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Design, Synthesis, and Biological Evaluation of 3-Benzazepin-1-ols as NR2B-Selective NMDA Receptor Antagonists

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Cleavage and reconstitution of a bond in the piperidine ring of ifenprodil (**1**) leads to 7-methoxy-2,3,4,5-tetrahydro-1*H*-3-benzazepin-1-ols, a novel class of NR2B-selective NMDA receptor antagonists. The secondary amine 7-methoxy-2,3,4,5-tetrahydro-1*H*-3-benzazepin-1-ol (**12**), which was synthesized in six steps starting from 2-phenylethylamine **3**, represents the central building block for the introduction of several N-linked residues. A distance of four methylene units between the basic nitrogen atom and the phenyl residue in the side chain results in high NR2B affinity. The 4-phenylbutyl derivative **13** (WMS-1405, $K_i = 5.4$ nM) and the conformationally restricted 4-phenylcyclohexyl derivative **31** ($K_i = 10$ nM) represent the most potent NR2B ligands of this series. Whereas **13** shows excellent selectivity, the 4-phenylcyclohexyl derivative **31** also interacts with σ_1 ($K_i = 33$ nM) and σ_2 receptors ($K_i = 82$ nM). In the excitotoxicity assay the phenylbutyl derivative **13** inhibits the glutamate-induced cytotoxicity with an IC_{50} value of 360 nM, indicating that **13** is an NMDA antagonist.

Introduction

The class of ionotropic glutamate receptors can be divided into three subtypes: *N*-methyl-D-aspartate (NMDA), 2-amino-3-(3-hydroxy-5-methylisooxazol-4-yl)propionate (AMPA), and kainate receptors.[1] The NMDA receptor mediates a number of important physiological and pathophysiological processes[2] in the mammalian central nervous system (CNS). Its key role in these processes renders the NMDA receptor a potential target for the treatment of neurological disorders.[3]

The NMDA receptor is a heteromeric complex of four subunits. Three types of subunits have been identified thus far: the NR1 subunit with eight splice variants (NR1a–h), the NR2 subunit, which has four distinct subtypes (NR2A–D) encoded by four distinct genes, and the NR3 subunits NR3A and NR3B (two genes).[4,5] A functional NMDA receptor comprises at least one NR1 subunit bearing the glycine binding site and one NR2 subunit responsible for glutamate binding.[6,7] Whereas the NR1 subunit is ubiquitously expressed in the CNS, the density of the different NR2 subunits varies depending on the region of the CNS. The NR2A subunit is found throughout the whole CNS, but the NR2B subunit is highly expressed only in the cortex and hippocampus with a rather low density in the cerebellum and hypothalamus. The NR2C subunit predominates in the cerebellum and both the NR2C and NR2D subunits are preferentially formed in the brain stem and the spinal cord.[8] NR3 subunits are expressed predominantly in the developing CNS and seem to be of no relevance in the adult brain.

Compared with classical unselective NMDA receptor antagonists (such as (+)-MK-801,[9] dexoxadrol[10]), NR2B-selective antagonists show decreased cognitive side effects due to the reduced expression of this subunit in the cerebellum. This results in an improved safety profile of these antagonists. Thus NR2B-selective NMDA antagonists have good potential for the treatment of Parkinson's disease,[11] traumatic brain injury,[12] stroke,[13] migraine,[14] alcohol withdrawal,[15] and chronic and neuropathic pain.[16]

In 1971 ifenprodil (**1**, Figure 1) was developed as an α_1 adrenoceptor antagonist.[17] However, **1** has been found to interact with several other receptors and ion channels including 5-HT_{1A}, 5-HT_2, σ, and NMDA receptors.[18] Subsequently, **1** was shown

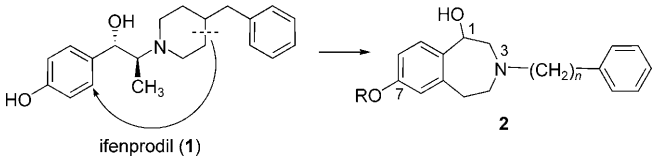

Figure 1. Design of 3-benzazepines **2** as NR2B-selective NMDA receptor antagonists from **1**.

to be a selective NR2B antagonist.[19] At first it was generally accepted that **1** interacts with the polyamine binding site of the NMDA receptor.[20,21] However, site-directed mutagenesis experiments have shown that a discrete binding site for **1** exists on the NR2B subunit.[22] However, **1** is a nonselective

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drug inducing many side effects such as hypotension, cognitive problems, and neurotoxicity.[23] In addition to these side effects, the bioavailability of **1** is rather low because of its fast and extensive metabolism.[24,25]

With the aim of increasing the selectivity of **1** without losing affinity toward NR2B subunit containing NMDA receptors (K_i = 10 nM), a new class of NR2B-selective NMDA receptor antagonists with reduced conformational flexibility was designed (Figure 1). To decrease the conformational freedom of the ethylene spacer between the phenol and the piperidine moiety, the C3–C4 bond of the piperidine ring was formally cleaved and the resulting open chain was connected to the phenol leading to a 3-benzazepine scaffold (**2**). Herein we report the synthesis, structural modification, and NR2B receptor affinity of novel 3-benzazepines of type **2**. After establishing structure–affinity relationships, the receptor selectivities and intrinsic activities of the most promising ligands were investigated.

Chemistry

The designed 3-benzazepin-1-ols **2** were synthesized from 2-(3-methoxyphenyl)ethan-1-amine (**3**, Scheme 1). Primary amine **3** was protected with a toluenesulfonyl group according to published procedures.[26] The *N*-tolylsulfonyl protecting group eliminates the basicity of the nitrogen atom in **4**, thereby allowing the planned ring closure to be performed via Friedel–Crafts acylation. Sulfonamide **4** was then treated with excess ethyl bromoacetate in the presence of potassium carbonate to give ester **5**, which was saponified with sodium hydroxide to afford carboxylic acid **6**.[27]

An intramolecular Friedel–Crafts acylation of carboxylic acid **6** should provide the 3-benzazepine scaffold. To enhance the electrophilicity of the carboxy group, carboxylic acid **6** was converted with thionyl chloride into acid chloride **7**, which was directly treated with aluminum trichloride. Surprisingly, in contrast to reported observations,[27] tetrahydroisoquinoline **8** was formed during this reaction instead of the described 3-benzazepine **9** (Scheme 1).

The mechanism for the formation of tetrahydroisoquinoline **8** is outlined in the Supporting Information. An analogous mechanism for the formation of 2-benzazepines has already been described.[28] It is assumed that after coordination of the Lewis acid aluminum trichloride with acid chloride **7**, a fast decarbonylation occurred instead of acylation of the benzene ring. The resulting iminium ion then reacted with the benzene ring to afford tetrahydroisoquinoline **8**.

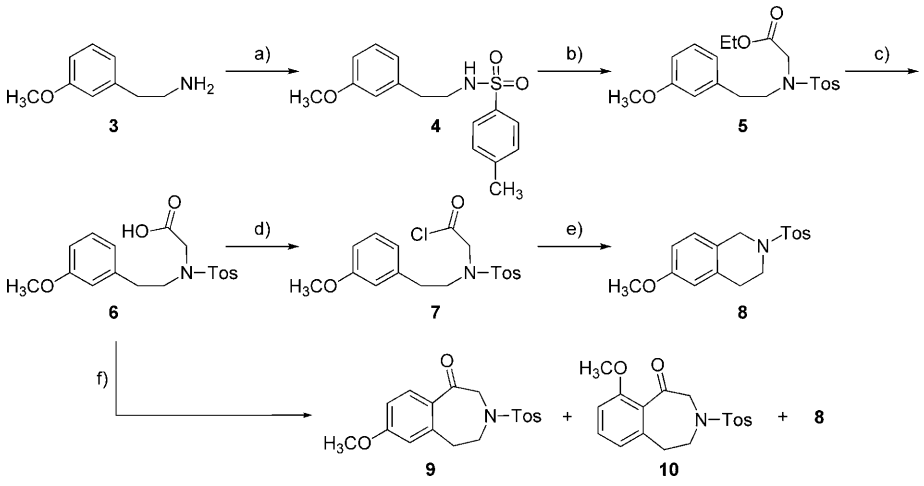
As all cyclization reactions with acid chloride **7** led exclusively to tetrahydroisoquinoline **8**, the carboxylic acid **6** was used directly for the intramolecular Friedel–Crafts acylation. The reaction of **6** with phosphorus pentoxide provided 3-benzazepin-1-one **9** as the major product, whilst the regioisomer **10** and the tetrahydroisoquinoline **8** were the main side products. The ratio of regioisomers **9** and **10** was controlled by the reaction temperature with higher temperatures decreasing the preference for the regioisomer **9**. The reaction of **6** with phosphorus pentoxide at 0 °C yielded the best ratio of **9**/**10** (90:10; Table 1). The regioisomers **9** and **10** were separated by recrystallization with ethanol. Whereas the reaction temperature strongly influenced the ratio of regioisomers **9**/**10**, the amount of tetrahydroisoquinoline **8** was almost constant at different reaction temperatures.

Table 1. Optimization of the intramolecular Friedel–Crafts acylation of **6** using P_2O_5.

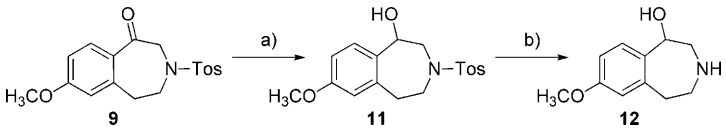
T [°C]	Yield **9**/**10** [%]	Yield **8** [%]	Ratio **9**:**10**
0	49	19	90:10
10	46	20	80:20
RT	47	28	70:30

In the next step ketone **9** was reduced with sodium borohydride to yield 3-benzazepinol **11**.[27] The *N*-tolylsulfonyl protecting group of **11** was removed with activated[29] magnesium in boiling methanol[30] to get the secondary amine **12** (Scheme 2).

The secondary amine **12** represents the central building block for the synthesis of 3-benzazepin-1-ols with various N-linked residues. A large number with differently substituted 3-benzazepines is required for the establishment of reliable structure–affinity relationships. In the first series of compounds the distance between the basic nitrogen atom of the 3-benzazepine scaffold and phenyl residue in the side chain was systematically increased from one to five methylene units. As the 4-phenylbutyl derivative **13**, which has the same N–Ph distance as the lead compound **1** (see Figure 1), showed the highest affinity in this series (see Figure 2), substituents with a spacer of 4–5 methylene groups (or heteroa-



Scheme 1. Reagents and conditions: a) *p*-toluenesulfonyl chloride, pyridine, RT, 1.5 h; b) ethyl bromoacetate, acetone, K_2CO_3, reflux, 20 h; c) NaOH, 50% EtOH, reflux, 6 h; d) $SOCl_2$, benzene, reflux, 8 h; e) $AlCl_3$, 1,2-dichloroethane, −65 °C, 20 h; f) P_2O_5, 1,2-dichloroethane, 0 °C, 24 h.



Scheme 2. Reagents and conditions: a) $NaBH_4$, CH_3OH, RT, 2 h; b) Mg^0, CH_3OH, reflux, 16 h.

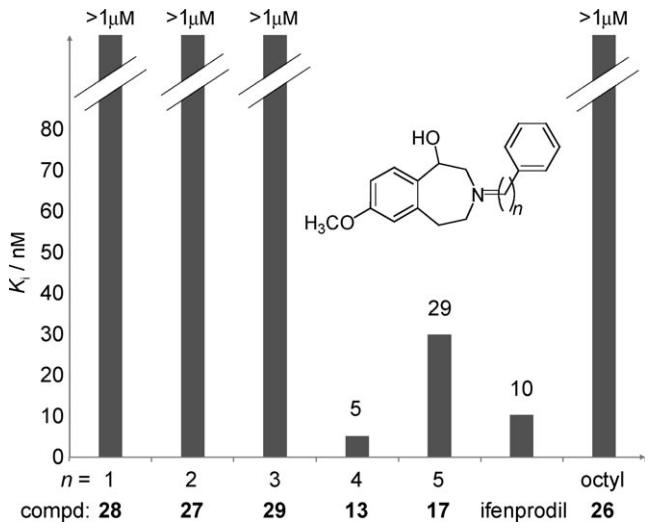
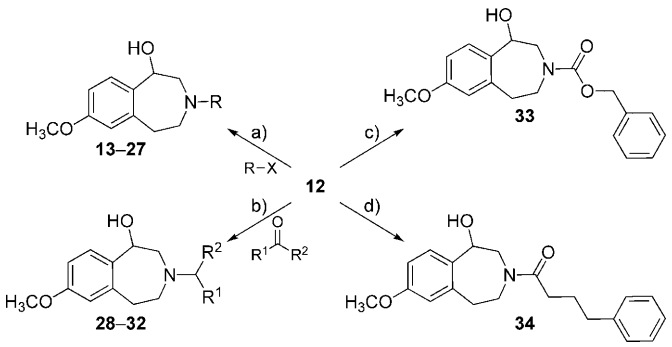


Figure 2. Correlation between NR2B affinity and the distance between the nitrogen atom and phenyl residue in the side chain.

tom equivalents) were preferentially selected. 3-Benzazepines **13**–**27** were synthesized by nucleophilic substitution of secondary amine **12** with various haloalkanes (Scheme 3 and Table 2). The transformations were performed in acetonitrile at reflux. In the case of chloroalkanes, tetra(*n*-butyl)ammonium iodide was added to the reaction mixture to activate the chloroalkanes through in situ generation of iodoalkanes (Finkelstein reaction[31]).

3-Benzazepines **28**–**32** were synthesized by reductive alkylation of secondary amine **12** with different aldehydes and a ketone in the presence of sodium triacetoxyborohydride[32] (Scheme 3 and Table 3). For the reductive alkylation of **12** with



Scheme 3. Reagents and conditions: a) RX (see Table 2), CH_3CN, K_2CO_3, $(nBu)_4NI$, reflux, 8–72 h; b) various aldehydes and a ketone (see Table 3), $NaBH(OAc)_3$, 1,2-dichloroethane, RT, 3 h; c) $ClCO_2Bn$, CH_2Cl_2, NEt_3, RT, 16 h; d) 4-phenylbutyric acid, EDC·HCl, CH_2Cl_2, RT, 6 h.

Table 2. Synthesis of 3-benzazepines by alkylation of **12** with RX.

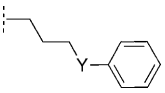
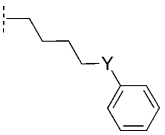
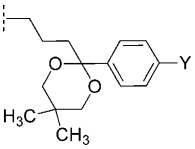
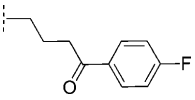
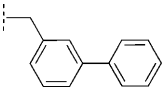
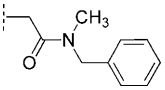
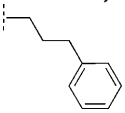
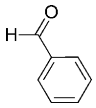
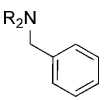
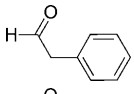
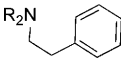
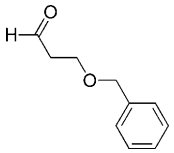
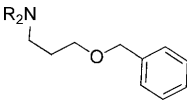
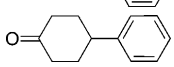
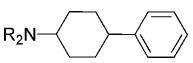
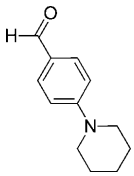
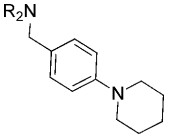
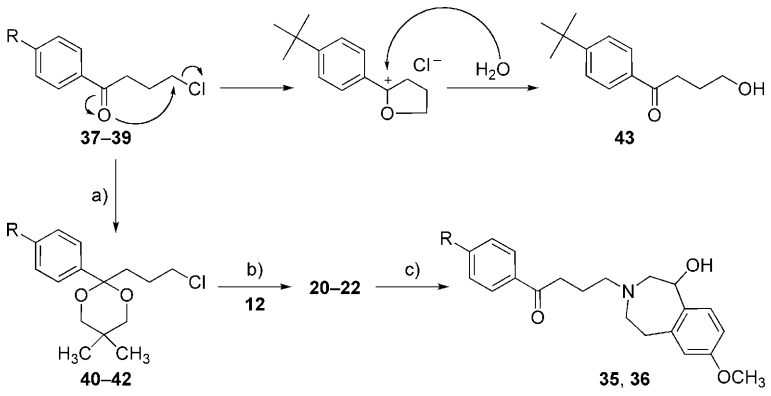
Compd	R	Y	X	Yield [%]
13 (WMS-1405)		CH_2	Cl	45
14		O	Br	79
15		S	Cl	95
16		SO_2	Cl	68
17		CH_2	Cl	93
18		S	Cl	96
19		SO_2	Cl	92
20		*t*Bu	Cl	80
21		OCH_3	Cl	79
22		F	Cl	74
23		–	Cl	34
24		–	Br	61
25		–	Cl	81
26	octyl	–	Br	91
27		–	Br	91

Table 3. Synthesis of 3-benzazepines by reductive alkylation of **12**.

Compd			Yield [%]
28			76
29			81
30			82
31			69
32			97

4-phenylcyclohexanone, acetic acid was added to the reaction mixture to increase the activity of the ketone. The ^{1}H NMR spectrum of the resulting *N*-(4-phenylcyclohexyl) derivative **31** shows the signals of two diastereomers, *cis*-**31** and *trans*-**31**, at a ratio of 70:30, which were used in the receptor binding studies without separation.

A further variation of the N-linked substituent was achieved upon hydrolysis of the cyclic ketals **20** and **21** with 1 M hydrochloric acid to obtain ketones **35** and **36**, respectively (Scheme 4). The direct reaction of the secondary amine **12** with the commercially available γ-chloroketones **37**–**39** resulted in very low yields of the desired ketones **23**, **35**, and **36** but with a major side product. After reaction of the *tert*-butyl ketone **37**, the ketone **43** bearing a hydroxy moiety instead of a chloro group was isolated and identified as the major product. We assume that the intermediate formation of a five-membered oxonium ion is the driving force for the formation of hydroxy ketone side products such as **43**. The electron-donating *tert*-butyl moiety of **37** supports the formation of the oxonium ion and ultimately the formation of **43**. The electron-withdrawing fluoro substituent of **39** inhibits oxonium ion formation and explains the moderate yield of **23** (34%) after direct reaction with the secondary amine **12**. Thus, acetalization of ketones **37**–**39** to get ketals **40**–**42**[33,34] completely suppressed the formation of hydroxy ketones such as **43** and provided the substitution products **20**–**22** in high yields.



Scheme 4. Reagents and conditions: a) 2,2-dimethylpropane-1,3-diol, toluene, *p*-toluenesulfonic acid, reflux, 4–8 h; b) **12**, CH_3CN, K_2CO_3, $(nBu)_4NI$, reflux, 50–72 h; c) 1 M HCl, Et_2O, RT, 16 h.

Potent and selective NR2B antagonists without a basic amine have been described.[35,36] Therefore, carbamate **33** and amide **34** were synthesized and their NR2B receptor affinities were determined (Scheme 3). 3-Benzazepine **33** was prepared by acylation of secondary amine **12** with benzyl chloroformate. The acylation of secondary amine **12** with 4-phenylbutyric acid was performed using the coupling reagent *N*-ethyl-*N*′-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl).[37]

Receptor Affinity

Affinity toward NR2B-containing NMDA receptors

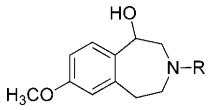
The NR2B receptor affinity of the synthesized 3-benzazepines **12**–**36** was determined in a competitive receptor binding assay recently developed in our group.[38] In this assay tritium-labeled [^{3}H]ifenprodil was employed as the radioligand. Membrane homogenates prepared upon ultrasonic irradiation of L(tk−) cells stably expressing recombinant human NR1a/NR2B receptors served as receptor material.[39] The high density of NMDA receptors renders this system selective. The expression of NMDA receptors at the cell surface was induced by addition of dexamethasone to the growth medium. During this period cell death was inhibited by addition of the NMDA antagonist ketamine (phencyclidine binding site) to the growth medium.

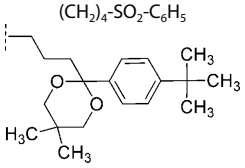
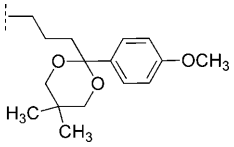
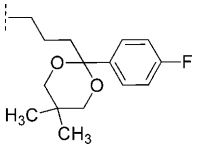
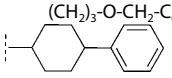
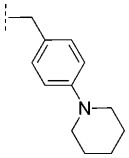
The receptor affinities of 3-benzazepines are summarized in Table 4. The secondary amine **12** does not exhibit considerable affinity toward NR2B containing NMDA receptors. However, substitution of **12** with various arylalkyl moieties led to derivatives with high NR2B affinity (for example, **13**, **17**, **21**, **31**, and **35**). In Figure 2 the NR2B affinity of compounds **13**, **17**, and **26**–**29** is correlated with the distance between the basic nitrogen atom and the phenyl group in the side chain. A distance of 4–5 methylene units proved to be optimal and resulted in the potent NR2B antagonists **13** (WMS-1405, $K_i = 5.4$ nM) with a 4-phenylbutyl residue and **17** ($K_i = 29$ nM) with a 5-phenylpentyl residue. A simple octyl chain (compound **26**) was not tolerated by the NR2B receptor. Consequently, *N*-arylalkyl substituents with four or five methylene groups (or heteroatom equivalents) in the side chain were selected for the establishment of structure–affinity relationships.

The replacement of the methylene group at position 4 of the 4-phenylbutyl side chain of **13** with an oxygen (in **14**) or sulfur atom (in **15**) led to a drastic decrease in affinity for NR2B by a factor of 41 (compound **14**) or even 346 (compound **15**). The same effect was observed with the homologous 5-phenylpentyl derivative **17**. Reasons for the decreased NR2B affinity of these derivatives might be the high electron density of the phenyl group, the increased polarity of the spacer or an alternate conformation of the spacer.

The butyrophenone derivatives **23**, **35**, and **36** together with the corresponding ketals **20**–**22** were synthesized to investigate the influence of different aromatic substituents (F, OCH_3, *t*Bu) on NR2B affinity. Compound **35**, with the bulky *tert*-butyl substituent, displays the highest NR2B affinity in this group ($K_i = 19$ nM), demonstrating that the NR2B receptor can tolerate a sterically large group at the *para* position of the phenyl group. The slightly lower affinities of the corresponding ketals **20**–**22** ($K_i = 32$–83 nM) indicate that the bulky 5,5-dimethyl-1,3-dioxane ring at position 4 of the side chain is also tolerated by the NR2B binding site.

Table 4. Affinities of 3-benzazepin-1-ols for the ifenprodil binding site of NR2B-containing NMDA receptors, the PCP binding site of the NMDA receptor, and for σ_1 and σ_2 receptors.



Compd	R	$K_i \pm$SEM [nM] NR2B	PCP	σ_1	σ_2
12	H	15%[a]	23%[b]	23%[a]	0%[a]
13	$(CH_2)_4$-C_6H_5	5.4±0.4	22%[b]	182±38	554±127
(WMS-1405)					
14	$(CH_2)_3$-O-C_6H_5	222±49.0	29%[a]	178±14	376
15	$(CH_2)_3$-S-C_6H_5	1880	35%[a]	155	1520
16	$(CH_2)_3$-SO_2-C_6H_5	934	0%[a]	21%[a]	32%[a]
17	$(CH_2)_5$-C_6H_5	29±2.0	35%[a]	65±7.1	305
18	$(CH_2)_4$-S-C_6H_5	1090	33%[a]	95±29	308±67
19	$(CH_2)_4$-SO_2-C_6H_5	1410	32%[a]	249	0%[a]
20		48±14	0%[a]	69	179
21		32±3.6	32%[a]	602	1260
22		83±32	46%[a]	46	103
23	$(CH_2)_3$-CO-C_6H_4-4-F	150±57	0%[a]	26%[a]	29%[a]
24	CH_2-C_6H_4-3-C_6H_5	12%[a]	0%[a]	452	1140
25	CH_2-CO-N(CH_3)-CH_2-C_6H_5	22%[a]	1100	8%[a]	14%[a]
26	$(CH_2)_7CH_3$	0%[a]	6%[a]	45±12	108±52
27	$(CH_2)_3$-C_6H_5	3400	1%[b]	280	497
28	CH_2-C_6H_5	25%[a]	34%[a]	33±3.1	6540
29	$(CH_2)_2$-C_6H_5	31%[a]	1%*[a]	234	1450
30	$(CH_2)_3$-O-CH_2-C_6H_5	19%[a]	29%[a]	434±88	1000
31		10±1.5	23%[a]	33±17	82±26
32		91±8.0	0%[a]	135±50	238
33	CO_2-CH_2-C_6H_5	1180	28%[a]	0%[a]	0%[a]
34	CO-$(CH_2)_3$-C_6H_5	19%[a]	44%[a]	43%[a]	0%[a]
35	$(CH_2)_3$-CO-C_6H_4-4-C(CH_3)$_3$	19±6.0	0%[a]	44±8.1	267±39
36	$(CH_2)_3$-CO-C_6H_4-4-OCH_3	341±64	9%[b]	12%[a]	2230
1	ifenprodil	10±0.7	–	125±24	98.3±34
	eliprodil	13±2.5	–	–	–
	dexoxadrol	–	32±7.4	–	–
	haloperidol	–	–	6.3±1.6	78.1±2.3
	di-*o*-tolylguanidine (DTG)	–	–	89±29	57.5±18

[a] Due to low affinity, only the inhibition at a concentration of 1 μM is given. [b] Due to low affinity, only the inhibition at a concentration of 10 μM is given.

Next, we investigated the NR2B affinity of 3-benzazepines with conformationally constrained side chains. Whereas the biphenylmethyl derivative **24** and compound **25** with an amide in the side chain did not reveal significant NR2B interaction, the 4-phenylcyclohexyl derivative **31** (*cis*/*trans* = 70:30) was highly potent (K_i = 10 nM). The high affinity of the 4-phenylcyclohexyl derivative **31** indicates that the conformationally restricted side chain fits well without adaptation to the ifenprodil binding pocket of the NR2B containing NMDA receptor.

The carbamate **33** and the amide **34** with an *N*-aryl distance of four carbon atoms were prepared as it has been shown that a basic amine is not essential for high NR2B affinity.[33,34] However, the negligible affinity of **33** and **34** indicates that in the 3-benzazepine class of NR2B antagonists the basic amine cannot be omitted.

Considering the affinity for the NR2B receptor, phenylbutyl compound **13** (K_i = 5.2 nM), phenylcyclohexyl compound **31** (K_i = 10 nM), phenyloxobutyl compound **36** (K_i = 19 nM), phenylpentyl compound **17** (K_i = 29 nM), and dioxane derivative **21** (K_i = 32 nM) represent the five most potent compounds of this series.

Receptor Selectivity

To investigate the selectivity of this new class of NR2B ligands, the compounds were tested against the phencyclidine (PCP) binding site of the NMDA receptor[40] and both σ receptor subtypes (σ_1 and σ_2)[40,41] in receptor binding studies with radioligands. In Table 4 the PCP, σ_1, and σ_2 receptor affinities of 3-benzazepines **12**–**36** are compared with their NR2B receptor affinities. The 3-benzazepines do not reveal significant interactions with the PCP binding site of the NMDA receptor, indicating high selectivity for the polyamine binding site over the phencyclidine binding site of the NMDA receptor. The affinities at the σ_2 receptor are also generally very low. In particular, the most potent NR2B ligands show excellent selectivity against σ_2 receptors. Whereas the NR2B/σ_2 selectivity factors for **31** (8),

17 (11), and **35** (14) are approximately 10, the corresponding selectivity factors of **13** and **21** are 102 and 40, respectively.

The σ_1 receptor affinity of the 3-benzazepine compound class has to be discussed separately. With NR2B/σ_1 selectivity factors of 34 and 19, the very potent NR2B antagonists **13** and **21** reveal high selectivity for the NR2B receptor over the σ_1 receptor. However, the σ_1 affinities of phenylpentyl derivative **17**, phenylcyclohexyl derivative **31**, and ketone **35** are in the same range as their NR2B affinities [NR2B/σ_1: 2 (**17**), 3 (**31**), 2 (**35**)].

In addition to selective and unselective NR2B ligands, compounds with a preference for the σ_1 receptor were uncovered in the 3-benzazepine compound class. In particular, **15** and **18** with a sulfur atom in the side chain as well as **27**, **28**, and **29** with side chains shorter than butyl display a greater than tenfold preference for the σ_1 receptor over the NR2B receptor. These results indicate that small structural modifications can shift the receptor profile from an NR2B-selective ligand to a σ_1-selective ligand. Therefore, investigation of both the NR2B and σ_1 affinity is required during the development of novel selective NR2B and σ_1 receptor ligands.

The receptor binding profile of the phenylbutyl derivative **13** was investigated because of the promising NR2B affinity ($K_i = 5.4$ nM) and selectivity toward the σ and the PCP receptors. In competition experiments, the interaction of **13** with 15 relevant receptor systems including α_1, D_1, D_2, NMDA (PCP binding site), NMDA (glycine binding site), κ-opioid, μ-opioid, δ-opioid, 5-HT_{1A}, 5-HT_2, σ_1, and σ_2 receptors, with the noradrenalin and serotonin transporter, as well as with monoamine oxidase A was investigated. At a concentration of 100 nM **13** did not compete significantly with the radioligands employed.

These results are of particular interest, as low receptor selectivity is one of the major drawbacks of **1**. Clearly, the decreased conformational flexibility of the ethylene spacer in the 3-benzazepine **13** significantly reduces interaction with α_1, 5-HT_{1A}, 5-HT_2, σ_1, σ_2, and PCP receptors.

Functional Activity[42]

To verify the antagonistic activity of the potent NR2B-selective ligand **13** the inhibition of excitotoxicity was investigated. In this assay L(tk−)cells stably expressing NR1a/NR2B receptors were employed. After addition of (*S*)-glutamate and glycine the excitotoxicity was determined by the amount of lactate dehydrogenase (LDH) released from the cells into the culture supernatant. Different concentrations of the test compound **13** were added 30 min before addition of (*S*)-glutamate and glycine and the release of LDH was measured.

In Figure 3 the excitotoxicity at different compound concentrations is shown. With an IC_{50} value of 360 nM, **13** inhibits the cytotoxic effects of (*S*)-glutamate and glycine. This result proves the NMDA receptor antagonistic activity of **13** and shows that **13** not only binds at the polyamine binding site, but also produces antagonistic effects.

The same experiment was performed with L12-G10 cells stably expressing NR1a/NR2A receptors. At a concentration of 10 μM, excitotoxicity inhibited by compound **13** was lower than 10%. Clearly, **13** does not interact with NMDA receptors consisting of NR2A subunits.

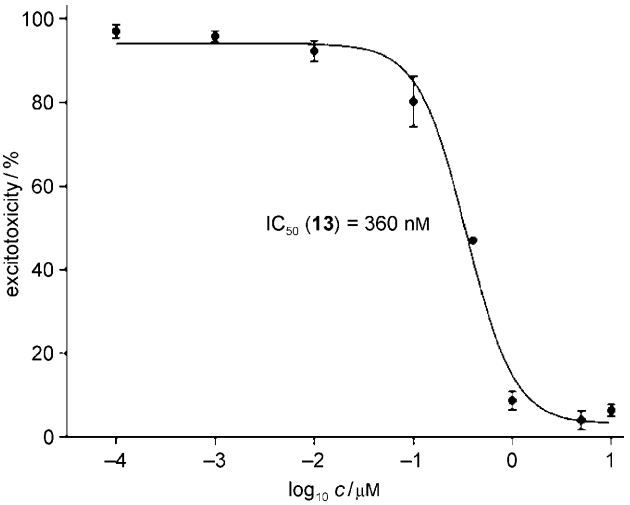


Figure 3. Inhibition of NMDA receptor-mediated cell toxicity by compound **13**. Different concentrations of the potent and selective ligand **13** were applied to L13-E6 cells, and NMDA receptors were activated by the addition of (*S*)-glutamate and glycine. Excitotoxicity was determined by the amount of lactate dehydrogenase (LDH) released. Compound **13** has an IC_{50} value of 360 nM.

Conclusions

A new compound class of NR2B-selective NMDA receptor antagonists has been identified. The most promising compound is 7-methoxy-3-(4-phenylbutyl)-2,3,4,5,-tetrahydro-1*H*-3-benzazepin-1-ol (**13**) showing high affinity ($K_i = 5.4$ nM) toward NR2B receptors. In contrast to the lead compound **1**, benzazepine **13** does not interact with α_1, NMDA (PCP binding site), 5-HT_{1A}, 5-HT_2, σ_1, and σ_2 receptors. In an assay using cells stably expressing only NR1a and NR2B subunits, **13** was able to inhibit ($IC_{50} = 360$ nM) the excitotoxicity caused by (*S*)-glutamate and glycine, indicating the NMDA antagonistic activity of **13**.

3-Benzazepine **13** represents a very promising new lead, which will be further optimized by introduction of crucial structural elements of the lead compound ifenprodil. At first the methoxy group of **13** will be converted into a hydroxy moiety, as a hydrogen bond donor at the aromatic system is generally favorable for potent NR2B ligands. Secondly, the methyl group of ifenprodil, which has been omitted in the first series of NR2B ligands, will be added at position 8 of the 3-benzazepine system. At the final stage, the most promising ligands will be prepared in enantiomerically pure form.

Experimental Section

General synthesis protocols

Unless otherwise noted, moisture-sensitive reactions were conducted under dry N_2. Flash chromatography (FC): silica gel 60, 40–64 μm (Merck); parentheses include diameter of the column, eluent, fraction size, R_f value. ^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz): Unity Mercury Plus 400 spectrometer (Varian); δ in ppm

relative to $(CH_3)_4Si$; coupling constants are given at a resolution of 0.5 Hz. According to HPLC analysis, the purity of all test compounds is >95%.

6-Methoxy-2-(4-methylphenylsulfonyl)-1,2,3,4-tetrahydroisoquinoline (8); 7-methoxy-3-(4-methylphenylsulfonyl)-2,3,4,5-tetrahydro-3-benzazepin-1-one (9); and 9-methoxy-3-(4-methylphenylsulfonyl)-2,3,4,5-tetrahydro-3-benzazepin-1-one (10): Under N_2 the acid **6** (1.0 g, 2.76 mmol) was dissolved in CH_2Cl_2 (20 mL) and cooled to 0 °C. Afterward P_2O_5 (1.96 g, 13.8 mmol) was added, and the mixture was stirred for 24 h at 0 °C. Subsequently 3% NaOH was added to the suspension to give pH 13–14. The mixture was extracted with CH_2Cl_2 (3×40 mL); the CH_2Cl_2 layer was washed with H_2O (3×50 mL), dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by FC (4 cm, *n*-hexane/EtOAc 7:3 and 2% *N*,*N*-dimethylethylamine, 50 mL, R_f (**8**) = 0.57) and R_f (**9** and **10**) = 0.23). **9** and **10** were separated by recrystallization from EtOH.

8 (R_f = 0.57): Colorless solid, mp: 113 °C, yield 0.12 g (14%); ^{1}H NMR ($CDCl_3$): δ = 2.41 (s, 3H, Ph-CH_3), 2.89 (t, *J* = 5.9 Hz, 2H, 4-H), 3.32 (t, *J* = 5.9 Hz, 2H, 3-H), 3.74 (s, 3H, OCH_3), 4.17 (s, 2H, 1-H), 6.59 (d, *J* = 2.3 Hz, 1H, 5-H), 6.71 (dd, *J* = 8.5/2.6 Hz, 1H, 7-H), 6.92 (d, *J* = 8.5 Hz, 1H, 8-H), 7.31 (d, *J* = 8.0 Hz, 2H, 3-H toluenesulfonyl and 5-H toluenesulfonyl), 7.71 ppm (d, *J* = 8.3 Hz, 2H, 2-H toluenesulfonyl and 6-H toluenesulfonyl); $C_{17}H_{19}NO_3S$ (317.4).

9 (R_f = 0.23): Colorless solid (EtOH), mp: 168 °C, yield 0.37 g (39%); ^{1}H NMR ($CDCl_3$): δ = 2.37 (s, 3H, Ph-CH_3), 2.95 (t, *J* = 6.6 Hz, 2H, 5-H), 3.67 (t, *J* = 6.6 Hz, 2H, 4-H), 3.84 (s, 3H, OCH_3), 4.17 (s, 2H, 2-H), 6.62 (d, *J* = 2.7 Hz, 1H, 6-H), 6.75 (dd, *J* = 8.6/2.7 Hz, 1H, 8-H), 7.12 (d, *J* = 8.2 Hz, 2H, 3-H toluenesulfonyl and 5-H toluenesulfonyl), 7.45 (d, *J* = 8.3 Hz, 2H, 2-H toluenesulfonyl and 6-H toluenesulfonyl), 7.48 ppm (d, *J* = 8.8 Hz, 1H, 9-H); $C_{18}H_{19}NO_4S$ (345.1).

7-Methoxy-3-(4-methylphenylsulfonyl)-2,3,4,5-tetrahydro-1*H*-3-benzazepin-1-ol (11): Ketone **9** (1.0 g, 2.90 mmol) was suspended in abs CH_3OH (15 mL) and $NaBH_4$ (0.230 g, 6.1 mmol) was added in several portions. After stirring for 2 h at room temperature, the solvent was evaporated in vacuo. H_2O (30 mL) was added to the residue, and the mixture was extracted with $CHCl_3$ (4×30 mL). The organic layer was washed with H_2O (3×40 mL), dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by FC (6 cm, CH_2Cl_2/Et_2O 9.5:0.5, 50 mL, R_f = 0.15) to afford **11** (0.98 g, 97%) as a colorless solid, mp: 98 °C; ^{1}H NMR ($CDCl_3$): δ = 2.39 (s, 3H, Ph-CH_3), 2.83 (dd, *J* = 15.1/7.2 Hz, 1H, 5-H), 3.08 (m, 1H, 4-H), 3.23 (d, *J* = 13.2 Hz, 1H, 2-H), 3.29 (m, 1H, 5-H), 3.61 (m, 1H, 4-H), 3.70 (dd, *J* = 12.9/7.0 Hz, 1H, 2-H), 3.76 (s, 3H, OCH_3), 4.83 (d, *J* = 6.3 Hz, 1H, 1-H), 6.62 (d, *J* = 2.4 Hz, 1H, 6-H), 6.69 (dd, *J* = 8.4/2.5 Hz, 1H, 8-H), 7.21 (d, *J* = 8.2 Hz, 1H, 9-H), 7.28 (d, *J* = 8.2 Hz, 2H, 3-H toluenesulfonyl and 5-H toluenesulfonyl), 7.65 ppm (d, *J* = 8.2 Hz, 2H, 2-H toluenesulfonyl and 6-H toluenesulfonyl) a signal for the OH proton is not visible; $C_{18}H_{21}NO_4S$ (347.1).

7-Methoxy-2,3,4,5-tetrahydro-1*H*-3-benzazepin-1-ol (12): Before use the surface of Mg turnings was activated by short treatment with 0.01 M HCl to remove MgO and subsequent washing with H_2O, dry CH_3OH, and dry Et_2O.[30] Mg turnings (2.83 g, 0.12 mol) were added to a solution of **11** (1.85 g, 5.33 mmol) in abs CH_3OH (100 mL), and the mixture was heated at reflux for 5 h. Then, under cooling with ice concd H_2SO_4 (6.55 mL) was added. The mixture was filtered and adjusted with NaOH to pH 9–10. The aqueous layer was extracted with CH_2Cl_2 (5×30 mL) and dried (Na_2SO_4). The solvent was evaporated in vacuo, and the residue was purified by FC (4.5 cm, CH_2Cl_2/CH_3OH 19:1 and 2% NH_3, 65 mL, R_f = 0.11) to afford **12** (0.80 g, 78%) as a colorless solid, mp: 123 °C; ^{1}H NMR ($CDCl_3$): δ = 2.58 (dd, *J* = 15.3/5.9 Hz, 1H, 5-H), 2.72 (t, *J* = 12.1 Hz, 1H, 4-H), 2.79 (d, *J* = 12.2 Hz, 1H, 2-H), 3.12–3.27 (m, 3H, 2-H, 4-H, 5-H), 3.71 (s, 3H, OCH_3), 4.52 (d, *J* = 5.9 Hz, 1H, 1-H), 6.55–6.61 (m, 2H, 6-H and 8-H), 7.02–7.08 ppm (m, 1H, 9-H) signals for the NH and OH protons are not visible; ^{13}C NMR ($CDCl_3$): δ = 39.5 (1C, C5), 49.7 (1C, C4), 54.0 (1C, C2), 55.4 (1C, O*C*H$_3$), 74.4 (1C, C1), 110.3 (1C, C8), 117.1 (1C, C6), 130.2 (1C, C9), 135.9 (1C, C9a), 142.1 (1C, C5a), 159.1 ppm (1C, C7); $C_{11}H_{15}NO_2$ (193.2).

7-Methoxy-3-(4-phenylbutyl)-2,3,4,5,-tetrahydro-1*H*-3-benzazepin-1-ol (13 = WMS-1405): A mixture of the secondary amine **12** (100.0 mg, 0.52 mmol), CH_3CN (10 mL), $(n$Bu$)_4$NI (11.1 mg, 0.03 mmol), K_2CO_3 (575.0 mg, 4.16 mmol), and 1-chloro-4-phenylbutane (110.7 μL, 0.62 mmol) was heated at reflux for 48 h. It was then filtered, and the solvent was evaporated in vacuo. The residue was purified by FC (2 cm, *n*-hexane/EtOAc 8:2 and 1% *N*,*N*-dimethylethylamine, 10 mL, R_f = 0.15) to afford **13** (76.1 mg, 45%) as colorless solid, mp: 76 °C; ^{1}H NMR ($CDCl_3$): δ = 1.54–1.69 (m, 4H, N-CH_2-C*H*$_2$-C*H*$_2$-CH_2-Ph), 2.39 (t, *J* = 11.9 Hz, 1H, 4-H), 2.50 (d, *J* = 12.1 Hz, 1H, 2-H), 2.58–2.66 (m, 5H, N-C*H*$_2$-CH_2-CH_2-C*H*$_2$-Ph and 5-H), 2.99 (dd, *J* = 12.2/6.0 Hz, 1H, 4-H), 3.14–3.18 (m, 1H, 2-H), 3.26 (br t, *J* = 13.4 Hz, 1H, 5-H), 3.77 (s, 3H, OCH_3), 4.57 (d, *J* = 6.7 Hz, 1H, 1-H), 6.64–6.66 (m, 2H, 6-H and 8-H), 7.09 (d, *J* = 7.8 Hz, 1H, 9-H), 7.17–7.20 (m, 3H, 3-H phenyl, 4-H phenyl and 5-H phenyl), 7.27–7.04 ppm (m, 2H, 2-H phenyl and 6-H phenyl) a signal for the OH proton is not visible; ^{13}C NMR ($CDCl_3$): δ = 26.8 (1C, N-CH_2-*C*H$_2$-CH_2-CH_2-Ph), 29.4 (1C, N-CH_2-*C*H$_2$-CH_2-CH_2-Ph), 36.0 (1C, N-CH_2-CH_2-*C*H$_2$-CH_2-Ph), 37.1 (1C, C5), 55.4 (1C, O*C*H$_3$), 56.3 (1C, C4), 59.9 (1C, N-*C*H$_2$-CH_2-CH_2-CH_2-Ph), 61.0 (1C, C2), 72.6 (1C, C1), 110.4 (1C, C6), 116.8 (1C, C8), 126.0 (1C, C4 phenyl), 128.6 (2C, C3 phenyl and C5 phenyl), 128.6 (2C, C2 phenyl and C6 phenyl), 130.0 (1C, C9), 135.7 (1C, C9a), 141.4 (1C, C1 phenyl), 142.5 (1C, C5a), 159.1 ppm (1C, C7); HRMS (ESI): calcd for $C_{21}H_{28}NO_2$ 326.2052, found 326.2115 $[M+H]^+$; $C_{21}H_{27}NO_2$ (325.2).

7-Methoxy-3-(5-phenylpentyl)-2,3,4,5-tetrahydro-1*H*-3-benzazepin-1-ol (17): A mixture of **12** (103.9 mg, 0.54 mmol), CH_3CN (10 mL), K_2CO_3 (431.2 mg, 3.12 mmol), TBAI (144.1 mg, 0.39 mmol), and 1-chloro-5-phenylpentane (104.5 μL, 0.58 mmol) was heated at reflux for 39 h. It was then filtered, and the solvent was evaporated in vacuo. The residue was purified by FC (3 cm, *n*-hexane/EtOAc 7:3 and 1% *N*,*N*-dimethylethylamine, 30 mL, R_f = 0.28) to afford **17** (170.3 mg, 93%) as colorless oil; ^{1}H NMR ($CDCl_3$): δ = 1.31–1.38 (m, 2H, N-CH_2-CH_2-C*H*$_2$-CH_2-CH_2-Ph), 1.48–1.56 (m, 2H, N-CH_2-CH_2-CH_2-C*H*$_2$-CH_2-Ph), 1.58–1.67 (m, 2H, N-CH_2-C*H*$_2$-CH_2-CH_2-CH_2-Ph), 2.38 (t, *J* = 11.8 Hz, 1H, 4-H), 2.48 (d, *J* = 12.0 Hz, 1H, 2-H), 2.53–2.65 (m, 5H, 5-H and N-C*H*$_2$-CH_2-CH_2-CH_2-C*H*$_2$-Ph), 2.98 (ddt, *J* = 12.3/6.1/2.1 Hz, 1H, 4-H), 3.14 (ddd, *J* = 12.0/6.8/1.9 Hz, 1H, 2-H), 3.24 (br t, *J* = 12.5 Hz, 1H, 5-H), 3.76 (s, 3H, OCH_3), 4.55 (d, *J* = 6.7 Hz, 1H, 1-H), 6.62–6.65 (m, 2H, 6-H and 8-H), 7.10 (d, *J* = 7.8 Hz, 1H, 9-H), 7.14–7.18 (m, 3H, 3-H phenyl, 4-H phenyl and 5-H phenyl), 7.25–7.29 ppm (m, 2H, 2-H phenyl and 6-H phenyl) a signal for the OH proton is not visible; $C_{22}H_{29}NO_2$ (339.2).

3-Benzyl-7-methoxy-2,3,4,5-tetrahydro-1*H*-3-benzazepin-1-ol (28): A solution of **12** (100.0 mg, 0.52 mmol) in 1,2-dichloroethane (1 mL) was treated with benzaldehyde (188.9 μL, 0.52 mmol) and $NaBH(OAc)_3$ (164.8 mg, 0.78 mmol). The mixture was stirred for 3 h at room temperature. A saturated solution of $NaHCO_3$ (10 mL) and H_2O (10 mL) were then added, the layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (3×10 mL). The combined organic layers were dried (Na_2SO_4), the solvent was evaporated in vacuo, and the residue was purified by FC (3 cm, *n*-hexane/EtOAc 8:2 and 1% *N*,*N*-dimethylethylamine, 20 mL, R_f = 0.25) to afford **28** (111.9 mg, 76%) as a colorless oil; ^{1}H NMR ($CDCl_3$): δ = 2.43 (t, *J* = 11.9 Hz, 1H, 4-H), 2.57 (d, *J* = 12.0 Hz, 1H, 2-H), 2.71 (dd, *J* = 15.2/

5.8 Hz, 1H, 5-H), 3.11 (dd, J=12.2/6.1 Hz, 1H, 4-H), 3.21–3.32 (m, 2H, 2-H and 5-H), 3.78 (s, 2H, N-CH_2-Ph), 3.83 (s, 3H, OCH_3), 4.64 (d, J=6.7 Hz, 1H, 1-H), 6.69–6.72 (m, 2H, 6-H and 8-H), 7.15 (d, J=8.1 Hz, 1H, 9-H), 7.27–7.29 (m, 1H, 4-H phenyl), 7.41–7.43 ppm (m, 4H, 2-H phenyl, 3-H phenyl, 5-H phenyl and 6-H phenyl) a signal for the OH proton is not visible; $C_{18}H_{21}NO_2$ (283.2).

***cis*- and *trans*-7-Methoxy-3-(4-phenylcyclohexyl)-2,3,4,5-tetrahydro-1*H*-3-benzazepin-1-ol (*cis*-31/*trans*-31):** $NaBH(OAc)_3$ (136.1 mg, 0.64 mmol) was added to a solution of **12** (82.7 mg, 0.43 mmol) in 1,2-dichloroethane (2 mL), 4-phenylcyclohexanone (61.3 μL, 0.52 mmol), and CH_3CO_2H (36.6 μL, 0.64 mmol), and the reaction mixture was stirred for 3 h at room temperature. A saturated solution of $NaHCO_3$ (10 mL) and H_2O (10 mL) were added, the layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (3×20 mL). The combined organic layers were dried (Na_2SO_4), and the solvent was evaporated in vacuo. The residue was purified by FC (2 cm, *n*-hexane/EtOAc 7:3 and 1% *N*,*N*-dimethylethylamine, 10 mL, R_f=0.15) to afford **31** (104.0 mg, 69%) as a colorless oil; ^{1}H NMR ($CDCl_3$): δ=1.39–1.54 (m, 4×0.3H, CH_2 cyclohexyl$^{\square}$), 1.56–1.67 (m, 3×0.7H, CH_2 cyclohexyl$^{\bullet}$), 1.69–1.80 (m, 3×0.7H, CH_2 cyclohexyl$^{\bullet}$), 1.85–1.98 (m, 4×0.3H, CH_2 cyclohexyl$^{\square}$), 2.16–2.22 (m, 2×0.7H, CH_2 cyclohexyl$^{\bullet}$), 2.42 (brt, J=11.9 Hz, 0.7H, 4-H$^{\bullet}$), 2.46 (d, J=12.0 Hz, 0.7H, 2-H$^{\bullet}$), 2.45–2.50 (m, 0.3H, 4-H$^{\square}$), 2.56 (d, J=12.1 Hz, 0.3H, 2-H$^{\square}$), 2.60–2.68 (m, 2.3H, 1-H cyclohexyl$^{\square}$, 1-H cyclohexyl$^{\bullet}$, 4-H cyclohexyl$^{\square}$, 5-H$^{\square}$ and 5-H$^{\bullet}$), 2.92 (quint, J=4.9 Hz, 0.7H, 4-H cyclohexyl$^{\bullet}$), 3.08 (ddt, J=12.4/5.8/2.2 Hz, 0.7H, 4-H$^{\bullet}$), 3.05–3.13 (m, 0.3H, 4-H$^{\square}$), 3.17–3.28 (m, 2H, 2-H and 5-H), 3.75 (s, 3×0.7H, $OCH_3{}^{\bullet}$), 3.76 (s, 3×0.3H, $OCH_3{}^{\square}$), 4.51 (d, J=6.7 Hz, 0.7H, 1-H$^{\bullet}$), 4.54 (d, J=6.7 Hz, 0.3H, 1-H$^{\square}$), 6.59–6.60 (m, 1H, 6-H$^{\bullet}$ and 6-H$^{\square}$), 6.61 (dd, J=7.8/2.5 Hz, 0.7H, 8-H$^{\bullet}$), 6.63 (dd, J=9.0/2.5 Hz, 0.3H, 8-H$^{\square}$), 7.06 (d, J=7.8 Hz, 0.7H, 9-H$^{\bullet}$), 7.09 (d, J=9.0 Hz, 0.3H, 9-H$^{\square}$), 7.10–7.19 (m, 1.6H, 4-H phenyl$^{\bullet}$, 4-H phenyl$^{\square}$, 2-H phenyl$^{\square}$ and 6-H phenyl$^{\square}$), 7.25–7.28 (m, 0.6H, 3-H phenyl$^{\square}$ and 5-H phenyl$^{\square}$), 7.29–7.30 ppm (m, 2.8H, 2-H phenyl$^{\bullet}$, 3-H phenyl$^{\bullet}$, 5-H phenyl$^{\bullet}$ and 6-H phenyl$^{\bullet}$) a signal for the OH proton is not visible; the ratio of *cis*-**31**($^{\bullet}$)/*trans*-**31**($^{\square}$) is 70:30; $C_{23}H_{29}NO_2$ (351.2).

Pharmacological studies

Materials and general procedures: *Centrifuge*: High-speed cooling centrifuge model Sorvall RC-5C plus (Thermo Finnigan). *Filter*: Printed Filtermat Type B (PerkinElmer), pre-soaked in 0.5% aqueous polyethylenimine for 2 h at room temperature before use. The filtration was carried out with a MicroBeta FilterMate-96 Harvester (PerkinElmer). Scintillation analysis was performed using a Meltilex (Type A) solid scintillator (PerkinElmer). The scintillation was measured using a MicroBeta Trilux scintillation analyzer (PerkinElmer). The overall counting efficiency was 20%.

Cell culture and preparation of membrane homogenates for the NR2B assay:[38] In the assay, mouse L(tk−) cells stably transfected with the dexamethasone-inducible eukaryotic expression vectors pMSG NR1a, pMSG NR2B at a 1:5 ratio were used. The transformed L(tk−) cells were grown in modified Earl's medium (MEM) containing 10% standardized fetal calf serum (FCS; Biochrom AG, Berlin, Germany). The expression of the NMDA receptor at the cell surface was induced after the adherent growing cells had reached a confluency of ~90%. For induction, the original growth medium was replaced by growth medium containing 4 μM dexamethasone and 4 μM ketamine (final concentrations). After 24 h the cells were harvested by scraping and centrifugation (10 min, 5000 *g*, Hettich Rotina 35R centrifuge, Tuttlingen, Germany).

For the binding assay, the cell pellet was resuspended in phosphate-buffered saline (PBS), and the number of cells was determined using an improved Neubauer's counting chamber (VWR, Darmstadt, Germany). The cells were subsequently lysed by sonication (4 °C, 6×10 s cycles with breaks of 10 s, device: Soniprep 150, MSE, London, UK). The resulting cell fragments were centrifuged with a high-performance cooled centrifuge (20000 *g*, 4 °C, Sorvall RC-5 plus, Thermo Scientific). The supernatant was discarded, and the pellet was resuspended in a defined volume of PBS yielding cell fragments of ~500000 cells mL^{-1}. The suspension of membrane homogenates was sonicated again (4 °C, 2×10 s cycles with a break of 10 min) and stored at −80 °C.

NR2B binding assay:[38] The competitive binding assay was performed with the radioligand [^{3}H]ifenprodil (60 Ci mmol^{-1}; Perkin–Elmer) using standard 96-well multiplates (Diagonal, Muenster, Germany). The thawed cell membrane preparation (~20 μg protein) was incubated with six different concentrations of test compounds, 5 nM [^{3}H]ifenprodil, and Tris/EDTA buffer (5 mM/1 mM, pH 7.5) in a total volume of 200 μL for 120 min at 37 °C. The incubation was terminated by rapid filtration through the presoaked filter mats using the cell harvester. After washing each well (5×300 μL H_2O), the filter mats were dried at 95 °C. Subsequently, the solid scintillator was placed on the filter mat and melted at 95 °C. After 5 min, the solid scintillator was allowed to solidify at room temperature. The bound radioactivity trapped on the filters was counted in the scintillation analyzer. The nonspecific binding was determined with 10 μM unlabeled **1**. The K_d value of **1** is 7.6 nM.[38]

Affinity toward σ_1 and σ_2 receptors and the PCP binding site of the NMDA receptor: The receptor binding studies were performed as previously described.[40,41]

Excitotoxicity assay:[42] Briefly, L(tk−) cells stably expressing either NR1–1a/NR2A (L12-G10 cells) or NR1–1a/NR2B (L13-E6 cells) were seeded in 96-well microtiter plates in MEM containing 10% FCS, 0.5 mM sodium pyruvate, penicillin/streptomycin (100 U/100 μg mL^{-1}), 100 μM ketamine, and 160 μM G418 in a humidified incubator at 37 °C and 5% CO_2. To perform excitotoxicity assays ketamine was removed by washing the cells in MEM lacking phenol red. Then compounds (volume: 200 μL; concentration range: 1 nM–10 μM, dissolved in DMSO) diluted in MEM without phenol red were added to each well (final DMSO content: 0.1%). After 30 min a mixture of (*S*)-glutamate and glycine (10 μM each) was added, and the cells were further incubated for 4 h. Excitotoxicity was determined by detection of LDH release from the cells into the culture supernatant using the Cytotoxicity Detection Kit® (Roche Diagnostics, Mannheim, Germany). Optical density was measured at 492 nm using the HTS 7000 Bioassay Reader (Perkin–Elmer) with background subtraction. Relative excitotoxicity values were calculated relative to a negative control (0% excitotoxicity is defined as LDH release in the absence of NMDA receptor agonist and in the presence 100 μM ketamine) and a positive control (100% excitotoxicity is defined as LDH release after addition of (*S*)-glutamate/glycine in the absence of ketamine). Experiments were repeated at least three times with six replicates per test concentration. IC_{50} values were calculated using GraphPad Prism v5.0. A direct inhibitory effect of compound **13** on LDH activity was excluded by performing the excitotoxicity assay in the presence of the test compound as described above; however, all cells were lysed by addition of 1% Triton X-100 prior to quantification of LDH activity. No effect of **13** was observed under this condition. Under standard assay conditions **13** had no effect on L(tk−) untransfected cells.

Supporting Information available: Purity data for all test compounds, physical and spectroscopic data for all new compounds, mechanism of the formation of tetrahydroisoquinoline **8**, general chemistry methods, and details of the pharmacological assays.

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