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Synthesis, pharmacological activity and structure affinity relationships of spirocyclic σ_1 receptor ligands with a (2-fluoroethyl) residue in 3-position

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ABSTRACT

In order to develop a fluorinated radiotracer for imaging of σ_1 receptors in the central nervous system a series of (2-fluoroethyl) substituted spirocyclic piperidines **3** has been prepared. In the key step of the synthesis 2-bromocinnamaldehyde acetal **5** was added to piperidones **6** with various substituents at the N-atom. Unexpectedly, this reaction led to 2-benzoxepines **8**, which were contracted with acid to afford the spirocyclic 2-benzofuranacetaldehydes **9**. The best yields were obtained, when the transformations up to the alcohols **10** were performed without isolation of intermediates. Generally the (2-fluoroethyl) derivatives **3** have higher σ_1 affinity and σ_1/σ_2 selectivity than the corresponding (3-fluoropropyl) derivatives **2**. The most promising candidate for the development as radiotracer is the (2-fluoroethyl) derivative **3a** (WMS-1828, fluspidine, 1′-benzyl-3-(2-fluoroethyl)-3*H*-spiro[[2]benzofuran-1,4′-piperidine]), which shows subnanomolar σ_1 affinity (K_i = 0.59 nM) and excellent selectivity over the σ_2 subtype (1331-fold) as well as some other receptor systems. The novel synthetic strategy also allows the systematic pharmacological evaluation of intermediate alcohols **10**. Despite their high σ_1 affinity (K_i = 6–32 nM) and selectivity the alcohols **10** are 10–30-fold less potent than the bioisosteric fluoro derivatives **3**.

1. Introduction

After initially being classified as subtype of opioid receptors,[1] σ receptors are today characterized as specific, non-opioid, non-phencyclidine but haloperidol-sensitive binding structures.[2] Two distinct subtypes of σ receptors, termed σ_1 and σ_2 receptors, are postulated,[3] but only the σ_1 receptor protein has been identified and characterized so far. The human σ_1 receptor is a unique protein consisting of 223 amino acids, which was first cloned and functionally expressed by Kekuda et al.[4] The σ_1 receptor is expressed in neuronal, immune and endocrine systems with especially high density in limbic and motor brain structures as well as in peripheral organs such as heart, lung, liver, pancreas, and sexual and immune glands.[4,5]

It has been shown that ligands interacting with the σ_1 receptor subtype are of particular interest for the treatment of acute and chronic neurological disorders, including depression,[6,7] Alzheimer's Disease and Parkinson's Disease,[8,9] pain, in particular neuropathic pain,[10,11] as well as alcohol and cocaine abuse.[12,13] However, the signal transduction pathway after activation of σ_1 receptors is not completely understood so far and thus the above mentioned pharmacological effects cannot be correlated directly to a biochemical mechanism.

Various experiments have demonstrated that σ_1 receptors are involved in the modulation of some neurotransmitter systems including the glutamatergic,[14] dopaminergic[15] and cholinergic[16] neurotransmission. Additionally, the influence on the regulation and activity of K^+ channels[17,18] and Ca^{2+} channels[19,20] is an important feature of σ_1 receptors. σ_1 Receptors are found in neuronal cell bodies and dendrites with particular accumulation within mitochondrial membranes, the endoplasmatic reticulum and postsynaptic membranous thickening.[21,22] As these subcellular compartments are known to be involved in the regulation of intracellular Ca^{2+} concentration, these ultrastructural together with pharmacological data on σ_1 receptor activation[23] indicate σ_1 receptor-mediated modulation of various neurotransmitter systems via Ca^{2+}-dependent cell signaling cascades.

This project aims at the development of a fluorinated high affinity σ_1 ligand, which will be used for non-invasive in vivo imaging of cerebral σ_1 receptors by positron emission tomography (PET). A fluorinated σ_1 receptor PET tracer is of particular value for target validation, that is, investigation of the correlation between σ_1 receptor occupancy and efficacy of a novel drug. Additionally, σ_1 receptor expression in the healthy and diseased brain will be quantified. Moreover, a fluorinated σ_1 receptor PET tracer should provide new information about the complex neurotransmitter

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balance in the central nervous system. The most commonly used σ_1 receptor PET tracer is [^{11}C]SA4503, which shows only moderate selectivity against the σ_2 subtype and requires a bedside cyclotron due to the short half-life of [^{11}C] of 20 min.[24–28] In order to become independent on a bedside cyclotron a fluorinated PET tracer is envisaged, since [^{18}F]fluorine has a half-life of 110 min and can be produced at a different place.

Recently, we have reported on the synthesis and pharmacological evaluation of a new class of spirocyclic piperidines, which interact with high affinity and selectivity with σ_1 receptors.[29–34] The spiro[benzofuran-piperidine] **1** with a methoxy moiety in position 3 (Fig. 1) represents an extraordinarily potent σ_1 receptor antagonist (K_i = 1.1 nM) with high selectivity against the σ_2 subtype (σ_1:σ_2 >1000-fold) and more than 60 other receptors, ion channels (including the hERG channel) and transporters. In the capsaicin assay, **1** was able to reduce the neuropathic pain reaction of mice.[35]

Replacement of the 3-methoxy group of **1** by a (3-fluoropropyl) residue resulted in the very potent (K_i = 1.4 nM) and selective (σ_1:σ_2 = 620) σ_1 receptor ligand **2a**, which has been evaluated as PET tracer.[36] Herein the synthesis, pharmacological properties and relationships between the structure and σ_1 receptor affinity of the corresponding (2-fluoroethyl) derivatives **3** are described (Fig. 1).

2. Synthesis

2-Bromobenzaldehyde (**4**) served as starting material for the synthesis of the (2-fluoroethyl) substituted spirocyclic σ_1 receptor ligands **3**. At first a homologation of the benzaldehyde by two carbon atoms using the Wittig reagent [(1,3-dioxolan-2-yl)methyl]triphenylphosphonium bromide [$(CH_2O)_2CHCH_2PPh_3Br$], was performed. (Scheme 1) Generation of the non-stabilized P-ylide with K_2CO_3 in the presence of the phase transfer catalyst TDA-1 (tris[2-(2-methoxyethoxy)ethyl]amine)[37,38] led to the diastereomeric cinnamaldehyde acetals **5** in 95% yield. The (*E*)- and (*Z*)-configured acetals (*E*)-**5** and (*Z*)-**5** were formed in the ratio 1:1. This mixture of diastereomers was treated with *n*-BuLi at −78 °C and after a reaction time of 10 min the formed aryllithium intermediate was trapped with piperidone **6a**. The reaction mixture was stirred at −78 °C for 1 h and at room temperature for 17 h. Instead of the expected hydroxy acetals (*E*)-**11a** and (*Z*)-**11a** these reaction conditions provided the 2-benzoxepine **8a**, which was isolated in 67% yield. It is assumed that the Li-cation of the intermediate alcoholate **7a** coordinated with an O-atom of the dioxolane moiety and subsequent opening of the dioxolane ring afforded the 2-benzoxepine **8a**.

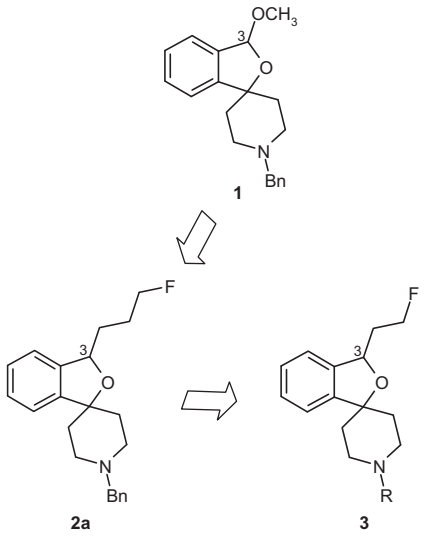


Figure 1. Structural development of the novel class of (2-fluoroethyl) substituted spirocyclic σ_1 receptor ligands **3** from **1** and **2**.

In order to get more insight into this unexpected ring closing reaction, the diastereomeric acetals (*E*)-**5** and (*Z*)-**5** were separated by flash chromatography and the halogen metal exchange as well as the subsequent addition to piperidone **6a** were performed with pure stereoisomers. At first the reaction time was reduced from 17 h to 3 h. Whereas stirring of (*Z*)-**5** for 3 h at room temperature yielded exclusively the 2-benzoxepine **8a**, the same reaction conditions applied on (*E*)-**5** led predominantly to formation of the (*E*)-configured hydroxy acetal (*E*)-**11a**. (Scheme 2). Prolongation of the reaction time of (*E*)-**5** to 18 h resulted in the formation of 2-benzoxepine **8a** as major product (Scheme 2). These observations support our hypothesis of a coordination of the Li-cation of **7a** with an O-atom of the dioxolane moiety, since only in the fast reacting (*Z*)-configured isomer (*Z*)-**7a** a close relationship between the Li-cation and a dioxolane O-atom exists. The slower cyclization of the (*E*)-configured derivative (*E*)-**7a** is explained with a slow isomerization of (*E*)-**7a** to (*Z*)-**7a**, which starts with a coordination of the Li-cation with the double bond.

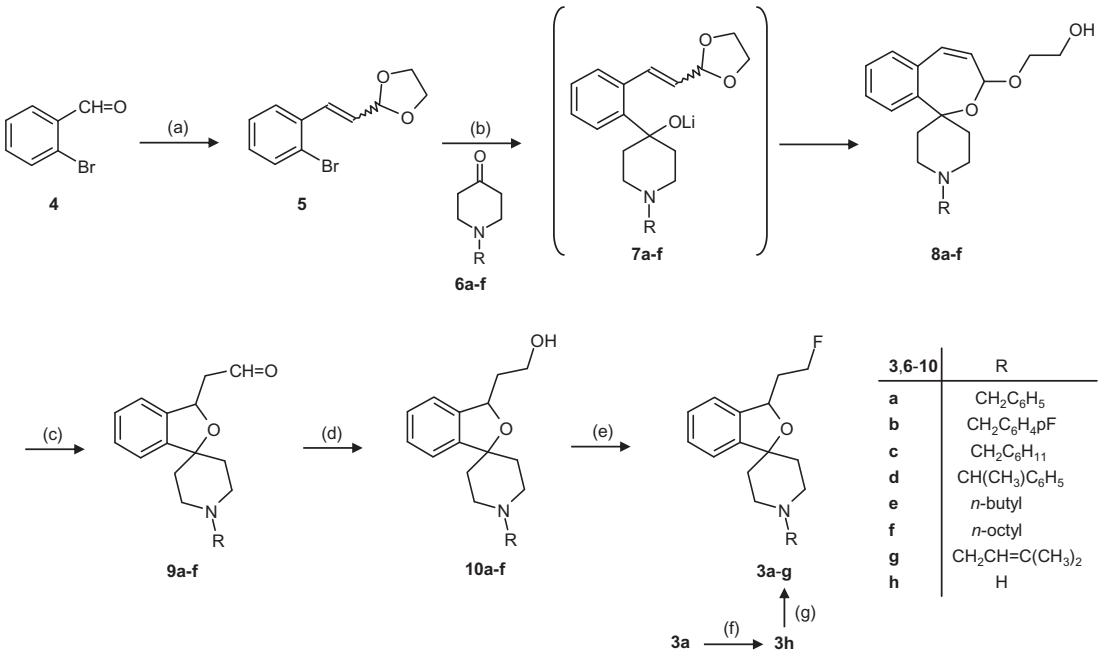
In the next step the 2-benzoxepine **8a** was heated with diluted HCl, which led to hydrolysis and ring contraction to end up with 2-benzofuranacetaldehyde **9a** (Scheme 1). Due to instability of the aldehyde **9a** only a small sample of **9a** was purified and most of the resulting product was directly reduced with $NaBH_4$ to provide the stable alcohol **10a** in 37% yield. Treatment of the 2-benzoxepine **8a** with methanol and *p*-toluenesulfonic acid instead of diluted HCl led to the ring contracted dimethyl acetal **14a** in 65% yield, which could be hydrolyzed with diluted HCl to give the aldehyde **9a** (Scheme 3).

The transformation of the 2-benzoxepine **8a** into the ring contracted 2-benzofuran **9a** can be explained by hydrolysis of the acetalic group of **8a** to give an α,β-unsaturated aldehyde and subsequent conjugate addition of the tertiary alcohol to the double bond. The formation of the dimethyl acetal **14a** starts with replacement of the hydroxyethoxy group of **8a** with methanol and acid catalyzed opening of the seven-membered ring to afford an allylic cation, which cyclizes to the 2-benzofuran system with an enol ether in the side chain. Final addition of methanol to the enol ether leads to the dimethyl acetal **14a**.

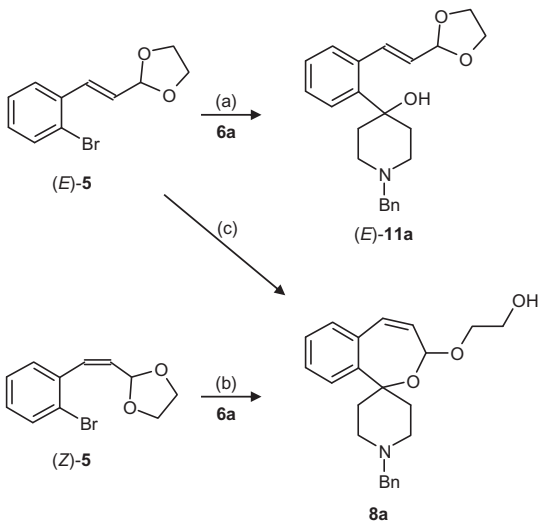
In the last step of the synthesis the alcohol **10a** was treated with diethylamino sulfur trifluoride (DAST)[39] to provide the fluoroethyl derivative **3a**. Generally this transformation gave 50–56% yields, when high quality reagent DAST was used. However DAST with lower quality afforded substantial amounts of the chloroethyl derivative **13a** as side product (ratio **13a**:**3a** up to 1:10) (Scheme 3). We assume that the chloroethyl derivative **13a** originated from small amounts of a chloride source in the reagent. Due to the similar properties of the fluoroethyl and chloroethyl derivatives **3a** and **13a** a preparative HPLC was necessary for the isolation and subsequent identification and characterization of **13a**.

The 4-butylpiperidin-4-ol **16a** represents a potential side product, resulting upon addition of an excess of *n*-BuLi to the ketone **6a**. After hydrolysis of the 2-benzoxepine **8a** the piperidinol **16a** was isolated and characterized in some samples (Scheme 3).

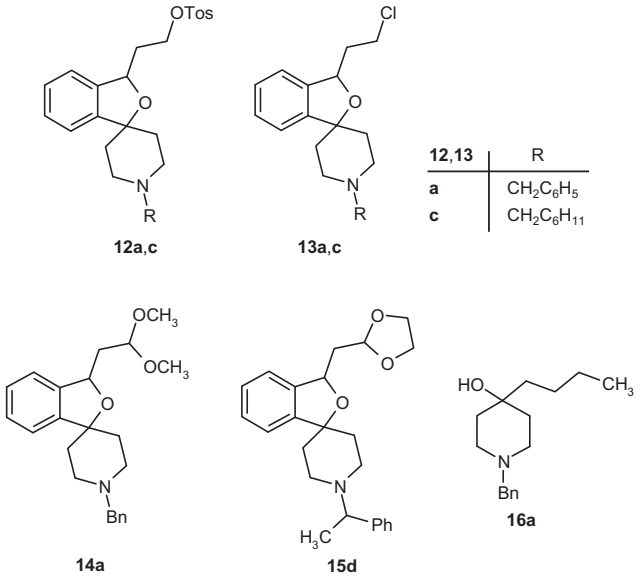
The residue at the N-atom of the spirocyclic piperidines **3** was modified by reacting different piperidones **6b**–**f** with the aryl bromide **5**. The following reaction steps were the same as described for the *N*-benzyl derivatives (**a**-series, see Scheme 1). Compared with the removal of the *N*-benzyl protective group of the final product **3a** and subsequent attachment of various substituents, this linear strategy with differently substituted piperidones **6b**–**f** was shorter by two reaction steps, since hydrogenolysis and N-substitution were omitted. Moreover, various intermediates



Scheme 1. Synthesis of spirocyclic σ_1 receptor ligands with a (2-fluoroethyl) residue in 3-position. Reagents and conditions: (a) [(1,3-dioxolan-2-yl)methyl]triphenylphosphonium bromide [$(CH_2O)_2CHCH_2PPh_3$ Br], K_2CO_3, TDA-1 [tris(methoxyethoxyethyl)amine, CH_2Cl_2, reflux, 6 d, 95%; (b) *n*-BuLi, THF, −78 °C, 10 min, then addition of piperidone **6**, −78 °C, 1 h, rt 16–18 h; (c) HCl, 2 M, THF, rt, 2 h; (d) $NaBH_4$, CH_3CN, 0 °C, 15 min, rt, 16 h; (e) DAST, CH_2Cl_2, −78 °C, 30 min, rt, 18 h; (f) NH_4HCO_2, Pd/C (10%), CH_3OH, reflux, 2 h; (g) 1-bromo-3-methylbut-2-ene, K_2CO_3, CH_3CN, reflux, 16 h.



Scheme 2. Investigation of the formation of 2-benzoxepine **8a** starting with stereoisomerically pure aryl bromides (*E*)-**5** and (*Z*)-**5**. Reagents and conditions: (a) *n*-BuLi, THF, −78 °C, 10 min, then addition of piperidone **6a**, −78 °C, 3.5 h, rt, 3 h, 31%; (b) *n*-BuLi, THF, −78 °C, 10 min, then addition of piperidone **6a**, −78 °C, 3.5 h, rt, 3 h, 51%; (c) *n*-BuLi, THF, −78 °C, 10 min, then addition of piperidone **6a**, −78 °C, 3.5 h, rt, 18 h, mixture of **11a** and **8a**.



Scheme 3. Intermediates and side products formed during the synthesis of (2-fluoroethyl) substituted σ_1 ligands.

with different N-substituents, for example, alcohols **10**, tosylates **12**, were also available according to this strategy and could be included into the structure affinity relationship study. The required N-substituted piperidones **6b**–**f** (Scheme 1) were obtained by alkylation of unsubstituted piperidin-4-one.

The rather labile dimethylallyl residue (**3g**) was introduced into the spirocyclic system by alkylation of the secondary amine **3h** with 1-bromo-3-methylbut-2-ene. A transfer hydrogenolysis of the *N*-benzyl derivative **3a** with ammonium formate and Pd/C[40] provided the secondary amine **3h**.

In order to achieve high diversity various alkyl, arylalkyl and cycloalkylalkyl residues were selected. Additionally, N-substituents of lead compounds with high σ_1 affinity were considered, for example, the dimethylallyl residue of **3g**. The branched 1-phenylethyl residue of **3d** was selected to increase the metabolic stability by inhibition of N-dealkylation during biotransformation.

Hydrolysis of 2-benzoxepine **8d** with diluted HCl led to the aldehyde **9d** and around 10% of the dioxolane **15d**, which was isolated after $NaBH_4$ reduction of the aldehyde **9d**. The formation of the ethylene acetal **15d** followed the same route as the formation of the dimethyl acetal **14a**, but without previous transacetalization with methanol.

Treatment of the cyclohexylmethyl derivative **10c** with a low quality sample of the reagent DAST led to the fluoroethyl derivative **3c** as main product but also to the chloroethyl derivative **13c** as side product (compare **13a**). In order to prove the structure of the chloroethyl derivatives the tosylates **12a** and **12c** were prepared by reaction of the alcohols **10a** and **10c** with *p*-toluenesulfonyl chloride. Additionally, the tosylates **12** represent the precursors for a potential radiosynthesis. Nucleophilic substitution of the tosylate **12c** with KCl in the presence of [18]-crown-6 led to the chloroethyl derivative **13c** in 59% yield (Scheme 3).

3. Receptor affinity

The σ receptor affinities of the spirocyclic compounds were determined in competition experiments with radioligands. In the σ_1 assay homogenates of guinea pig brains were used as receptor material and the σ_1 selective ligand [^{3}H]-(+)-pentazocine was employed as radioligand. 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]-1,3-di(*o*-tolyl)guanidine was employed in the presence of an excess of non-tritiated (+)-pentazocine, which selectively occupies σ_1 receptors.[29,33]

In Table 1 the σ_1 and σ_2 receptor affinities of the spirocyclic piperidines with various substituents in the ethyl side chain and at the N-atom are summarized. The σ_1 receptor affinity of the (2-fluoroethyl) derivative **3a** (WMS-1828, K_i = 0.59 nM), which was termed fluspidine, is about twofold increased compared with the σ_1 affinity of the homologous (3-fluoropropyl) compound **2a** (WMS-1813, K_i = 1.4 nM).[36]

Moreover, the σ_1 affinities of all (2-fluoroethyl) derivatives **3b**–**g** of this study are in the low nanomolar or even subnanomolar range (0.57–3.9 nM). Only **3f** with an unusual long lipophilic octyl substituent at the N-atom shows a reduced σ_1 affinity of K_i = 15 nM. In general the σ_1 affinities of these (2-fluoroethyl) derivatives **3** are higher than the σ_1 affinities of the corresponding (3-fluoropropyl) derivatives **2** with the same N-residue, respectively.[36] Obviously, the reduction of the side chain length by one methylene moiety leads to an increased σ_1 receptor affinity.

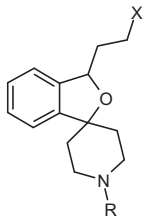
The σ_2 receptor affinities of the (2-fluoroethyl) derivatives **3** are generally rather low indicating high σ_1/σ_2 selectivity. Exemplarily the very potent *N*-benzyl and *N*-(*p*-fluorobenzyl) derivatives **3a** and **3b** show σ_1/σ_2 selectivity of 1331 and 844, respectively. Again the σ_1/σ_2 selectivity of all (2-fluoroethyl) compounds **3** is higher than the σ_1/σ_2 selectivity of the corresponding (3-fluoropropyl) derivatives **2**.[36] The high σ_1 affinity (K_i = 1.0 nM) together with the high σ_1/σ_2 selectivity (>1000) of the 1-phenylethyl derivative **3d** has to be emphasized, since this compound had been designed to inhibit N-dealkylation during the biotransformation by introduction of a branched side chain. However, due to the additional centre of chirality in the N-residue two diastereomeric pairs of enantiomers resulted in the synthesis and were tested.

A relatively high σ_2 receptor affinity (K_i = 57 nM) and thus a low σ_1/σ_2 selectivity of 80 was found for the potent σ_1 ligand **3c** with an *N*-cyclohexylmethyl residue. Similar effects were observed in other compound classes, when a cyclohexylmethyl residue was introduced.[36,41] Generally, compounds with an *N*-cyclohexylmethyl residue appear to have very high σ_1 and σ_2 receptor affinities, but reduced σ_1/σ_2 selectivity.

The lowest σ_1/σ_2 selectivity (factor 8) was observed for the *N*-octyl derivative **3f**. The low selectivity of **3f** is due to the relatively low σ_1 affinity (K_i = 15 nM) accompanied with moderate σ_2 affinity (K_i = 118 nM).

The novel synthetic strategy, using appropriately substituted piperidones, allowed the pharmacological evaluation of the synthetic precursors, too. The σ_1 and σ_2 receptor affinities of the

Table 1
σ_1 and σ_2 receptor affinities of spirocyclic piperidines with substituted ethyl residues in 3-position



Compd	R	X	K_i ± SEM (nM) (n = 3)		σ_1/σ_2 selectivity
			σ_1	σ_2	
3a (WMS-1828, fluspidine)	$CH_2C_6H_5$	F	0.59 ± 0.20	785	1331
10a	$CH_2C_6H_5$	OH	14.2	20%*	>70
12a	$CH_2C_6H_5$	OTos	1.5 ± 0.64	25%*	>685
13a	$CH_2C_6H_5$	Cl	1.6 ± 0.54	0%*	>620
3b	$CH_2C_6H_4$*p*F	F	0.57 ± 0.04	481 ± 63	844
10b	$CH_2C_6H_4$*p*F	OH	6.2 ± 2.5	16%*	>160
3c	$CH_2C_6H_{11}$	F	0.71 ± 0.20	57 ± 14	80
10c	$CH_2C_6H_{11}$	OH	6.8 ± 1.7	29%*	>145
13c	$CH_2C_6H_{11}$	Cl	2.2 ± 0.63	421	190
3d	$CH(CH_3)C_6H_5$	F	1.0 ± 0.27	27%*	>1000
10d	$CH(CH_3)C_6H_5$	OH	27 ± 1.6	18%*	>37
3e	*n*-Butyl	F	3.9 ± 1.0	878	228
10e	*n*-Butyl	OH	32 ± 2.5	29%*	>31
3f	*n*-Octyl	F	15 ± 1.6	118	8.0
10f	*n*-Octyl	OH	22 ± 7.6	82 ± 7.5	3.8
3g	$CH_2CH{=}C(CH_3)_2$	F	1.5 ± 0.82	0%*	>670
15d	$CH(CH_3)C_6H_5$	##	22 ± 3.8	0%*	>45
2a (WMS-1813)[33]	$CH_2C_6H_5$	CH_2F	1.4 ± 0.82	0%*	>670
Haloperidol			6.3 ± 1.6	78 ± 2.3	12
(+)-Pentazocine			5.7 ± 2.2	—	

* Inhibition of the radioligand binding at a concentration of the test compound of 1 μM.
$CH_2X = CH(OCH_2)_2$ (compare Scheme 3).

alcohols **10a**–**f** were investigated systematically. Generally, the σ_1 affinity of the alcohols **10** is 10–30-fold lower than the σ_1 affinity of the corresponding (2-fluoroethyl) derivatives **3**. Nevertheless, the most potent alcohols **10a**–**c** bind with K_i-values of 6–14 nM indicating very high σ_1 affinity. Obviously the σ_1 receptor protein tolerates the bioisosteric replacement of the fluorine atom with the polar OH-moiety, although the affinity is reduced.

In contrast the σ_2 receptor does not accept the polar hydroxy moiety, which is reflected by the negligible competition of the alcohols **10** with the radioligand [^{3}H]di-o-tolylguanidine (s. Table 1). The only exception is **10f** with a σ_2 affinity of 82 nM. It is assumed that the long lipophilic octyl residue is able to compensate the polarity of the hydroxy moiety during interaction with σ_2 receptors.

In addition to the alcohols **10** the tolsylate **12a** (precursor for the radiosynthesis), the (2-chloroethyl) derivatives **13a** and **13c** (side products during fluorination) and the dioxolane **15d** (side product) were considered in the receptor binding studies. Surprisingly, not only the (2-chloroethyl) derivatives **13a** and **13c** but also the tosylate **12a** revealed high σ_1 affinity and σ_1/σ_2 selectivity. The tolerance of the very large tosyloxyethyl group in 3-position of the spirocyclic system had never been expected. Usually aliphatic tosylates and chlorides are not considered as drugs due to their high alkylation potential. Nevertheless, irreversibly binding σ_1 ligands will be exploited for labeling of the binding pocket of σ_1 receptors.

According to its high σ_1 affinity and σ_1/σ_2 selectivity the benzyl derivative **3a** (WMS-1828, fluspidine) represents the most promising compound of this series for the development of a radiotracer by nucleophilic introduction of [^{18}F]fluoride.[42] Therefore the affinities of fluspidine **3a** towards the phencyclidine binding site of the NMDA receptor[43,44] as well as the opioid receptors (μ, κ, δ receptors)[45,46] were investigated. At a concentration of 1 μM **3a** did not compete significantly with the employed radioligands in the NMDA and δ assay. In the μ and κ assay K_i-values of 456 nM and 372 nM were determined, respectively, indicating low affinity and thus very high σ_1 selectivity (σ_1/μ selectivity = 774; σ_1/κ selectivity = 631).

4. Conclusion

A series of spirocyclic piperidines **3** with a (2-fluoroethyl) residue in 3-position and various substituents at the piperidine N-atom was prepared according to a novel strategy. The best yields were obtained, when the reaction sequence from the 2-bromocinnamaldehyde **5** up to the alcohols **10** was performed without purification of intermediates. The (2-fluoroethyl) derivatives **3** show higher σ_1 affinity and σ_1/σ_2 selectivity than the corresponding (3-fluoropropyl) derivatives **2**. With respect to σ_1 affinity and receptor selectivity the *N*-benzyl derivative **3a** (K_i = 0.59 nM, σ_1/σ_2 = 1331), which was termed fluspidine, represents the most promising compound of this series and will be developed as fluorinated radiotracer.[42] The alcohols **10**, which were available by the novel synthetic strategy, interact also with high affinity with σ_1 receptors, albeit they reveal a slightly reduced σ_1 affinity compared with the fluoro bioisosteres **3**. The unexpected high σ_1 affinity and selectivity of the potential alkylation agents **12** (tosylate) and **13** (chlorides) will be exploited for a covalent labeling of the binding pocket of σ_1 receptors.

5. Experimental, chemistry

5.1. 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-400BB 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%): 0–4 min: 90%, 4 min: 90%, 4–29 min: gradient from 90% to 0%, 29–31 min: 0%, 31–31.5 min: gradient from 0% to 90%, 31.5–40 min: 90%. Method 2: column: LiChrospher® 100 RP 18 (5 μm) 25 cm; 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) 25 cm; flow rate: 0.6 mL/min; temperature: rt; detection at λ = 235 nm; solvent: acetonitrile/water 85:15 with 0.1% triethylamine. The purity of all test compounds was greater than 95%, which was determined by one of the given HPLC methods.

5.2. (*E*)/(*Z*)-2-[2-(2-Bromophenyl)vinyl]-1,3-dioxolane ((*E*)-5/(*Z*)-5)[38]

2-Bromobenzaldehyde (**4**, 640 μL, 5.56 mmol) and tris[2-(2-methoxyethoxy)ethyl]amine (TDA-1, 1.950 g, 6.03 mmol) were dissolved in CH_2Cl_2 (70 mL). Then a saturated solution of K_2CO_3 (70 mL) and (1,3-dioxolan-2-ylmethyl)triphenylphosphonium bromide (2.627 g, 5.926 mmol) were added. The mixture was heated to reflux for 6 d. The organic layer was separated, the aqueous layer was extracted with CH_2Cl_2 (4×) and the combined organic layers were dried (Na_2SO_4). The solvent was removed in vacuo and the residue was purified by fc (5 cm, cyclohexane: ethyl acetate 95:5, 30 mL, R_f ((*Z*)-**5**) 0.29, R_f ((*E*)-**5**) 0.22). Pale yellow oil, yield 1.341 g (95%). $C_{11}H_{11}BrO_2$ (255.1). MS (EI): *m/z* = 257/255 [MH], 256/254 [M], 175 [M−Br]. IR: $\bar{\nu}$ (cm^{-1}) = 2883 (C–H), 1682 (C=C), 1055 (C–O), 742 (C–H, 1,2-disub. arom). FC purification of a small sample led to almost complete separation of the diastereomers.

Compound (*Z*)-**5** (R_f = 0.29): ^{1}H NMR ($CDCl_3$): δ (ppm) = 3.87–3.94 (m, 2H, OC_2H_4O), 4.02–4.10 (m, 2H, OC_2H_4O), 5.36 (d, *J* = 7.8 Hz, 1H, CHC*H*(OR)$_2$), 5.79 (dd, *J* = 11.6/7.8 Hz, 1H, CH=C*H*CH(OR)$_2$), 6.88 (d, *J* = 11.6 Hz, 1H, C*H*=CHCH(OR)$_2$), 7.16 (broad t, *J* = 7.7 Hz, 1H, 4-H arom), 7.30 (broad t, *J* = 7.5 Hz, 1H, 5-H arom), 7.47 (dd, *J* = 7.6/1.4 Hz, 1H, 6-H arom), 7.58 (dd, *J* = 8.0/1.0 Hz, 3-H arom).

Compound (*E*)-**5** (R_f = 0.22): ^{1}H NMR ($CDCl_3$): δ (ppm) = 3.95–4.01 (m, 2H, OC_2H_4O), 4.04–4.09 (m, 2H, OC_2H_4O), 5.48 (dd, *J* = 5.9/0.7 Hz, 1H, CHC*H*(OR)$_2$), 6.11 (dd, *J* = 15.9/5.9 Hz, 1H, CH=C*H*CH(OR)$_2$), 7.13 (broad t, *J* = 7.6 Hz, 1H, 4-H arom), 7.14 (d, *J* = 15.9 Hz, 1H, C*H*=CHCH(OR)$_2$), 7.28 (broad t, *J* = 7.6 Hz, 1H, 5-H arom), 7.55 (dd, *J* = 8.0/1.4 Hz, 2H, 6-H, 3-H arom).

5.3. 2-(1′-Benzyl-3*H*-spiro[[2]-benzoxepine-1,4′-piperidin]-3-yl-oxyl)ethanol (8a)

Under N_2 a solution of *n*-BuLi in *n*-hexane (1.6 M, 3.2 mL, 5.12 mmol) was added slowly to a cold (−78 °C) solution of (*E*)-**5**/(*Z*)-**5** (986 mg, 3.86 mmol) in THF (25 mL). After 10 min a solution of 1-benzylpiperidin-4-one (**6a**, 831.6 mg, 4.39 mmol) in

THF (2 mL) was added slowly. The mixture was stirred for 1 h at −78 °C and then overnight at rt. Then H_2O (7 mL) was added, after addition of CH_2Cl_2 the layers were separated and the aqueous layer was extracted with CH_2Cl_2. The organic layer was washed with a solution of $NaHSO_3$ (10%) and a saturated solution of NaCl. The organic layer was dried (Na_2SO_4), the solvent was removed in vacuo and the residue was purified by fc (5 cm, cyclohexane/ethyl acetate 7:3, 30 mL, R_f (cyclohexane:ethyl acetate 5:5) 0.15). Pale yellow oil, yield 0.441 g (67%). $C_{23}H_{27}NO_3$ (365.5). MS (EI): *m/z* = 365 [M], 320 [M−CH_2CH_2OH], 274 [M−CH_2Ph], 91 [$PhCH_2$]. IR: $\bar{\nu}$ (cm^{-1}) = 3440 (O–H), 2936 (C–H), 1664, 1648 (C=C), 1601 (aromat. C=C), 1045 (C–O), 754 (C–H, 1,2-disub. aromat), 698 (C–H, monosub. aromat). ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.74 (d, *J* = 12.9 Hz, 2H, N(CH_2CH_2)$_2$), 1.91 (td, *J* = 12.9/3.8 Hz, 1H, N(CH_2CH_2)$_2$), 2.14 (td, *J* = 13.6/4.6 Hz, 1H, N(CH_2CH_2)$_2$), 2.46–2.54 (m, 2H, N(CH_2CH_2)$_2$), 2.81–2.86 (m, 2H, N(CH_2CH_2)$_2$), 3.59 (s, 2H, NCH_2Ph), 3.81–3.98 (m, 4H, OCH_2-CH_2OH), 4.84 (dd, *J* = 12.6/9.1 Hz, 1H, CH=*CH*CH(OR)$_2$), 5.50 (d, *J* = 9.0 Hz, 1H, CH=CH*CH*(OR)$_2$), 6.72 (d, *J* = 12.6 Hz, 1H, *CH*=CHCH(OR)$_2$), 7.09–7.11 (m, 1H, aromat. H), 7.14–7.17 (m, 1H, aromat. H), 7.23–7.38 (m, 7H, aromat. H). A signal for the OH proton is not seen in the spectrum.

5.4. 2-(1′-Benzyl-3*H*-spiro[[2]benzofuran-1,4′-piperidin]-3-yl)-acetaldehyde (9a)

Method 1: A mixture of dimethyl acetal **14a** (48.6 mg, 0.13 mmol), THF (5 mL) and HCl (2 M, 5 mL) was heated to reflux for 3 h. Then NaOH (2 M) was added and the mixture was extracted with CH_2Cl_2. The organic layer was dried (Na_2SO_4), the solvent was removed in vacuo and the residue was purified by fc (1.5 cm, cyclohexane/ethyl acetate 7:3, R_f (CH_2Cl_2/MeOH 9:1) 0.5). Pale yellow oil, yield 10.6 mg (25%).

Method 2: 2-Benzoxepine **8a** (1.02 g, 2.8 mmol) was dissolved in THF (8 mL) and HCl (2 M, 8 mL) was added. After stirring for 2 h at rt, NaOH (2 M) was added and the aqueous layer was extracted several times with CH_2Cl_2. The organic layer was dried (Na_2SO_4) and the solvent was removed in vacuo to obtain a colorless oil (828.4 mg). A small part of the residue (29.6 mg) was purified by fc (0.5 cm, cyclohexane/ethyl acetate 7:3, R_f (CH_2Cl_2/MeOH 9:1) 0.5). Pale yellow oil, yield 17.0 mg (37%). $C_{21}H_{23}NO_2$ (321.4). MS (EI): *m/z* = 321 [M], 292 [M−CHO], 230 [M−CH_2Ph], 91 [$PhCH_2$]. IR: $\bar{\nu}$ (cm^{-1}) = 2916 (C–H), 1723 (C=O), 1603 (aromat. C=C), 1047 (C–O), 756 (C–H, 1,2-disub. aromat), 740, 698 (C–H, monosub. aromat). ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.66–1.76 (m, 2H, N(CH_2CH_2)$_2$), 1.91 (td, *J* = 12.9/4.5 Hz, 1H, N(CH_2CH_2)$_2$), 2.12 (td, *J* = 13.1/4.5 Hz, 1H, N(CH_2CH_2)$_2$), 2.44 (td, *J* = 11.8/2.5 Hz, 2H, N(CH_2CH_2)$_2$), 2.80–2.85 (m, 2H, N(CH_2CH_2)$_2$), 2.81 (ddd, *J* = 16.3/7.1/2.1 Hz, 1H, CHCH_2CH=O), 2.92 (ddd, *J* = 16.3/4.7/2.1 Hz, 1H, CHCH_2CH=O), 3.58 (s, 2H, NCH_2Ph), 5.67 (dd, *J* = 6.9/4.8 Hz, 1H, ArC*H*O), 7.14–7.17 (m, 2H, aromat. H), 7.28–7.38 (m, 7H, aromat. H), 9.84 (t, *J* = 2.3 Hz, 1H, C*H*=O). Purity (HPLC, method 1): 97.8%, t_R = 15.22 min.

5.5. 2-(1′-Benzyl-3*H*-spiro[[2]benzofuran-1,4′-piperidin]-3-yl)-ethan-1-ol (10a, WMS-1826)

A mixture of 2-benzoxepine **8a** (948 mg, 2.58 mmol), THF (9 mL) and HCl (1 M, 8 mL) was stirred at rt for 1.75 h. Then NaOH (2 M) was added and the mixture was extracted with CH_2Cl_2. The organic layer was dried (Na_2SO_4), the solvent was removed in vacuo, the residue (**9a**) was dissolved in CH_3CN (15 mL) and $NaBH_4$ (192.0 mg, 5.08 mmol) was added under ice cooling. The mixture was stirred at rt overnight. Excess of $NaBH_4$ was destroyed by addition of HCl (1 M). After 20 min NaOH (2 M) was added (pH 9–10) and the product was extracted with CH_2Cl_2 (4 × 4 mL). The organic layer was concentrated in vacuo and the residue was purified by fc (4 cm, cyclohexane/ethyl acetate 7:3, 20 mL; 3 cm, cyclohexane/ethyl acetate 8:2, 10 mL, R_f (cyclohexane/ethyl acetate 5:5) 0.20). Pale yellow oil, yield 311.7 mg (37%). $C_{21}H_{25}NO_2$ (323.4). MS (EI): *m/z* = 323 [M], 246 [M−Ph], 232 [M−CH_2Ph], 91 [CH_2Ph]. IR: $\bar{\nu}$ (cm^{-1}) = 3410 (w, O–H), 2940 (C–H), 1045 (C–O), 754 (C–H, 1,2-disub. aromat), 740, 698 (C–H, monosub. aromat). ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.74 (ddd, *J* = 12.9/5.2/2.6 Hz, 2H, N(CH_2CH_2)$_2$), 1.86–1.96 (m, 2H, N(CH_2CH_2)$_2$ (1), CH_2CH_2OH (1)), 2.09–2.23 (m, 2H, N(CH_2CH_2)$_2$ (1), CH_2CH_2OH (1)), 2.36 (td, *J* = 11.7/2.4 Hz, 1H, N(CH_2CH_2)$_2$), 2.41 (td, *J* = 11.7/2.4 Hz, 1H, N(CH_2CH_2)$_2$), 2.79–2.89 (m, 2H, N(CH_2CH_2)$_2$), 3.57 (s, 2H, NCH_2Ph), 3.84 (ddd, *J* = 10.4/6.1/3.6 Hz, 1H, CH_2CH_2OH), 3.93 (ddd, *J* = 10.4/6.1/3.6 Hz, 1H, CH_2CH_2OH), 5.40 (dd, *J* = 8.7/3.0 Hz, 1H, ArC*H*O), 7.08–7.17 (m, 2H, aromat. H), 7.24–7.37 (m, 7H, aromat. H). A signal for the OH-proton is not seen in the spectrum. Purity (HPLC, method 1): 98.6%, t_R = 14.61 min.

5.6. 1′-Benzyl-3-(2-fluoroethyl)-3*H*-spiro[[2]benzofuran-1,4′-piperidine] (3a, WMS-1828, fluspidine)

Under N_2 diethylaminosulfur trifluoride (DAST, 0.21 mL, 1.62 mmol) was dissolved in CH_2Cl_2 (23 mL) at −78 °C. After 5 min a solution of the alcohol **10a** (255.7 mg, 0.79 mmol) in CH_2Cl_2 was added slowly. The mixture was stirred for 30 min at −78 °C and for 18 h at rt. Then NaOH (2 M) was added under ice cooling, the aqueous layer was separated and extracted with CH_2Cl_2 (4 × 4–6 mL). The combined organic layers were dried (Na_2SO_4), concentrated in vacuo and the residue was purified by fc (3 cm, cyclohexane/ethyl acetate 8:2, 20 mL, R_f (cyclohexane/ethyl acetate 5:5) 0.55). Pale yellow oil, yield 144.7 mg (56%). $C_{21}H_{24}FNO$ (325.4). MS (EI): *m/z* = 325 [M], 306 [M−F], 234 [M−CH_2Ph], 91 [$PhCH_2$]. IR: $\bar{\nu}$ (cm^{-1}) = 2941 (C–H), 1603 (aromat. C=C), 1045 (C–O), 756 (C–H, 1,2-disub. aromat), 738, 698 (C–H, monosub. aromat). ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.65 (ddd, *J* = 13.5/5.5/2.7 Hz, 1H, N(CH_2CH_2)$_2$), 1.70 (ddd, *J* = 13.6/5.6/2.8 Hz, 1H, N(CH_2CH_2)$_2$), 1.87–2.03 (m, 2H, N(CH_2CH_2)$_2$ (1), CH_2CH_2F (1)), 2.09 (td, *J* = 13.0/4.5 Hz, 1H, N(CH_2CH_2)$_2$), 2.27–2.32 (m, 1H, CH_2CH_2F), 2.45 (td, *J* = 12.1/4.6 Hz, 2H, N(CH_2CH_2)$_2$), 2.79–2.85 (m, 2H, N(CH_2CH_2)$_2$), 3.58 (s, 2H, NCH_2Ph), 4.63 (dddd, *J* = 46.9/9.1/6.5/4.7 Hz, 1H, CH_2CH_2F), 4.75 (dddd, *J* = 46.9/9.1/6.5/4.7 Hz, 1H, CH_2CH_2F), 5.32 (dd, *J* = 8.5/3.2 Hz, 1H, ArC*H*O), 7.11–7.17 (m, 2H, aromat. H), 7.25–7.38 (m, 7H, aromat. H). ^{13}C NMR ($CDCl_3$): δ (ppm) = 37.4 (1C, N(CH_2CH_2)$_2$), 38.4 (d, *J* = 18.8 Hz, 1C, CH_2CH_2F), 38.8 (1C, N(CH_2CH_2)$_2$), 50.2 (1C, N(CH_2CH_2)$_2$), 50.5 (1C, N(CH_2CH_2)$_2$), 63.7 (1C, NCH_2Ph), 77.7 (d, *J* = 6.8 Hz, 1C, ArCHO), 81.5 (d, *J* = 163.9 Hz, 1C, CH_2F), 84.3 (1C, ArCO), 121.2 (1C, aromat. CH), 121.4 (1C, aromat. CH), 127.2 (1C, aromat. CH), 128.0 (1C, aromat. CH), 128.0 (1C, aromat. CH), 128.4 (2C, aromat. CH), 129.6 (2C, aromat. CH), 138.8 (1C, aromat. C), 141.6 (1C, aromat. C), 146.2 (1C, aromat. C). Purity (HPLC, method 2): 97.9%, t_R = 14.95 min. Elemental Anal. Calcd. C, 77.51; H, 7.43; N, 4.30. Found: C, 77.34; H, 7.55; N, 4.32.

5.7. (*E*)-1-Benzyl-4-{2-[2-(1,3-dioxolan-2yl)vinyl]phenyl}piperidin-4-ol ((*E*)-11a)

Under N_2 a solution of *n*-BuLi (1.6 M in *n*-hexane, 0.7 mL, 1.12 mmol) was added slowly to a cold (−78 °C) solution of (*E*)-**5** (206.8 mg, 0.81 mmol) in THF (8 mL). After 5 min a solution of 1-benzylpiperidin-4-one (**6a**. 173.8 mg, 0.92 mmol) in THF (1 mL) was added slowly. After stirring the mixture for 3.5 h at −78 °C and for 3 h at rt, the reaction was stopped by addition of H_2O. Then CH_2Cl_2 was added, the layers were separated and the organic layer was extracted with a solution of $NaHSO_3$ (10%) and a saturated solution of NaCl. The aqueous layer was extracted with CH_2Cl_2 (3×), the combined organic layers were dried (Na_2SO_4), concentrated in vacuo and the residue was purified by fc (2 cm, cyclohexane/ethyl acetate 4:6, 10 mL, R_f (cyclohexane/ethyl acetate 5:5)

0.14). Pale yellow oil, yield 93.1 mg (31%). $C_{23}H_{27}NO_3$ (365.5). MS (EI): *m/z* = 365 [M], 348 [M−OH], 321 [M−CH_2CH_2O], 274 [M−CH_2Ph], 91 [$PhCH_2$]. IR: $\bar{\nu}$ (cm^{-1}) = 2937 (C–H), 1650 (C=C), 1601 (aromat. C=C), 1040 (C–O), 743 (C–H, 1,2-disub. aromat), 699 (C–H, monosub. aromat). ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.95 (dd, *J* = 14.0/2.4 Hz, 2H, N(CH_2CH_2)$_2$), 2.17 (td, *J* = 12.9/4.4 Hz, 2H, N(CH_2CH_2)$_2$), 2.51 (td, *J* = 11.8/2.2 Hz, 2H, N(CH_2CH_2)$_2$), 2.77 (broad d, *J* = 11.3 Hz, 2H, N(CH_2CH_2)$_2$), 3.57 (s, 2H, NCH_2Ph), 3.92–4.07 (m, 4H, OC_2H_4O), 5.44 (dd, *J* = 6.3/0.5 Hz, 1H, CHCH(OR)$_2$), 5.88 (dd, *J* = 15.9/6.3 Hz, 1H, CH=CHCH(OR)$_2$), 7.22–7.36 (m, 7H, aromat. H), 7.38–7.41 (m, 1H, aromat. H), 7.47–7.50 (m, 1H, aromat. H), 7.48 (d, *J* = 15.9 Hz, 1H, CH=CHCH(OR)$_2$); a signal for the OH-proton is not seen in the spectrum.

5.8. [2-(1′-Benzyl-3H-spiro[[2]benzofuran-1,4′-piperidin]-3-yl)-ethyl] *p*-Toluenesulfonate (12a, WMS-1835)

Under N_2 a solution of **10a** (103.4 mg, 0.32 mmol), 4-(dimethylamino)pyridine (DMAP, 13.1 mg, 0.11 mmol) and triethylamine (0.22 mL, 1.60 mmol) in CH_2Cl_2 (15 mL) was cooled down to −25 °C. A solution of *p*-toluenesulfonyl chloride (136.9 mg, 0.72 mmol) in CH_2Cl_2 (ca. 2 mL) was added and the mixture was stirred for 1.5 h at −25 °C and for 13.5 h at rt. Then NaOH (1 M) was added, the aqueous layer was separated and extracted with CH_2Cl_2 (4×). The organic layer was dried (Na_2SO_4), concentrated in vacuo and the residue was purified by fc (0.7 cm, cyclohexane/ethyl acetate 7:3, 5 mL, R_f (cyclohexane/ethyl acetate 5:5) 0.44). Colorless oil, yield 95.6 mg (63%). $C_{28}H_{31}NO_4S$ (477.6). MS (EI): *m/z* = 477 [M], 386 [M−CH_2Ph], 345 [M−(C_2H_3)NCH_2Ph], 331 [M−(C_3H_6)NCH_2Ph], 322 [M−SO_2PhCH_3], 91 [$PhCH_2$]. IR: $\bar{\nu}$ (cm^{-1}) = 2922 (C–H), 1597 (aromat. C=C), 1361, 1175 (O_2S=O), 813 (C–H, 1,4-disub. aromat), 756 (C–H, 1,2-disub. aromat), 740, 698 (C–H, monosub. aromat). ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.52 (ddd, *J* = 13.5/5.2/2.6 Hz, 1H, N(CH_2CH_2)$_2$), 1.63 (ddd, *J* = 13.2/5.1/2.4 Hz, 1H, N(CH_2CH_2)$_2$), 1.81–1.94 (m, 2H, N(CH_2CH_2)$_2$ (1), CH_2CH_2OTos (1H)), 2.02 (td, *J* = 13.0/4.4 Hz, 1H, N(CH_2CH_2)$_2$), 2.23–2.36 (m, 3H, N(CH_2CH_2)$_2$ (2), CH_2CH_2OTos (1)), 2.44 (s, 3H, ArCH_3), 2.71–2.81 (m, 2H, N(CH_2CH_2)$_2$), 3.53 (d, *J* = 13.1 Hz, 1H, NCH_2Ph), 3.56 (d, *J* = 13.1 Hz, 1H, NCH_2Ph), 4.22 (ddd, *J* = 9.8/7.3/4.9 Hz, 1H, CH_2CH_2OTos), 4.30 (ddd, *J* = 9.8/7.9/6.6 Hz, 1H, CH_2CH_2OTos), 5.19 (dd, *J* = 8.4/3.2 Hz, 1H, ArCHO), 7.05–7.11 (m, 2H, aromat. H), 7.23–7.38 (m, 9H, aromat. H), 7.78 (broad d, *J* = 8.3 Hz, 2H, aromat. H). Purity (HPLC, method 1): 99.0%, t_R = 17.57 min.

5.9. 1′-Benzyl-3-(2-chloroethyl)-3*H*-spiro[[2]benzofuran-1,4′-piperidine] (13a, WMS-1831)

As described above the alcohol **10a** (207 mg, 0.64 mmol) was treated with an old, low quality sample of DAST (0.17 mL, 1.4 mmol) and the product was worked-up and purified as described above. After fc purification the product contained in addition to the (2-fluoroethyl) derivative **3a** the (2-chloroethyl) compound **13a** (**3a**:**13a** = 90:10 according to HPLC analysis). **13a** was isolated by preparative HPLC: Merck Hitachi 7000 series; UV detector L-7400; autosampler L-7200; pump L-7150; interface D-7000; data analysis HSM D-7000 HPLC System Manager Version 4.1; mobile phase MeOH with 0.1% trifluoroacetic acid/H_2O with 0.1% trifluoroacetic acid 60: 40; flow rate 21.2 mL/min; column Agilent® Präp C18 (10 μm, 21.2 × 250 mm); temp. rt; detection 254 nm; t_R = 5.06 min (**3a**), t_R = 9.52 min (**13a**).

Compound **3a:** Colorless oil, yield 61.5 mg (30%).

Compound **13a:** Colorless oil, yield 5.4 mg (2.5%). $C_{21}H_{24}ClNO_2$ (341.9). MS (EI): *m/z* = 343/341 [M], 306 [M−Cl], 252/250 [M−CH_2Ph], 91 [$PhCH_2$]. IR: $\bar{\nu}$ (cm^{-1}) = 2938 (C–H), 1048 (C–O), 756 (C–H, 1,2-disub. aromat), 733, 698 (C–H, monosub. aromat). ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.64 (ddd, *J* = 13.4/5.1/2.5 Hz, 1H, N(CH_2CH_2)$_2$), 1.70 (ddd, *J* = 13.8/5.4/2.7 Hz, 1H, N(CH_2CH_2)$_2$), 1.91 (td, *J* = 12.7/4.2 Hz, 1H, N(CH_2CH_2)$_2$), 2.02–2.13 (m, 2H, N(CH_2CH_2)$_2$ (1), CH_2CH_2Cl (1)), 2.33 (dtd, *J* = 14.1/8.0/3.3 Hz, 1H, CH_2CH_2Cl), 2.45 (broad t, *J* = 11.4 Hz, 2H, N(CH_2CH_2)$_2$), 2.81–2.86 (m, 2H, N(CH_2CH_2)$_2$), 3.58 (s, 2H, NCH_2Ph), 3.66 (ddd, *J* = 10.7/8.2/4.7 Hz, 1H, CH_2CH_2Cl), 3.79 (dt, *J* = 10.6/7.7 Hz, 1H, CH_2CH_2Cl), 5.33 (dd, *J* = 8.4/3.2 Hz, 1H, ArCHO), 7.12–7.16 (m, 2H, aromat. H), 7.24–7.38 (m, 7H, aromat. H). Purity (HPLC, method 1): 1: 94.8%, t_R = 19.73 min.

5.10. 2-(1′-Benzyl-3*H*-spiro[[2]benzofuran-1,4′-piperidin]-3-yl)acetaldehyd dimethyl acetal (14a)

A mixture of 2-benzoxepine **8a** (157.7 mg, 0.43 mmol), methanol (10 mL) and *p*-toluenesulfonic acid (159 mg, 0.83 mmol) was stirred at rt for 2 d. NaOH (2 M) was added (pH 10) and the mixture was extracted with CH_2Cl_2 (4×). The organic layer was dried (Na_2SO_4), the solvent was removed in vacuo and the residue was purified by fc (1 cm, cyclohexane/ethyl acetate 8:2, 5 mL, R_f (cyclohexane/ethyl acetate 5:5) 0.44). Pale yellow oil, yield 104 mg (65%). $C_{23}H_{29}NO_3$ (367. 5). MS (EI): *m/z* = 367 [M], 91 [$PhCH_2$]. IR: $\bar{\nu}$ (cm^{-1}) = 2923 (C–H), 1603 (aromat. C=C), 1047 (C–O), 755 (C–H, 1,2-disub. aromat), 738, 698 (C–H, monosub. aromat). ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.66–1.73 (m, 2H, N(CH_2CH_2)$_2$), 1.84 (ddd, *J* = 13.9/9.7/3.3 Hz, 1H, CHCH_2CH(OR)$_2$) 1.91 (td, *J* = 13.3/4.5 Hz, 1H, N(CH_2CH_2)$_2$), 2.09 (td, *J* = 13.1/4.5 Hz, 1H, N(CH_2CH_2)$_2$), 2.16 (ddd, *J* = 13.9/8.2/3.2 Hz, 1H, CHCH_2CH(OR)$_2$), 2.45 (td, *J* = 12.3/2.7 Hz, 1H, N(CH_2CH_2)$_2$), 2.47 (td, *J* = 12.3/2.7 Hz, 1H, N(CH_2CH_2)$_2$), 2.77–2.86 (m, 2H, N(CH_2CH_2)$_2$), 3.31 (s, 3H, OCH_3), 3.47 (s, 3H, OCH_3), 3.58 (s, 2H, NCH_2Ph), 4.78 (dd, *J* = 8.2/3.3 Hz, 1H, CH_2CH(OR)$_2$), 5.25 (dd, *J* = 9.7/3.1 Hz, 1H, ArCHO), 7.12–7.16 (m, 2H, aromat. H), 7.24–7.39 (m, 7H, aromat. H). Purity (HPLC, method 1): 98.5%, t_R = 17.52 min.

5.11. 1-Benzyl-4-butylpiperidin-4-ol (16a)

The side product, which had been formed during the synthesis of the 2-benzoxepine **8a**, was separated after transformation of the 2-benzoxepine **8a** into the aldehyde **9a**. The structure of **16a** has been determined by ^{1}H NMR spectroscopy. $C_{16}H_{25}NO$ (247.4). ^{1}H NMR ($CDCl_3$): δ (ppm) = 0.90 (t, *J* = 7.0 Hz, 3H, $C_3H_6CH_3$), 1.25–1.36 (m, 4H, $CH_2C_2H_4CH_3$), 1.42–1.47 (m, 2H, $CH_2(C_2H_2)_2CH_3$), 1.54 (d, *J* = 12.4 Hz, 2H, N(CH_2CH_2)$_2$), 1.66 (td, *J* = 12.5/4.3 Hz, 2H, N(CH_2CH_2)$_2$), 2.32 (td, *J* = 11.5/2.5 Hz, 2H, N(CH_2CH2)$_2$), 2.62 (broad d, *J* = 11.6 Hz, 2H, N(CH_2CH_2)$_2$), 3.52 (s, 2H, NCH_2Ph), 7.22–7.35 (m, 5H, aromat. H). A signal for the OH-proton is not seen in the spectrum.

5.12. 2-[1′-(4-Fluorobenzyl)-3*H*-spiro[[2]benzoxepine-1,4′-piperidin]-3-yloxyl]ethanol (8b)

Under N_2 a solution of *n*-BuLi in *n*-hexane (1.6 M, 1.6 mL, 2.56 mmol) was added slowly at −78 °C to a solution of bromobenzene **5** (528 mg, 2.07 mmol) in THF (10 mL). After 10 min a solution of 1-(4-fluorobenzyl)piperidin-4-one (**6b**, 450 mg, 2.17 mmol) in THF (4 mL) was added slowly and the mixture was stirred at −78 °C for 1 h and at rt overnight. Then H_2O was added, after addition of CH_2Cl_2 the layers were separated and the aqueous layer was extracted with CH_2Cl_2. The organic layer was washed with a solution of $NaHSO_3$ (10%) and a saturated solution of NaCl, dried (Na_2SO_4), the solvent was removed in vacuo and the residue was purified by fc (4 cm, cyclohexane/ethyl acetate 5:5, 30 mL, R_f 0.10). Pale yellow oil, yield 466.2 mg (62%). $C_{23}H_{26}FNO_3$ (383.5). MS (EI): *m/z* = 383 [M], 338 [M−CH_2CH_2OH], 274 [M−CH_2PhF], 109 [CH_2PhF]. IR: $\bar{\nu}$ (cm^{-1}) = 3440 (w, O–H), 2925 (C–H), 1663,

1649 (C=C), 1603 (aromat. C=C), 1046 (C–O), 832 (C–H, 1,4-disub. aromat), 754 (C–H, 1,2-disub. aromat). ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.73 (d, J = 12.9 Hz, 2H, N(CH_2CH_2)$_2$), 1.88 (td, J = 12.6/3.6 Hz, 1H, N(CH_2CH_2)$_2$), 2.11 (td, J = 12.8/4.2 Hz, 1H, N(CH_2CH_2)$_2$), 2.44–2.51 (m, 2H, N(CH_2CH_2)$_2$), 2.76–2.85 (m, 2H, N(CH_2CH_2)$_2$), 3.54 (s, 2H, NCH_2Ph), 3.80–3.88 (m, 4H, OCH_2CH_2OH), 4.85 (dd, J = 12.6/9.1 Hz, 1H, CH=C*H*CH(OR)$_2$), 5.49 (d, J = 9.1 Hz, 1H, CH=CHC*H*(OR)$_2$), 6.72 (d, J = 12.7 Hz, 1H, C*H*=CHCH(OR)$_2$), 7.01 (t, J = 8.7 Hz, 2H, aromat. H), 7.09–7.11 (m, 1H, aromat. H), 7.13–7.15 (m, 1H, aromat. H), 7.25–7.33 (m, 4H, aromat. H). A signal for the OH-proton is not seen in the spectrum.

5.13. 2-[1′-(4-Fluorobenzyl)-3*H*-spiro[[2]benzofuran-1,4′-piperidin]-3yl]ethanol (10b, WMS-1825)

A mixture of 2-benzoxepine **8b** (466 mg, 1.21 mmol) in THF (7 mL) and HCl (1 M, 1 mL) was stirred for 1.75 h at rt. NaOH (2 M) was added and the mixture was extracted with CH_2Cl_2 (4×). The organic layer was dried (Na_2SO_4) and the solvent was removed in vacuo. The residue containing the aldehyde **9b** was dissolved in CH_3CN (10 mL) and $NaBH_4$ (91.8 mg, 2.43 mmol) was added under cooling with ice. The mixture was stirred at rt overnight. Then excess of $NaBH_4$ was destroyed with HCl (1 M). After 20 min NaOH (2 M) was added (pH 9–10) and the aqueous layer was extracted with CH_2Cl_2 (4 × 4 mL). The solvent was evaporated in vacuo and the residue was purified by fc (first fc: 3 cm, cyclohexane/ethyl acetate 5:5, 20 mL; second fc: 1.5 cm, cyclohexane/ethyl acetate 7:3, 10 mL, R_f (cyclohexane/ethyl acetate 5:5) 0.18). Pale yellow oil, yield 149 mg (36%). $C_{21}H_{24}FNO_2$ (341.4). MS (EI): m/z = 341 [M], 322 [M−F], 232 [M−CH_2PhF], 109 [CH_2PhF]. IR: $\bar{\nu}$ (cm^{-1}) = 3410 (w, O–H), 2921 (C–H), 1602 (aromat. C=C), 1046 (C–O), 829 (C–H, 1,4-disub. aromat), 754 (C–H, 1,2-disub. aromat). ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.69–1.78 (m, 2H, N(CH_2CH_2)$_2$), 1.84–1.95 (m, 2H, N(CH_2CH_2)$_2$ (1), CH_2CH_2OH (1)), 2.12–2.22 (m, 2H, N(CH_2CH_2)$_2$ (1), CH_2CH_2OH (1)), 2.31–2.41 (m, 2H, N(CH_2CH_2)$_2$), 2.78–2.85 (m, 2H, N(CH_2CH_2)$_2$), 3.52 (s, 2H, NCH_2Ph), 3.84 (ddd, J = 11.0/5.6/3.8 Hz, 1H, CH_2CH_2OH), 3.94 (ddd, J = 11.0/8.4/3.1 Hz, 1H, CH_2CH_2OH), 5.40 (dd, J = 8.8/3.0 Hz, 1H, ArC*H*O), 7.00 (t, J = 7.7 Hz, 2H, aromat. H), 7.09–7.12 (m, 2H, aromat. H), 7.27−7.34 (m, 4H, aromat. H). A signal for the OH-proton is not seen in the spectrum. Purity (HPLC, method 1): 95.0%, t_R = 15.46 min.

5.14. 2-[1′-(4-Fluorobenzyl)-3*H*-spiro[[2]benzofuran-1,4′-piperidin]-3-yl]acetaldehyde (9b)

To identify and characterize the aldehyde **9b** a small sample of the above mentioned intermediate was purified by fc (3 cm, cyclohexane/ethyl acetate 8:2, 20 mL, R_f (CH_2Cl_2/MeOH 9:1) 0.68). Colorless solid, mp 109–112 °C. $C_{21}H_{22}FNO_2$ (339.4). MS (EI): m/z = 339 [M], 296 [M−C_2H_3O], 230 [M−CH_2PhF], 109 [CH_2PhF]. IR: $\bar{\nu}$ (cm^{-1}) = 2920 (C–H), 1727 (C=O), 1601 (aromat. C=C), 1215 (C–F), 1050 (C-O), 754 (C–H, 1,2-disub. aromat). ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.65–1.75 (m, 2H, N(CH_2CH_2)$_2$), 1.89 (td, J = 13.1/4.0 Hz, 1H, N(CH_2CH_2)$_2$), 2.10 (td, J = 13.2/3.9 Hz, 1H, N(CH_2CH_2)$_2$), 2.41 (broad t, J = 11.8 Hz, 2H, N(CH_2CH_2)$_2$), 2.77–2.82 (m, 2H, N(CH_2CH_2)$_2$), 2.80 (ddd, J = 16.3/7.1/2.5 Hz, 1H, ArCHCH_2CH=O), 2.91 (ddd, J = 16.3/4.6/2.1 Hz, 1H, ArCHCH_2CH=O), 3.53 (s, 2H, NCH_2Ph), 5.67 (dd, J = 7.0/4.7 Hz, 1H, ArC*H*O), 7.01 (t, J = 8.7 Hz, 2H, aromat. H), 7.14–7.17 (m, 2H, aromat. H), 7.28–7.38 (m, 4H, aromat. H), 9.83 (t, J = 2.3 Hz, 1H, C*H*=O).

5.15. 1′-(4-Fluorobenzyl)-3-(2-fluoroethyl)-3*H*-spiro[[2]benzofuran-1,4′-piperidine] (3b, WMS-1829)

Under N_2 diethylaminosulfur trifluoride (DAST, 0.055 mL, 0.45 mmol) and 5 min later a solution of alcohol **10b** (65.1 mg, 0.19 mmol) in CH_2Cl_2 were added to a cold (−78 °C) CH_2Cl_2 (6 mL). The mixture was stirred for 30 min at −78 °C and at rt for 8.5 h. Then NaOH (2 M) was carefully added, the layers were separated and the aqueous layer was extracted with CH_2Cl_2 (4×). The organic layer was dried (Na_2SO_4), concentrated in vacuo and the residue was purified by fc (0.5 cm, cyclohexane/ethyl acetate 7:3, 10 mL, R_f (cyclohexane/ethyl acetate 5:5) 0.50). Colorless solid, mp 96 °C, yield 23.0 mg (35%). $C_{21}H_{23}F_2NO$ (343.4). MS (EI): m/z = 343 [M], 324 [M−F], 234 [M−CH_2PhF], 109 [CH_2PhF]. IR: $\bar{\nu}$ (cm^{-1}) = 2941 (C–H), 1603 (aromat. C=C), 1046 (C–O), 830 (C–H, 1,4-disub. aromat), 755 (C–H, 1,2-disub. aromat). ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.66 (ddd, J = 13.4/5.1/2.6 Hz, 1H, N(CH_2CH_2)$_2$), 1.70 (ddd, J = 13.4/5.3/2.5 Hz, 1H, N(CH_2CH_2)$_2$), 1.85–2.03 (m, 2H, N(CH_2CH_2)$_2$ (1), CH_2CH_2F (1)), 2.08 (td, J = 13.0/4.3 Hz, 1H, N(CH_2CH_2)$_2$), 2.28–2.39 (m, 1H, CH_2CH_2F), 2.44 (broad t, J = 12.1 Hz, 2H, N(CH_2CH_2)$_2$), 2.75–2.83 (m, 2H, N(CH_2CH_2)$_2$), 3.54 (s, 2H, NCH_2Ph), 4.55–4.84 (m, 2H, CH_2CH_2F), 5.32 (dd, J = 8.4/3.8 Hz, 1H, ArC*H*O), 7.01 (t, J = 8.8 Hz, 2H, aromat. H), 7.13–7.17 (m, 2H, aromat. H), 7.26–7.35 (m, 4H, aromat. H). Purity (HPLC, method 1): 98.0%, t_R = 18.46 min.

5.16. 1-(Cyclohexylmethyl)piperidin-4-one (6c)

1-(Bromomethyl)cyclohexane (2.09 g, 11.8 mmol) and K_2CO_3 (6.65 g, 48.1 mmol) were added to a solution of piperidin-4-one·1H_2O·1HCl (1.47 g, 9.57 mmol) in a mixture of CH_3CN/H_2O (9:1, 40 mL). The mixture was heated to reflux for 19 h. Subsequently the product was extracted with CH_2Cl_2. The organic layer was dried (Na_2SO_4), concentrated in vacuo and the residue was purified by fc (6 cm, cyclohexane/ethyl acetate 7:3, 30 mL, R_f (CH_2Cl_2:MeOH 9:1) 0.76). Pale yellow oil, yield 0.881 g (47%). $C_{12}H_{21}NO$ (195.3). MS (EI): m/z = 196 [MH], 112 [M−C_6H_{11}]. IR: $\bar{\nu}$ (cm^{-1}) = 2920 (C–H), 1718 (C=O). ^{1}H NMR ($CDCl_3$): δ (ppm) = 0.85–0.93 (m, 2H, C_6H_{11}), 1.12–1.29 (m, 3H, C_6H_{11}), 1.42–1.52 (m, 1H, C_6H_{11}), 1.64–1.52 (m, 3H, C_6H_{11}), 1.76–1.83 (m, 2H, C_6H_{11}), 2.22 (d, J = 7.2 Hz, 2H, N$CH_2C_6H_{11}$), 2.43 (t, J = 6.2 Hz, 4H, N(CH_2CH_2)$_2$), 2.68 (t, J = 6.2 Hz, 4H, N(CH_2CH_2)$_2$).

5.17. 2-[1′-(Cyclohexylmethyl)-3*H*-spiro[[2]-benzoxepine-1,4′-piperidin]-3-yl-oxyl]ethanol (8c)

Under N_2 a solution of *n*-BuLi in *n*-hexane (1.6 M, 2.3 mL, 3.68 mmol) was added slowly at −78 °C to a solution of bromobenzene **5** (601 mg, 2.35 mmol) in THF (15 mL). After stirring at −78 °C for 10 min, a solution of piperidone **6c** (606 mg, 3.10 mmol) in THF (5 mL) was added slowly. The mixture was stirred at −78 °C for 1 h and at rt overnight. The reaction was terminated by addition of H_2O. The mixture was extracted with CH_2Cl_2, the organic layer was washed with a solution of $NaHSO_3$ (10%) and a saturated solution of NaCl, dried (Na_2SO_4), concentrated in vacuo and the residue was purified by fc (5 cm, cyclohexane/ethyl acetate 7:3, 20 mL, R_f (cyclohexane/ethyl acetate 5:5) 0.10). Pale yellow oil, yield 787 mg (90%). $C_{23}H_{33}NO_3$ (371.5). MS (EI): m/z = 371 [M], 326 [M−CH_2CH_2OH], 288 [M−C_6H_{11}]. IR: $\bar{\nu}$ (cm^{-1}) = 3400 (w, O–H), 2920 (C–H), 1667, 1650 (C=C), 1046 (C–O), 755 (C–H, 1,2-disub. aromat). ^{1}H NMR ($CDCl_3$): δ (ppm) = 0.85–0.95 (m, 2H, C_6H_{11}), 1.15–1.35 (m, 3H, C_6H_{11}), 1.48–1.82 (m, 8H, N(CH_2CH_2)$_2$ (2), C_6H_{11} (6)), 1.91 (td, J = 12.9/3.6 Hz, 1H, N(CH_2CH_2)$_2$), 2.15 (td, J = 12.7/4.5 Hz, 1H, N(CH_2CH_2)$_2$), 2.22 (d, J = 7.0 Hz, 2H, $CH_2C_6H_{11}$), 2.35–2.44 (m, 2H, N(CH_2CH_2)$_2$), 2.76–2.84 (m, 2H, N(CH_2CH_2)$_2$), 3.81–3.89 (m, 4H, OCH_2CH_2OH), 4.87 (dd, J = 12.6/9.1 Hz, 1H, CH=C*H*CH(OR)$_2$), 5.50 (d, J = 9.1 Hz, 1H, CH=CHC*H*(OR)$_2$), 6.72 (d, J = 12.6 Hz, 1H, C*H*=CHCH(OR)$_2$), 7.09–7.12 (m, 1H, aromat. H), 7.14–7.17 (m, 1H, aromat. H), 7.26–7.30 (m, 2H, aromat. H). A signal for the OH-proton is not seen in the spectrum.

5.18. 2-[1′-(Cyclohexylmethyl)-3*H*-spiro[[2]benzofuran-1,4′-piperidin-3-yl])ethan-1-ol (10c, WMS-1832)

A mixture of 2-benzoxepine **8c** (787 mg, 2.12 mmol), THF (10 mL) and HCl (1 M, 10 mL) was stirred at rt for 1.75 h. Then NaOH (2 M) was added and the mixture was extracted with CH_2Cl_2 (4 × 4 mL). The organic layer was dried (Na_2SO_4), concentrated in vacuo and the residue (**9c**) was dissolved in CH_3CN (20 mL). $NaBH_4$ (171 mg, 4.5 mmol) was added under cooling with ice and the mixture was stirred for 2 h at rt. Excess of $NaBH_4$ was destroyed by addition of HCl (1 M). After 20 min NaOH (2 M) was added (pH 9–10). The product was extracted with CH_2Cl_2, the CH_2Cl_2 layer was dried (Na_2SO_4), concentrated in vacuo and the residue was purified by fc (first fc: 3 cm, cyclohexane/ethyl acetate 7:3, 20 mL; second fc: 2 cm, cyclohexane/ethyl acetate 8:2, 10 mL, R_f (cyclohexane/ethyl acetate 5:5) 0.28). Pale yellow oil, yield 150 mg (31%). $C_{21}H_{31}NO_2$ (329.5). MS (EI): *m/z* = 329 [M], 246 [M−C_6H_{11}]. IR: $\bar{\nu}$ (cm^{-1}) = 3400 (w, O–H), 2917 (C–H), 1046 (C–O), 754 (C–H, 1,2-disub. aromat). ^1H NMR ($CDCl_3$): δ (ppm) = 0.81–0.93 (m, 2H, C_6H_{11}), 1.15–1.25 (m, 3H, C_6H_{11}), 1.48–1.56 (m, 1H, C_6H_{11}), 1.65–1.81 (m, 7H, N(CH_2CH_2)$_2$ (2), C_6H_{11} (5)), 1.86–1.96 (m, 2H, N(CH_2CH_2)$_2$ (1), CH_2CH_2OH (1)), 2.10–2.35 (m, 6H, N(CH_2CH_2)$_2$ (1), N(CH_2CH_2)$_2$ (2), CH_2CH_2OH (1), N$CH_2C_6H_{11}$ (2)), 2.77–2.84 (m, 2H, N(CH_2CH_2)$_2$), 3.84 (ddd, *J* = 10.6/5.9/3.8 Hz, 1H, CH_2CH_2OH), 3.93 (ddd, *J* = 11.4/8.6/3.3 Hz, 1H, CH_2CH_2OH), 5.40 (dd, *J* = 8.8/3.0 Hz, 1H, ArC*H*O), 7.10–7.16 (m, 2H, aromat. H), 7.26–7.31 (m, 2H, aromat. H). A signal for the OH-proton is not seen in the spectrum. Purity (HPLC, method 1): 95.8%, t_R = 18.85 min.

5.19. 2-[1′-(Cyclohexylmethyl)-3*H*-spiro[[2]benzofuran-1,4′-piperidin]-3-yl]acetaldehyde (9c)

To identify and characterize the aldehyde **9c** a small sample of the above mentioned intermediate was purified by fc (2 cm, cyclohexane/ethyl acetate 7:3, 10 mL, R_f (CH_2Cl_2/MeOH 9:1) 0.49). $C_{21}H_{29}NO_2$ (327.5). MS (EI): *m/z* = 327 [M], 244 [M−C_6H_{11}]. IR: $\bar{\nu}$ (cm^{-1}) = 2919 (C–H), 1725 (C=O), 1049 (C–O), 755 (C–H, 1,2-disub. aromat). ^1H NMR ($CDCl_3$): δ (ppm) = 0.86–0.94 (m, 2H, $CH_2C_6H_{11}$), 1.14–1.27 (m, 3H, $CH_2C_6H_{11}$), 1.48–1.57 (m, 1H, $CH_2C_6H_{11}$), 1.65–1.84 (m, 7H, N(CH_2CH_2)$_2$ (2), $CH_2C_6H_{11}$ (5)), 1.88–1.98 (m, 1H, N(CH_2CH_2)$_2$), 2.07–2.24 (m, 3H, N(CH_2CH_2)$_2$ (1), $CH_2C_6H_{11}$ (2)), 2.34 (broad t, *J* = 11.5 Hz, 2H, N(CH_2CH_2)$_2$), 2.69–2.81 (m, 2H, N(CH_2CH_2)$_2$), 2.80 (ddd, *J* = 16.3/7.0/2.5 Hz, 1H, $CHCH_2CHO$), 2.91 (ddd, *J* = 16.3/4.7/2.1 Hz, 1H, $CHCH_2CHO$), 5.66 (dd, *J* = 7.0/4.8 Hz, 1H, ArC*H*O), 7.13–7.17 (m, 2H, aromat. H), 7.26–7.33 (m, 2H, aromat. H), 9.83 (t, *J* = 2.3 Hz, 1H, C*H*O).

5.20. 1′-(Cyclohexylmethyl)-3-(2-fluoroethyl)-3*H*-spiro[[2]-benzofuran-1,4′-piperidine] (3c, WMS-1833)

Under N_2 at −78 °C DAST (0.082 mL, 0.67 mmol) and 5 min later a solution of alcohol **10c** (103 mg, 0.31 mmol) in CH_2Cl_2 were successively added to −78 °C cold CH_2Cl_2 (10 mL). After stirring for 30 min at −78 °C the mixture was kept for 27.5 h at rt. Then NaOH (2 M) was added under cooling with ice, the layers were separated, 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 (0.7 cm, cyclohexane/ethyl acetate 7:3, 5 mL, R_f (cyclohexane/ethyl acetate 5:5) 0.58). Pale yellow oil, yield 37.7 mg (37%). $C_{21}H_{30}FNO$ (331.5). MS (EI): *m/z* = 331 [M], 248 [M−C_6H_{11}]. IR: $\bar{\nu}$ (cm^{-1}) = 2918 (C–H), 1046 (C–O), 755 (C–H, 1,2-disub. aromat). ^1H NMR ($CDCl_3$): δ (ppm) = 0.85–0.95 (m, 2H, C_6H_{11}), 1.14–1.30 (m, 3H, C_6H_{11}), 1.47–1.56 (m, 1H, C_6H_{11}), 1.61–1.74 (m, 5H, N(CH_2CH_2)$_2$ (2), C_6H_{11} (3)), 1.76–1.80 (m, 2H, C_6H_{11}), 1.86–2.03 (m, 2H, N(CH_2CH_2)$_2$ (1), CH_2CH_2F (1)), 2.08 (td, *J* = 13.3/4.7 Hz, 1H, N(CH_2CH_2)$_2$), 2.20 (d, *J* = 7.0 Hz, 2H, N$CH_2C_6H_{11}$), 2.27–2.40 (m, 3H, N(CH_2CH_2)$_2$ (2), CH_2CH_2F (1)), 2.74–2.81 (m, 2H, N(CH_2CH_2)$_2$), 4.55–4.84 (m, 2H, CH_2CH_2F), 5.32 (dd, *J* = 8.6/3.4 Hz, 1H, ArC*H*O), 7.13–7.17 (m, 2H, aromat. H), 7.25–7.30 (m, 2H, aromat. H). Purity (HPLC, method 1): 98.9%, t_R = 22.01 min.

5.21. {2-[1′-(Cyclohexylmethyl)-3*H*-spiro[[2]benzofuran-1,4′-piperidin]-3-yl]ethyl} *p*-toluenesulfonate (12c)

Under N_2 the alcohol **10c** (46.0 mg, 0.14 mmol), DMAP (6.1 mg, 0.05 mmol) and triethylamine (0.072 mL, 0.52 mmol) were dissolved in CH_2Cl_2 (15 mL). The solution was cooled down to −25 °C and a solution of *p*-toluenesulfonyl chloride (64.4 mg, 0.34 mmol) in CH_2Cl_2 (2 mL) was added. The mixture was stirred at 25 °C for 1.5 h and after addition of NH_4Cl (9.6 mg, 0.18 mmol) for additional 72 h at rt. Then H_20 was added, the aqueous layer was separated and 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 (1 cm, cyclohexane/ethyl acetate 5:5, 5 mL, R_f 0.41). Pale yellow oil, yield 29.0 mg (43%). $C_{28}H_{37}NO_4$ (483.7). MS (EI): *m/z* = 483 [M], 400 [M−C_6H_{11}], 171 [SO_3PhCH_3], 91 [CH_2Ph]. IR: $\bar{\nu}$ (cm^{-1}) = 2920 (C–H), 1598 (aromat. C=C), 1361, 1175 (O_2S=O), 813 (C–H, 1,4-disub. aromat), 757 (C–H, 1,2-disub. aromat). ^1H NMR ($CDCl_3$): δ (ppm) = 0.85–0.94 (m, 2H, $CH_2C_6H_{11}$), 1.14–1.28 (m, 3H, $CH_2C_6H_{11}$), 1.44–1.55 (m, 1H, $CH_2C_6H_{11}$), 1.48 (ddd, *J* = 13.4/6.4/3.7 Hz, 1H, N(CH_2CH_2)$_2$), 1.59 (ddd, *J* = 13.5/5.1/2.5 Hz, 1H, N(CH_2CH_2)$_2$), 1.63–1.83 (m, 5H, $CH_2C_6H_{11}$), 1.83–1.92 (m, 2H, N(CH_2CH_2)$_2$ (1), CH_2CH_2OTos (1)), 1.97–2.06 (m, 1H, N(CH_2CH_2)$_2$), 2.14–2.31 (m, 3H, N(CH_2CH_2)$_2$ (2), CH_2CH_2OTos (1)), 2.16 (d, *J* = 7.1 Hz, 2H, N$CH_2C_6H_{11}$), 2.44 (s, 3H, $ArCH_3$), 2.67–2.76 (m, 2H, N(CH_2CH_2)$_2$), 4.21 (ddd, *J* = 9.8/7.3/5.0 Hz, 1H, CH_2CH_2O-Tos), 4.28 (ddd, *J* = 9.6/7.9/6.5 Hz, 1H, CH_2CH_2OTos), 5.18 (dd, *J* = 8.6/3.2 Hz, 1H, ArC*H*O), 7.02–7.10 (m, 2H, aromat. H), 7.21–7.30 (m, 2H, aromat. H), 7.32 (broad d, *J* = 7.8 Hz, 2H, aromat. H), 7.77 (broad d, *J* = 8.3 Hz, 2H, aromat. H).

5.22. 3-(2-Chloroethyl)-1′-(cyclohexylmethyl)-3*H*-spiro[[2]-benzofuran-1,4′-piperidine] (13c, WMS-1838)

A solution of tosylate **12c** (29.0 mg, 0.06 mmol) in CH_3CN was added to a solution of KCl (13.5 mg, 0.21 mmol) and [18]-crown-6 (21.1 mg, 0.08 mmol) in CH_3CN. The mixture was heated under reflux overnight. Then NaOH was added and the product was extracted with CH_2Cl_2 (4×). The organic layer was dried (Na_2SO_4), concentrated in vacuo and the residue was purified by fc (0.5 cm, cyclohexane/ethyl acetate 5:5, 5 mL, R_f 0.50). Pale yellow oil, yield 19.2 mg (39%). $C_{21}H_{30}ClNO$ (347.9). MS (EI): *m/z* = 350/348 [M+H], 266/264 [M−C_6H_{11}]. IR: $\bar{\nu}$ (cm^{-1}) = 2919 (C–H), 1049 (C–O), 754 (C–H, 1,2-disub. aromat). ^1H NMR ($CDCl_3$): δ (ppm) = 0.85–0.95 (m, 2H, $CH_2C_6H_{11}$), 1.11–1.30 (m, 3H, $CH_2C_6H_{11}$), 1.48–1.58 (m, 1H, $CH_2C_6H_{11}$), 1.60–1.74 (m, 5H, N(CH_2CH_2)$_2$ (2H), $CH_2C_6H_{11}$ (3H)), 1.76–1.84 (m, 2H, $CH_2C_6H_{11}$), 1.90 (td, *J* = 11.7/4.7 Hz, 1H, N(CH_2CH_2)$_2$), 2.02–2.12 (m, 2H, CH_2CH_2Cl (1), N(CH_2CH_2)$_2$ (1)), 2.20 (d, *J* = 7.0 Hz, 2H, N$CH_2C_6H_{11}$), 2.28–2.38 (m, 3H, CH_2CH_2Cl (1), N(CH_2CH_2)$_2$ (2)), 2.73–2.83 (m, 2H, N(CH_2CH_2)$_2$), 3.66 (ddd, *J* = 10.7/8.2/4.8 Hz, 1H, CH_2CH_2Cl), 3.79 (dt, *J* = 10.7/7.7 Hz, 1H, CH_2CH_2Cl), 5.32 (dd, *J* = 8.3/3.3 Hz, 1H, ArC*H*O) 7.12–7.16 (m, 2H, aromat. H), 7.26–7.30 (m, 2H, aromat. H). Purity (HPLC, method 2): 95.3%, t_R = 16.22 min.

5.23. 2-[1′-(1-Phenylethyl)-3*H*-spiro[[2]benzoxepine-1,4′-piperidin]-3-yloxy]ethanol (8d)

Under N_2 a solution of *n*-BuLi in *n*-hexane (1.6 M, 3.5 mL, 5.6 mmol) was added slowly at −78 °C to a solution of **5** (1.10 g, 4.33 mmol) in THF (20 mL). After 10 min a solution of **6d**

(943 mg, 4.64 mmol) in THF (4 mL) was added slowly and the mixture was stirred at −78 °C for 1 h and at rt overnight. Then H_2O and CH_2Cl_2 were added, the layers were separated, the aqueous layer was extracted with CH_2Cl_2 (3×), the organic layer was washed with a saturated solution of NaCl, dried (Na_2SO_4), concentrated in vacuo and the residue was purified by fc (6 cm, cyclohexane/ethyl acetate 7:3, 50 mL, R_f (cyclohexane/ethyl acetate 5:5) 0.18). Pale yellow oil, yield 1152 mg (70%). $C_{24}H_{29}NO_3$ (379.5). MS (EI): *m/z* = 379 [M], 364 [M−CH_3], 334 [M−CH_2CH_2OH]. IR: $\bar{\nu}$ (cm^{-1}) = 3455 (O–H), 2933 (C–H), 1667, 1649 (C=C), 1043 (C–O), 757 (C–H, 1,2-disub. aromat), 701 (C–H, monosub. aromat). ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.42 (d, *J* = 6.8 Hz, 3H, C*H*$_3$CHNAr), 1.67 (ddd, *J* = 13.5/5.3/2.6 Hz, 1H, N(CH_2CH_2)$_2$), 1.75–1.84 (m, 1.5 H, N(CH_2CH_2)$_2$), 1.93 (td, *J* = 12.8/4.3 Hz, 0.5H, N(CH_2CH_2)$_2$), 2.04 (td, *J* = 13.0/4.3 Hz, 0.5H, N(CH_2CH_2)$_2$), 2.16 (td, *J* = 13.0/4.8 Hz, 0.5 H, N(CH_2CH_2)$_2$), 2.33–2.52 (m, 2H, N(CH_2CH_2)$_2$), 2.70 (broad d, *J* = 10.7 Hz, 1H, N(CH_2CH_2)$_2$), 3.04 (broad d, *J* = 10.9 Hz, 1H, N(CH_2CH_2)$_2$), 3.48 (q, *J* = 6.7 Hz, 1H, CH_3CHNAr), 3.79–3.87 (m, 4H, OCH_2CH_2O), 4.84 (dt, *J* = 12.6/9.4 Hz, 1H, ArCH=C*H*CH), 5.47 (t, *J* = 8.7 Hz, 1H, CH=CHC*H*O$_2$), 6.69 (d, *J* = 12.6 Hz, 1H, ArC*H*=CH), 7.07–7.10 (m, 1H, aromat. H), 7.13–7.17 (m, 1H, aromat. H), 7.21–7.37 (m, 7H, aromat. H). A signal for the OH-proton is not seen in the spectrum.

5.24. 2-[1′-(1-Phenylethyl)-3*H*-spiro[[2]benzofuran-1,4′-piperidin]-3-yl]acetaldehyde (9d)

A solution of 2-benzoxepine **8d** (1.15 g, 3.08 mmol) in THF (10 mL) and HCl (1 M, 8 mL) was stirred for 5 h at rt. Then NaOH (2 M) was added and the mixture was extracted with CH_2Cl_2. The organic layer was dried (Na_2SO_4), the solvent was evaporated in vacuo and the residue was purified by fc (5 cm, cyclohexane/ethyl acetate 7:3, 30 mL, 10 cm, R_f (CH_2Cl_2: MeOH 9:1) 0.66). Colorless oil, yield 581 mg (57%). According to ^{1}H NMR spectrum the product contained **9d** and **15d** (see **10d**) in the ratio 88: 12. **9d**: Colorless oil. $C_{22}H_{25}NO_2$ (335.4). MS (EI): *m/z* = 335 [M], 320 [M−CH_3], 258 [M−Ph], 91 [CH_2Ph]. IR: $\bar{\nu}$ (cm^{-1}) = 2936 (C–H), 1723 (C=O), 1601 (aromat. C=C), 1057 (C–O), 756 (C–H, 1,2-disub. aromat), 701 (C–H, monosub. aromat). ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.41 (d, *J* = 6.8 Hz, 3H, C*H*$_3$CHNAr), 1.56–1.81 (m, 2H, N(CH_2CH_2)$_2$), 1.84–2.19 (m, 2H, N(CH_2CH_2)$_2$), 2.26–2.44 (m, 2H, N(CH_2CH_2)$_2$), 2.64–2.71 (m, 1H, N(CH_2CH_2)$_2$), 2.78 (ddd, *J* = 16.4/7.1/2.5 Hz, 1H, CHCH_2CH=O), 2.85 (ddd, *J* = 16.3/4.8/2.2 Hz, 1H, CHCH_2CH=O) 2.98–3.07 (m, 1H, N(CH_2CH_2)$_2$), 3.46 (q, *J* = 6.6 Hz, 1H, CH_3CHNAr), 5.60–5.66 (m, 1H, ArC*H*O), 7.12–7.17 (m, 2H, aromat. H), 7.21–7.36 (m, 7H, aromat. H), 9.79 (t, *J* = 2.3 Hz, 0.5H, CHCH_2C*H*=O), 9.81 (t, *J* = 2.3 Hz, 0.5H, CHCH_2C*H*=O).

5.25. 2-[1′-(1-Phenylethyl)-3*H*-spiro[[2]benzofuran-1,4′-piperidin]-3-yl]ethan-1-ol (10d, WMS-1839) and 2-[1′-(1-phenylethyl)-3*H*-spiro[[2]benzofuran-1,4′-piperidin]-3-yl]acetaldehyde ethylene acetal (15d, WMS-1844)

Under cooling with ice $NaBH_4$ (192 mg, 5.08 mmol) was added to a solution of the aldehyde **9d** (581 mg, 1.73 mmol) in CH_3CN (15 mL). After 15 min the reaction mixture was warmed to rt and stirred overnight. Excess of $NaBH_4$ was destroyed by addition of HCl (1 M).Then NaOH (2 M) was added (pH 9–10), the mixture was extracted with CH_2Cl_2 (4×), the organic layer was dried (Na_2SO_4), concentrated in vacuo and the residue was purified by fc (1. fc: 4 cm, cyclohexane/ethyl acetate 7:3, 30 mL; 2. fc: 3 cm, cyclohexane/ethyl acetate 8:2, 20 mL R_f (cyclohexane/ethyl acetate 5:5) 0.26 (**15d**), 0.17 (**10d**)).

Compound **10d:** Colorless solid, mp 99 °C–100 °C, yield 79.6 mg (14%). $C_{22}H_{27}NO_2$ (337.5). MS (EI): *m/z* = 337 [M], 322 [M−CH_3], 260 [M−Ph], 91 [PhCH_2]. IR: $\bar{\nu}$ (cm^{-1}) = 3410 (w, O–H), 2925 (C–H), 1043 (C–O), 756 (C–H, 1,2-disub. aromat), 700 (C–H, monosub. aromat). ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.40 (d, *J* = 6.7 Hz, 1.5H, C*H*$_3$CHNAr), 1.41 (d, *J* = 7.0 Hz, 1.5H, C*H*$_3$CHAr), 1.62–1.70 (m, 1H, N(CH_2CH_2)$_2$), 1.75–1.92 (m, 3H, N(CH_2CH_2)$_2$ (2), CH_2CH_2OH (1)), 2.05 (td, *J* = 12.8/4.4 Hz, 0.5 H, N(CH_2CH_2)$_2$), 2.13–2.43 (m, 3.5 H, N(CH_2CH_2)$_2$ (0.5), N(CH_2CH_2)$_2$ (2), CH_2CH_2OH (1)), 2.66–2.78 (m, 1H, N(CH_2CH_2)$_2$), 3.00–3.13 (m, 1H, N(CH_2CH_2)$_2$), 3.41–3.49 (m, 1H, CH_3CHNAr), 3.78–3.85 (m, 1H, CH_2CH_2OH), 3.87–3.94 (m, 1H, CH_2CH_2OH), 5.35–5.40 (m, 1H, ArC*H*O), 7.09–7.11 (m, 1H, aromat. H), 7.14–7.16 (m, 1H, aromat. H), 7.22–7.37 (m, 7H, aromat. H). A signal for the OH-proton is not seen in the spectrum. Purity (HPLC, method 2): 98.3%, t_R = 13.34 min.

Compound **15d:** Pale yellow oil, yield 28.7 mg (4.4%). $C_{24}H_{29}NO_3$ (379.5). MS (ESI): *m/z* = 380 [M+H]. MS (EI): *m/z* = 364 [M−CH_3], 274 [M−CH_3CHPh], 105 [CH_3CHPh], 91 [CH_2Ph]. IR: $\bar{\nu}$ (cm^{-1}) = 2925 (C–H), 1134, 1058 (C–O), 756 (C–H, 1,2-disub. aromat), 701 (C–H, monosub. aromat). ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.40 (d, *J* = 7.3 Hz, 3H, C*H*$_3$CHNAr), 1.55–1.66 (m, 1H, N(CH_2CH_2)$_2$), 1.70–1.85 (m, 1.5 H, N(CH_2CH_2)$_2$), 1.90–2.13 (m, 3.5H, N(CH_2CH_2)$_2$ (1.5), CH_2CH(OR)$_2$ (2)), 2.35 (td, *J* = 12.2/2.5 Hz, 1H, N(CH_2CH_2)$_2$), 2.44 (td, *J* = 12.3/2.4 Hz, 1H, N(CH_2CH_2)$_2$), 2.63–2.71 (m, 1H, N(CH_2CH_2)$_2$), 2.98–3.05 (m, 1H, N(CH_2CH_2)$_2$), 3.46 (q, *J* = 6.5 Hz, 1H, CH_3CHNAr), 3.85–3.94 (m, 2H, OCH_2CH_2O), 3.97–4.05 (m, 2H, OCH_2CH_2O), 5.18 (td, *J* = 7.0/3.4 Hz, 1H, CH_2CH(OR)$_2$), 5.30–5.35 (m, 1H, ArC*H*O), 7.12–7.17 (m, 2H, aromat. H), 7.21–7.36 (m, 7H, aromat. H). Purity (HPLC, method 2): 95.4%, t_R = 15.25 min.

5.26. 3-(2-Fluoroethyl)-1′-(1-phenylethyl)-3*H*-spiro[[2]benzofuran-1,4′-piperidine] (3d, WMS-1840)

Under N_2 CH_2Cl_2 (10 mL) was cooled down to −78 °C. DAST (0.050 mL, 0.41 mmol) and approximately 5 min later a solution of alcohol **10d** (63.3 mg, 0.19 mmol) in CH_2Cl_2 (3 mL) were added slowly. The reaction mixture was stirred at −78 °C for 30 min and for 22 h at rt. Then NaOH (2 M) was carefully added under ice cooling to decompose the excess of DAST. The aqueous layer was extracted with CH_2Cl_2 (4 × 4 mL), the organic layer was dried (Na_2SO_4), the solvent was removed in vacuo and the residue was purified by fc (1 cm, cyclohexane/ethyl acetate 8:2, 5 mL, R_f (cyclohexane/ethyl acetate 5:5) 0.50). Pale yellow oil, yield 38.9 mg (61%). $C_{22}H_{26}FNO$ (339.5). MS (EI): *m/z* = 339 [M], 324 [M−CH_3], 262 [M−Ph], 91 [PhCH_2]. IR: $\bar{\nu}$ (cm^{-1}) = 2937 (C–H), 1604 (aromat. C=C), 1042 (C–O), 756 (C–H, 1,2-disub. aromat), 700 (C–H, monosub. aromat). ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.42 (d, *J* = 6.7 Hz, 3H, C*H*$_3$CHNAr), 1.58–1.80 (m, 2H, N(CH_2CH_2)$_2$), 1.81–2.05 (m, 2.5 H, N(CH_2CH_2)$_2$ (1.5), CH_2CH_2F (1)), 2.12 (td, *J* = 12.8/4.4 Hz, 0.5 H, N(CH_2CH_2)$_2$), 2.22–2.47 (m, 3H, N(CH_2CH_2)$_2$ (2), CH_2CH_2F (1)), 2.64–2.73 (m, 1H, N(CH_2CH_2)$_2$), 2.99–3.06 (m, 1H, N(CH_2CH_2)$_2$), 3.47 (q, *J* = 6.6 Hz, 1H, CH_3CHNAr), 4.48–4.84 (m, 2H, CH_2CH_2F), 5.28 (dd, *J* = 8.6/3.4 Hz, 0.5 H, ArC*H*O), 5.30 (dd, *J* = 8.7/3.4 Hz, 0.5 H, ArC*H*O), 7.12–7.17 (m, 2H, aromat. H), 7.21–7.38 (m, 7H, aromat. H). Purity (HPLC, method 1): 96.2% t_R = 18.96 min.

5.27. 2-(1′-Butyl-3*H*-spiro[[2]benzofuran-1,4′-piperidin]-3-yl)-ethan-1-ol (10e, WMS-1824)

Under N_2 at −78 °C a solution of *n*-BuLi in *n*-hexane (1.6 M, 1.7 mL, 2.72 mmol) was added slowly to a solution of **5** (548 mg, 2.15 mmol) in THF (10 mL). After 10 min a solution of **6e** (367 mg, 2.36 mmol) in THF (5 mL) was added slowly. The mixture was stirred at −78 °C for 1.25 h and at rt overnight. Then H_2O and CH_2Cl_2 were added, the organic layer was washed with $NaHSO_3$ solution (10%) and saturated solution of NaCl, dried (Na_2SO_4) and concentrated in vacuo to obtain the 2-benzoxepine **8e**. Without further purification the residue was dissolved in THF (6 mL) and HCl (1 M, 6 mL) and the mixture was stirred at rt for 5 h. Then NaOH (2 M) was added and the mixture was extracted with CH_2Cl_2

(4×). The organic layer was dried (Na_2SO_4) and the solvent was evaporated in vacuo to yield the aldehyde **9e**. Without further purification the residue was dissolved in CH_3CN (15 mL) and under cooling with ice $NaBH_4$ (143 mg, 3.8 mmol) was added. The mixture was stirred at rt for 2.5 h. Then, excess of $NaBH_4$ was destroyed by addition of HCl (1 M). After 20 min NaOH (2 M) was added (pH 9–10), the mixture was extracted with CH_2Cl_2 (4×), the solvent was evaporated in vacuo and the residue was purified by fc (first fc: 4 cm, cyclohexane/ethyl acetate 7:3, 20 mL; second fc: 1 cm, CH_2Cl_2/MeOH 9:1, 10 mL, R_f (cyclohexane/ethyl acetate 5:5) 0.04, R_f (CH_2Cl_2/MeOH 9:1) 0.18). Pale yellow oil, yield 66.8 mg (11%). $C_{18}H_{27}NO_2$ (289.4). MS (EI): *m/z* = 289 [M], 246 [M−C_3H_7]. IR: $\bar{\nu}$ (cm^{-1}) = 3358 (w, O–H), 2933 (C–H), 1046 (C–O), 754 (C–H, 1,2-disub. aromat). ^{1}H NMR ($CDCl_3$): δ (ppm) = 0.93 (t, *J* = 7.3 Hz, 3H, $(CH_2)_3CH_3$), 1.35 (sext, *J* = 7.5 Hz, 2H, $(CH_2)_2CH_2CH_3$), 1.53–1.60 (m, 2H, $CH_2CH_2CH_2CH_3$), 1.72–1.81 (m, 2H, $N(CH_2CH_2)_2$), 1.90–2.05 (m, 1H, $N(CH_2CH_2)_2$), 1.91 (dtd, *J* = 14.5/8.5/3.9 Hz, 1H, $CHCH_2CH_2OH$), 2.19 (ddt, *J* = 14.4/6.4/3.2 Hz, 1H, $CHCH_2CH_2OH$), 2.20–2.30 (m, 1H, $N(CH_2CH_2)_2$), 2.34–2.52 (m, 4H, $N(CH_2CH_2)_2$ (2), $CH_2(CH_2)_2CH_3$ (2)), 2.90–3.02 (m, 2H, $N(CH_2CH_2)_2$), 3.84 (ddd, *J* = 10.3/5.9/3.9 Hz, 1H, CH_2CH_2OH), 3.93 (ddd, *J* = 11.0/8.3/3.4 Hz, 1H, CH_2CH_2OH), 5.41 (dd, *J* = 8.8/3.0 Hz, 1H, ArCHO), 7.09–7.18 (m, 2H, aromat. H), 7.27–7.31 (m, 2H, aromat. H). A signal for the OH-proton is not seen in the spectrum. Purity (HPLC, method 1): 94.9%, t_R = 13.03 min.

5.28. 2-(1′-Butyl-3*H*-spiro[[2]-benzoxepine-1,4′-piperidin]-3-yl-oxy)ethanol (8e)

In order to identify and characterize the 2-benzoxepine **8e**, a small sample of the above described residue was purified by fc (1 cm, cyclohexane/ethyl acetate 4:6, 10 mL, R_f (cyclohexane/ethyl acetate 5:5) 0.04). Pale yellow oil. $C_{20}H_{29}NO_3$ (331.5). MS (EI): *m/z* = 331 [M], 288 [M−C_3H_7], 286 [M−C_2H_2OH]. IR: $\bar{\nu}$ (cm^{-1}) = 3410 (w, O–H), 2925 (C–H), 1663, 1649 (C=C), 1046 (C–O), 755 (C–H, 1,2-disub. aromat). ^{1}H NMR ($CDCl_3$): δ (ppm) = 0.93 (t, *J* = 7.3 Hz, 3H, $(CH_2)_3CH_3$), 1.33 (sext, *J* = 7.4 Hz, 2H, $(CH_2)_2CH_2CH_3$), 1.49–1.57 (m, 2H, $CH_2CH_2CH_2CH_3$), 1.75 (broad d, *J* = 12.9 Hz, 2H, $N(CH_2CH_2)_2$), 1.92 (td, *J* = 13.3/4.2 Hz, 1H, $N(CH_2CH_2)_2$), 2.16 (td, *J* = 13.3/4.4 Hz, 1H, $N(CH_2CH_2)_2$), 2.40–2.48 (m, 4H, $N(CH_2CH_2)_2$ (2), $CH_2CH_2CH_2CH_3$ (2)), 2.86–2.90 (m, 2H, $N(CH_2CH_2)_2$), 3.81–3.90 (m, 4H, OCH_2CH_2OH), 4.86 (dd, *J* = 12.6/9.1 Hz, 1H, CH=CHCH(OR)$_2$), 5.49 (d, *J* = 9.1 Hz, 1H, CH=CHCH(OR)$_2$), 6.71 (d, *J* = 12.6 Hz, 1H, CH=CHCH(OR)$_2$), 7.08–7.10 (m, 1H, aromat. H), 7.13–7.15 (m, 1H, aromat. H), 7.26–7.30 (m, 2H, aromat. H). A signal for the OH-proton is not seen in the spectrum.

5.29. 2-(1′-Butyl-3*H*-spiro[[2]benzofuran-1,4′-piperidin]-3-yl)-acetaldehyde (9e)

In order to identify and characterize the aldehyde **9e**, a small sample of the above described residue was purified by fc (1 cm, cyclohexane/ethyl acetate 8:2, 10 mL, R_f (CH_2Cl_2/MeOH 9:1) 0.53). $C_{18}H_{25}NO_2$ (287.4). MS (EI): *m/z* = 288 [M+H], 244 [M−C_3H_7]. IR: $\bar{\nu}$ (cm^{-1}) = 2923 (C–H), 1726 (C=O), 1050 (C=O), 756 (C–H, 1,2-disub. aromat). ^{1}H NMR ($CDCl_3$): δ (ppm) = 0.93 (t, *J* = 7.3 Hz, 3H, $(CH_2)_2CH_3$), 1.34 (sext, *J* = 7.4 Hz, 2H, $(CH_2)_2CH_2CH_3$), 1.49–1.57 (m, 2H, $CH_2CH_2CH_2CH_3$), 1.67–1.76 (m, 2H, $N(CH_2CH_2)_2$), 1.93 (td, *J* = 12.9/4.4 Hz, 1H, $N(CH_2CH_2)_2$), 2.14 (td, *J* = 13.0/4.2 Hz, 1H, $N(CH_2CH_2)_2$), 2.34–2.44 (m, 4H, $N(CH_2CH_2)_2$ (2), $CH_2(CH_2)_2CH_3$ (2)), 2.81 (ddd, *J* = 16.3/7.1/2.5 Hz, 1H, $CHCH_2CHO$), 2.81–2.90 (m, 2H, $N(CH_2CH_2)_2$), 2.92 (ddd, *J* = 16.3/4.7/2.1 Hz, 1H, $CHCH_2CHO$), 5.67 (dd, *J* = 7.0/4.7 Hz, 1H, ArCHO), 7.14–7.16 (m, 2H, aromat. H), 7.27–7.32 (m, 2H, aromat. H), 9.84 (t, *J* = 2.3 Hz, 1H, CHO).

5.30. 1′-Butyl-3-(2-fluoroethyl)-3*H*-spiro[[2]benzofuran-1,4′-piperidine] (3e, WMS-1830)

Under N_2 CH_2Cl_2 (7 mL) was cooled down to −78 °C. DAST (0.055 mL, 0.44 mmol) and 5 min later a solution of alcohol **10e** (58.2 mg, 0.20 mmol) in CH_2Cl_2 (4 mL) were added. The mixture was stirred for 30 min at −78 °C and 16 h at rt. Under cooling with ice NaOH (2 M) was carefully added to decompose the DAST reagent. Then the organic layer was separated and 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 (1 cm, cyclohexane/ethyl acetate 7:3, 10 mL, R_f (cyclohexane/ethyl acetate 5:5) 0.13). Pale yellow oil, yield 15.1 mg (26%). $C_{18}H_{26}FNO$ (291.4). MS (EI): *m/z* = 291 [M], 248 [M−C_3H_7]. IR: $\bar{\nu}$ (cm^{-1}) = 2933 (C–H), 1035 (C–O), 755 (C–H, 1,2-disub. aromat). ^{1}H NMR ($CDCl_3$): δ (ppm) = 0.94 (t, *J* = 7.3 Hz, 3H, $(CH_2)_3CH_3$), 1.36 (sext, *J* = 7.2 Hz, 2H, $(CH_2)_2CH_2CH_3$), 1.50–1.57 (m, 2H, $CH_2CH_2CH_2CH_3$), 1.68 (ddd, *J* = 13.3/5.1/2.5 Hz, 1H, $N(CH_2CH_2)_2$), 1.73 (ddd, *J* = 13.3/5.3/2.8 Hz, 1H, $N(CH_2CH_2)_2$), 1.88–2.03 (m, 2H, $N(CH_2CH_2)_2$ (1), $CHCH_2CH_2F$ (1)), 2.11 (td, *J* = 13.1/4.4 Hz, 1H, $N(CH_2CH_2)_2$), 2.28–2.44 (m, 5H, $N(CH_2CH_2)_2$ (2), $CH_2(CH_2)_2CH_3$ (2)), $CHCH_2CH_2F$ (1)), 2.82–2.90 (m, 2H, $N(CH_2CH_2)_2$), 4.59–4.84 (m, 2H, CH_2CH_2F), 5.32 (dd, *J* = 8.3/3.4 Hz, 1H, ArCHO), 7.11–7.18 (m, 2H, aromat. H), 7.25–7.31 (m, 2H, aromat. H). Purity (HPLC, method 1): 96.6%, t_R = 17.50 min.

5.31. 2-(1′-Octyl-3*H*-spiro[[2]benzoxepin-1,4′-piperidin]-3-yl-oxy)ethanol (8f)

Under N_2 a solution of *n*-BuLi in *n*-hexane (1.6 M, 1.7 mL, 2.72 mmol) was added slowly at −78 °C to a solution of **5** (555 mg, 2.17 mmol) in THF (10 mL). After 10 min at −78 °C a solution of 1-octylpiperidin-4-one (**6f**, 445 mg, 2.1 mmol) in THF (5 mL) was added slowly. The mixture was stirred at −78 °C for 1 h and at rt overnight. Then H_2O and CH_2Cl_2 were added, the organic layer was separated, washed with $NaHSO_3$ solution (10%) and saturated solution of NaCl, dried (Na_2SO_4), concentrated in vacuo and the residue was purified by fc (4 cm, cyclohexane/ethyl acetate 5:5, 30 mL, R_f 0.08). Pale yellow oil, yield 247 mg, (29%). $C_{23}H_{36}NO_3$ (387.6). MS (EI): *m/z* = 387 [M], 342 [M−CH_2CH_2OH], 288 [M−C_7H_{15}]. IR: $\bar{\nu}$ (cm^{-1}) = 3408 (w, O–H), 2924 (C–H), 1672, 1650 (C=C), 1046 (C–O), 755 (C–H, 1,2-disub. aromat). ^{1}H NMR ($CDCl_3$): δ (ppm) = 0.87 (t, *J* = 6.8 Hz, 3H, $C_7H_{14}CH_3$), 1.21–1.35 (m, 10H, $C_2H_4(CH_2)_5CH_3$), 1.48–1.57 (m, 2H, $CH_2CH_2C_6H_{13}$), 1.76 (d, *J* = 12.8 Hz, 2H, $N(CH_2CH_2)_2$), 1.92 (td, *J* = 12.9/3.6 Hz, 1H, $N(CH_2CH_2)_2$), 2.16 (td, *J* = 12.7/4.5 Hz, 1H, $N(CH_2CH_2)_2$), 2.39–2.49 (m, 4H, $N(CH_2CH_2)_2$ (2), $CH_2C_7H_{15}$ (2)), 2.84–2.92 (m, 2H, $N(CH_2CH_2)_2$), 3.82–3.85 (m, 4H, OCH_2CH_2OH), 4.86 (dd, *J* = 12.6/9.1 Hz, 1H, CH=CHCH(OR)$_2$), 5.49 (d, *J* = 9.1 Hz, 1H, CH=CHCH(OR)$_2$), 6.72 (d, *J* = 12.6 Hz, 1H, CH=CHCH(OR)$_2$), 7.08–7.11 (m, 1H, aromat. H), 7.13–7.15 (m, 1H, aromat. H), 7.25–7.30 (m, 2H, aromat. H). A signal for the OH-proton is not seen in the spectrum.

5.32. 2-(1′-Octyl-3*H*-spiro[[2]benzofuran-1,4′-piperidin]-3-yl)-ethanol (10f, WMS-1827)

The 2-benzoxepine **8f** (247 mg, 0.64 mmol) was dissolved in THF (7 mL), HCl (1 M, 7 mL) was added and the mixture was stirred for 2 h at rt. NaOH (2 M) was added, the mixture was extracted with CH_2Cl_2, the organic layer was dried (Na_2SO_4) and concentrated in vacuo. The residue (aldehyde **9f**) was dissolved in CH_3CN (8 mL) and $NaBH_4$ (51 mg, 1.3 mmol) was added. After 15 min the mixture was warmed to rt and subsequently stirred at rt for 3 h. Excess of $NaBH_4$ was destroyed by addition of HCl (1 M). After 20 min NaOH

(2 M) was added under ice cooling (pH 9–10) and the mixture was extracted with CH_2Cl_2 (4 × 6–8 mL). The solvent was removed in vacuo and the residue was purified by fc (first fc: 1.5 cm, cyclohexane/ethyl acetate 7:3, 10 mL; second fc: 3 cm, cyclohexane/ethyl acetate 8:2, 10 mL, R_f (cyclohexane/ethyl acetate 5:5) 0.32). Pale yellow oil, yield 59 mg (27%). $C_{22}H_{35}NO_2$ (345.5). MS (EI): *m/z* = 345 [M], 246 [M−C_7H_{15}]. IR: $\bar{\nu}$ (cm^{-1}) = 3425 (w, O–H), 2922 (C–H), 1046 (C–O), 754 (C–H, 1,2-disub. aromat). ^{1}H NMR ($CDCl_3$): δ (ppm) = 0.91 (t, *J* = 6.8 Hz, 3H, $C_7H_{14}CH_3$), 1.30–1.34 (m, 10H, $C_2H_4(CH_2)_5CH_3$), 1.50–1.57 (m, 2H, $CH_2CH_2C_6H_{13}$), 1.72–1.81 (m, 2H, $N(CH_2CH_2)_2$), 1.91–2.00 (m, 2H, $N(CH_2CH_2)_2$ (1), CH_2CH_2OH (1)), 2.15–2.26 (m, 2H, $N(CH_2CH_2)_2$ (1), CH_2CH_2OH (1)), 2.30–2.44 (m, 4H, $N(CH_2CH_2)_2$ (2), $NCH_2C_7H_{15}$ (2)), 2.88–2.96 (m, 2H, $N(CH_2CH_2)_2$), 3.87 (ddd, *J* = 10.3/6.0/3.7 Hz, 1H, CH_2CH_2OH), 3.97 (ddd, *J* = 11.3/8.4/3.3 Hz, 1H, CH_2CH_2OH), 5.44 (dd, *J* = 8.8/3.0 Hz, 1H, ArCHO), 7.14–7.21 (m, 2H, aromat. H), 7.27–7.36 (m, 2H, aromat. H). A signal for the OH-proton is not seen in the spectrum. Purity (HPLC, method 1): 97.6%, t_R = 18.93 min.

5.33. 2-(1′-Octyl-3*H*-spiro[[2]benzofuran-1,4′-piperidin]-3-yl)-acetaldehyde (9f)

In order to identify and characterize the aldehyde **9f** a small sample of the above described residue was purified by fc (2 cm, cyclohexane/ethyl acetate 8:2, 10 mL, R_f (CH_2Cl_2/MeOH 9:1) 0.53). $C_{22}H_{33}NO_2$ (343.5). MS (EI): *m/z* = 343 [M], 244 [M−$(CH_2)_6CH_3$]. IR: $\bar{\nu}$ (cm^{-1}) = 2923 (C–H), 1726 (C=O), 1050 (C–O), 756 (C–H, 1,2-disub. aromat). ^{1}H NMR ($CDCl_3$): δ (ppm) = 0.88 (t, *J* = 6.9 Hz, 3H, $(CH_2)_7CH_3$), 1.27–1.33 (m, 10H, $(CH_2)_2(CH_2)_5CH_3$), 1.41–1.55 (m, 2H, $CH_2CH_2C_6H_{13}$), 1.67–1.77 (m, 2H, $N(CH_2CH_2)_2$), 1.92 (td, *J* = 12.9/4.1 Hz, 1H, $N(CH_2CH_2)_2$), 2.13 (td, *J* = 13.0/4.0 Hz, 1H, $N(CH_2CH_2)_2$), 2.34–2.42 (m, 4H, $N(CH_2CH_2)_2$ (2), $CH_2(C_7H_{15})$ (2)), 2.81 (ddd, *J* = 16.3/7.1/2.5 Hz, 1H, $ArCHCH_2CH$=O), 2.80–2.90 (m, 2H, $N(CH_2CH_2)_2$), 2.91 (ddd, *J* = 16.3/4.7/2.1 Hz, 1H, $ArCHCH_2CH$=O), 5.67 (dd, *J* = 7.0/4.7 Hz, 1H, ArCHO), 7.13–7.16 (m, 2H, aromat. H), 7.27–7.32 (m, 2H, aromat. H), 9.83 (t, *J* = 2.3 Hz, 1H, CH=O).

5.34. 3-(2-Fluoroethyl)-1′-octyl-3*H*-spiro[[2]benzofuran-1,4′-piperidine] (3f, WMS-1834)

Under N_2 CH_2Cl_2 (10 mL) was cooled down to −78 °C. DAST (0.045 mL, 0.37 mmol) and 5 min later a solution of alcohol **10f** (49.2 mg, 0.14 mmol) in CH_2Cl_2 (4 mL) were added. The mixture was stirred for 30 min at −78 °C and for 28 h at rt. Under cooling with ice NaOH (2 M) was added. 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 purified by fc (0.7 cm, cyclohexane/ethyl acetate 7:3, 5 mL, R_f (cyclohexane/ethyl acetate 5:5) 0.31). Pale yellow oil, yield 26.1 mg (53%). $C_{22}H_{34}FNO$ (347.5). MS (EI): *m/z* = 347 [M], 248 [M−C_7H_{15}]. IR: $\bar{\nu}$ (cm^{-1}) = 2924 (C–H), 1037 (C–O), 755 (C–H, 1,2-disub. aromat). ^{1}H NMR ($CDCl_3$): δ (ppm) = 0.88 (t, *J* = 6.7 Hz, 3H, $C_7H_{14}CH_3$), 1.25–1.34 (m, 10H, $C_2H_4(CH_2)_5CH_3$), 1.50–1.57 (m, 2H, $CH_2CH_2C_6H_{13}$), 1.64–1.75 (m, 2H, $N(CH_2CH_2)_2$), 1.88–2.04 (m, 2H, $N(CH_2CH_2)_2$ (1), CH_2CH_2F (1)), 2.10 (td, *J* = 13.0/4.4 Hz, 1H, $N(CH_2CH_2)_2$), 2.27–2.42 (m, 5H, $N(CH_2CH_2)_2$ (2), $NCH_2C_7H_{15}$ (2), CH_2CH_2F (1)), 2.82–2.89 (m, 2H, $N(CH_2CH_2)_2$), 4.55–4.84 (m, 2H, CH_2CH_2F), 5.32 (dd, *J* = 8.6/3.4 Hz, 1H, ArCHO), 7.12–7.18 (m, 2H, aromat. H), 7.26–7.30 (m, 2H, aromat. H). Purity (HPLC, method 1): 95.3%, t_R = 19.78 min.

5.35. 3-(2-Fluoroethyl)-1′-(3-methylbut-2-en-1-yl)-3*H*-spiro[[2]-benzofuran-1,4′-piperidine] (3g, WMS-1843)

The unpurified secondary amine **3h** was dissolved in CH_3CN (8 mL). K_2CO_3 (95.7 mg, 0.69 mmol) and 1-bromo-3-methylbut-2-ene (0.013 mL, 0.11 mmol) were added and the mixture was heated to reflux overnight. The mixture was filtered over Celite®, the solvent was removed in vacuo and the residue was purified by fc (0.5 cm, cyclohexane/ethyl acetate 4:6, 5 mL, R_f (cyclohexane/ethyl acetate 5:5) 0.10). Colorless oil, yield 4.5 mg (13% calculated from **3a**). $C_{19}H_{26}FNO$ (303.4). MS (EI): *m/z* = 304 [MH], 288 [M−CH_3], 248 [M−C_4H_7], 235 [M−C_5H_9]. IR: $\bar{\nu}$ (cm^{-1}) = 2914 (C–H), 1676 (C=C), 1045 (C–O), 755 (C–H, 1,2-disub. aromat). ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.64–1.71 (m, 5H, $N(CH_2CH_2)_2$ (2), $CHC(CH_3)_2$ (3)), 1.75 (s, 3H, $CHC(CH_3)_2$), 1.86–2.06 (m, 2H, $N(CH_2CH_2)_2$ (1), CH_2CH_2F (1)), 2.12 (td, *J* = 13.2/4.4 Hz, 1H, $N(CH_2CH_2)_2$), 2.26–2.44 (m, 3H, $N(CH_2CH_2)_2$ (2), CH_2CH_2F (1)), 2.84–2.93 (m, 2H, $N(CH_2CH_2)_2$), 3.05 (d, *J* = 7.1 Hz, 2H, $NCH_2CHC(CH_3)_2$), 4.52–4.87 (m, 2H, CH_2F), 5.30–5.35 (m, 2H, ArCHO (1), $CH_2CHC(CH_3)_2$ (1)), 7.11–7.18 (m, 2H, aromat. H), 7.26–7.31 (m, 2H, aromat. H). Purity (HPLC, method 1): 95.3%, t_R = 18.25 min.

5.36. 3-(2-Fluoroethyl)-3*H*-spiro[[2]benzofuran-1,4′-piperidine] (3h)

Under N_2 a mixture of *N*-benzyl derivative **3a** (37.4 mg, 0.11 mmol), Pd/C (8.9 mg, 10% (m/m)) and dried ammonium formate (51 mg, 1.37 mmol) in CH_3OH (10 mL) was heated under reflux for 2 h. After filtration over Celite® the solvent was removed in vacuo. For the characterization only a small part of the residue was purified by fc (ethyl acetate/methanol: ammonia 9:1: 0.2, R_f 0.04). Colorless oil. $C_{14}H_{18}FNO$ (235.4). MS (EI): *m/z* = 236 [MH], 216 [M−F], 188 [M−C_2H_4F]. IR: $\bar{\nu}$ (cm^{-1}) = 3294 (N–H), 2932 (C–H), 1676 (C=C), 1035 (C–O), 755 (C–H, 1,2-disub. aromat). ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.62–2.05 (m, 5H, $N(CH_2CH_2)_2$ (4), CH_2CH_2F (1)), 2.26–2.43 (m, 1H, CH_2CH_2F), 3.00–3.13 (m, 4H, $N(CH_2CH_2)_2$), 4.48 (dddd, *J* = 46.8/9.1/6.7/4.6 Hz, 1H, CH_2CH_2F), 4.55 (dddd, *J* = 47.1/9.1/8.3/5.4 Hz, 1H, CH_2CH_2F), 5.33 (dd, *J* = 8.7/3.4 Hz, 1H, ArCHO), 7.12–7.18 (m, 2H, aromat. H), 7.26–7.30 (m, 2H, aromat. H). A signal for the NH-proton is not seen in the spectrum. Purity (HPLC, method 2): 92.2%, t_R = 11.11 min.

6. Receptor binding studies

6.1. 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 radioactivity bound to the filter was measured using a MicroBeta Trilux scintillation analyzer (Perkin Elmer). The overall counting efficiency was 20%.

6.2. Membrane preparation for the σ_1 assay[29,33]

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 1200x*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 23500x*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[47] 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.

6.3. Performing of the σ_1 assay[29,33]

The test was performed with the radioligand [^{3}H]-(+)-pentazocine (22 Ci/mmol; Perkin Elmer). The thawed membrane preparation (about 75 μg of the protein) was incubated with various concentrations of test compounds, 2 nM [^{3}H]-(+)-pentazocine, and buffer (50 mM TRIS, pH 7.4) in a total volume of 200 μL for 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.[48]

6.4. Membrane preparation for the σ_2 assay[29,33]

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[47] 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.

6.5. Performing of the σ_2 assay[29,33]

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.[49]

6.6. 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.[50] The K_i-values of potent compounds are given as mean values + SEM from three independent experiments.

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