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Do GluN2B subunit containing NMDA receptors tolerate a fluorine atom in the phenylalkyl side chain?†‡

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The influence of an F-atom in the side chain of benzo[7]annulen-7-amines on the affinity towards GluN2B subunit containing NMDA receptors and the selectivity over related receptors was investigated. The synthesis of **5a** and **5b** was performed by reductive amination of the ketone **6** with primary alkanamines **14a** and **14b** bearing an F-atom in β-position. The GluN2B affinities of non-fluorinated and fluorinated ligands **4** and **5** are almost identical. The low impact of the F-atom on GluN2B affinity was unexpected, as it influences several chemical and physicochemical properties of the ligands. However, introduction of the F-atom led to reduced selectivity over σ receptors. Whereas **5a** and **5b** display still a 2–3-fold preference for GluN2B over σ_1 receptors, they show almost the same affinity to GluN2B and σ_2 receptors.

1. Introduction

The amino acid (*S*)-glutamate is the most important excitatory neurotransmitter in the central nervous system. It interacts with G-protein coupled receptors (mGlu receptors), which are differentiated into 8 subtypes termed mGlu1-8, and ligand gated ion channels (iGlu receptors). Three subtypes of (*S*)-glutamate-gated ion channels are known, which are termed according to their prototypical ligands AMPA (2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionic acid), kainate (according to the natural product kainic acid) and NMDA (*N*-methyl-D-aspartate).[1,2]

The NMDA receptor is composed of four subunits forming the heterotetrameric ion channel. Cloning has resulted in seven genes encoding these subunits, which are classified into three types: GluN1 (with 8 splice variants GluN1a–h), GluN2 (with four types GluN2A–D) and GluN3 (with two types GluN3A and GluN3B).[3–5]

We are particularly interested in the modulation of the activity of NMDA receptors containing the GluN2B subunit. The GluN2B subunit is found only in some regions of the central nervous system (*e.g.* cortex, hippocampus, striatum), other regions (*e.g.* cerebellum) do not express the GluN2B subunit. NMDA receptor antagonists inhibiting only GluN2B subunit containing receptors should lead to a better side effect profile, since the NMDA receptors are addressed in only some regions of the central nervous system.[6] Moreover, the GluN2B subunit offers an additional binding site, the so-called ifenprodil binding site. Ligands interacting with this binding site in the periphery of the ion channel (N-terminal part of the receptor) lead to a mere modulation than complete inhibition of the ion channel, which also contributes to a more favorable side effect profile of such type of ligands.[7–13]

NMDA receptor antagonists blocking the ion channel by interaction with the ifenprodil binding site at the N-terminal domain of the GluN2B subunit could be used for the treatment of ischemia/stroke, neurodegenerative disorders (Parkinson's and Alzheimer's disease), neuropathic pain,[9] migraine, and alcohol withdrawal symptoms.[3,6,7]

The aminopropanol Ro 25-6981 (**1**) represents a promising GluN2B selective NMDA receptor antagonist, which is able to protect cultured cortical neurons against glutamate-induced toxicity[14] (Fig. 1). Very recently we have reported on novel benzo[7]annulen-7-amines[15–17] resulting from rearrangement of the substructures of Ro 25-6981. Both the alcohol **2** (*cis*-isomer) and the unsubstituted benzo[7]annulenamine **3** showed low K_i values (GluN2B binding) of 16 nM (*cis*-isomer) and 11 nM, respectively. Moreover, the alcohol **2** (*cis*-isomer) was active as GluN2B antagonist in the low nanomolar range (IC_{50} = 12 nM).[15] Removal of the 2-methoxy group of **3** led to the benzo[7]annulenamines **4** displaying unexpectedly high

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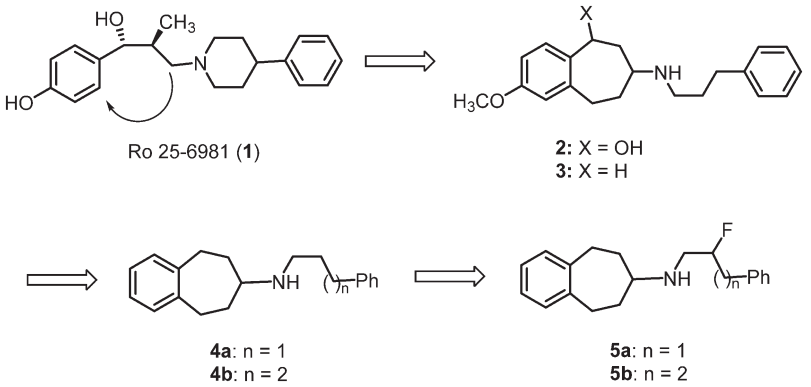


Fig. 1 Design of GluN2B ligands with a fluorine atom in the phenylalkyl side chain.

GluN2B affinity. **4a** and **4b** differing in the length of the phenylalkyl moiety bind with similar affinity towards GluN2B containing NMDA receptors (K_i = 16 and 17 nM).[16]

Starting from the phenylalkyl derivatives **4**, it should be investigated whether the introduction of a fluorine atom in the side chain (**5**) will be tolerated by GluN2B subunit containing NMDA receptors. The results of this study should be used for the development of a fluorinated PET tracer labeling selectively NMDA receptors with the GluN2B subunit. The fluorine atom with its high electronegativity will reduce the basicity of an amino moiety in β-position by approx. two log units. Thus the formation of ionic and H-bond interactions of the basic amino moiety will be modulated by the fluorine atom. On the other hand, the fluorine atom in the side chain modulates the lipophilicity and is able to form H-bonds with H-bond donating functional groups.[18,19] Moreover, a fluorine atom should influence the conformation of the alkyl side chain, as a gauche orientation of the fluorine atom and the basic amino moiety in β-position represents a preferred conformation.[20,21]

2. Synthesis

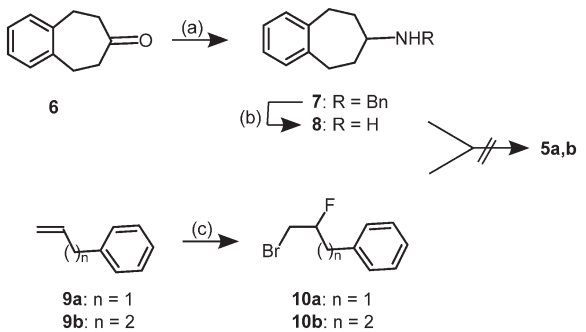
In the first approach the fluorinated benzo[7]annulen-7-amines **5** should be prepared by alkylation of amines **7** or **8** with fluorinated phenylalkyl bromides **10**. Reductive amination of ketone **6** with benzylamine and $NaBH(OAc)_3$ provided the benzylamine **7** (ref. 16) which was debenzylated with H_2 and Pd/C to give the primary amine **8** (Scheme 1).

According to a literature protocol,[22] allylbenzene **9a** was reacted with $NEt_3{\cdot}3$ HF and *N*-bromosuccinimide (NBS) to form the bromofluoropropane **10a**. (Scheme 1) As the regioselectivity of this addition was poor, an 80 : 20-mixture of **10a** and its regioisomer was isolated in 64% yield, which was used for alkylation of amines **7** and **8**. The same reagents ($NEt_3{\cdot}3$ HF, NBS) were employed to transform the homologous butenylbenzene **9b** into the bromofluorobutane **10b**. The reaction of **9b** took place with high regioselectivity resulting only in the desired regioisomer **10b**.

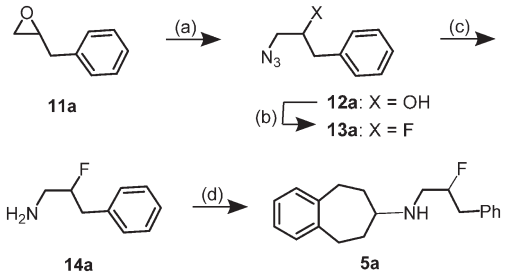
All attempts to react the bromofluoroalkanes **10a** and **10b** with the primary amine **8** or the secondary amine **7** failed to produce the fluoroalkylated amines **5a** and **5b**. Even heating of a solution of primary amine **8** and bromofluoroalkanes **10** in DMSO to reflux did not lead to any transformation. Therefore, a reductive amination of ketone **6** with fluorinated primary amines **14** was envisaged instead of alkylation of amine **8**.

For the synthesis of the 2-fluoro-3-phenylpropyl derivative **5a**, oxirane **11a**[23] was opened regioselectively with NaN_3 in aqueous CH_3OH to yield the azidoalcohol **12a**[24] (Scheme 2). Transformation of **12a** into fluoroazide **13a** was performed with DAST (diethylaminosulfur trifluoride) at −78 °C. Reduction of the azido moiety of **13a** with H_2 led to the fluorinated primary amine **14a**. In contrast to the alkylation of amine **8** with alkyl bromides **10a**, the reaction of the ketone **6** with the primary amine **14a** in the presence of $NaBH(OAc)_3$ (ref. 25) led to a clean transformation and produced the 2-fluoro-3-phenylpropyl derivative **5a** in 50% yield.

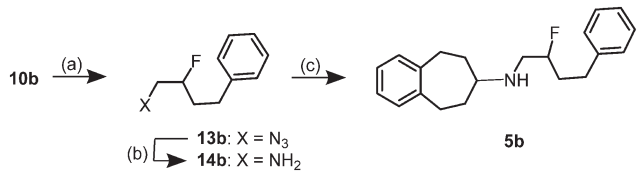
The bromofluorobutyl derivative **10b**, which was obtained with high regioselectivity (see Scheme 1), served as starting material for the synthesis of the fluorobutyl derivative **5b** (Scheme 3). Reaction of **10b** with NaN_3 afforded the fluoroazide **13b**,[26] which was reduced with H_2 and $Pd(OH)_2/C$ to give the primary amine **14b**. Reductive amination[25] of ketone **6** with primary amine **14b** provided the 2-fluorobutylamine **5b** in 34% yield.



Scheme 1 Alkylation with β-fluoroalkyl bromides **10**. Reagents and reaction conditions: (a) $BnNH_2$, $NaBH(OAc)_3$, CH_2Cl_2, rt, 16 h, 81%. (b) H_2 (balloon), Pd/C, CH_3OH, rt, 21 h, 82%. (c) $NEt_3{\cdot}3$ HF, NBS, CH_2Cl_2, rt, 3–23 h, 64% (**10a**), 63% (**10b**).



Scheme 2 Synthesis of (2-fluoro-3-phenylpropyl)amine **5a**. Reagents and reaction conditions: (a) NaN_3, CH_3OH, H_2O, reflux, 1 h, 88%. (b) DAST, CH_2Cl_2, −78 °C to rt, 3 h, 30%. (c) H_2 (balloon), Pd/C, CH_3OH, rt, 4 h, 86%. (d) ketone **6**, $NaBH(OAc)_3$, CH_2Cl_2, rt, 16 h, 50%.



Scheme 3 Synthesis of (2-fluoro-4-phenylbutyl)amine **5b**. Reagents and reaction conditions: (a) NaN_3, DMSO, 65 °C, 2 h, 99%. (b) H_2 (balloon), $Pd(OH)_2/C$, CH_3OH, HCl, rt, 7 h, 51%. (c) ketone **6**, $NaBH(OAc)_3$, CH_2Cl_2, rt, 24 h, 34%.

3. Receptor affinity

The affinity towards GluN2B subunit containing NMDA receptors was determined in receptor binding studies using the radioligand [^{3}H]ifenprodil.[27,28] The receptor material was obtained from L(tk−) cells stably transfected with a vector containing the genetic information of GluN1a and GluN2B subunits. The synthesis of NMDA receptor subunits was induced by addition of dexamethasone. Finally, membrane preparations were prepared and standardized.

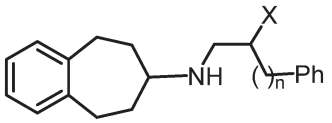
Table 1 clearly indicates that the introduction of an F-atom is well tolerated by the GluN2B receptor: the phenylpropylamines **4a** and **5a** without and with an F-atom in β-position to the amino moiety show almost the same GluN2B affinity. The GluN2B affinity of the homologous phenylbutylamine **5b** with an F-atom in the side chain is slightly reduced compared to its non-fluorinated analog **4b**. It can be concluded that the GluN2B affinity of this type of ligands is only slightly changed by introduction of an F-atom in the phenylalkyl side chain.

In addition to the interaction with the ifenprodil binding site of the NMDA receptor, the affinity of the fluorinated benzo[7]annulenamines **5** towards the PCP (phencyclidine) binding site within the channel pore of the NMDA receptor was also determined.[29,30] As it can be seen from Table 1, the amines **4** and **5** did not interact considerably with the PCP binding site at a concentration of 1 μM indicating high selectivity over the PCP binding site.

Since the prototypical lead compound ifenprodil[31,32] and the benzo[7]annulenamines **4** interact with σ_1 and σ_2 receptors as well (see Table 1), the affinity of the fluorinated compounds **5** towards both σ receptor subtypes was also recorded.[33–35] Compared to ifenprodil and the lead compounds **4** the σ_1 affinity of the fluorinated compounds **5** is increased. Both fluorinated compounds **5a** and **5b** show σ_1 receptor affinity, which is approx. 3-fold lower than their GluN2B affinity. Overall, the fluorinated compounds **5** show still a preference for the GluN2B receptor, which is however reduced compared to the non-fluorinated ligands **4**.

A similar observation was made in the σ_2 field. Both fluorinated ligands **5** reveal increased σ_2 affinity compared to ifenprodil and the non-fluorinated analogs **4**. Whereas compounds **4** show a 2–3-fold preference for GluN2B receptors over σ_2 receptors, the GluN2B and the σ_2 affinity of the fluorinated ligands **5** are almost identical. Obviously, the selectivity

Table 1 Receptor affinities of fluorinated benzo[7]annulenamines **5** compared with the affinities of lead compounds **4** without F atom and some reference compounds



			K_i ± SEM [nM] (n = 3)			
Compd.	X	n	GluN2B	PCP[a]	σ_1	σ_2
4a[16]	H	1	16 ± 4.0	0%	150	27 ± 12
4b[16]	H	2	17 ± 2.0	2%	216	55 ± 7.0
5a	F	1	17 ± 1.0	2%	54 ± 6.0	14 ± 5.0
5b	F	2	27 ± 10[b]	3%	82 ± 24[b]	19 ± 2.0[b]
Ifenprodil			10 ± 0.7	—	125 ± 24	98 ± 34
Eliprodil			13 ± 2.5	—	—	—
Dexoxadrol			—	32 ± 7.4	—	—
(+)-MK-801			—	3.4 ± 0.8	—	—
Haloperidol			—	—	6.3 ± 1.6	78.1 ± 2.3
Di-o-tolylguanidine			—	—	89 ± 29	57.5 ± 18

[a] Due to the very low affinity towards the PCP binding site of the NMDA receptor, only the inhibition of radioligand binding (%) at the high test compound concentration of 1 μM is given in the table. [b] The K_i values were determined four times (n = 4). —: not determined.

or preference of GluN2B ligands with a benzo[7]annulenamine scaffold over σ_2 receptors was lost upon introduction of a F-atom into the phenylalkyl side chain.

4. Conclusion

The effect of introduction of an F-atom into the phenylalkyl side chain of GluN2B ligands was investigated. Both fluorinated ligands **5** and non-fluorinated ligands **4** possess almost identical GluN2B affinity in the low nanomolar range and show high selectivity over the PCP binding site of the NMDA receptor. Introduction of an F-atom into the side chain resulted in reduced selectivity of **5a** and **5b** over both σ receptor subtypes. They still display a preference for GluN2B over σ_1 receptors, but do not have any selectivity over the σ_2 subtype. Although only two compounds have been synthesized and biologically evaluated, the study proves the principle of F-tolerance, which will be further exploited with additional examples.

5. Experimental

5.1. Chemistry, general

Unless otherwise noted, moisture sensitive reactions were conducted under dry nitrogen. Acetonitrile was dried over molecular sieves 3 Å. CH_2Cl_2 was distilled over CaH_2. 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 (*d*), fraction size (*v*), eluent, R_f value. Melting point: melting point apparatus Mettler Toledo MP50 Melting Point System, uncorrected. MS: microOTOF-Q II (Bruker Daltonics); APCI, atmospheric pressure chemical ionization. IR: FT-IR spectrophotometer MIRacle 10 (Shimadzu) equipped with ATR technique. 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 tetramethylsilane 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)); trichlorofluoromethane (CCl_3F) was used as reference compound in ^{19}F NMR spectroscopy; coupling constants are given with 0.5 Hz resolution; the assignments of ^{13}C and ^{1}H NMR signals were supported by 2-D NMR techniques where necessary. 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: 1.0 mL min^{-1}; 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: 90 → 0%, 29–31 min: 0%, 31–31.5 min: 0 → 90%, 31.5–40 min: 90%. The purity of all compounds was determined by this method. The purity of all test compounds is higher than 95%.

5.2. Synthetic procedures

5.2.1. 6,7,8,9-Tetrahydro-5*H*-benzo[7]annulen-7-amine ·HOAc (8·HOAc). A mixture of benzylamine **7** (126 mg, 0.50 mmol), HOAc (2 drops), Pd/C (10% w/w, 31.7 mg) and CH_3-OH (10 mL) was stirred under H_2 (balloon) at rt for 21 h. The suspension was filtered over Celite® and the filtrate was concentrated *in vacuo*. The compound was used without further purification. Colorless solid, mp 170 °C, yield 91.2 mg (82%). $C_{13}H_{19}NO_2$ (221.3). Purity (HPLC): 95%, t_R = 12.8 min. Exact MS (APCI): *m/z* = calcd. for $C_{11}H_{16}N$ [MH^+] 162.1277, found 162.1281. FT-IR (neat): ν [cm^{-1}] = 3013 (NH_3^+), 2936, 2847 (C–H_{alkyl}), 1381 (COO^-).

5.2.2. (3-Bromo-2-fluoropropyl)benzene (10a). $Et_3N{\cdot}3HF$ (2.7 mL, 17 mmol) was added to a solution of **9a** (591 mg, 5.0 mmol) in CH_2Cl_2 (25 mL). The solution was cooled to 0 °C and subsequently NBS (980 mg, 5.5 mmol) was added. The mixture was stirred at 0 °C for 30 min and at rt for 16 h. Ice (*ca.* 50 mL) and 28% NH_4OH (30 mL) were added, the organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (3 × 30 mL). The combined organic layers were washed with 1 M HCl (2 × 25 mL) and saturated $NaHCO_3$ solution (50 mL). The organic layer was dried (Na_2SO_4), filtered, concentrated *in vacuo* and the residue was purified by fc (4 cm, hexane, 30 mL, R_f = 0.28). Colorless oil, yield 699 mg (64%). Mixture of regioisomers 8 : 2 (NMR). The mixture was used without further purification. $C_9H_{10}BrF$ (217.1). Purity (HPLC): 71%, t_R = 22.1 min. Exact MS (APCI): *m/z* = calcd. for $C_9H_{11}{}^{79}BrF$ [MH^+] 217.0023, found 216.9979. ^{1}H NMR ($CDCl_3$): δ (ppm) = 3.00 (dd, *J* = 24/6.1 Hz, 2H, CH_2-Br,), 3.31–3.46 (m, 2H, CH_2Ph), 4.78 (dq, *J* = 47/6.2 Hz, 1H, CHF), 7.15–7.30 (m, 5H, arom.). ^{19}F NMR ($CDCl_3$): δ (ppm) = −175.2. FT-IR (neat): ν [cm^{-1}] = 3063 (CH_2–Br), 2962, 2905 (C–H_{alkyl}), 1605, 1586, 1497 (C–H_{arom}).

5.2.3. (4-Bromo-3-fluorobutyl)benzene (10b). $Et_3N{\cdot}3HF$ (9.3 mL, 56.8 mmol) was added to a solution of **9b** (3000 mg, 22.7 mmol) in CH_2Cl_2 (25 mL). The solution was cooled to 0 °C and subsequently NBS (4450 mg, 25 mmol) was added. The mixture was stirred at 0 °C for 30 min and at rt for 16 h. Ice (*ca.* 100 mL) and 28% NH_4OH (30 mL) were added, the organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (3 × 30 mL). The combined organic layers were washed with 1 M HCl (2 × 25 mL) and saturated $NaHCO_3$ solution (50 mL). The organic layer was dried (Na_2SO_4), filtered, concentrated *in vacuo* and the residue was purified by fc (6 cm, l = 16 cm, hexane, 50 mL, R_f = 0.15). Colorless oil, yield 3332 mg (63%). $C_{10}H_{12}BrF$ (231.1). Purity (HPLC): 88%, t_R = 23.2 min. Exact MS (APCI): *m/z* = calcd. for $C_{10}H_{12}{}^{81}BrF$ [M] 232.0086, found 232.0082. ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.91–2.07 (m, 2H, C$H_2$$CH_2Ph$), 2.60–2.82 (m, 2H, CH_2Ph), 3.41 (dd, *J* = 19.6/5.1 Hz, 2H, CH_2Br), 4.64 (dquint, *J* = 49/5.2 Hz, 1H, CHF), 7.19–7.24 (m, 3H, 2-CH_{ar}, 4-CH_{ar}, 6-CH_{ar}), 7.29–7.33 (m, 2H, 3-CH_{ar}, 5-CH_{ar}). ^{13}C NMR ($CDCl_3$): δ (ppm) = 31.1 (d, 1C, *J* = 4.2 Hz, CH_2Ph), 33.7 (d, 1C, *J* = 25.2 Hz, CH_2Br), 35.3 (d, 1C, *J* = 20.8 Hz, C$H_2$$CH_2Ph$), 91.1 (d, 1C, *J* = 175.4 Hz, CHF), 126.4

(1C, C-4_{ar}), 128.6 (2C, C-2_{ar}, C-6_{ar}), 128.7 (2C, C-3_{ar}, C-5_{ar}), 140.7 (1C, C-1_{ar}). ^{19}F NMR ($CDCl_3$): δ (ppm) = −179.95. FT-IR (neat): ν [cm^{-1}] = 3063 (CH_2–Br), 2951, 2928, 2862 (C–H_{alkyl}), 1601, 1586, 1497 (C–H_{arom}).

5.2.4. 1-Azido-3-phenylpropan-2-ol (12a)[24]. A solution of oxirane **11a** (670 mg, 5.6 mmol), NaN_3 (975 mg, 15 mmol) and NH_4Cl (401 mg, 7.5 mmol) in H_2O (3 mL) and CH_3OH (15 mL) was heated to reflux for 1 h. Ethyl acetate (50 mL) and H_2O (50 mL) were added, the organic layer was separated and the aqueous layer was extracted with ethyl acetate (3 × 50 mL). The combined organic layers were washed with brine (50 mL), dried (Na_2SO_4), filtered, concentrated *in vacuo* and the residue was purified by fc (4 cm, cyclohexane/ethyl acetate = 1 : 1, 20 mL, R_f = 0.25). Colorless oil, yield 782 mg (79%). $C_9H_{11}N_3O$ (177.2). Purity (HPLC): 87%, t_R = 17.4 min. Exact MS (APCI): *m/z* = calcd. for $C_9H_{12}NO$ [$MH^+ - N_2$] 150.0913, found 150.914. ^{1}H NMR ($CDCl_3$): δ (ppm) = 2.71–2.84 (m, 2H, CH_2N_3), 3.24 (dd, *J* = 12/6.8 Hz, 1H, CH_2Ph), 3.32 (dd, *J* = 12/6.8 Hz, 1H, CH_2Ph), 3.90–3.97 (m, 1H, C*H*OH), 7.14–7.21 (m, 3H, arom.), 7.23–7.28 (m, 2H, arom.). FT-IR (neat): ν [cm^{-1}] = 3395 (O–H), 2099 (–N_3), 1601, 1586, 1493 (C–H_{arom}), 1080 (C–O).

5.2.5. (3-Azido-2-fluoropropyl)benzene (13a). At −78 °C, a solution of DAST (806 mg, 5.0 mmol) in CH_2Cl_2 (5 mL) was added to a solution of **12a** (709 mg, 4.0 mmol) in CH_2Cl_2 (5 mL). The cooling bath was removed and the mixture was stirred for 3 h. H_2O (10 mL) was added and the mixture was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic layers were dried (Na_2SO_4), filtered, concentrated *in vacuo* and the residue was purified by fc (4 cm, hexane/ethyl acetate = 10 : 1, 20 mL, R_f = 0.18). Colorless oil, yield 213 mg (30%). $C_9H_{10}FN_3$ (179.2). Purity (HPLC): 86%, t_R = 21.7 min. Exact MS (APCI): *m/z* = calcd. for $C_9H_{11}FN$ [$MH - N_2$] 152.0876, found 152.0857. ^{1}H NMR ($CDCl_3$): δ (ppm) = 2.83–3.05 (m, 2H, CH_2N_3), 3.25–3.39 (m, 2H, CH_2Ph), 4.67–4.86 (m, 1H, CHF), 7.13–7.27 (m, 5H, arom.), ^{19}F NMR ($CDCl_3$): δ (ppm) = −182.1. FT-IR (neat): ν [cm^{-1}] = 3028 (Aryl-H), 2936 (C–H_{alkyl}), 2099 (–N_3), 1605, 1585, 1497 (C–H_{arom}).

5.2.6. (4-Azido-3-fluorobutyl)benzene (13b)[26]. A solution of **10b** (3265 mg, 14.1 mmol) and NaN_3 (1378 mg, 21.2 mmol) in DMSO (20 mL) was heated to 65 °C for 2 h. Then, ethyl acetate (60 mL) was added, and the mixture was washed with H_2O (2 × 40 mL) and brine (40 mL). The organic layer was dried (Na_2SO_4), filtered, concentrated *in vacuo* and the residue was purified by fc (6 cm, l = 8 cm, hexane/ethyl acetate = 20 : 1, 60 mL, R_f = 0.25). Colorless oil, yield 2720 mg (99%). $C_{10}H_{12}FN_3$ (193.2). Purity (HPLC): 99%, t_R = 22.6 min. Exact MS (APCI): *m/z* = calcd. for $C_{10}H_{13}FN$ [$MH - N_2$] 166.1032, found 166.1035. ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.77–2.15 (m, 1H, C*H*$_2$CH$_2$Ph), 2.68–2.88 (m, 1H, CH$_2$C*H*$_2$Ph), 3.31–3.47 (m, 2H, CH_2N_3), 4.54–4.72 (m, 1H, CHF), 7.19–7.24 (m, 3H, 2-CH_{ar}, 4-CH_{ar}, 6-CH_{ar}), 7.29–7.33 (m, 2H, 3-CH_{ar}, 5-CH_{ar}). ^{13}C NMR ($CDCl_3$): δ (ppm) = 31.1 (d, 1C, *J* = 4.5 Hz, CH_2Ph), 34.0 (d, 1C, *J* = 20.5 Hz, *C*H_2CH_2Ph), 54.5 (d, 1C, *J* = 21.8 Hz, CH_2N_3), 91.9 (d, 1C, *J* = 173.5 Hz, CHF), 126.4 (1C, C-4_{ar}), 128.6 (2C, C-2_{ar}, C-6_{ar}), 128.7 (2C, C-3_{ar}, C-5_{ar}), 140.7 (1C, C-1_{ar}). ^{19}F NMR ($CDCl_3$): δ (ppm) = −186.1. FT-IR (neat): ν [cm^{-1}] = 3028 (Aryl-H), 2928 (C–H_{alkyl}), 2095 (–N_3), 1601, 1586, 1497 (C–H_{arom}).

5.2.7. 2-Fluoro-3-phenylpropan-2-amine (14a). A mixture of **13a** (89.6 mg, 0.50 mmol), Pd/C (10% w/w, 50 mg) and CH_3OH (4 mL) was stirred under H_2 (balloon) at rt for 4 h. The suspension was filtered over Celite® and the filtrate was concentrated *in vacuo*. Colorless solid, yield 71.5 mg (86%). $C_9H_{12}FN$ (153.2). Since the purity of compound **14a** was very high, it was used for the reductive amination of ketone **6** without further purification.

5.2.8. 2-Fluoro-4-phenylbutan-1-amine (14b). A mixture of **13b** (2640 mg, 13.7 mmol), $Pd(OH)_2$/C (20% w/w, 270 mg), 1 M HCl (12 mL) and CH_3OH (30 mL) was stirred under H_2 (balloon) at rt for 7 h. The suspension was filtered over Celite® and the filtrate was concentrated *in vacuo*. The product was purified by fc (6 cm, l = 12 cm, CH_2Cl_2/CH_3OH/Et_3N = 93.5 : 5 : 1.5, 60 mL, R_f = 0.17). Colorless oil, yield 1165 mg (51%). $C_{10}H_{15}FN$ (167.2). Purity (HPLC): 99%, t_R = 13.1 min. Exact MS (APCI): *m/z* = calcd. for $C_{10}H_{15}FN$ [MH^+] 168.1183, found 168.1172. ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.76–2.04 (m, 2H, C*H*$_2$CH$_2$Ph), 2.68–2.73 (m, 1H, CH_2Ph), 2.80–2.90 (m, 3H, CH_2Ph, CH_2NH_2), 4.39–4.51 (m, 1H, CHF), 7.18–7.22 (m, 3H, 2-CH_{ar}, 4-CH_{ar}, 6-CH_{ar}), 7.28–7.31 (m, 2H, 3-CH_{ar}, 5-CH_{ar}). ^{13}C NMR ($CDCl_3$): δ (ppm) = 31.4 (d, 1C, *J* = 4.4 Hz, CH_2Ph), 34.3 (d, 1C, *J* = 20.7 Hz, *C*H_2CH_2Ph), 46.4 (d, 1C, *J* = 21.7 Hz, CH_2NH_2), 94.9 (d, 1C, *J* = 168.5 Hz, CHF), 126.2 (1C, C-4_{ar}), 128.57 (2C, C-2_{ar}, C-6_{ar}), 128.61 (2C, C-3_{ar}, C-5_{ar}), 141.3 (1C, C-1_{ar}). ^{19}F NMR ($CDCl_3$): δ (ppm) = −190.2. FT-IR (neat): ν [cm^{-1}] = 3028 (Aryl-H), 3001 (NH_3^+), 2955, 2920 (C–H_{alkyl}), 1597, 1512, 1497 (C–H_{arom}).

5.2.9. *N*-(2-Fluoro-3-phenylpropyl)-6,7,8,9-tetrahydro-5*H*-benzo[7]annulen-7-amine (5a). A mixture of ketone **6** (64.1 mg, 0.40 mmol), primary amine **14a** (65.9 mg, 0.43 mmol), $NaBH(OAc)_3$ (424 mg, 2.0 mmol) and CH_2Cl_2 (14 mL) was stirred at rt for 24 h. Saturated NH_4Cl solution (10 mL) was added and the mixture was extracted with CH_2Cl_2 (3 × 30 mL). The combined organic layers were dried (Na_2SO_4), filtered, concentrated *in vacuo* and the residue was purified by fc (2 cm, CH_2Cl_2/CH_3OH/Et_3N = 200 : 1 : 1, 7 mL, R_f = 0.22) and preparative tlc (CH_2Cl_2/CH_3OH/Et_3N = 50 : 1 : 1). Colorless solid, mp 52 °C, yield 44.3 mg (37%). $C_{20}H_{24}FN$ (297.4). Purity (HPLC): 96%, t_R = 18.9 min. Exact MS (APCI): *m/z* = calcd. for $C_{20}H_{25}FN$ [MH^+] 298.1966, found 298.1948. ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.34 (q, *J* = 11 Hz, 2H, 6-CH_2, 8-CH_2), 2.07 (m, 2H, 6-CH_2, 8-CH_2), 2.66–2.86 (m, 5H, 5-CH_2, 9-CH_2, 7-CH), 2.85–2.91 (m, 2H, NCH_2), 2.91–3.06 (m, 2H, CH_2Ph), 4.81–4.94 (m, 1H, CHF), 7.10 (m, 4H, 1-CH, 2-CH, 3-CH, 4-CH), 7.22–7.27 (m, 3H, 1-CH_{aryl}, 3-CH_{aryl}, 5-CH_{aryl}), 7.30–7.34 (m, 2H, 2-CH_{aryl}, 4-CH_{aryl}). ^{13}C NMR ($CDCl_3$): δ (ppm) = 32.2 (2C, C-5, C-9), 34.1, 34.2 (2C, C-6, C-8), 39.6 (d, 1C, *J* = 21.2 Hz, CH_2Ar), 50.1 (d, 1C, *J* = 21.0 Hz, $NHCH_2$), 61.2 (1C, C-7), 94.1 (d, 1C, *J* = 171.4 Hz, CHF), 126.37, 126.38 (2C, C-2, C-3), 126.8 (1C, C-4_{aryl}), 128.7 (2C, C-3_{aryl}, C-5_{aryl}), 129.02, 129.03 (2C, C-1, C-4), 129.5 (2C, C-2_{aryl}, C-6_{aryl}), 136.83 (d, *J* = 5.5 Hz, 1C, C-1_{aryl}), 142.45, 142.48 (2C, C-1a, C-4a). ^{19}F NMR ($CDCl_3$): δ (ppm) =

−184.24. FT-IR (neat): ν [cm^{-1}] = 3325 (NH), 3021 (Aryl-H), 2928, 2882 (C–H_{alkyl}), 1601, 1510, 1493 (C–H_{arom}).

5.2.10. *N*-(2-Fluoro-4-phenylbutyl)-6,7,8,9-tetrahydro-5*H*-benzo[7]annulen-7-amine (5b). A mixture of ketone **6** (100 mg, 0.60 mmol), primary amine **14b**·HCl (201 mg, 1.20 mmol), $NaBH(OAc)_3$ (255 mg, 1.20 mmol) and CH_2Cl_2 (10 mL) was stirred at rt for 24 h. Saturated NH_4Cl solution (10 mL) was added and the mixture was extracted with CH_2Cl_2 (3 × 30 mL). The combined organic layers were dried (Na_2SO_4), filtered, concentrated *in vacuo* and the residue was purified by fc (2 cm, l = 31 cm, $CH_2Cl_2/CH_3OH/Et_3N$ = 196 : 1 : 3, 7 mL, R_f = 0.18). Colorless oil, yield 64 mg (34%). $C_{21}H_{26}FN$ (311.4). Purity (HPLC): 99%, t_R = 20.0 min. Exact MS (APCI): *m/z* = calcd. for $C_{21}H_{27}FN$ [MH^+] 312.2122, found 312.2103. ^1H NMR ($CDCl_3$): δ (ppm) = 1.38 (m, 2H, 6-CH_2, 8-CH_2), 1.82–2.04 (m, 2H, C$H_2$$CH_2$Ph), 2.14 (m, 2H, 6-$CH_2$, 8-$CH_2$), 2.68–2.95 (m, 9H, 5-$CH_2$, 9-$CH_2$, CH_2CH_2Ph, NHCH_2, 7-CH), 4.66–4.79 (m, 1H, CHF), 7.08–7.13 (m, 4H, 1-CH, 2-CH, 3-CH, 4-CH), 7.18–7.21 (m, 3H, 1-CH_{aryl}, 3-CH_{aryl}, 5-CH_{aryl}), 7.27–7.30 (m, 2H, 2-CH_{aryl}, 4-CH_{aryl}). ^{13}C NMR ($CDCl_3$): δ (ppm) = 31.4 (d, 1C, *J* = 4.6 Hz, CH_2CH_2Ph), 32.2 (2C, C-5, C-9), 33.4, 33.8 (2C, C-6, C-8), 35.0 (d, 1C, *J* = 20.5 Hz, CH_2CH_2Ph), 50.4 (d, 1C, *J* = 20.8 Hz, NHCH_2), 61.2 (1C, C-7), 92.9 (d, 1C, *J* = 167.8 Hz, CHF), 126.3 (1C, C-4_{aryl}), 126.45 (2C, C-2, C-3), 128.58 (2C, C-2_{aryl}, C-6_{aryl}), 128.64 (2C, C-3_{aryl}, C-5_{aryl}), 129.05, 129.07 (2C, C-1, C-4), 141.2 (1C, C-1_{aryl}), 142.29, 142.32 (2C, C-1a, C-4a). ^{19}F NMR ($CDCl_3$): δ (ppm) = −187.4. FT-IR (neat): ν [cm^{-1}] = 3021 (Aryl-H), 2927, 2847 (C–H_{alkyl}), 1601, 1586, 1493 (C–H_{arom}).

5.3. Receptor binding studies

5.3.1. Materials. The recombinant L(tk−) cells stably expressing the GluN2B receptor were a generous donation of Prof. Steinhilber (Frankfurt, Germany). Cell incubator: Heracell 120 (Thermo Fisher Scientific, Langenselbold, Germany). Homogenizers: Elvehjem Potter (B. Braun Biotech International, Melsungen, Germany) and Soniprep 150, MSE, London, UK). Centrifuges: 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.

5.3.2. Cell culture and preparation of membrane homogenates for the GluN2B assay[27]. Mouse L(tk−) cells stably transfected with the dexamethasone-inducible eukaryotic expression vectors pMSG NR1-1a, pMSG GluN2B (1 : 5 ratio) were grown in Modified Earl's Medium (MEM) containing 10% of standardized FCS (Biochrom AG, Berlin, Germany). The expression of the NMDA receptor at the cell surface was induced after the cell density of the adherent growing cells had reached approximately 90% of confluency. For the induction, the original growth medium was replaced by a growth medium containing 4 μM dexamethasone and 4 μM ketamine (final concentration). After 24 h the cells were harvested by trypsination and pelleted (10 min, 5000 × *g*).

For the binding assay, the cell pellet was resuspended in PBS buffer and the number of cells was determined using an improved Neubauer's counting chamber (VWR, Darmstadt, Germany). Subsequently, the cells were lysed by sonication (4 °C, 6 × 10 s cycles with breaks of 10 s). The resulting cell fragments were centrifuged with a high performance cool centrifuge (20 000 × *g*, 4 °C). The supernatant was discarded and the pellet resuspended in a defined volume of phosphate buffer saline (PBS) yielding cell fragments of approximately 500 000 cells per mL. The suspension of membrane homogenates was sonicated again (4 °C, 2 × 10 s cycles with breaks of 10 s) and stored at −80 °C.

5.3.3. Protein determination. The protein concentration was determined by the method of Bradford,[36] modified by Stoscheck.[37] 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^{-1}). 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).

5.3.4. General protocol for the binding assay[27]. 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^{-1}), 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.[38] The K_i-values are given as mean value ± SEM from three independent experiments.

5.3.5. Performing of the GluN2B assay[27]. The competitive binding assay was performed with the radioligand [^{3}H]-ifenprodil (60 Ci mmol^{-1}; Perkin Elmer). The thawed cell membrane preparation from the transfected L(tk–) cells (about 20 μg protein) was incubated with various concentrations of test compounds, 5 nM [^{3}H]-Ifenprodil, and TRIS/EDTA-buffer (5 mM/1 mM, pH 7.5) at 37 °C. The non-specific binding was determined with 10 μM unlabeled ifenprodil. The K_d value of ifenprodil is 7.6 nM.

5.3.6. Affinity toward σ_1 and σ_2 receptors and the PCP binding site of the NMDA receptor. The affinity toward the PCP binding site of the NMDA receptor[29,30] and the affinity toward the σ_1 and σ_2 receptors[33–35] were recorded as previously described.

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