Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry 16 (2008) 2992–3001
Bioorganic & Medicinal Chemistry

Synthesis and structure–affinity relationships of novel spirocyclic σ receptor ligands with furopyrazole structure

Torsten Schläger,[a] Dirk Schepmann,[a] Ernst-Ulrich Würthwein[b] and Bernhard Wünsch[a,*]

[a]*Institut für Pharmazeutische und Medizinische Chemie, Westfälische Wilhelms-Universität Münster, Hittorfstrasse 58-62, D-48149 Münster, Germany*
[b]*Organisch-chemisches Institut, Westfälische Wilhelms-Universität Münster, Corrensstrasse 40, D-48149 Münster, Germany*

Received 24 August 2007; revised 18 December 2007; accepted 20 December 2007
Available online 25 December 2007

Abstract—The synthesis of novel spirocyclic σ receptor ligands with high affinity is described. The cyclization of the hydroxy acetal **8**, which represents a key step in the synthesis of the spirocyclic compounds **3**, was supported by theoretical considerations. The affinity of the spirocyclic furopyrazoles **3a**–**c** to the σ receptors was determined in receptor binding studies with radioligands. The *N*-benzyl (**3b**) and *N*-butyl (**3c**) derivatives display very high σ_1 receptor affinity (**3b**, K_i = 0.50 nM; **3c**, K_i = 1.28 nM) and high selectivity toward the σ_2 receptor and some other receptor systems. Calculation of crucial distances of the spirocyclic furopyrazole derivatives **3b** and **3c** shows good correlation with the pharmacophore model of Glennon.

1. Introduction

Originally, Martin and co-workers postulated the σ receptor as a subtype of the opioid receptors to explain the psychotomimetic effects of benzomorphan-derived opioids, particularly those of SKF-10,047 [(±)-*N*-allylnormetazocine].[1] However, this hypothesis was discarded because most of the σ effects caused by typical σ ligands were not blocked by the opioid antagonist naloxone.[2]

Some years later, the σ receptor was considered to be identical with the phencyclidine binding site at the NMDA receptor.[3] However, it was recognized that some ligands (e.g., haloperidol) exhibit high affinity to the σ receptor but no affinity to the PCP binding site of the NMDA receptor.[4]

Today, σ receptors are well accepted as unique pharmacological entities with a specific drug selectivity pattern and a characteristic distribution within the central nervous system (CNS) as well as in many tissues outside the CNS (e.g., liver, kidney, and lung).[5,6] A relationship between σ receptors and opioid receptors or the PCP binding site of the NMDA receptor is no longer considered.

The σ receptors play an important role in several physiological and pathophysiological processes. Modulatory effects on several physiological and cellular events by σ_1 receptors have been described. σ_1 ligands are involved in the regulation of various ion channels and receptors, e.g., potassium channels, voltage-dependent calcium channels, inositol-1,4,5-triphosphate (IP_3) receptors,[7] NMDA receptors,[7] dopamine receptors,[8] and GABA receptors.[9] It has also been reported that σ_1 receptors modulate the neuronal activity and the release of different neurotransmitters, for example, serotonin, dopamine, noradrenalin, glutamate, and GABA.[10,11]

σ Ligands have a potential for the treatment of epileptic disorders,[12] depression,[13] and drug abuse.[14] They also show neuroprotective,[15] antiamnesic,[10] analgesic,[10] and antineoplastic[16] activity, and may also be used for tumor imaging purposes.[17]

The existence of two different σ receptor subtypes (σ_1 and σ_2) was shown by biochemical and pharmacological analysis. The rat brain gene of the σ_1 receptor codes for a protein with 223 amino acids. Two transmembrane domains with the amino and carboxy termini located on the intracellular side of the membrane are postulated.[10]

Keywords: Spirocyclic piperidines; Pharmacophore model; Molecular modeling experiments; Theoretical considerations.
* Corresponding author. Tel.: +49 (0)251 83 33311; fax: +49 (0)251 83 32144; e-mail: wuensch@uni-muenster.de

0968-0896/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2007.12.045

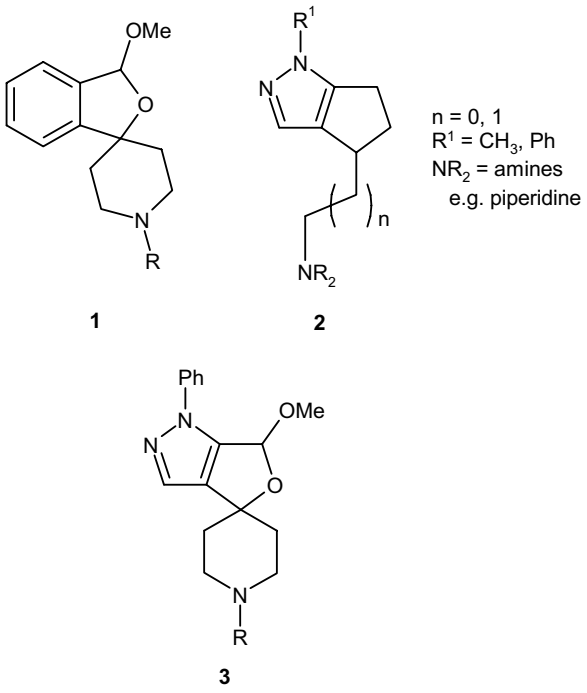


Figure 1. Lead compounds in comparison with novel pyrazole based spirocyclic σ receptor ligands.

In order to get a better understanding of the physiological and pathopysiological roles of σ_1 and σ_2 receptors, the development of potent and highly selective σ ligands is necessary. Recently, the synthesis of spirocyclic σ_1 receptor ligands **1** was described. In particular the *N*-benzyl derivative **1a** (R = Bn) displays very high σ_1 receptor affinity (K_i = 1.14 nM) and extraordinarily high selectivity against more than 80 receptors and reuptake systems.[18–20] Very recently it was shown that pyrazole derivatives **2** also bind with high affinity and selectivity at σ_1 receptors[21] (Fig. 1).

Herein, we wish to report on the synthesis and pharmacological evaluation of the spirocyclic compounds **3**, which represent a combination of the σ_1 ligands **1** and **2**. Instead of the benzene moiety of **1** the bioisosteric pyrazole heterocycle from **2** should be introduced into the spirocyclic system. In the designed σ_1 ligands **3** and the lead compound **1** the distances between the basic amino moiety and the aromatic system (benzene in **1**, pyrazole in **3**) are almost identical. Compared with the σ_1 ligands **2**, which contain a flexible aminoalkyl side chain in position 4, the *N*-aryl distance of the conformationally restricted spirocyclic derivatives **3** is exactly defined. Generally, ligands with restricted conformational flexibility are valuable tools in the exploration of pharmacophore models since they provide information about the exact three dimensional structure and electronic properties of the complementary binding site.

2. Chemistry

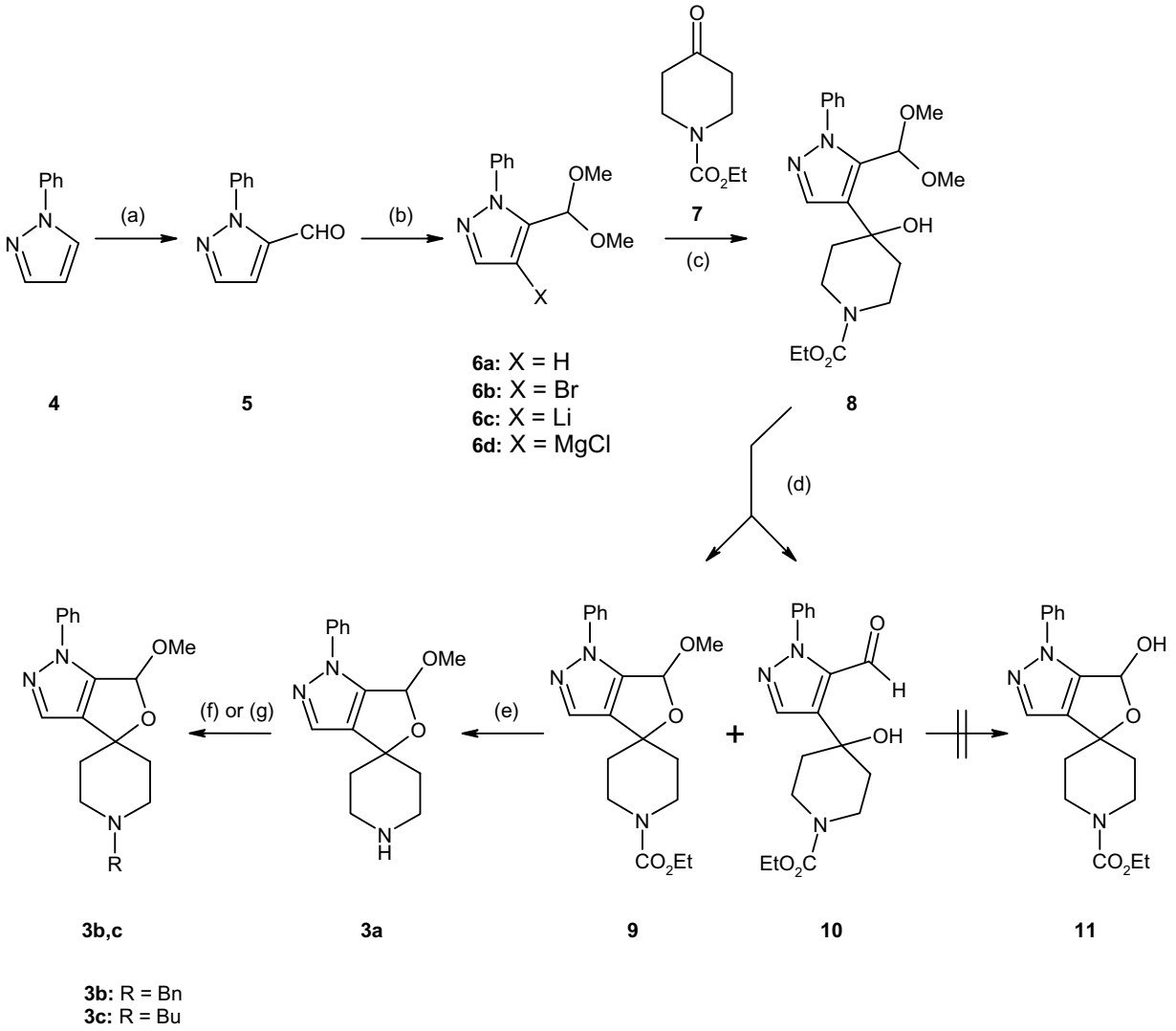
The synthesis of the spirocyclic furopyrazoles **3** started with 1-phenylpyrazole (**4**). According to the reported method pyrazole **4** was α-metalated with *n*-BuLi at −78 °C to generate regioselectively the pyrazol-5-yl lithium intermediate, which was trapped with *N*,*N*-dimethylformamide to afford the pyrazole-5-carbaldehyde **5**[22] (Scheme 1).

Acetalization of the aldehyde **5** with methanol and trimethyl orthoformate provided the dimethyl acetal **6a** and subsequent bromination with pyridinium bromide perbromide led to the brominated acetal **6b**. However, the overall yield of **6b** was considerably improved by a one-pot-transformation of the aldehyde **5** using methanol and pyridinium bromide perbromide in the presence of *p*-toluenesulfonic acid and trimethyl orthoformate. The regioselective bromination of **6a** can be explained by the high electron density of the pyrazole system at position 4. Whereas the formyl group of **5** decreases the electron density in position 4 the acetalic substructure of **6a** increases the C-4 electron density and promotes the bromination. The chemical shift of the C-4 proton in the ^{1}H NMR spectra (**5**: 7.11 ppm; **6a**: 6.57 ppm) and the C-4 carbon in the ^{13}C NMR spectra (**5**: 112.5 ppm; **6a**: 107.4 ppm) clearly demonstrates the activation of the 4 position by the acetal group.

Next the brominated acetal **6b** was treated with *n*-BuLi to yield the aryl lithium intermediate **6c** which should be reacted with N-protected piperidin-4-one derivatives. Unfortunately, the reaction of the pyrazolyl lithium intermediate **6c** with 1-benzylpiperidin-4-one did not afford the corresponding addition product. The only product, which was detected and isolated, was the debrominated acetal **6a**, which proved the successful halogen/metal exchange. Therefore, the *N*-ethoxycarbonyl protected piperidinone **7** was used instead of benzylpiperidone. In fact, the corresponding addition product **8** was isolated in 25% yield. In order to improve the yield of **8** the halogen/metal exchange of **6b** was performed with *i*-PrMgCl at 20 °C to produce the aryl magnesium intermediate **6d**. Again the pyrazolyl magnesium intermediate **6d** did not react with 1-benzylpiperidin-4-one. The addition of **6d** to the 1-ethoxycarbonyl protected piperidin-4-one **7**, however, provided the hydroxy acetal **8** in 32% yield.

In the next step the spirocyclic ring system was established by intramolecular transacetalization of the hydroxy acetal **8**. However, treatment of **8** with a 1.5 equivalents of *p*-toluenesulfonic acid in THF led to the spirocyclic furan derivative **9** and the hydroxy aldehyde **10** in a 3:1-ratio. Even when using absolute solvents and a dried solution of *p*-toluenesulfonic acid the hydroxy aldehyde **10** was formed as side product. The yield of the spirocyclic acetal **9** did not exceed 40 % in spite of several optimization attempts. The facile hydrolysis of the dimethyl acetal **8** and/or the cyclic acetal **9** is most likely due to the adjacent electron-donating pyrazole heterocycle.

Surprisingly, the γ-hydroxy aldehyde **10** did not form the intramolecular hemiacetal **11**. In the ^{1}H NMR spectrum a signal for the lactol proton was not seen. The equilibrium at least in $CDCl_3$ is quantitatively shifted to-



Scheme 1. Synthesis of spirocyclic σ_1 receptor ligands with pyrazole substructure. Reagents and conditions: (a) *n*-BuLi, THF, −78 °C, 2 h then DMF, THF, −78 °C, 1 h, rt, 18 h, yield 90%; (b) TosOH·H_2O, $HC(OMe)_3$, pyridinium bromide perbromide, MeOH, rt, 90 h, yield **6b**: 93%; (c) *i*-PrMgCl, THF, rt, 90 h then **7**, THF, rt, 24 h, yield 32% or *n*-BuLi, THF, −78 °C, 15 min then **7**, THF, −78 °C, 2 h, yield 25%; (d) TosOH·H_2O, THF, rt, 24 h, yield **9**: 40%; yield **10**: 12%; (e) KOH 2 M, dioxane/H_2O, 100 °C, 18 h, yield 77%; (f) **3b**: Benzyl bromide, K_2CO_3, THF, reflux, 24 h, yield 71 %, **3c**: Butyl bromide, K_2CO_3, THF, reflux, 49 h, yield 59%.

ward the hydroxy aldehyde form **10**. This is in sharp contrast to the corresponding benzaldehyde derivative **12**, which easily forms the respective hemiacetal **13** (Fig. 2).[18,19]

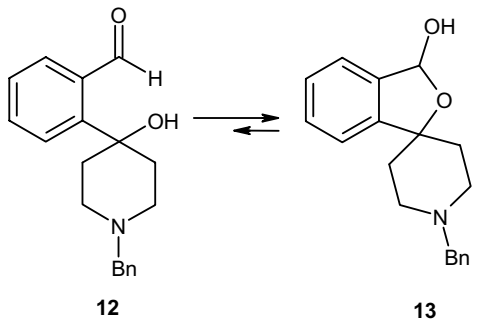


Figure 2. Equilibrium between hydroxy aldehyde **12** and hemiacetal **13**.

In order to gain a better understanding of these intramolecular acetal and hemiacetal formations, theoretical calculations for these cyclization reactions were performed for the N-methylated model compounds **10a**–**13a**. DFT-geometry optimization using the B3LYP/6-31G(d) method were performed for the corresponding N-methyl analogues **10a**–**13a** with the GAUSSIAN 03 package of programs.[23] Relative energies of the pairs of isomers (hydroxy aldehyde versus hemiacetal) were calculated using the SCS-MP2-method of S. Grimme[24] (SCS-MP2/6-31G(d)//B3LYP/6-31G(d) including DFT zero point correction), which is known to give very reliable results for structurally differing isomers.[25,26] Thus, the N-methylated hydroxy aldehyde **10a** was calculated to be lower in energy by 7.5 kcal/mol compared to the hemiacetal **11a**, therefore excluding the formation of

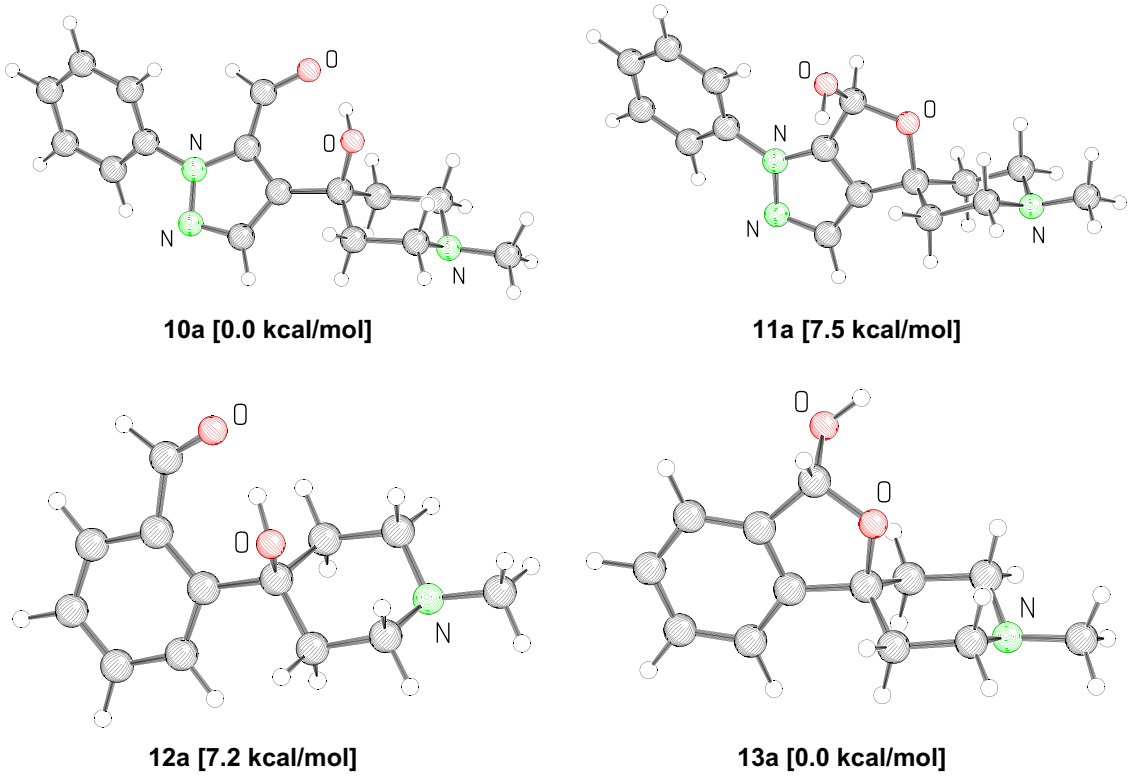


Figure 3. Molecular structures and relative energies of compounds **10a**, **11a** and **12a**, **13a** as obtained by DFT calculations (SCS-MP2/6-31G(d)// B3LYP/6-31G(d) including DFT zero point correction).

11a for thermodynamic reasons. This is quite in contrast to the hydroxy aldehyde **12a**, which is 7.2 kcal/mol higher in energy than the hemiacetal **13a**, allowing exothermic cyclization (see Fig. 3). This difference may be traced back to the higher angle strain of the two fused five-membered rings present in **11a**, in contrast to the five six-ring system of **13a** with smaller outer C–C–C(OH)– and C–C–C(OC)– angles at the bridging sp^2-carbon atoms (**11a**: 140.5°, 144.9°; **13a**: 130.0°, 130.2°). These calculations with the N-methylated model systems **10a**–**13a** explain the unexpected different cyclization behavior of the N-ethoxycarbonyl derivatives **10/11** and *N*-benzyl derivatives **12/13**.

Cleavage of the carbamate protective group of **9** with KOH in refluxing dioxane/water led to the secondary amine **3a**. Finally the tertiary amines **3b** and **3c** were prepared by alkylation of the secondary amine **3a** with benzyl bromide and butyl bromide, respectively.

3. σ Receptor affinity

The σ_1 and σ_2 receptor affinity of the spirocyclic compounds **3a**–**c** was determined in competition experiments with radioligands. In the σ_1 assay guinea pig brain membrane preparations were used as receptor material and [^{3}H]-(+)-pentazocine as radioligand. The non-specific binding was determined in the presence of a large excess of non-tritiated (+)-pentazocine. Homogenates of rat liver served as source for σ_2 receptors in the σ_2 assay. Since a σ_2 selective radioligand is not available, the non-selective radioligand [^{3}H]-ditolylguanidine was employed in the presence of an excess of non-labeled (+)-pentazocine (500 nM) for selective masking of σ_1 receptors. An excess of non-tritiated ditolylguanidine was used for determination of the non-specific binding.[18,19,27]

The K_i-values were calculated according to the equation of Cheng and Prussoff.[28] The σ receptor binding data are summarized in Table 1.

4. Discussion

Table 1 shows that the benzyl derivative **3b** represents an extraordinary potent σ_1 receptor ligand with a subnanomolar affinity (K_i = 0.50 nM). Even the butyl derivative **3c** without a second aromatic residue is a very potent σ_1 receptor ligand with a K_i value of 1.28 nM. Apparently the σ_1 receptor is capable of tolerating an additional phenyl moiety at the hydrophobic aromatic system (compare **1a** with R = benzyl and **3b**, see Table 1).

The affinity of the secondary amine **3a** toward both σ receptor subtypes is rather low. The lack of σ_1 receptor affinity is due to the absence of a hydrophobic substituent at the N-atom for the interaction with the secondary hydrophobic binding site of the receptor protein.

The receptor interaction of the tertiary amines **3b** and **3c** is explained with the pharmacophore model of Glennon[29] shown in Figure 4. According to this model a basic amine is necessary, which is located between two hydrophobic binding sites with defined distances.

Table 1. Affinities of **3a–c**, lead and reference compounds toward σ_1 and σ_2 receptors

Compound	R	$K_i \pm$ SEM (nM) ($n = 3$)		σ_1/σ_2 selectivity
		σ_1([^{3}H]-(+)-pentazocine)	σ_2([^{3}H]-ditolyguanidine)	
1a	Bn	1.14 ± 0.22[18–20]	1280 ± 137[18–20]	1130
2	$n = 1$; R^1 = Ph; NR_2 = piperidine	1.1[21]	36.2[21]	33
3a	H	1200	12[a]	
3b	Bn	0.50 ± 0.13	1750	3500
3c	Bu	1.28 ± 0.16	1050	820
Haloperidol		1.9 ± 0.23	78.1 ± 1.4	
Ditolylguanidine		177.3 ± 3.8	20.2 ± 1.3	

[a] Percent inhibition at a concentration of 1 μM of the test compound.

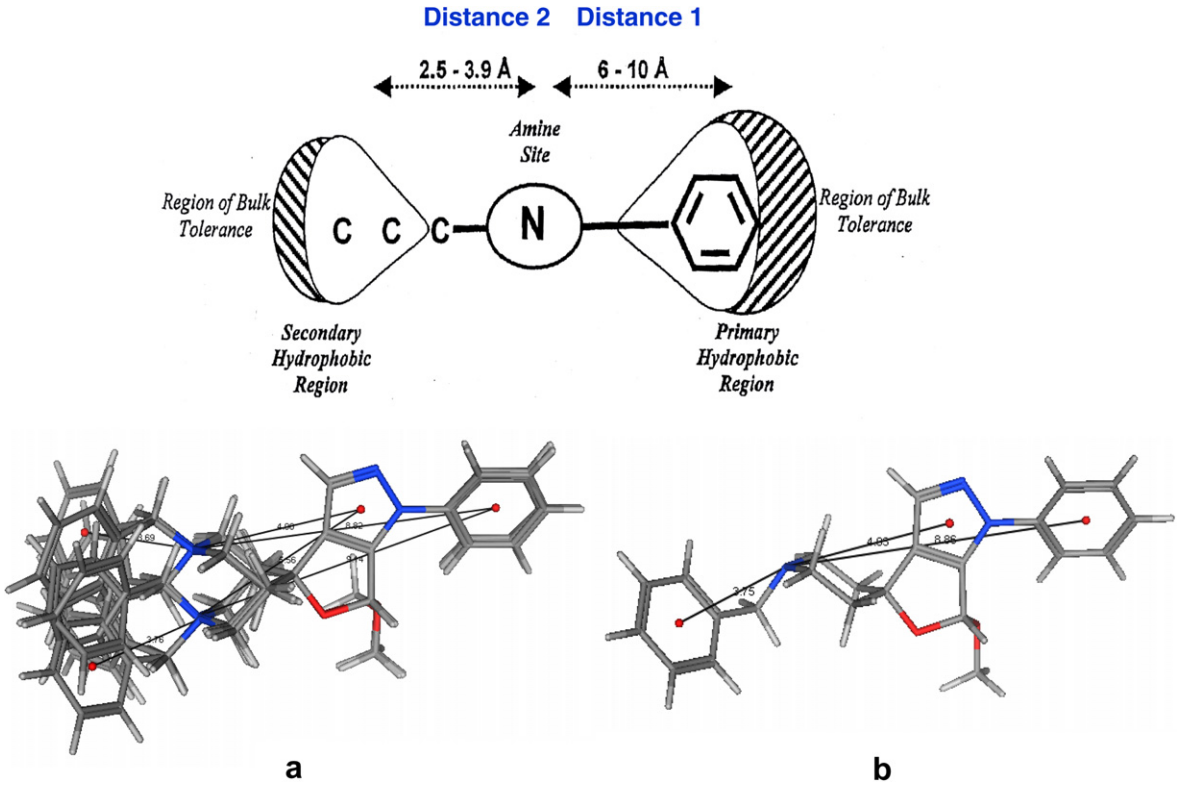


Figure 4. Pharmacophore model of Glennon[29] and results of the modeling experiments, (a) eight superposed conformers with given distances, (b) energetically most favored conformer with given distances.

In the spirocyclic compounds **1** and **3a–c** the piperidine N represents the basic center, whereas the tertiary amine fulfills the same task in the rather flexible compounds **2**. In order to explain the high σ_1 receptor affinity of the spirocyclic furopyrazole derivatives, the distances between the basic center and the hydrophobic residues of the most potent compound **3b** were compared with the proposed model of Glennon.[29] Furthermore these calculations should give an idea whether the phenyl or the pyrazolyl residue occupies the primary hydrophobic binding region of the σ_1 receptor.

The geometry of **3b** was optimized by molecular modeling operations using the molecular modeling program package MOE (Molecular Operating Environment).[30] The calculations were performed with the semiempirical method AM1. A stochastic conformation analysis afforded eight energetically very similar conformations which exist within a small energy range of 1.76 kcal/mol. In Figure 3(a) the eight superposed conformers show that conformers with an axial as well as an equatorial orientation of the pyrazole moiety with respect to the piperidine ring are included. Distance 1, which depends on the orientation of the pyrazole moiety is bigger in the case of equatorial orientation (N–phenyl: 9.14 Å; N–pyrazolyl: 5.56 Å) than in the case of axial orientation (N–phenyl: 8.82 Å; N–pyrazolyl: 4.90 Å) (Fig. 4(a)).

Exemplarily, the energetically most favorable conformer of **3b** with axial orientation of the pyrazole moiety is shown in Figure 4(b). The calculated distances point out, that **3b** is in good accordance with the pharmacophore model of Glennon because distance 1 (N–phenyl) with 8.82 Å and distance 2 with 3.69 Å agree well with the required distances of the model. It is assumed that the N–phenyl distance (8.82 Å) determines the σ_1 recep-

Table 2. Affinities of **3b** and **3c** toward other receptors

Compound	NMDA ([^{3}H]-MK-801)	IC_{50} (nM)				hERG
		α_1	α_2	5-HT_{1A}	5-HTT	
3b	17[a]	>1000	>1000	>10,000	>10,000	0.41
3c	30[a]	1218	>1000	>10,000	>10,000	0.65

[a] Percent inhibition at a concentration of 1 μM of the test compound.

tor affinity of **3b** and **3c**, since the N–pyrazolyl distance (4.90 Å) is too short to fit into the primary hydrophobic region.

According to the pharmacophore model the distance between the basic N and the secondary hydrophobic region requires a chain length of 3–4 carbon atoms. Longer residues are also tolerated by the region of bulk tolerance but are not necessary.

The extraordinarily high σ_1 affinity and selectivity of these spirocyclic pyrazole derivatives stimulated us to do further pharmacological evaluation. Therefore the affinity toward some related receptor systems and the hERG-channel was investigated. The results are summarized in Table 2. They clearly indicate that **3b** and **3c** do not interact significantly with α_1, α_2, 5-HT_{1A} receptors, with the phencyclidine binding site of the NMDA receptor and with the 5-HT transporter. Unfortunately, they exhibit unfavorable interaction with the hERG channel, a cardiac K^+ channel, which is responsible for the development of arrhythmia. Interaction with the hERG channel is a 'knockout criterion' for the further clinical development of the spirocyclic furopyrazole derivatives **3b** and **3c**.

5. Conclusions

Herein we have described the synthesis of the novel spirocyclic furopyrazole derivatives **3b** and **3c** which display σ_1 affinity in the low nanomolar and subnanomolar range. In comparison to the lead compound **1a** (R = Bn) the exchange of the benzene ring against the bioisosteric pyrazole moiety leads to an increase of affinity. The additional phenyl group of **3b** and **3c** seems to be favorable for high σ_1 affinity, presumably because of the extension of the primary hydrophobic region and thus distance 1 compared with the benzofuran derivatives **1**. Molecular modeling studies demonstrate that the spirocyclic furopyrazoles **3b** and **3c** fit well into the pharmacophore model described by Glennon.[29]

6. Experimental

6.1. Chemistry general

Unless otherwise noted, moisture sensitive reactions were conducted under dry nitrogen. THF was dried with sodium/benzophenone and was freshly distilled before use. Methanol was dried with magnesium and iodine, distilled, and stored over molecular sieves 4 Å. DMF was dried with CaH_2, filtered, distilled, and stored over molecular sieves 3 Å. The concentration of *n*-BuLi was determined by titration with 1,3-Diphenyl-2-propanone *p*-toluenesulfonylhydrazone in THF under N_2 atmosphere.[31] 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 and eluent. Melting point, Melting point apparatus SMP 3 (Stuart Scientific), uncorrected. MS, MAT GCQ (Thermo-Finnigan); EI, electron impact; ESI, electrospray ionisation. 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; the assignments of ^{13}C and ^{1}H NMR signals were supported by 2D NMR techniques. Elemental analysis: CHN-Rapid Analysator (Fons-Heraeus).

6.2. 1-Phenylpyrazole-5-carbaldehyde (5)[22]

Under N_2 a solution of *n*-BuLi in heptane (2.61 M, 5.3 mL, 13.9 mmol) was added dropwise to a cooled (−78 °C) solution of **4** (2.0 g, 13.9 mmol) in THF (60 mL). The mixture was stirred at −78 °C for 2 h. Then a solution of dry DMF (1.1 mL, 13.9 mmol) in THF (8 mL) was added dropwise at −78 °C and stirred for 1 h at −78 °C. After stirring at rt for 18 h the solution was hydrolyzed with H_2O (10 mL) and extracted with CH_2Cl_2. The organic layer was dried (K_2CO_3), filtered, concentrated in vacuo, and the residue was purified by fc (Ø = 6 cm, *n*-hexane/EtOAc, 8/2). Yellow solid, yield 2.15 g (90 %), mp 31 °C, R_f = 0.24 (*n*-hexane/EtOAc, 8/2). IR (neat): 3063 (C—$H_{aromat.}$), 2923 (C—$H_{aliphat.}$), 2854 (C—H), 1683 (C=O), 1596, 1517, 1499 (C=C), 763, 694 (C—H). ^{1}H NMR ($CDCl_3$): δ 7.11 (d, J = 2.1 Hz, 1H, Pyr-4-C*H*), 7.46–7.56 (m, 5H, Phenyl-C*H*), 7.76 (d, J = 2.0 Hz, 1H, Pyr-3-C*H*), 9.88 (s, 1 H, C*H*O). ^{13}C NMR ($CDCl_3$): δ 112.5 (Pyr-*C*-4), 125.8 (Phenyl-*C*H, *ortho*), 129.4 (Phenyl-*C*H, *para*), 129.6 (Phenyl-*C*H, *meta*), 139.0 (Phenyl-*C*, q), 140.3 (Pyr-*C*-5), 140.7 (Pyr-*C*-3), 180.2 (*C*HO). $C_{10}H_8N_2O$ (172.2). MS (EI): *m*/*e* (rel. int.) 172 (M^+, 100), 144 (M−CO, 69).

6.3. 1-Phenylpyrazole-5-carbaldehyde dimethyl acetal (6a)

The aldehyde **5** (2.0 g, 11.6 mmol), trimethyl orthoformate (1.4 mL, 12.8 mmol) und *p*-toluenesulfonic acid monohydrate (110 mg, 0.58 mmol) were dissolved in MeOH (120 mL). The solution was stirred for 20 h at rt. Then the mixture was neutralized with a saturated solution of $NaHCO_3$, diluted with H_2O, and extracted with CH_2Cl_2. The organic layer was dried (K_2CO_3), filtered, and concentrated in vacuo to yield a pale yellow solid

(2.45 g, 97 %), mp 49 °C, R_f = 0.23 (*n*-hexane/EtOAc, 8:2). IR (neat): 3064 (C—$H_{aromat.}$), 2938 (C—$H_{aliphat.}$), 2830 (C—H), 1599, 1538, 1503 (C=C), 1123, 1096 (C—O), 764, 695 (C—H). ^{1}H NMR ($CDCl_3$): δ 3.31 (s, 6 H, ArCH(OC*H*$_3$)$_2$), 5.43 (s, 1 H, ArC*H*(OCH$_3$)$_2$), 6.57 (d, J = 1.9 Hz, 1H, Pyrazol-4-C*H*), 7.39 (tt, J = 7.3/1.6 Hz, 1H, Phenyl-C*H*, *para*), 7.44–7.49 (m, 2H, Phenyl-C*H*, *meta*), 7.54–7.58 (m, 2 H, Phenyl-C*H*, *ortho*), 7.65 (d, J = 1.8 Hz, 1H, Pyr-3-C*H*).^{13}C NMR ($CDCl_3$): δ 52.8 (ArCH(O*C*H$_3$)$_2$), 96.9 (Ar*C*H(OCH$_3$)$_2$), 107.4 (Pyr-*C*-4), 125.2 (Phenyl-*C*H, *ortho*), 128.3 (Phenyl-*C*H, *para*), 129.3 (Phenyl-*C*H, *meta*), 139.6 (Phenyl-*C*, q), 139.9 (Pyr-*C*-3), 140.0 (Pyr-*C*-5). $C_{12}H_{14}N_2O_2$ (218.3). MS (ESI): *m*/*e* (rel. int.) 219 [MH$^+$, 71], 459 [2M+Na$^+$, 100].

6.4. 4-Bromo-1-phenylpyrazole-5-carbaldehyde dimethyl acetal (6b)

6.4.1. Method 1. To a solution of the acetal **6a** (100 mg, 0.46 mmol) in methanol (5 mL) pyridinium bromide perbromide (PBB) (147 mg, 0.46 mmol) was added in portions at 0 °C. The solution was stirred for 1 h at 0 °C and then for 19 h at rt. After addition of a saturated solution of $NaHCO_3$ (~5 mL) and dilution with H_2O the mixture was extracted with CH_2Cl_2. The organic layer was dried (K_2CO_3), filtered, concentrated in vacuo, and the residue was purified by fc (Ø = 4 cm, *n*-hexane/EtOAc, 9:1). Colorless solid, yield 119 mg (87 %), mp 33 °C, R_f = 0.20 (*n*-hexane/EtOAc, 9:1).

6.4.2. Method 2. The aldehyde **5** (2.30 g, 13.4 mmol), trimethyl orthoformate (4.4 mL, 40.1 mmol) and *p*-toluenesulfonic acid monohydrate (127.1 mg, 0.67 mmol) were dissolved in methanol (5 mL). After addition of pyridinium bromide perbromide (PBB) (4.3 g, 13.4 mmol) in portions, the solution was stirred for 90 h at rt. Then water, a saturated solution of $NaHCO_3$ and brine were added and the aqueous solution was extracted with CH_2Cl_2 (3×). The organic layer was dried (K_2CO_3), filtered, concentrated in vacuo, and the residue was purified by fc (Ø = 8 cm, *n*-hexane/EtOAc, 9:1). Colorless solid, yield 3.67 g (93 %), mp 33 °C, R_f = 0.20 (*n*-hexane/EtOAc, 9:1). IR (neat): 3062 (C—$H_{aromat.}$), 2933 (C—$H_{aliphat.}$), 2830 (C—H), 1597, 1501 (C=C), 1090, 1065 (C—O), 761, 692 (C—H). ^{1}H NMR ($CDCl_3$): δ 3.37 (s, 6 H, ArCH(OC*H*$_3$)$_2$), 5.36 (s, 1 H, ArC*H*(OCH$_3$)$_2$), 7.39–7.49 (m, 3 H, Phenyl-C*H*), 7.53–7.59 (m, 2 H, Phenyl-C*H*, *ortho*), 7.63 (s, 1 H, Pyr-3C*H*). ^{13}C NMR ($CDCl_3$): δ 54.7 (ArCH(O*C*H$_3$)$_2$), 95.9 (Pyr-*C*-4), 98.5 (Ar*C*H(OCH$_3$)$_2$), 125.6 (Phenyl-*C*H, *ortho*), 128.7 (Phenyl-*C*H, *para*), 129.1 (Phenyl-*C*H, *meta*), 136.3 (Phenyl-*C*, q), 140.1 (Pyr-*C*-5), 141.2 (Pyr-*C*-3). MS (EI): *m*/*e* (rel. int.) 298 [^{81}Br–M$^+$, 28], 296 [^{79}Br–M$^+$, 25], 267 [^{81}Br–M−OCH$_3$, 93], 265 [^{79}Br–M−OCH$_3$, 100], 235 [^{81}Br–M−OCH$_3$−HOCH$_3$, 49], 233 [^{79}Br–M−OCH$_3$−HOCH$_3$, 47]. Anal. Calcd for $C_{12}H_{13}BrN_2O_2$ (297.2): C, 48.5; H, 4.41; N, 9.43; found C, 48.5; H, 4.17; N, 9.32.

6.5. Ethyl 4-[5-(dimethoxymethyl)-1-phenylpyrazole-4-yl]-4-hydroxypiperidine-1-carboxylate (8)

6.5.1. Method 1. Under N_2 a solution of *i*-PrMgCl in THF (2 M, 2.8 mL, 5.60 mmol) was added dropwise to a solution of **6b** (1.50 g, 5.05 mmol) in THF (100 mL). The mixture was stirred for 90 h at rt. Then a solution of the ketone **7** (1.12 g, 6.56 mmol) in THF (65 mL) was added dropwise. After stirring for 24 h at rt. H_2O was added until precipitation stopped. The suspension was filtered and the solution was extracted with CH_2Cl_2 (3×). The organic layer was dried (K_2CO_3), filtered, concentrated in vacuo, and the residue was purified by fc (Ø = 8 cm, cyclohexane/EtOAc, 5:5). Colorless oil, yield 623 mg (32 %), R_f = 0.13 (cyclohexane/EtOAc, 5:5).

6.5.2. Method 2. Under N_2 a solution of *n*-BuLi in heptane (2.61 M, 130.0 µL, 0.34 mmol) was added dropwise at −78 °C to a solution of the brominated acetal **6b** (100 mg, 0.34 mmol) in THF (7 mL). The mixture was stirred for 15 min before a solution of the ketone **7** (58.2 mg, 0.34 mmol) in THF (7 mL) was added dropwise at −78 °C. After stirring for 2 h at −78 °C H_2O was added at rt until the formed precipitate was dissolved. After extraction with CH_2Cl_2 (3×) the organic layer was dried (K_2CO_3), filtered, concentrated in vacuo, and the residue was purified by fc (Ø = 3 cm, *n*-hexane/EtOAc, 3:7). Colorless oil, yield 33 mg (25 %), R_f = 0.23 (*n*-hexane/EtOAc, 3:7). IR (neat): 3448 (O—H), 2980, 2933 (C—$H_{aliphat.}$), 1693 (C=O), 1598, 1545, 1500 (C=C), 1093, 1072 (C—O), 765, 696 (C—H). ^{1}H NMR (DMSO-d_6): δ 1.17 (t, J = 7.1 Hz, 3H, OCH$_2$C*H*$_3$), 1.77 (br d, J = 12.5 Hz, 2H, N(CH$_2$C*H*$_2$)$_2$), 1.88 (td, J = 12.9/4.5 Hz, 2H, N(CH$_2$C*H*$_2$)$_2$), 3.10–3.21 (m, 2H, N(C*H*$_2$CH$_2$)$_2$),[†] 3.20 (s, 6H, ArCH(OC*H*$_3$)$_2$), 3.74–3.87 (m, 2H, N(C*H*$_2$CH$_2$)$_2$), 4.02 (q, J = 7.1 Hz, 2H, OC*H*$_2$CH$_3$), 5.09 (s, 1H, ArC*H*(OCH$_3$)$_2$), 7.37 (tt, J = 7.2/1.8 Hz, 1H, Phenyl-C*H*, *para*), 7.40–7.45 (m, 2H, Phenyl-C*H*, *meta*), 7.48–7.52 (m, 2H, Phenyl-C*H*, *ortho*), 7.58 (s, 1H, Pyr-3-C*H*). $C_{20}H_{27}N_3O_5$ (389.5). MS (EI): *m*/*e* (rel. int.) 358 [M−OCH$_3$, 44], 343 [M−CH$_3$CH$_2$OH, 55], 326 [M−CH$_3$−HOCH$_3$, 100].

6.6. Ethyl 6-methoxy-1-phenyl-4,6-dihydrospiro[1*H*-furo[3,4-*c*]pyrazole-4,4′-piperidine]-1′-carboxylate (9) and Ethyl 4-(1-phenyl-5-formyl-pyrazole-4-yl)-4-hydroxypiperidine-1-carboxylate (10)

Under N_2 a dried (Na_2SO_4) solution of *p*-toluenesulfonic acid (0.1 M in THF, 3.9 mL) was added at rt to a solution of the hydroxy acetal **8** (100 mg, 0.26 mmol) in THF (6 mL). The mixture was stirred for 24 h at rt. The pH was adjusted to pH 10 by addition of a 0.1-M NaOH solution and the solution was extracted with CH_2Cl_2 (3×). The organic layer was dried (K_2CO_3), filtered, concentrated in vacuo, and the residue was purified by fc (Ø = 2 cm, *n*-hexane/EtOAc, 5:5).

6.6.1. Compound 9. Colorless oil, yield 37 mg (40%), R_f = 0.43 (*n*-hexane/EtOAc, 5/5). IR (neat): 2954, 2930 (C—$H_{aliphat.}$), 2829 (C—H), 1692 (C=O), 1600, 1566, 1514 (C=C), 1098, 1083 (C—O), 756, 711 (C—H). ^{1}H NMR (DMSO-d_6): δ 1.18 (t, J = 7.1 Hz, 3H, OCH$_2$C*H*$_3$), 1.66–1.90 (m, 4H, N(CH$_2$C*H*$_2$)$_2$), 3.34 (s, 3H, OC*H*$_3$), 3.43–3.65 (m, 4H, N(C*H*$_2$CH$_2$)$_2$), 4.05 (q,

[†] This signal is superposed by the signal of the methoxy group. It is detected by recording the NMR spectrum at 50 °C.

J = 7.1 Hz, 2H, OCH_2CH_3), 6.38 (s, 1H, Ar-C*H*), 7.31 (t, J = 7.1 Hz, 1H, Phenyl-C*H*, *para*), 7.46–7.53 (m, 2H, Phenyl-C*H*, *meta*), 7.66–7.71 (m, 2H, Phenyl-C*H*, *ortho*), 7.79 (s, 1H, Pyr-3-C*H*). $C_{19}H_{23}N_3O_4$ (357.5). MS (EI): *m/e* (rel. int.) 357 [M^+, 61], 342 [$M-CH_3$, 70], 325 [$M-HOCH_3$, 100], 297 [$M-OCH_3$–CH_3CH_2, 41].

6.6.2. Compound 10. Colorless oil, yield 11 mg (12 %), R_f = 0.22 (*n*-hexane/EtOAc, 5:5). IR (neat): 3416 (O—H), 2925 (C—$H_{aliphat.}$), 1694 (C=O). ^{1}H NMR (DMSO-d_6): δ 1.18 (t, J = 7.1 Hz, 3H, $CO_2CH_2CH_3$), 1.74–1.81 (m, 2H, $N(CH_2CH_2)_2$), 1.99 (td, J = 13.0/4.6 Hz, 2H, $N(CH_2CH_2)_2$), 3.09–3.25 (m, 2H, $N(CH_2CH_2)_2$), 3.81–3.90 (m, 2H, $N(CH_2CH_2)_2$), 4.05 (q, J = 7.0 Hz, 2H, $COOCH_2CH_3$), 5.57 (s, 1H, O*H*), 7.42–7.56 (m, 5H, Phenyl-C*H*), 7.84 (s, 1H, Pyr-3-C*H*), 10.17 (s, 1H, C*H*O). $C_{18}H_{21}N_3O_4$ (343.4). MS (ESI): *m/e* (rel. int.) 343 [MH^+, 100].

6.7. 6-Methoxy-1-phenyl-4,6-dihydrospiro[1*H*-furo[3,4-*c*]pyrazole-4,4′-piperidine] (3a)

The carbamate **9** (343 mg, 0.96 mmol) was dissolved in dioxane/H_2O (96 mL). Then a solution of KOH (2 M, 30 mL) was added and the mixture was refluxed for 18 h. After dilution with H_2O the solution was extracted with EtOAc (3×), the organic layer was dried (K_2CO_3), filtered, concentrated in vacuo, and the residue was purified by fc (Ø = 4 cm, methanol + 2% NH_{3concd}). Pale yellow solid, yield 210 mg (77 %), mp 81 °C, R_f = 0.21 (methanol + 2% $NH_{3conc.}$). IR (neat): 3309 (N—H), 3063 (C—$H_{aromat.}$), 2942 (C—$H_{aliphat.}$), 2826 (C—H), 1599, 1563, 1512 (C=C), 1080, 1061 (C—O), 755, 710 (C—H). ^{1}H NMR (DMSO-d_6): δ 1.65–1.77 (m, 4H, $N(CH_2CH_2)_2$), 2.67–2.78 (m, 2H, $N(CH_2CH_2)_2$), 2.87–2.97 (m, 2H, $N(CH_2CH_2)_2$), 3.34 (s, 3H, OCH_3), 6.35 (s, 1H, ArC*H*), 7.30 (tt, J = 7.4/1.2 Hz, 1H, Phenyl-C*H*, *para*), 7.45–7.52 (m, 2H, Phenyl-C*H*, *meta*), 7.66–7.71 (m, 2H, Phenyl-C*H*, *ortho*), 7.73 (s, 1H, Pyr-3-C*H*). MS (EI): m/e (rel. int.) 285 [M^+, 60], 270 [$M-CH_3$, 100], 77 [Phenyl, 10]. Anal. Calcd for $C_{16}H_{19}N_3O_2$ (285.4) C, 67.4; H, 6.71; N, 14.7; found C, 67.1; H, 6.73; N, 14.5.

6.8. 1′-Benzyl-6-methoxy-1-phenyl-4,6-dihydrospiro[1*H*-furo[3,4-*c*]pyrazole-4,4′-piperidine] (3b)

To a solution of the secondary amine **3a** (60.0 mg, 0.21 mmol) in THF (7 mL) benzyl bromide (29.9 μL, 0.25 mmol) and K_2CO_3 (232.2 mg, 1.68 mmol) were added. After heating of the mixture to reflux for 24 h the suspension was filtered and concentrated in vacuo. The residue was purified by fc (Ø = 3 cm, *n*-hexane/EtOAc, 5:5). Pale yellow resin, yield 56 mg (71 %), R_f = 0.20 (*n*-hexane/EtOAc, 5/5). IR (neat): 3061 (C—$H_{aromat.}$), 2923 (C—$H_{aliphat.}$), 2804 (C—H), 1600, 1565, 1512 (C=C), 1080, 1061 (C—O), 755, 738 (C—H). ^{1}H NMR ($CDCl_3$): δ 1.86–2.12 (m, 4H, $N(CH_2CH_2)_2$), 2.49–2.80 (m, 4H, $N(CH_2CH_2)_2$), 3.46 (s, 3H, OCH_3), 3.62 (s, 2H, NCH_2Ph), 6.16 (s, 1H, ArC*H*), 7.24–7.46 (m, 9H, aromat. C*H*), 7.73 (dd, J = 8.4/0.8 Hz, 2H, Phenyl-C*H*, *ortho*). MS (EI): *m/e* (rel. int.) 375 [M^+, 60], 360 [$M-CH_3$, 100], 344 [$M-OCH_3$, 17], 284 [M−Benzyl, 17], 91 [Benzyl, 93]. Anal. Calcd for $C_{23}H_{25}N_3O_2$ (375.5) C, 73.6; H, 6.71; N, 11.2; found C, 73.6; H, 6.82; N, 11.1.

6.9. 1′-Butyl-6-methoxy-1-phenyl-4,6-dihydrospiro[1*H*-furo[3,4-*c*]pyrazole-4,4′-piperidine] (3c)

To a solution of the secondary amine **3a** (106.0 mg, 0.37 mmol) in THF (12 mL) butyl bromide (48.4 μL, 0.45 mmol) and K_2CO_3 (410.5 mg, 2.97 mmol) were added. After heating of the mixture to reflux for 49 h the suspension was filtered and the solvent was evaporated. The residue was purified by fc (Ø = 3 cm, EtOAc + 10% methanol). Yellow oil, yield 74 mg (59 %), R_f = 0.18 (EtOAc + 10% methanol). IR (neat): 3064 (C—$H_{aromat.}$), 2927 (C—$H_{aliphat.}$), 2804 (C—H), 1601, 1564, 1513 (C=C), 1086, 1062 (C—O), 754, 712 (C—H). ^{1}H NMR ($CDCl_3$): δ 0.94 (t, J = 7.3 Hz, 3H, $NCH_2CH_2CH_2CH_3$), 1.30–1.41 (m, 2H, $NCH_2CH_2CH_2CH_3$), 1.48–1.60 (m, 2H, $NCH_2CH_2CH_2CH_3$), 1.90–2.12 (m, 4H, $N(CH_2CH_2)_2$), 2.38–2.50 (m, 2H, $NCH_2CH_2CH_2CH_3$), 2.51–2.81 (m, 4H, $N(CH_2CH_2)_2$), 3.46 (s, 3H, OCH_3)), 6.16 (s, 1H, ArC*H*), 7.25 (t, J = 7.4 Hz, 1H, Phenyl-C*H*, *para*), 7.41–7.44 (m, 2H, Phenyl-C*H*, *meta*), 7.46 (s, 1H, Pyr-3-C*H*), 7.74 (d, J = 7.7 Hz, 2H, Phenyl-C*H*, *ortho*). MS (EI): *m/e* (rel. int.) 341 [M^+, 5], 298 [M–Propyl, 100]. Anal. calcd. for $C_{20}H_{27}N_3O_2$ (341.5) C, 70.4; H, 7.97; N, 12.3; found C, 70.3; H, 8.01; N, 12.2.

7. Receptor binding studies

7.1. General information

The guinea pig brains and rat livers were commercially available (Harlan-Winkelmann, Borchen, Germany). Homogenizer: Elvehjem Potter (B. Braun Biotech International, Melsungen, Germany). Centrifuge: High-speed cooling centrifuge model Sorvall RC-5C plus (Thermo Fischer Scientific, Whatham, MA, USA). Filter: Printed Filtermat Typ B (Perkin Elmer, Whatham, MA, USA), 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, Whatham, MA, USA). The scintillation analysis was performed using Meltilex (Typ A) solid scintillator (Perkin-Elmer, Whatham, MA, USA). 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, Whatham, MA, USA). The overall counting efficiency was 20%, the counting time 5 min. All experiments were carried out in duplicate using standard 96-well multiplates (Diagonal, Münster, Germany). The IC_{50} values were determined in competition experiments with at least six concentrations of the test compounds and were calculated with the program GraphPad Prism® 3.0 (GraphPad Software) by non-linear regression analysis. The K_i values were calculated according to Cheng and Prusoff.[28] The K_i values are given as mean values ± SEM from three independent assays.

7.2. Membrane preparation for the σ_1 assay (modified according to Refs. 18,19,27)

Five guinea pig brains were homogenized with the potter (500–800 rpm, 10 up-and-down strokes) in six 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 23,500*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 23,500*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[32] 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 (modified according to Refs. 18,19,27)

The test was performed with the radioligand [^{3}H]-(+)-pentazocine (42.5 Ci/mmol; Perkin-Elmer, Whatham, MA, USA). The thawed membrane preparation (about 75 μg of the protein) was incubated with various concentrations of test compounds, 3 nM [^{3}H]-(+)-pentazocine, and buffer (50 mM Tris, pH 7.4) in a total volume of 200 μL for 120 min at 37 °C. The incubation was terminated by rapid filtration through the presoaked filtermats using the cell harvester. After washing each well five times with 300 μL of water, the filtermats were dried at 95 °C. Subsequently, 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.[33]

7.4. Membrane preparation for the σ_2 assay (modified according to Refs. 18,19,27)

Two rat livers were cut into smaller 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 31,000*g* for 20 min at 4 °C. The pellet was resuspended in buffer (50 mM Tris, pH 8.0) and incubated at room temperature for 30 min. After the incubation, the suspension was centrifuged again at 31,000*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[32] 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 (modified according to Refs. 18,19,27)

The test was performed with the radioligand [^{3}H]-ditolylguanidine (50 Ci/mmol; ARC, St.Louis, MO, USA). The thawed membrane preparation (about 100 μg of the protein) was incubated with various concentrations of test compounds, 3 nM [^{3}H]-ditolylguanidine, 500 nM (+)-pentazocine and buffer (50 mM Tris, pH 8.0) in a total volume of 200 μL for 120 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 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.[34]

7.6. NMDA assay

The preparation of the receptor material and the assay were performed according to Ref.27.

Acknowledgments

We thank the Laboratorios del Dr. Esteve, S.A., Barcelona for financial support and carrying out the additional binding studies and Prof. Dr. Thomas Schmidt for performing the conformation analysis.

References and notes

1. Martin, W. R.; Eades, C. G.; Thompson, J. A.; Huppler, R. R.; Gilbert, P. E. *J. Pharmacol. Exp. Ther.* **1976**, *197*, 517.
2. (a) Iwamoto, E. T. *J. Pharmacol. Exp. Ther.* **1981**, *217*, 451; (b) Vaupel, D. B. *Eur. J. Pharmacol.* **1983**, *92*, 269.
3. (a) Holtzman, S. G. *Psychopharmacology* **1982**, *77*, 295; (b) Zukin, S. R. *Life Sci.* **1982**, *31*, 1307.
4. Quirion, R. *Trends Neurosci.* **1987**, *10*, 444.
5. Walker, J. M.; Bowen, W. D.; Walker, F. O.; Matsumoto, R. R.; De Costa, B.; Rice, K. C. *Pharmacol. Rev.* **1990**, *42*, 353.
6. Kaiser, C.; Pontecorvo, J.; Mewshaw, R. E. *Neurotransmissions* **1991**, *7*, 1.
7. Martina, M.; Turcotte, M. E.; Halman, S.; Bergeron, R. *J. Physiol.* **2007**, *578*, 143.
8. (a) Peeters, M.; Romien, P.; Maurice, T.; Su, T. P.; Maloteaux, J. M.; Hermans, E. *Eur. J. Neurosci.* **2004**, *19*, 2212; (b) Feng, X. Q.; Dong, Y.; Fu, Y. M.; Zhu, Y. H.; Sun, J. L.; Wang, Z.; Sun, F. Y.; Zheng, P. *Neuropharmacology* **2004**, *46*, 211.
9. Mtchedlishvili, Z.; Kapur, J. *Mol. Pharmacol.* **2003**, *64*, 857.
10. Guitart, X.; Codony, X.; Monroy, X. *Psychopharmacology* **2004**, *174*, 301.
11. Monnet, F. P.; De Costa, B. R.; Bowen, W. D. *Br. J. Pharmacol.* **1996**, *119*, 65.
12. Lin, Z.; Kadaba, P. K. *Med. Res. Rev.* **1997**, *17*, 537.
13. Monograph Igmesine Hydrochloride *Drugs Future* **1999**, *24*, 133.
14. Ujike, H.; Kuroda, S.; Otsuki, S. *Eur. J. Pharmacol.* **1996**, *296*, 123.
15. Maurice, T.; Lockhart, B. P. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry* **1997**, *21*, 69.
16. Bowen, W. D. *Pharm. Acta Helv.* **2000**, *74*, 211.
17. John, C. S.; Lim, B. B.; Vilner, B. J.; Geyer, B. C.; Bowen, W. D. *J. Med. Chem.* **1998**, *41*, 2445.
18. Maier, C.A. Ph.D. Thesis, University of Freiburg, Germany, **2002**.

19. Maier, C. A.; Wünsch, B. *J. Med. Chem.* **2002**, *45*, 438.
20. Maier, C. A.; Wünsch, B. *J. Med. Chem.* **2002**, *45*, 4923.
21. Corbera, J.; Vano, D.; Martinez, D.; Vela, J. M.; Zamanillo, D.; Dordal, A.; Andreu, F.; Hernandez, E.; Perez, R.; Escriche, M.; Salgado, L.; Yeste, S.; Serafini, M. T.; Pasqual, R.; Alegre, J.; Calvet, C.; Cano, N.; Carvo, M.; Buschmann, H.; Holenz, J. *Chem. Med. Chem.* **2006**, *1*, 140.
22. Medio-Simon, M.; Alvarez de Laviada, M. J.; Sequlveda-Arques, J. *J. Chem. Soc., Perkin Trans. 1* **1990**, 2749.
23. Gaussian 03, Revision C.02, Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, Jr., J. A.; Vreven, T.; Kudin, K. N.; Burant, J. C.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Gonzalez, C.; and Pople, J. A.; Gaussian, Inc., Wallingford CT, **2004**. Details of the quantum chemical calculations may be obtained from E.-U. Würthwein upon request.
24. Grimme, S. *J. Chem. Phys.* **2003**, *118*, 9095.
25. Goumans, T. P. M.; Ehlers, A. W.; Lammertsma, K.; Würthwein, E.-U.; Grimme, S. *Chem. Eur. J.* **2004**, 6468.
26. Grimme, S.; Steinmetz, M.; Korth, M. *J. Org. Chem.* **2007**, *72*, 2118.
27. Wirt, U.; Schepmann, D.; Wünsch, B. *Eur. J. Org.Chem.* **2007**, 462.
28. Cheng, Y.; Prusoff, W. H. *Biochem. Pharmacol.* **1973**, *22*, 3099.
29. Glennon, R. A. *Mini-Rev. Med. Chem.* **2005**, *5*, 927.
30. Chemical Computing Group AG, MOE **2006**.08.
31. Suffert, J. *J. Org. Chem.* **1989**, *54*, 509.
32. Bradford, M. M. *Anal. Biochem.* **1976**, *72*, 248.
33. DeHaven-Hudkins, D. L.; Fleissner, L. C.; Ford-Rice, F. Y. *Eur. J. Pharmacol.* **1992**, *227*, 371.
34. Mach, R. H.; Smith, C. R.; Childers, S. R. *Life Sci.* **1995**, *57*, PL 57.