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Original article

Microwave assisted synthesis of spirocyclic pyrrolidines – σ_1 receptor ligands with modified benzene-*N*-distance

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ABSTRACT

Two series of σ_1 ligands with a spiro[[2]benzopyran-1,3′-pyrrolidine] (**3**) and a spiro[[2]benzofuran-1,3′-pyrrolidine] (**4**) framework were synthesized and pharmacologically evaluated. Several reaction steps were considerably improved by microwave irradiation. The σ_1 affinity of the spirocyclic ligands correlates nicely with the benzene-*N*-distance, i.e. **2** < **3** < **4** < **1**. The σ_1 affinity of both compound classes could be increased with large *N*-substituents (e.g. 2-phenylethyl, octyl). Nevertheless the benzyl derivative **4a** represents the most promising σ_1 ligand (K_i = 25 nM) due to its high selectivity against the σ_2 subtype (>40-fold), the NMDA receptor and 5-HT_6 and 5-HT_7 receptors. Moreover, **4a** did not inhibit the hERG channel in the heart.

1. Introduction

After the first postulation of σ receptors as opioid receptor subtype [1], it has been shown that σ receptors represent a unique, non-opioid, non-phencyclidine but haloperidol sensitive receptor family. σ Receptors have a characteristic distribution in the central nervous system but they are also found in some organs and tissues in the periphery including lung, liver, kidney and heart. Today two subtypes are known, which are termed σ_1 and σ_2 receptor [2–4].

The amino acid sequence of the σ_1 receptor subtype was determined by successful cloning [5–9]. According to the amino acid sequence (223 amino acids, molecular weight 25.3 kDa), the membrane-bound σ_1 receptor containing two transmembrane domains represents a unique receptor type differing from classical G-protein coupled receptors, ion channel receptors and tyrosine kinase receptors. Whereas a similarity to a mammalian protein could not be detected, a 30% homology to the yeast enzyme sterol-Δ^8/Δ^7 isomerase was found [6]. The σ_2 receptor subtype is less characterized, it has not been cloned yet, but its molecular weight is approximately 21.5 kDa [10].

The unique structure of the σ_1 receptor protein renders the discovery of the mechanism of signal transduction rather difficult. It has been shown that the σ_1 receptor is involved in the modulation of some neurotransmitter systems including the cholinergic, dopaminergic and glutamatergic neurotransmission [11]. However, more importantly the σ_1 receptor is able to regulate some ion channels (e.g. Na^+, K^+, and Ca^{2+}-channels) [12–15].

σ_1 Receptor ligands are considered as promising drug candidates for the treatment of various neurological and psychiatric disorders [15–19]. There is a particular potential of σ_1 ligands for the treatment of depression [20–22], anxiety [17], memory disorders [23], Alzheimer's disease as well as alcohol and cocaine abuse [16,17]. Chen and coworkers have described the σ_1 system as an endogenous anti-opioid system. The well-known σ_1 agonist (+)-pentazocine is able to antagonize μ-, κ-, and δ-opioid receptor mediated analgesia, whereas σ_1 antagonists enhance opioid induced antinociception [24]. Furthermore, opioid mediated analgesia was potentiated by down regulation of σ_1 receptors [18]. Therefore σ_1 antagonists possess a high potential as innovative analgesics with reduced side effects. The development of novel σ ligand-based antitumor drugs and tumor diagnostics is stimulated by the high density of σ_1 (and σ_2 receptors) in some human tumor cell lines (e.g. breast, lung, prostate cancer cells) [25,26].

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Since we are interested in conformationally restricted receptor ligands, we have developed potent σ_1 ligands with a spirocyclic framework [27–41]. Compounds of type **1** containing a spirocyclic piperidine moiety represent a typical example of this class of σ_1 ligands (Fig. 1). As an example the *N*-benzyl derivative **1a** (R = Bn) displays a σ_1 affinity in the low nanomolar range (K_i = 1.3 nM) [27]. Recently we have shown that shifting of the *N*-atom to the adjacent position (**2**) led to 1000-fold decrease of σ_1 affinity (**2a**, R = Bn, K_i > 1400 nM) [30]. The reduced σ_1 affinity was explained with a reduced distance between the benzene ring of the benzopyran ring and the basic *N*-atom (benzene-*N*-distance).

In Fig. 2 the piperidine chair conformations of the spirocyclic piperidine **1a** are shown. Since the energies are very similar, both conformations **1a**–**1** and **1a**–**2** have to be taken into account. The benzene-*N*-distance of conformer **1a**–**1** bearing the phenyl moiety in equatorial orientation of the piperidine ring amounts 5.7 Å. The corresponding conformer **1a**–**2** with axial position of the phenyl moiety shows a considerably shorter distance of 5.1 Å. A similar situation is observed for the regioisomeric spirocyclic piperidine **2a**. In conformer **2a**–**1** with equatorially oriented phenyl ring the benzene-*N*-distance is about 0.5 Å longer than in conformer **2a**–**2**.

A comparison of **1a** and **2a** clearly demonstrates that the benzene-*N*-distance of **2a** (4.4–4.9 Å) is considerably shorter than the benzene-*N*-distance of **1a** (5.1–5.7 Å). The explanation of the reduced σ_1 affinity of **2a** (K_i > 1400 nM) with the reduced benzene-*N*-distance is in good accordance with established σ_1 pharmacophore models [41–46]. In order to learn more about the crucial benzene-*N*-distance in this type of spirocyclic σ_1 ligands, replacement of the piperidine moiety of **1** and/or **2** with a pyrrolidine ring (compounds **3** and **4** in Fig. 1) was envisaged. Since the five-membered pyrrolidine ring is more flexible than the piperidine ring, several energetically favored conformations have to be considered. Analysis of the conformations of benzopyran **3a** (R = Bn) led to benzene-*N*-distance in the range of 4.6–5.0 Å. In the benzofuran **4a** (R = Bn) this distance is slightly increased to 4.8–5.1 Å (Fig. 2).

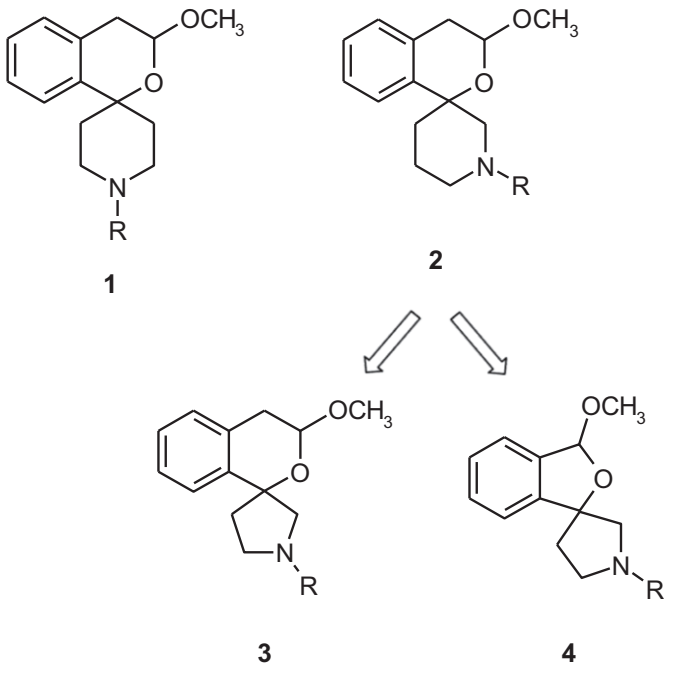


Fig. 1. Development of spirocyclic pyrrolidines **3** and **4** from spirocyclic piperidines with symmetric (**1**) and unsymmetrical piperidine connection (**2**).

2. Chemistry

Exchange of the bromine atom of the bromoacetal **5** [47] with *n*-BuLi at −78 °C followed by trapping of the resulting aryllithium intermediate with 1-benzylpyrrolidin-3-one provided the hydroxyacetal **6** in 33% isolated yield. Treatment of the hydroxyacetal **6** with 1.2 equivalents of p-toluenesulfonic acid in methanol led to cyclization via intramolecular transacetalization. Whereas a reaction time of 4 d at room temperature was required to achieve a yield of 67%, microwave irradiation during the cyclization step afforded a yield of 73% within 15 min. However, the best yield over two steps (41%) was obtained by consecutive conversions without isolation of the unstable intermediate **6** (Scheme 1).

The *N*-benzyl moiety of **3a** was removed by a transfer hydrogenolysis using ammonium formate and Pd/C [48]. The resulting secondary amine **3b** was alkylated with various arylalkyl and alkyl halides to obtain the tertiary amines **3c**–**f**. Microwave irradiation reduced the reaction times of the transformations and increased the yields of the products **3c**–**f**. The spirocyclic pyrrolidines **3** were formed as 1:1-mixtures of *cis*- and *trans*-configured diastereomers. The stereodescriptors *cis*/*trans* refer to the orientation of the OCH_3 group and the N–CH_2–moiety on the same side/on opposite sides of the benzopyran plane. For the present the compounds were tested as 1:1-mixtures.

In order to investigate the effect of a ring contracted *O*-heterocycle on the σ_1 receptor affinity, the corresponding spirocyclic benzofuran derivatives **4** were synthesized in the same manner starting with 2-bromobenzaldeyhde acetal **7** (Scheme 2). Halogen-metal exchange followed by reaction with 1-benzylpyrrolidin-3-one led to hydroxyacetal **8** in 51% yield. The acid-catalyzed cyclization of **8** was supported by microwave irradiation providing the spirocyclic benzofuran **4a** in 92%. The *N*-benzyl substituent of **4a** was exchanged by transfer hydrogenolysis [48] and subsequent alkylation of the secondary amine **4b**. For the introduction of *N*-substituents a consecutive two-step procedure was used consisting of a microwave supported *Finkelstein* reaction of the corresponding arylalkyl chlorides or alkyl bromides with NaI in acetone and a microwave assisted alkylation of the secondary amine **4b** with the *in situ* formed (aryl)alkyl iodides.

Reaction of the secondary amine **4b** with 1,4-dibromobutane led to a spirocyclic quaternary ammonium ion, which did not react with further nucleophiles. Therefore, the secondary amine **4b** was reacted with 1-(4-chlorobutyl)imidazole, which was available by alkylation of imidazole with 1-bromo-4-chlorobutane, under microwave irradiation to form the imidazolylbutyl derivative **4g** in 65% yield.

As described for the spirocyclic benzopyrans **3** the spirocyclic benzofurans **4** were produced and tested as 1:1-mixtures of *cis*- and *trans*-configured diastereomers.

3. Pharmacological evaluation

At first the σ_1 and σ_2 receptor affinities of the spirocyclic pyrrolidines **3** and **4** were determined in competition experiments with radioligands. In the σ_1 assay homogenates of guinea pig brains served as receptor material and the σ_1 selective ligand [^{3}H]-(+)-pentazocine as radioligand. The non-specific binding was determined in the presence of a large excess of non-labeled (+)-pentazocine [27,34]. Rat liver membrane preparations were the source of σ_2 receptors in the σ_2 assay. Since a σ_2 selective radioligand is not commercially available, the non-selective σ ligand [^{3}H]-di(*o*-tolyl)guanidine was employed and the present σ_1

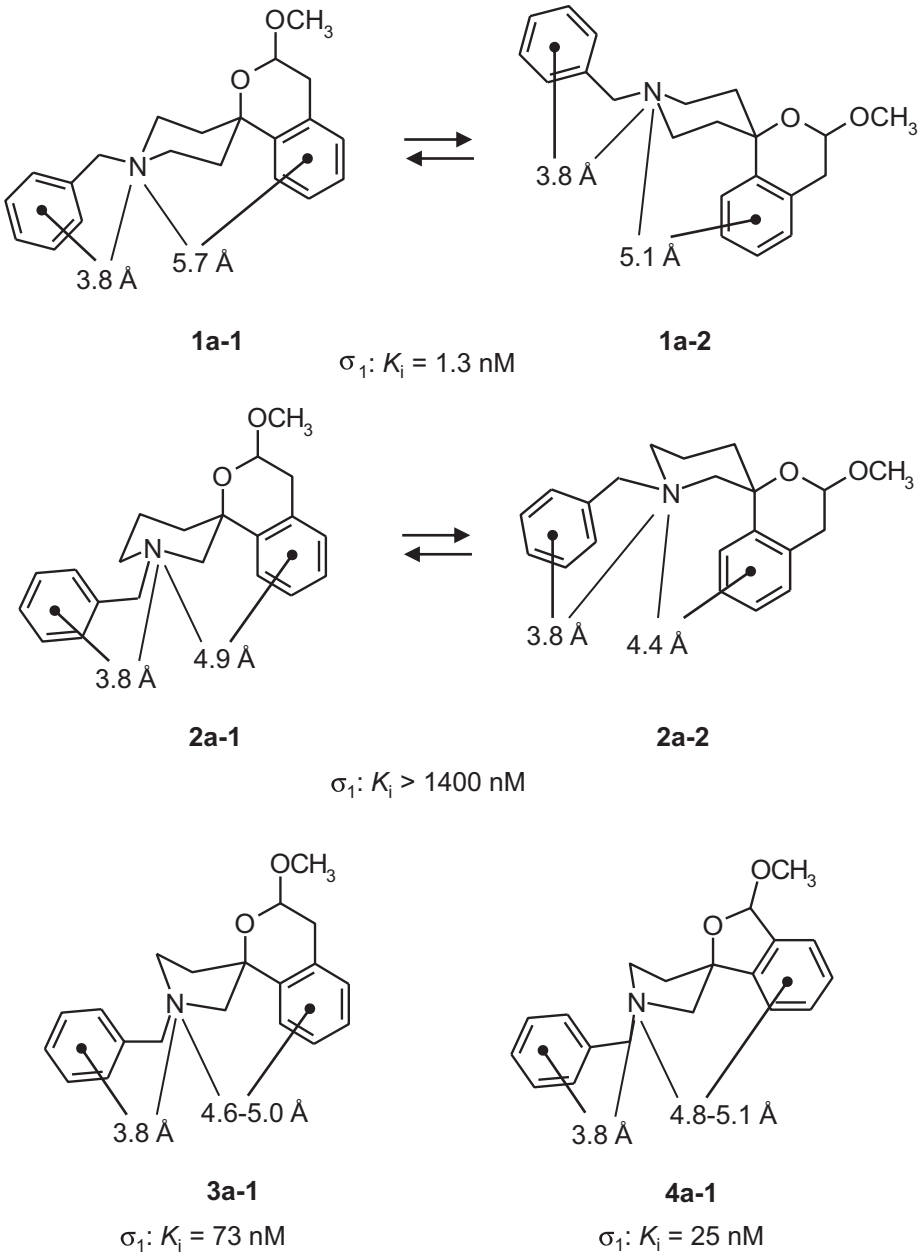


Fig. 2. Comparison of the crucial benzene-*N*-distances of spirocyclic compounds **1**–**4** and correlation with their σ_1 affinities.

receptors were masked with an excess of the non-radiolabeled σ_1 selective ligand (+)-pentazocine. Performing of the σ_2 assay in the presence of an excess of non-tritiated 1,3-di(*o*-tolyl)guanidine provided the non-specific binding of the radioligand [27,34].

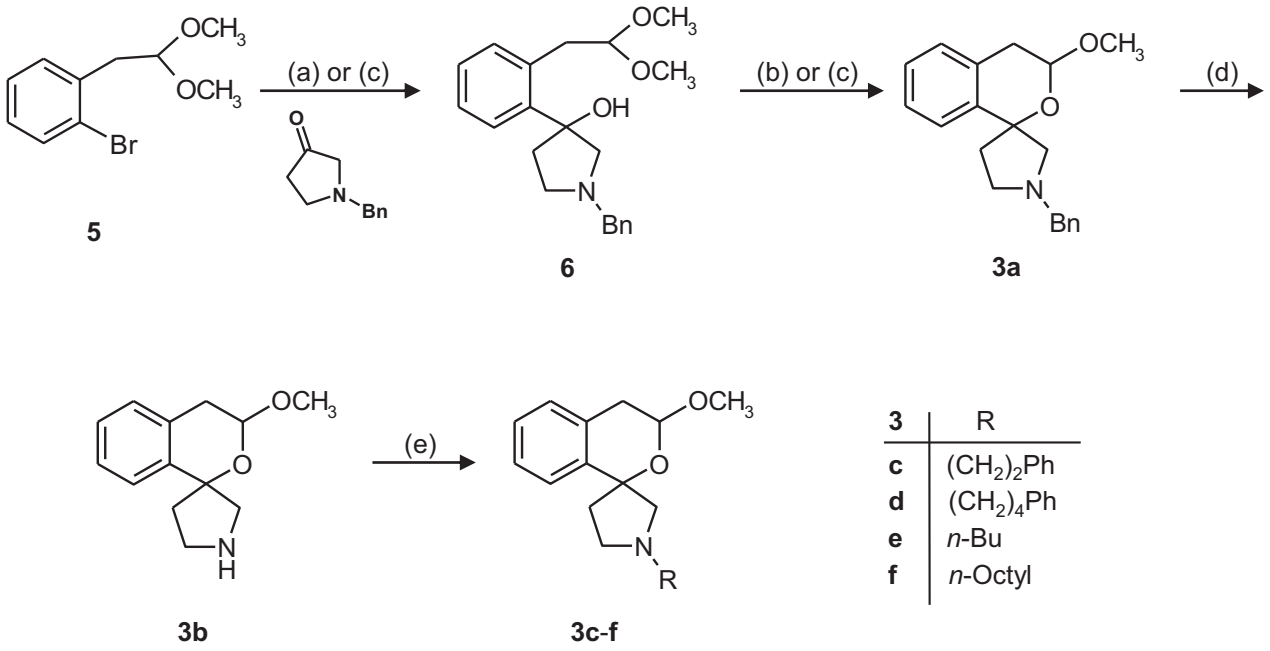
Since the structures of σ and NMDA receptor ligands are sometimes very similar, the affinity of the spirocyclic pyrrolidines **3** and **4** toward the phencyclidine binding site of the NMDA receptor was recorded with pig brain cortex membrane preparations and [^{3}H]-(+)-MK-801 as radioligand [49]. Furthermore, the affinities of **3** and **4** toward the serotonin receptors 5-HT$_6$ and 5-HT$_7$ were recorded.

The hERG (human ether-a-go-go related gene) channel in the heart represents an antitarget for the development of novel drugs. In a fluorescence-based assay the blockade of the hERG channel by the spirocyclic pyrrolidines **3** and **4** was tested [26,50,51]. The remaining hERG channel activity after incubation with 10 μM of test compounds is summarized in Table 1.

4. Results and discussion

4.1. σ_1 Receptor affinity

In Table 1 the σ_1 receptor affinities of the spirocyclic pyrrolidines **3** and **4** are given and compared with those of lead and reference compounds. The spiro[[2]benzopyran-1,3′-pyrrolidine] **3a** with an *N*-benzyl residue displays a 55-fold reduced σ_1 affinity ($K_i = 73$ nM) compared with the σ_1 affinity of the lead compound **1a**. Increasing of the length of the *N*-residue led to the 2-phenylethyl derivative **3c** with increased σ_1 affinity ($K_i = 25$ nM). A further enlargement of the chain length of the *N*-residue reduced the σ_1 affinity to 53 nM (**3d**). Replacement of the *N*-benzyl residue of **3a** with the aliphatic butyl moiety (**3e**) slightly reduced the σ_1 affinity. A similar trend was observed after replacement of the *N*-benzyl moiety of the lead compound **1a** with an *N*-butyl residue (**1b**, $K_i = 2.3$ nM). The most



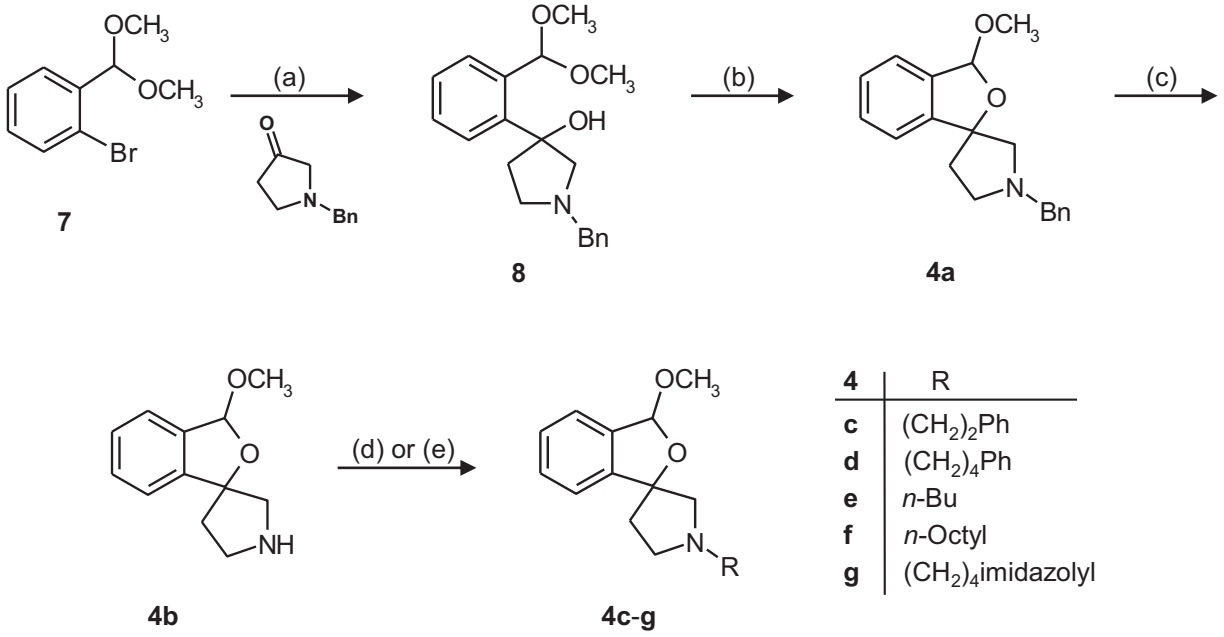
Scheme 1. Synthesis of spiro[[2]benzopyran-1,3′-pyrrolidines] **3**. Reagents and reaction conditions: (a) *n*-BuLi, THF, −78 °C, 2 h, then rt, 16 h, 33%. (b) *p*-TolSO$_3$H 1.2 equiv., H$_3$COH, rt, 4 d, 67%; microwave irradiation 73%. (c) *n*-BuLi, THF, −78 °C, 2 h, then rt, 12 h; HCl 5%, rt, 41%. (d) NH_4HCO_2, Pd/C, CH_3OH, 65 °C, 2 h, 82%. (e) R–Cl or R–Br, K_2CO_3, acetonitrile, microwave irradiation, 45–69%.

potent σ_1 ligand of this series of compounds is the *N*-octyl derivative **3f** ($K_i = 9.4$ nM).

A similar relationship between the σ_1 affinity and the structure of the *N*-residues was observed for the spirocyclic benzofuran derivatives **4**, although the level of σ_1 affinity is considerably higher. In particular the *N*-phenylalkyl (**4a**, **4c**, **4d**) and the *N*-octyl derivatives **4f** represent very potent σ_1 receptor ligands interacting in the low nanomolar range with σ_1 receptors.

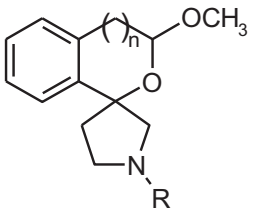
Similar effects of the *N*-residue on the σ_1 affinity have been described for analogous spirocyclic σ_1 receptor ligands [27,28,33,34,39].

In Fig. 2 the σ_1 receptor affinities of the spirocyclic compounds **1a**–**4a** are correlated with the distance of the basic amino group and the benzene moiety of the benzopyran or benzofuran ring (benzene-*N*-distance). The data in Fig. 2 indicate high σ_1 receptor affinity for **1a** with a long benzene-*N*-distance (5.1–5.7 Å).



Scheme 2. Synthesis of spiro[[2]benzofuran-1,3′-pyrrolidines] **4**. Reagents and reaction conditions: (a) *n*-BuLi, THF, −78 °C, 2 h, then rt, 16 h, 51%. (b) *p*-TolSO$_3$H 1.2 equiv., H$_3$COH, microwave irradiation, 92%. (c) NH_4HCO_2, Pd/C, CH_3OH, 65 °C, 2 h, 87%. (d) 1. R–Cl or R–Br, NaI, acetone, microwave irradiation, filtration; 2. **4b**, K_2CO_3, acetone, microwave irradiation, 35–75% (**4c**–**f**). (e) 1-(4-chlorobutyl)imidazole, K_2CO_3, acetonitrile, microwave irradiation, 65%.

Table 1
Affinities of the spirocyclic pyrrolidines **3** and **4** toward σ_1 and σ_2 receptors and inhibition of hERG channel activity.



Compd.	R	*n*	σ_1 $K_i \pm$ SEM (nM)[a]	σ_2 $K_i \pm$ SEM (nM)[a]	Selectivity σ_1/σ_2	hERG (%)[b]
1a [27]	CH_2Ph	–	1.3 ± 0.2	3500	2708	92
1b [30]	*n*-Bu	–	2.3 ± 0.8	1020	443	87
2a [30]	CH_2Ph	–	>1400	>1500	–	n.d.
2b [30]	*n*-Bu	–	1000	>1500	–	n.d.
3a	CH_2Ph	1	73 ± 5.1	>1000	>14	93
3c	$(CH_2)_2Ph$	1	25 ± 4.3	>1000	>40	23
3d	$(CH_2)_4Ph$	1	53 ± 24	428 ± 34	8	24
3e	*n*-Bu	1	104 ± 30	1180	11	98
3f	*n*-Octyl	1	9.4 ± 3.1	290 ± 30	31	6
4a	CH_2Ph	0	25 ± 3.8	>1000	>40	99
4c	$(CH_2)_2Ph$	0	22 ± 4.4	>1000	>45	99
4d	$(CH_2)_4Ph$	0	21 ± 13	127 ± 38	6	6
4e	*n*-Bu	0	55 ± 16	964	18	97
4f	*n*-Octyl	0	8.3 ± 2.6	84 ± 10	10	25
4g	$(CH_2)_4$imidazolyl	0	1520	453 ± 167	0.30	n.d.
(+)-Pentazocine			4.2 ± 1.1	–	–	n.d.
Haloperidol			3.9 ± 1.5	78 ± 2.3	20	n.d.
Di-*o*-tolylguanidine			61 ± 18	42 ± 17	0.7	n.d.

n.d. = not determined.
[a] The standard error of the mean (SEM) is given, when three independent experiments have been performed. For compounds with low affinity (K_i > 1000 nM) the K_i value was recorded only once and, then, a SEM is not given.
[b] Remaining hERG channel activity (%) after incubation with 10 μM test compound.

Decreasing of the benzene-*N*-distance to 4.4–4.9 Å in the regioisomeric piperidine **2a** results in almost complete loss of σ_1 affinity. Increasing of this distance to 4.6–5.0 Å (**3a**) and 4.8–5.1 Å (**4a**) led to considerably increased σ_1 affinities with K_i = 73 nM and K_i = 25 nM, respectively.

4.2. Receptor selectivity

In addition to the σ_1 affinity the selectivity of this new class of potent σ_1 ligands toward related receptor systems was investigated. At first the σ_2 affinity was recorded. Table 1 clearly demonstrates low σ_2 affinity of the spirocyclic pyrrolidines **3** and **4** indicating high selectivity for the σ_1 over the σ_2 subtype. However, large residues at the pyrrolidine *N*-atom like a 4-phenylbutyl (**3d**, **4d**) or octyl (**3f**, **4f**) residue increased the σ_2 affinity and thus reduced the σ_1/σ_2 selectivity. This tendency is in good accordance with observations made with analogous spirocyclic σ_1 ligands [27,28,33,34,39].

The spirocyclic pyrrolidine **4g** with a 4-imidazolylbutyl residue at the pyrrolidine *N*-atom shows special properties, since it is the only compound of this series with a preference for the σ_2 subtype. Replacement of the phenyl moiety of the 4-phenylbutyl derivative **4d** with the heterocyclic imidazolyl residue (**4g**) led to a slightly reduced σ_2 affinity (K_i = 453 nM), but a strongly reduced σ_1 affinity (K_i = 1520 nM).

The affinity of the spirocyclic pyrrolidines **3** and **4** toward the phencyclidine binding site of the NMDA receptor, the 5-HT_6 and 5-HT_7 receptors was recorded in competition experiments with radioligands. At test compound concentrations of 10 μM (NMDA assay) and 100 nM (5-HT assays) the inhibition of the radioligand binding was always lower than 40% indicating rather low affinities (IC_{50} > 10 μM for NMDA receptor, IC_{50} > 100 nM for 5-HT receptors).

4.3. Inhibition of the hERG channel

The hERG (human ether-a-go-go related gene) channel is a voltage gated K^+ channel in the heart. Blockade of this channel prolongs the QT interval in the electrocardiogram. Drugs blocking the hERG channel in the heart increase the probability of ventricular arrhythmia, which may degenerate into ventricular fibrillation and acute sudden death. Therefore the inhibition of the hERG channel by new drug candidates should be taken into account at a very early stage of drug development [52].

At a concentration of 10 μM only the *N*-benzyl (**3a**) and *N*-butyl (**3e**) derivatives of the spirocyclic benzopyran series **3** did not influence the K^+-ion permeability through the hERG channel (Table 1). Compounds with larger *N*-substituents (**3c**, **3d**, **3f**) led to a reduced permeability of 6–24%.

However, the spirocyclic benzofuran derivatives **4**, which are more potent σ_1 receptor ligands than the benzopyran derivatives **3**, showed reduced interactions with the hERG channel. With exception of **4f** with the very large *N*-octyl residue, the spirocyclic benzofurans **4** did not block the hERG channel permeability at a concentration of 10 μM.

5. Conclusion

The σ_1 affinity of the spirocyclic compounds increases in the order **2** < **3** < **4** < **1**. This order correlates nicely with the distance between the basic amino moiety and the benzene ring of the benzopyran/benzofuran system: **2** < **3** < **4** < **1**. The *N*-benzyl moiety represents the most promising *N*-substituent in terms of σ_1 affinity, σ_1/σ_2-selectivity and potential hERG channel inhibition. Although replacement of the benzyl moiety with larger phenylalkyl or linear aliphatic octyl residue increased the σ_1 affinity, these modifications also led to reduced σ_1/σ_2-selectivity and to potential heart problems via hERG channel inhibition. Altogether the spiro[[2] benzofuran-1,3′-pyrrolidine] **4a** is a potent σ_1 ligand (K_i = 25 nM) with high selectivity against the σ_2 subtype (>40-fold), the phencyclidine binding site of the NMDA receptor and 5-HT_6 and 5-HT_7 receptors. Moreover, inhibition of the hERG channel in the heart was excluded.

6. Experimental

6.1. Chemistry, general methods

Unless otherwise noted, moisture sensitive reactions were conducted under dry nitrogen. THF was distilled from sodium/benzophenone ketyl prior to use. CH_3OH was distilled from magnesium/iodine prior to use. Microwave irradiation: discover synthesis microwave apparatus (CEM); the following parameters are given in parenthesis: program; max. power; max. pressure; temperature; the reaction time is divided into ramp time, hold time and cool off time. 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. Elemental analyses: Vario EL (Elementaranalysesysteme GmbH). MS: MAT GCQ (Thermo Finnigan); mode of ionization: electron impact (EI). 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

and the assignments of ^1H and ^{13}C NMR signals were supported by 2D NMR techniques (COSY, HETCOR). Compound **5** was prepared according to Ref. [47].

6.2. 2-(1-Benzyl-3-hydroxypyrrolidin-3-yl)phenylacetaldehyde dimethyl acetal (**6**)

Under N_2 bromoacetal **5** (252 mg, 1.03 mmol) was dissolved in THF (4.5 mL) and the solution was cooled to −78 °C. A 1.6 M solution of *n*-BuLi in *n*-hexane (0.75 mL, 1.2 mmol) was added slowly. After 20 min at −78 °C, a solution of 1-benzylpyrrolidin-3-one (178 mg, 1.1 mmol) in THF (2 mL) was added dropwise and the mixture was stirred at −78 °C for 2 h. After stirring at rt for another 16 h a saturated NH_4Cl solution was added. The organic layer was separated, washed with $NaHSO_3$ solution (10%), dried (Na_2SO_4) and filtered. The solvent was removed in vacuo, and the residue was purified by fc (2 cm, petroleum ether/ethyl acetate 1:2, 10 mL, $R_f = 0.35$). Pale yellow oil, yield 116 mg (33%). $C_{21}H_{27}NO_3$ (341.5). MS (EI): $m/z = 327$ [$MH^+ - CH_3$], 248 [$M^+ - OH_2$, $-CH(OCH_3)_2$]. IR: $\tilde{\nu}$ (cm^{-1}) = 2924 (ν C–H). ^1H NMR ($CDCl_3$): δ (ppm) = 1.52–1.77 (m, 4H, CH_2CH_2N), 2.45–2.57 (m, 1H, $(CH_2)_2NCH_2$), 2.90–3.03 (m, 1H, $(CH_2)_3NCH_2$), 3.11–3.19 (m, 2H, $ArCH_2C$–H), 3.30 (s, 3H, $CHOCH_3$), 3.34 (s, 3H, $CHOCH_3$), 3.60–3.74 (m, 2H, NCH_2Ph), 4.58–4.62 (m, 1H, $ArCH_2CH$), 7.18–7.21 (m, 9H, arom. H). A signal for the OH proton is not seen in the spectrum.

6.3. cis/trans-1′-Benzyl-3-methoxy-3,4-dihydrospiro[[2]benzopyran-1,3′-pyrrolidine] (**3a**)

Under N_2 bromoacetal **5** (1.18 g, 4.82 mmol) was dissolved in THF (15 mL) and cooled to −78 °C. A 1.6 M solution of *n*-BuLi in *n*-hexane (3.7 mL, 5.92 mmol) was added slowly. After 20 min at −78 °C, a solution of 1-benzylpyrrolidin-3-one (846 mg, 5.3 mmol) in THF (10 mL) was added dropwise and the mixture was stirred at −78 °C for 2 h and at rt for 12 h. A saturated NH_4Cl solution was added, the mixture was extracted with Et_2O (6×), and the Et_2O layer was washed with H_2O (2×). The organic layer was extracted with aqueous HCl (5%, 6×) and the acidic aqueous layer was washed with Et_2O (2×). Solid KOH was added to the aqueous layer (pH > 9). Then it was extracted with Et_2O (6×). The organic layer was washed with H_2O (2×) and with $NaHSO_3$ solution (10%, 2×), then it was dried (Na_2SO_4), filtered, the filtrate was concentrated in vacuo and the residue was purified by fc (5 cm, CH_2Cl_2:methanol = 98:2, 30 mL, $R_f = 0.41$). Pale yellow oil, yield 617 mg (41%). $C_{20}H_{23}NO_2$ (309.4). Anal. calcd. C 77.64 H 7.49 N 4.53 found C 77.34 H 7.57 N 4.37. MS (EI): m/z = 309 [M^+], 278 [$M^+ - OCH_3$], 249 [$M^+ - OCHOCH_3$]. IR: $\tilde{\nu}$ (cm^{-1}) = 2910 (ν C–H), 1075 (ν C–O), 754, 697 (δ C–H monosubst. benzene). ^1H NMR ($CDCl_3$): δ (ppm) = 2.13–2.24 (m, 0.5H, CH_2CH_2N (*cis* or *trans*)), 2.32–2.45 (m, 1.5H, CH_2CH_2N (*cis*, *trans*)), 2.71–2.80 (q, $J = 7.2$ Hz, 0.5H, $CH_2CH_2NCH_2$ (*cis* or *trans*)), 2.87–3.05 (m, 5H, $CH_2CH_2NCH_2$ (1.5H; *cis*, *trans*), $CH_2CH_2NCH_2$ (2H), $ArCH_2CHOCH_3$ (1.5H; *cis*, *trans*)), 3.10–3.18 (m, 0.5H, $ArCH_2CHOCH_3$ (*cis* or *trans*)), 3.50, 3.54 (2s, together 3H, OCH_3 (*cis*, *trans*)), 3.69–3.80 (m, 2H, NCH_2Ph), 4.73, 4.82 (2 t, $J = 5.2$ Hz, $J = 3.3$ Hz, together 1H, CH_2CHOCH_3 (*cis*, *trans*)), 7.03 (d, $J = 7.2$ Hz, 1H, arom. H), 7.13–7.48 (m, 8H, arom. H). Ratio of diastereomers 52:48.

6.4. cis/trans-3-Methoxy-3,4-dihydrospiro[[2]benzopyran-1,3′-pyrrolidine] (**3b**)

Under N_2 a mixture of **3a** (1.04 g, 3.38 mmol), ammonium formate (1.09 g, 17.3 mmol), Pd/C (10%, 213 mg) and methanol (20 mL) was heated to reflux for 2 h. Then it was filtered through Celite®, the solvent was removed in vacuo and the residue was purified by fc (4 cm, methanol/ammonia 98:2, 30 mL, $R_f = 0.48$). Colorless oil, which solidified upon storage at 4 °C, yield 604 mg (82%). $C_{13}H_{17}NO_2$ (219.3). MS (EI): m/z = 219 [M^+], 188 [$M^+ - OCH_3$], 159 [$M^+ - OCHOCH_3$], 145 [$M^+ - OCH_3$, $-NH(CH_2)_2$]. IR: $\tilde{\nu}$ (cm^{-1}) = 2939/2873 (ν C–H), 1073 (ν C–O), 754 (δ C–H). ^1H NMR ($CDCl_3$): δ (ppm) = 2.03–2.41 (m, 2H, CH_2CH_2N), 2.59 (s, 1H, CH_2NHCH_2), 2.88–3.01 (m, 2.5H, CH_2CH_2N (2H), $ArCH_2CHOCH_3$ (0.5H; *cis* or *trans*)), 3.13 (d, $J = 12.5$ Hz, 0.5H, $ArCH_2CHOCH_3$ (*cis* or *trans*)), 3.20–3.30 (m, 1H, $CH_2CH_2NCH_2$), 3.32–3.43 (m, 2H, $ArCH_2CHOCH_3$ (1H; *cis* or *trans*), $CH_2CH_2NCH_2$ (1H)), 3.52/3.53 (2s, together 3H, OCH_3 (*cis*, *trans*)), 4.77–4.85 (m, 1H, $ArCH_2CHOCH_3$), 7.09–7.26 (m, 4H, arom. H). Ratio of diastereomers 57:43.

6.5. cis/trans-3-Methoxy-1′-(2-phenylethyl)-3,4-dihydrospiro[[2]benzopyran-1,3′-pyrrolidine] (**3c**)

1-Chloro-2-phenylethane (57.0 mg, 0.41 mmol) and K_2CO_3 (298 mg, 2.16 mmol) were added to a solution of the secondary amine **3b** (58.6 mg, 0.27 mmol) in acetonitrile (10 mL). The mixture was filled into a 10 mL-microwave pressure vial and irradiated with microwaves (program: standard; max. power 180 W; max. pressure 5 bar; temperature 140 °C; time program: 5 min ramp time; 25 min hold time; 5 min cool off time). The mixture was filtered through Celite®, washed with CH_2Cl_2, concentrated in vacuo and the residue was purified by fc (1 cm, petroleum ether/ethyl acetate 7:3, 5 mL, $R_f = 0.22$). Pale yellow oil, yield 40 mg (45%). $C_{21}H_{25}NO_2$ (323.4). Anal. calcd. C 77.99 H 7.79 N 4.33 found C 77.73 H 7.85 N 4.24. MS (EI): m/z = 292 [$M^+ - OCH_3$], 232 [$M^+ - CH_2Ph$]. IR: $\tilde{\nu}$ (cm^{-1}) = 2939 (ν C–H), 1076 (ν C–O), 752/698 (δ C–H monosubst. benzene). ^1H NMR ($CDCl_3$): δ (ppm) = 2.11–2.20 (m, 0.5H, CH_2CH_2N (*cis* or *trans*)), 2.29–2.46 (m, 1.5H, CH_2CH_2N (*cis*, *trans*)), 2.77–3.06 (m, 9H, $N(CH_2)_2Ph$ (4H), $CH_2CH_2NCH_2$ (4H), $ArCH_2CHOCH_3$ (1.5H; *cis*, *trans*)), 3.19–3.24 (m, 0.5H, $ArCH_2CHOCH_3$), 3.53/3.54 (2s, together 3H, OCH_3 (*cis*, *trans*)), 4.80–4.85 (m, 1H, $ArCH_2CHOCH_3$), 7.03–7.08 (m, 1H, arom. H), 7.15–7.39 (m, 8H, arom. H). Ratio of diastereomers 52:48.

6.6. cis/trans-3-Methoxy-1′-(4-phenylbutyl)-3,4-dihydrospiro[[2]benzopyran-1,3′-pyrrolidine] (**3d**)

A mixture of the secondary amine **3b** (58.0 mg, 0.26 mmol), 1-chloro-4-phenylbutane (60.7 mg, 0.36 mmol), K_2CO_3 (298 mg, 2.16 mmol) and acetonitrile (2 mL) was filled into a 10 mL-microwave pressure vial and irradiated with microwaves (program: standard; max. power 180 W; max. pressure 5 bar; temperature 140 °C; time program: 5 min ramp time; 25 min hold time; 5 min cool off time). The mixture was filtered through Celite®, washed with CH_2Cl_2, concentrated in vacuo and the residue was purified by fc (1 cm, petroleum ether/ethyl acetate 6:4, 5 mL, $R_f = 0.38$). Pale yellow oil, yield 60 mg (56%). $C_{23}H_{29}NO_2$ (351.5). Anal. calcd. C 78.60 H 8.32 N 3.98 found C 78.13 H 8.36 N 3.95. MS: m/z = 351 [M^+], 320 [$M^+ - OCH_3$], 232 [$M^+ - (CH_2)_3Ph$], 201 [$M^+ - OCH_3$, $-(CH_2)_3Ph$]. IR: $\tilde{\nu}$ (cm^{-1}) = 2933 (ν C–H), 1076 (ν C–O), 748/698 (δ C–H monosubst. benzene). ^1H NMR ($CDCl_3$): δ (ppm) = 1.49–1.53 (m, 2H, $NCH_2CH_2CH_2CH_2Ph$ (2H)), 1.62–1.66 (m, 2H, $NCH_2CH_2CH_2CH_2Ph$ (2H)), 2.03–2.10 (m, 0.5H, CH_2CH_2N (*cis* or *trans*)), 2.20–2.38 (m, 1.5H, CH_2CH_2N (*cis*, *trans*)), 2.47–2.51 (m, 2H, $N(CH_2)_3CH_2Ph$), 2.55–2.59 (m, 2H, CH_2CH_2N), 2.61–2.67 (m, 0.5H, $NCH_2(CH_2)_3Ph$ (*cis* or *trans*)), 2.75–2.92 (m, 5H, $NCH_2(CH_2)_3Ph$ (1.5H; *cis*, *trans*), $ArCH_2CHOCH_3$ (2H), $CH_2CH_2NCH_2$ (1.5H; *cis*, *trans*)), 3.05–3.09 (m, 0.5H, $CH_2CH_2NCH_2$ (*cis* or *trans*)), 3.46 (s, 3H, OCH_3), 4.70–4.78 (m, 1H, $ArCH_2CHOCH_3$), 6.95–7.02 (m, 1H, arom. H), 7.08–7.38 (m, 8H, arom. H). Ratio of diastereomers 54:46.

6.7. cis/trans-1′-Butyl-3-methoxy-3,4-dihydrospiro[[2]benzopyran-1,3′-pyrrolidine] (**3e**)

1-Bromobutane (88.0 mg, 0.64 mmol) and K_2CO_3 (483 mg, 3.50 mmol) were added to a solution of the secondary amine **3b** (92.3 mg, 0.42 mmol) in acetonitrile (3 mL). The mixture was filled into a 10 mL-microwave pressure vial and irradiated with microwaves (program: standard; max. power 180 W; max. pressure 5 bar; temperature 140 °C; time program: 5 min ramp time; 40 min hold time; 5 min cool off time). The mixture was filtered through Celite®, washed with CH_2Cl_2, concentrated in vacuo and the residue was purified by fc (1 cm, petroleum ether/ethyl acetate 7:3, 5 mL, $R_f = 0.31$). Pale yellow oil, yield 76 mg (69%). $C_{17}H_{25}NO_2$ (275.4). Anal. calcd. C 74.14 H 9.15 N 5.09 found C 74.10 H 9.60 N 4.60. MS (EI): $m/z = 275$ [M^+], 244 [$M^+ - OCH_3$], 232 [$M^+ - (CH_2)_2CH_3$], 201 [$M^+ - OCH_3$, $-(CH_2)_2CH_3$]. IR: $\tilde{\nu}$ (cm^{-1}) = 2929 (ν C—H), 1076 (ν C—O), 758 (δ C—H). ^{1}H NMR ($CDCl_3$): δ (ppm) = 0.85, 0.87 (2 t, $J = 7.2$ Hz, together 3H, $N(CH_2)_3CH_3$ (*cis*, *trans*)), 1.31 (m, 2H, $N(CH_2)_2CH_2CH_3$), 1.43–1.47 (m, 2H, $NCH_2CH_2CH_2CH_3$), 2.03–2.12 (m, 0.5H, CH_2CH_2N (*cis* or *trans*)), 2.21–2.36 (m, together 1.5H, CH_2CH_2N (*cis*, *trans*)), 2.44–2.51 (m, 2H, CH_2CH_2N), 2.62–2.70 (m, 0.5H, $NCH_2(CH_2)_2CH_3$), 2.78–2.95 (m, together 5H, $NCH_2(CH_2)_2CH_3$ (1.5H; *cis*, *trans*), $ArCH_2CHOCH_3$ (2H), $CH_2CH_2NCH_2$ (1.5H; *cis*, *trans*)), 3.08–3.13 (m, 0.5H, $CH_2CH_2NCH_2$ (*cis* or *trans*)), 3.47 (s, 3H, OCH_3), 4.72–4.76 (m, 1H, $ArCH_2CHOCH_3$), 6.98–7.00 (m, 1H, arom. H), 7.09–7.35 (m, 3H, arom. H). Ratio of diastereomers 52:48.

6.8. cis/trans-3-Methoxy-1′-octyl-3,4-dihydrospiro[[2]benzopyran-1,3′-pyrrolidine] (**3f**)

The secondary amine **3b** (91.0 mg, 0.42 mmol) was dissolved in acetonitrile (3 mL). 1-Bromooctane (111 mg, 0.57 mmol) and K_2CO_3 (452 mg, 3.28 mmol) were added, the mixture was filled into a 10 mL-microwave pressure vial and the mixture was irradiated with microwaves (program: standard; max. power 180 W; max. pressure 5 bar; temperature 140 °C; time program: 5 min ramp time; 40 min hold time; 5 min cool off time). The mixture was filtered through Celite®, washed with CH_2Cl_2, concentrated in vacuo and the residue was purified by fc (1 cm, petroleum ether/ethyl acetate 6:4, 5 mL, $R_f = 0.33$). Pale yellow oil, yield 85 mg (64%). $C_{21}H_{33}NO_2$ (331.5). Anal. calcd, C 76.09 H 10.03 N 4.23 found C 76.42 H 10.05 N 4.17. MS (EI): $m/z = 331$ [M^+], 300 [$M^+ - OCH_3$], 232 [$M^+ - (CH_2)_6CH_3$], 201 [$M^+ - OCH_3$, $-(CH_2)_6CH_3$]. IR: $\tilde{\nu}$ (cm^{-1}) = 2924 (ν C—H), 1077 (ν C—O), 757 (δ C—H). ^{1}H NMR ($CDCl_3$): δ (ppm) = 0.86, 0.89 (2 t, $J = 6.6$ Hz, together 3H, CH_2CH_3 (*cis*, *trans*)), 1.22–1.40 (m, 10H, $CH_2(CH_2)_5CH_3$), 1.48–1.58 (m, 2H, $CH_2(CH_2)_5CH_3$), 2.10–2.19 (m, 0.5H, CH_2CH_2N (*cis* or *trans*)), 2.28–2.43 (m, 1.5H, CH_2CH_2N (*cis*, *trans*)), 2.53–2.55 (m, 2H, CH_2CH_2N), 2.69–2.77 (m, 0.5H, $NCH_2(CH_2)_6CH_3$), 2.83–3.02 (m, 5H, $NCH_2(CH_2)_6CH_3$ (1.5H), $ArCH_2CHOCH_3$ (2H), $CH_2CH_2NCH_2$ (1.5H)), 3.14–3.19 (m, 0.5H, $CH_2CH_2NCH_2$ (*cis* or *trans*)), 3.54 (s, 3H, OCH_3), 4.79–4.83 (m, 1H, $ArCH_2CHOCH_3$), 7.03–7.08 (m, 1H, arom. H), 7.16–7.42 (m, 3H, arom. H). Ratio of diastereomers 51:49.

6.9. 2-(1-Benzyl-3-hydroxypyrrolidin-3-yl)benzaldehyde dimethyl acetal (**8**)

Under N_2 2-bromobenzaldehyde dimethyl acetal (**7**, 2.31 g, 10.0 mmol) was dissolved in THF (22 mL) and cooled to −78 °C. A 1.6 M solution of *n*-BuLi in *n*-hexane (7.5 mL, 12.0 mmol) was added slowly and the mixture was stirred at −78 °C for 20 min. A solution of 1-benzylpyrrolidin-3-one (1.75 g, 5.0 mmol) in THF (12 mL) was added dropwise and the mixture was stirred at −78 °C for 2 h and at rt for 16 h. After addition of water, the mixture was extracted with CH_2Cl_2. The combined organic layer was extracted with $NaHSO_3$ solution (10%, 2×), dried (Na_2SO_4), filtered, concentrated in vacuo and the residue was purified by fc (6 cm, petroleum ether/ethyl acetate = 1:2, 30 mL, $R_f = 0.38$). Pale yellow oil, yield 1.65 g (51%). $C_{20}H_{25}NO_3$ (327.4). MS (EI): $m/z = 327$ [M^+], 312 [$M^+ - CH_3$], 296 [$M^+ - OCH_3$]. IR: $\tilde{\nu}$ (cm^{-1}) = 3437 (ν —OH), 2929/2799 (ν C—H), 1070 (ν C—O), 752/698 (δ C—H monosubst. benzene). ^{1}H NMR ($CDCl_3$): δ (ppm) = 2.28–2.42 (m 2H, CH_2CH_2N), 2.60–2.68 (m, 1H, $CH_2CH_2NCH_2$), 3.00–3.09 (m, 3H, $CH_2CH_2NCH_2$ (2H), $CH_2CH_2NCH_2$ (1H)), 3.33 (s, 3H, $CH(OCH_3)_2$), 3.37 (s, 3H, $CH(OCH_3)_2$), 3.77 (s, 2H, NCH_2Ph), 5.98 (s, 1H, $ArCH(OCH_3)_2$), 7.26–7.39 (m, 8H, arom. H), 7.68–7.71 (m, 1H, arom. H). A signal for the OH proton is not seen in the spectrum.

6.10. cis/trans-1′-Benzyl-3-methoxy-3H-spiro[[2]benzofuran-1,3′-pyrrolidine] (**4a**)

Hydroxyacetal **8** (476 mg, 1.45 mmol) and *p*-toluenesulfonic acid (360 mg, 1.89 mmol) were dissolved in methanol (3 mL). The solution was filled into a 10 mL-microwave pressure vial and irradiated with microwaves (program: standard; max. power 220 W; max. pressure 2 bar; temperature 80 °C; time program: 5 min ramp time; 15 min hold time; 5 min cool off time). Then CH_2Cl_2 was added and the mixture was washed with 2 M NaOH. The organic layer was dried (Na_2SO_4), filtered, concentrated in vacuo and the residue was purified by fc (3 cm, petroleum ether/ethyl acetate 3:1, 20 mL, $R_f = 0.31$). Pale yellow oil, yield 380 mg (92%). $C_{19}H_{21}NO_2$ (295.4). Anal. calcd. C 77.26 H 7.17 N 4.64 found C 77.03 H 7.11 N 4.74. MS (EI): $m/z = 295$ [M^+], 264 [$M^+ - OCH_3$], 131 [$M^+ - OCH_3$, $-(CH_2)_2NBn$]. IR: $\tilde{\nu}$ (cm^{-1}) = 2905 (ν C—H), 1081 (ν C—O), 750/697 (δ C—H monosubst. benzene). ^{1}H NMR ($CDCl_3$): δ (ppm) = 2.24–2.35 (m, 2H, CH_2CH_2N), 2.78–2.98 (m, 4H, $CH_2CH_2NCH_2$), 3.43/3.46 (2s, together 3H, OCH_3 (*cis*, *trans*)), 3.66–3.77 (m, 2H, NCH_2Ph), 6.06/6.07 (2s, together 1H, $ArCHOCH_3$ (*cis*, *trans*)), 7.23–7.40 (m, 9H, arom. H). Ratio of diastereomers 51:49.

6.11. cis/trans-3-Methoxy-3H-spiro[[2]benzofuran-1,3′-pyrrolidine] (**4b**)

Under N_2 a mixture of **4a** (856 mg, 2.90 mmol), ammonium formate (914 mg, 14.5 mmol), 10% Pd/C (178 g) and methanol (45 mL) was heated to reflux for 2 h. Then it was filtered through Celite®, the solvent was removed in vacuo and the residue was purified by fc (3 cm, methanol/ammonia 98:2, 20 mL, $R_f = 0.48$). Colorless oil, which solidified upon storage at 4 °C, yield 520 mg (87%). $C_{12}H_{15}NO_2$ (205.3). MS (EI): $m/z = 205$ [M^+], 174 [$M^+ - OCH_3$], 131 [$M^+ - OCH_3$, $-(CH_2)_2NH$]. IR: $\tilde{\nu}$ (cm^{-1}) = 2932/2878 (ν C—H), 1081 (ν C—O), 750 (δ C—H). ^{1}H NMR ($CDCl_3$): δ (ppm) = 2.12–2.33 (m, 2H, CH_2CH_2N), 3.00–3.50 (m, 5H, CH_2NHCH_2), 3.43/3.48 (2s, together 3H, OCH_3 (*cis*, *trans*)), 6.05/6.10 (2s, together 1H, $ArCHOCH_3$ (*cis*, *trans*)), 7.20–7.27 (2 d, $J = 7.4$ Hz, together 1H, arom. H (*cis*, *trans*)), 7.32–7.43 (m, 3H, arom. H). Ratio of diastereomers 52:48.

6.12. cis/trans-3-Methoxy-1′-(2-Phenylethyl)-3H-spiro[[2]benzofuran-1,3′-pyrrolidine] (**4c**)

A solution of 1-chloro-2-phenylethane (210 mg, 1.50 mmol) and NaI (152 mg, 1.80 mmol) in acetone (3 mL) was filled into a 10 mL-microwave pressure vial and irradiated with microwaves (program: standard; max. power 180 W; max. pressure 4 bar; temperature 90 °C; time program: 5 min ramp time; 10 min hold time; 5 min cool off time). The precipitate (NaCl) was filtered off and the clear solution containing 1-iodo-2-phenylethane was treated with **4b** (205 mg, 1.0 mmol), K_2CO_3 (1.10 g, 8.0 mmol) and a small amount of acetone (1 mL). The mixture was irradiated with microwaves

(program: standard; max. power 180 W; max. pressure 4 bar; temperature 90 °C; time program: 5 min ramp time; 30 min hold time; 5 min cool off time). After filtration through Celite® (CH_2Cl_2), the solvent was removed in vacuo and the residue was purified by fc (3 cm, cyclohexane/ethyl acetate 7:3, 20 mL, R_f = 0.28). Pale yellow oil, yield 171 mg (55%). $C_{20}H_{23}NO_2$ (309.4). Anal. calcd. C 77.24 H 7.56 N 4.47 found C 77.64 H 7.49 N 4.53. MS (EI): m/z = 278 [$M^+ - OCH_3$], 218 [$M^+ - CH_2Ph$]. IR: $\tilde{\nu}$ (cm^{-1}) = 2927 (ν C–H), 1081 (ν C–O), 749/698 (δ C–H monosubst. benzene). ^{1}H NMR ($CDCl_3$): δ (ppm) = 2.29–2.36 (m, 2H, CH_2CH$_2$N), 2.86–3.03 (m, 4H, CH$_2$CH_2NCH_2), 3.03–3.12 (m, 4H, NCH_2CH_2Ph), 3.48/3.49 (2s, together 3H, OCH_3 (*cis*, *trans*)), 6.07/6.09 (2s, together 1H, ArC*H*-OCH$_3$ (*cis*, *trans*)), 7.20–7.40 (m, 9H, arom. H). Ratio of diastereomers 53:47.

6.13. *cis/trans-3-Methoxy-1′-(4-phenylbutyl)-3H-spiro[[2]benzofuran-1,3′-pyrrolidine]* (**4d**)

A solution of 1-chloro-4-phenylbutane (253 mg, 1.50 mmol) and NaI (152 mg, 1.80 mmol) in acetone (3 mL) was filled into a 10 mL-microwave pressure vial and irradiated with microwaves (program: standard; max. power 180 W; max. pressure 4 bar; temperature 90 °C; time program: 5 min ramp time; 10 min hold time; 5 min cool off time). The precipitate (NaCl) was filtered off and the clear solution containing 1-iodo-4-phenylbutane was treated with **4b** (205 mg, 1.0 mmol) and K_2CO_3 (1.10 g, 8.0 mmol) and a small amount of additional acetone (1 mL). The mixture was irradiated with microwaves (program: standard; max. power 180 W; max. pressure 4 bar; temperature 90 °C; time program: 5 min ramp time; 30 min hold time; 5 min cool off time). After filtration through Celite® (CH_2Cl_2), the solvent was removed in vacuo and the residue was purified by (3 cm, cyclohexane/ethyl acetate 7:3, 20 mL, R_f = 0.29). Pale yellow oil, yield 253 mg (75%). $C_{22}H_{27}NO_2$ (337.5). Anal. calcd. C 78.30 H 8.06 N 4.15 found C 77.92 H 8.12 N 4.02. MS (EI): m/z = 337 [M^+], 306 [$M^+ - OCH_3$], 218 [$M^+ - (CH_2)_3Ph$]. IR: $\tilde{\nu}$ (cm^{-1}) = 2932 (ν C–H), 1082 (ν C–O), 747/698 (δ C–H monosubst. benzene). ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.51–1.53 (m, 2H, NCH$_2$CH_2(CH$_2$)$_2$Ph), 1.60–1.63 (m, 2H, N(CH$_2$)$_2$CH_2CH$_2$Ph), 2.15–2.30 (m, 2H, CH_2CH$_2$N), 2.49–2.59 (m, 4H, CH$_2$CH_2NCH_2), 2.60–2.91 (m, 4H, NCH_2(CH$_2$)$_2$CH_2Ph), 3.39/3.40 (2s, together 3H, OCH_3 (*cis*, *trans*)), 5.98/6.00 (2s, together 1H, ArC*H*OCH$_3$ (*cis*, *trans*)), 7.10–7.32 (m, 9H, arom. H). Ratio of diastereomers 51:49.

6.14. *cis/trans-1′-Butyl-3-methoxy-3H-spiro[[2]benzofuran-1,3′-pyrrolidine]* (**4e**)

A solution of 1-bromobutane (205 mg, 1.50 mmol) and NaI (152 mg, 1.80 mmol) in acetone (3 mL) was filled into a 10 mL-microwave pressure vial and irradiated with microwaves (program: standard; max. power 180 W; max. pressure 4 bar; temperature 90 °C; time program: 5 min ramp time; 10 min hold time; 5 min cool off time). The precipitate (NaBr) was filtered off and the clear solution containing 1-iodobutane was treated with **4b** (205 mg, 1.0 mmol), K_2CO_3 (1.10 g, 8.0 mmol) and acetone (1 mL). The mixture was irradiated with microwaves (program: standard; max. power 180 W; max. pressure 4 bar; temperature 90 °C; time program: 5 min ramp time; 30 min hold time; 5 min cool off time). After filtration through Celite® (CH_2Cl_2), the solvent was removed in vacuo and the residue was purified by fc (3 cm, cyclohexane/ethyl acetate 5:5, 20 mL, R_f = 0.19). Pale yellow oil, yield 128 mg (49%). $C_{16}H_{23}NO_2$ (261.4). Anal. calcd. C 73.66 H 8.89 N 5.04 found C 73.53 H 8.87 N 5.36. MS (EI): m/z = 261 [M^+], 230 [$M^+ - OCH_3$], 218 [$M^+ - (CH_2)_2CH_3$]. IR: $\tilde{\nu}$ (cm^{-1}) = 2929 (ν C–H), 1085 (ν C–O), 754 (δ C–H). ^{1}H NMR ($CDCl_3$): δ (ppm) = 0.92/0.93 (2 t, J = 3.5 Hz, together 3H, CH$_2$CH_3 (*cis*, *trans*)), 1.25–1.41 (m, 2H, CH$_2$CH_2CH$_3$ (*cis*, *trans*)), 1.44–1.60 (m, 2H, CH_2CH$_2$CH$_3$), 2.27 (t, J = 7.0 Hz, 2H, CH_2CH$_2$N), 2.50–2.60 (m, 2H, CH$_2$CH_2NCH$_2$), 2.75–2.87 (m, 1H, NCH_2(CH$_2$)$_2$CH$_3$), 2.93–3.03 (m, 3H, NCH_2(CH$_2$)$_2$CH$_3$ (1H), CH$_2$CH$_2$NCH_2 (2H)), 3.46/3.48 (2s, together 3H, OCH_3 (*cis*, *trans*)), 6.05/6.07 (2s, together 1H, ArC*H*OCH$_3$ (*cis*, *trans*)), 7.32–7.39 (m, 4H, arom. H). Ratio of diastereomers 52:48.

6.15. *cis/trans-3-Methoxy-1′-octyl-3H-spiro[[2]benzofuran-1,3′-pyrrolidine]* (**4f**)

A solution of 1-bromooctane (289 mg, 1.50 mmol) and NaI (152 mg, 1.80 mmol) in acetone (3 mL) was filled into a 10 mL-microwave pressure vial and irradiated with microwaves (program: standard; max. power 180 W; max. pressure 4 bar; temperature 90 °C; time program: 5 min ramp time; 10 min hold time; 5 min cool off time). The precipitate (NaBr) was filtered off and the clear solution containing 1-iodooctane was treated with **4b** (205 mg, 1.0 mmol), K_2CO_3 (1.10 g, 8.0 mmol) and acetone (1 mL). The mixture was irradiated with microwaves (program: standard; max. power 180 W; max. pressure 4 bar; temperature 90 °C; time program: 5 min ramp time; 30 min hold time; 5 min cool off time). After filtration through Celite® (CH_2Cl_2), the solvent was removed in vacuo and the residue was purified by fc (3 cm, cyclohexane/ethyl acetate 7:3, 20 mL, R_f = 0.36). Pale yellow oil, yield 111 mg (35%). $C_{20}H_{31}NO_2$ (317.5). Anal. calcd. C 75.67 H 9.84 N 4.41 found C 75.03 H 10.01 N 4.26. MS (EI): m/z = 317 [M^+], 286 [$M^+ - OCH_3$], 218 [$M^+ - (CH_2)_6CH_3$]. IR: $\tilde{\nu}$ (cm^{-1}) = 2925 (ν C–H), 1082 (ν C–O), 752 (δ C–H). ^{1}H NMR ($CDCl_3$): δ (ppm) = 0.85–0.89 (2 t, J = 6.7 Hz, together 3 H, CH$_2$CH_3 (*cis*, *trans*)), 1.22–1.38 (m, 10 H, CH$_2$(CH_2)$_5$CH$_3$), 1.50–1.60 (m, 2H, CH_2(CH$_2$)$_5$CH$_3$), 2.22–2.28 (m, 2H, CH_2CH$_2$N), 2.49–2.60 (m, 2H, CH$_2$CH_2NCH$_2$), 2.72–2.83 (m, 1H, NCH_2(CH$_2$)$_6$CH$_3$), 2.90–3.03 (m, 3H, NCH_2(CH$_2$)$_6$CH$_3$ (1H), CH$_2$CH$_2$NCH_2 (2H)), 3.46/3.48 (2s, together 3H, OCH_3 (*cis*, *trans*)), 6.05/6.07 (2s, together 1H, ArC*H*OCH$_3$ (*cis*, *trans*)), 7.32–7.39 (m, 4H, arom. H). Ratio of diastereomers 57:43.

6.16. *1-(4-Chlorobutyl)imidazole*

NaH (60% dispersion, 804 mg, 20.1 mmol) was added to a solution of imidazole (683 mg, 10.0 mmol) in acetone (40 mL). The mixture was stirred at rt for 2 h, then a solution of 1-bromo-4-chlorobutane (1.72 g, 10.0 mmol) in acetone (10 mL) was added dropwise and the reaction mixture was stirred for 6 h at 70 °C. The solvent was removed in vacuo, the residue was dissolved in CH_2Cl_2 (100 mL) and washed with H_2O. The combined organic layer was dried (Na_2SO_4), filtered, concentrated in vacuo and the residue was purified by fc (5 cm, ethyl acetate:methanol = 9:1, 30 mL, R_f = 0.31). Pale yellow oil, yield 1.31 g (83%). $C_7H_{11}ClN_2$ (158.6). MS (EI): m/z (%) = 159 [M^+], 131 [$M^+ - CHN$], 123 [$M^+ - Cl$], 82 [$M^+ - (CH_2)_3Cl$]. IR: $\tilde{\nu}$ (cm^{-1}) = 2945 (ν C–H), 1699 (ν C=N), 737 (ν C–Cl). ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.72–1.78 (m, 2H, NCH$_2$CH_2CH$_2$CH$_2$Cl), 1.91–1.96 (m, 2H, NCH$_2$CH$_2$CH_2CH$_2$Cl), 3.52 (t, J = 6.3 Hz, 2H, CH_2Cl), 3.98 (t, J = 7.0 Hz, 2H, NCH_2), 6.90 (s, 1H, CH$_2$NC*H*CH), 7.05 (s, 1H, CH$_2$NCHC*H*), 7.50 (s, 1H, NC*H*N).

6.17. *cis/trans-1′-[4-(Imidazol-1-yl)butyl]-3-methoxy-3H-spiro[[2]benzofuran-1,3′-pyrrolidine]* (**4g**)

A mixture of **4b** (227 mg, 1.11 mmol), 1-(4-chlorobutyl)imidazole (167 mg, 1.05 mmol), K_2CO_3 (319 mg, 2.32 mmol), a catalytic amount of KI and acetonitrile (4 mL) was filled into a 10 mL-microwave pressure vial and irradiated with microwaves (program: standard; max. power 220 W; max. pressure 4 bar; temperature 100 °C; time program: 5 min ramp time; 40 min hold time; 5 min cool off time). After filtration through Celite® (CH_2Cl_2), the solvent

was removed in vacuo and the residue was purified by fc (3 cm, methanol:ammonia = 98:2, 10 mL, R_f = 0.41). Colorless oil, yield 223 mg (65%). $C_{19}H_{25}N_3O_2$ (327.4). Anal. calcd. C 69.70 H 7.70 N 12.83 found C 69.60 H 7.82 N 11.79. MS (EI): m/z = 327 [M^+], 296 [$M^+ - OCH_3$], 218 [$M^+ - (CH_2)_3 - C_3H_3N_2$]. IR: $\tilde{\nu}$ (cm^{-1}) = 2935 (ν C–H), 1080 (ν C–O), 756 (δ C–H). ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.49–1.58 (m, 2H, CH_2(CH_2)$_2$-imid), 1.81–1.92 (m, 2H, CH_2CH_2-imid), 2.18–2.35 (m, 2H, CH_2CH_2N), 2.53–2.57 (m, 2H, CH_2CH_2N), 2.66–2.78 (m, 1H, CH_2(CH_2)$_3$-imid), 2.82–2.95 (m, 3H, CH_2(CH_2)$_3$-imid (1H), CH_2CH_2NCH_2 (2H)), 3.45/3.47 (2s, together 3H, OCH_3 (*cis*, *trans*)), 3.92–3.99 (m, 2H, (CH_2)$_3$CH_2-imid), 6.04/6.07 (2s, together 1H, ArC*H*OCH_3 (*cis*, *trans*)), 6.91 (s, 1H, CH_2NC*H*CH), 7.04 (s, 1H, CH_2NCHC*H*), 7.25–7.46 (m, 4H, arom. H), 7.50 (s, 1H, NC*H*N).

7. Pharmacological evaluation

7.1. Receptor binding studies, materials and general procedures

Guinea pig brains and rat livers were commercially available (Harlan-Winkelmann, Germany). Homogenizer: Elvehjem Potter (B. Braun Biotech International). Centrifuge: High-speed cooling centrifuge model Sorvall RC-5C plus (Thermo Finnigan). Filter: Printed Filtermat Type A (Perkin Elmer), presoaked in 0.5% aqueous polyethylenimine for 2 h at rt before use. The filtration was carried out with a MicroBeta FilterMate-96 Harvester (Perkin Elmer). The scintillation analysis was performed using Meltilex (Type A) solid scintillator (Perkin Elmer). The 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 counting efficiency was 20%.

7.2. Membrane preparation for the σ_1 assay [27,34]

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 [53] 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.

7.3. Performing of the σ_1 assay [27,34]

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 [54].

7.4. Membrane preparation for the σ_2 assay [27,34]

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 [53] 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.

7.5. Performing of the σ_2 assay [27,34]

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 [55].

7.6. Affinity at the phencyclidine binding site of the NMDA receptor

This assay was performed as described in Ref. [49].

7.7. 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 [56]. For potent compounds the K_i-values are given as mean values + SEM from three independent experiments. For compounds with low affinity (K_i value > 1 μM) the K_i value is recorded only once. When the competition curve does not provide a clear correlation between the test compound concentration and the inhibition of radioligand binding only the inhibition at a test compound concentration of 1 μM is given.

7.8. Interaction with the hERG channel [36]

The effects of spirocyclic pyrrolidines **3** and **4** on hERG-potassium channel activity were investigated using a fluorescence assay. The study was done by evotec AG (Schnackenburgallee 114, D-22525 Hamburg, Germany). The fluorescence-based microplate assay was performed using CHO-cells stably transfected with the hERG-potassium channel, and wild-type CHO-cells for control. The assay is based on the observation that inhibition of potassium channel activity is correlated to a positive shift in membrane potential. Changes of the membrane potential were measured using potentiometric dyes. Fluorescence read-out was performed with a Tecan

Safire-fluorescence reader (Tecan, Crailsheim, Germany) on 384-well plates. Spirocyclic pyrrolidines **3** and **4** were tested at a concentration of 10 μM and mean fluorescence increase was correlated to remaining hERG channel activity. A full block of hERG channel-mediated activity in cell-based assay thereby results in a remaining activity of 0%. No effect of the compounds on the ion channel-mediated activity results in a remaining activity of at least 1oo %.

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References

[1] W.R. Martin, C.G. Eades, J.A. Thompson, R.E. Huppler, P.E. Gilbert, J. Pharmacol. Exp. Ther. 197 (1976) 517–532.
[2] U.B. Vaupel, Eur. J. Pharmacol. 92 (1983) 269–274.
[3] S.W. Tam, Proc. Natl. Acad. Sci. U S A 80 (1983) 6703–6707.
[4] R. Quirion, W.D. Bowen, Y. Itzhak, J.L. Junien, J.M. Misacchio, R.B. Rothman, T. Su, W. Tam, D.P. Taylor, Trends Pharmacol. Sci. 13 (1992) 85–86.
[5] M. Hanner, F.F. Moebius, A. Flandorfer, H. Knaus, J. Striessnig, E. Kempner, H. Glossmann, Proc. Natl. Acad. Sci. U S A 93 (1996) 8072–8077.
[6] P. Seth, Y. Fei, H.W. Li, W. Huang, F. Leibach, V.J. Ganapathy, Neurochem 70 (1998) 922–931.
[7] R. Kekuda, P.D. Prasad, Y. Fei, F.H. Leibach, V. Ganapathy, Biochem. Biophys. Res. Commun. 229 (1996) 553–558.
[8] Y.X. Pan, J. Mei, J. Xu, B.-L. Wan, A. Zuckerman, G.W.J. Pasternak, Neurochem 70 (1998) 2279–2285.
[9] P.D. Prasad, H.W. Li, Y.J. Fei, M.E. Ganapathy, T. Fujita, L.H. Plumley, T.L. Yang-Feng, F.H. Leibach, V.J. Ganapathy, Neurochem 70 (1998) 443–451.
[10] S.B. Hellewell, A. Bruce, G. Feinstein, J. Orringer, W. Williams, W.D. Bowen, Eur. J. Pharmacol. 268 (1994) 9–18.
[11] K. Kitaichi, J.G. Chabot, F.F. Moebius, A. Flandorfer, H. Glossmann, R. Quirion, J. Chem. Neuroanat. 20 (2000) 375–387.
[12] P.J. Lupardus, R.A. Wilke, E. Aydar, C.P. Palmer, Y. Chen, A.E. Ruoho, M.B. Jackson, J. Physiol. (Lond) 526 (2000) 527–539.
[13] E. Aydar, C.P. Palmer, V.A. Klyachko, M.B. Jackson, Neuron 34 (2002) 399–410.
[14] C. Ela, J. Barg, Z. Vogel, Y. Hasin, Y. Eilam, J. Pharmacol. Exp. Ther. 269 (1994) 1300–1309.
[15] M. Johannessen, S. Ramachandran, L. Riemer, A. Ramos-Serrano, A.E. Ruoho, M.B. Jackson, Am. J. Physiol. Cell Physiol. 296 (2009) C1049–C1057.
[16] T. Hayashi, T.P. Su, CNS Drugs 18 (2004) 269–284.
[17] E.J. Cobos, J.M. Entrena, F.R. Nieto, C.M. Cendan, E. DelPezo, Curr. Neuropharmacol. 6 (2008) 344–366.
[18] T. Maurice, T.P. Su, Pharmacol. Ther 124 (2009) 195–206.
[19] M. Ishikawa, K.J. Hashimoto, Receptor Ligand Channel Res. 3 (2010) 25–36.
[20] V. Sabino, P. Cottone, S.L. Parylak, L. Steardo, E.P. Zorilla, Behav. Brain Res. 198 (2009) 472–476.
[21] A. Urani, F.J. Roman, V. Phan, T. Su, T.J. Maurice, Pharmacol. Exp. Ther. 298 (2001) 1269–1279.
[22] K. Hashimoto, Centr. Nerv. Syst. Agents Med. Chem. 9 (2009) 197–204.
[23] J. Meunier, M. Gue, M. Recasens, T. Maurice, Br. J. Pharmacol. 142 (2004) 689–700.
[24] C.-C. Chien, G.W. Pasternak, J. Pharmacol. Exp. Ther. 271 (1994) 1583–1590.
[25] K. Hashimoto, K. Ishiwata, Curr. Pharm. Des. 12 (2006) 3857–3876.
[26] J. Simony-Lafontaine, M. Esslomani, E. Bribes, S. Gougou, N. Lequeux, R. Lavail, J. Grenier, A. Kramar, P. Casellas, Br. J. Cancer 82 (2000) 1958–1966.
[27] C.A. Maier, B. Wünsch, J. Med. Chem. 45 (2002) 438–448.
[28] C.A. Maier, B. Wünsch, J. Med. Chem. 45 (2002) 4923–4930.
[29] C.A. Maier, B. Wünsch, Eur. J. Org. Chem. (2003) 714–720.
[30] A. Jasper, D. Schepmann, K. Lehmkuhl, J.M. Vela, H. Buschmann, J. Holenz, B. Wünsch, Eur. J. Med. Chem. 44 (2009) 4306–4314.
[31] E. Rack, R. Fröhlich, D. Schepmann, B. Wünsch, Bioorg. Med. Chem. 19 (2011) 3141–3151.
[32] T. Schläger, D. Schepmann, E.U. Würthwein, B. Wünsch, Bioorg. Med. Chem. 16 (2008) 2992–3001.
[33] T. Schläger, D. Schepmann, J. Holzenz, J.M. Vela, H. Buschmann, B. Wünsch, J. Med. Chem. 54 (2011) 6704–6713.
[34] C. Oberdorf, D. Schepmann, J.M. Vela, J.L. Diaz, B. Wünsch, J. Med. Chem. 51 (2008) 6531–6537.
[35] C. Meyer, B. Neue, D. Schepmann, S. Yynagisawa, J. Yamaguchi, E.U. Würthwein, K. Itami, B. Wünsch, Org. Biomol. Chem. 9 (2011) 8016–8029.
[36] C. Wiese, E. Große Maestrup, D. Schepmann, J.M. Vela, J. Holenz, H. Buschmann, B. Wünsch, J. Pharm. Pharmacol. 61 (2009) 631–640.
[37] E. Große Maestrup, C. Wiese, D. Schepmann, P. Brust, B. Wünsch, Bioorg. Med. Chem. 19 (2011) 393–405.
[38] S. Fischer, C. Wiese, E. Große Maestrup, A. Hiller, W. Deuther-Conrad, M. Scheunemann, D. Schepmann, J. Steinbach, B. Wünsch, P. Brust, Eur. J. Nucl. Med. Mol. Imaging 38 (2011) 540–551.
[39] E. Große Maestrup, S. Fischer, C. Wiese, D. Schepmann, A. Hiller, W. Deuther-Conrad, J. Steinbach, B. Wünsch, P. Brust, J. Med. Chem. 52 (2009) 6062–6072.
[40] C. Wiese, Große E. Maestrup, D. Schepmann, S. Grimme, H.U. Humpf, P. Brust, B. Wünsch, Chirality 23 (2011) 148–154.
[41] B. Wünsch, Curr. Pharmaceut. Des. 18 (2012) 930–937.
[42] R.A. Glennon, S.Y. Ablordeppey, A.M. Ismaiel, M.B. El-Ashmawy, J.B. Fischer, K.B. Howie, J. Med. Chem. 37 (1994) 1214–1219.
[43] R.A. Glennon, Mini. Rev. Med. Chem. 5 (2005) 927–940.
[44] C. Laggner, C. Schieferer, B. Fiechtner, G. Poles, R.D. Hoffmann, H. Glossmann, T. Langer, F.F. Moebius, J. Med. Chem. 48 (2005) 4754–4764.
[45] D. Zampieri, M.G. Mamolo, E. Laurini, C. Florio, C. Zanette, M. Fermeglia, P. Posocco, M.S. Paneni, S. Pricl, L. Vio, J. Med. Chem. 52 (2009) 5380–5393.
[46] C. Oberdorf, T.J. Schmidt, B. Wünsch, Eur. J. Med. Chem. 45 (2010) 3116–3124.
[47] B. Wünsch, Arch. Pharm. (Weinheim) 323 (1990) 493–499.
[48] S. Ram, L.D. Spicer, Tetrahedron Lett 28 (1987) 515–516.
[49] U. Wirt, D. Schepmann, B. Wünsch, Eur. J. Org. Chem. (2007) 462–475.
[50] J. Plásek, K. Sigler, J. Photochem. Photobiol. 33 (1996) 101–124.
[51] J.W. Warnke, B. Ganetzky, Proc. Nat. Acad. Sci. 91 (1994) 3438–3442.
[52] M. Recanatini, E. Poluzzi, M. Masetti, A. Cavalli, F. de Ponti, Med. Res. Rev. 25 (2005) 133–166.
[53] M.M. Bradford, Anal. Biochem. 72 (1976) 248–254.
[54] D.L. De-Haven-Hudkins, L.C. Fleissner, F.Y. Ford-Rice, Eur. J. Pharmacol. Mol. Pharmacol. Sect. 227 (1992) 371–378.
[55] H. Mach, C.R. Smith, S.R. Childers, Life Sci. 57 (1995) 57–62.
[56] Y.C. Cheng, W.H. Prusoff, Biochem. Pharmacol. 22 (1973) 3099–3108.