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Synthesis and NMDA receptor affinity of fluorinated dioxadrol analogues

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ABSTRACT

A series of dioxadrol analogues with fluorine substituents in position 4 of the piperidine ring has been synthesized and pharmacologically evaluated. The key step in the synthesis was the fluorination of diastereomeric piperidones **6a** and **6c** as well as diastereomeric alcohols **9a** and **9c** with DAST. The reaction of the alcohols **9a** and **9c** took place with inversion of configuration. After removal of the Cbz-protective group, the NMDA receptor affinities of the resulting secondary amines **8a**, **8c**, **12b**, and **12d** were investigated in receptor binding studies. It was shown that the *like*-configuration of the ring junction was crucial for high NMDA receptor affinity. An axially oriented fluorine atom in position 4 led to 2-(2,2-diphenyl-1,3-dioxolan-4-yl)-4-fluoropiperidine (**12d**, WMS-2517) with a K_i-value of 27 nM. The NMDA receptor affinity of **8c** (WMS-2513) with an additional fluorine atom in equatorial 4-position was slightly reduced (K_i = 81 nM). Both fluorinated dioxadrol derivatives **8c** and **12d** showed high selectivity against σ_1 and σ_2 receptors as well as the polyamine binding site of NR2B receptors.

1. Introduction

(*S*)-Glutamate represents the most important excitatory neurotransmitter in the central nervous system (CNS). It mediates its effects through two types of receptors, which are termed metabotropic and ionotropic receptors. We are particularly interested in ligands interacting with the NMDA (*N-m*ethyl-D-*a*spartate) receptor, which is the pharmacologically best characterized type of the ionotropic receptors.[1]

The NMDA receptor is a ligand gated ion channel, which controls the influx of Ca^{2+}-ions into the neuron. This unique feature renders NMDA receptors suitable for synaptic plasticity and long term potentiation (LTP), which are thought to be cellular correlates for learning and memory.[2,3]

However, overactivation of NMDA receptors leads to an increased influx of Ca^{2+}-ions into neurons resulting in an uncontrolled activation of several Ca^{2+}-dependent enzymes and at the end in damage of neuronal cells (excitotoxicity).[4] Therefore, compounds blocking the excessive influx of Ca^{2+}-ions through the NMDA receptor associated ion channel into neurons are of major interest as neuroprotective agents, which may be used for the therapy of cerebral ischemia, stroke, epilepsy and trauma (brain injury). Moreover, permanent increased activation of NMDA receptors has been discussed to be involved in the development of chronic neurodegenerative disorders like Parkinson's disease, Alzheimer's disease, amyotrophic lateral sclerosis and alcohol dependence. Altogether the NMDA receptor represents an interesting target for the development of novel drugs for the therapy of neurological disorders.[5]

Opening of the NMDA receptor associated cation channel is controlled by various ligands interacting with different binding sites at the receptor protein. The receptor comprises binding sites for (*S*)-glutamate, glycine, polyamines, Zn^{2+}, Mg^{2+}, H^+ and phencyclidine (1-(1-phenylcyclohexyl)piperidine, PCP, **1**, Fig. 1).[6]

Our interest has been focused on the PCP binding site, which is located within the cation channel. Compounds interacting with

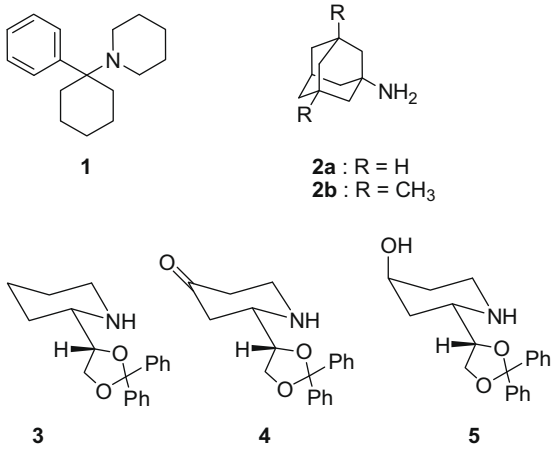

Figure 1. Structures of some important NMDA receptor antagonists: phencyclidine (PCP, **1**); amantadine (**2a**); memantine (**2b**); dexoxadrol (**3**); racemic 4-oxodioxadrol (**4**), racemic 4-hydroxydioxadrol (**5**).

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the PCP binding site work as non-competitive NMDA receptor antagonists by blocking the cation channel and inhibiting the influx of Ca^{2+}-ions. Up to now, memantine (**2b**, Fig. 1) is the only NMDA receptor antagonist with moderate NMDA receptor affinity (K_i = 1.2 μM),[7] which is used for the treatment of severe Alzheimer's disease.[8] The particular advantage of memantine is the fast off rate kinetic preventing the drug from accumulation in the ion channel.[9,10] Therefore memantine enters the open channel preferentially when it is pathologically activated for longer periods of time. The physiological neurotransmission is not disturbed by memantine resulting in minimal adverse side effects.[7,9] Parkinson's disease can be treated with the desmethyl derivative amantadine (**2a**).

In contrast to memantine (**2b**), the piperidine derivative dexoxadrol (**3**, (*S*,*S*)-enantiomer) binds with high affinity (K_i = 11 nM) at the phencyclidine (PCP) binding site within the NMDA receptor associated cation channel. Dexoxadrol was synthesized by Hardie et al. in the 1960s and revealed local anesthetic, spasmolytic, and central nervous system activity.[11] Later phencyclidine like dissociative anesthetic activities were found.[12] Unfortunately, clinical trials of dexoxadrol had to be stopped because of the psychotomimetic side effects. Since the severe side effects of dexoxadrol are attributed to its high NMDA receptor affinity and unfavorable kinetic properties, novel analogues with moderate affinities between those of dexoxadrol (K_i = 11 nM) and memantine (K_i = 1.2 μM) should display the desired pharmacological activity with an improved side effect profile.

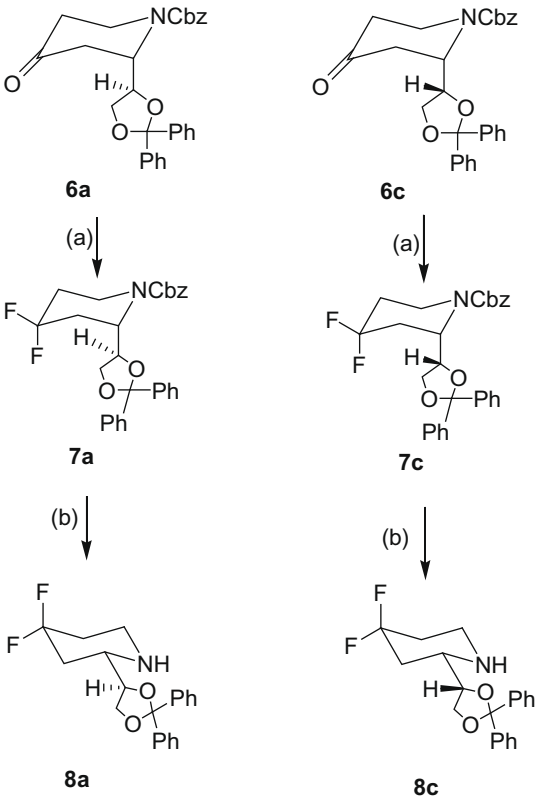
Some derivatives of dexoxadrol have been synthesized in order to get more insight into the structure–affinity relationships within this compound class. In particular the substituents, the size and the heteroatoms in the oxygen heterocycle and furthermore the distance between the basic amino moiety and the oxygen heterocycle have been modified.[13–15] In addition to various N-substituents the piperidine ring has been exchanged for simple primary, secondary and tertiary amines.[16,17]

In order to broaden the structure–affinity relationships of this class of NMDA antagonists we became interested in the influence of substituents in the piperidine moiety on the NMDA receptor affinity. Recently we have reported on the synthesis of racemic 4-oxo-,[18] 4-hydroxy-, 4-methoxy, and 4-amino-substituted dioxadrol derivatives.[19] Racemic 4-oxodioxadrol (**4**) and 4-hydroxydioxadrol (**5**) with the same relative configuration as dexoxadrol (**3**) showed promising NMDA receptor affinity of 470 nM and 44 nM, respectively.[18,19] The affinity of both compounds **4** and **5** is in an interesting range between the affinities of enantiomerically pure dexoxadrol and memantine.

Herein we report on the synthesis and NMDA receptor affinity of fluorinated dioxadrol analogues with two fluorine atoms (**8**) or one fluorine atom (**12**) in position 4 of the piperidine ring. The fluorinated compounds **8** and **12** were considered as bioisosteres of oxodioxadrol **4** (C=O/CF_2-exchange) and hydroxydioxadrol **5** (CHOH/CHF-exchange). Introduction of fluorine atoms into lead compounds is a common strategy in Medicinal Chemistry. The high electronegativity and the small size of the fluorine atom as well as its very different chemical reactivity with respect to a proton, hydroxy or carbonyl moiety will affect the pharmacodynamic (e.g., receptor affinity, receptor selectivity) as well as the pharmacokinetic properties (e.g., absorption, distribution, passage of barriers, metabolism) of pharmacologically active compounds.[20,21]

2. Chemistry

The synthesis was started with the diastereomeric piperidones **6a** and **6c**,[19,22] which have been prepared by an imino-Diels–Alder reaction as key step.[23,24] Reaction of the ketones **6a** and **6c** with diethylaminosulfur trifluoride (DAST)[25] at room temperature provided the difluoro derivatives **7a** and **7c**, respectively. Hydrogenolytic removal of the Cbz protective group led to the diastereomeric difluoro dioxadrol derivatives **8a** and **8c** (Scheme 1). The relative configuration of the products **8a** and **8c** was derived from the relative configuration of the starting ketones **6a** and **6c**.

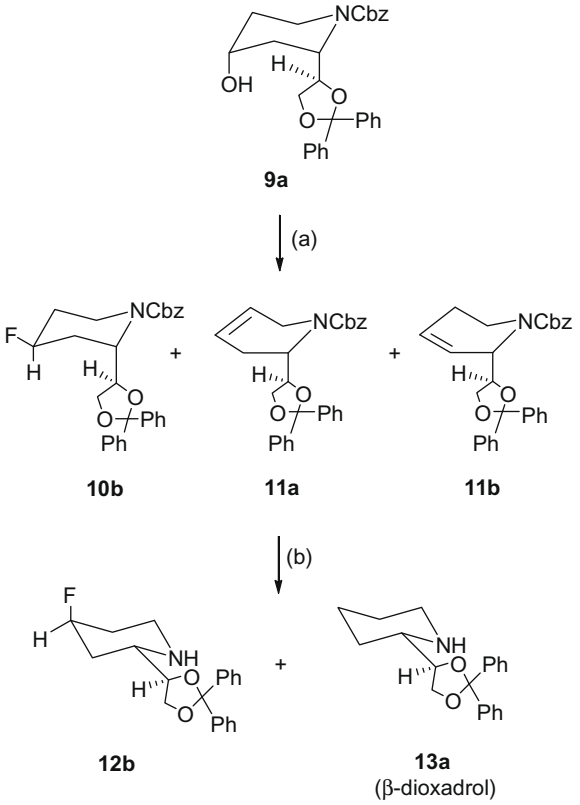


Scheme 1. Synthesis of difluorodioxadrol derivatives (only one enantiomer is shown, all compounds are racemic). Reagents and conditions: (a) DAST, CH_2Cl_2, 16 h, rt, 39% (**7a**), 70% (**7c**); (b) H_2, Pd/C 10%, MeOH, 4 h, rt, 75% (**8a**), 85% (**8c**).

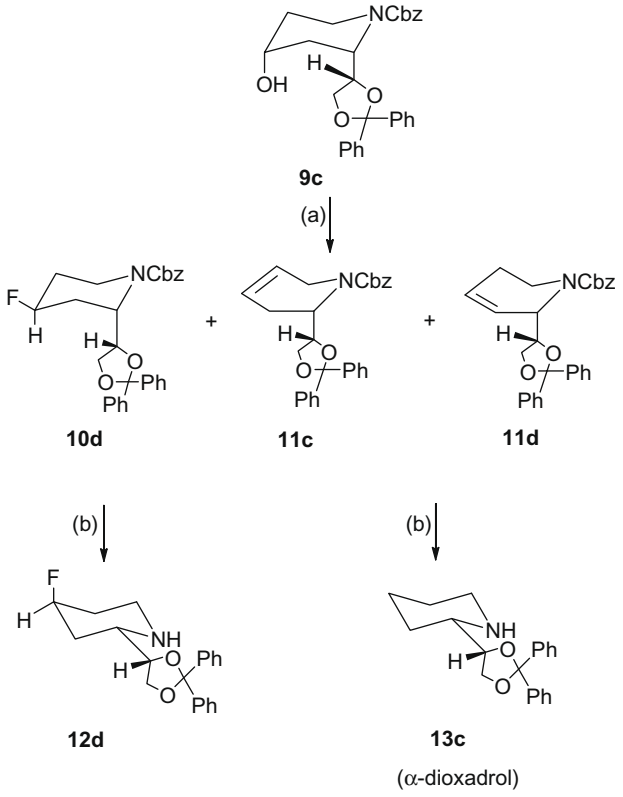
For the synthesis of the monofluoro derivatives **12** the diastereomeric alcohols **9a**–**d**[19,22] were reacted with DAST[25] at −78 °C. This transformation was strongly dependent on the orientation of the hydroxy moiety in position 4 of the piperidine ring. Whereas the alcohols **9b** and **9d** with equatorially oriented hydroxy moiety gave upon reaction with DAST several unidentified products, the diastereomeric alcohols **9a** and **9c** with the hydroxy group in axial position led to the inverted monofluoro derivatives **10b** and **10d** together with considerable amounts of regioisomeric elimination products **11a**/**11b** and **11c**/**11d**, respectively (Schemes 2 and 3). After hydrogenolytic removal of the Cbz-protective group the monofluoro derivatives **12b** and **12d** with axially oriented fluorine substituents were isolated in 15% and 24% yield (two steps from **9a** and **9c**), respectively. In addition to the monofluoro derivatives racemic β-dioxadrol (**13a**)[26] and racemic α-dioxadrol (**13c**)[26] were obtained after hydrogenation in 64% and 47% yield (two steps from **9a** and **9c**), respectively (Schemes 2 and 3).

For the correct assignment of the configuration of the newly formed center of chirality in position 4 of the piperidine ring the change of the conformation of the piperidine chair has to be taken into account. Whereas piperidines with an N-Cbz group (**6**, **7**, **9**, **10**, **11**) adopt the $^1C_4$-conformation due to allylic strain, the corresponding piperidines with a proton at the N-atom (**8**, **12**, **13**) exist in the $_1C^4$-conformation (see Schemes 1–3).

For example, the dtt (*J* = 49.0/10.0/4.9 Hz, 4-H) at 4.62 ppm in the ^1H NMR spectrum of the Cbz-protected monofluoro derivative **10d** clearly proves the axial orientation of 4-H. The large coupling



Scheme 2. Synthesis of monofluorodioxadrol derivatives and β-dioxadrol (only one enantiomer is shown, all compounds are racemic). Reagents and conditions: (a) DAST, CH_2Cl_2, 3 h, −78 °C; (b) H_2, Pd/C 10%, MeOH, 4 h, rt, 15% (**12b** from **9a**), 64% (**13a** from **9a**).



Scheme 3. Synthesis of monofluorodioxadrol derivatives and α-dioxadrol (only one enantiomer is shown, all compounds are racemic). Reagents and conditions: (a) DAST, CH_2Cl_2, 3 h, −78 °C, 29% (**10d**), 52% (**11c** and **11d**); (b) H_2, Pd/C 10%, MeOH, 4 h, rt, 81% (**12d**), 91% (**13c**).

constant of 49.0 Hz is coming from 4-H/4-F-coupling. The triplet with two large couplings to the axially oriented protons in 3- and 5-position and the triplet with two small couplings with the corresponding equatorially positioned protons in 3- and 5-position can only be explained by an axial orientation of 4-H. After removal of the Cbz-protective group a quintet of doublet (dquint, J = 47.9/2.7 Hz) at 4.97 ppm is observed for the proton in 4-position. The small coupling constants of this signal demonstrate almost identical couplings with all neighboring protons, which is only possible in an equatorial orientation of 4-H.

In order to get the diastereomeric monofluoro derivatives **12a** and **12c** with equatorially oriented fluoro substituent, several modifications of the fluorination with DAST were investigated. In particular variation of the temperature (e.g., −100 °C and −60 °C) and the solvent (e.g., CH_2Cl_2, DMF) for the fluorination of **9b** and **9d** did not result in the corresponding Cbz-protected monofluoro derivatives **10a** or **10c** with an axially oriented fluorine atom. In a further experiment the nucleophilic substitution of the mesylate **14d**, which had been prepared by mesylation of the alcohol **9d**, with KF and the crown ether [18]crown-6 was investigated (Scheme 4). However, instead of the expected substitution product **10c** the elimination products **11c**/**11d** were isolated as sole products in 78% yield. Obviously the high basicity of fluoride led to elimination instead of substitution. Since it has been shown that the α-dioxadrol analogues with an axially oriented 4-hydroxy or 4-methoxy moiety are generally the more potent stereoisomers we decided to skip the equatorially oriented fluoro derivatives **12a** and **12c**.

The efficient synthesis of the alkenes **11c**/**11d** from the mesylate **14d** increased the total yield of α-dioxadrol ('racemic dexoxadrol') **13c** considerably and renders this alternate α-dioxadrol synthesis very attractive. Starting from the alcohol **9c** the total yield of α-dioxadrol (**13c**) was 66%.

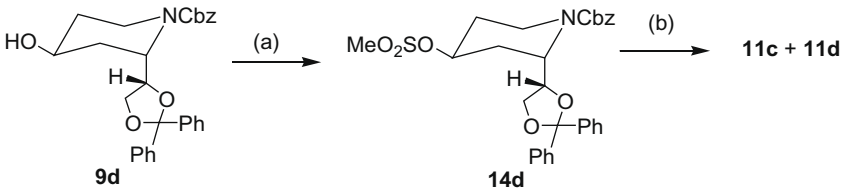
3. Receptor affinities

3.1. General description of the assay systems

The affinities of the novel dexoxadrol analogues to the PCP binding site of the NMDA receptor were determined in competition experiments using the potent and selective radioligand [^{3}H]-(+)-MK-801. A membrane preparation from pig brain cortex was employed as receptor material.[27,28] In addition to the affinity towards the PCP binding site the affinity of selected compounds towards the ifenprodil binding site of NR2B containing NMDA receptors was investigated. For this purpose the competitive receptor binding assay recently developed in our group was used. Briefly, in this assay tritium labeled [^{3}H]ifenprodil was employed as radioligand. Membrane homogenates prepared from L(tk-) cells stably expressing recombinant human NR1a/NR2B receptors served as receptor material. The high density of NMDA receptors renders this system selective.[29]

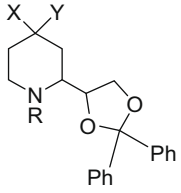
Since some potent NMDA receptor antagonists also interact with σ receptors and vice versa,[30,31] the σ_1 and σ_2 receptor affinity of the piperidines was also determined. In the σ assays the radioligands [^{3}H]-(+)-pentazocine (σ_1) and [^{3}H]-di-*o*-tolylguanidine (σ_2) and membrane preparations from guinea pig brains (σ_1) and rat livers (σ_2) were used. In the σ_2 assay, the σ_1 binding sites were selectively masked with an excess of non-tritiated (+)-pentazocine.[32–34]

For compounds with high NMDA affinity the competition curves with six compound concentrations were recorded three



Scheme 4. Only one enantiomer is shown, all compounds are racemic. Reagents and conditions: (a) CH_3SO_2Cl, NEt_3, CH_2Cl_2, 2 h, 0 °C, 79%; (b) KF, [18]crown-6, DMF, 1 h, 160 °C, 78%.

Table 1
Affinities of the dexoxadrol analogues towards NMDA (PCP), σ_1 and σ_2 receptors



Compound	4-CXY	Stereochemistry[b]	K_i ± SEM[a] (nM)		
			NMDA (PCP) ([^{3}H]-(+)-MK-801)	σ_1 ([^{3}H]-(+)-pentazocine)	σ_2 ([^{3}H]-di-*o*-tolylguanidine)
4	C=O	2*S*-4′*S*	470 ± 173	0%[c]	36%[c]
5 (WMS-2508)	CHOH	2*S*,4*S*-4′*S*	44 ± 13	971 (n = 1)	13%[c]
8a	CF_2	2*S*-4′*R*	58%[c]	0%[c]	137 (n = 1)
8c (WMS-2513)	CF_2	2*S*-4′*S*	81 ± 17	810 (n = 1)	17%[c]
12b	CHF	2*S*,4*S*-4′*R*	0%[c]	5%[c]	19%[c]
12d (WMS-2517)	CHF	2*S*,4*S*-4′*S*	27 ± 3.1	5%[c]	19%[c]
13a (β-dioxadrol)	CH_2	2*S*-4′*R*	38%[c]	35 ± 13	25%[c]
13c (α-dioxadrol)	CH_2	2*S*-4′*S*	20 ± 4.8 (n = 6)	0%[c]	0%[c]
Dexoxadrol			11 ± 0.3	—	—
(+)-MK-801			2.8 ± 1.8	—	—
(+)-Pentazocine			—	4.2 ± 1.1	—
Di-*o*-tolylguanidine			—	89 ± 29	58 ± 18

[a] The K_i-values were determined in three independent experiments (n = 3) unless otherwise noted.
[b] All compounds were tested as racemic mixtures. In the column stereochemistry the relative configuration of the three centers of chirality is given.
[c] Percent inhibition at a concentration of 1 μM of the test compound.

times and the resulting K_i-values with SEM are given. For compounds showing only low NMDA receptor interaction in the first screening experiment only the inhibition (%) at a compound concentration of 1 μM is given in Table 1. Some compounds indicated promising σ_1 and/or σ_2 affinities in the first screening. However, recording of the complete competition curves revealed only low affinity, and therefore the experiments were performed only once (n = 1).

3.2. NMDA receptor affinity

The receptor binding data are summarized in Table 1. The data clearly show that the NMDA receptor affinity is strongly dependent on the stereochemistry. Whereas the difluoride **8c**, the axial monofluoride **12d**, and α-dioxadrol (**13c**) having the same relative configuration as dexoxadrol, interact with high affinity with the PCP binding site of the NMDA receptor, the corresponding diastereomers **8a**, **12b** and **13a** (β-dioxadrol) reveal only very low NMDA receptor affinity.

Bioisosteric replacement of the carbonyl moiety of **4** (K_i = 470 nM) by a difluoromethyl group (**8c**, K_i = 81 nM) led to a sixfold increase of NMDA receptor affinity. This example represents one of the rare bioisosteric C=O/CF_2 replacements resulting in an improved receptor affinity.[35] The racemic alcohol **5** with (*S*,*S*,*S*)- or (*R*,*R*,*R*)-configuration of the centers of chirality has a 10-fold higher NMDA affinity than the ketone **4**. Also in the case of alcohol **5** (K_i = 44 nM) the bioisosteric replacement of the OH-moiety by a fluorine atom (**12d**, K_i = 27 nM) led to an increase of NMDA receptor affinity. The higher NMDA affinity of the monofluoro derivative **12d** compared with the affinity of the difluoro derivative **8c** indicates that the axially oriented fluorine in position 4 is more important than the equatorial one.

The diastereomeric racemic dioxadrol derivatives **13a** (β-dioxadrol) and **13c** (α-dioxadrol) were also included into this study. The more potent diastereomer α-dioxadrol (**13c**) showed a K_i-value of 20 nM, which is only slightly lower than the K_i-value of the monofluoride **12d**. Obviously the axially oriented proton in position 4 of α-dioxadrol (**13c**) can be replaced bioisosterically by a fluorine atom without loss of affinity.

Altogether, it was shown that the NMDA receptor protein tolerates a small electronegative substituent (OH, F) axially attached at position 4 of the piperidine ring, which increases the electron density at that position and is able to accept a proton in an H-bond bridge.

3.3. Receptor selectivity

Only negligible affinity towards the ifenprodil binding site of the NMDA receptor was detected for the test compounds. Obviously, at least the most potent ligands **8c** and **12d** reveal high

selectivity for the PCP binding site over the ifenprodil binding site of the NMDA receptor.

The monofluoro derivatives **12b** and **12d** did not interact considerably with σ_1 and σ_2 receptors indicating high selectivity against these receptor types. Whereas a weak σ_1 affinity was found for the *like*-configured difluoro derivative **8c** (K_i = 810 nM) a moderate σ_2 affinity was detected for the *unlike*-configured difluoro derivatives **8a** (K_i = 137 nM). Very interestingly β-dioxadrol (**13a**), which does not interact with the NMDA receptor, displays high σ_1 receptor affinity of 35 nM. Obviously the configuration of the two centers of chirality at the junction of the two heterocycles determines the receptor affinity and selectivity of these dioxadrol derivatives.

4. Conclusion

Herein compounds with one (**12**) and two (**8**) fluorine atoms in position 4 were synthesized and pharmacologically evaluated. It was shown that the chiral centers of the ring junction (2-position of the piperidine ring, 4-position of the 1,3-dioxolane ring) should have the same configuration to attain high NMDA receptor affinity. Electronegative substituents like a hydroxy group (**5**) or a fluorine atom (**12d**) in position 4 of the piperidine moiety are well tolerated, when they are axially oriented. A second fluorine atom in position 4 (**8c**) leads to a slightly reduced NMDA receptor affinity.

5. Experimental

5.1. Chemistry general

Unless otherwise noted, moisture and oxygen sensitive reactions were conducted in dry glassware (Schlenk flask sealed with a rubber septum) under N_2 (dried with phosphorous pentoxide (Granusic® A, Baker)). 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 (cm), length of the stationary phase (cm), eluent, fraction size (mL) and retention factor R_f. IR: IR spectrophotometer 480Plus FT-ATR-IR (Jasco). ^{1}H NMR (400 MHz, 300 MHz, 600 MHz), ^{13}C NMR (100 MHz) and ^{19}F NMR (300 MHz, 600 MHz) spectra were recorded on a Unity Mercury Plus 400 (400 MHz) NMR spectrometer (Varian), Brucker AV 300 (300 MHz), and Varian Unity Plus 600 (600 MHz) operating at 23 °C. High temperature NMR was recorded in Unity Mercury Plus 400 (400 MHz) NMR spectrometer (Varian) at 100 °C. Chemical shifts δ are reported in parts per million (ppm) against the reference compound tetramethylsilane and calculated using the chemical shift of the signal of the residual non-deuterated solvent, for ^{19}F NMR chemical shifts δ are reported in parts per million (ppm) against the reference compound $CFCl_3$ and calculated using the chemical shift of the signal of the solvent. High temperature ^{1}H NMR spectra were recorded using $C_6D_5NO_2$ as solvent. MS: HRMS (ESI): Finnigan MAT 4200s, Brucker Daltonics Micro Tof and Waters Micromass Quatro LCZ, peaks are given in *m/z* (% of basis peak). EI, electron impact, MAT GCQ (Thermo-Finnigan). HPLC: Merck Hitachi Equipment; UV detector: L-7400; autosampler:L-7200; pump: L-7100; degasser: L-7614; column: LiChrospher® 60 RP-select B (5 μm); LiChroCART® 250–4 mm cartridge; flow rate: 1.000 mL/min; injection volume: 5.0 μL; detection at λ = 210 nm; Method A: 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: 0% to 90%, 31.5–40 min: 90%; Method B: solvents: A: water with 0.05% (v/v) trifluoroacetic acid; B: methanol with 0.05% (v/v) trifluoroacetic acid: gradient elution: (A%): 0–1 min: 80%, 1–22 min: gradient from 80% to 0%, 22–30 min: 0%, 30–31.5 min: 0% to 80%, 31.5–40 min: 80%. The purity of all test compounds was greater than 95%, which was determined by one of the given HPLC methods.

5.2. General procedures

5.2.1. General procedure A: reaction of ketones 6 with DAST

Under N_2 the ketone **6** was dissolved in CH_2Cl_2 (2 mL). Then, diethylaminosulfur trifluoride (DAST, 2 equiv) was added and the mixture was stirred at rt overnight. Then, water and CH_2Cl_2 were added, the organic layer was separated and the aqueous layer was extracted twice with CH_2Cl_2. The combined organic layers were dried (Na_2SO_4), filtered and concentrated in vacuo. The residue was purified by fc (2 cm, 12 cm, petroleum ether/EtOAc = 9:1, 10 mL).

5.2.2. General procedure B: reaction of alcohols 9 with DAST

Under N_2 the alcohol **9** was dissolved in CH_2Cl_2 (2 mL), cooled down to −78 °C and diethylaminosulfur trifluoride (DAST, 1 equiv) was added and the mixture was stirred at −78 °C for 3 h. Then, water and CH_2Cl_2 were added and the mixture was warmed to rt. The organic layer was separated and the aqueous layer was extracted twice with CH_2Cl_2. The organic layer was dried (Na_2SO_4), filtered and concentrated in vacuo. The residue was purified by fc (2 cm, 12 cm, petroleum ether/EtOAc = 9:1, 10 mL).

5.2.3. General procedure C: removal of the Cbz protective group

The Cbz-protected piperidine was dissolved in MeOH (6 mL), Pd/C (10%) was added and the suspension was stirred under H_2 atmosphere (balloon) at rt for 4 h. The reaction mixture was filtered through Celite®, which was rinsed with MeOH and the filtrate was evaporated to dryness. The product was purified by fc (2 cm, 12 cm, EtOAc/MeOH/$NEtMe_2$ = 94:5:1, 10 mL).

5.3. (±)-Benzyl (2*RS*)-2-[(4*SR*)-2,2-diphenyl-1,3-dioxolan-4-yl]-4,4-difluoropiperidine-1-carboxylate (7a)

According to General Procedure A ketone **6a**[19] (81.6 mg, 0.18 mmol) was dissolved in CH_2Cl_2 (2 mL), DAST (0.042 mL, 0.36 mmol) was added and the reaction mixture was stirred overnight at rt. The product was purified by fc. Compound **7a** (R_f = 0.30): Colorless oil, yield 33.2 mg (39%). IR (neat): ν (cm^{-1}) = 1698 (s, Cbz C=O), 1205 (s, C–O–C). ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.73–2.05 (m, 4H, 3-H + 5-H), 3.17 (td, *J* = 13.6/2.3 Hz, 1H, 6-H), 3.66–3.70 (m, 1H, 2-H), 4.03–4.06 (m, 1H, 6-H), 4.27 (s broad, 1H, OCH_2), 4.40–4.46 (m, 1H, OCH), 4.55 (m broad, 1H, OCH_2) 4.83 (s broad, 1H, OCH_2Ph), 5.12 (d, *J* = 12.3 Hz, 1H, OCH_2Ph), 7.15–7.27 (m, 10H, Ph), 7.39–7.41 (m, 5H, Ph). HRMS: calcd for $C_{28}H_{27}F_2NO_4Na$ 502.1806, found 502.1800. HPLC (Method A): Purity 99%, t_R = 23.9 min.

5.4. (±)-Benzyl (2*RS*)-2-[(4*RS*)-2,2-diphenyl-1,3-dioxolan-4-yl]-4,4-difluoropiperidine-1-carboxylate (7c)

According to General Procedure A ketone **6c**[19] (130 mg, 0.28 mmol) was dissolved in CH_2Cl_2 (2 mL), DAST (0.070 mL, 0.56 mmol) was added and the reaction mixture was stirred overnight at rt. The product was purified by fc. Compound **7c** (R_f = 0.30): Colorless oil, yield 91.2 mg (70%). IR (neat): ν (cm^{-1}) = 1698 (s, Cbz C=O), 1203 (s, C–O–C). ^{1}H NMR ($C_6D_5NO_2$, 100 °C): δ (ppm) = 1.43–1.64 (m, 4H, 3-H + 5-H), 2.22–2.30 (m, 1H, 6-H), 2.68–2.76 (m, 1H, 6-H), 3.59–3.63 (m, 1H, 2-H), 3.69 (dd, *J* = 8.0/4.4 Hz, 1H, OCH_2), 3.87 (ddd, *J* = 8.0/5.1/2.4 Hz 1H, OCH), 4.16 (s broad, 1H, OCH_2) 4.72–4.80 (m, 2H, OCH_2Ph), 6.82–6.92 (m, 9H, Ph), 7.14–7.16 (m, 6H, Ph). HRMS: calcd for $C_{28}H_{27}F_2NO_4Na$

502.1806, found 502.1800. HPLC (Method A): Purity 99%, t_R = 24.6 min.

5.5. (±)-(2*RS*)-2-[(4*SR*)-2,2-Diphenyl-1,3-dioxolan-4-yl]-4,4-difluoropiperidine (8a)

According to General Procedure C **7a** (28.1 mg, 0.06 mmol) was dissolved in methanol (5 mL) and Pd/C (10%, 3.0 mg) was added. The product was purified by fc. Compound **8a** (R_f = 0.34): Colorless oil, yield 15.3 mg (75%). IR (neat): ν (cm^{-1}) = 1203 (s, C–O–C). ^1H NMR ($CDCl_3$, 500 MHz): δ (ppm) = 1.62 (dtd, J = 33.4/12.4/4.1 Hz, 1H, 3-H_{ax}), 1.73–1.85 (m, 1H, 5-H), 1.86–1.94 (m, 2H, 3-H + NH), 2.00–2.07 (m, 1H, 5-H), 2.79 (td, J = 12.7/2.8 Hz, 1H, 6-H), 2.84–2.89 (m, 1H, 2-H), 3.14 (ddt, J = 11.9/5.2/2.6 Hz, 1H, 6-H), 3.94–3.97 (m, 1H, OCH_2), 4.01–4.07 (m, 2H, OCH_2 + OCH), 7.27–7.36 (m, 6H, Ph), 7.45–7.54 (m, 4H, Ph). ^{13}C NMR ($CDCl_3$, 150 MHz): δ (ppm) = 34.9 (dd, J = 23.8/20.4 Hz, C-5), 37.1 (dd, J = 24.3/21.3 Hz, C-3), 42.1 (d, J = 10.2 Hz, C-6), 56.2 (d, J = 9.5 Hz, C-2), 66.7 (OCH_2), 78.9 (OCH), 110.1 (OCO), 121.9 (dd, J = 266.3/239.3 Hz, C-4), 125.8 (Ph-C), 126.0 (Ph-C), 126.2 (Ph-C), 128.1 (Ph-C), 128.2 (Ph-C), 128.3 (Ph-C), 141.7 (Ph-C), 141.8 (Ph-C). ^{19}F NMR ($CDCl_3$, 600 MHz): δ (ppm) = −100.9 (dtt, J = 235.6/34.3/11.7 Hz, 1F, 4-F), −87.9 (d, J = 235.5 Hz, 1F, 4-F). HRMS: calcd for $C_{20}H_{21}F_2NO_2H$ 346.1619, found 346.1613. HPLC (Method A): Purity 95%, t_R = 18.73 min.

5.6. (±)-(2*RS*)-2-[(4*RS*)-2,2-Diphenyl-1,3-dioxolan-4-yl]-4,4-difluoropiperidine (8c, WMS-2513)

According to General Procedure C **7c** (73.6 mg, 0.15 mmol) was dissolved in methanol (5 mL) and Pd/C (10%, 7.0 mg) was added. The product was purified by fc. Compound **8c** (R_f = 0.30): Colorless oil, yield 44.3 mg (85%). IR (neat): ν (cm^{-1}) = 1205 (s, C–O–C). ^1H NMR ($CDCl_3$, 500 MHz): δ (ppm) = 1.50 (dtd, J = 33.8/12.6/3.8 Hz, 1H, 3-H_{ax}), 1.69–1.83 (m, 2 H, 5-H + NH), 1.98–2.05 (m, 1H, 5-H), 2.12–2.19 (m, 1H, 3-H), 2.78 (td, J = 12.7/2.9 Hz, 1H, 6-H), 3.02–3.05 (m, 1H, 2-H), 3.14 (ddt, J = 12.6/5.3/2.2 Hz, 1H, 6-H), 3.93–3.98 (m, 1H, OCH_2), 4.08–4.13 (m, 2H, OCH_2 + OCH), 7.25–7.35 (m, 6H, Ph), 7.45–7.54 (m, 4H, Ph). ^{13}C NMR ($CDCl_3$, 150 MHz): δ (ppm) = 34.8 (dd, J = 24.0/20.5 Hz, C-5), 36.6 (dd, J = 24.2/21.4 Hz, C-3), 42.3 (d, J = 10.1 Hz, C-6), 55.2 (d, J = 9.6 Hz, C-2), 65.9 (OCH_2), 78.3 (OCH), 109.9 (OCO), 122.3 (dd, J = 244.5/239.2 Hz, C-4), 125.9 (Ph-C), 126.1 (Ph-C), 128.1 (Ph-C), 128.2 (Ph-C), 128.3 (Ph-C), 141.6 (Ph-C), 141.8 (Ph-C). ^{19}F NMR ($CDCl_3$, 600 MHz): δ (ppm) = −101.5 (dtt, J = 236.2/34.2/11.5 Hz, 1F, 4-F), −87.7 (d, J = 236.3 Hz, 1F, 4-F). HRMS: calcd for $C_{20}H_{21}F_2NO_2H$ 346.1619, found 346.1613. HPLC (Method A): Purity 97%, t_R = 18.07 min.

5.7. (±)-(2*RS*,4*RS*)-2-[(4*SR*)-2,2-Diphenyl-1,3-dioxolan-4-yl]-4-fluoropiperidine (12b) and (±)-(2*RS*)-2-[(4*SR*)-2,2-diphenyl-1,3-dioxolan-4-yl]piperidine (13a, β-dioxadrol)

According to General Procedure B **9a**[19] (41.9 mg, 0.13 mmol) was reacted with DAST (0.016 ml, 0.13 mmol). The product was purified by fc, R_f = 0.78 (petroleum ether/ethyl acetate, 9:1), colorless oil, yield 33.1 mg. The colorless oil was dissolved in methanol (5 mL) and Pd/C (10%, 3.0 mg) was added according to General Procedure C. The product was purified by fc (petroleum ether/EtOAc/$NEtMe_2$, 49:49:2).

Compound **12b** (R_f = 0.65): Colorless oil, yield 6.2 mg (15%, over two steps). IR (neat): ν (cm^{-1}) = 1068 (s, C–O–C). ^1H NMR ($CDCl_3$): δ (ppm) = 1.38 (dddd, J = 43.6/13.4/11.7/2.1 Hz, 1H, 3-H_{ax}), 1.53–1.64 (m, 1H, 5-H), 1.67–1.80 (m, 2H, 3-H + NH), 1.85–1.96 (m, 2H, 5-H + 6-H), 2.92 (ddd, J = 11.6/5.3/2.1 Hz, 1H, 2-H), 2.95–3.03 (m, 1H, 6-H), 3.86 (t, J = 6.1 Hz, 1H, OCH_2), 3.91–4.02 (m, 2H, OCH_2 + OCH), 4.92 (dquint, J = 47.9/2.7 Hz, 1H, 4-H), 7.27–7.34 (m, 6H, Ph), 7.42–7.51 (m, 4H, Ph). ^{13}C NMR ($CDCl_3$): δ (ppm) = 30.8 (d, J = 19.1 Hz, C-3), 33.3 (d, J = 19.1 Hz, C-5), 40.6 (C-6), 53.5 (C-2), 66.6 (OCH_2), 79.9 (OCH), 87.0 (d, J = 143.0 Hz, C-4), 109.7 (OCO), 126.2 (Ph-C).126.3 (Ph-C), 128.0 (Ph-C), 128.1 (Ph-C), 128.2 (Ph-C), 141.9 (Ph-C), 142.1 (Ph-C). ^{19}F NMR ($CDCl_3$): δ (ppm) = −184.11 (ddt, J = 92.0/44.2/11.5 Hz, 4-F). HRMS: calcd for $C_{20}H_{22}FNO_2H$ 328.1713, found 328.1707. HPLC (Method B): Purity 97%, t_R = 15.7 min.

Compound **13a** (R_f = 0.54): Colorless oil, yield 18.2 mg (64%, over two steps). IR (neat): ν (cm^{-1}) = 1068 (s, C–O–C). ^1H NMR ($CDCl_3$): δ (ppm) = 1.06 (qd, J = 12.3/3.8 Hz, 1H, 3-H_{ax}), 1.22 (qt, J = 12.3/3.7 Hz, 1H, 5-H_{ax}), 1.31–1.43 (m, 2H, 4-H), 1.52 (d broad, J = 12.9 Hz, 1H, 5-H), 1.70 (d broad, J = 12.5 Hz, 1H, 3-H), 1.92 (s, 1H, NH), 2.49–2.56 (m, 2H, 6-H + 2-H), 3.01(d broad, J = 11.5 Hz, 1H, 6-H), 3.76 (t, J = 6.9 Hz, 1H, OCH_2), 3.89 (dt, J = 8.2/6.6 Hz, 1H, OCH), 3.96 (t, J = 7.0 Hz, 1H, OCH_2), 7.19–7.27 (m, 6H, Ph), 7.38–7.45 (m, 4H, Ph). ^{13}C NMR ($CDCl_3$): δ = 24.3 (C-4), 26.1 (C-5), 28.5 (C-3), 46.6 (C-6), 59.9 (C-2), 67.5 (OCH_2), 80.6 (OCH), 109.9 (OCO), 126.4 (Ph-C).126.5 (Ph-C), 128.2 (Ph-C), 128.3 (Ph-C), 128.4 (Ph-C), 142.2 (Ph-C), 142.5 (PhC). HRMS: calcd for $C_{20}H_{23}NO_2H$ 310.1807, found 310.1804. HPLC (Method B): Purity 95%, t_R = 15.23 min.

5.8. (±)-Benzyl (2*RS*,4*RS*)-2-[(4*RS*)-2,2-diphenyl-1,3-dioxolan-4-yl]-4-fluoropiperidine-1-carboxylate (10d) and (±)-(*RS*)-benzyl 2-[(4*RS*)-2,2-diphenyl-1,3-dioxolan-4-yl]-1,2,3,6-tetrahydropyridine-1-carboxylate (11c) and (±)-(*RS*)-benzyl 2-[(4*RS*)-2,2-diphenyl-1,3-dioxolan-4-yl]-1,2,5,6-tetrahydropyridine-1-carboxylate (11d)

According to General Procedure B **9c**[19] (78.9 mg, 0.17 mmol) was reacted with DAST (0.020 ml, 0.17 mmol). The product was purified by fc.

Compound **10d** (R_f = 0.32): Colorless oil, yield 23.2 mg (29%). IR (neat): ν (cm^{-1}) = 1698 (s, Cbz C=O), 1205 (s, C–O–C). ^1H NMR ($C_6D_5NO_2$, 100 °C): δ (ppm) = 1.13–1.35 (m, 2H, 3-H + 5-H), 1.58 (s broad, 1H, 3-H), 1.60–1.65 (m, 1H, 5-H), 2.07–2.14 (m, 1H, 6-H), 2.68–2.75 (m, 1H, 6-H), 3.60–3.63(m, 1H, 2-H), 3.84–3.87 (m,1H, OCH_2), 3.99–4.05 (m, 1H, OCH), 4.17 (broad s, 1H, OCH_2), 4.62 (dtt, J = 49.0/10.0/4.9 Hz, 1H, 4-H), 4.71–4.82 (m, 2H, OCH_2Ph), 6.81–7.64 (m, 15H, Ph). HRMS: calcd for $C_{20}H_{28}FNO_4Na$ 484.1900, found 484.1889. HPLC (Method B): Purity 95%, t_R = 21.69 min.

Compounds **11c** and **11d** (R_f = 0.43): Colorless oil, yield 39.5 mg (52%). IR (neat): ν (cm^{-1}) = 1698 (s, Cbz C=O), 1205 (s, C–O–C). ^1H NMR ($C_6D_5NO_2$, 100 °C): δ (ppm) = 1.07 (d broad, J = 15.7 Hz, 0.7H, 3°-H), 1.28–1.37 (m, 0.7H, 3°-H), 1.49 (ddd, J = 17.4/5.9/2.9 Hz, 0.3H, 5^x-H), 1.76 (d broad, J = 17.6 Hz, 0.3 H, 5^x-H), 2.23–2.30 (m, 0.7H, 6°-H), 2.75 (d broad, J = 18.0 Hz, 0.3H, 6^x-H), 3.16–3.25 (m, 1H, 6°-H + 6^x-H), 3.38–3.42 (m, 1H, 2°-H + 2^x-H), 3.52 (ddd, J = 8.7/7.2/2.9 Hz, 0.7H, OCH_2°), 3.60 (ddd, J = 12.5/6.3/3.3 Hz, 0.3H, OCH_2^x), 3.67 (m broad, 0.7H, OCH°), 3.73–3.76 (m, 0.3H, OCH^x), 3.81 (m broad, 1H, $OCH_2^\circ + OCH_2^x$), 4.33–4.41 (m, 2H, OCH_2Ph), 4.81 (d broad, J = 10.8 Hz, 0.3H, 3^xH), 4.91–4.95 (m, 0.3H, 4^xH), 5.03–5.12 (m, 1.4H, 4°H + 5°H), 6.42–7.24 (m, 15H, Ph). The ratio of **11c**:**11d** = 7:3; ° = index for the major regiomer **11c**, ^{x} = index for the minor regiomer **11d**. HRMS: calcd for $C_{28}H_{27}NO_4Na$ 464.1832, found 464.1836. HPLC (Method B): Purity 96%, t_R = 22.13 min.

5.9. (±)-(2*RS*,4*RS*)-2-[(4*RS*)-2,2-Diphenyl-1,3-dioxolan-4-yl]-4-fluoropiperidine (12d, WMS-2517)

According to General Procedure C **10d** (44.2 mg, 0.09 mmol) was dissolved in methanol (5 mL) and Pd/C (10%, 4.0 mg) was added. The product was purified by fc. Compound **12d** (R_f = 0.67): Colorless oil, yield 17.6 mg (81%). IR (neat): ν (cm^{-1}) = 1067 (s, C–O–C). ^1H NMR ($CDCl_3$): δ (ppm) = 1.36 (dddd, J = 43.9/13.6/12.1/2.2 Hz, 1H, 3-

H_{ax}), 1.53–1.73 (m, 2H, 5-H + NH), 1.87–1.95 (m, 1H, 3-H), 1.96–2.05 (m, 1H, 5-H), 2.90 (ddd, J = 12.2/5.3/1.9 Hz, 1H, 2-H), 2.99 (td, J = 12.4/2.8 Hz, 1H, 6-H), 3.24 (dt, J = 12.2/3.3 Hz, 1H, 6-H), 3.94 (t, J = 6.5 Hz, 1H, OCH_2), 4.04–4.12 (m, 2H, OCH_2 + OCH), 4.97 (dquint, J = 47.9/2.7 Hz, 1H, 4-H), 7.27–7.36 (m, 5H, Ph), 7.47–7.54 (m, 5H, Ph). ^{13}C NMR ($CDCl_3$): δ (ppm) = 31.2 (d, J = 20.4 Hz, C-3), 33.0 (d, J = 20.4 Hz, C-5), 40.6 (C-6), 52.0 (C-2), 65.7 (OCH_2), 79.2 (OCH), 87.4 (d, J = 168.3 Hz, C-4), 109.7 (OCO), 126.0 (Ph-C), 126.2 (Ph-C), 128.0 (Ph-C), 128.1 (Ph-C), 128.2 (Ph-C), 128.3 (Ph-C), 141.8 (Ph-C), 142.0 (Ph-C). ^{19}F NMR (300 MHz, $CDCl_3$): δ (ppm) = −184.80 (dtt, J = 47.8/44.2/11.3 Hz, 4-F). HRMS: calcd for $C_{20}H_{22}FNO_2H$ 328.1713, found 328.1707. HPLC (Method A): Purity 96%, t_R = 17.03 min.

5.10. (±)-(2*RS*)-2-[(4*RS*)-2,2-Diphenyl-1,3-dioxolan-4-yl]piperidine (13c, α-dioxadrol)

According to General Procedure C the mixture of **11c** and **11d** (39.5 mg, 0.09 mmol) was dissolved in methanol (5 mL) and Pd/C (10%, 4.0 mg) was added. The product was purified by fc. Compound **13c** (R_f = 0.46): Colorless oil, yield 25.1 mg (91%). IR (neat): ν (cm^{-1}) = 1066 (s, C–O–C). ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.01 (qd, J = 12.3/3.7 Hz, 1H, 3-H_{ax}), 1.18–1.26 (m, 1H, 4-H), 1.33 (qt, J = 12.3/3.7 Hz, 1H, 5-H_{ax}), 1.50 (d broad, J = 12.8 Hz, 1H, 5-H), 1.61 (d broad, J = 13.6 Hz, 1H, 3-H), 1.72 (d broad, J = 14.2 Hz, 1H, 4-H), 1.90 (s, 1H, NH), 2.51 (td, J = 11.7/2.8 Hz, 1H, 6-H), 2.75 (ddd, J = 11.3/4.3/2.5 Hz, 1H, 2-H), 2.97(d broad, J = 11.9 Hz, 1H, 6-H), 3.86 (t, J = 7.2 Hz, 1H, OCH_2), 3.96 (td, J = 6.6/4.5 Hz, 1H, OCH), 4.06 (t, J = 6.9 Hz, 1H, OCH_2), 7.17–7.27 (m, 5H, Ph), 7.39–7.46 (m, 5H, Ph). ^{13}C NMR ($CDCl_3$): δ = 24.7 (C-4), 26.6 (C-5), 28.8 (C-3), 46.9 (C-6), 58.2 (C-2), 66.2 (OCH_2), 79.9 (OCH), 109.7 (OCO), 126.4 (Ph-C).126.5 (Ph-C), 128.2 (Ph-C), 128.3 (Ph-C), 128.4 (Ph-C), 128.5 (Ph-C), 142.2 (Ph-C), 142.5 (Ph-C). HRMS: calcd for $C_{20}H_{23}NO_2H$ 310.1807, found 310.1803. HPLC (Method A): Purity 95%, t_R = 17.17 min.

5.11. (±)-Benzyl (2*RS*,4*RS*)-2-[(4*RS*)-2,2-diphenyl-1,3-dioxolan-4-yl]-4-methylsulfonyloxy piperidine-1-carboxylate (14d)

Under N_2 **9d** (65.5 mg, 0.14 mmol) was dissolved in CH_2Cl_2 (1 mL), the solution was cooled down to 0 °C and NEt_3 (0.03 mL, 0.21 mmol) was added followed by methanesulfonyl chloride (0.02 mL, 0.21 mmol). The reaction mixture was stirred for 2 h at 0 °C. Then a saturated solution of $NaHCO_3$ was added and the reaction mixture was warmed to rt. Then CH_2Cl_2 was added, the organic layer was separated, the aqueous layer was extracted twice with CH_2Cl_2, the organic layer was dried (Na_2SO_4), filtered and concentrated in vacuo. The residue was purified by fc (2 cm, 12 cm, petroleum ether/EtOAc = 7:3, 10 mL). Compound **14d** (R_f = 0.58): Colorless oil, yield 60 mg (79%). IR (neat): ν (cm^{-1}) = 1696 (s, Cbz C=O), 1205 (s, C–O–C), 1331(s)/1175 (s, S(=O)$_2$). ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.64–1.72 (m, 1H, 3-H), 1.74–1.79 (m, 1H, 5-H), 2.20 (d broad, J = 12.1 Hz, 1H, 3-H), 2.38 (d broad, J = 11.3 Hz, 1H, 5-H), 2.66 (s, 3H, SO_2CH_3), 3.11–3.28 (m, 1H, 6-H), 3.89 (s broad, 1H, 2-H), 3.99 (s broad, 1H, 6-H), 4.19–4.23 (m broad, 1H, OCH_2), 4.30 (q, J = 6.3 Hz, 1H, OCH), 4.34–4.51 (m, 1H, OCH_2), 5.06 (tt, J = 11.5/5.2 Hz, 1H, 4-H), 5.11 (s, 2H, OCH_2Ph), 7.24–7.36 (m, 10H, Ph), 7.49–7.51 (m, 5H, Ph). HRMS: calcd for $C_{29}H_{31}NO_7SNa$ 560.1713, found 560.1717. HPLC (Method B): Purity 92%, t_R = 20.3 min.

6. Receptor binding studies

6.1. Materials and general procedures

Pig brains were a donation of the local slaughterhouse (Coesfeld, Germany). The guinea pig brains and rat livers were commercially available (Harlan-Winkelmann, Borchen, Germany). Homogenizer: Elvehjem Potter (B. Braun Biotech International, Melsungen, Germany). Centrifuge: High-speed cooling centrifuge model Sorvall RC-5C plus (Thermo Fisher Scientific, Langenselbold, Germany). Filter: Printed Filtermat Typ A and B (Perkin Elmer LAS, Rodgau-Jügesheim, Germany), presoaked in 0.5% aqueous polyethylenimine for 2 h at room temperature before use. The filtration was carried out with a MicroBeta FilterMate-96 Harvester (Perkin Elmer). The scintillation analysis was performed using Meltilex (Typ A or B) solid scintillator (Perkin Elmer) and a MicroBeta Trilux scintillation analyzer (Perkin Elmer). The overall counting efficiency was 20%. All experiments were carried out in triplicates using standard 96-well-multiplates (Diagonal, Muenster, Germany). The IC_{50}-values were determined in competition experiments with at least six concentrations of the test compounds and were calculated with the program GRAPHPAD PRISM® 3.0 (GraphPad Software, San Diego, CA, USA) by non-linear regression analysis. The K_i-values were calculated according to the formula of Cheng and Prusoff.[36] The K_i-values are given as mean value ± SEM from three independent experiments.

6.2. Determination of the affinity to the phencyclidine binding site of the NMDA receptor, modified according to Ref. 28

6.2.1. Preparation of the tissue

Fresh pig brain cortex was 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 23,500 *g* for 20 min at 4 °C. The pellet was resuspended in 5–6 volumes of TRIS/EDTA-buffer (5 mM/1 mM, pH 7.5) and centrifuged again at 31,000 *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[37] using bovine serum albumin as standard, and subsequently the preparation was frozen (−80 °C) in 1.5 mL portions containing about 0.8 mg protein/mL.

6.2.2. Performance of the assay

The test was performed with the radioligand [^{3}H]-(+)-MK-801 (22.0 Ci/mmol; Perkin Elmer). The thawed membrane preparation (about 100 μg of the protein) was incubated with various concentrations of test compounds, 2 nM [^{3}H]-(+)-MK-801, and TRIS/EDTA-buffer (5 mM/1 mM, pH 7.5) in a total volume of 200 μL for 150 min at rt. The incubation was terminated by rapid filtration through the presoaked filtermats using a cell harvester. After washing each well five times with 300 μL of water, the filtermats were dried at 95 °C. Subsequently, the solid scintillator was 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 non-specific binding was determined with 10 μM unlabeled (+)-MK-801. The K_d-value of (+)-MK-801 is 2.26 nM.[38]

6.3. Determination of σ_1,σ_2 and NF2B affinity

Details on the σ_1 and σ_2 assay are given Refs. 32–34, details on the NR2B assay in Ref. 29.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.04.002.

References and notes

1. Brauner-Osborne, H.; Egebjerg, J.; Nielsen, E. O.; Madsen, U.; Krogsgaard-Larsen, P. *J. Med. Chem.* **2000**, *43*, 2609.
2. Maren, S.; Baudry, M. *Neurobiol. Learn. Mem.* **1995**, *63*, 1.
3. Rebola, N.; Srikumar, B. N.; Mulle, C. *J. Physiol.* **2010**, *588*, 93.
4. Lipton, S. A.; Nicotera, P. *Cell Calcium* **1998**, *23*, 165.
5. Danysz, W.; Parsons, C. G.; Bresink, I.; Quack, G. *Drug News Perspect.* **1995**, 261.
6. McBain, C. J.; Mayer, M. L. *Physiol. Rev.* **1994**, *74*, 723.
7. Lipton, S. A. *Nat. Rev. Drug Disc.* **2006**, *5*, 160.
8. Parsons, C. G.; Stoeffler, A.; Danysz, W. *Neuropharmacology* **2007**, *53*, 699.
9. Rammes, G.; Danysz, W.; Parsons, C. G. *Curr. Neuropharmacol.* **2008**, *6*, 55.
10. Gilling, K. E.; Jatzke, C.; Parsons, C. G. *Neuropharmacology* **2007**, *53*, 415.
11. Hardie, W. R.; Hidalgo, J.; Halverstadt, I. F.; Allen, R. E. *J. Med. Chem.* **1966**, *9*, 127.
12. Jacobson, A. E.; Harrison, E. A., Jr.; Mattson, M. V.; Rafferty, M. F.; Rice, K. C.; Woods, J. H.; Winger, G.; Solomon, R. E.; Lessor, R. A.; Silverton, J. V. *J. Pharmacol. Exp. Ther.* **1987**, *243*, 110.
13. Thurkauf, A.; Mattson, M. V.; Richardson, S.; Mirsadeghi, S.; Ornstein, P. L.; Harrison, E. A., Jr.; Rice, K. C.; Jacobson, A. E.; Monn, J. A. *J. Med. Chem.* **1992**, *35*, 1323.
14. Sax, M.; Fröhlich, R.; Schepmann, D.; Wünsch, B. *Eur. J. Org. Chem.* **2008**, 6015.
15. Aepkers, M.; Wünsch, B. *Arch. Pharm. Pharm. Med. Chem.* **2004**, *337*, 67.
16. Sax, M.; Wünsch, B. *Curr. Top. Med. Chem.* **2006**, *6*, 723.
17. Aepkers, M.; Wünsch, B. *Bioorg. Med. Chem.* **2005**, *13*, 6836.
18. Sax, M.; Ebert, K.; Schepmann, D.; Wibbeling, B.; Wünsch, B. *Bioorg. Med. Chem.* **2006**, *14*, 5955.
19. Banerjee, A.; Fröhlich, R.; Schepmann, D.; Wünsch, B. *Med. Chem. Comm.* **2010**, doi:10.1039/comd00017e.
20. Schlosser, M. *Tetrahedron* **1978**, *34*, 3.
21. Hagmann, W. K. *J. Med. Chem.* **2008**, *51*, 4359.
22. The small roman letters **a**–**d** are describing the relative configuration at the three centers of chirality: **a** refers to (2*RS*,4*SR*,4′*SR*)-configuration; **b** refers to (2*RS*,4*RS*,4′*SR*)-configuration; **c** refers to (2*RS*,4*SR*,4′*RS*)-configuration; **d** refers to (2*RS*,4*RS*,4′*RS*)-configuration.
23. Buonora, P.; Olsen, J. C.; Oh, T. *Tetrahedron* **2001**, *57*, 6099.
24. Sax, M.; Berning, S.; Wünsch, B. *Tetrahedron* **2005**, *61*, 205.
25. Middleton, W. J. *J. Org. Chem.* **1975**, *40*, 574.
26. Thurkauf, A.; Zenk, P. C.; Balster, R. L.; May, E. L.; George, C.; Carroll, F. I.; Mascarella, S. W.; Rice, K. C.; Jacobson, A. E.; Mattson, M. V. *J. Med. Chem.* **1988**, *31*, 2257.
27. McKernan, R. M.; Castro, S.; Poat, J. A.; Wong, E. H. F. *J. Neurochem.* **1989**, *52*, 777.
28. Wirt, U.; Schepmann, D.; Wünsch, B. *Eur. J. Org. Chem.* **2007**, 462.
29. Schepmann, D. T. B.; Frehland, B.; Wünsch, B. *J. Pharm. Biomed. Anal.*, in press.
30. Carroll, F. I.; Abraham, P.; Parham, K.; Bai, X.; Zhang, X.; Brine, G. A.; Mascarella, S. W.; Martin, B. R.; May, E. L.; Sauss, C.; Di Paolo, L.; Wallace, P.; Walker, J. M.; Bowen, W. D. *J. Med. Chem.* **1992**, *35*, 2812.
31. May, E. L.; Aceto, M. D.; Bowman, E. R.; Bentley, C.; Martin, B. R.; Harris, L. S.; Medzihradsky, F.; Mattson, M. V.; Jacobson, A. E. *J. Med. Chem.* **1994**, *37*, 3408.
32. Maier, C. A.; Wünsch, B. *J. Med. Chem.* **2002**, *45*, 438.
33. Maier, C. A.; Wünsch, B. *J. Med. Chem.* **2002**, *45*, 4923.
34. Holl, R.; Schepmann, D.; Fröhlich, R.; Grünert, R.; Bednarski, P. J.; Wünsch, B. *J. Med. Chem.* **2009**, *52*, 2126.
35. Barber, C. G.; Blakemore, D. C.; Chiva, J.-Y.; Eastwood, R. L.; Middleton, D. S.; Paradowski, K. A. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 1075.
36. Cheng, Y.-C.; Prusoff, W. H. *Biochem. Pharmacol.* **1973**, *22*, 3099.
37. Bradford, M. M. *Anal. Biochem.* **1976**, *72*, 248.
38. Ueda, Y.; Nakanishi, H.; Yoshida, K. *Life Sci.* **1999**, *65*, 1477.