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Pyridine analogues of spirocyclic σ_1 receptor ligands

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ABSTRACT

Spirocyclic benzopyrans **2** interact with high affinity and selectivity with σ_1 receptors. Bioisosteric replacement of the benzene ring of the benzopyran substructure with the electron rich thiophene ring (**3**) led to increased σ_1 affinity. Herein the synthesis and pharmacological evaluation of electron deficient pyridine bioisosteres **4** are reported. Homologation of the aldehyde **6** to afford the pyridylacetaldehyde derivative **8** was performed by a Wittig reaction. Bromine lithium exchange of the bromopyridine **8**, addition to 1-benzylpiperidin-4-one and cyclization led to the spirocyclic pyrranopyridine **10**. Hydrogenolytic removal of the *N*-benzyl moiety of **10** provided the secondary amine **11**, which allowed the introduction of various *N*-substituents (**12a–d**). Cyclization of the hydroxy acetal **9** with HCl led to various modifications of the substituent in 3′-position. Generally the σ_1 affinity of the pyridine derivatives is reduced compared with those of the benzene and thiophene derivatives **2** and **3**. However, the relationships between the structure and the σ_1 affinity follow the same rules as for the benzene and thiophene derivatives. The most promising σ_1 ligand within this class of compounds is the pyranopyridine **15** with a double bond in the pyran ring revealing a K_i-value of 4.6 nM and a very high selectivity (>217-fold) over the σ_2 subtype.

1. Introduction

The class of non-opioid, non-PCP but haloperidol sensitive σ receptors consists of two subtypes, σ_1 and σ_2 receptors, which are distinguished by their molecular weight, ligand binding profile and distribution in the organism. The σ_1 receptor is found in the central nervous system (CNS),[1–3] the endocrine and immune system as well as in many peripheral tissues.[4–8] In 1996, Hanner et al. succeeded in cloning of the σ_1 receptor from guinea pig liver.[9] After this report, σ_1 receptors were also cloned from various human,[10] mouse,[11,12] and rat tissues.[13] The human σ_1 receptor gene encodes for a protein consisting of 223 amino acids with a molecular weight of 25.3 kDa. Although the σ_2 receptor has not been cloned yet, its molecular weight is approximately 21.5 kDa.[14] Recently the identity of the σ_2 receptor and the progesterone receptor membrane component 1 (pgrmc1) was postulated.[15] In 1996 the pgrmc1 was cloned resulting in a protein of 194 amino acids with a molecular weight of 21.7 kDa.[16] Endogenous ligands for both σ receptor subtypes have not been identified unequivocally. However, it has been postulated that *N*,*N*-dimethyltryptamine, sphingosine, and neurosteroids (e.g., pregnenolone, progesterone) might function as endogenous activators of the σ_1 receptor.[17–19]

Antagonists at the σ_1 receptor can be used for the treatment of various CNS diseases. A particular application of σ_1 receptor antagonists is the treatment of neuropathic pain, which is defined as spontaneous hypersensitive pain response persisting even when the painful stimuli have disappeared.[20,21] Medical treatments of neuropathic pain situations is rather difficult, due to its diffuse origin. The role of σ_1 receptors and σ_1 receptor antagonists in neuropathic pain conditions has been shown with the help of σ_1 receptor knockout mice.[22] These efforts resulted in the potent and selective σ_1 receptor antagonist S1RA, which has entered phase II clinical trials for the treatment of neuropathic pain.[23–25] In addition to the treatment of neuropathic pain, σ_1 receptor ligands have a potential for the treatment of various neurological and psychiatric diseases[26–29] including major depression,[26,30,31] schizophrenia,[26,32] and Alzheimer's disease.[33]

In 2002 we reported on the synthesis and pharmacological evaluation of spirocyclic benzofurans **1** and benzopyrans **2**, which represent very potent σ_1 receptor antagonists (Fig. 1).[34,35] Moreover, it was shown that the spirocyclic benzofuran **1** (R^1 = Bn, R^2 = OCH_3) is very active in the capsaicin model of neuropathic pain.[36] Very recently it was shown that bioisosteric replacement of the benzene ring of **2** with a thiophene ring (**3**) increased the

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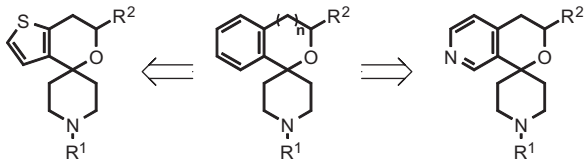


Figure 1. Bioisosteric replacement of the benzene ring of potent σ_1 receptor antagonists **1** and **2** with electron rich thiophene (**3**) and electron deficient pyridine ring (**4**).

σ_1 receptor affinity up to subnanomolar range (**3a**: R^1 = Bn, R^2 = OCH_3, $K_i(\sigma_1)$ = 0.32 nM).[37] The thienopyran **3a** (R^1 = Bn, R^2 = OCH_3) was also analgesically active in the capsaicin assay. The ring size of benzene and thiophene are very similar, but the thiophene ring has a higher electron density representing an electron rich aromatic system. It might be speculated that the higher electron density of thiophene is responsible for the increased σ_1 receptor affinity.

In order to gain further information about the effect of electron density of the aromatic ring on the σ_1 receptor affinity, the synthesis of the pyridine derivatives **4** was envisaged. Whereas the size of the pyridine, benzene and thiophene ring is very similar, the pyridine ring represents an electron deficient aromatic system. Herein the synthesis, σ receptor affinity and structure affinity relationships of pyridine analogous spirocyclic compounds are reported.

2. Chemistry

The pyridine analogous σ receptor ligands **4** were prepared from 3-bromopyridine (**5**) (Scheme 1). Treatment of **5** with LDA led regioselectively to metalation in 4-position adjacent to the bromine atom.[38] The resulting pyridyllithium species reacted with *N*-formylpiperidine to form pyridine-4-carbaldehyde **6** in 48% yield.[38] Wittig reaction of aldehyde **6** with (methoxymethyl)triphenylphosphonium chloride and *t*-BuOK forming the reactive P-ylide intermediate led to the enol ether **7** as mixture of (*E*)- and (*Z*)-isomers in the ratio 70:30.[39] The dimethyl acetal **8** was obtained in 94% yield by addition of methanol to the enol ether **7** in the presence of *p*-toluenesulfonic acid.

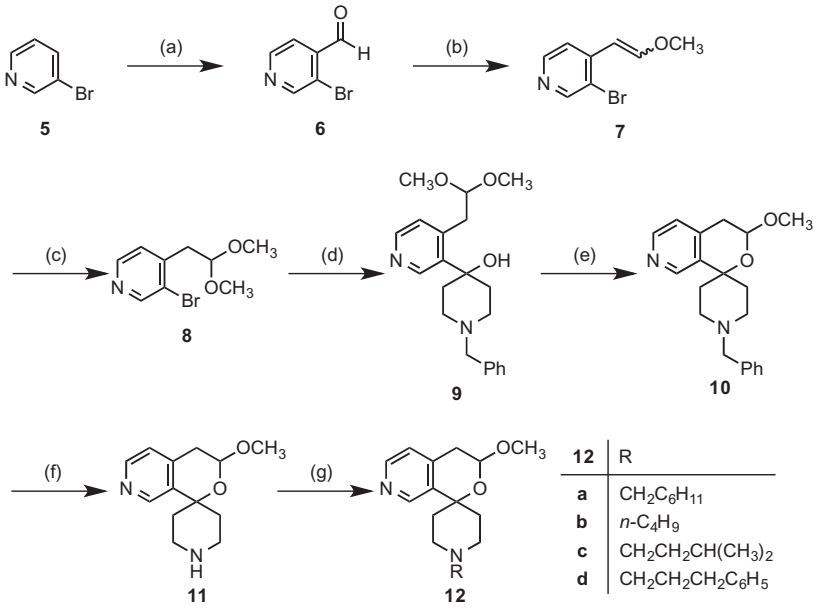
The halogen-metal exchange of the bromo acetal **8** with *n*-butyllithium at −78 °C afforded a pyridyllithium intermediate, which was trapped with 1-benzylpiperidin-4-one to yield the hydroxy acetal **9**. Intramolecular transacetalization of **9** was performed with *p*-toluenesulfonic acid in methanol to provide the spirocyclic pyranopyridine **10**.

The benzyl group of **10** was removed hydrogenolytically with hydrazine and catalytic amounts of Pd/C. Alkylation of the secondary amine **11** with various alkyl bromides provided the *N*-alkylated spirocyclic piperidines **12a–d**. *N*-residues leading to high σ_1 receptor affinity and σ_1/σ_2 selectivity in the benzopyran (**2**) and thienopyran (**3**) series were selected.

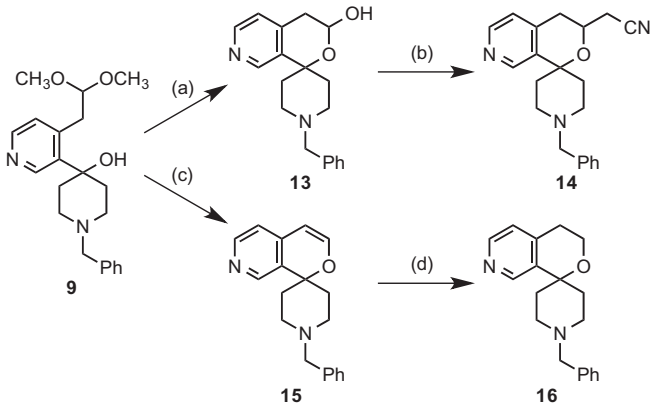
The hydroxy acetal **9** was used for the synthesis of pyridine analogous spirocyclic σ ligands with various substituents in 3′-position of the pyran ring (Scheme 2). Treatment of the hydroxy acetal **9** with diluted HCl at 60 °C gave the lactol **13** in 50% yield. However, increasing the reaction temperature to 80 °C led predominantly to the elimination product **15**, which was isolated in 87% yield. The cyanomethyl derivative **14** was obtained by a tandem Wittig reaction of the hemiacetal **13** with Ph_3P = CHCN and subsequent conjugate addition induced by Cs_2CO_3. Hydrogenation of the alkene **15** was performed with H_2 (1 bar) and Pd/C as catalyst to afford **16** without substituent in the pyranopyridine ring system.

3. Receptor affinity

The affinity of the spirocyclic pyranopyridines **10–16** towards σ_1 and σ_2 receptors was investigated in receptor binding studies using the tritium labeled radioligands [^{3}H]-(+)-pentazocine and [^{3}H]-di-(*o*-tolyl)guanidine, respectively. In the σ_2 assay an excess of non-labeled (+)-pentazocine was added to mask the σ_1 receptors. Membrane preparations from guinea pig brains and rat liver were employed as receptor material, respectively.[40–43] In Table 1 the σ_1 and σ_2 affinity data of the pyranopyridines **10–16** are



Scheme 1. Synthesis of spirocyclic compounds **10–12**. Reagents and reaction conditions: (a) *n*-BuLi, diisopropylamine, *N*-formylpiperidine, THF, −78 °C, 50 min then rt, 1.5 h, 48%; (b) $(Ph_3PCH_2OCH_3)Cl$, *t*-BuOK, THF, −50 °C, 30 min then rt, 20 min, 64%; (c) *p*-TsOH·H_2O, CH_3OH, reflux, 16 h, 94%; (d) *n*-BuLi, 1-benzylpiperidin-4-one, THF, −78 °C, 5 min then rt, 1.5 h, 44%; (e) *p*-TsOH·H_2O, $CH(OCH_3)_3$, CH_3OH, rt, 17 h, 78%; (f) H_2NNH_2·H_2O, Pd/C, CH_3OH, 60 °C, 1 h, 87%; (g) RBr, K_2CO_3, CH_3CN, 70–80 °C, 1–22 h; **12a**, 64%; **12b**, 27%; **12c**, 30%; **12d**, 49%.



Scheme 2. Synthesis of spirocyclic compounds **13**–**16** with various substituents in 3-position. Reagents and reaction conditions: (a) 6 M HCl, CH_3CN, 60 °C, 2.5 h, 50%; (b) Ph_3P = CHCN, Cs_2CO_3, THF, reflux, 17 h, 42%; (c) 6 M HCl, CH_3CN, 80 °C, 3.5 h, 87%; (d) H_2, Pd/C, CH_3OH, rt, 3 h, 54%.

summarized and compared with the σ receptor affinities of the corresponding benzene and thiophene analogs **2** and **3**.

Compound **10** with a benzyl residue at the piperidine *N*-atom and a methoxy moiety at 3′-position of the pyran ring shows moderate σ_1 affinity of 44 nM. The corresponding benzene and thiophene derivatives **2a** and **3a** display 34- and 138-fold higher σ_1 affinity compared to the pyranopyridine **10**. This result indicates that the electron density of the aromatic system has a great impact on the σ_1 receptor affinity.

Replacement of the *N*-benzyl residue with a cyclohexylmethyl moiety led to **12a** with 4-fold higher σ_1 receptor affinity (K_i = 11 nM). The σ_1 receptor affinity of the branched isopentyl substituted spirocyclic pyridine **12c** is slightly increased (K_i = 23 nM). Elongation of *N*-phenyl distance to three methylene moieties (**12d**) did not influence the σ_1 receptor affinity. However, a 3-fold reduced σ_1 affinity was observed for the *n*-butyl substituted derivative **12b** (K_i = 120 nM). It can be concluded that an aromatic residue within the piperidine *N*-substituent is not essential, but a considerable bulkiness of this *N*-substituent is required to achieve high σ_1 receptor affinity.

The lactol **13** with a polar hydroxy group in 3′-position displayed 4-fold reduced σ_1 affinity (K_i = 179 nM) compared to the methoxy derivative **10**. A larger substituent such as the cyanomethyl moiety in 3′-position is not tolerated by the σ_1 receptor. However, removal of the C-38-substituent increased the σ_1 affinity (**16**; K_i = 19 nM). A further increase of the σ_1 affinity was attained with the alkene **15** containing a double bond between C-3′ and C-4′ of the pyran ring. With a K_i-value of 4.6 nM **15** represents the most potent σ_1 receptor ligand of this series of spirocyclic pyranopyridines. Obviously very small substituents in 3′-position are favorable for high σ_1 receptor affinity. These relationships between the nature of substituents in 3′-position and at the piperidine N-atom follow the same trend as the SAR of spirocyclic benzopyrans **2** and thienopyrans **3**.

The pyranopyridines **10**–**16** do not interact significantly with the σ_2 receptor subtype indicating high σ_1/σ_2 selectivity. Very high σ_1/σ_2 selectivity is observed for the spirocyclic compounds **15** and **16** without a substituent in 3′-position. Although the σ_1 affinity of **15** and **16** is lower than the σ_1 affinity of the corresponding thiophene derivative **3d**, the σ_1/σ_2 selectivity of both pyranopyridines is higher than that of **3d**.

4. Conclusion

In this project the benzene ring of the potent spirocyclic σ_1 ligands **2** was replaced by an electron deficient pyridine ring (**4**). Generally, the σ_1 affinity of the spirocyclic pyranopyridines **10**–**16** is lower than the σ_1 affinity of the corresponding benzopyran and thienopyran derivatives **2** and **3**. Therefore it can be concluded that an electron deficient pyridine ring is less tolerated by the σ_1 receptor than the neutral benzene ring or an electron rich thiophene ring. Nevertheless, compounds with very high σ_1 affinity (e.g., **15**: K_i = 4.6 nM) are found within this new class of spirocyclic pyranopyridines. In general the pyridine analogs reveal high σ_1/σ_2 selectivity and the pyranopyridines **15** and **16** without a substituent in 3′-position show higher σ_1/σ_2 selectivity than the very potent thienopyran **3d**.

Table 1
σ receptor affinity of spirocyclic pyranopyridines compared with analogous benzopyrans and thienopyrans

Compd	R^1	R^2	K_i ± SEM (nM) (n = 3)[a]		σ_1/σ_2
			σ_1 ([^{3}H]-(+)-pentazocine)	σ_2 ([^{3}H]di-*o*-tolylguanidine)	Selectivity
2a[34]	$CH_2C_6H_5$	OCH_3	1.3 ± 0.18	3500	2708
2b[49]	*n*-C_4H_9	OCH_3	2.3 ± 0.55	1020	443
2c[35]	$CH_2C_6H_5$	CH_2CN	16 ± 4.04	4700	295
2d[34]	$CH_2C_6H_5$	H	0.69 ± 0.17	99.7	146
3a[37]	$CH_2C_6H_5$	OCH_3	0.32 ± 0.10	1260	3940
3b[37]	*n*-C_4H_9	OCH_3	4.3 ± 0.10	246	57
3c[50]	$CH_2C_6H_5$	CH_2CN	64 ± 16	>1000	>15
3d[50]	$CH_2C_6H_5$	H	0.31 ± 0.06	13 ± 2.5	42
10	$CH_2C_6H_5$	OCH_3	44 ± 12	>1000	>22
12a	$CH_2C_6H_{11}$	OCH_3	11 ± 4.5	>1000	>91
12b	*n*-C_4H_9	OCH_3	120	>1000	>8
12c	$(CH_2)_2CH(CH_3)_2$	OCH_3	23 ± 9.5	>1000	>43
12d	$(CH_2)_3C_6H_5$	OCH_3	44 ± 4	>1000	>22
13	$CH_2C_6H_5$	OH	179	>1000	>5
14	$CH_2C_6H_5$	CH_2CN	>1000	>1000	–
15	$CH_2C_6H_5$	$C_{3'}$=$C_{4'}$	4.6 ± 1.3	>1000	>217
16	$CH_2C_6H_5$	H	19 ± 3.8	>1000	>51
Haloperidol			6.3 ± 1.6	78 ± 2.3	12
Di-*o*-tolylguanidine			89 ± 29	58 ± 18	0.7
(+)-Pentazocine			5.7 ± 2.2	–	–

[a] Triplicates were recorded only for high affinity compounds. The affinity of compounds, which did not reduce significantly the radioligand binding in the first experiment, was recorded only once. The affinity of low affinity compounds is given as K_i >1000 nM.

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5. Experimental

5.1. Chemistry

5.1.1. General

THF was dried with sodium/benzophenone and was freshly distilled before use. Thin layer chromatography (tlc): Silica gel 60 F254 plates (Merck). Flash chromatography (fc): Silica gel 60, 40–64 μm (Merck); parentheses include: diameter of the column, eluent. Melting point: Melting point apparatus SMP 10 (Stuart Scientific), uncorrected. Nuclear magnetic resonance (NMR) spectra were recorded on Agilent 600-MR (600 MHz for ^{1}H, 151 MHz for ^{13}C) or Agilent 400-MR spectrometer (400 MHz for ^{1}H, 101 MHz for ^{13}C); δ in ppm related to $(CH_3)_4Si$ and measured referring to $CHCl_3$ (δ 7.26 ppm (^{1}H NMR) and δ 77.2 ppm (^{13}C NMR)) and CHD_2OD (δ 3.31 ppm (^{1}H NMR) and δ 49.0 ppm (^{13}C NMR)); 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. MS: micrOTOF-Q II (Bruker Daltronics); APCI, atmospheric pressure chemical ionization. IR: FT-IR spectrophotometer MIRacle 10 (Shimadzu) equipped with ATR technique. HPLC (Merck Hitachi): Equipment; UV detector: L-7400; autosampler: L-7200; pump: L-7100; degasser: L-7614; Method: column: LiChrospher® 60 RP-select B (5 μm), 250–4 mm cartridge; flow rate: 1.00 mL/min; injection volume: 5.0 μL; detection at λ = 210 nm; solvents: A: water with 0.05% (v/v) trifluoroacetic acid; B: acetonitrile with 0.05% (v/v) trifluoroacetic acid: gradient elution: (A%): 0–4 min: 90%, 4–29 min: gradient from 90% to 0%, 29–31 min: 0%, 31–31.5 min: gradient from 0% to 90%, 31.5–40 min: 90%. The purity of all test compounds was determined by this method. The purity of all test compounds is higher than 95%.

5.1.2. 3-Bromopyridine-4-carbaldehyde (6)[38]

Under an N_2 atmosphere, a 1.6 M solution of *n*-butyllithium in hexane (7.06 mL, 11.3 mmol) was added slowly to a cooled (−78 °C) solution of diisopropylamine (1.25 g, 1.73 mL, 12.4 mmol) in THF (25 mL). After 30-min stirring at 0 °C, the mixture was cooled to −78 °C and a solution of 3-bromopyridine (**5**, 1.63 g, 1.00 mL, 10.3 mmol) in THF (5 mmol) was added slowly. The mixture was stirred at −78 °C for 15 min, and then a solution of *N*-formylpiperidine (5.24 g, 5.14 mL, 46.4 mmol) was added slowly. The solution was stirred at −78 °C for 50 min. Then it was allowed to warm to room temperature and stirred for another 1.5 h. Saturated NH_4Cl solution (40 mL) was added. The aqueous layer was extracted with EtOAc (3 × 30 mL). The combined organic layers were washed with brine (60 mL) and dried over Na_2SO_4. Filtration and evaporation afforded crude product, which was purified by fc (5.5 cm, EtOAc/cyclohexane 1:4). Yellow solid (EtOAc/cyclohexane2:1, R_f = 0.55), yield 914 mg (48%). ^{1}H NMR (600 MHz, $CDCl_3$): δ (ppm) = 7.22 (d, *J* = 4.9 Hz, 1H, 5-H-Py), 8.72 (d, *J* = 4.9 Hz, 1H, 6-H-Py), 8.92 (s, 1H, 2-H-Py), 10.37 (s, 1H, CHO).

5.1.3. 3-Bromo-4-(2-methoxyvinyl)pyridine (7)[39]

Under an N_2 atmosphere, $(Ph_3PCH_2OCH_3)Cl$ was suspended in THF (12 mL). The suspension was cooled to −50 °C, and then 1 M *t*-BuOK solution in THF (2.39 mL, 2.39 mmol) was added. After 3-min stirring at −50 °C, **6** (295 mg, 1.59 mmol) in THF (2 mL) was added. The solution was stirred at −50 °C for 30 min. Then it was allowed to warm to room temperature and stirred for another 20 min. The reaction mixture was partitioned between water (12 mL) and EtOAc (10 mL). The aqueous layer was extracted with EtOAc (3 × 6 mL). The combined organic layers were washed with brine (10 mL) and dried over Na_2SO_4. Filtration and evaporation afforded crude product, which was purified by fc (2 cm, EtOAc/cyclohexane 1:2 to EtOAc/cyclohexane 1:1). Pale yellow liquid ((*E*):(*Z*) = 70:30, EtOAc/cyclohexane 2:1, R_f = 0.52 and 0.46), yield 217 mg (64%). ^{1}H NMR (600 MHz, $CDCl_3$): δ (ppm) = 3.79 (s, 3 × 0.7H, OCH_3, (*E*)), 3.90 (s, 3 × 0.3H, OCH_3, (*Z*)), 5.61 (d, *J* = 7.1 Hz, 0.3H, PyC*H* = CH, (*Z*)), 6.02 (d, *J* = 12.9 Hz, 0.7H, PyC*H* = CH, (*E*)), 6.51 (d, *J* = 7.1 Hz, 0.3H, PyCH = C*H*, (*Z*)), 7.25 (d, *J* = 5.3 Hz, 0.7H, 5-H-Py, (*E*)), 7.28 (d, *J* = 12.9 Hz, 0.7H, PyCH = C*H*, (*E*)), 8.02 (d, *J* = 5.4 Hz, 0.3H, 5-H-Py, (*Z*)), 8.32 (d, *J* = 5.3 Hz, 0.7H, 6-H-Py, (*E*)), 8.37 (d, *J* = 5.4 Hz, 0.3H, 6-H-Py, (*Z*)), 8.62 (s, 0.7H, 2-H-Py, (*E*)), 8.64 (s, 0.3H, 2-H-Py, (*Z*)).

5.1.4. 2-(3-Bromopyridin-4-yl)acetaldehyde dimethyl acetal (8)

A solution of **7** ((*E*):(*Z*) = 70:30, 211 mg, 0.984 mmol) and *p*-TsOH·H_2O (206 mg, 1.08 mmol) in CH_3OH (15 mL) was heated to reflux for 16 h. After concentration of the mixture in vacuo, the residue was partitioned between saturated $NaHCO_3$ solution (5 mL) and EtOAc (4 mL). The aqueous layer was extracted with EtOAc (5 × 3 mL). The combined organic layers were washed with brine (15 mL) and dried over Na_2SO_4. Filtration and evaporation afforded crude product, which was purified by fc (2 cm, EtOAc/cyclohexane 1:1). Orange oil (EtOAc/cyclohexane 2:1, R_f = 0.56), yield 227 mg (94%). ^{1}H NMR (400 MHz, CD_3OD): δ (ppm) = 3.10 (d, *J* = 5.6 Hz, 2H, PyCH_2CH), 3.35 (s, 6H, OCH_3), 4.67 (t, *J* = 5.6 Hz, 1H, PyCH_2C*H*), 7.41 (d, *J* = 4.9 Hz, 1H, 5-H-Py), 8.39 (s broad, 1H, 6-H-Py), 8.64 (s broad, 1H, 2-H-Py). ^{13}C NMR (101 MHz, CD_3OD): δ (ppm) = 40.0 (1C, PyCH_2CH), 54.3 (2C, OCH_3), 104.5 (1C, PyCH_2C*H*), 124.7 (1C, C-3-Py), 128.5 (1C, C-5-Py), 148.0 (1C, C-4-Py), 148.6 (1C, C-6-Py), 152.2 (1C, C-2-Py). $C_9H_{12}BrNO_2$ (245.0). Exact mass (APCI): *m*/*z* = 246.0129 (calcd 246.0124 for $C_9H_{13}BrNO_2$ [M+H$^+$]). IR (neat): ν (cm^{-1}) = 2936 (C—H), 2832 (C—H), 1582 (C—H), 1115 (C—O).

5.1.5. 2-[3-(1-Benzyl-4-hydroxypiperidin-4-yl)pyridin-4-yl]acetaldehyde dimethyl acetal (9)

Under an N_2 atmosphere, a 1.6 M solution of *n*-butyllithium in hexane (2.27 mL, 3.63 mmol) was added slowly to a cooled (−78 °C) solution of **8** (812 mg, 3.30 mmol) in THF (17 mL). After 3-min stirring at −78 °C, 1-benzylpiperidin-4-one (937 mg, 4.95 mmol) in THF (3 mL) was added slowly. The solution was stirred at −78 °C for 5 min. Then it was allowed to warm to room temperature and stirred for another 1.5 h. Then water (30 mL) was added and the aqueous layer was extracted with CH_2Cl_2 (4 × 20 mL). The combined organic layers were dried over Na_2SO_4. Filtration and evaporation afforded crude product, which was purified by fc (5 cm, EtOAc/cyclohexane 1:1 to EtOAc to EtOAc/CH_3OH 10:1). Pale orange solid (EtOAc/cyclohexane 4:1, R_f = 0.07), yield 522 mg (44%). Melting point: 143 °C. ^{1}H NMR (400 MHz, CD_3OD): δ (ppm) = 1.93–1.99 (m, 2H, N(CH_2CH_2)$_2$), 2.15–2.23 (m, 2H, N(CH_2CH_2)$_2$), 2.65–2.72 (m, 2H, N(CH_2CH_2)$_2$), 2.77–2.83 (m, 2H, N(CH_2CH_2)$_2$), 3.35 (s, 6H, OCH_3), 3.39 (d, *J* = 5.5 Hz, 2H, PyCH_2CH), 3.63 (s, 2H, NCH_2Ph), 4.63 (t, *J* = 5.5 Hz, 1H, PyCH_2C*H*), 7.26–7.39 -(m, 6H, Ph-H, 5-H-Py), 8.29 (d, *J* = 5.1 Hz, 1H, 6-H-Py), 8.53 (s, 1H, 2-H-Py). ^{13}C NMR (101 MHz, CD_3OD): δ (ppm) = 38.3 (2C, N(CH_2CH_2)$_2$), 38.3 (1C, PyCH_2CH), 50.0 (2C, N(CH_2CH_2)$_2$), 54.5 (2C, OCH_3), 64.0 (1C, NCH_2Ph), 71.7 (1C, PyCOH), 106.8 (1C, PyCH_2CH), 128.6 (1C, C-4-Ph), 129.0 (1C, C-5-Py), 129.4 (2C, C-3-Ph/C-5-Ph), 130.9 (2C, C-2-Ph/C-6-Ph), 138.25 (1C, C-1-Ph), 143.7 (1C, C-3-Py), 147.3 (1C, C-2-Py), 147.9 (1C, C-6-Py), 148.1 (1C, C-4-Py). $C_{21}H_{28}N_2O_3$ (356.2). Exact mass (APCI): *m*/*z* = 357.2213 (calcd357.2173 for $C_{21}H_{29}N_2O_3$ [M+H$^+$]). IR (neat): ν (cm^{-1}) = 3221 (O—H), 2932 (C—H), 2824 (C—H), 1589 (C—H), 1111 (C—O), 1061 (C—N), 741 (C—H), 698 (C—H).

5.1.6. 1-Benzyl-3′-methoxy-3′,4′-dihydrospiro[piperidine-4,1′-pyrano[3,4-*c*]pyridine] (10)

A solution of **9** (200 mg, 0.561 mmol), *p*-TsOH·H_2O (427 mg, 2.24 mmol) and trimethyl orthoformate (1 mL) in CH_3OH (8 mL)

was stirred at room temperature for 17 h. After concentration of the mixture in vacuo, the residue was partitioned between saturated $NaHCO_3$ solution (15 mL) and CH_2Cl_2 (15 mL). The aqueous layer was extracted with CH_2Cl_2 (2 × 15 mL) and the combined organic layers were dried over Na_2SO_4. Filtration and evaporation afforded crude product, which was purified by fc (2 cm, EtOAc/CH_3OH 50:1 + 2% $(CH_3)_2NC_2H_5$). Yellow oil (EtOAc/CH_3OH 50:1 + 2% $(CH_3)_2NC_2H_5$, R_f = 0.49 (3-time-developed)), yield 142 mg (78%). ^1H NMR (400 MHz, CD_3OD): δ (ppm) = 1.85 (ddd, J = 13.6/5.5/2.7 Hz, 1H, $N(CH_2CH_2)_2$), 1.93–2.09 (m, 2H, $N(CH_2CH_2)_2$), 2.24 (ddd, J = 13.5/12.6/4.5 Hz, 1H, $N(CH_2CH_2)_2$), 2.54–2.67 (m, 2H, $N(CH_2CH_2)_2$), 2.79–2.88 (m, 3H, $N(CH_2CH_2)_2$, $PyCH_2CH$), 2.99 (dd, J = 16.7/3.2 Hz, 1H, $PyCH_2CH$), 3.49 (s, 3H, OCH_3), 3.64 (s, 2H, NCH_2Ph), 4.97 (dd, J = 6.5/3.2 Hz, 1H, $PyCH_2CH$), 7.19 (d, J = 5.1 Hz, 1H, 5′-H-Py), 7.26–7.40 (m, 5H, Ph-H), 8.28 (d, J = 5.1 Hz, 1H, 6′-H-Py), 8.40 (s, 1H, 8′-H-Py). ^{13}C NMR (101 MHz, CD_3OD): δ (ppm) = 35.3 (1C, $PyCH_2CH$), 37.6 (1C, $N(CH_2CH_2)_2$), 39.1 (1C, $N(CH_2CH_2)_2$), 49.98 (1C, $N(CH_2CH_2)_2$), 50.00 (1C, $N(CH_2CH_2)_2$), 56.5 (1C, OCH_3), 64.2 (1C, NCH_2Ph), 74.1 (1C, PyCO), 97.2 (1C, $PyCH_2CH$), 125.5 (1C, C-5′-Py), 128.5 (1C, C-4-Ph), 129.4 (2C, C-3-Ph/C-5-Ph), 130.9 (2C, C-2-Ph/C-6-Ph), 138.2 (1C, C-1-Ph), 139.0 (1C, C-8′a-Py), 143.8 (1C, C-4′a-Py), 146.8 (1C, C-8′-Py), 147.8 (1C, C-6′-Py). $C_{20}H_{24}N_2O_2$ (324.2). Exact mass (APCI): m/z = 325.1924 (calcd 325.1911 for $C_{20}H_{25}N_2O_2$ $[M+H^+]$). IR (neat): ν (cm^{-1}) = 2928 (C—H), 2820 (C—H), 1593 (C—H), 1115 (C—O), 1061 (C—N), 737 (C—H), 698 (C—H). Purity (HPLC): 98.8% (t_R = 6.5 min).

5.1.7. 3′-Methoxy-3′,4′-dihydrospiro[piperidine-4,1′-pyrano[3,4-*c*]pyridine] (11)

Hydrazine hydrate (86.4 mg, 84 μL, 1.73 mmol) was added to a stirred mixture of **10** (280 mg, 0.863 mmol) and 10 wt % Pd/C (91.8 mg, 86.3 μmol) in CH_3OH (10 mL). This mixture was stirred at 60 °C for 1 h. Then it was filtered through Celite® and concentrated in vacuo. The crude product (175 mg, 87% yield) was used for next alkylation-reaction without purification. ^1H NMR (400 MHz, CD_3OD): δ (ppm) = 1.80–1.88 (m, 2H, $N(CH_2CH_2)_2$), 2.00–2.15 (m, 2H, $N(CH_2CH_2)_2$), 2.84 (dd, J = 16.6/6.4 Hz, 1H, $PyCH_2CH$), 2.91–3.03 (m, 3H, $N(CH_2CH_2)_2$, $PyCH_2CH$), 3.08–3.21 (m, 2H, $N(CH_2CH_2)_2$), 3.53 (s, 3H, OCH_3), 5.00 (dd, J = 6.4/3.3 Hz, 1H, $PyCH_2CH$), 7.20 (d, J = 5.1 Hz, 1H, 5′-H-Py), 8.29 (d, J = 5.1 Hz, 1H, 6′-H-Py), 8.42 (s, 1H, 8′-H-Py). ^{13}C NMR (101 MHz, CD_3OD): δ (ppm) = 35.1 (1C, $PyCH_2CH$), 36.0 (1C, $N(CH_2CH_2)_2$), 37.0 (1C, $N(CH_2CH_2)_2$), 41.6 (1C, $N(CH_2CH_2)_2$), 41.7 (1C, $N(CH_2CH_2)_2$), 56.4 (1C, OCH_3), 72.6 (1C, PyCO), 97.9 (1C, $PyCH_2CH$), 125.7 (1C, C-5′-Py), 137.7 (1C, C-8′a-Py), 143.5 (1C, C-4′a-Py), 146.5 (1C, C-8′-Py), 148.3 (1C, C-6′-Py). $C_{13}H_{18}N_2O_2$ (234.1). Exact mass (APCI): m/z = 235.1456 (calcd 235.1441 for $C_{13}H_{19}N_2O_2$ $[M+H^+]$).

5.1.8. 1-(Cyclohexylmethyl)-3′-methoxy-3′,4′-dihydrospiro[piperidine-4,1′-pyrano[3,4-*c*]pyridine] (12a)

(Bromomethyl)cyclohexane (71.7 mg, 0.405 mmol) and potassium carbonate (112 mg, 0.810 mmol) were added to a solution of **11** (63.2 mg, 0.270 mmol) in CH_3CN (4 mL). This mixture was stirred at 75 °C for 17 h. Then it was filtered and concentrated in vacuo. The crude product was purified by fc (2 cm, EtOAc/CH_3OH 10:1). Pale yellow solid (EtOAc/CH_3OH 4:1, R_f = 0.39), yield 56.8 mg (64%). Melting point: 119 °C. ^1H NMR (600 MHz, CD_3OD): δ (ppm) = 0.94–1.00 (m, 2H, *Cy*-hex-H), 1.19–1.35 (m, 3H, *Cy*-hex-H), 1.51–1.64 (m, 1H, *Cy*-hex-H), 1.68–1.77 (m, 3H, *Cy*-hex-H), 1.83–1.87 (m, 3H, *Cy*-hex-H, $N(CH_2CH_2)_2$), 1.97–2.07 (m, 2H, $N(CH_2CH_2)_2$), 2.24–2.29 (m, 3H, $N(CH_2CH_2)_2$, NCH_2Cy-hex), 2.45–2.56 (m, 2H, $N(CH_2CH_2)_2$), 2.81–2.87 (m, 3H, $N(CH_2CH_2)_2$, $PyCH_2CH$), 3.00 (dd, J = 16.6/3.3 Hz, 1H, $PyCH_2CH$), 3.52 (s, 3H, OCH_3), 4.98 (dd, J = 6.5/3.3 Hz, 1H, $PyCH_2CH$), 7.20 (d, J = 5.1 Hz, 1H, 5′-H-Py), 8.29 (d, J = 5.1 Hz, 1H, 6′-H-Py), 8.41 (s, 1H, 8′-H-Py). ^{13}C NMR (101 MHz, CD_3OD): δ (ppm) = 27.2 (2C, *Cy*-hex-C), 27.7 (1C, *Cy*-hex-C), 33.2 (2C, *Cy*-hex-C), 35.4 (1C, $PyCH_2CH$), 36.4 (1C, *Cy*-hex-C), 37.5 (1C, $N(CH_2CH_2)_2$), 39.1 (1C, $N(CH_2CH_2)_2$), 50.86 (1C, $N(CH_2CH_2)_2$), 50.89 (1C, $N(CH_2CH_2)_2$), 56.6 (1C, OCH_3), 67.2 (1C, NCH_2Cy-hex), 74.2 (1C, PyCO), 97.2 (1C, $PyCH_2CH$), 125.5 (1C, C-5′-Py), 139.1 (1C, C-8′a-Py), 143.8 (1C, C-4′a-Py), 146.8 (1C, C-8′-Py), 147.7 (1C, C-6′-Py). $C_{20}H_{30}N_2O_2$ (330.2). Exact mass (APCI): m/z = 331.2399 (calcd 331.2380 for $C_{20}H_{31}N_2O_2$ $[M+H^+]$). IR (neat): ν (cm^{-1}) = 2924 (C—H), 2898 (C—H), 2851 (C—H), 2805 (C—H), 1593 (C—H), 1123 (C—O), 1045 (C—N). Purity (HPLC): 98.6% (t_R = 9.8 min).

5.1.9. 1-Butyl-3′-methoxy-3′,4′-dihydrospiro[piperidine-4,1′-pyrano[3,4-*c*]pyridine] (12b)

1-Bromobutane (43.9 mg, 0.320 mmol) and potassium carbonate (88.3 mg, 0.639 mmol) were added to a solution of **11** (50.0 mg, 0.213 mmol) in CH_3CN (4 mL). This mixture was stirred at 70 °C for 1 h. Then it was filtered and concentrated in vacuo. The crude product was purified by fc (2 cm, EtOAc/CH_3OH 10:1 to 5:1). Yellow oil (EtOAc/CH_3OH 3:1, R_f = 0.15), yield 16.5 mg (27%). ^1H NMR (400 MHz, CD_3OD): δ (ppm) = 0.98 (t, J = 7.3 Hz, 3H, $NCH_2CH_2CH_2CH_3$), 1.34–1.44 (m, 2H, $NCH_2CH_2CH_2CH_3$), 1.55–1.63 (m, 2H, $NCH_2CH_2CH_2CH_3$), 1.86–2.11 (m, 3H, $N(CH_2CH_2)_2$), 2.24 (ddd, J = 13.8/12.6/4.4 Hz, 1H, $N(CH_2CH_2)_2$), 2.49–2.67 (m, 4H, $N(CH_2CH_2)_2$, $NCH_2CH_2CH_2CH_3$), 2.83 (dd, J = 16.6/6.3 Hz, 1H, $PyCH_2CH$), 2.89–2.97 (m, 2H, $N(CH_2CH_2)_2$), 3.01 (dd, J = 16.6/3.3 Hz, 1H, $PyCH_2CH$), 3.51 (s, 3H, OCH_3), 4.99 (dd, J = 6.3/3.3 Hz, 1H, $PyCH_2CH$), 7.20 (d, J = 5.0 Hz, 1H, 5′-H-Py), 8.29 (d, J = 5.0 Hz, 1H, 6′-H-Py), 8.40 (s, 1H, 8′-H-Py). ^{13}C NMR (101 MHz, CD_3OD): δ (ppm) = 14.3 (1C, $NCH_2CH_2CH_2CH_3$), 21.9 (1C, $NCH_2CH_2CH_2CH_3$), 29.8 (1C, $NCH_2CH_2CH_2CH_3$), 35.3 (1C, $PyCH_2CH$), 37.5 (1C, $N(CH_2CH_2)_2$), 39.0 (1C, $N(CH_2CH_2)_2$), 50.26 (1C, $N(CH_2CH_2)_2$), 50.28 (1C, $N(CH_2CH_2)_2$), 56.6 (1C, OCH_3), 59.7 (1C, $NCH_2CH_2CH_2CH_3$), 73.9 (1C, PyCO), 97.3 (1C, $PyCH_2CH$), 125.6 (1C, C-5′-Py), 138.8 (1C, C-8′a-Py), 143.8 (1C, C-4′a-Py), 146.7 (1C, C-8′-Py), 147.8 (1C, C-6′-Py). $C_{17}H_{26}N_2O_2$ (290.2). Exact mass (APCI): m/z = 291.2074 (calcd 291.2067 for $C_{17}H_{27}N_2O_2$ $[M+H^+]$). IR (neat): ν (cm^{-1}) = 2947 (C—H), 2928 (C—H), 2812 (C—H), 1597 (C—H), 1123 (C—O), 1053 (C—N). Purity (HPLC): 97.0% (t_R = 4.4 min).

5.1.10. 1-Isopentyl-3′-methoxy-3′,4′-dihydrospiro[piperidine-4,1′-pyrano[3,4-*c*]pyridine] (12c)

1-Bromo-3-methylbutane (56.3 mg, 0.373 mmol) and potassium carbonate (103 mg, 0.747 mmol) were added to a solution of **11** (58.3 mg, 0.249 mmol) in CH_3CN (5 mL). This mixture was stirred at 80 °C for 21.5 h. Then it was filtered and concentrated in vacuo. The crude product was purified by fc (2 cm, EtOAc/CH_3OH 10:1 to 5:1). Brown oil (EtOAc/CH_3OH 4:1, R_f = 0.13), yield 22.4 mg (30%). ^1H NMR (400 MHz, CD_3OD): δ (ppm) = 0.97 (d, J = 6.6 Hz, 6H, $NCH_2CH_2CH(CH_3)_2$), 1.50–1.56 (m, 2H, $NCH_2CH_2CH(CH_3)_2$), 1.59–1.69 (m, 1H, $NCH_2CH_2CH(CH_3)_2$), 1.91–2.14 (m, 3H, $N(CH_2CH_2)_2$), 2.27 (ddd, J = 13.7/12.7/4.4 Hz, 1H, $N(CH_2CH_2)_2$), 2.60–2.78 (m, 4H, $N(CH_2CH_2)_2$, $NCH_2CH_2CH(CH_3)_2$), 2.85 (dd, J = 16.5/6.2 Hz, 1H, $PyCH_2CH$), 3.00–3.05 (m, 3H, $N(CH_2CH_2)_2$, $PyCH_2CH$), 3.52 (s, 3H, OCH_3), 5.01 (dd, J = 6.2/3.3 Hz, 1H, $PyCH_2CH$), 7.22 (d, J = 5.1 Hz, 1H, 5′-H-Py), 8.31 (d, J = 5.1 Hz, 1H, 6′-H-Py), 8.41 (s, 1H, 8′-H-Py). ^{13}C NMR (101 MHz, CD_3OD): δ (ppm) = 23.0 (2C, $NCH_2CH_2CH(CH_3)_2$), 28.0 (1C, $NCH_2CH_2CH(CH_3)_2$), 35.3 (1C, $PyCH_2CH$), 36.4 (1C, $NCH_2CH_2CH(CH_3)_2$), 37.4 (1C, $N(CH_2CH_2)_2$), 38.8 (1C, $N(CH_2CH_2)_2$), 50.27 (1C, $N(CH_2CH_2)_2$), 50.29 (1C, $N(CH_2CH_2)_2$), 56.6 (1C, OCH_3), 58.1 (1C, $NCH_2CH_2CH(CH_3)_2$), 73.7 (1C, PyCO), 97.4 (1C, $PyCH_2CH$), 125.6 (1C, C-5′-Py), 138.6 (1C, C-8′a-Py), 143.8 (1C, C-4′a-Py), 146.7 (1C, C-8′-Py), 147.9 (1C, C-6′-Py). $C_{18}H_{28}N_2O_2$ (304.2). Exact mass (APCI): m/z = 305.2216 (calcd 305.2224 for $C_{18}H_{29}N_2O_2$ $[M+H^+]$). IR (neat): ν (cm^{-1}) = 2951 (C—H), 2924 (C—H), 2866 (C—H), 2812 (C—H), 1597 (C—H), 1141 (C—O), 1057 (C—N). Purity (HPLC): 96.2% (t_R = 6.2 min).

5.1.11. 3′-Methoxy-1-(3-phenylpropyl)-3′,4′-dihydrospiro[piperidine-4,1′-pyrano[3,4-*c*]-pyridine] (12d)

1-Bromo-3-phenylpropane (74.3 mg, 0.373 mmol) and potassium carbonate (103 mg, 0.747 mmol) were added to a solution of **11** (58.3 mg, 0.249 mmol) in CH_3CN (7 mL). This mixture was stirred at 70 °C for 14.5 h. Then it was filtered and concentrated in vacuo. The crude product was purified by fc (2 cm, EtOAc/CH_3OH 10:1 to 5:1). Pale orange oil (EtOAc/CH_3OH 5:1, R_f = 0.21), yield 43.0 mg (49%). ^1H NMR (600 MHz, CD_3OD): δ (ppm) = 1.88–2.01 (m, 4H, $N(CH_2CH_2)_2$, $NCH_2CH_2CH_2Ph$), 2.06–2.10 (m, 1H, $N(CH_2CH_2)_2$), 2.25 (ddd, J = 13.9/12.8/4.4 Hz, 1H, $N(CH_2CH_2)_2$), 2.56–2.70 (m, 6H, $N(CH_2CH_2)_2$, $NCH_2CH_2CH_2Ph$), 2.83 (dd, J = 16.5/6.3 Hz, 1H, $PyCH_2CH$), 2.92–3.02 (m, 3H, $N(CH_2CH_2)_2$, $PyCH_2CH$), 3.51 (s, 3H, OCH_3), 4.98 (dd, J = 6.3/3.3 Hz, 1H, $PyCH_2CH$), 7.15–7.29 (m, 6H, Ph-H, 5′-H-Py), 8.30 (d, J = 5.1 Hz, 1H, 6′-H-Py), 8.40 (s, 1H, 8′-H-Py). ^{13}C NMR (151 MHz, CD_3OD): δ (ppm) = 29.4 (1C, $NCH_2CH_2CH_2Ph$), 34.7 (1C, $NCH_2CH_2CH_2Ph$), 35.3 (1C, $PyCH_2CH$), 37.5 (1C, $N(CH_2CH_2)_2$), 38.9 (1C, $N(CH_2CH_2)_2$), 50.2 (1C, $N(CH_2CH_2)_2$), 50.3 (1C, $N(CH_2CH_2)_2$), 56.6 (1C, OCH_3), 59.1 (1C, $NCH_2CH_2CH_2Ph$), 73.8 (1C, PyCO), 97.4 (1C, $PyCH_2CH$), 125.6 (1C, C-4-Ph), 127.0 (2C, C-3-Ph/C-5-Ph), 129.4 (3C, C-2-Ph/C-6-Ph/C-5′-Py), 138.7 (1C, C-8′a-Py), 143.0 (1C, C-1-Ph), 143.8 (1C, C-4′a-Py), 146.7 (1C, C-8′-Py), 147.9 (1C, C-6′-Py). $C_{22}H_{28}N_2O_2$ (352.2). Exact mass (APCI): m/z = 353.2241 (calcd 353.2224 for $C_{22}H_{29}N_2O_2$ [M+H$^+$]). IR (neat): ν (cm^{-1}) = 2924 (C—H), 2812 (C—H), 1597 (C—H), 1119 (C—O), 1053 (C—N), 744 (C—H), 698 (C—H). Purity (HPLC): 98.7% (t_R = 11.4 min).

5.1.12. 1-Benzyl-3′,4′-dihydrospiro[piperidine-4,1′-pyrano[3,4-*c*]pyridin]-3′-ol (13)

An aqueous HCl solution (6 M, 1 mL) was added to a solution of **9** (200 mg, 0.561 mmol) in CH_3CN (5 mL). The mixture was stirred at 60 °C for 2.5 h. After addition of saturated $NaHCO_3$ solution (ca. 8 mL, pH >8), the mixture was extracted with CH_2Cl_2 (3 × 8 mL). The combined organic layers were dried over Na_2SO_4. Filtration and evaporation afforded crude product, which was purified two times by fc (1.2 cm, EtOAc/CH_3OH 50:1 + 2% $(CH_3)_2NC_2H_5$ to EtOAc/CH_3OH 20:1 +2% $(CH_3)_2NC_2H_5$, 2. 2 cm, EtOAc/CH_3OH 4:1). Pale yellow oil (EtOAc/CH_3OH 5:1, R_f = 0.22 (3-time-developed)), yield 86.4 mg (50%). ^1H NMR (600 MHz, CD_3OD): δ (ppm) = 1.86–1.96 (m, 2H, $N(CH_2CH_2)_2$), 2.01–2.05 (m, 1H, $N(CH_2CH_2)_2$), 2.22 (ddd, J = 13.5/12.5/4.5 Hz, 1H, $N(CH_2CH_2)_2$), 2.57–2.68 (m, 2H, $N(CH_2CH_2)_2$), 2.75–2.82 (m, 3H, $N(CH_2CH_2)_2$, $PyCH_2CH$), 2.97 (dd, J = 16.6/3.0 Hz, 1H, $PyCH_2CH$), 3.60 (s, 2H, NCH_2Ph), 5.29 (dd, J = 7.2/3.0 Hz, 1H, $PyCH_2CH$), 7.18 (d, J = 5.0 Hz, 1H, 5′-H-Py), 7.26–7.28 (m, 1H, Ph-H), 7.32–7.38 (m, 4H, Ph-H), 8.27 (d, J = 5.0 Hz, 1H, 6′-H-Py), 8.40 (s, 1H, 8′-H-Py). ^{13}C NMR (101 MHz, CD_3OD): δ (ppm) = 36.8 (1C, $PyCH_2CH$), 36.9 (1C, $N(CH_2CH_2)_2$), 39.1 (1C, $N(CH_2CH_2)_2$), 49.8 (1C, $N(CH_2CH_2)_2$), 49.9 (1C, $N(CH_2CH_2)_2$), 63.9 (1C, NCH_2Ph), 74.2 (1C, PyCO), 89.6 (1C, $PyCH_2CH$), 125.7 (1C, C-5′-Py), 128.8 (1C, C-4-Ph), 129.5 (2C, C-3-Ph/C-5-Ph), 131.0 (2C, C-2-Ph/C-6-Ph), 137.6 (1C, C-1-Ph), 138.9 (1C, C-8′a-Py), 144.3 (1C, C-4′a-Py), 146.8 (1C, C-8′-Py), 147.7 (1C, C-6′-Py). $C_{19}H_{22}N_2O_2$ (310.2). Exact mass (APCI): m/z = 311.1782 (calcd 311.1754 for $C_{19}H_{23}N_2O_2$ [M+H$^+$]). IR (neat): ν (cm^{-1}) = 3183 (O—H), 2940 (C—H), 2820 (C—H), 1601 (C—H), 1057 (C—O), 1018 (C—N), 741 (C—H), 698 (C—H). Purity (HPLC): 97.8% (t_R = 4.18 min).

5.1.13. 2-(1-Benzyl-3′,4′-dihydrospiro[piperidine-4,1′-pyrano[3,4-*c*]pyridin]-3′-yl)acetonitrile (14)

(Triphenylphosphoranylidene)acetonitrile (116 mg, 0.386 mmol) and Cs_2CO_3 (31.5 mg, 96.6 μmol) were added to a solution of **13** (30.0 mg, 96.6 μmol) in THF (3 mL). The mixture was heated to reflux for 17 h. Then it was filtered and concentrated in vacuo. The crude product was purified two times by fc (1. 2 cm, EtOAc/CH_3OH 50:1 to 5:1, 2. 2 cm, EtOAc/CH_3OH 50:1 + 2% $(CH_3)_2NC_2H_5$). Colorless oil (EtOAc/CH_3OH 50:1 + 2% $(CH_3)_2NC_2H_5$, R_f = 0.24 (twice-developed)), yield 13.6 mg (42%). ^1H NMR (400 MHz, CD_3OD): δ (ppm) = 1.75 (ddd, J = 13.6/5.3/2.7 Hz, 1H, $N(CH_2CH_2)_2$), 1.93 (ddd, J = 14.5/12.7/4.5 Hz, 1H, $N(CH_2CH_2)_2$), 2.19 (ddd, J = 14.5/5.5/2.7 Hz, 1H, $N(CH_2CH_2)_2$), 2.29 (ddd, J = 13.6/12.7/4.5 Hz, 1H, $N(CH_2CH_2)_2$), 2.59–2.67 (m, 2H, $N(CH_2CH_2)_2$), 2.75–2.85 (m, 5H, $N(CH_2CH_2)_2$, $PyCH_2CH$, CH_2CN), 2.91 (dd, J = 16.8/3.9 Hz, 1H, $PyCH_2CH$), 3.60 (s, 2H, NCH_2Ph), 4.13–4.19 (m, 1H, $PyCH_2CH$), 7.20 (d, J = 5.2 Hz, 1H, 5′-H-Py), 7.25–7.40 (m, 5H, Ph-H), 8.28 (d, J = 5.2 Hz, 1H, 6′-H-Py), 8.42 (s, 1H, 8′-H-Py). ^{13}C NMR (101 MHz, CD_3OD): δ (ppm) = 24.6 (1C, $PyCH_2CH$), 34.4 (1C, CH_2CN), 35.3 (1C, $N(CH_2CH_2)_2$), 39.1 (1C, $N(CH_2CH_2)_2$), 49.82 (1C, $N(CH_2CH_2)_2$), 49.84 (1C, $N(CH_2CH_2)_2$), 64.1 (1C, NCH_2Ph), 65.4 (1C, $PyCH_2CH$), 74.4 (1C, PyCO), 119.0 (1C, CN), 125.3 (1C, C-5′-Py), 128.7 (1C, C-4-Ph), 129.4 (2C, C-3-Ph/C-5-Ph), 130.9 (2C, C-2-Ph/C-6-Ph), 138.2 (1C, C-1-Ph), 138.9 (1C, C-8′a-Py), 144.6 (1C, C-4′a-Py), 147.3 (1C, C-8′-Py), 147.6 (1C, C-6′-Py). $C_{21}H_{23}N_3O$ (333.2). Exact mass (APCI): m/z = 334.1910 (calcd 334.1914 for $C_{21}H_{24}N_3O$ [M+H$^+$]). IR (neat): ν (cm^{-1}) = 2928 (C—H), 2816 (C—H), 2253 (C≡N), 1601 (C—H), 1099 (C—O), 1065 (C—N), 745 (C—H), 698 (C—H). Purity (HPLC): 96.6% (t_R = 6.5 min).

5.1.14. 1-Benzylspiro[piperidine-4,1′-pyrano[3,4-*c*]pyridine] (15)

An aqueous HCl solution (6 M, 1.5 mL) was added to a solution of **9** (150 mg, 0.421 mmol) in CH_3CN (5 mL). The mixture was stirred at 80 °C for 3.5 h. After addition of saturated $NaHCO_3$ solution (ca. 10 mL, pH >8), the mixture was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic layers were dried over Na_2SO_4. Filtration and evaporation afforded crude product, which was purified by fc (2 cm, EtOAc/CH_3OH 10:1). Pale yellow oil (EtOAc/CH_3OH 3:1, R_f = 0.40), yield 107 mg (87%), which solidified on standing at −20 °C. Melting point: 96 °C. ^1H NMR (600 MHz, CD_3OD): δ (ppm) = 1.99 (ddd, J = 13.8/12.6/4.6 Hz, 2H, $N(CH_2CH_2)_2$), 2.24–2.27 (m, 2H, $N(CH_2CH_2)_2$), 2.50 (ddd, J = 14.6/12.6/2.5 Hz, 1H, $N(CH_2CH_2)_2$), 2.78–2.81 (m, 2H, $N(CH_2CH_2)_2$), 3.59 (s, 2H, NCH_2Ph), 5.81 (d, J = 5.7 Hz, 1H, $PyCH=CH$), 6.75 (d, J = 5.7 Hz, 1H, $PyCH=CH$), 6.95 (d, J = 5.1 Hz, 1H, 5′-H-Py), 7.24–7.27 (m, 1H, Ph-H), 7.30–7.36 (m, 4H, Ph-H), 8.23 (s, 1H, 8′-H-Py), 8.27 (d, J = 5.1 Hz, 1H, 6′-H-Py). ^{13}C NMR (151 MHz, CD_3OD): δ (ppm) = 35.1 (2C, $N(CH_2CH_2)_2$), 50.0 (2C, $N(CH_2CH_2)_2$), 64.0 (1C, NCH_2Ph), 76.7 (1C, PyCO), 103.4 (1C, $PyCH=CH$), 118.9 (1C, C-5′-Py), 128.5 (1C, C-4-Ph), 129.4 (2C, C-3-Ph/C-5-Ph), 130.8 (2C, C-2-Ph/C-6-Ph), 131.3 (1C, C-8′a-Py), 138.5 (1C, C-1-Ph), 139.2 (1C, C-4′a-Py), 144.1 (1C, C-8′-Py), 149.7 (1C, $PyCH=CH$), 149.7 (1C, C-6′-Py). $C_{19}H_{20}N_2O$ (292.2). Exact mass (APCI): m/z = 293.1641 (calcd 293.1648 for $C_{19}H_{21}N_2O$ [M+H$^+$]). IR (neat): ν (cm^{-1}) = 2947 (C—H), 2832 (C—H), 1616 (C=C), 1585 (C—H), 1049 (C—N), 741 (C—H), 698 (C—H). Purity (HPLC): 95.1% (t_R = 6.1 min).

5.1.15. 1-Benzyl-3′,4′-dihydrospiro[piperidine-4,1′-pyrano[3,4-*c*]pyridine] (16)

10 wt % Pd/C (20.1 mg, 18.8 μmol) was added to a solution of **15** (55.0 mg, 0.188 mmol) in CH_3OH (6 mL). The mixture was stirred under H_2 pressure (1.0 bar) for 3 h at rt. Then it was filtered through Celite® and concentrated in vacuo. The residue was purified by fc (2 cm, EtOAc/CH_3OH 10:1). Pale brown oil (EtOAc/CH_3OH 2:1, R_f = 0.26), yield 29.7 mg (54%). ^1H NMR (600 MHz, CD_3OD): δ (ppm) = 1.94–1.98 (m, 2H, $N(CH_2CH_2)_2$), 2.09 (ddd, J = 13.9/12.6/4.5 Hz, 1H, $N(CH_2CH_2)_2$), 2.56–2.60 (m, 2H, $N(CH_2CH_2)_2$), 2.83–2.85 (m, 4H, $N(CH_2CH_2)_2$, $PyCH_2CH$), 3.67 (s, 2H, NCH_2Ph), 7.19 (d, J = 5.1 Hz, 1H, 5′-H-Py), 7.29–7.31 (m, 1H, Ph-H), 7.34–7.40 (m, 4H, Ph-H), 8.25 (d, J = 5.1 Hz, 1H, 6′-H-Py), 8.38 (s, 1H, 8′-H-Py). ^{13}C NMR (151 MHz, CD_3OD): δ (ppm) = 29.8 (1C, $PyCH_2CH_2$), 37.0 (2C, $N(CH_2CH_2)_2$), 49.9 (2C, $N(CH_2CH_2)_2$), 59.1 (1C, $PyCH_2CH_2$),

64.0 (1C, NCH_2Ph), 72.6 (1C, PyCO), 125.3 (1C, C-5′-Py), 128.8 (1C, C-4-Ph), 129.5 (2C, C-3-Ph/C-5-Ph), 131.0 (2C, C-2-Ph/C-6-Ph), 137.7 (1C, C-1-Ph), 139.7 (1C, C-8′a-Py), 146.0 (1C, C-4′a-Py), 147.2 (1C, C-6′-Py), 147.4 (1C, C-8′-Py). $C_{19}H_{22}N_2O$ (294.2). Exact mass (APCI): m/z = 295.1842 (calcd 295.1805 for $C_{19}H_{23}N_2O$ [M+H$^+$]). IR (neat): ν (cm^{-1}) = 2936 (C—H), 2812 (C—H), 1593 (C—H), 1088 (C—O), 1065 (C—N), 741 (C—H), 698 (C—H). Purity (HPLC): 96.6% (t_R = 6.0 min).

5.2. Receptor binding studies[40–43]

5.2.1. Materials

The guinea pig brains and rat liver for the σ_1 and σ_2 receptor binding assays were commercially available (Harlan-Winkelmann, Borchen, Germany). Homogenizer: Elvehjem Potter (B. Braun Biotech International, Melsungen, Germany). Cooling centrifuge model Rotina 35R (Hettich, Tuttlingen, Germany) and High-speed cooling centrifuge model Sorvall RC-5C plus (Thermo Fisher Scientific, Langenselbold, Germany). Multiplates: standard 96-well multiplates (Diagonal, Muenster, Germany). Shaker: self-made device with adjustable temperature and tumbling speed (scientific workshop of the institute). Vortexer: Vortex Genie 2 (Thermo Fisher Scientific, Langenselbold, Germany). Harvester: MicroBeta FilterMate-96 Harvester. Filter: Printed Filtermat Typ A and B. Scintillator: Meltilex (Typ A or B) solid state scintillator. Scintillation analyzer: MicroBeta Trilux (all Perkin Elmer LAS, Rodgau-Jügesheim, Germany). Chemicals and reagents were purchased from different commercial sources and of analytical grade.

5.2.2. Preparation of membrane homogenates from guinea pig brain

5 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 1200g for 10 min at 4 °C. The supernatant was separated and centrifuged at 23,500g 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 23,500g (20 min, 4 °C). This procedure was repeated twice. The final pellet was resuspended in 5–6 volumes of buffer and frozen (−80 °C) in 1.5 mL portions containing about 1.5 mg protein/mL.

5.2.3. Preparation of membrane homogenates from rat liver

Two rat livers were cut into small pieces and 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 1200g for 10 min at 4 °C. The supernatant was separated and centrifuged at 31,000g for 20 min at 4 °C. The pellet was resuspended in 5–6 volumes of buffer (50 mM TRIS, pH 8.0) and incubated at room temperature for 30 min. After the incubation, the suspension was centrifuged again at 31,000g for 20 min at 4 °C. The final pellet was resuspended in 5–6 volumes of buffer and stored at −80 °C in 1.5 mL portions containing about 2 mg protein/mL.

5.2.4. Protein determination

The protein concentration was determined by the method of Bradford,[44] modified by Stoscheck.[45] The Bradford solution was prepared by dissolving 5 mg of Coomassie Brilliant Blue G 250 in 2.5 mL of EtOH (95%, v/v). 10 mL deionized H_2O and 5 mL phosphoric acid (85%, m/v) were added to this solution, the mixture was stirred and filled to a total volume of 50.0 mL with deionized water. The calibration was carried out using bovine serum albumin as a standard in 9 concentrations (0.1, 0.2, 0.4, 0.6, 0.8, 1.0, 1.5, 2.0 and 4.0 mg /mL). In a 96-well standard multiplate, 10 μL of the calibration solution or 10 μL of the membrane receptor preparation were mixed with 190 μL of the Bradford solution, respectively. After 5 min, the UV absorption of the protein–dye complex at λ = 595 nm was measured with a platereader (Tecan Genios, Tecan, Crailsheim, Germany).

5.2.5. General protocol for the binding assays

The test compound solutions were prepared by dissolving approximately 10 μmol (usually 2–4 mg) of test compound in DMSO so that a 10 mM stock solution was obtained. To obtain the required test solutions for the assay, the DMSO stock solution was diluted with the respective assay buffer. The filtermats were presoaked in 0.5% aqueous polyethylenimine solution for 2 h at room temperature before use. All binding experiments were carried out in duplicates in 96-well multiplates. The concentrations given are the final concentrations in the assay. Generally, the assays were performed by addition of 50 μL of the respective assay buffer, 50 μL test compound solution in various concentrations (10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}, 10^{-9} and 10^{-10} mol/L), 50 μL of corresponding radioligand solution and 50 μL of the respective receptor preparation into each well of the multiplate (total volume 200 μL). The receptor preparation was always added last. During the incubation, the multiplates were shaken at a speed of 500–600 rpm at the specified temperature. Unless otherwise noted, the assays were terminated after 120 min by rapid filtration using the harvester. During the filtration each well was washed five times with 300 μL of water. Subsequently, the filtermats were dried at 95 °C. The solid scintillator was melted on the dried filtermats at a temperature of 95 °C for 5 min. After solidifying of the scintillator at room temperature, the trapped radioactivity in the filtermats was measured with the scintillation analyzer. Each position on the filtermat corresponding to one well of the multiplate was measured for 5 min with the [^{3}H]-counting protocol. The overall counting efficiency was 20%. The IC_{50}-values were calculated with the program GraphPad Prism® 3.0 (GraphPad Software, San Diego, CA, USA) by non-linear regression analysis. Subsequently, the IC_{50} values were transformed into K_i-values using the equation of Cheng and Prusoff.[46] The K_i-values are given as mean value ± SEM from three independent experiments.

5.2.6. Protocol of the σ_1 receptor binding assay

The assay was performed with the radioligand [^{3}H]-(+)-Pentazocine (22.0 Ci/mmol; Perkin Elmer). The thawed membrane preparation of guinea pig brain cortex (about 100 μg of protein) was incubated with various concentrations of test compounds, 2 nM [^{3}H]-(+)-Pentazocine, and TRIS buffer (50 mM, pH 7.4) at 37 °C. The non-specific binding was determined with 10 μM unlabeled (+)-Pentazocine. The K_d-value of (+)-pentazocine is 2.9 nM.[47]

5.2.7. Protocol of the σ_2 receptor binding assay

The assays were performed with the radioligand [^{3}H]DTG (specific activity 50 Ci/mmol; ARC, St. Louis, MO, USA). The thawed membrane preparation of rat liver (about 100 μg of protein) was incubated with various concentrations of the test compound, 3 nM [^{3}H]DTG and buffer containing (+)-pentazocine (500 nM (+)-pentazocine in 50 mM TRIS, pH 8.0) at room temperature. The non-specific bind was determined with 10 μM non-labeled DTG. The K_d value of [^{3}H]DTG is 17.9 nM.[48]

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