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Combination of Two Pharmacophoric Systems: Synthesis and Pharmacological Evaluation of Spirocyclic Pyranopyrazoles with High σ_1 Receptor Affinity

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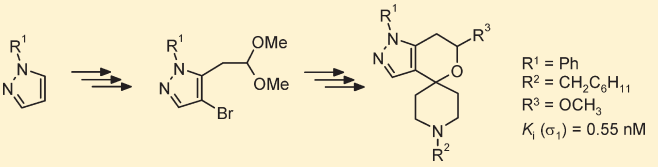
Supporting Information

ABSTRACT: The novel class of spirocyclic σ_1 ligands **3** (6′,7′-dihydro-1′*H*-spiro[piperidine-4,4′-pyrano[4,3-c]pyrazoles]) was designed by the combination of the potent σ_1 ligands **1** and **2** in one molecule. Thorough structure affinity relationships were derived by the variation of the substituents in position 1′, 1, and 6′. Whereas the small electron rich methylpyrazole heterocycle was less tolerated by the σ_1 receptor protein, the introduction of a phenyl substituent instead of the methyl group led to ligands with a high σ_1 affinity. It is postulated that the additional phenyl substituent occupies a previously unrecognized hydrophobic region of the σ_1 receptor resulting in additional lipophilic interactions. The spirocyclic pyranopyrazoles are very selective against the σ_2 subtype, the PCP binding site of the NMDA receptor, and further targets. Despite high σ_1 affinity, the cyclohexylmethyl derivative **17i** (K_i (σ_1) = 0.55 nM) and the isopentenyl derivative **17p** (K_i (σ_1) = 1.6 nM) showed only low antiallodynic activity in the capsaicin assay.



INTRODUCTION

Originally the class of σ receptors was identified as an opioid receptor subtype due to the particular effects caused by benzomorphans (e.g., SKF-10,047, pentazocine).[1] Then, σ receptors were discussed to be identical with the phencyclidine (PCP) binding site of the *N*-methyl-D-aspartate (NMDA) receptor,[2] until they were recognized as specific, nonopioid, non-PCP but haloperidol-sensitive binding structures. Two subtypes of σ receptors have been identified, which are termed σ_1 and σ_2 receptors.[3]

The σ_1 receptor was cloned from different species (guinea pig, mouse, rat, and human) and different tissues (liver, human placental choriocarcinoma cell line, and brain),[4−8] Whereas the gene (≈7 kbp) and the amino acid sequence (223 amino acids) of the σ_1 receptor is well-known, the σ_2 receptor has not been cloned yet.

The influence of σ_1 receptors on several signal transduction pathways has been investigated. It was shown that the σ_1 receptor modulates some ion channels like Kv 1.4 K^+-channels in nerve terminals,[9,10] Ca^{2+}-channels in cultured cardiac myocytes,[11] and voltage-gated Na^+-channels in cardiac myocytes.[12] Additionally, some neurotransmitter systems like NMDA receptors,[13] inositoltriphosphate (IP_3) receptors in the endoplasmatic reticulum,[14] and ankyrin, a cytoskeletal adaptor protein, which regulates Ca^{2+}-influx at IP_3 receptors, are modulated.[15] A chaperone activity of σ_1 receptors was also postulated.[16] Nevertheless, further investigations are necessary to learn more about the σ_1 receptor pharmacology, in particular to find clear correlations between ligand binding properties, signal transduction pathways, and pharmaceutical properties.

σ_1 receptors are expressed in high density in the central nervous system (CNS), in particular in brain regions involved in memory, emotion, sensoric, and motor functions. Additionally, they are found in some peripheral organs (e.g., liver, kidney, heart, lung, intestine, and pancreas)[5,17,18] and, moreover, in some human tumor cell lines.[19,20] Because of their involvement in different neurological processes, σ_1 receptors represent an attractive target for the development of novel drugs for CNS diseases, including depression, schizophrenia, anxiety, cocaine, alcohol and methamphetamine addiction, amnesia, and neuropathic pain as well as some neurodegenerative disorders (e.g., Alzheimer's Disease and Parkinson's Disease).[21−24]

Neuropathic pain is a special kind of pain, which is characterized by a spontaneous hypersensitive pain response and which can typically persist long after the original nerve injury has healed.[25,26] The treatment of neuropathic pain is very difficult due to its diffuse origin. It has been shown with σ_1 receptor knockout mice that σ_1 receptor antagonists can be used for the therapy of neuropathic pain situations.[27] Very recently, it has been reported that indazole derivatives of type **1** (Figure 1) are potent and selective σ_1 receptor antagonists, which are

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analgesically active in the capsaicin model of neuropathic pain. Compound **1a** (R^1 = CH_3, NR_2^2 = 4-phenylpiperidin-1-yl) represents a typical example of a highly potent σ_1 ligand (K_i (σ_1) = 7.0 nM, K_i (σ_2) = 39.7 nM, see Table 1) with analgesic activity. At a dose of 0.5 mg/kg body weight, **1a** led to more than 50% analgesia in the late phase of the capsaicin assay (neuropathic pain model).[28−30]

We have shown that spirocyclic piperidines of type **2** and analogues interact with high affinity and selectivity with σ_1 receptors.[31−35] In particular, the benzofuran derivative **2a** (n = 0) and the benzopyran derivative **2b** (n = 1) represent σ_1 receptor antagonists with low nanomolar σ_1 affinity and extraordinarily high σ_1/σ_2 selectivity[31] (see Table 1). Moreover, the benzofuran derivative **2a** was also active in the mouse capsaicin assay indicating its potential as a drug for the treatment of neuropathic pain.[35] The high σ_1/σ_2 selectivity of the compounds **2** is attributed to the reduced conformational flexibility of the rigid spirocyclic ring system.

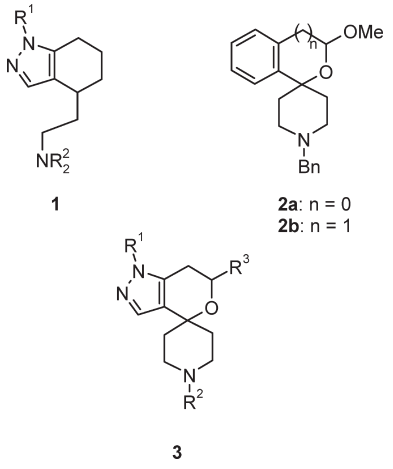


Figure 1. Development of novel σ_1 receptor ligands with spirocyclic pyranopyrazole substructure.

In order to combine the excellent σ_1 affinity and σ_1/σ_2 selectivity of the spirocyclic compounds **2** with the high analgesic activity of the indazole derivatives **1**, the structures of **1** and **2** should be combined in the new spirocyclic pyranopyrazole derivatives **3**. (Figure 1) The incorporation of the flexible aminoethyl side chain of **1** into the rather rigid piperidine moiety of **3** should result in an increased σ_1/σ_2 selectivity of the mixed spirocyclic ligands. It has already been shown that the replacement of the benzene ring of spirocyclic compounds **2** by an electron rich thiophene ring leads to very potent and selective σ_1 ligands.[36,37] Herein, we report on the synthesis and pharmacological evaluation of spirocyclic pyranopyrazole derivatives **3** bearing various substituents R^1, R^2, and R^3.

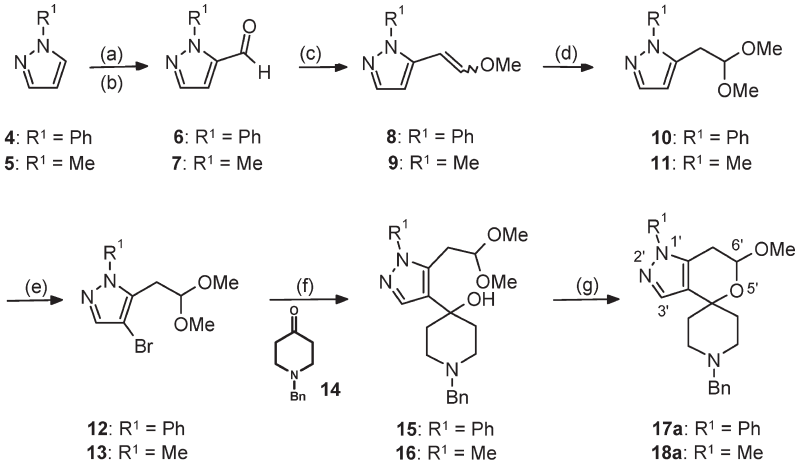
SYNTHESIS

The synthesis of the spirocyclic pyranopyrazoles **3** started with 1-phenylpyrazole (**4**). As described in the literature, pyrazole **4** was α-metalated with n-butyllithium at −78 °C to generate regioselectively the pyrazol-5-yllithium intermediate, which was trapped with N,N-dimethylformamide to afford the pyrazole-5-carbaldehyde **6**[37,38] (Scheme 1). In order to avoid the formation of the thermodynamically more stable phenyllithium intermediate by intramolecular deprotonation, the complete transformation had to be performed at low temperature (< −65 °C) in the solvent tetrahydrofuran (THF).[39,40]

The methylated pyrazole-5-carbaldehyde **7** was prepared in the same way by metalation of 1-methylpyrazole (**5**) with n-butyllithium and subsequent trapping of the anion with N,N dimethylformamide.[41] However, in this case, the methyl group of the pyrazole moiety was deprotonated first. In order to obtain high yields of the ring substitution product **7**, the intermediate anion was stirred for 30 min at 0 °C to induce a transmetalation from the methylllithium intermediate to the thermodynamically more stable pyrazol-5-yllithium derivative.[42,43]

The aldehyde **6** was homologated by a Wittig reaction using the phosphonium salt $Ph_3PCH_2OCH_3^+$ Cl^-, which was deprotonated by KO^{t}Bu,[44] to obtain the enol ether **8** in 92% yield.

Scheme 1[a]



[a] Reagents and reaction conditions: (a) **4**: n-BuLi, THF, −78 °C, 2 h then DMF, THF, −78 °C, 1 h, then rt, 18 h, 90%. (b) **5**: n-BuLi, THF, −60 °C, then 30 min at 0 °C, then DMF, THF, −60 °C, 1 h, then 0 °C, 2 h. (c) $Ph_3PCH_2OCH_3^+$ Cl^-, KOtBu, THF, −50 °C, then rt, overnight, 92% (**8**). (d) MeOH, TosOH·H_2O, rt, 72 h, 90% (**10**). (e) Pyridinium bromide perbromide, MeOH, $HC(OMe)_3$, 0 °C, 1 h, then rt, 4 h, 95% (**12**), 29% over 4 steps (**13**). (f) n-BuLi, THF, −78 °C, 15 min then **14**, THF, −78 °C, 4.5 h, then rt, 67% (**15**), 67% (**16**). (g) TosOH·H_2O, MeOH, rt, 73% (**17a**), 41% (**18a**) together with 30% (**22b**).

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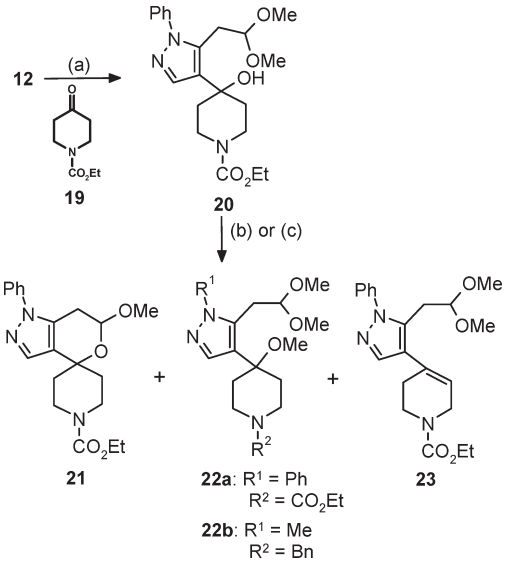
Scheme 2[a]



[a] Reagents and reaction conditions: (a) *n*-BuLi, THF, −78 °C, 15 min, then **19**, −78 °C, 4.5 h, then rt, 85%. (b) TosOH·H_2O, MeOH, rt, 21 h, 9% (**21**), 81% (**22a**). (c) TosOH·H_2O, THF, rt, 70 h, 28% (**21**), 39% (**23**).

Addition of methanol to the enol ether **8** provided the acetaldehyde dimethyl acetal **10**, which was brominated with pyridinium bromide perbromide ($PyH^+ Br_3^-$). Because of the high electron density at the pyrazole 4-position, the bromination took place with high regioselectivity. The bromo acetal **12** was obtained in 95% yield.

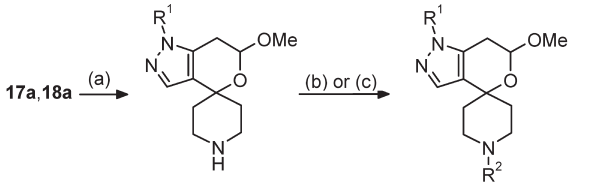
In order to prove the position of the acetalic side chain unequivocally, a nuclear Overhauser effect (NOE) difference spectrum of compound **12** was recorded. After irradiation at 2.95 ppm (CH_2), the signal at 7.45−7.58 ppm (C_6H_5) was increased showing the neighborhood of the acetalic side chain and the phenyl moiety. A metalation of pyrazole **4** in the 3-position would lead to a regioisomeric product, which could not give a positive NOE between side chain and phenylic protons.

The methylated derivatives were synthesized in the same manner. However, because of the high volatility of the methylpyrazole derivatives **7**, **9**, and **11**, these intermediates were not isolated but directly converted into the brominated derivative **13**, which was isolated in 29% yield over four steps starting from methylpyrazole (**5**).

Treatment of bromopyrazoles **12** and **13** with *n*-BuLi led to the pyrazolyllithium derivatives, which were trapped with 1-benzylpiperidone **14** to afford the hydroxy acetals **15** and **16** in 67% yield, respectively. In order to increase the yields, benzylpiperidone **14** was replaced with ethoxycarbonyl protected piperidone **19**, which provided the addition product **20** in 85% yield. (Scheme 2) Therefore, initial experiments for the establishment of the spirocyclic ring system were performed starting with the hydroxy acetal **20**.

Reaction of hydroxy acetal **20** with *p*-toluenesulfonic acid in the solvent methanol[44] provided the desired spirocyclic pyranopyrazole **21** (9%) and the methyl ether **22a** (82%). In order to avoid methyl ether formation, the cyclization of **20** was performed in THF leading to the spirocyclic pyranopyrazole **21** (28%) and the elimination product **23** (39%). The formation of the side products **22a** and **23** is explained by protonation of the tertiary alcohol **20**, elimination of water, and subsequent trapping of the tertiary carbenium ion by methanol (→ **22a**) or deprotonation (→ **23**). This result is in sharp contrast to the formation of the corresponding benzopyran derivatives, which does not lead to substitution or elimination side products.[31] We assume that the high electron density of the electron rich pyrazole moiety is responsible for the good stabilization of the tertiary carbenium ion, initiating the formation of the side products **22a** and **23**.

Scheme 3[a]



[a] Reagents and reaction conditions: (a) $NH_4^+ HCO_2^-$, Pd/C, MeOH, reflux, 10−25 min, 88% (**17b**), 96% (**18b**). (b) R−X, acetonitrile, K_2CO_3, ($Bu_4N^+ I^-$), reflux. (c) RCH═O, $NaBH(OAc)_3$, 1,2-dichloroethane, rt.

Next, the cyclization of the hydroxy acetal **15** was performed using 2.5 equivalents of *p*-toluenesulfonic acid (Scheme 1). The excess of acid should inhibit the formation of the tertiary carbenium ion by protonation of both the pyrazole system (reducing the electron donating properties) and the piperidine N-atom (destabilization of the second cation in position 3 to the first cation). Indeed, cyclization of **15** in methanol and THF led to the spirocyclic pyranopyrazole **17a** in 73% and 60% yields, respectively.

Applying these optimized reaction conditions on the cyclization of the hydroxy acetal **16** using 2.5 equivalents of *p*-toluenesulfonic acid provided the spirocyclic pyranopyrazole **18a** in only 30% yield together with large amounts of the corresponding methyl ether **22b** (R^1 = CH_3, R^2 = Bn, 56%). Increasing the amount of *p*-toluenesulfonic acid to 10 equivalents resulted in increased yields of **18a** (41%) and reduced yields of the methyl ether **22b** (30%).

For the generation of broad structure affinity relationships, the *N*-benzyl residue of the pyranopyrazoles **17a** and **18a** was cleaved off by hydrogenolysis (Scheme 3). A phase transfer hydrogenolysis using ammonium formate as an H_2 source and Pd/C as a catalyst[45] provided the secondary amines **17b** and **18b** in 88% and 96% yields, respectively. Various residues were introduced at the secondary amines **17b** and **18b** by alkylation with alkyl halides or reductive alkylation with aldehydes and $NaBH(OAc)_3$.[46,47]

In order to modify the substitution pattern of the pyran moiety, the hydroxy acetals **15** and **16** were hydrolyzed with diluted HCl to form the cyclic hemiacetals **24** and **25** (Scheme 4). Elimination of water was achieved upon treatment of the lactols **24** and **25** with methanesulfonyl chloride and an excess of triethylamine. Careful hydrogenation (H_2, 1 bar, rt, 1 h) of the cyclic enol ethers **26** and **27** resulted in the spirocyclic pyranopyrazoles **28a** and **29a**. Spirocyclic pyranopyrazoles **28c**−**f** and **29c**−**e** were prepared by hydrogenolytic removal of the *N*-benzyl protecting group and subsequent alkylation of the secondary amines **28b** and **29b**, respectively.

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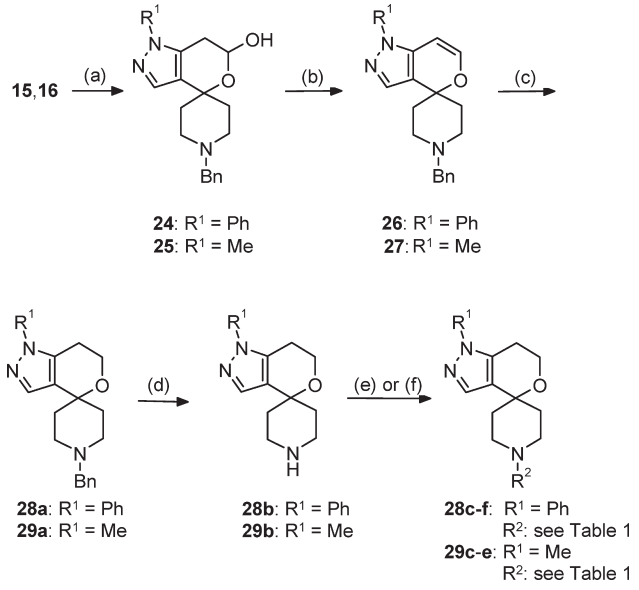
Scheme 4[a]



[a] Reagents and reaction conditions: (a) HCl 2M, rt, 82% (**24**), 77% (**25**). (b) $MeSO_2Cl$, NEt_3, CH_2Cl_2, rt, 2 h, then reflux, 1 h, 65% (**26**), 79% (**27**). (c) H_2, Pd/C, HOAc, rt, 1 h, 62% (**28a**), 74% (**29a**). (d) NH_4^+ HCO_2^-, Pd/C, MeOH, reflux, 25−39 min, 93% (**28b**), 90% (**29b**). (e) R−X, acetonitrile, K_2CO_3, reflux. (f) RCH=O, $NaBH(OAc)_3$, 1,2-dichloroethane, rt.

σ_1 RECEPTOR AFFINITY

The σ_1 receptor affinity of the spirocyclic pyranopyrazoles of type **3** was determined in competition experiments with the potent and σ_1 selective radioligand [^{3}H]-(+)-pentazocine. Guinea pig brain membrane preparations were used as receptor material, and the nonspecific binding was determined in the presence of a large excess of nontritiated (+)-pentazocine.[31−33,48]

The σ_1 receptor affinities of the spirocyclic pyranopyrazoles are summarized in Table 1. Whereas the *N*-benzyl derivative **17a** with the phenylpyrazole framework shows a very similar σ_1 receptor affinity (K_i = 1.5 nM) as the corresponding benzopyran derivative **2b** (K_i = 1.3 nM), the methylpyrazole derived analogue **18a** has a considerably reduced σ_1 affinity (K_i = 21 nM). It can be concluded from these results that replacement of the benzene ring of **2b** with the polar and electron rich pyrazole moiety leads to a decreased σ_1 affinity, which can be compensated by the enlargement of the aromatic system by an additional phenyl moiety at the pyrazole moiety (**17a**).

Replacement of the *N*-benzyl moiety of **17a** with an electron rich arylmethyl residue (methoxybenzyl (**17d**), furan-2-ylmethyl (**17e**)) resulted in the same or slightly reduced σ_1 affinity. However, an electron poor benzyl moiety (4-fluorobenzyl (**17c**)) led to an increased σ_1 affinity. This observation is in good accordance with analogous spirocyclic piperidine σ_1 ligands.[36]

Systematic extension of the aryl-N distance from one methylene moiety (**17a**) to two (**17f**), three (**17g**), and four (**17h**) methylene moieties led to a slightly reduced σ_1 affinity from 1.5 nM (**17a**) to 3.2 nM (**17h**). Replacement of the terminal phenyl moiety of the potent benzyl derivative **17a** with a hydrogenated cyclohexyl group (**17i**) afforded a σ_1 ligand with subnanomolar affinity (K_i = 0.55 nM). This result indicates that the aromatic system of **17a** can be replaced by a saturated system, which even increases σ_1 affinity. Therefore, smaller N-substituents were included into this study.

The secondary amine **17b** was almost inactive at the σ_1 receptor. However, the σ_1 receptor affinity was enhanced with increasing size of the N-substituent. In this series, compounds containing N-residues with five carbon atoms (**17n**−**p**) represent the highest affinity σ_1 ligands with K_i-values in the range of 1 nM. Even **17q** with the very large octyl residue showed a σ_1 receptor affinity of 2.9 nM.

Replacement of the methoxy moiety in position 6′ with an OH moiety (**24**) led to a 20-fold decrease of the σ_1 receptor affinity. However, the compound with a double bond between C-6′ and C-7′ (**26**) and the unsubstituted system (**28a**) showed the same σ_1 receptor affinity as the parent methoxy derivative **17a**. Variation of the N-substituent (R^2) of the unsubstituted compound **28a** resulted in the same trends as those observed for the methoxy derivatives **17**. In particular, the very high σ_1 affinity of the cyclohexylmethyl derivative **28d** should be emphasized (K_i = 0.43 nM).

Despite the high σ_1 affinity and analgesic potency of the methyl substituted indazole derivatives **1** (R^1 = CH_3), the corresponding spirocyclic pyranopyrazoles with a methyl substituent showed considerably lower σ_1 receptor affinities than the phenyl derivatives. As a rule, the σ_1 affinity of the methyl substituted derivatives is about 15−20-fold lower than the σ_1 affinity of the phenyl substituted derivatives (e.g., **17a**, K_i = 1.5 nM; **18a**, K_i = 21 nM; **17o**, K_i = 0.97 nM; **18f**, K_i = 18 nM; **28c**, K_i = 0.81 nM; **29c**, K_i = 17 nM). However, the structure affinity relationships within the class of methyl derivatives correlate nicely with those derived for the phenyl substituted derivatives, although at a lower level of σ_1 affinity.

σ_1 PHARMACOPHORE MODELS

Various pharmacophore models have been developed as tools for the explanation of the affinity of known σ_1 ligands and for the design of novel σ_1 ligands.[49−53] A basic N-atom together with hydrophobic regions are important features according to these models. The first 3D computer-based σ_1 pharmacophore model was developed by Langer et al. and consists of four hydrophobic groups and one positive ionizable group (Figure 2, top). The postulated distances between the basic amino moiety and the hydrophobic groups are 4.1 Å, 6.3 Å, and 9.8 Å, respectively.[51] These distances are in good accordance with the distances defined in the Glennon model.[49,50]

In order to define the corresponding distances in the spirocyclic pyranopyrazoles **28a** and **29a** (achiral without the 6′-OCH_3 group), a stochastic conformational analysis (Molecular Operating Environment (MOE)) was performed with subsequent AM1 minimization of the resulting conformations. For both compounds, six energetically favored conformations were found in the energy range <1.66 kcal/mol. In all conformations, the *N*-benzyl substituent is equatorially oriented at the piperidine ring. For the spirocyclic framework, two types of conformations were found with the pyrazole ring in an equatorial or axial orientation related to the piperidine chair. The distance between the basic N-atom and the pyrazole ring is greater (6.5 Å) for an equatorially oriented pyrazole ring than for an axially oriented one (5.9 Å).

In Figure 2, energetically favored spirocyclic pyranopyrazoles **28a** and **29a** with an equatorially oriented *N*-benzyl moiety and pyrazole ring are compared with the pharmacophore model

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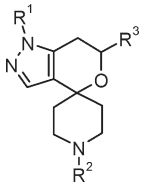
Table 1. σ_1 and σ_2 Receptor Affinities of Spirocyclic Pyranopyrazoles Compared with Those of Lead Compounds and Reference Compounds



compd	R^1	R^2	R^3	$\sigma_1 K_i \pm$ SEM (nM)	$\sigma_2 K_i \pm$ SEM (nM)	selectivity σ_1/σ_2
1a[a,28]				7.0	39.7	5.7
2a[31]		Ph-CH_2	OCH_3	1.1 ± 0.22	1280	1130
2b[31]		Ph-CH_2	OCH_3	1.3 ± 0.18	3500	2708
17a	Ph	Ph-CH_2	OCH_3	1.5 ± 0.08	>1 μM	>680
17b	Ph	H	OCH_3	>1 μM	>1 μM	
17c	Ph	*p*-F-C_6H_4-CH_2	OCH_3	0.94 ± 0.21	687	730
17d	Ph	*p*-H_3CO-C_6H_4-CH_2	OCH_3	1.5 ± 0.29	925	600
17e	Ph	furan-2-yl-CH_2	OCH_3	2.2 ± 0.37	>1 μM	>450
17f	Ph	Ph-$(CH_2)_2$	OCH_3	2.7 ± 0.54	570	209
17g	Ph	Ph-$(CH_2)_3$	OCH_3	3.2 ± 0.70	833	260
17h	Ph	Ph-$(CH_2)_4$	OCH_3	3.2 ± 0.29	428	134
17i	Ph	C_6H_{11}-CH_2	OCH_3	0.55 ± 0.17	109 ± 13	200
17j	Ph	*n*-propyl	OCH_3	33 ± 4.3	>1 μM	>30
17k	Ph	isopropyl	OCH_3	210 ± 35	>1 μM	>5
17l	Ph	*n*-butyl	OCH_3	8.0 ± 2.2	752	94
17m	Ph	isobutyl	OCH_3	6.3 ± 1.1	933	150
17n	Ph	*n*-pentyl	OCH_3	0.82 ± 0.06	340 ± 32	415
17o	Ph	isopentyl	OCH_3	0.97 ± 0.16	316 ± 55	326
17p	Ph	isopentenyl	OCH_3	1.6 ± 0.33	>1 μM	610
17q	Ph	octyl	OCH_3	2.9 ± 0.47	209 ± 22	73
18a	CH_3	Ph−CH_2	OCH_3	21 ± 2.3	>1 μM	>48
18c	CH_3	*p*-F-C_6H_4-CH_2	OCH_3	21 ± 6.3	>1 μM	>48
18d	CH_3	Ph-$(CH_2)_3$	OCH_3	93 ± 19	>1 μM	>11
18e	CH_3	*n*-propyl	OCH_3	>1 μM	>1 μM	
18f	CH_3	isopentyl	OCH_3	18 ± 6.3	>1 μM	>56
24	Ph	Ph-CH_2	OH	27 ± 6.7	>1 μM	>37
25	CH_3	Ph-CH_2	OH	190 ± 9.5	>1 μM	>5
26	Ph	Ph-CH_2	$HC^6{=}C^7H^7$	1.48 ± 0.27	557	376
27	CH_3	Ph-CH_2	$HC^6{=}C^7H$	12 ± 3.7	429	36
28a	Ph	Ph-CH_2	H	1.71 ± 0.08	773	452
28c	Ph	Ph-$(CH_2)_3$	H	0.81 ± 0.15	102 ± 11	125
28d	Ph	C_6H_{11}-CH_2	H	0.43 ± 0.09	43 ± 4.8	100
28e	Ph	isopentyl	H	0.98 ± 0.17	83 ± 22	85
28f	Ph	isopentenyl	H	0.97 ± 0.07	269	297
29a	CH_3	Ph-CH_2	H	9.2 ± 2.8	191	21
29c	CH_3	Ph-$(CH_2)_3$	H	17 ± 11	>1 μM	>59
29d	CH_3	isopentyl	H	30 ± 11	>1 μM	>33
29e	CH_3	isopentenyl	H	14 ± 2.9	>1 μM	>71
(+)-pentazocine				4.2 ± 1.1		
haloperidol				3.9 ± 1.5	78 ± 2.3	20
di-*o*-tolylguanidine				61 ± 18	42 ± 15	0.7
progesterone				660 ± 115		

[a] Compound **1a**: $R^1 = CH_3$, $NR_2{}^2$ = 4-phenylpiperidin-1-yl.

developed by Langer. The corresponding distances were calculated using the end points of the hydrophobic substituents. The distances determined for the spirocyclic systems fit nicely to the distances of the model. The higher σ_1 affinity of the phenyl

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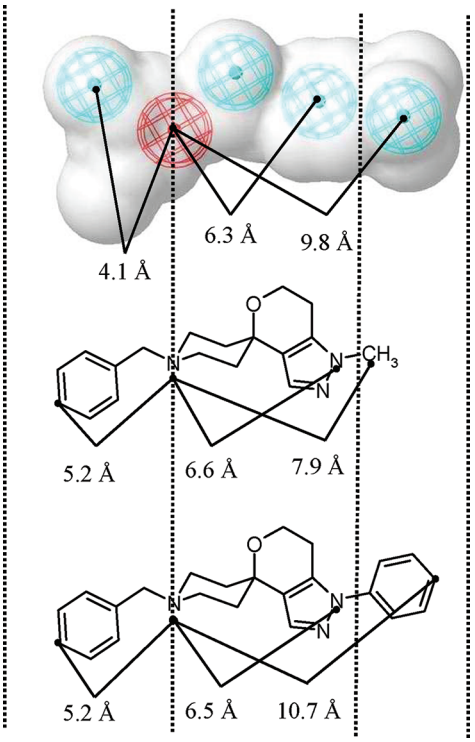

Figure 2. Comparison of spirocyclic pyranopyrazole derivatives **28a** and **29a** with the σ_1 pharmacophore model of Langer et al.[51] Top: σ_1 pharmacophore model with indicated distances;[51] middle and bottom: distances between the basic N-atom and hydrophobic regions of the energetically most favored conformations of **28a** and **29a** calculated with AM1.

derivative **28a** (K_i = 1.7 nM) compared with that of the methyl derivative **29a** (K_i = 9.2 nM) is attributed to the additional phenyl residue. Whereas the methyl group of **29a** is too small to produce high lipophilic interactions with the σ_1 receptor protein, the phenyl moiety of **28a** is able to occupy an additional hydrophobic pocket resulting in increased lipophilic interactions (lower K_i value).

RECEPTOR SELECTIVITY

The selectivity of the spirocyclic pyranopyrazoles against related receptor systems was investigated. At first, the affinity toward the σ_2 subtype was determined using homogenates of rat liver as the σ_2 receptor source. Since a σ_2 selective radioligand is not commercially available, the nonselective radioligand [^{3}H]-ditolylguanidine was employed in the presence of an excess of nonlabeled (+)-pentazocine (500 nM) for the selective masking of σ_1 receptors. An excess of nontritiated ditolylguanidine was used for determining the nonspecific binding.[31−33,48]

Generally, all spirocyclic pyranopyrazoles reveal high selectivity for the σ_1 receptor subtype over the σ_2 subtype. The σ_1/σ_2 selectivity is particularly high for very potent σ_1 ligands (e.g., **17c**, K_i = 0.94 nM, selectivity 730; **28a**, K_i = 1.71 nM, selectivity 452), but compounds with reduced σ_1 affinity display lower σ_1/σ_2 selectivity.

Relatively high σ_2 affinities were observed for the cyclohexylmethyl derivatives **17i** (K_i = 109 nM) and **28d** (K_i = 43 nM). Because of the very high σ_1 affinities of these compounds, the σ_1/σ_2 selectivities are still greater than 100.

The removal of the methoxy group in position 6′ led in most cases to an increased σ_2 affinity (e.g., **17a**, K_i > 1 μM; **28a**, K_i = 773 nM; **17o**, K_i = 316 nM; **28e**, K_i = 83 nM). This result indicates that high σ_1 affinity and selectivity against the σ_2 subtype can be achieved by introducing a substituent in position 6′ of the pyranopyrazole system.

Since some potent σ ligands also interact with NMDA receptors and vice versa,[54,55] the affinity of all synthesized pyranopyrazoles toward the NMDA receptor was also included into this study. The affinity to the PCP binding site of the NMDA receptor was determined in competition experiments using fresh pig brain cortex membrane preparations as the receptor material and the radioligand [^{3}H]-(+)-MK-801.[56] At a concentration of 1 μM, all synthesized pyranopyrazoles showed no interaction with the PCP binding site of the NMDA receptor.

Additionally, the affinities of the highly affine σ_1 ligands **17a**, **17i**, **17m**, **17o**, and **17q** toward α_1, α_2, and 5-HT$_{1A}$ receptors as well as the 5-HT-transporter were investigated. The corresponding IC$_{50}$-values were higher than 1 μM for all five test compounds indicating a more than 1000-fold selectivity of these compounds against these targets.

ANTIALLODYNIC ACTIVITY

Sensitization by subplantar capsaicin injection was used to assess the effect of the cyclohexylmethyl derivative **17i** (K_i (σ_1) = 0.55 nM) and the isopentenyl derivative **17p** (K_i (σ_1) = 1.6 nM) on mechanical allodynia of mice.[27,28] In this assay, capsaicin (8-methyl-*N*-vanillylnon-6-enamide) was injected subplantarly to evoke a nocifensive behavior that is characterized by lifting and guarding the injected paw and typically lasts up to 5 min following injection. Afterward, hypersensitivity to both thermal and mechanical stimuli is evidenced.[27,28,57]

Mice were treated with the σ_1 ligands **17i** and **17p** 30 min before the capsaicin injection into the midplantar surface of the right hind paw. Withdrawal latencies to mechanical stimuli by a von Frey filament (1 g) were determined 15 min after the capsaicin injection. Whereas the indazole derivative **1a** (K_i (σ_1) = 7.0 nM) was very potent in the capsaicin assay,[28] the more potent σ_1 ligands **17i** and **17p** showed only low antiallodynic activity. Even at the highest dose tested (32 mg/kg body weight), the antiallodynic effect of **17i** and **17p** was only 70% and 25%, respectively, indicating low in vivo activity. Although the antiallodynic activity is rather low, both compounds can be considered as partial σ_1 receptor antagonists.

CONCLUSIONS

The combination of potent indazole-based (**1**) and spirocyclic (**2**) σ_1 ligands resulted in the spirocyclic pyranopyrazoles **3**, which interact with high affinity and selectivity with σ_1 receptors. The core structure was extensively modified in positions 1, 1′, and 6′. In contrast to the indazole class of σ_1 ligands **1**, spirocyclic pyranopyrazoles **18** with a methyl group (R^1) showed lower σ_1 affinities than their phenyl (R^1) substituted analogues **17**. The lower σ_1 affinities of compounds containing the methylpyrazole substructure (e.g., **18**) compared with those of analogous benzene derivatives **2** were attributed to the small, electron rich pyrazole heterocycle. However, this effect was compensated by an additional phenyl ring at the pyrazole heterocycle, which is able to occupy an additional hydrophobic region of the σ_1 receptor protein. This observation is in good accordance with σ_1 pharmacophore models. Despite the high σ_1 affinity and

selectivity, the in vivo activity of two promising representatives (**17i**, **17p**) in the capsaicin neuropathic pain model was rather low. Nevertheless, the low antiallodynic activity of **17i** and **17p** indicates at least a partial σ_1 receptor antagonistic activity.

EXPERIMENTAL SECTION

General. Unless otherwise noted, moisture sensitive reactions were conducted under dry nitrogen. THF was dried with sodium/benzophenone and was freshly distilled before use. The concentration of *n*-BuLi marked with an asterisk (*) was determined by titration with 1,3-diphenylpropan-2-one *p*-toluenesulfonylhydrazone. Flash chromatography (fc), Silica gel 60, 40−64 μm (Merck); parentheses include diameter of the column, eluent, fraction size, and R_f value. Melting point: melting point apparatus SMP 3 (Stuart Scientific), uncorrected. ^{1}H NMR (400 MHz) and ^{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). The purity of all test compounds was proved by elemental analysis; all values are within ±0.4%. The purity of four test compounds was proved by HPLC analysis (purity >95%).

5-(2-Methoxyvinyl)-1-phenylpyrazole (8). Under N_2, dry $Ph_3PCH_2OCH_3^+$ Cl^- (6.22 g, 18.1 mmol) was suspended in THF (60 mL) for 30 min. The suspension was cooled down to −50 °C, and then a solution of KO^{t}Bu in THF (1 M, 16.5 mL, 16.5 mmol) was added dropwise. After stirring for 15 min, a solution of **6** (1.42 g, 8.3 mmol) in abs. THF (40 mL) was added dropwise. The mixture was allowed to warm to rt overnight. After the addition of water (∼25 mL), the mixture was extracted with EtOAc. The organic layer was dried (K_2CO_3) and filtered, the solvent was removed in vacuo, and the residue was purified by fc (Ø = 8 cm, *n*-hexane:EtOAc = 9:1, 40 mL, R_f = 0.10). Colorless oil, yield 1.53 g (92%). $C_{12}H_{12}N_2O$ (200.2). ^{1}H NMR (DMSO-D_6): δ (ppm) = 3.56 (s, 3 × 0.75H, OCH_3, trans), 3.77 (s, 3 × 0.25H, OCH_3, cis), 5.18 (d, *J* = 6.8 Hz, 0.25H, C*H*═CHOCH$_3$, cis), 5.59 (d, *J* = 12.8 Hz, 0.75H, C*H*═CHOCH$_3$, trans), 6.37 (d, *J* = 6.8 Hz, 0.25H, CH═C*H*OCH$_3$, cis), 6.47 (d, *J* = 1,6 Hz, 0.75H, pyrazole-4-C*H*, trans), 6.63 (d, *J* = 1.6 Hz, 0.25H, pyrazole-4-C*H*, cis), 7.26 (d, *J* = 12.8 Hz, 0.75H, CH═C*H*OCH$_3$, trans), 7.39−7.54 (m, 5H, phenyl-C*H*), 7.55 (d, *J* = 1.6 Hz, 0.75H, pyrazole-3-C*H*, trans), 7.58 (d, *J* = 1.2 Hz, 0.25H, pyrazole-3-C*H*, cis). The ratio of *cis*-**8**:*trans*-**8** is 25:75.

2-(1-Phenylpyrazol-5-yl)acetaldehyde dimethyl acetal (10). A solution of the enol ether **8** (1.0 g, 5.0 mmol) and *p*-toluenesulfonic acid monohydrate (475 mg, 2.5 mmol) in MeOH (80 mL) was stirred at rt for 72 h. Then, a solution of saturated $NaHCO_3$ was added. The mixture was extracted with CH_2Cl_2, the organic layer was dried (K_2CO_3), the solvent was removed in vacuo, and the residue was purified by fc (Ø = 6 cm, *n*-hexane:EtOAc = 8:2, 40 mL, R_f = 0.12). Colorless oil, yield 1.04 g (90%). $C_{13}H_{16}N_2O_2$ (232.3). ^{1}H NMR (DMSO-D_6): δ (ppm) = 2.92 (d, *J* = 5.9 Hz, 2H, CH_2CH(OCH$_3$)$_2$), 3.39 (s, 6H, CH$_2$CH(OCH_3)$_2$), 4.55 (t, *J* = 5.5 Hz, 1H, CH$_2$C*H*-(OCH$_3$)$_2$), 6.39 (d, *J* = 1.6 Hz, 1H, pyrazole-4-C*H*), 7.40−7.55 (m, 5H, phenyl-C*H*), 7.59 (d, *J* = 1.6 Hz, 1H, pyrazole-3-C*H*). ^{13}C NMR (DMSO-D_6): δ (ppm) = 30.5 (1C, CH$_2$CH(OCH$_3$)$_2$), 53.8 (2C, CH$_2$CH(OCH$_3$)$_2$), 103.6 (1C, CH$_2$CH(OCH$_3$)$_2$), 107.3 (1C, pyrazole-4-CH), 126.0 (2C, phenyl-CH, ortho), 128.6 (1C, phenyl-CH, para), 129.9 (2C, phenyl-CH, meta), 139.1 (1C, phenyl-C), 140.2 (1C, pyrazole-5-CH), 140.3 (1C, pyrazole-3-CH).

2-(4-Bromo-1-phenylpyrazol-5-yl)acetaldehyde dimethyl acetal (12). Trimethyl orthoformate (7.0 mL, 64.3 mmol) and pyridinium bromide perbromide (PBB) (13.7 g, 42.8 mmol) were added in portions to a solution of **10** (9.9 g, 42.8 mmol) in MeOH (500 mL) at 0 °C. After stirring for 1 h at 0 °C, the mixture was allowed to warm to rt and stirred for 4 h. Then, water and a saturated solution of $NaHCO_3$ were added, and the aqueous solution was extracted with CH_2Cl_2. The organic layer was dried (K_2CO_3), the solvent was removed in vacuo, and the residue was purified by fc (Ø = 8 cm, *n*-hexane:EtOAc = 8:2, 40 mL, R_f = 0.30). Colorless oil, yield 12.7 g (95%). Anal. ($C_{13}H_{15}BrN_2O_2$, 311.2) C, H, N. ^{1}H NMR (DMSO-D_6): δ (ppm) = 2.95 (d, *J* = 5.9 Hz, 2H, CH_2CH(OCH$_3$)$_2$), 3.10 (s, 6H, CH$_2$CH(OCH_3)$_2$), 4.40 (t, *J* = 5.7 Hz, 1H, CH$_2$C*H*(OCH$_3$)$_2$), 7.45−7.58 (m, 5H, phenyl-C*H*), 7.76 (s, 1H, pyrazole-3-C*H*). NOE difference spectrum (CDCl$_3$): irrad. at 2.95 ppm (CH_2CH(OCH$_3$)$_2$), increase of signals at 4.40 ppm (CH$_2$C*H*-(OCH$_3$)$_2$), 7.45−7.58 ppm (C$_6$H$_5$). ^{13}C NMR (DMSO-D_6): δ (ppm) = 29.6 (1C, *C*H$_2$CH(OCH$_3$)$_2$), 54.2 (2C, CH$_2$CH(OCH$_3$)$_2$), 95.8 (1C, pyrazole-4C), 102.7 (1C, CH$_2$*C*H(OCH$_3$)$_2$), 126.3 (2C, phenyl-CH, ortho), 129.3 (1C, phenyl-CH, para), 130.0 (2C, phenyl-CH, meta), 137.4 (1C, phenyl-C), 140.2 (1C, pyrazole-5C), 140.5 (1C, pyrazole-3-CH).

2-[4-(1-Benzyl-4-hydroxypiperidin-4-yl)-1-phenylpyrazol-5-yl]acetaldehyde dimethyl acetal (15). Under N_2, a 1.53 M* solution of *n*-butyllithium in hexane (10.5 mL, 16.1 mmol) was added slowly to a cooled (−78 °C) solution of **12** (5.0 g, 16.1 mmol) in THF (80 mL). The mixture was stirred at −78 °C for 15 min, then a solution of piperidone **14** (3.3 g, 17.7 mmol) in THF (10 mL) was added slowly, and the mixture was stirred at −78 °C for 4.5 h and for 1 h at rt. Then, water (∼40 mL) was added until no more precipitate was formed. The mixture was extracted with CH_2Cl_2, the organic layer was dried (K_2CO_3) and concentrated in vacuo, and the residue was purified by fc (Ø = 8 cm, *n*-hexane:EtOAc = 2:8 + 2% *N*,*N*-dimethylethanamine, 80 mL, R_f = 0.23). Pale yellow oil, which solidified slowly upon standing. Pale yellow solid, mp 107 °C, yield 4.6 g (67%). $C_{25}H_{31}N_3O_3$ (421.5). ^{1}H NMR (DMSO-D_6): δ (ppm) = 1.77 (d broad, *J* = 12.5 Hz, 2H, N(CH$_2$C*H*$_2$)$_2$), 1.92 (td, *J* = 12.5/3.1 Hz, 2H, N(CH$_2$C*H*$_2$)$_2$), 2.42 (t, *J* = 10.6 Hz, 2H, N(C*H*$_2$CH$_2$)$_2$), 2.58 (d broad, *J* = 11.0 Hz, 2H, N(C*H*$_2$CH$_2$)$_2$), 3.04 (s, 6H, CH$_2$CH(OCH_3)$_2$), 3.12 (d, *J* = 5.5 Hz, 2H, CH_2CH(OCH$_3$)$_2$), 3.49 (s, 2H, NCH_2Ph), 4.50 (t, *J* = 5.8 Hz, 1H, CH$_2$C*H*(OCH$_3$)$_2$), 4.66 (s, 1H, O*H*), 7.21−7.27 (m, 1H, aromat. C*H*, para), 7.32 (m, 4H, aromat. C*H*, meta), 7.40−7.46 (m, 3H, aromat. C*H*, para, ortho), 7.49 (d, *J* = 7.0 Hz, 2H, aromat. C*H*, ortho), 7.50 (s, 1H, pyrazole-3-C*H*).

1-Benzyl-6′-methoxy-1′-phenyl-6′,7′-dihydro-1′*H*-spiro-[piperidine-4,4′-pyrano[4,3-c]pyrazole] (17a). A solution of **15** (4.5 g, 10.7 mmol) and *p*-toluenesulfonic acid monohydrate (4.5 g, 23.5 mmol) in MeOH (150 mL) was stirred at rt for 21 h. After the addition of NaOH (0.5 M, 5 mL), the mixture was extracted with CH_2Cl_2. The organic layer was dried (K_2CO_3) and concentrated in vacuo, and the residue was purified by fc (Ø = 8 cm, EtOAc, 80 mL, R_f = 0.27). Colorless solid, mp 151 °C, yield 3.0 g (73%). Anal. ($C_{24}H_{27}N_3O_2$, 389.5) C, H, N. ^{1}H NMR (CDCl$_3$): δ (ppm) = 1.92 (dd, *J* = 14.1/3.1 Hz, 2H, N(CH$_2$C*H*$_2$)$_2$), 1.98−2.05 (m, 1H, N(CH$_2$C*H*$_2$)$_2$), 2.09 (td, *J* = 12.5/3.7 Hz, 1H, N(CH$_2$C*H*$_2$)$_2$), 2.45 (t broad, *J* = 11.7 Hz, 1H, N(C*H*$_2$CH$_2$)$_2$), 2.55 (t broad, *J* = 11.7 Hz, 1H, N(C*H*$_2$CH$_2$)$_2$), 2.77 (m, 2H, N(C*H*$_2$CH$_2$)$_2$), 2.88 (dd, *J* = 15.6/7.0 Hz, 1H, CH_2CHOCH$_3$), 2.96 (dd, *J* = 15.7/3.9 Hz, 1H, CH_2CHOCH$_3$), 3.53 (s, 3H, OCH_3), 3.58 (s, 2H, NCH_2Ph), 4.84 (dd, *J* = 7.0/3.9 Hz, 1H, CH$_2$C*H*OCH$_3$), 7.23−7.28 (m, 1H, phenyl-C*H*), 7.29−7.39 (m, 5H, phenyl-C*H*), 7.40−7.47 (m, 4H, phenyl-C*H*), 7.49 (s, 1H, pyrazole-3-C*H*). ^{13}C NMR (CDCl$_3$): δ (ppm) = 31.2 (1C, CH$_2$CHOCH$_3$), 36.7 (1C, N(CH$_2$CH$_2$)$_2$), 39.5 (1C, N(CH$_2$CH$_2$)$_2$), 49.5 (1C, N(CH$_2$CH$_2$)$_2$), 49.6 (1C, N(CH$_2$CH$_2$)$_2$), 56.9 (1C, OCH$_3$), 63.7 (1C, NCH$_2$Ph), 71.9 (1C, spiro-C), 77.5 (1C, pyrazole-4-C), 96.8 (1C, CH$_2$CHOCH$_3$), 122.8 (2C, phenyl-CH), 124.4 (1C, phenyl-C), 127.3, 128.5, 129.5 (8C, phenyl-CH), 133.8 (1C, phenyl-C), 135.9 (1C, pyrazole-3-CH), 139.5 (1C, pyrazole-5-C).

6′-Methoxy-1′-phenyl-6′,7′-dihydro-1′*H*-spiro[piperidine-4,4′-pyrano[4,3-c]pyrazole] (17b). Dry ammonium formate (64.8 mg, 1.28 mmol) was added to a stirred suspension of **17a**

(80 mg, 0.21 mmol) and 10% Pd/C (16 mg) in MeOH (5 mL). This mixture was heated to reflux for 10 min. Then, it was filtered and concentrated in vacuo, and the residue was purified by fc (Ø = 2 cm, methanol + 2% $NH_{3\ (conc.)}$, 10 mL, R_f = 0.10). After removing the solvent of the respective fractions, the residue was dissolved in CH_2Cl_2, the solution was filtered, and the solvent was evaporated in vacuo. Pale yellow oil, yield 55 mg (88%). Anal. ($C_{17}H_{21}N_3O_2$, 299.4) H, N; C calcd. 68.2; found, 67.2. Purity (HPLC): 100% (t_R = 14.8 min). ^{1}H NMR (DMSO-D_6): δ (ppm) = 1.55−1.68 (m, 2H, $N(CH_2CH_2)_2$), 1.79−1.92 (m, 2H, $N(CH_2CH_2)_2$), 2.75 (t broad, J = 11.0 Hz, 2H, $N(CH_2CH_2)_2$), 2.80−2.95 (m, 2H, $N(CH_2CH_2)_2$), 2.82 (dd, J = 15.7/7.0 Hz, 1H, CH_2CHOCH_3), 2.91 (dd, J = 15.7/3.9 Hz, 1H, CH_2CHOCH_3), 3.31 (s broad, 1H, NH), 3.39 (s, 3H, OCH_3), 4.88 (dd, J = 6.7/3.5 Hz, 1H, CH_2CHOCH_3), 7.35 (t, J = 7.4 Hz, 1H, phenyl-CH, para), 7.44−7.55 (m, 4H, phenyl-CH, meta, ortho), 7.59 (s, 1H, pyrazole-3-CH).

1-(4-Fluorobenzyl)-6′-methoxy-1′-phenyl-6′,7′-dihydro-1′*H*-spiro[piperidine-4,4′-pyrano[4,3-c]pyrazole] (17c). *p*-Fluorobenzaldehyde (36.0 μL, 0.33 mmol) and $NaBH(OAc)_3$ (106 mg, 0.50 mmol) were added to a stirred solution of **17b** (100 mg, 0.33 mmol) in dichloroethane (2 mL). The mixture was stirred at rt for 19 h. After the addition of a saturated solution of $NaHCO_3$ (10 mL), the mixture was extracted with CH_2Cl_2. The organic layer was dried (K_2CO_3) and concentrated in vacuo, and the residue was purified by fc (Ø = 3 cm, *n*-hexane:EtOAc 5:5 + 2% *N*,*N*-dimethylethanamine, 20 mL, R_f = 0.20). Colorless solid, mp 158 °C, yield 107 mg (79%). Anal. ($C_{24}H_{26}FN_3O_2$, 407.5) C, H, N. ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.86−1.98 (m, 2H, $N(CH_2CH_2)_2$), 2.00−2.06 (m, 1H, $N(CH_2CH_2)_2$), 2.09 (td, J = 12.9/4.4 Hz, 1H, $N(CH_2CH_2)_2$), 2.45 (t broad, J = 11.0 Hz, 1H, $N(CH_2CH_2)_2$), 2.56 (t broad, J = 11.0 Hz, 1H, $N(CH_2CH_2)_2$), 2.71−2.81 (m, 2H, $N(CH_2CH_2)_2$), 2.90 (dd, J = 15.3/6.7 Hz, 1H, CH_2CHOCH_3), 2.98 (dd, J = 15.3/3.5 Hz, 1H, CH_2CHOCH_3), 3.55 (s, 5H, OCH_3 (3H), NCH_2Ph (2H)), 4.86 (dd, J = 6.7/3.5 Hz, 1H, CH_2CHOCH_3), 7.03 (t, J = 8.6 Hz, 2H, phenyl-CH), 7.30−7.38 (m, 3H, phenyl-CH), 7.41−7.48 (m, 4H, phenyl-CH), 7.50 (s, 1H, pyrazole-3-CH). ^{13}C NMR ($CDCl_3$): δ (ppm) = 31.2 (1C, CH_2CHOCH_3), 36.7 (1C, $N(CH_2CH_2)_2$), 39.4 (1C, $N(CH_2CH_2)_2$), 49.3 (1C, $N(CH_2CH_2)_2$), 49.4 (1C, $N(CH_2CH_2)_2$), 56.9 (1C, OCH_3), 62.7 (1C, NCH_2Ph), 71.8 (1C, spiro-C), 77.4 (1C, pyrazole-4-C), 96.8 (1C, CH_2CHOCH_3), 115.1, 115.3 (2C, benzyl-2′,6′-CH), 122.8 (2C, phenyl-CH, ortho), 124.3 (1C, benzyl-4′-C), 127.3 (1C, phenyl-CH, para), 129.4 (2C, phenyl-CH, meta), 130.9 (2C, benzyl-3′5′-CH), 133.7 (1C, phenyl-C), 134.3 (1C, benzyl-1′-C), 135.8 (1C, pyrazole-3-CH), 139.4 (1C, pyrazole-5-C).

1-(Cyclohexylmethyl)-6′-methoxy-1′-phenyl-6′,7′-dihydro-1′*H*-spiro[piperidine-4,4′-pyrano[4,3-c]pyrazole] (17i). (Bromomethyl)cyclohexane (48.5 μL, 0.35 mmol) and K_2CO_3 (295 mg, 2.14 mmol) were added to a solution of **17b** (80 mg, 0.27 mmol) in acetonitrile (5 mL). This mixture was heated to reflux for 26 h. Then, it was filtered and concentrated in vacuo, and the residue was purified by fc (Ø = 2.5 cm, *n*-hexane:EtOAc 7:3 + 1% *N*,*N*-dimethylethanamine, 10 mL, R_f = 0.18). Colorless solid, mp 151 °C, yield 82 mg (77%). Anal. ($C_{24}H_{33}N_3O_2$, 395.6) C, H, N. ^{1}H NMR ($CDCl_3$): δ (ppm) = 0.84−0.97 (m, 2H, $NCH_2C_6H_{11}$), 1.18−1.29 (m, 4H, $NCH_2C_6H_{11}$), 1.47−1.59 (m, 1H, $NCH_2C_6H_{11}$), 1.62−1.87 (m, 4H, $NCH_2C_6H_{11}$), 1.90−1.98 (m, 2H, $N(CH_2CH_2)_2$), 2.02−2.15 (m, 2H, $N(CH_2CH_2)_2$), 2.21 (d, J = 7.0 Hz, 2H, $NCH_2C_6H_{11}$), 2.37 (td, J = 11.4/2.4 Hz, 1H, $N(CH_2CH_2)_2$), 2.45 (td, J = 11.7/2.4 Hz, 1H, $N(CH_2CH_2)_2$), 2.70−2.79 (m, 2H, $N(CH_2CH_2)_2$), 2.90 (dd, J = 15.7/7.0 Hz, 1H, CH_2CHOCH_3), 2.97 (dd, J = 15.7/3.5 Hz, 1H, CH_2CHOCH_3), 3.56 (s, 3H, OCH_3), 4.84 (dd, J = 6.7/3.5 Hz, 1H, CH_2CHOCH_3), 7.30−7.35 (m, 1H, phenyl-CH, para), 7.41−7.48 (m, 4H, phenyl-CH), 7.51 (s, 1H, pyrazole-3-CH).

1-Benzyl-1′-phenyl-1′*H*-spiro[piperidine-4,4′-pyrano-[4,3-c]pyrazole] (26). Under N_2, lactol **24** (510 mg, 1.36 mmol) was dissolved in CH_2Cl_2 (12 mL). The mixture was cooled to 0 °C, and NEt_3 (454 μL, 3.26 mmol) and $MeSO_2Cl$ (127 μL, 1.63 mmol) were added. The solution was stirred at rt for 2 h and then heated to reflux for 1 h. After the addition of a saturated solution of $NaHCO_3$ (5 mL), the mixture was extracted with CH_2Cl_2 (3×). The organic layer was dried (K_2CO_3), filtered, and concentrated in vacuo, and the residue was purified by fc (Ø = 5 cm, *n*-hexane:EtOAc 6:4 + 2% *N*,*N*-dimethylethanamine, 65 mL, R_f = 0.15). Colorless solid, mp 135 °C, yield 315 mg (65%). Anal. ($C_{23}H_{23}N_3O$, 357.5) C, H, N. ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.92−2.02 (m, 2H, $N(CH_2CH_2)_2$), 2.19−2.26 (m, 2H, $N(CH_2CH_2)_2$), 2.47 (td, J = 11.5/1.7 Hz, 2H, $N(CH_2CH_2)_2$), 2.70−2.76 (m, 2H, $N(CH_2CH_2)_2$), 3.58 (s, 2H, NCH_2Ph), 5.82 (dd, J = 6.1/0.8 Hz, 1H, ArCH═CHO), 6.50 (d, J = 5.9 Hz, 1H, ArCH═CHO), 7.26−7.29 (m, 1H, phenyl-CH), 7.30−7.39 (m, 6H, phenyl-CH), 7.43−7.52 (m, 4H, phenyl-CH (3H), pyrazole-3-CH (1H)).

1-Benzyl-1′-phenyl-6′,7′-dihydro-1′*H*-spiro[piperidine-4,4′-pyrano[4,3-c]pyrazole] (28a). Ten percent Pd/C (35 mg) was added to a solution of **26** (50 mg, 0.14 mmol) in HOAc (5 mL). The mixture was stirred under H_2 (balloon) for 1 h. Then, the catalyst was filtered off, and the filtrate was alkalized with NaOH (2 M) and extracted with CH_2Cl_2 (3×). The organic layer was dried (K_2CO_3) and filtered, the solvent was removed in vacuo, and the residue was purified by fc (Ø = 2.5 cm, *n*-hexane:EtOAc 6:4 + 2% *N*,*N*-dimethylethanamine, 10 mL, R_f = 0.23). Colorless solid, mp 141 °C, yield 31 mg (62%). Anal. ($C_{23}H_{25}N_3O$, 359.5) C, H, N. ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.88−1.95 (m, 2H, $N(CH_2CH_2)_2$), 1.98 (td, J = 13.5/4.3 Hz, 2H, $N(CH_2CH_2)_2$), 2.42 (td, J = 11.4/3.2 Hz, 2H, $N(CH_2CH_2)_2$), 2.73 (d broad, J = 11.3 Hz, 2H, $N(CH_2CH_2)_2$), 2.82 (t, J = 5.5 Hz, 2H, $ArCH_2CH_2O$), 3.57 (s, 2H, NCH_2Ph), 3.88 (t, J = 5.4 Hz, 2H, $ArCH_2CH_2O$), 7.23−7.38 (m, 6H, phenyl-CH), 7.42−7.52 (m, 5H, phenyl-CH (4H), pyrazole-3-CH (1H)).

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 a Meltilex (Type A) solid scintillator (Perkin-Elmer). The radioactivity bound to the filter was measured using a MicroBeta Trilux scintillation analyzer (Perkin-Elmer). The overall counting efficiency was 20%.

Membrane Preparation for the σ_1 Assay[31−33,48]. 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[58] using bovine serum albumin as a standard, and, subsequently, the preparation was frozen (−80 °C) in 1.5 mL portions containing about 1.5 mg protein/mL.

Performing of the σ_1 Assay[31−33,48]. The test was performed with the radioligand [^{3}H]-(+)-pentazocine (22 Ci/mmol; Perkin-Elmer). The thawed membrane preparation (about 75 μg of the protein) was incubated with various concentrations of test compounds, 2 nM [^{3}H]-(+)-pentazocine, and buffer (50 mM TRIS, pH 7.4) in a total volume of 200 μL for 180 min at 37 °C. The incubation was terminated by rapid filtration through the presoaked filtermats by using the cell harvester. After washing each well five times with 300 μL of water, the filtermats were dried at 95 °C. Subsequently, the solid scintillator was placed 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 nonspecific binding was determined with 10 μM unlabeled (+)-pentazocine. The K_d-value of the radioligand [^{3}H]-(+)-pentazocine is 2.9 nM.[59]

Data Analysis. Usually, all experiments were carried out in triplicate 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 nonlinear regression analysis. The K_i-values were calculated according to Cheng and Prusoff.[60] The K_i-values of highly affine compounds are given as mean values ± SEM from three independent experiments.

Experimental Details for the σ_2 Assay. See refs 31−33 and 48.

Experimental Details for the NMDA Assay. See ref 56.

■ ASSOCIATED CONTENT

Supporting Information. Physical and spectroscopic data of all new compounds, purity data of all test compounds, general chemistry methods, and details of the pharmacological assays. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ABBREVIATIONS USED

NMDA, *N*-methyl-D-aspartate; IP_3, inositoltriphosphate; CNS, central nervous system; THF, tetrahydrofuran; MOE, molecular operating system; NOE, nuclear Overhauser effect

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