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Original article

1,4-Diazepanes derived from (*S*)-serine – Homopiperazines with improved σ_1 (sigma) receptor affinity and selectivity

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Abstract

Starting from the proteinogenic amino acid (*S*)-serine chiral non-racemic 1,4-diazepanes **4** with a hydroxymethyl residue in position 2 are synthesized and pharmacologically evaluated. The key step in the synthesis is the formation of the bicyclic system **8** by consecutive nucleophilic substitution of the chloropropionamide **7** with primary amines and intramolecular aminolysis. Both reaction steps require catalysis with the Lewis acid Ti(O-*i*Pr)$_4$. Homologation of the piperazine to the 1,4-diazepane ring results in a remarkable improvement of σ_1 receptor affinity and σ_1/σ_2 selectivity. The 1,4-dibenzyl derivative **4a** interacts with a K_i value of 7.4 nM with σ_1 receptors and shows a 53-fold selectivity for σ_1 receptors over σ_2 receptors.

Keywords: σ Receptor ligands; 1,4-Diazepanes; Homopiperazines

1. Introduction

The class of σ receptors is subdivided into at least two subtypes, which are termed σ_1 and σ_2 receptor. Whereas the σ_1 receptor has been cloned from various tissues and species [1–4] the σ_2 receptor subtype is not cloned so far. Both receptor subtypes are found in the central nervous system as well as in tissues of the periphery (e.g. liver, lung, heart). The exact understanding of the physiological role of both σ receptor subtypes still remains to be elucidated [5,6]. However, it is well established that σ receptors are involved in several physiological and pathophysiological processes. Therefore, ligands interacting with σ receptors are of particular interest for the development of novel antipsychotics [7,8], antidepressants [9], anti-cocaine agents [10–12], and antitumor agents [6,13,14].

In literature some σ_1 receptor ligands based on the piperazine heterocycle are described. These σ_1 receptor ligands include the very potent 1,4-disubstituted piperazines **1** [15]

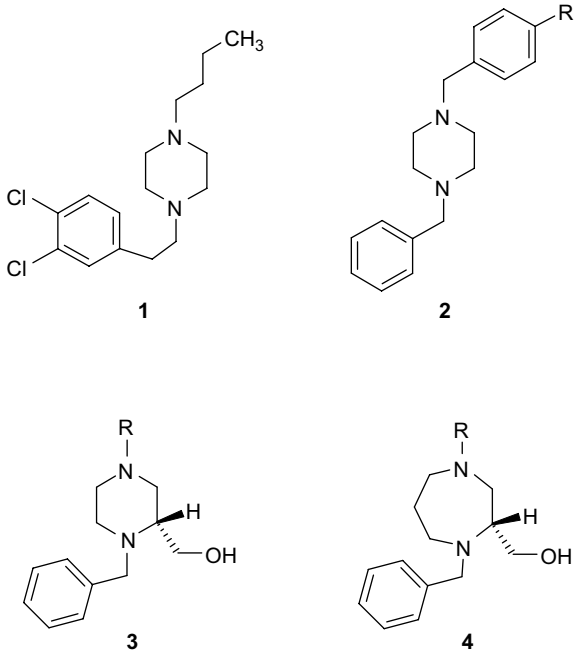

Fig. 1. Comparison of hydroxymethyl substituted 1,4-diazepanes (homopiperazines) **4** with known piperazine based σ_1 receptor ligands **1**–**3**.

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and **2** [10] (compare Fig. 1). Recently we have shown that piperazines **3** with a hydroxymethyl residue in position 2 also interact with σ_1 receptors. High σ_1 receptor affinity is achieved for derivatives with a second aromatic system within the residue R in position 4. Thus, the dibenzyl derivative **3a** ($R = CH_2Ph$, $K_i = 38$ nM) represents one of the most potent compounds of this series [16].

In order to broaden the relationships between the structure of piperazine derivatives and their σ_1 receptor affinity homologous ligands were envisaged. Herein we wish to report on the synthesis of 2-hydroxymethyl substituted 1,4-diazepanes (homopiperazines) **4**, which represent ring homologs of piperazines **3**. The ring enlargement results in an increased σ_1 receptor affinity and, moreover, a considerable improvement of selectivity against the σ_2 receptor.

2. Chemistry

The synthesis of the hydroxymethyl substituted 1,4-diazepanes **4** started with the methyl ester **5** of the proteinogenic amino acid (*S*)-serine [16]. At first the primary amine of **5** was acylated with chloropropionyl chloride to obtain the chloropropionamide **6** in 74% yield. Reaction of the hydroxyamide **6** with benzaldehyde dimethyl acetal led to an inseparable mixture of diastereomeric oxazolidines *cis*-**7** and *trans*-**7** (ratio 30:70) (Scheme 1).

In contrast to our experience with the corresponding chloroacetamides [16] the chloropropionamides **7** did not react with benzylamine upon simple heating to reflux. Only when the Lewis acid Ti(O-*i*Pr)$_4$ was added the conversion of the chloropropionamide **7** into the bicyclic system **8a** took place (Scheme 1). Also, the synthesis of the (dimethoxyethyl) substituted derivatives **8b** by reaction of **7** with aminoacetaldehyde dimethyl acetal was only possible in the presence of the Lewis acid Ti(O-*i*Pr)$_4$. We assume that the Lewis acid activates the chloropropionamide substructure for the nucleophilic substitution as well as the ester moiety for the intramolecular aminolysis. The diastereomeric bicyclic compounds *cis*-**8a**,**b** and *trans*-**8a**,**b** were separated by flash column chromatography and, subsequently, the relative configuration of the products was assigned by nuclear Overhauser effect.

It should be noted that the direct intramolecular aminolysis of linear dipeptides made up of an α and a β-amino acid to form 1,4-diazepanediones is very difficult [17]. However, the preformation of the oxazolidine ring in **7** reduces the conformational flexibility and induces a favorable orientation of the crucial functional groups for the intramolecular aminolysis.

In the last step the bicyclic systems **8a** and **8b** were reduced with $LiAlH_4$. During this reduction both lactam carbonyl moieties as well as the oxazolidine ring were reduced to give directly the hydroxymethyl substituted 1,4-diazepanes **4a** and **4b**, respectively. Since the chiral center in position 10 of the bicyclic systems **8a** and **8b** was destroyed in this reaction step, a mixture of diastereomers *cis*-**8** and *trans*-**8** was employed for the $LiAlH_4$ reduction, respectively.

3. Receptor binding studies

The σ receptor affinities of the 1,4-diazepanes **4** were determined in competition experiments with radioligands. In the σ_1 assay, homogenates of guinea pig brains served as receptor material. The σ_1 selective ligand [^{3}H]-(+)-pentazocine was employed as radioligand, and the non-specific binding was determined in the presence of a large excess of haloperidol [18,19]. Rat liver was the source for σ_2 receptors in the σ_2 assay. Since a σ_2 selective radioligand is not commercially available, the non-selective radioligand [^{3}H]-ditolylguanidine was used in the presence of an excess of non-radiolabeled (+)-pentazocine (100 nM) for selective labeling of σ_1 receptors. Performing the σ_2 assay in the presence of an excess of non-tritiated 1,3-di(*o*-tolyl)guanidine led to the non-specific binding of the radioligand [18,19].

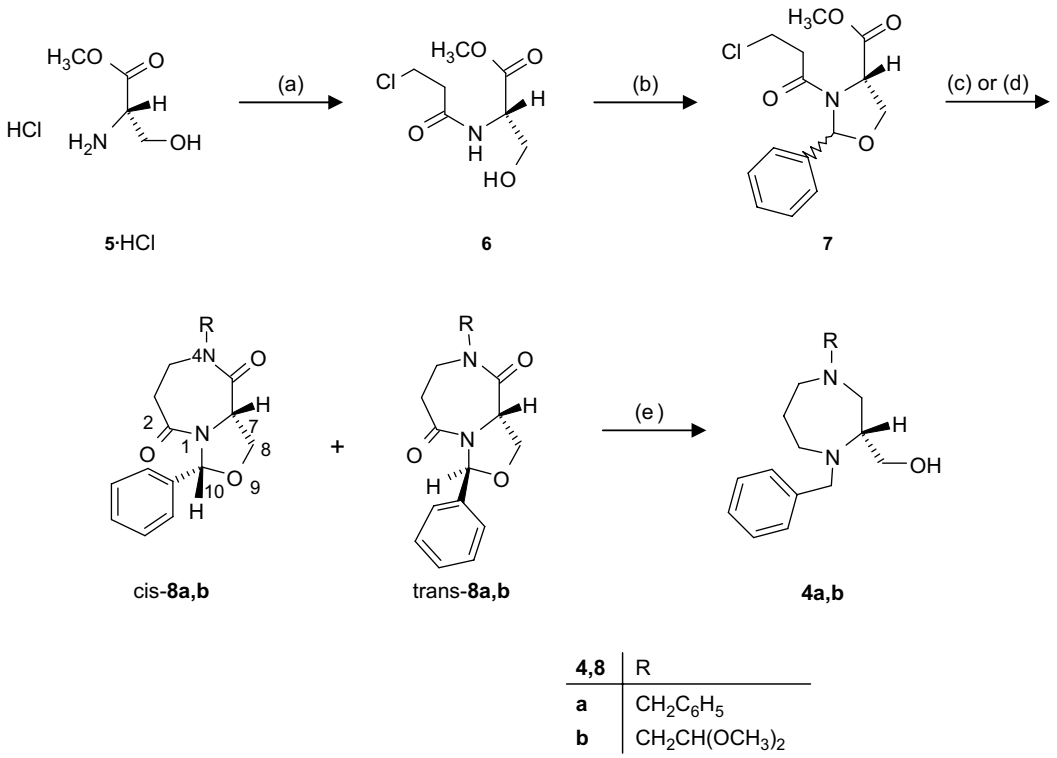
In addition to the σ_1 and σ_2 receptor affinities the interactions with μ-opioid and κ-opioid receptors as well as with the phencyclidine binding site of the NMDA receptor were determined [20]. At first a screening with two rather high concentrations (1 and 10 μM) of the test compounds was performed. Only when considerable inhibition of the radioligand binding was observed at a concentration of 10 μM the exact K_i values were determined.

4. Results and discussion

In Table 1 the σ receptor affinities of the 1,4-diazepaes **4a** and **4b** are compared with the σ receptor affinities of the corresponding piperazine derivatives **3a** and **3b** and reference compounds. The K_i value of the 1,4-dibenzyl-1,4-diazepane **4a** towards the σ_1 receptor is 7.4 nM indicating a 5-fold increase of its σ_1 receptor affinity compared with the 1,4-dibenzylpiperazine **3a** ($K_i = 38$ nM). Additionally the σ_2 receptor affinity of the 1,4-diazepane **4a** is 2-fold lower than the σ_2 receptor affinity of the piperazine **3a**. Taking the increased σ_1 and decreased σ_2 receptor affinity of the 1,4-diazepane **4a** together results in a considerable improvement of the σ_1/σ_2 selectivity (selectivity factor 53 instead of 5).

This observation was confirmed by synthesis and pharmacological evaluation of the homologous 1,4-diazepane **4b** of the (dimethoxyethyl) substituted piperazine **3b**, which showed negligible affinity to both σ receptor subtypes (compare Table 1, entry 2). For the homologous 1,4-diazepane **4b** a σ_1 receptor affinity of 320 nM was determined, which demonstrates again a considerable affinity increase compared with the corresponding piperazine derivative **3b** ($K_i > 10{,}000$ nM). The σ_2 receptor affinity of **4b** ($K_i = 9010$ nM) is in the same range as the σ_2 receptor affinity of the piperazine **3b** ($K_i > 10{,}000$ nM). These data for **4b** support the idea that ring enlargement of the piperazine ring to a 1,4-diazepane ring leads to increase of the σ_1 receptor affinity and σ_1/σ_2 selectivity.

In order to get a rough impression of the selectivity of the 1,4-diazepanes **4** the affinity to μ-opioid and κ-opioid receptors as well as the phencyclidine binding site of the NMDA



Scheme 1. (a) Cl–CH_2CH_2COCl, NEt_3, CH_2Cl_2, 1 h, −5 °C, 1 h, rt, 74%. (b) $PhCH(OCH_3)_2$, TosOH, toluene, 2 h, reflux, 74%. (c) $BnNH_2$, NEt_3, CH_3CN, 24 h, reflux, then Ti(O-*i*Pr)$_4$, 24 h, reflux, 25% (*cis*-**8a**), 67% (*trans*-**8a**). (d) $H_2NCH_2CH(OCH_3)_2$, NEt_3, CH_3CN, 24 h, reflux, then Ti(O-*i*Pr)$_4$, 96 h, reflux, 8% (*cis*-**8b**), 34% (*trans*-**8b**). (e) $LiAlH_4$, THF, 96 h, reflux, 46% (**4a**), 40% (**4b**).

receptor was investigated. In all experiments with concentrations of 10 μM of the test compounds **4a** and **4b** the inhibition of the radioligand binding was lower than 50% indicating IC_{50} values greater than 10 μM. Thus, both 1,4-diazeoanes **4a** and **4b** interact selectively with σ_1 receptors.

5. Conclusion

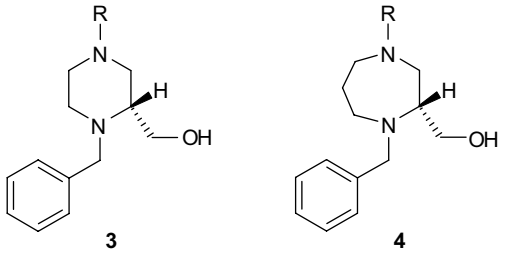
In this communication a method for the preparation of chiral non-racemic 2-hydroxymethyl substituted 1,4-diazepanes **4** is presented. When compared with the piperazines **3** the homologous 1,4-diazepanes **4** show considerable increase of σ_1 receptor affinity and σ_1/σ_2 selectivity.

6. Experimental

6.1. Chemistry, general

Unless otherwise noted, moisture sensitive reactions were conducted under dry nitrogen. Thin layer chromatography

Table 1
σ Receptor affinities of hydroxymethyl substituted 1,4-diazepanes **4** compared with the corresponding piperazines **3** and reference compounds



Entry	Compd	R	$K_i \pm$ SEM (3) (nM)		
			σ_1 ([^{3}H]-(+)-pentazocine)	σ_2 ([^{3}H]-ditolylguanidine)	σ_1/σ_2 Selectivity
1	**3a** [16]	CH_2Ph	38 ± 2.4	177 ± 16	5
2	**3b** [16]	$CH_2CH(OCH_3)_2$	>10,000	>10,000	–
3	**4a**	CH_2Ph	7.4 ± 0.9	374 ± 59	53
4	**4b**	$CH_2CH(OCH_3)_2$	320 ± 18	9010 ± 641	28
5	Haloperidol		2.2 ± 0.3	34 ± 2.3	16
6	Ditolylguanidine		164 ± 47	64 ± 11	0.39
7	BMY 14802		265 ± 32	391 ± 62	1.5

(tlc): Silica gel 60 F_{254} plates (Merck). Flash chromatography (fc): Silica gel 60, 0.040–0.063 mm (Merck); parentheses include: diameter of the column [cm], eluent, fraction size [mL], R_f – optical rotation: polarimeter 241 (Perkin–Elmer); 1.0 dm tube; concentration c [g/100 mL]; temperature 20 °C. Elemental analyses: Vario EL (Elementaranalysesysteme GmbH). MS: MAT 312, MAT 8200, MAT 44, and TSQ 7000 (Finnigan); EI = electron impact, CI = chemical ionization. High resolution MS (HRMS): MAT 8200 (Finnigan). IR: IR spectrophotometer 1605 FT-IR (Perkin–Elmer). (br = broad, m = medium, s = strong). ^{1}H NMR (300 MHz), ^{13}C NMR (75 MHz): Unity 300 FT NMR 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.

6.2. (+)-Methyl (2S)-2-(3-chloropropanoylamino)-3-hydroxypropanoate (**6**)

A suspension of (*S*)-serine methyl ester hydrochloride [16] (**5**·HCl, 10.0 g, 64.3 mmol) in CH_2Cl_2 (100 mL) was cooled (−5 °C). Triethylamine (17.9 mL, 128.6 mmol) and subsequently a solution of 3-chloropropanoyl chloride (5.9 mL, 61.1 mmol) in CH_2Cl_2 (15 mL) were added dropwise. The reaction mixture was stirred for 1 h at −5 °C and for 1 h at rt. Then Et_3N·HCl was filtered off, the solvent was evaporated in vacuo and the residue was purified by fc (2 × 8 cm, ethyl acetate, 100 mL, R_f = 0.52) to yield **6** (9.95 g, 74%) as a pale yellow, viscous oil. $[\alpha]_{589}$ = +21.7 (c = 0.85, CH_2Cl_2). $C_7H_{12}ClNO_4$ (209.6) Calcd. C 40.11, H 5.77, N 6.68, Found C 40.04, H 5.97, N 6.45. MS (EI): *m/z* (%) = 152/150 (M − CO_2CH_3, 12/39), 118 (M − ClC_2H_4CO, 2). MS (CI): *m/z* (%) = 229/227 (M + NH_4^+, 33/100), 212/210 (MH^+, 16/47). IR (film): ν (cm^{-1}) = 3312 (br, OH), 2956 (m, C–H), 1737 (s, C=O ester), 1649 (s, C=O amide), 1535 (s, C–N). ^{1}H NMR ($CDCl_3$): δ = 2.56 (t, J = 6.0 Hz, 1H, O*H*), 2.71–2.77 (m, 2H, $ClCH_2CH_2CO$ and/or $ClCH_2CH_2CO$), 3.79–3.85 (m, 2H, $ClCH_2CH_2CO$ and/or $ClCH_2CH_2CO$), 3.81 (s, 3H, CO_2CH_3), 3.91–4.05 (m, 2H, 3-H), 4.71 (dt, J = 7.3/3.6 Hz, 1H, 2-H), 6.64 (s broad, 1H, N*H*).

6.3. Methyl (2R,4S)- and (2S,4S)-3-(3-chloropropanoyl)-2-phenyl-1,3-oxazolidine-4-carboxylate (cis-**7** and trans-**7**)

A solution of benzaldehyde dimethyl acetal (2.15 mL, 14.31 mmol), **6** (1.0 g, 4.77 mmol) and *p*-toluenesulfonic acid monohydrate (21 mg, 0.11 mmol) in toluene (20 mL) was heated to reflux for 2 h in a Dean–Stark trap. The reaction mixture was concentrated to a volume of about 5 mL. After addition of Et_2O (20 mL) to the cooled solution, the organic layer was washed with a saturated solution of $NaHCO_3$ (2 × 10 mL), water (10 mL) and brine (10 mL). The organic layer was dried (Na_2SO_4), evaporated in vacuo and the residue was purified by fc (5 cm, CH_2Cl_2/ethyl acetate 9:1, 30 mL, R_f = 0.55) to provide a mixture of *cis*-**7**/*trans*-**7** (yield 1.046 g, 74%) as a pale yellow, viscous oil, ratio *cis*-**7**:*trans*-**7** = 30:70. $C_{14}H_{16}ClNO_4$ (297.7) Calcd. C 56.97, H 5.41, N 4.70, Found C 57.68, H 5.67, N 4.22. MS (EI): *m/z* (%) = 299/297 (M, 1/3), 240/238 (M − CO_2CH_3, 3/8), 206 (M − ClC_2H_4CO, 26), 148 (2-phenyl-1,3-oxazolidine, 100), 105 (PhCHO, 67). MS (CI): *m/z* (%) = 300/298 (MH^+, 20/59). IR (film): ν (cm^{-1}) = 2953 (m, C–H), 1749 (s, C=O ester), 1663 (s, C=O amide), 1431 (s, C–H). ^{1}H NMR ($CDCl_3$): δ = 2.31 (dt, J = 16.5/6.6 Hz, 0.7H, $ClCH_2CH_2CO$, *trans*), 2.45–2.54 (m, 0.3H, $ClCH_2CH_2CO$, *cis*), 2.59 (dt, 16.6/6.8 Hz, 0.7H, $ClCH_2CH_2CO$, *trans*), 2.72–2.82 (m, 0.3H, $ClCH_2CH_2CO$, *cis*), 3.54–3.79 (m, 2H, $ClCH_2CH_2CO$), 3.81 (s, 3 × 0.23H, OCH_3, *cis*, rotamer 1), 3.83 (s, 3 × 0.70H, OCH_3, *trans*), 3.86 (s, 3 × 0.07H, OCH_3, *cis*, rotamer 2), 4.07–4.14 (m, 0.23H, 5-H_A, *cis*, rotamer 1), 4.12 (dd, J = 14.3/7.1 Hz, 0.17H, 5-H_A, *trans*, rotamer 2), 4.19–4.29 (m, 0.07H, 5-H_A, *cis*, rotamer 2, and 0.53H, 5-H_A, *trans*, rotamer 1, and 0.3H, 5-H_B, *cis*, rotamer 1 and 2, and 0.53H, 5-H_B, *trans* rotamer1), 4.44–4.47 (m, 0.17H, 5-H_B, *trans*, rotamer 2), 4.68–4.72 (m, 0.3H, 4-H, *cis*), 4.81–4.89 (m, 0.7H, 4-H, *trans*), 6.11 (s, 0.53H, 2-H, *trans*, rotamer 1), 6.27 (s, 0.17H, 2-H, *trans*, rotamer 2), 6.32 (s, 0.23H, 2-H, *cis*, rotamer 1), 6.50 (s, 0.07H, 2-H, *cis*, rotamer 2), 7.35–7.53 (m, 4H, arom), 7.71–7.74 (m, 1H, arom).

6.4. (−)-(7S,10R)-5-Benzyl-10-phenyl-9-oxa-1,5-diazabicyclo[5.3.0]decane-2,6-dione (cis-**8a**) and (+)-(7S,10S)-5-benzyl-10-phenyl-9-oxa-1,5-diazabicyclo[5.3.0]decane-2,6-dione (trans-**8a**)

A solution of **7** (*cis*-**7**:*trans*-**7** = 30:70, 0.278 g, 0.93 mmol), benzylamine (0.10 mL, 0.93 mmol) and triethylamine (0.13 mL, 0.93 mmol) in acetonitrile (35 mL) was heated to reflux for 24 h. Then titanium(IV) tetraisopropylate (0.41 mL, 1.4 mmol) was added and the mixture was heated to reflux for additional 24 h. Removal of the solvent in vacuo followed by fc (3 cm, ethyl acetate, 8 mL) gave *cis*-**8a** (R_f = 0.65) and *trans*-**8a** (R_f = 0.39).

cis-**8a**. Colorless viscous oil, yield 79 mg (25%). $[\alpha]_{589}$ = −32.6 (c = 0.50, CH_2Cl_2). $C_{20}H_{20}N_2O_3$ (336.4). HRMS: Calcd. 336.1474, Found 336.1472 (−0.2 ppm). MS (EI): *m/z* (%) = 336 (M, 24), 259 (M − phenyl, 3), 245 (M − benzyl, 21), 105 (PhCHO, 30), 91 (benzyl, 100). IR (film): ν (cm^{-1}) = 2927 (m, C–H), 1642 (s, C=O), 1430 (m, C–H). ^{1}H NMR ($CDCl_3$): δ = 2.51 (ddd, J = 18.1/12.8/4.3 Hz, 1H, 3-H), 2.65 (dt, J = 18.3/3.2 Hz, 1H, 3-H), 3.29 (dt, J = 15.9/4.0 Hz, 1H, 4-H), 3.92 (ddd, J = 15.7/12.7/2.9 Hz, 1H, 4-H), 4.32 (dd, J = 10.8/8.5 Hz, 1H, 8-H), 4.53 (d, J = 14.7 Hz, 1H, $PhCH_2N$), 4.78–4.83 (m, 2H, 7-H and 8-H), 4.81 (d, J = 14.6 Hz, 1H, $PhCH_2N$), 6.47 (s, 1H, 10-H), 7.27–7.43 (m, 10H, arom). ^{1}H NOE: after irradiation at δ = 6.47 ppm (10-H) a NOE was found at δ = 4.8 ppm (7-H and 8-H). ^{13}C NMR ($CDCl_3$): δ = 35.9 (1C, C-3), 41.6 (1C, C-4), 50.2 (1C, $PhCH_2N$), 56.6 (1C, C-7), 68.6 (1C, C-8), 91.9 (1C, C-10), 126.7 (2C, C-4 [Bn] and C-4 [Ph]), 128.1 (1C, C-2 or C-3 or C-5 or C-6 arom), 128.3 (2C, C-2 and/or C-3 and/or C-5 and/or C-6 arom), 128.4 (2C, C-2 and/or

C-3 and/or C-5 and/or C-6 arom), 128.8 (1C, C-2 or C-3 or C-5 or C-6 arom), 128.9 (2C, C-2 and/or C-3 and/or C-5 and/or C-6 arom), 136.2 (1C, C-1 [Bn] or C-1 [Ph]), 137.8 (1C, C-1 [Bn] or C-1 [Ph]), 167.3 (1C, C-2 or C-6), 167.4 (1C, C-6 or C-2).

*trans-**8a***. Colorless viscous oil, yield 209 mg (67%). $[\alpha]_{589} = +76.2$ ($c = 0.55$, CH_2Cl_2). $C_{20}H_{20}N_2O_3$ (336.4). HRMS: Calcd. 336.1474, Found 336.1472 (−0.2 ppm). MS (EI): *m/z* (%) = 336 (M, 22), 259 (M − phenyl, 5), 245 (M − benzyl, 13), 105 (PhCHO, 26), 91 (benzyl, 100). IR (film): ν (cm^{-1}) = 2928 (m, C−H), 1644 (s, C═O), 1429 (m, C−H). ^{1}H NMR ($CDCl_3$): $\delta = 2.53$ (ddd, $J = 17.4/11.0/4.2$ Hz, 1H, 3-H), 2.78 (ddd, $J = 17.4/6.4/3.0$ Hz, 1H, 3-H), 3.43 (ddd, $J = 15.5/6.5/4.7$ Hz, 1H, 4-H), 3.83 (ddd, $J = 15.3/11.0/3.0$ Hz, 1H, 4-H), 4.39 (dd, $J = 9.5/7.1$ Hz, 1H, 8-H), 4.57 (d, $J = 14.7$ Hz, 1H, PhCH_2N), 4.59 (t, $J = 9.2$ Hz, 1H, 8-H), 4.82 (d, $J = 14.3$ Hz, 1H, PhCH_2N), 4.81−4.86 (m, 1H, 7-H), 6.49 (s, 1H, 10-H), 7.27−7.38 (m, 10H, arom). ^{1}H NOE: after irradiation at $\delta = 6.49$ ppm (10-H) a NOE at $\delta = 4.85$ ppm (7-H) was not detected.

*6.5. (−)-(7S,10R)-5-(2,2-Dimethoxyethyl)-10-phenyl-9-oxa-1,5-diazabicyclo[5.3.0]decane-2,6-dione (cis-**8b**) and (+)-(7S,10S)-5-(2,2-dimethoxyethyl)-10-phenyl-9-oxa-1,5-diazabicyclo[5.3.0]decane-2,6-dione (trans-**8b**)*

A solution of **7** (*cis*-**7**:*trans*-**7** = 30:70, 0.218 g, 0.73 mmol), aminoacetaldehyde dimethyl acetal (79 μL, 0.73 mmol) and triethylamine (0.10 mL, 0.73 mmol) in acetonitrile (25 mL) was heated to reflux for 24 h. Then titanium(IV) tetraisopropylate (0.32 mL, 1.1 mmol) was added and heating was continued for additional 96 h. The solvent was removed in vacuo and the residue was purified by fc (3 cm, ethyl acetate, 8 mL) to yield *cis*-**8b** ($R_f = 0.30$) and *trans*-**8b** ($R_f = 0.18$).

*cis-**8b***. Colorless viscous oil, yield 20 mg (8%). $[\alpha]_{589} = -28.3$ ($c = 0.375$, CH_2Cl_2). $C_{17}H_{22}N_2O_5$ (334.4). HRMS: Calcd. 334.1529, Found 334.1530 (+0.1 ppm). MS (EI): *m/z* (%) = 334 (M, 4), 303 (M − OCH_3, 7), 153 (M − PhCHO − $CH(OCH_3)_2$, 9), 105 (PhCHO, 5), 91 (benzyl, 6), 75 ($CH(OCH_3)_2$, 100). IR (film): ν (cm^{-1}) = 2938 (m, C−H), 2835 (m, C−H), 1639 (s, C═O), 1428 (m, C−H). ^{1}H NMR ($CDCl_3$): $\delta = 2.71$ (dt, $J = 18.3/2.9$ Hz, 1H, 3-H), 2.88 (ddd, $J = 18.3/13.2/4.6$ Hz, 1H, 3-H), 3.41−3.47 (m, 1H, 4-H), 3.43 (s, 6H, 2 × OCH_3), 3.48 (dd, $J = 13.8/5.5$ Hz, 1H, NCH_2CH$(OCH_3)_2$), 3.65 (dd, $J = 13.9/4.8$ Hz, 1H, NCH_2CH$(OCH_3)_2$), 4.05 (ddd, $J = 15.9/13.2/2.7$ Hz, 1H, 4-H), 4.22−4.29 (m, 1H, 8-H), 4.47 (t, $J = 5.0$ Hz, 1H, C$H(OCH_3)_2$), 4,72−4.79 (m, 2H, 7-H and 8-H), 6.48 (s, 1H, 10-H), 7.32−7.43 (m, 5H, arom). ^{1}H NOE: after irradiation at $\delta = 6.48$ ppm (10-H) a NOE was found at $\delta = 4.75$ ppm (7-H and 8-H).

*trans-**8b***. Colorless viscous oil, yield 82 mg (34%). $[\alpha]_{589} = +22.2$ ($c = 0.58$, CH_2Cl_2). $C_{17}H_{22}N_2O_5$ (334.4). HRMS: Calcd. 334.1529, Found 334.1530 (+ 0.1 ppm). MS (EI): *m/z* (%) = 334 (M, 1), 105 (PhCHO, 1), 75 ($CH(OCH_3)_2$, 100). MS (CI): *m/z* (%) = 335 (MH^+, 2), 303 (M − OCH_3, 82). IR (film): ν (cm^{-1}) = 2938 (m, C−H), 2834 (m, C−H), 1638 (s, C═O), 1428 (m, C−H). ^{1}H NMR ($CDCl_3$): $\delta = 2.78$ (ddd, $J = 17.7/5.3/3.3$ Hz, 1H, 3-H), 2.91 (ddd, $J = 17.7/11.3/4.2$ Hz, 1H, 3-H), 3.39 (dd, $J = 14.1/5.2$ Hz, 1H, NCH_2CH$(OCH_3)_2$), 3.42 (s, 3H, OCH_3), 3.43 (s, 3H, OCH_3), 3.55 (dt, $J = 15.5/4.8$ Hz, 1H, 4-H), 3.84 (dd, $J = 13.9/5.0$ Hz, 1H, NCH_2CH$(OCH_3)_2$), 4.01 (ddd, $J = 15.1/11.6/3.5$ Hz, 1H, 4-H), 4.30−4.36 (m, 1H, C$H(OCH_3)_2$), 4.48−4.55 (m, 2H, 8-H), 4.80 (t, $J = 7.6$ Hz, 1H, 7-H), 6.51 (s, 1H, 10-H), 7.27−7.38 (m, 5H, arom). ^{1}H NOE: after irradiation at $\delta = 6.51$ ppm (10-H) a NOE at $\delta = 4.80$ ppm (7-H) was not detected.

*6.6. (−)-[(2R)-(1,4-Dibenzyl)-1,4-diazepan-2-yl]methanol (**4a**)*

Under N_2 atmosphere $LiAlH_4$ powder (0.271 g, 7.13 mmol) was added to a stirred solution of **8a** (mixture of *cis*/*trans* isomers, 0.240 g, 0.713 mmol) in THF (40 mL). The reaction mixture was heated to reflux for 96 h. Under N_2 atmosphere and cooling (ice bath) water (0.5 mL), 3 M NaOH (0.5 mL) and water (0.5 mL) were successively added. The suspension was refluxed for 30 min. After cooling down to rt, the precipitate was filtered off and the solvent was removed in vacuo. Purification of the residue by fc (2 cm, ethyl acetate, 3 mL, $R_f = 0.34$) furnished **4a** as a colorless oil, yield 0.101 g (46%). $[\alpha]_{589} = -8.3$ ($c = 0.585$, CH_2Cl_2). $C_{20}H_{26}N_2O$ (310.4) Calcd. C 77.37, H 8.44, N 9.02, Found C 76.83, H 7.94, N 8.33. HRMS: Calcd. 310.2045, Found 310.2044 (−0.1 ppm). MS (EI): *m/z* (%) = 310 (M, 1), 279 (M − CH_2OH, 45), 219 (M − benzyl, 4), 91 (benzyl, 100). MS (CI): *m/z* (%) = 311 (MH^+, 100). IR (film): ν (cm^{-1}) = 3416 (br, OH), 2932 (m, C−H), 1452 (m, C−H). ^{1}H NMR ($CDCl_3$): $\delta = 1.72-1.83$ (m, 2H, 6-H), 2.54 (ddd, $J = 12.2/7.9/4.3$ Hz, 1H, 5-H or 7-H), 2.64−2.81 (m, 4H, 3-H, 5-H, and 7-H), 2.86−2.92 (m, 1H, 2-H), 2.99 (ddd, $J = 14.3/7.4/3.2$ Hz, 1H, 7-H or 5-H), 3.46 (d, $J = 5.5$ Hz, 2H, CH_2OH), 3.64 (s, 2H, PhCH_2N^4), 3.84 (d, $J = 13.7$ Hz, 1H, PhCH_2N^1), 3.93 (d, $J = 13.7$ Hz, 1H, PhCH_2N^1), 7.27−7.37 (m, 10H, arom). The signal for the proton of the OH group could not be detected. ^{13}C NMR ($CDCl_3$): $\delta = 27.2$ (1C, C-6), 49.8 (1C, C-5 or C-7), 56.3 (1C, C-3, C-5 or C-7), 56.4 (1C, C-3, C-5 or C-7), 57.0 (1C, N^1CH_2Ph), 62.5 (1C, C-2), 62.9 (1C, CH_2OH), 63.9 (1C, N^4CH_2Ph), 127.1 and 127.2 (2C, 2 × C-4 arom), 128.30 and 128.32 (4C, 2 × C-3 and 2 × C-5 arom), 128.78 and 128.82 (4 C, 2 × C-2 and 2 × C-6 arom), 139.2 and 139.7 (2C, 2 × C-1 arom).

*6.7. (−)-[(2R)-1-Benzyl-4-(2,2-dimethoxyethyl)-1,4-diazepan-2-yl]methanol (**4b**)*

Under N_2 atmosphere $LiAlH_4$ powder (0.233 g, 6.13 mmol) was added to a stirred solution of **8b** (mixture of *cis*/*trans* isomers, 0.205 g, 0.613 mmol) in THF (40 mL). The reaction mixture was heated to reflux for 96 h. Under N_2 atmosphere and cooling (ice bath) water (0.5 mL), 3 M NaOH (0.5 mL)

and water (0.5 mL) were successively added. The suspension was refluxed for 30 min. After cooling down to rt, the precipitate was filtered off and the solvent was removed in vacuo. Purification of the residue by fc (2 cm, ethyl acetate/ethanol, 5:1, 3 mL, $R_f = 0.35$) furnished **4b** as pale yellow oil, yield 75 mg (40%.). $[\alpha]_{589} = -3.9$ ($c = 0.505$, CH_2Cl_2). $C_{17}H_{28}N_2O_3$ (308.4). HRMS: Calcd. 308.2099, Found 308.2092 (−0.7 ppm). MS (EI): *m/z* (%) = 308 (M, 3), 277 (M − CH_2OH, 75), 233 (M − $CH(OCH_3)_2$, 49), 186 (M − benzyl − CH_2OH, 28), 91 (benzyl, 100). MS (CI): *m/z* (%) = 309 (MH^+, 100), 277 (M − CH_2OH, 31). IR (film): ν (cm^{-1}) = 3306 (br, O−H), 2937 (m, C−H), 2871 (m, C−H), 1446 (m, C−H). ^{1}H NMR ($CDCl_3$): $\delta = 1.67-1{,}86$ (m, 2H, 6-H), 2.53−2.67 (m, 4H, 3-H, 5-H and 7-H), 2.68−2.82 (m, 3H, $CH_2CH(OCH_3)_2$, and 3-H or 5-H or 7-H), 2.85−2.93 (m, 1H, 2-H), 2.95 (ddd, $J = 13.9/7.0/3.1$ Hz, 1H, 3-H, 5-H or 7-H), 3.37 (s, 3H, OCH_3), 3.39 (s, 3H, OCH_3), 3.51 (dd, $J = 11.1/6.1$ Hz, CH_2OH), 3.64 (dd, $J = 11.0/6.2$ Hz, 1H, CH_2OH), 3.83 (d, $J = 15.1$ Hz, 1H, $PhCH_2N$), 3.85 (d, $J = 14.7$ Hz, 1H, $PhCH_2N$), 4.49 (t, $J = 5.4$ Hz, 1H, $CH(OCH_3)_2$), 7.24−7.32 (m, 5H, arom). The signal for the proton of the OH group could not be detected. ^{13}C NMR ($CDCl_3$): $\delta = 27.6$ (1C, C-6), 49.5 (1C, C-3, C-5 or C-7), 52.9 and 53.8 (2C, 2 × OCH_3), 55.8 (1C, $NCH_2CH(OCH_3)_2$), 57.6 (1C, C-3, C-5 or C-7), 57.9 (1C, NCH_2Ph), 60.3 (1C, C-3, C-5 or C-7), 62.7 (1C, C-2), 63.1 (1C, CH_2OH), 103.0 (1C, $CH(OCH_3)_2$), 127.0 (1C, C-4′), 128.3 (2C, C-3′ and C-5′), 128.8 (2C, C-2′ and C-6′), 139.7 (1C, C-1′).

7. Receptor binding studies

7.1. General

Homogenizer: Potter®S (B. Braun Biotech International). Ultraturrax: Euroturrax® T20 (Ika Labortechnik). Centrifuge: High speed cooling centrifuge model J2-HS (Beckman). Filter: Whatman glass fiber filters GF/B, presoaked in the medium described below before use. Filtration was performed with a Brandel 24-well cell harvester. Scintillation cocktail: Rotiscint Eco Plus (Roth). Liquid scintillation analyzer: Tri-Carb 2100 TR (Canberra Packard), counting efficiency 66%. All experiments were carried out in triplicates. IC_{50} values were determined from competition experiments with at least 6 concentrations of test compounds and were calculated with the program GraphPad Prism® 3.0 (GraphPad Software) by non-linear regression analysis. K_i values were calculated according to Cheng and Prusoff [21]. The K_i values are given as mean value ± SEM from three independent experiments.

7.2. σ_1 Receptor binding assay [18]

For the σ_1 assay guinea pig brain membranes were prepared as described in Ref. [18]. The test was performed with the radioligand [^{3}H]-pentazocine (1036 GBq/mmol; NEN™ Life Science Products). The thawed membrane preparation (about 150 μg of protein) was incubated with various concentrations of the test compound, 3 nM [^{3}H]-pentazocine, and buffer (50 mM Tris−HCl, pH 7.4) in a total volume of 500 μL for 120 min at 37 °C. The incubation was terminated by rapid filtration through presoaked Whatman GF/B filters (0.5% polyethylenimine in water for 2 h at 4 °C) using a cell harvester. After washing four times with 2 mL of cold buffer 3 mL of scintillation cocktail were added to the filters. After at least 8 h bound radioactivity trapped on the filters was counted in a liquid scintillation analyzer. Non-specific binding was determined with 10 μM haloperidol.

7.3. σ_2 Receptor binding assay [18]

For the σ_2 assay rat liver membranes were prepared as described in Ref. [18]. The membrane preparation (about 60 μg of protein) was incubated with 3 nM [^{3}H]-ditolylguanidine (2220 GBq/mmol, American Radiolabeled Chemicals, Inc.) and different concentrations of test compounds in buffer (50 mM Tris−HCl, pH 8.0) in the presence of 100 nM (+)-pentazocine. The total volume was 250 μL. The incubation (120 min, 25 °C) was stopped by addition of 2 mL of ice-cold buffer (10 mM Tris−HCl, pH 8.0) followed by rapid filtration through presoaked Whatman GF/B filters (0.5% polyethylenimine in water for 2 h at 4 °C) using a cell harvester. After the sample was washed three times with 2 mL of cold buffer, a total volume of 3 mL of scintillation cocktail was added to the filters. After at least 8 h bound radioactivity trapped on the filters was counted in a liquid scintillation analyzer. Non-specific binding was determined with 10 μM non-radiolabeled ditolylguanidine.

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