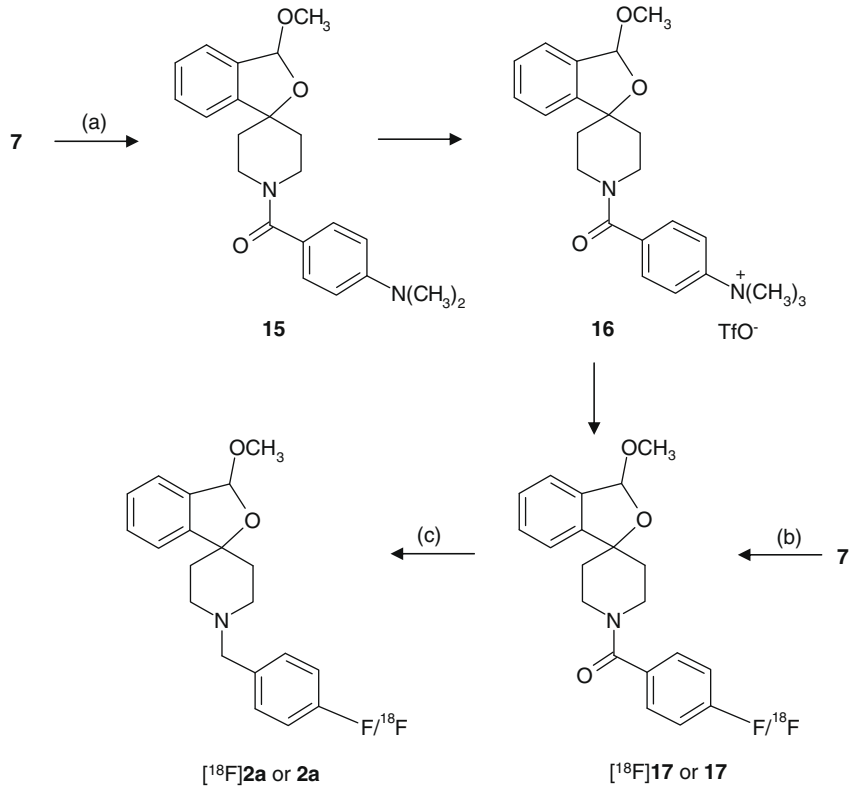
a σ_1 affinity of 0.18 nM. However, the very fast metabolic degradation renders **2d** less suitable as candidate for radiofluorination. Therefore, the methoxy derivative **2a** and the nitrile **2e** having also subnanomolar σ_1 affinities were selected for the radiochemical approach. It is assumed that both spirocyclic piperidines **2a** and **2e** are able to cross the blood brain barrier, since the defluoro analogue of **2a**, the lead compound **1**, displays centrally mediated analgesic activity in vivo.[23] Moreover, in an ex vivo autoradiography experiment the enrichment in the brain of a PET tracer derived from **1** bearing a 3-[^{18}F]fluoropropyl residue in position 3 instead of a methoxy group has been shown.[36]

The plan for the synthesis of [^{18}F]**2a** is outlined in Scheme 5. The trimethylanilinium triflate **16** should be an appropriate precursor for the introduction of a fluoro substituent into the benzyl moiety. The synthesis of **2a** was started with acylation of the secondary amine **7** with *p*-(dimethylamino)benzoyl chloride to give the benzamide **15**. Quarternization of the tertiary amine **15** with methyl triflate and subsequent nucleophilic aromatic substitution with F^- (or [^{18}F]F^-) should provide the fluorinated amide **17** (or [^{18}F]**17**), which has to be reduced to give **2a** (or [^{18}F]**2a**). In order to use optimized reaction conditions for the synthesis of the potential radiotracer [^{18}F]**2a,** the *p*-fluorobenzamide **17** (reference compound) was synthesized by acylation of the secondary amine **7** with *p*-fluorobenzoyl chloride and, subsequently, its reduction to the *p*-fluorobenzylamine **2a** was carefully investigated. The reduction step, however, turned out to be very problematic and numerous reducing agents and reaction conditions were tested. Finally, reduction of the benzamide **17** with $BH_3{\cdot}SMe_2$ gave the best result providing the *p*-fluorobenzyl derivative **2a** in up to 35% yield together with several side products. The sluggish reduction of the model benzamide **17** together with the low metabolic stability of **2a** (see Fig. 2) and, additionally, its potential labile acetal group prompted us to concentrate on the nitrile **2e** (K_i = 0.86 nM) as a potential [^{18}F] labeled radiotracer using another radiosynthetic strategy (see Scheme 6).

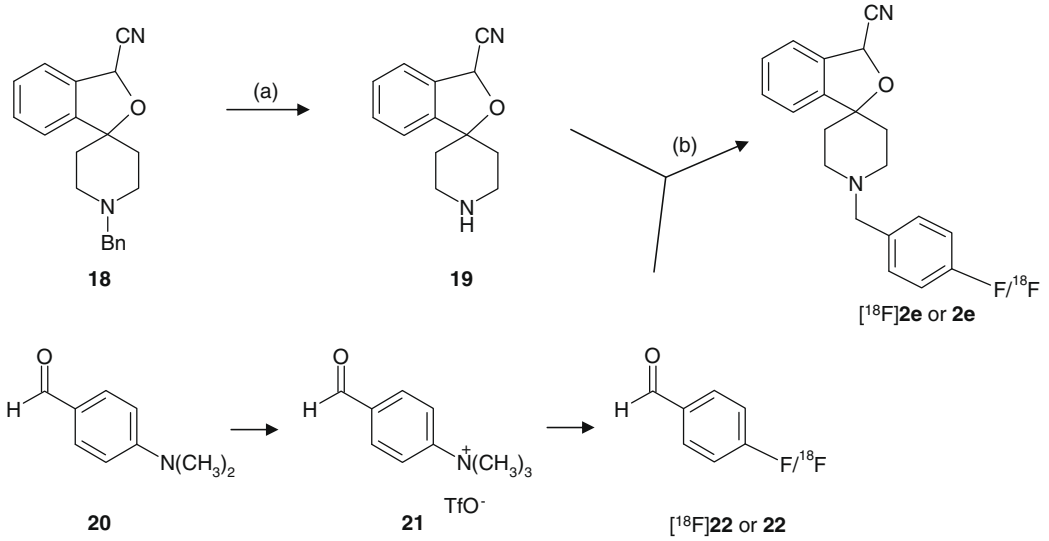
Accordingly, the secondary amine **19**, which was synthesized upon hydrogenolysis of the *N*-benzyl derivative **18**[21] with NH_4^+ HCO_2^- and Pd/C, should be coupled reductively with *p*-fluorobenzaldehyde (**22**). Whereas *p*-fluorobenzaldehyde (**22**) is commercially available, the ^{18}F-substituted benzaldehyde [^{18}F]**22** can be prepared by methylation of *p*-(dimethylamino)benzaldehyde (**20**) with methyl triflate and subsequent nucleophilic aromatic substitution of the quaternary ammonium triflate **21** with [^{18}F]F^-.[29,30] The reductive alkylation of the secondary amine **19** with the model compound **22** was performed using the reducing agents $NaBH(OAc)_3$ and $NaBH_3CN$. This reductive alkylation led to several side products providing the desired nitrile **2e** in only 20% yield after time consuming purification procedures. Several variations of the reductive alkylation conditions were investigated. Finally, $NaBH_3CN$ in methanol and addition of small amounts of acetic acid turned out to be the best method. We assume that the problems of this reaction are due to the instability of the secondary amine **19**, which led to very fast decomposition even when storing under N_2 at −20 °C. Therefore, the strategy of introducing [^{18}F] into the *N*-benzyl moiety was no longer pursued.

6. Conclusion

In conclusion, spirocyclic piperidines with a *p*-fluorobenzyl residue at the piperidine N-atom show very high σ_1 receptor affinity and σ_1/σ_2 selectivity. The most potent σ_1 antagonist is the unsubstituted derivative **2d** with a K_i-value of 0.18 nM. However, **2d** is very fast metabolized due to the active methylene moiety in position 3 of the benzofuran system. After an incubation period of 30 min only 13% of the parent compound **2d** remained unchanged. Therefore, **2d** was not selected for the development of a radiotracer for PET studies. The methyl acetal **2a** and the nitrile **2e** also interact in the subnanomolar range with σ_1 receptors (K_i = 0.79 nM and 0.86 nM). Both compounds were metabolically more stable than the unsubstituted derivative **2d**, in particular the nitrile **2e** was transformed very slowly. Two different approaches for the radiosynthesis of [^{18}F] labeled methyl acetal **2a** and nitrile **2e** were investigated.



Scheme 5. Strategy for the synthesis of [^{18}F]-labeled methyl acetal **2a**: (a) *p*-dimethylaminobenzoyl chloride, NEt_3, CH_2Cl_2, 6 h, rt. 31%; (b) *p*-fluorobenzoyl chloride, NEt_3, CH_2Cl_2, 1.5 h, rt, 42%; (c) $BH_3{\cdot}SMe_2$, THF, 30–35%.



Scheme 6. Strategy for the synthesis of [^{18}F]-labeled nitrile **2e**: (a) NH_4HCO_2, Pd/C, CH_3OH, 8 h, reflux, 44%; (b) $NaBH_3CN$, CH_3OH, HOAc, 10–20 min, 65 °C, 20%.

7. Experimental, chemistry

7.1. General

Unless otherwise noted, moisture sensitive reactions were conducted under dry nitrogen. THF was dried with sodium/benzophenone and was freshly distilled before use. Thin layer chromatography (tlc): Silica gel 60 F_{254} plates (Merck). Flash chromatography (fc): Silica gel 60, 40–64 μm (Merck); parentheses include: diameter of the column, eluent, fraction size, R_f value. Melting point: melting point apparatus SMP 3 (Stuart Scientific), uncorrected. MS: MAT GCQ (Thermo-Finnigan); EI = electron impact; Thermo Finnigan LCQ® ion trap mass spectrometer with an ESI = electrospray ionization interface. IR: IR spectrophotometer 480Plus FT-ATR-IR (Jasco). ^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz): Mercury-400 spectrometer (Varian); δ in ppm related to tetramethylsilane; coupling constants are given with 0.5 Hz resolution. HPLC: Merck Hitachi Equipment; UV detector: L-7400; autosampler: L-7200; pump: L-7100; degasser: L-7614; *Method 1*: column: LiChrospher® 60 RP-select B (5 μm), 250 × 4 mm; flow

rate: 1.00 mL/min; 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 min: 90%, 4 min: 90%, 29 min: 0%, 31 min: 0%, 31.5 min: 90%, 40 min: 90%. *Method 2*: column: LiChrospher® 100 RP 18 (5 μm) 250 × 4 mm; flow rate: 1.0 mL/min; temperature: rt; detection at λ = 254 nm; solvent: acetonitrile/water 50:50 with 0.1% triethylamine. *Method 3*: column: Supersphere® 100 RP 18 (5 μm) 250 × 4 mm; flow rate: 0.6 mL/min; temperature: rt; detection at λ = 235 nm; solvent: acetonitrile/water 85:15 with 0.1% triethylamine.

7.2. 1-(4-Fluorobenzyl)piperidin-4-one (5)

p-Fluorobenzyl chloride (190 mg, 1.31 mmol) and K_2CO_3 (835 mg, 6.04 mmol) were added to a solution of piperidin-4-one·1H_2O·1HCl (**4**·HCl, 162 mg, 1.06 mmol) in CH_3CN/H_2O (1:1, 15 mL). The mixture was heated to reflux for 2 h. After addition of CH_2Cl_2 (8 mL) the layers were separated and the aqueous layer was extracted with CH_2Cl_2 (4 × 4 mL). The organic layer was washed with saturated solution of NaCl (3 × 4 mL), dried (Na_2SO_4), concentrated in vacuo and the residue was purified by fc (1.5 cm, cyclohexane:ethyl acetate 7:3, 10 mL, R_f 0.16). Colorless oil, yield 147 mg (71%). $C_{12}H_{14}FNO$ (207.2). MS (EI): *m*/*z* = 316 [M+CH_2PhF], 208 [M+H], 109 [FPhCH_2]. IR: ν (cm^{-1}) = 2911 (C–H), 1715 (C=O), 1507 (aromat. C=C), 1218 (C–F), 1082 (C–O), 835 (C–H, 1,4 disubst. aromat.), 760 (C–H, 1,2 disubst. aromat.). ^{1}H NMR ($CDCl_3$): δ (ppm) = 2.45 (t, *J* = 6.1 Hz, 4H, N(CH_2CH_2)$_2$), 2.73 (t, *J* = 6.1 Hz, 4H, N(CH_2CH_2)$_2$), 3.58 (s, 2H, CH_2PhF), 7.02 (t, *J* = 8.7 Hz, 2H, FC(*CH*CH)$_2$), 7.29–7.34 (m, 2H, FC(CH*CH*)$_2$).

7.3. 2-[1-(4-Fluorobenzyl)-4-hydroxypiperidin-4-yl]benzaldehyde dimethyl acetal (6)

Under N_2 the aryl bromide **3** (303 mg, 1.31 mmol) was dissolved in THF (25 mL) and cooled down to −85 °C. Then, *n*-butyllithium (1.6 M in *n*-hexane, 1.1 mL, 1.76 mmol)) was slowly added and the mixture was stirred at −85 °C. After 5 min piperidin-4-one **5** (209 mg, 1.01 mmol) was slowly added. The solution was stirred for 1 h at −85 °C and for additional 3.5 h at rt. Then H_2O (10 mL) was added, the organic layer was separated and washed (4×) with a solution of $NaHSO_3$ (10%). The aqueous layer was extracted with CH_2Cl_2 (4×). The combined organic layers were dried (Na_2SO_4), concentrated in vacuo and the residue was purified by fc (2 cm, cyclohexane:ethyl acetate 7:3, 10 mL). At first the benzofuran **2a** (R_f 0.13), then the hydroxyacetal **6** (R_f 0.04) was eluted.

Compound **2a**: (R_f = 0.13): colorless oil, yield 12.1 mg (3.7%).

Compounds **2a/6** (mixture): pale yellow oil, yield 136 mg (38%).

Compound **6** (R_f = 0.04): $C_{21}H_{26}FNO_3$ (359.4). MS (ESI): *m*/*z* = 360 [M+H]. IR: ν (cm^{-1}) = 2923 (C–H), 1606 (aromat. C=C), 1220 (C–F), 1084 (C–O), 829 (C–H, 1,4-disubst. aromat.), 755 (C–H, 1,2-disubst. aromat.). ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.84 (d br, *J* = 12.1 Hz, 2H, N(CH_2CH_2)$_2$), 2.18 (td, *J* = 12.8/4.1 Hz, 2H, N(CH_2CH_2)$_2$), 2.54 (t br, *J* = 11.5 Hz, 2H, N(CH_2CH_2)$_2$), 2.74 (d br, *J* = 11.3 Hz, 2H, N(CH_2CH_2)$_2$), 3.34 (s, 6H, CH(OCH_3)$_2$), 3.54 (s, 2H, NCH_2Ph), 6.22 (s, 1H, ArC*H*(OCH_3)$_2$), 7.01 (t, *J* = 8.7 Hz, 2H, FC(*CH*CH)$_2$), 7.28–7.40 (m, 5H, aromat. *H*), 7.74 (dd, *J* = 7.4/1.8 Hz, 1H, aromat. *H*). A signal for the OH proton is not visible.

7.4. 1′-(4-Fluorobenzyl)-3-methoxy-3H-spiro[[2]benzofuran-1,4′-piperidine] (2a)

7.4.1. Method 1

p-Fluorobenzaldehyde (165 mg, 1.33 mmol) was slowly added to a solution of the secondary amine **7**[21] (57 mg, 0.26 mmol) CH_2Cl_2 (5 mL). Then $NaBH(OAc)_3$ (40 mg, 0.33 mmol) was added and the mixture was stirred for 5 h at rt. It was filtered with Celite®, the solvent was removed in vacuo and the residue was purified by fc (1.5 cm, cyclohexane:ethyl acetate 7:3, 5 mL, R_f 0.08). Colorless oil, yield 41 mg (48%).

7.4.2. Method 2

A mixture of **7** (53 mg, 0.24 mmol), *p*-fluorobenzyl chloride (47 mg, 0.33 mmol), K_2CO_3 (91 mg, 1.49 mmol) and CH_3CN (10 mL) was stirred under reflux for 16 h. Subsequently, it was filtered over Celite®, the solvent was removed in vacuo and the residue was purified by fc (1.5 cm, cyclohexane:ethyl acetate 7:3, 5 mL, R_f 0.08). Pale yellow oil, yield 66 mg (84%).

7.4.3. Method 3

The unpurified hydroxyacetal **6** (79 mg, 0.22 mmol) was dissolved in CH_3OH (ca. 10 mL) and *p*-toluenesulfonic acid·H_2O (88 mg, 0.47 mmol) was added. Under N_2 the mixture was stirred at rt for 6 d. Then 2 M NaOH (pH 10) was added and the solution was extracted with CH_2Cl_2 (4 × 8 mL). The combined organic layers were dried (Na_2SO_4), concentrated in vacuo and the residue was purified by fc (2 cm, cyclohexane:ethyl acetate 7:3, 10 mL, R_f 0.13). Pale yellow oil, yield 54 mg (75%).

$C_{20}H_{22}FNO_2$ (327.2): MS (EI): *m*/*z* = 327 [M], 218 [M−CH_2PhF], 186 [M−CH_2PhF–OCH_3], 109 [FPhCH_2]. IR: ν (cm^{-1}) = 2923 (C–H), 1507 (aromat. C=C), 1219 (C–F), 1084 (C–O), 828 (C–H, 1,4 disubst. aromat.), 754 (C–H, 1,2 disubst. aromat.). ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.64 (ddd, *J* = 13.4/5.5/2.7 Hz, 1H, N(CH_2CH_2)$_2$), 1.80 (ddd, *J* = 13.5/5.5/2.7 Hz, 1H, N(CH_2CH_2)$_2$), 2.00 (td, *J* = 13.0/4.5 Hz, 1H, N(CH_2CH_2)$_2$), 2.08 (td, *J* = 13.1/4.6 Hz, 1H, N(CH_2CH_2)$_2$), 2.47 (td, *J* = 12.2/2.5 Hz, 2H, N(CH_2CH_2)$_2$), 2.79–2.88 (m, 2H, N(CH_2CH_2)$_2$), 3.47 (s, 3H, OCH_3), 3.55 (s, 2H, NCH_2Ph), 6.07 (s, 1H, ArC*H*), 7.01 (t, *J* = 8.7 Hz, 2H, FC(*CH*CH)$_2$), 7.16–7.19 (m, 1H, aromat. *H*), 7.29–7.39 (m, 5H, aromat. *H*). Purity (HPLC, method 2): 99.9%.

7.5. 1′-(4-Fluorobenzyl)-3H-spiro[[2]benzofuran-1,4′-piperidin]-3-ol (2b)

7.5.1. Method 1

A solution of **2a** (58 mg, 0.18 mmol) in CH_3CN (6 mL) and 1 M HCl (1.5 mL) was stirred at rt for 1 h. After addition of 2 M NaOH (pH 10) the mixture was extracted with CH_2Cl_2 (4 × 4 mL). The combined CH_2Cl_2 layers were washed with saturated solution of NaCl (2 × 4 mL), dried (Na_2SO_4), concentrated in vacuo and the residue was recrystallized with CH_2Cl_2 and *n*-hexane. Colorless solid, mp 148–152 °C, yield 37 mg (64%).

7.5.2. Method 2

The unpurified hydroxyacetal **6** (54 mg, 0.15 mmol) was dissolved in a mixture of THF (5 mL) and 2 M HCl (5 mL) and heated to reflux for 3.5 h. After addition of 2 M NaOH (pH 10) the mixture was extracted with CH_2Cl_2. The organic layer was dried (Na_2SO_4), concentrated in vacuo and the residue was purified by fc (2 cm, CH_2Cl_2:MeOH = 96:4, 10 mL, R_f 0.18). Colorless solid, yield 42 mg (89%).

$C_{19}H_{20}FNO_2$ (313.2): MS (EI): *m*/*z* = 313 [M], 296 [M−OH], 204 [M−CH_2PhF], 186 [M−(CH_2PhF–OH)], 109 [FPhCH_2]. IR: ν (cm^{-1}) = 2922 (C–H), 1510 (aromat. C=C), 1220 (C–F), 1039 (C–O), 835 (C–H, 1,4 disubst. aromat.), 758 (C–H, 1,2 disubst. aromat.). ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.61 (ddd, *J* = 13.5/5.4/2.6 Hz, 1H, N(CH_2CH_2)$_2$), 1.79 (ddd, *J* = 13.5/5.4/2.6 Hz, 1H, N(CH_2CH_2)$_2$), 2.04 (td, *J* = 13.0/4.0 Hz, 1H, N(CH_2CH_2)$_2$), 2.08 (td, *J* = 13.0/4.0 Hz, 1H, N(CH_2CH_2)$_2$), 2.47 (td, *J* = 11.9/2.4 Hz, 1H, N(CH_2CH_2)$_2$), 2.49 (td, *J* = 11.9/2.5 Hz, 1H, N(CH_2CH_2)$_2$), 2.80–2.85 (m, 2H, N(CH_2CH_2)$_2$), 3.56 (s, 2H, NCH_2Ph), 6.44 (s, 1H, ArC*H*), 7.01 (t, *J* = 8.7 Hz, 2H, FC(*CH*CH)$_2$), 7.18–7.21 (m, 1H, aromat. *H*), 7.30–7.43 (m, 5H, aro-

mat. *H*). A signal for the OH proton is not visible in the spectrum. Purity (HPLC, method 3): 99.4%.

7.6. 1′-(4-Fluorobenzyl)spiro[[2]benzofuran-1,4′-piperidin]-3-one (2c)

Under N_2 tetrapropylammonium perruthenate ($Pr_4N\ RuO_4$, 2.7 mg, 0.008 mmol, 5 mol %) was added to a mixture of the lactol **2b** (62 mg, 0.20 mmol), *N*-methylmorpholin-N-oxide (NMMO, 51 mg, 0.44 mmol), powdered molecular sieves 4 Å (0.1 g) and CH_2Cl_2/CH_3CN (1:1, 8 mL). After stirring for 2 h at rt it was filtered and the residue was washed with CH_2Cl_2. A saturated solution of NaCl was added to the filtrate, the organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (4×). The organic layer was dried (Na_2SO_4), filtered, concentrated in vacuo and the residue was purified by fc (1.5 cm, cyclohexane:ethyl acetate 5:5, 5 mL, R_f 0.17). Colorless solid, mp 103–106 °C, yield 31 mg (50%). $C_{19}H_{18}FNO_2$ (311.1). MS (EI): *m*/*z* = 311 [M], 202 [M−CH_2PhF], 109 [FPhCH_2]. IR: ν (cm^{-1}) = 2920 (C–H), 1757 (C=O), 1510 (aromat. C=C), 1220 (C–F), 1068 (C–O), 836 (C–H, 1,4 disubst. aromat.), 759 (C–H, 1,2 disubst. aromat.). ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.64 (dd, *J* = 14.2/2.3 Hz, 2H, N(CH_2CH_2)$_2$), 2.14 (td, *J* = 13.2/4.5 Hz, 2H, N(CH_2CH_2)$_2$), 2.48 (td, *J* = 12.1/2.3 Hz, 2H, N(CH_2CH_2)$_2$), 2.82 (d, *J* = 11.1 Hz, 2H, N(CH_2CH_2)$_2$), 3.51 (s, 2H, NCH_2Ph), 6.96 (tt, *J* = 8.7/2.0 Hz, 2H, FC($CHCH$)$_2$), 7.23–7.29 (m, 2H, FC(CHC*H*)$_2$), 7.35 (d, *J* = 7.6 Hz, 1H, 7-*H*), 7.44 (td, *J* = 7.5/0.7 Hz, 1H, 5-*H*), 7.60 (td, 7.6/1.0 Hz, 1H, 6-*H*), 7.81 (d, 7.6 Hz, 1 H, 4-*H*). Purity (HPLC, method 1): 95.1%.

7.7. 1′-[4-Fluorobenzyl]-3H-spiro[[2]benzofuran-1,4′piperidine] (2d)

Under N_2 a solution of the acetal **2a** (0.194 g, 0.59 mmol) in CH_2Cl_2 (12 mL) was cooled to −25 °C. Then, Et_3SiH (0.75 mL, 4.8 mmol) and $BF_3{\cdot}Et_2O$ (0.18 mL, 1.4 mmol) were added and the mixture was stirred for 20 min at −25 °C and for 1 h at 0–5 °C. After addition of H_2O (7 mL) the organic layer was separated, washed with a saturated solution of NaCl (2×), dried (Na_2SO_4) and concentrated in vacuo to yield a **2d**·BF_3 adduct.

Compound **2d**·BF_3: colorless solid, mp 254–257 °C (decomp.), yield 174 mg (81%). $C_{19}H_{20}FNO{\cdot}BF_3$ (366.0). MS (EI): *m*/*z* = 297 [M(**2d**)], 188 [M−CH_2PhF], 109 [FPhCH_2]. IR: ν (cm^{-1}) = 2912 (C–H); 2498 (R_3NH^+); 1507 (aromat. C=C); 1220 (C–F); 1049 (C–O); 828 (C–H, 1,4 disubst. aromat.), 754 (C–H, 1,2 disubst. aromat.). ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.85 (d, *J* = 14.9 Hz, 2H, N(CH_2CH_2)$_2$), 2.81 (td, *J* = 14.0/4.4 Hz, 2H, N(CH_2CH_2)$_2$), 3.11 (t, *J* = 11.7 Hz, 1H, N(CH_2CH_2)$_2$), 3.14 (t, *J* = 11.7 Hz, 1H, N(CH_2CH_2)$_2$), 3.40 (d br, *J* = 13.4 Hz, 2H, N(CH_2CH_2)$_2$,), 4.14 (d, *J* = 5.3 Hz, 2H, NCH_2Ph), 5.05 (s, 2H, ArCH_2), 7.13 (t, *J* = 8.6 Hz, 2H, FC($CHCH$)$_2$), 7.16–7.18 (m, 1H, aromat. *H*), 7.27–7.31 (m, 3 H, aromat. *H*), 7.71 (m, 2 H, aromat. *H*). Purity (HPLC, method 1): 94.5%.

In order to isolate the free base **2d** the solid (**2d**·BF_3, 33 mg, 0.09 mmol) was dissolved in 2 M NaOH. The mixture was extracted with CH_2Cl_2, the organic layer was dried (Na_2SO_4) and concentrated in vacuo. Pale yellow oil, yield 26 mg (97%, referred to the amount of employed solid **2d**·BF_3, 79% referred to **2a**). $C_{19}H_{20}FNO$ (297.2). MS (EI): *m*/*z* = 297 [M], 188 [M−CH_2PhF], 109 [FPhCH_2]. IR: ν (cm^{-1}) = 2912 (C–H); 1507 (aromat. C=C); 1220 (C–F); 1049 (C–O); 828 (C–H, 1,4 disubst. aromat.), 754 (C–H, 1,2 disubst. aromat.). ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.74 (bd, *J* = 13.9 Hz, 2H, N(CH_2CH_2)$_2$), 1.95 (td, *J* = 13.0/4.4 Hz, 2H, N(CH_2CH_2)$_2$), 2.40 (td br, *J* = 12.0/2.3 Hz, 2H, N(CH_2CH_2)$_2$), 2.79 (d br, *J* = 11.0 Hz, 2H, N(CH_2CH_2)$_2$), 3.52 (s, 2H, NCH_2Ph), 5.05 (s, 2H, ArCH_2O), 6.99 (t, *J* = 8.7 Hz, 2H, FC($CHCH$)$_2$), 7.11–7.14 (m, 1H, aromat. *H*), 7.16–7.21 (m, 1H, aromat. *H*), 7.23–7.28 (m, 2H, aromat. *H*), 7.28–7.33 (m, 2H, aromat. *H*). Purity (HPLC, method 1): 93.1%.

7.8. 1′-(4-Fluorobenzyl)-3H-spiro[[2]benzofuran-1,4′-piperidine]-3-carbonitrile (2e)

Under N_2 a solution of the acetal **2a** (94 mg, 0.29 mmol) in CH_2Cl_2 (7 mL) was cooled to −25 °C. Then, Me_3SiCN (0.22 mL, 1.76 mmol) and $BF_3{\cdot}Et_2O$ (0.04 mL, 0.32 mmol) were added. The mixture was stirred for 20 min at −25 °C and for 1 h at 0–5 °C. Afterwards CH_3OH (1 mL) and 2 M NaOH (5 mL) were added. The organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (4 × 4 mL). The combined organic layers were dried (Na_2SO_4), the solvent was removed in vacuo and the residue was purified by fc (2 cm, cyclohexane:ethyl acetate 7:3, 10 mL, R_f 0.13). Pale yellow solid, mp 111–113 °C, yield 70 mg (75%). $C_{20}H_{19}FN_2O$ (322.4). MS (EI): *m*/*z* = 322 [M], 227 [M−PhF], 213 [M−CH_2PhF], 109 [FPhCH_2]. IR: ν (cm^{-1}) = 2909 (C–H), 2210 (CN), 1508 (aromat. C=C), 1216 (C–F), 1033 (C–O), 840 (C–H, 1,4-disubst. aromat.), 753 (C–H, 1,2-disubst. aromat.). The signal for the CN-group at 2210 cm^{-1} is rather weak. ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.69 (ddd, *J* = 13.7/5.0/2.5 Hz, 1H, N(CH_2CH_2)$_2$), 1.89–2.09 (m, 3H, N(CH_2CH_2)$_2$), 2.38 (td, *J* = 12.1/2.5 Hz, 1H, N(CH_2CH_2)$_2$), 2.47 (td, *J* = 11.2/4.4 Hz, 1H, N(CH_2CH_2)$_2$), 2.75–2.84 (m, 2H, N(CH_2CH_2)$_2$), 3.53 (s, 2H, NCH_2Ph), 5.84 (s, 1H, ArC*H*), 7.00 (t, *J* = 8.7 Hz, 2H, FC($CHCH$)$_2$), 7.16–7.20 (m, 1H, aromat. *H*), 7.28–7.33 (m, 2H, aromat. *H*), 7.36–7.42 (m, 3H, aromat. H). ^{13}C NMR ($CDCl_3$): δ (ppm) = 37.1 (1C, N(CH_2CH_2)$_2$), 37.4 (1C, N(CH_2CH_2)$_2$), 49.8 (1C, N(CH_2CH_2)$_2$), 50.0 (1C, N(CH_2CH_2)$_2$), 62.7 (1C, NCH_2Ph), 69.1 (1C, ArCHCN), 88.3 (1C, ArCO), 115.2 (1C, FC($CHCH$)$_2$), 115.4 (1C, FC($CHCH$)$_2$), 118.7 (1C, CN), 121.6 (1C, aromat.CH), 122.2 (1C, aromat.CH), 129.1 (1C, aromat.CH), 130.0 (1C, aromat.CH), 130.9 (2C, aromat.CH), 134.0 (1C, aromat.C), 134.4 (1C, aromat.C), 145.6 (1C, aromat. C), 162.2 (d, *J* = 244.6 Hz, 1C, CF). Purity (HPLC, method 1): 98.3%.

7.9. 3-Allyl-1′-(4-fluorobenzyl)-3H-spiro[[2]benzofuran-1,4′-piperidine] (2f)

Under N_2 a solution of the acetal **2a** (71 mg, 0.22 mmol) in CH_2Cl_2 (6 mL) was cooled to −25 °C. Then, $Me_3SiCH_2CH{=}CH_2$ (0.26 mL, 1.2 mmol) and $BF_3{\cdot}Et_2O$ (0.15 mL, 1.2 mmol) were added. The mixture was stirred for 20 min at −25 °C, for 6.5 h at 0–5 °C and for 3.5 h at rt. After addition of H_2O (4 mL) the organic layer was separated and washed with a saturated solution of NaCl. The aqueous layer was extracted with CH_2Cl_2 (4×). The combined organic layers were dried (Na_2SO_4), the solvent was removed in vacuo and the residue was recrystallized with a mixture of CH_2Cl_2 and *n*-hexane to yield a **2f** BF_3 adduct.

Compound ***2f***·BF_3: colorless solid, mp 211–216 °C (decomp.), yield 56 mg (63%). $C_{22}H_{24}FNO^+BF_3^-$ (406.0). MS (EI): *m*/*z* = 337 [M(**2f**)], 296 [M−$CH_2CH{=}CH_2$], 228 [M−CH_2PhF], 109 [FPhCH_2]. IR: ν (cm^{-1}) = 2921 (C–H), 2500 (R_3NH^+), 1641 (C=C), 1512 (aromat. C=C), 1226 (C–F), 1038 (C–O), 840 (C–H, 1,4 disubst. aromat.), 753 (C–H, 1,2 disubst. aromat.). ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.80–1.84 (m, 2H, N(CH_2CH_2)$_2$), 2.46–2.54 (m, 1H, CH_2–CH=CH_2)), 2.56–2.64 (m, 1H, CH_2–CH=CH_2)), 2.75 (td, *J* = 14.0/4.4 Hz, 1H, N(CH_2CH_2)$_2$), 2.90 (td, *J* = 13.9/4.2 Hz, 1H, N(CH_2CH_2)$_2$), 3.09–3.21 (m, 2H, N(CH_2CH_2)$_2$), 3.36–3.45 (m, 2H, N(CH_2CH_2)$_2$), 4.15 (s, 2H, NCH_2Ph), 5.06–5.14 (m, 2H, CH_2–CH=CH_2), 5.28 (t, *J* = 5.8 Hz, 1H, ArC*H*), 5.71–5.83 (m, 1H, CH_2–C*H*=CH_2), 7.11–7.18 (m, 3H, aromat. *H*), 7.28–7.34 (m, 3H, aromat. *H*), 7.72–7.77 (m, 2H, aromat. *H*). Purity (HPLC, method 1): 95.3%.

In order to obtain the free base **2f** the solid (**2f**·BF_3, 23 mg, 0.06 mmol) was dissolved in 2 M NaOH and CH_2Cl_2, the organic layer was separated, dried (Na_2SO_4) and concentrated in vacuo. Pale yellow oil, yield 20 mg (99%, referred to the employed amount of **2f**·BF_3; 62% referred to the starting compound **2a**). $C_{22}H_{24}FNO$ (337.2). MS (EI): *m*/*z* = 337 [M], 296 [M−$CH_2CH{=}CH_2$], 228

[M−CH_2PhF], 109 [FPhCH_2]. IR: ν (cm^{-1}) = 2913 (C–H), 1641 (C=C), 1507 (aromat. C=C), 1219 (C–F), 1090 (C–O), 827 (C–H, 1,4 disubst. aromat.), 754 (C–H, 1,2 disubst. aromat.). ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.65–1.73 (m, 2H, N(CH_2CH_2)$_2$,), 1.88 (td, J = 13.1/4.5 Hz, 1H, N(CH_2CH_2)$_2$), 2.03 (td, J = 13.0/4.5 Hz,1H, N(CH_2CH_2)$_2$), 2.39–2.47 (m, 2H, N(CH_2CH_2)$_2$), 2.49–2.61 (m, 2H, CH_2–CH=CH_2), 2.77 (d, J = 11.0 Hz, 2H, N(CH_2CH_2)$_2$), 3.52 (s, 2H, NCH_2Ph), 5.04–5.13 (m, 2H, CH_2–CH=CH_2), 5.24 (t, J = 5.8 Hz, 1H, ArC*H*), 5.78–5.90 (m, 1H, CH_2–C*H*=CH_2), 6.99 (t, J = 8.7 Hz, 2H, FC(*CH*CH)$_2$), 7.08–7.16 (m, 2H, aromat. *H*), 7.21–7.33 (m, 4H, aromat. *H*). Purity (HPLC, method 1): 95.8%.

7.10. 1′-[4-(*N*,*N*-Dimethylamino)benzoyl]-3-methoxy-3H-spiro[[2]benzofuran-1,4′-piperidine] (15)

A mixture of secondary amine **7** (99 mg, 0.45 mmol), *p*-(dimethylamino)benzoyl chloride (107 mg, 0.65 mmol) and NEt_3 (0.15 mL) in CH_2Cl_2 (20 mL) was stirred for 6 h at rt. Then, 2 M NaOH was added (pH 10) and after 1 h the organic layer was separated. The aqueous layer was extracted twice with CH_2Cl_2, the combined organic layers were dried (Na_2SO_4), concentrated in vacuo and the residue was purified by fc (2 cm, cyclohexane:ethyl acetate 7:3, 10 mL, R_f 0.09). Colorless viscous oil, yield 50 mg (31%). $C_{22}H_{26}N_2O_3$ (366.5). MS (EI): m/z = 366 [M], 218 [M−OCPhN(CH_3)$_2$), 186 [M−OCPhN(CH_3)$_2$–OCH_3], 148 [OCPhN(CH_3)$_2$]. IR: ν (cm^{-1}) = 2923 (C–H), 1627 (C=O amide), 1523 (aromat. C=C), 1357 (C-N amide), 1083 (C–O), 823 (C–H, 1,4 disubst. aromat.), 757 (C–H, 1,2 disubst. aromat.). ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.50–1.72 (m, 2H, N(CH_2CH_2)$_2$), 1.76–2.06 (m, 2H, N(CH_2CH_2)$_2$), 3.02 (s, 6H, N(CH_3)$_2$), 3.27–3.56 (m, 2H, N(CH_2CH_2)$_2$), 3.50 (s, 3H, OCH_3), 6.09 (s, 1H, ArC*H*), 6.75–6.93 (m, 2H, (CH_3)$_2$NC(*CH*CH)$_2$), 7.13–7.18 (m, 1H, aromat. *H*), 7.32–7.47 (m, 5H, aromat. *H*). The signals of the piperidine ring protons are very broad, signals for two protons adjacent to the N-atom are not seen. ^{1}H NMR ($C_6H_5NO_2$, rt): δ (ppm) = 1.75 (dd, J = 13.4/1.9 Hz, 1H, N(CH_2CH_2)$_2$), 1.91 (d br, J = 11.9 Hz, 1H, N(CH_2CH_2)$_2$), 2.11 (td, J = 13.0/4.7 Hz, 1H, N(CH_2CH_2)$_2$), 2.22 (td, J = 13.1/4.8 Hz, 1H, N(CH_2CH_2)$_2$), 2.89 (s, 6H, N(CH_3)$_2$), 3.51–3.65 (m, 2H, N(CH_2CH_2)$_2$), 3.57 (s, 3H, OCH_3), 4.40–4.67 (m, 2H, N(CH_2CH_2)$_2$), 6.21 (s, 1H, ArC*H*), 6.66 (d, J = 8.9 Hz, 2H, (CH_3)$_2$NC(*CH*CH)$_2$), 7.28 (d, J = 8.3 Hz, 1H, aromat. *H*), 7.34–7.46 (m, 3H, aromat. *H*), 7.51 (d, J = 8.9 Hz, 2H, (CH_3)$_2$NC(CHC*H*)$_2$). The signals of the protons adjacent to the N-atom are very broad. ^{1}H NMR ($C_6H_5NO_2$, 100 °C): δ (ppm) = 1.73 (ddd, J = 13.4/5.3/2.6 Hz, 1H, N(CH_2CH_2)$_2$), 1.93 (ddd, J = 13.4/5.3/2.7 Hz, 1H, N(CH_2CH_2)$_2$), 2.10 (td, J = 13.1/4.7 Hz, 1H, N(CH_2CH_2)$_2$), 2.16 (td, J = 13.1/4.7 Hz, 1H, N(CH_2CH_2)$_2$), 2.93 (s, 6H, N(CH_3)$_2$), 3.54–3.62 (m, 2H, N(CH_2CH_2)$_2$), 3.55 (s, 3H, OCH_3), 4.41–4.53 (m, 2H, N(CH_2CH_2)$_2$), 6.21 (s, 1H, ArC*H*), 6.72 (d, J = 8.9 Hz, 2H, (CH_3)$_2$NC(*CH*CH)$_2$), 7.25–7.29 (m, 1H, aromat. *H*), 7.32–7.43 (m, 3H, aromat. *H*), 7.57 (d, J = 8.9 Hz, 2H, (CH_3)$_2$NC(CHC*H*)$_2$). Purity (HPLC, method 2): 92.7%.

7.11. 1′-(4-Fluorobenzoyl)-3-methoxy-3H-spiro[[2]benzofuran-1,4′-piperidine] (17)

A solution of the secondary amine **7** (104 mg, 0.47 mmol), *p*-fluorobenzoyl chloride (108 mg, 0.68 mmol) and NEt_3 (0.16 mL) in CH_2Cl_2 (12 mL) was stirred for 1.5 h at rt. After addition of 2 M NaOH (8 mL) the mixture was stirred for 1 h. The aqueous layer was separated, extracted with CH_2Cl_2 (2×), the combined organic layers were dried (Na_2SO_4) concentrated in vacuo and the residue was purified by fc (3 cm, cyclohexane:ethyl acetate 7:3, 15 mL, R_f 0.20). Colorless solid, mp 109 °C, yield 142 mg (42%). $C_{20}H_{20}FNO_3$ (341.4). MS (EI): m/z = 341 [M], 310 [M–OCH_3], 292 [M−OCH_3–F], 218 [M−O=CPhF], 123 [O=CPhF]. IR: ν (cm^{-1}) = 2913 (C–H), 1620 (C=O amide), 1509 (aromat. C=C), 1219 (C–F), 1037 (C–O), 827 (C–H, 1,4 disubst. aromat.), 757 (C–H, 1,2 disubst. aromat.). ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.69–2.10 (m, 4H, N(CH_2CH_2)$_2$), 3.22–3.42 (m, 1H, N(CH_2CH_2)$_2$), 3.50 (s, 3H, OCH_3), 3.52–3.65 (m, 1H, N(CH_2CH_2)$_2$) 3.69–3.84 (m, 1H, N(CH_2CH_2)$_2$), 4.63–4.81 (m, 1H, N(CH_2CH_2)$_2$), 6.01 (s, 1H, ArC*H*), 7.11 (t, J = 8.7 Hz, 2H, FC(*CH*CH)$_2$), 7.13–7.18 (m, 1H, aromat. *H*), 7.33–7.42 (m, 3H, aromat. *H*), 7.47 (2d, J = 8.7 Hz, 2H, FC(CHC*H*)$_2$). ^{1}H NMR ($C_6H_5NO_2$, rt): δ (ppm) = 1.64–1.80 (m, 1H, N(CH_2CH_2)$_2$), 1.80–2.00 (m, 1H, N(CH_2CH_2)$_2$), 2.09 (td, J = 13.0/4.5 Hz, 1H, N(CH_2CH_2)$_2$), 2.20 (td, J = 13.1/4.8 Hz, 1H, N(CH_2CH_2)$_2$), 3.33–3.54 (m, 1H, N(CH_2CH_2)$_2$), 3.51 (s, 3H, OCH_3), 3.48–3.75 (m, 1H, N(CH_2CH_2)$_2$), 3.77–4.08 (m, 1H, N(CH_2CH_2)$_2$), 4.71–5.08 (m, 1H, N(CH_2CH_2)$_2$), 6.19 (s, 1H, ArC*H*), 7.16 (t, J = 8.8 Hz, 2H, FC(*CH*CH)$_2$), 7.30 (d, J = 8.3 Hz, 1H, aromat. *H*), 7.33–7.45 (m, 3H, aromat. *H*), 7.64–7.71 (m, 2H, FC(CHC*H*)$_2$). The signals of the protons adjacent to the N-atom are very broad. ^{1}H NMR ($C_6H_5NO_2$, 100 °C): δ (ppm) = 1.71 (d, J = 13.5 Hz, 1H, N(CH_2CH_2)$_2$), 1.90 (d, J = 13.5 Hz, 1H, N(CH_2CH_2)$_2$), 2.07 (td, J = 13.1/4.8 Hz, 1H, N(CH_2CH_2)$_2$), 2.14 (td, J = 13.3/4.8 Hz, 1H, N(CH_2CH_2)$_2$), 3.53 (s, 3H, OCH_3), 3.53–3.63 (m, 2H, N(CH_2CH_2)$_2$), 4.24–4.42 (m, 2H, N(CH_2CH_2)$_2$), 6.19 (s, 1H, ArC*H*), 7.13 (t, J = 8.8 Hz, 2H, FC(*CH*CH)$_2$), 7.24–7.29 (m, 1H, aromat. *H*), 7.30–7.43 (m, 3H, aromat. *H*), 7.59–7.67 (m, 2H, FC(CHC*H*)$_2$). Purity (HPLC, method 2): 97.7%.

7.12. 3H-Spiro[[2]benzofuran-1,4′-piperidine]-3-carbonitrile (19)

Under N_2 the carbonitrile **18**[21] (100 mg, 0.33 mmol) was dissolved in CH_3OH (20 mL). Pd/C (25 mg, 10% (m/m)) and dried ammonium formate (104 mg, 1.64 mmol) were added. The mixture was heated to reflux for 8 h. After periods of 1.5 h and 7 h ammonium formate (12 mg) was added. After cooling to rt the mixture was filtered with Celite®. The solvent was removed in vacuo and the residue was purified by fc (3 cm, ethyl acetate:methanol:ammonia 90:10:2, 20 mL, R_f 0.05) several times. Pale yellow oil, yield 28 mg (44%). $C_{14}H_{16}N_2O$ (214.3). MS (EI): m/z = 215 [M], 171 [M–C_2H_5N], 115 [PhCH_2CN]. IR: ν (cm^{-1}) = 3333 (N-H), 2942 (C–H), 1607 (C=C), 1045 (C–O), 754 (C–H, 1,2 disubst. aromat.). ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.70 (ddd, 2H, J = 13.6/4.9/2.4 Hz, N(CH_2CH_2)$_2$), 1.87–1.98 (m, 2H, N(CH_2CH_2)$_2$), 3.03–3.14 (m, 4H, N(CH_2CH_2)$_2$), 5.84 (s, 1H, Ar-C*H*-O), 7.17–7.24 (m, 1H, aromat. H), 7.38–7.44 (m, 3H, aromat. H). ^{13}C NMR ($CDCl_3$): δ (ppm) = 36.8 (1C, N(CH_2CH_2)$_2$), 37.1 (1C, N(CH_2CH_2)$_2$), 42.3 (1C, N(CH_2CH_2)$_2$), 42.5 (1C, N(CH_2CH_2)$_2$), 69.2 (1C, ArCHCN), 88.2 (1C, ArCO), 118.5 (1C, CN), 121.7 (1C, aromat.CH), 122.3 (1C, aromat.CH), 129.2 (1C, aromat.CH), 130.2 (1C, aromat.CH), 133.8 (1C, aromat.C), 145.3 (1C, aromat.C). Purity (HPLC, method 1): 76.2%.

8. Receptor binding studies

8.1. 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%.

8.2. Membrane preparation for the σ_1 assay[21,28]

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[31] 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.

8.3. Performing of the σ_1 assay[21,28]

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.[32]

8.4. Membrane preparation for the σ_2 assay[21,28]

Two rat livers were cut into small pieces and homogenized with a potter (500–800 rpm, 10 up-and-down strokes) in 6 volumes of cold 0.32 M sucrose. The suspension was centrifuged at 1200*g* for 10 min at 4 °C. The supernatant was separated and centrifuged at 31000*g* for 20 min at 4 °C. The pellet was resuspended in buffer (50 mM TRIS, pH 8.0) and incubated at rt for 30 min. After the incubation, the suspension was centrifuged again at 31000*g* for 20 min at 4 °C. The final pellet was resuspended in buffer, the protein concentration was determined according to the method of Bradford[31] 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.

8.5. Performing of the σ_2 assay[21,28]

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.[33]

8.6. 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.[34] The K_i-values are given as mean values + SEM from three independent experiments.

9. Metabolism

9.1. Preparation of rat liver microsomes[35]

Frozen rat livers from Wistar rats were thawn in phosphate buffer pH 7.4 with 0.25 M sucrose and 5 mM EDTA, cut into small pieces and homogenized with a Potter (Elvehjem Potter, B. Braun Biotech International). The homogenization was carried out at 4 °C. The resulting suspension was centrifuged (high-speed cooling centrifuge model Sorvall, RC-5C plus, Thermo Finnigan) at 10,000*g* for 15 min at 4 °C. Fat was removed by cellulose. The pellet was resuspended in buffer, the mixture was centrifuged again and afterwards both supernatants were combined. This suspension was transferred into an ultracentrifuge (Beckmann L8-M with rotor Ti 70.1) and centrifuged for 60 min at 100,000*g* and 4 °C. The resulting supernatant (cytosol) was discarded, the pellet carefully washed with buffer, resuspended and the centrifugation repeated. Finally the supernatant was removed, the pellet resuspended in a small amount of phosphate buffer pH 7.4 and the microsome suspension was stored at −80 °C. The protein concentration was determined according to the method of Bradford[31] using bovine serum albumin as standard.

9.2. Incubation of 2a, 2d and 2e with rat liver microsomes

The incubation was carried out in phosphate buffer pH 7.4 at rt in a circular shaker (IKA vibrax VXR) and contained rat liver microsomes (1.5 mg/mL protein), 0.86 mM $MgCl_2$ and 2.6 mM NADPH/H^+. The concentration of **2a**, **2d** and **2e** was 260 μM in a final volume of 0.9 mL. Usually, the incubation was stopped after 90 min by addition of cold acetonitrile (−20 °C). The samples were stored for 30 min at −20 °C to complete protein precipitation. After thawing, the samples were centrifuged (10,000*g* in Hettich Mikro 20 benchtop centrifuge). The supernatant was decanted, filtered with a 0.45 μm (pore size) syringe filter made from regenerated cellulose and finally analyzed.

9.3. Degradation of 2a, 2d and 2e during 90 min

Seven incubations of **2a**, **2d** and **2e** were carried out in phosphate buffer pH 7.4 at rt in a circular shaker containing rat liver microsomes (1.5 mg/mL protein), 0.86 mM $MgCl_2$ and 2.6 mM NADPH/H^+. The concentrations of **2a**, **2d** and **2e** were 320 μM in a total volume of 0.9 mL. During the first hour every 10 min one incubation was stopped by addition of cold acetonitrile (−20 °C). 145 μL of a 0.6 mg/mL haloperidol solution were added as internal standard resulting in a final concentration of 220 μM in a total volume of 1.045 mL. After 90 min the last incubation was stopped and analyzed. The calibration was carried out with the same matrix except NADPH/H^+. All calibration standards were treated in the same way (90 min on the shaker, protein precipitation with acetonitrile, centrifugation, etc.).

9.4. Incubation of 2d and 2e with human liver microsomes (pooled human microsomes)

The pooled human liver microsomes were kindly provided by Bayer Healthcare. 20 μM of the respective compound **2** were incubated for 60 min at 37 °C with 0.5 mg/mL protein microsomes (from Cytonet, pooled donors, specially selected for Bayer) in phosphate buffer pH 7.4 and a NADPH regenerating system containing glucose-6-phosphate, NADPH and glucose-6-phosphate dehydrogenase (from leuconostoc mesenteroides). The final NADPH concentration was 1 mM. The incubations were stopped by addition of cold acetonitrile (−20 °C), cooled on ice for 30 min, centrifuged, filtered and analyzed afterwards.

9.5. Reactions of 2d and 2e with recombinant isoenzymes

The concentration of the isoenzymes CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6 and CYP3A4 (human P450 BD Supersomes® from Gentest) was 0.05 μM. The incubations were performed as described for the incubation with the pooled human microsomes.

9.6. HPLC–UV

The HPLC analysis was carried out with a Merck Hitachi equipment consisting of UV detector: L-7400; autosampler: L-7200; pump: L-7150; interface: D-7000. Twenty microliters of the prepared incubation solution were injected onto a Phenomenex Gemini® C18 column (5 μm, 250 × 4.6 mm) at a flow rate of 1.0 mL/min and UV detection wavelength of 210 nm. The mobile phase was composed of (A) 15% acetonitrile in water and (B) 60% acetonitrile in water. Trifluoroacetic acid (0.05%) was added to both components. The following gradient was applied (A%): 0 min: 100%, 20 min: 0%, 23 min: 0%, 24 min: 100%, 30 min: 100%.

9.7. LC–MS

The HPLC–UV method was transferred onto a HPLC system consisting of a Waters Alliance® 2690 separations module, a Waters 2487 dual λ absorbance detector and a Thermo Finnigan LCQ® ion trap mass spectrometer with an ESI interface. The ion spray voltage was 3 kV in positive mode at a sheath gas flow of 80 arbitrary units. Temperature of the capillary was set to 200 °C and the capillary voltage to 4 V. A 20 μL volume of the prepared incubation solution was injected onto a LiChrospher® RP Select B 5 μm (250 × 4 mm) column (Merck, Germany) at a flow rate of 1.0 mL/min. Solvents and gradient were identical with the HPLC–UV method described above. In addition to the MS spectra the UV absorption at 210 nm was recorded.

Acknowledgements

This work was performed within the International Research Training Group 'Complex Functional Systems in Chemistry: Design, Synthesis and Applications' in collaboration with the University of Nagoya. Financial support of the IRTG and this project by the *Deutsche Forschungsgemeinschaft* is gratefully acknowledged. The experiments with the human microsomes and the recombinant isoenzymes were supported by *Bayer HealthCare AG*, Wuppertal.

References and notes

1. Danso-Danquah, R.; Bai, X.; Zhang, X.; Mascarella, S. W.; Williams, W.; Sine, B.; Bowen, W. D.; Carroll, F. I. *J. Med. Chem.* **1995**, *38*, 2978.
2. Kaiser, C.; Pontecorvo, J.; Mewshaw, R. E. *Neurotransmission* **1991**, *7*, 1.
3. Walker, J. M.; Bowen, W. D.; Walker, F. O.; Matsumoto, R. R.; De Costa, B.; Rice, K. C. *Pharmacol. Rev.* **1990**, *42*, 353.
4. Rowley, M.; Bristow, L. J.; Hutson, P. H. *J. Med. Chem.* **2001**, *44*, 477.
5. Monograph Igmesine Hydrochloride, *Drugs Future* **1999**, *24*, 133.
6. Skuza, G. *Pol. J. Pharmacol.* **2003**, *55*, 923.
7. Maurice, T.; Lockhart, B. P. *Prog. Neuropsychopharmacol. Biol. Psychiat.* **1997**, *21*, 69.
8. Marrazzo, A.; Caraci, F.; Salinaro, E. T.; Su, T. P.; Copani, A.; Ronsisvalle, G. *Neuroreport* **2005**, *18*, 1223.
9. Gilchrist, H. D.; Allard, B. L.; Simone, D. A. *Pain* **1996**, *67*, 179.
10. Baeyens, J. M. PCT Int. Appl. **2006**, WO 2006/010587 A1.
11. Matsumoto, R. R.; Liu, Y.; Lerner, M.; Howard, E. W.; Brackett, D. J. *Eur. J. Pharmacol.* **2003**, *496*, 1.
12. Hayashi, T.; Su, T.-P. *CNS Drugs* **2004**, *18*, 269.
13. Meunier, J.; Ieni, J.; Maurice, T. *Br. J. Pharmacol.* **2006**, *149*, 998.
14. Bermack, J. E.; Debonnel, G. *Synapse* **2005**, *55*, 37.
15. Gudelsky, G. A. *J. Neural. Trans.* **1999**, *106*, 849.
16. Bowen, W. D. *Pharm. Acta Helv.* **2000**, *74*, 211.
17. Wilke, R. A.; Lupardus, P. J.; Grandy, D. K.; Rubinstein, M.; Low, M. J.; Jackson, M. B. *J. Physiol.* **1999**, *517*, 391.
18. Aydar, E.; Palmer, C. P.; Djamgoz, M. B. A. *Cancer Res.* **2004**, *64*, 5029.
19. Monnet, F. P. *Biol. Cell* **2005**, *97*, 873.
20. Zhang, H.; Cuevas, J. *J. Neurophysiol.* **2002**, *87*, 2867.
21. Maier, C. A.; Wünsch, B. *J. Med. Chem.* **2002**, *45*, 438.
22. Maier, C. A.; Wünsch, B. *J. Med. Chem.* **2002**, *45*, 4923.
23. Wiese, C.; Große Maestrup, E.; Schepmann, D.; Vela, J. M.; Holenz, J.; Buschmann, H.; Wünsch, B. *J. Pharm. Pharmacol.* **2009**, *61*, 631.
24. Ley, S. V.; Norman, J.; Griffith, W. P.; Marsden, S. P. *Synthesis* **1994**, 639.
25. Brückner, Reaktionsmechanismen, 3rd ed., Elsevier GmbH, **2004**, p 797.
26. Cohen, N.; Schaer, B.; Saucy, G.; Borer, R.; Todaro, L.; Chiu, A.-M. *J. Org. Chem.* **1989**, *54*, 3282.
27. Komatsu, N.; Uda, M.; Suzuki, H.; Takahashi, T.; Domae, T.; Wada, M. *Tetrahedron Lett.* **1997**, 7215.
28. Wirt, U.; Schepmann, D.; Wünsch, B. *Eur. J. Org. Chem.* **2007**, 462.
29. Haka, M. S.; Kilbourn, R.; Watkins, G. L.; Toorongian, S. A. *J. Label. Comp. Radiopharm.* **1989**, *27*, 823.
30. Wilson, A. A.; Dannanls, R. F.; Hayden, T. R.; Wagner, H. N. *J. Label. Comp. Radiopharm.* **1990**, *28*, 1189.
31. Bradford, M. M. *Anal. Biochem.* **1976**, *72*, 248.
32. De-Haven-Hudkins, D. L.; Fleissner, L. C.; Ford-Rice, F. Y. *Eur. J. Pharmacol. Mol. Pharmacol. Sect.* **1992**, *227*, 371.
33. Mach, R. H.; Smith, C. R.; Childers, S. R. *Life Sci.* **1995**, *57*, 57.
34. Cheng, Y.-C.; Prusoff, W. H. *Biochem. Pharmacol.* **1973**, *22*, 3099.
35. Dayer, P.; Gasser, R. *Biochem. Biophys. Res. Commun.* **1984**, *30*, 374.
36. Unpublished results, manuscript in preparation.