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Conformationally constrained NR2B selective NMDA receptor antagonists derived from ifenprodil: Synthesis and biological evaluation of tetrahydro-3-benzazepine-1,7-diols

Bastian Tewes [a], Bastian Frehland [a], Dirk Schepmann [a], Kai-Uwe Schmidtke [b], Thomas Winckler [b], Bernhard Wünsch [a,*]

[a] Institut für Pharmazeutische und Medizinische Chemie der Westfälischen Wilhelms-Universität Münster, Hittorfstraße 58-62, D-48149 Münster, Germany
[b] Institut für Pharmazie der Friedrich-Schiller-Universität Jena, Semmelweisstraße 10, D-07743 Jena, Germany

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ABSTRACT

NR2B selective NMDA receptor antagonists with tetrahydro-3-benzazepine-1,7-diol scaffold have been designed by formal cleavage and reconstitution of the piperidine ring of the lead compound ifenprodil (**1**). The secondary amine **10** represents the central building block for the synthesis of more than 25 tetrahydro-3-benzazepin-1-ols. Generally 7-hydroxy derivatives display higher NR2B receptor affinities than the corresponding 7-benzyloxy compounds. A distance of four atoms (five bond lengths) between the basic amino group and the terminal aryl moiety led to highest NR2B affinity. 3-(4-Phenylbutyl)-2,3,4,5-tetrahydro-1*H*-3-benzazepine-1,7-diol (WMS-1410, **25**) represents the most promising NR2B antagonist of this series showing a K_i-value of 14 nM. Compound **25** reveals excellent selectivity over more than 100 further relevant target proteins, antagonizes glutamate induced excitotoxicity (IC_{50} = 18.4 nM) and is metabolically more stable than ifenprodil. Up to a dose of 100 mg/kg **25** is well tolerated by mice and it shows dose dependent analgesic activity in the late neuropathic pain phase of the formalin assay.

1. Introduction

In the mammalian central nervous system (CNS), the *N*-methyl-D-aspartate (NMDA) receptor associated ion channel plays a central role in physiological processes, such as neuronal development, synaptic plasticity, learning, and memory. The NMDA receptor represents a ligand gated ion channel controlling the flow of Ca^{2+}-, Na^+-, and K^+-ions across neuronal cell membranes. According to the concentration gradient Ca^{2+}- and Na^+-ions penetrate into the neuron, whilst K^+-ions leave the cells. The NMDA receptor is activated by simultaneous binding of the physiological agonist (*S*)-glutamate and the coagonist glycine. In addition to binding of two agonists, a predepolarization of the cell membrane is required before opening of the ion channel takes place. This depolarization can be achieved by activation of further ionotropic glutamate receptors, like AMPA or kainate receptors, in the vicinity of the NMDA receptor. The depolarization opens the ion channel by removal of Mg^{2+}-ions, which are bound within the channel pore and block the passage of cations in particular the influx of Ca^{2+}-ions.[1–3]

Overactivation of the NMDA receptor caused by excessive glutamate release leads to an unphysiologically high intracellular Ca^{2+}-concentration, which results in uncontrolled activation of various Ca^{2+}-dependent enzymes, neuronal damage and at the end cell death. This process of excitotoxicity occurs during many acute adverse events (e.g., traumatic brain injury, stroke) and chronic neurodegenerative disorders (e.g., Alzheimer's disease, Parkinson's disease, Huntington's disease). Due to its central role in these processes the NMDA receptor represents an interesting target for the development of novel drugs.[1–3]

A functional NMDA receptor is formed by four subunits (heterotetramer).[4] Three types of subunits have been identified: The NR1 subunit with eight splice variants (NR1a-h), the NR2 subunit, which exists in four distinct subtypes (NR2A-D) encoded by four distinct genes, and the NR3 subunits NR3A and NR3B (two genes). At least one NR1 subunit bearing the glycine binding site and one NR2 subunit responsible for glutamate binding are required in a functional NMDA receptor. Whereas the NR1 subunit is expressed ubiquitously 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. NR3 subunits are expressed predominantly in the

* Corresponding author. Tel.: +49 251 8333311; fax: +49 251 8332144.
E-mail address: wuensch@uni-muenster.de (B. Wünsch).

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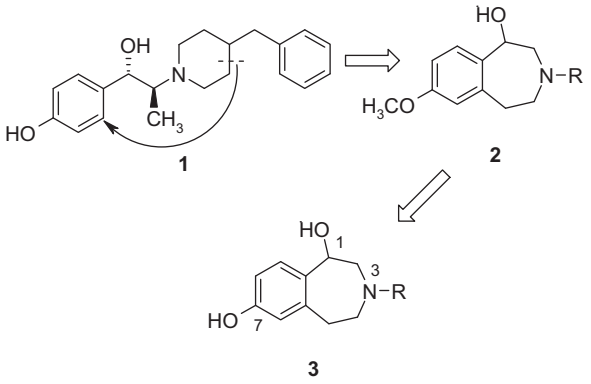


Figure 1. Ifenprodil (**1**) and tetrahydro-3-benzazepines **2** as lead compounds for the development of novel NR2B selective NMDA receptor antagonists of type **3**.

developing CNS and seem to be of low relevance in the adult brain.[5,6]

Non-selective NMDA receptor antagonists, like ketamine, phencyclidine, and CPP (3-(3-carboxypiperazin-1-yl)propane-1-phosphonic acid) have been reported to produce symptomatic relief in a number of neuropathic disorders, including postherpetic neuralgia, central pain caused by spinal cord injury and phantom limb pain.[7–10] However, at analgesic doses these drugs also induce inacceptable side effects, including hallucinations, dysphoria, and cognitive and motor dysfunctions, which limit their use.[11]

Due to the restricted expression of the NR2B subunit in the CNS, NMDA receptor antagonists selectively addressing NR2B subunit containing NMDA receptors should show an improved side effect profile. In particular, expression of the NR2B subunit in the cerebellum is rather low and, therefore, cognitive side effects should be minimized with NR2B selective antagonists.[11] Potential therapeutic applications of NR2B selective NMDA receptor antagonists are Parkinson's disease,[12] traumatic brain injury,[13] stroke,[14] migraine,[15] alcohol-withdrawal,[16] and chronic and neuropathic pain.[11]

The lead compound for this project was ifenprodil (**1**, Fig. 1), which blocks NMDA receptors by selective interaction with the ifenprodil binding site on the NR2B subunit. The ifenprodil binding site is close to the polyamine binding site, but not completely identical with it. Neither the NMDA receptor nor the NR2B subunit have been crystallized so far to prove the exact structure of the binding site of NR2B selective ligands. However a molecular modeling study using the X-ray crystal structure of the similar bacterial protein leucine, isoleucine, valine binding protein (LIVBP) as template, showed the NR2B receptor antagonist Ro 25-6981 docked into the modeled binding pocket of the NR2B subunit and the corresponding crucial interactions.[17]

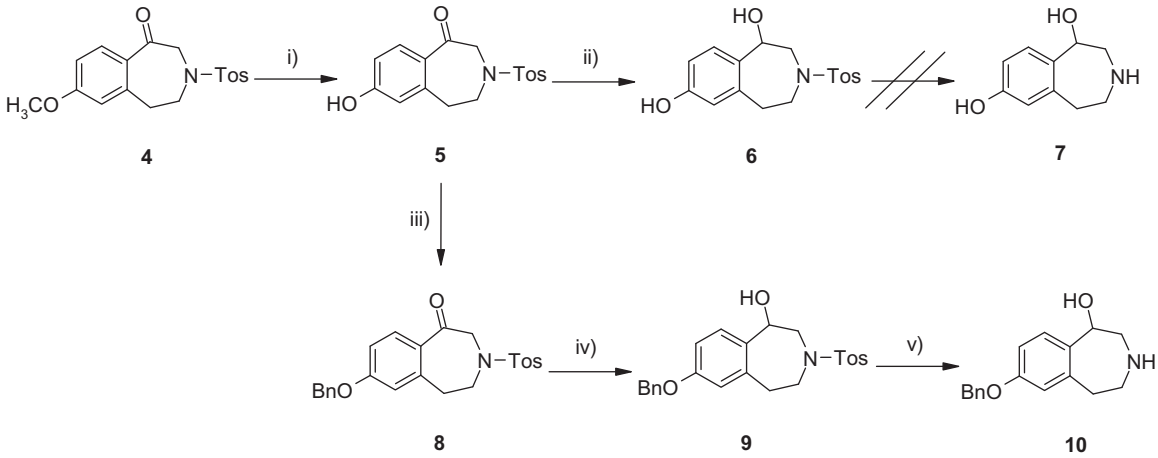
Originally, **1** has been developed as an α_1 adrenoceptor antagonist. However, it was shown that **1** also interacts with several other types of receptors and ion channels, including 5-HT_{1A}, 5-HT_2 and both σ_1 and σ_2 receptors.[3,18–20] Altogether, **1** is a nonselective drug with side effects such as hypotension, cognitive problems and neurotoxicity.[20] In addition to the side effects, the bioavailability of **1** is low due to the fast and extensive metabolic degradation.[21,22]

Formal cleavage and reconstitution of a bond in the piperidine ring of **1** lead to tetrahydro-3-benzazepines **2** (Fig. 1). Since the methyl moiety in the side chain of ifenprodil is not essential for the interaction with NR2B receptors, it was omitted in the first series of 3-benzazepines **2**. Very recently we have reported on the synthesis of tetrahydro-3-benzazepines **2** with a methoxy moiety in position 7.[23] The most potent compounds in this new class of NR2B antagonists are bearing ω-arylalkyl residues with preferably four methylene moieties between the basic N-atom of the 3-benzazepine ring and the terminal phenyl moiety. In particular, the NR2B affinity of **2a** (WMS-1405, R = 4-phenylbutyl, K_i = 5.4 nM) is higher than the NR2B affinity of **1** (K_i = 10 nM).[23] In contrast to **1**, the tetrahydro-3-benzazepine **2a** displayed high selectivity against α_1, 5-HT_{1A}, 5-HT_2, σ_1 and σ_2 receptors.

In order to come closer to the structure of the lead compound **1** the methoxy group in position 7 of the 3-benzazepines **2** should be replaced by a hydroxy group in a new series of NR2B antagonists. The resulting phenols **3** contain the same H-bond-donor group as **1**. Since an H-bond donor group in position 7 is described to be essential for high NR2B affinity[18] the phenols **3** should interact with high affinity with the NR2B subunit.

2. Chemistry

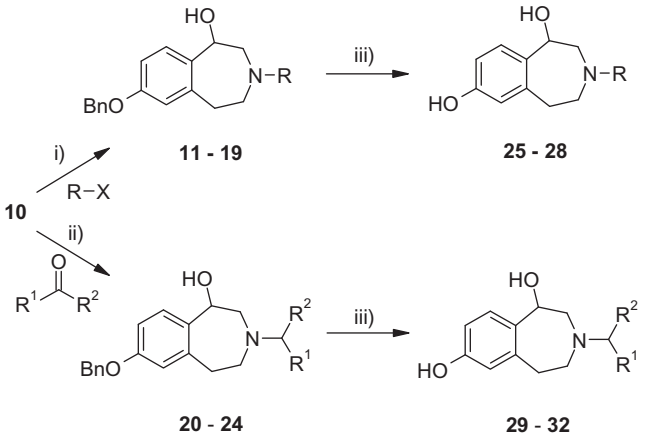
The designed phenols **3** were synthesized starting from 7-methoxy-3-benzazepin-1-one **4**[23] (Scheme 1). At first the methyl ether of **4** was cleaved with the Lewis acid $AlCl_3$ in refluxing CH_2Cl_2 to afford the phenol **5** in 68% yield. After reduction of the ketone **5** with $NaBH_4$ the diol **6** was obtained in 93% yield. However, all attempts to remove the tosyl protecting group of **6** failed to produce the secondary amine **7** (e.g., Mg in methanol, reflux, decomposition). Since the cleavage of the tosyl group of the corresponding methoxy derivative had been successful,[23] we assumed that the



Scheme 1. Reagents and conditions: (i) $AlCl_3$, CH_2Cl_2, reflux, 23 h, 68%; (ii) $NaBH_4$, CH_3OH, rt, 18 h, 93%; (iii) benzyl bromide, acetone, K_2CO_3, reflux, 4 h, 85%; (iv) $NaBH_4$, CH_3OH, rt, 6 h, 98%; (v) Mg, CH_3OH, reflux, 5 h, 83%.

unstable diol substructure of **6** (phenylogous hydrate) inhibits the removal of the N-protecting group.

Therefore, the phenolic hydroxy group had to be supplied with a new protecting group. For this purpose the benzyl group was selected, because it can easily be attached, is stable during various reaction conditions, and can be removed selectively upon hydrogenolysis. Reaction of phenol **5** with benzyl bromide in the presence of K_2CO_3 provided the benzyl ether **8**, which was reduced with



Scheme 2. Reagents and conditions: (i) R-X (see Table 1), CH_3CN, K_2CO_3, Bu_4NI, reflux, 8–72 h; (ii) different ketones and an aldehyde (see Table 2), $NaBH(OAc)_3$, 1,2-dichloroethane, rt, 16 h; (iii) H_2 (1 bar), Pd/C, CH_3OH or THF, rt, 30 min–16 h (see Table 3).

Table 1
Synthesis of 7-benzyloxy-3-benzazepines by alkylation of **10** with RX

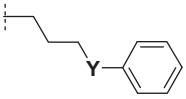
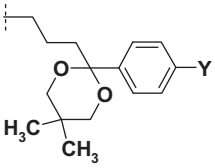
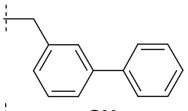
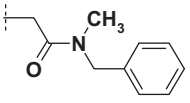
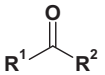
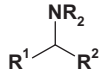
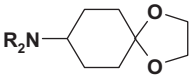
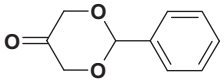
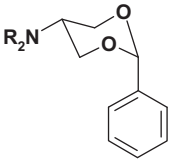
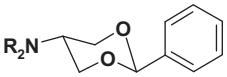
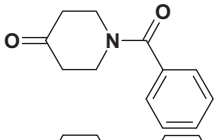
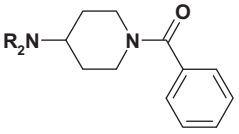
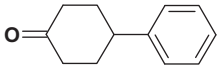
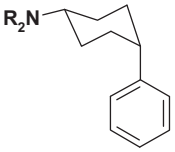
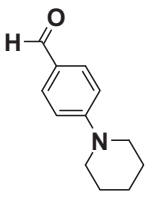
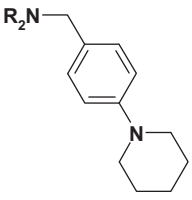
Compd	R	Y	X	Yield (%)
11 (WMS-1418)		CH_2	Cl	69
12		O	Br	79
13		S	Cl	91
14		SO_2	Cl	90
15		*tert*-Butyl	Cl	74
16		OCH_3	Cl	76
17		F	Cl	90
18		–	Br	65
19		–	Cl	93

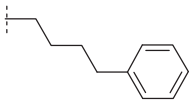
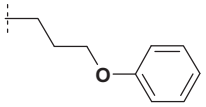
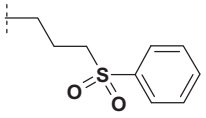
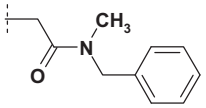
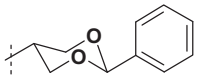
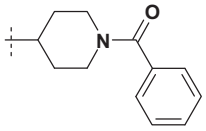
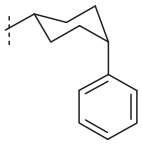
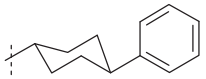
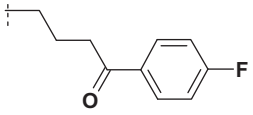
Table 2
Synthesis of 7-benzyloxy-3-benzazepines by reductive alkylation of **10** with $R^1C(=O)R^2$ and $NaBH(OAc)_3$

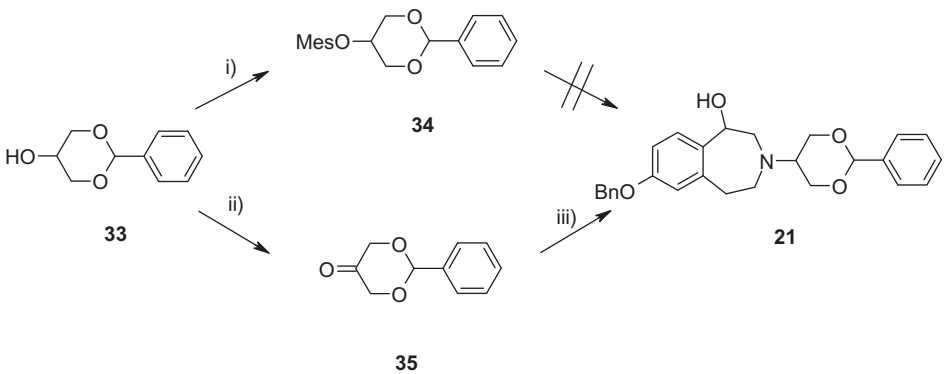
Compd			Yield (%)
20			80
cis-**21**			41
trans-**21**			
22			43
cis-**23**			71
trans-**23**			14
24			93

$NaBH_4$ to obtain the alcohol **9**. Treatment of the sulfonamide **9** with magnesium in boiling methanol gave the secondary amine **10** (83%), which represents the central building block of this project (Scheme 1).

In order to find reliable structure NR2B affinity relationships a large number of tetrahydro-3-benzazepines with various N-substituents was prepared. Since the 4-phenylbutyl derivative **2a** with the same N-Ph-distance as the lead compound **1** (see Fig. 1)

Table 3
Synthesis of 7-hydroxy-3-benzazepines by hydrogenolysis of 7-benzyloxy-3-benzazepines

Educt	Product	R	Reaction time	Solvent	Yield (%)
11	**25** (WMS-1410)		30 min	CH_3OH	67
12	**26**		2 h	CH_3OH	63
14	**27**		16 h	CH_3OH	75
19	**28**		1 h	CH_3OH	68
20	**29**		1 h	CH_3OH	63
cis-**21**/*trans*-**21** (1:1)	*trans*-**30**		1 h	CH_3OH	93
22	**31**		2 h	CH_3OH	60
cis-**23**	*cis*-**32**		2 h	CH_3OH	74
trans-**23**	*trans*-**32**		2 h	THF	65
38	**39**		1 h	CH_3OH	64



Scheme 3. Reagents and conditions: (i) methanesulfonyl chloride, NEt_3, CH_2Cl_2, rt, 16 h, 99%; (ii) Dess–Martin periodinane, CH_2Cl_2, rt, 3 h, 28%; (iii) **10**, $NaBH(OAc)_3$, 1,2-dichloroethane, rt, 16 h, 41%.

showed the highest NR2B affinity in the series of 7-methoxytetrahydro-3-benzazepines,[23] substituents with a spacer of 4–5 methylene groups (or heteroatom equivalents) were preferably selected. The tetrahydro-3-benzazepines **11**–**19** were synthesized by alkylation of **10** with various haloalkanes (Scheme 2 and Table 1). In case of chloro derivatives Bu_4NI was added to improve the conversion by in situ generation of iodoalkanes (Finkelstein reaction).

Alternatively, tetrahydro-3-benzazepines **20**–**24** were synthesized by reductive alkylation of secondary amine **10** with different ketones and an aldehyde in the presence of $NaBH(OAc)_3$ (Scheme 2 and Table 2).[24] In order to increase the reactivity of the ketone acetic acid was added to the reaction mixture during the reductive alkylation of **10** with 4-phenylcyclohexanone. In this case two diastereomers *cis*-**23** and *trans*-**23** were formed in the ratio 1:1, which were separated by fc. The relative configuration of *cis*-**23** and *trans*-**23** was unequivocally assigned by ^1H NMR spectroscopy.

For the synthesis of the 2-phenyl-1,3-dioxan-5-yl substituted 3-benzazepine **21** the 1,3-dioxan-5-ol **33** was converted into mesylate **34** (Scheme 3). However, all attempts to react the secondary amine **10** with the mesylate **34** failed to give the substitution product **21**. Therefore, the alcohol **33** was oxidized with Dess–Martin Periodinane[25] to produce the ketone **35**, which reacted smoothly with the secondary amine **10** and $NaBH(OAc)_3$ to yield the dioxanyl derivative **21** as 1:1-mixture of *cis*/*trans*-diastereomers.

In the final step the benzyl ether of the tetrahydro-3-benzazepines was cleaved by hydrogenolysis with H_2 (1 bar) and Pd/C to yield the phenols **25**–**32** (Table 3). After hydrogenolysis of the diastereomeric mixture *cis*-**21** and *trans*-**21** (1:1) the ^1H NMR spectrum of the product shows only one set of signals for *trans*-**30**. The *trans*-configuration was proved by NOE difference spectroscopy. The fact that after hydrogenolysis of a 1:1 mixture of diastereomers *cis*-**21** and *trans*-**21**, only the *trans*-isomer *trans*-**30** was isolated in a yield of 93%, can only be explained by a Pd catalyzed isomerization of the acetalic substructure to end up with the thermodynamically more stable *trans*-isomer *trans*-**30**.

In order to increase the diversity of substituents, the acetals **15**–**17** were hydrolyzed with dilute HCl to yield the ketones **36**–**38**. Hydrogenolytic cleavage of the benzyl protecting group of **38** led to phenol **39** (Scheme 4). In particular the haloperidol like N-residue of **38** and **39** was selected, because haloperidol also interacts with NR2B containing NMDA receptors.[26,27]

3. Receptor affinity

3.1. Affinity toward NR2B containing NMDA receptors

The NR2B receptor affinities of the synthesized tetrahydro-3-benzazepines **11**–**32** and **36**–**39** were determined in a competitive receptor binding assay recently developed in our group.[28] In this assay tritium labeled ifenprodil was employed as radioligand. Membrane homogenates prepared upon ultrasonication of L(tk-)-cells stably expressing recombinant human NR1a/NR2B receptors served as receptor material.[29] 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 receptor antagonist ketamine (phencyclidine binding site) to the growth medium.

The receptor affinities of 7-benzyloxy-3-benzazepines are summarized in Table 4. Most of the 7-benzyloxy-3-benzazepines display only moderate affinity to the ifenprodil binding site of the NMDA receptor. For example, the NR2B affinity of the 4-phenylbutyl derivative **11** (K_i = 187 nM) is about 35-fold lower than the NR2B affinity of the corresponding methoxy derivative **2a** (K_i = 5.4 nM).

The bioisosteric replacement of the methylene moiety in position 4 of the 4-phenylbutyl side chain of **11** with an O- (**12**), S-atom (**13**) or a SO_2-group (**14**) led to increased N2RB affinity. In case of the sulfone **14** (K_i = 37 nM) the NR2B affinity is fivefold increased compared with the affinity of 4-phenylbutyl derivative **11**.

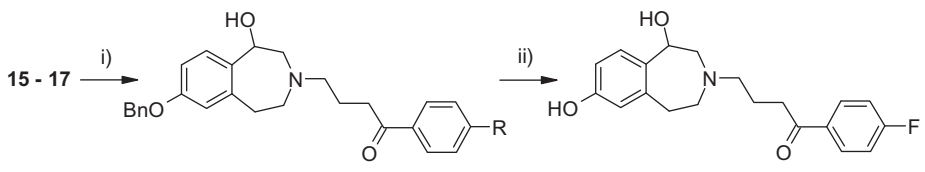
In the 4-phenylcyclohexyl derivatives **23** a conformational restriction of the flexible side chain of the 4-phenylbutyl derivative **11** is realized. Both diastereomers *cis*-**23** (K_i = 139 nM) and *trans*-**23** (K_i = 389 nM) show similar NR2B receptor affinities as the flexible 4-phenylbutyl derivative **11** do, although *cis*-**23** is slightly more potent than *trans*-**23**. The comparable affinities of **11** and **23** indicate that a conformationally constrained side chain is well accepted by the ifenprodil binding site of the NR2B subunit.

The receptor affinities of phenolic tetrahydro-3-benzazepines are summarized in Table 5. In general tetrahydro-3-benzazepines with a phenolic OH moiety in position 7 show considerably higher NR2B affinities than the corresponding benzyl ethers (Table 4) and methyl ethers.[23] This observation is in good accordance with reported results,[18] showing that an H-bond donor group, for example, a OH group, is favorable for high NR2B affinity.

The NR2B affinities of the 4-phenylbutyl derivative WMS-1410 (**25**, K_i = 14 nM) and its bioisosteric phenoxypropyl derivative **26** (K_i = 20 nM) are in the same range as the NR2B affinity of ifenprodil (K_i = 10 nM). Surprisingly the methyl ether **2a** (K_i = 5.4 nM) is slightly more potent than the phenols **25** and **26**.

Whereas introduction of a sulfonyl moiety (**27**) or a rigid amide substructure (**28**) into the N-side chain led to considerable loss of NR2B affinity, a carbonyl moiety in the side chain (**39**) is tolerated by the ifenprodil binding site. The N-residue of **29** without a phenyl group decreased the NR2B affinity.

The most promising NR2B receptor antagonists of this series of compounds are the diastereomeric 4-phenylcyclohexyl derivatives

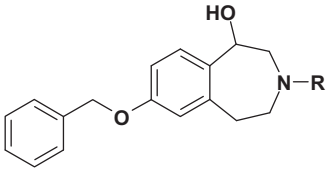


36 - 38 39

Compound	R
15, 36	*tert*-Butyl
16, 37	OCH_3
17, 38	F

Scheme 4. Reagents and conditions: (i) 1 M HCl, Et_2O, rt, 16 h, 69% (**36**), 80% (**37**), 76% (**38**); (ii) H_2 (1 bar), Pd/C, CH_3OH or THF, rt, 1 h, 64% (see Table 3).

Table 4
Affinities of 7-benzyloxy-3-benzazepin-1-ols to the ifenprodil binding site of NR2B containing NMDA receptors, the PCP binding site of the NMDA receptor as well as to σ_1 and σ_2 receptors



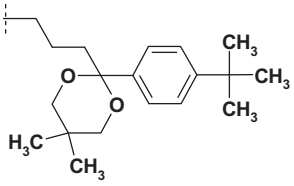
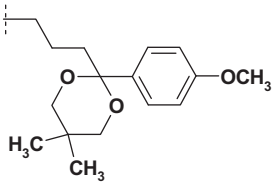
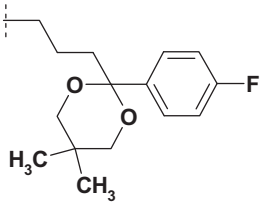
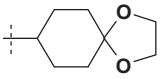
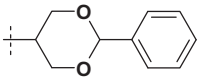
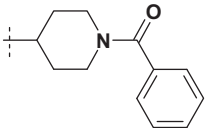
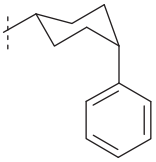
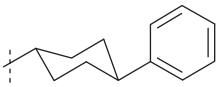
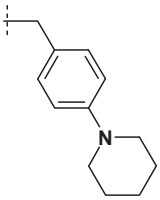
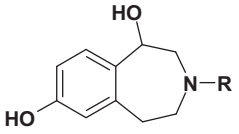
Compd	R	K_i ± SEM (nM)			
		NR2B	PCP	σ_1	σ_2
11	$-(CH_2)_4Ph$	187 ± 72	>1 μM	293 ± 58	1050
12	$-(CH_2)_3OPh$	93 ± 27	>1 μM	193	945
13	$-(CH_2)_3SPh$	41 ± 2.6	>1 μM	376	653
14	$-(CH_2)_3SO_2Ph$	37 ± 8.9	>1 μM	349	3040
15		>1 μM	>1 μM	>1 μM	900
16		661	>1 μM	>1 μM	>1 μM
17		1420	>1 μM	>1 μM	>1 μM
18	$-CH_2$-Ph-3-Ph	1210	>10 μM	>1 μM	>1 μM
19	$-CH_2CON(CH_3)CH_2Ph$	377 ± 53	>1 μM	>1 μM	2960
20		1120	>1 μM	183	753
21	 *cis*/*trans* (50:50)	1000	>1 μM	186 ± 74	>1 μM
22		>1 μM	>10 μM	>1 μM	>1 μM
cis-**23**		139 ± 29	>1 μM	10 ± 0.8	311
trans-**23**		388 ± 130	>1 μM	386	>1 μM

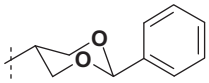
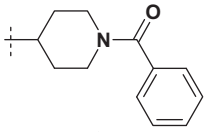
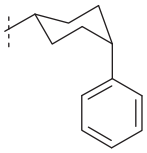
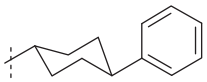
Table 4 (*continued*)

Compd	R	K_i ± SEM (nM)			
		NR2B	PCP	σ_1	σ_2
24		1310	>1 μM	604	6430
36	–$(CH_2)_3$CO-Ph-4-*tert*-butyl	644 ± 202	>1 μM	>1 μM	>1 μM
37	–$(CH_2)_3$CO-Ph-4-OCH_3	>1 μM	>1 μM	>1 μM	4500
38	–$(CH_2)_3$CO-Ph-4-F	151 ± 61	>1 μM	293 ± 58	1050
2a (WMS-1405)	–$(CH_2)_4$Ph 7-H_3CO instead of 7-BnO	5.4 ± 0.4	>10 μM	182 ± 38	554 ± 127
1	Ifenprodil	10 ± 0.7	—	125 ± 24	98.3 ± 34
	Eliprodil	13 ± 2.5	—	—	—
	Dexoxadrol	—	32 ± 7.4	—	—
	Haloperidol	—	—	6.3 ± 1.6	78 ± 2.3
	Di-*o*-tolylguanidine (DTG)	—	—	89 ± 29	58 ± 18

At a concentration of 1 μM or even 10 μM low-affinity compounds did not compete significantly with the radioligand. Therefore, the K_i-value is only estimated to be greater than 1 μM.

Table 5
Affinities of tetrahydro-3-benzazepine-1,7-diols to the ifenprodil binding site of NR2B containing NMDA receptors, the PCP binding site of the NMDA receptor as well as to σ_1 and σ_2 receptors



Compd	R	K_i ± SEM (nM)			
		NR2B	PCP	σ_1	σ_2
25	–$(CH_2)_4$Ph	14 ± 1.5	>1 μM	194	18,000
26	–$(CH_2)_3$OPh	20 ± 2.2	>1 μM	170 ± 51	5.9 ± 1.7
27	–$(CH_2)_3SO_2$Ph	>1 μM	>1 μM	>1 μM	653
28	–$CH_2CON(CH_3)CH_2$Ph	>1 μM	>1 μM	172	1580
29		>1 μM	>1 μM	>1 μM	658
trans-**30**		>1 μM	>1 μM	>1 μM	>1 μM
31		204 ± 95	>1 μM	>1 μM	98
cis-**32**		3.3 ± 1.0	>1 μM	123 ± 11	8.6 ± 2.5
trans-**32**		9.0 ± 3.7	>1 μM	200 ± 36	8.9 ± 1.5
39	$(CH_2)_3$CO-Ph-4-F	36 ± 6.1	>1 μM	1050	33 ± 12

At a concentration of 1 μM low-affinity compounds did not compete significantly with the radioligand. Therefore, the K_i-value is only estimated to be greater than 1 μM.

cis-**32** (K_i = 3.3 nM) and *trans*-**32** (K_i = 9.0 nM) binding in the low nanomolar range. As described for the benzyl ethers **23** the *cis*-isomer is slightly more potent than the trans-isomer. Obviously the conformationally restricted 4-phenylcyclohexyl residue is well tolerated by NR2B containing receptors. However, this system appeared to be rather sensitive, since replacement of two methylene moieties of the cyclohexane ring of *trans*-**32** by two O-atoms (*trans*-**30**) led to almost complete loss of NR2B affinity. The high electronegativity and/or the additional H-bond acceptor properties of the O-atoms may be responsible for the reduced affinity of *trans*-**30**. The benzamide **31** (K_i = 204 nM) with changed geometry and N-Ph-distance displays moderate NR2B affinity.

With respect to NR2B receptor affinity compounds **25** (K_i = 14 nM), **26** (K_i = 20 nM), *cis*-**32** (K_i = 3.3 nM), *trans*-**32** (K_i = 9 nM), and **39** (K_i = 36 nM) represent the five most potent tetrahydro-3-benzazepines of this series.

3.2. Receptor selectivity

In order to learn more about the receptor selectivity of this new class of NR2B antagonists (see also Ref. 23), the affinities of all test compounds to the phencyclidine (PCP) binding site of the NMDA receptor[30] and to both σ receptor subtypes (σ_1 and σ_2 receptor)[31–33] were investigated systematically in receptor binding studies with radioligands. In Tables 4 and 5 the PCP, σ_1 and σ_2 receptor affinities of the tetrahydro-3-benzazepines **11**–**32** and **36**–**39** are compared with their NR2B receptor affinities.

The 7-benzyloxy-3-benzazepines as well as the corresponding phenols do not interact significantly with the PCP binding site of the NMDA receptor. This result indicates a high selectivity for the ifenprodil binding site over the phencyclidine binding site of the NMDA receptor, at least for the most potent NR2B ligands.

The σ_1 receptor affinity of the tetrahydro-3-benzazepines has to be discussed differentially. Most of the compounds have low to moderate σ_1 affinity. However, the K_i-values (σ_1) of the benzyl ethers **11**, **12**, and *trans*-**23** are very similar to their K_i-values at the NR2B receptor indicating low receptor selectivity. Moreover, for the *cis*-configured phenylcyclohexyl derivative *cis*-**23** a σ_1 affinity of 10 nM was found, which is 14-fold higher than its NR2B affinity. On the other hand the most potent NR2B ligands with phenolic substructure, that is, **25**, **26**, *cis*-**32**, *trans*-**32**, and **39**, show a 10–40-fold selectivity for the NR2B receptor over the σ_1 receptor.

The σ_2 receptor affinities of most of the test compounds are very low. Unfortunately, the σ_2 receptor affinities of just the most potent NR2B ligands are remarkably high. Whereas the K_i-values (σ_2) of *trans*-**32** and **39** are identical with their NR2B K_i-values,

the σ_2 affinity of **26** even exceeds its NR2B affinity threefold (see Table 5). The most potent NR2B ligand of this series, that is, *trans*-**32**, shows only a slight preference for the NR2B receptor (2.5-fold). Apparently, conformational restriction of the flexible *N*-butyl side chain by a cyclohexane ring does not only increase the NR2B affinity but also the σ_2 (and σ_1) affinity, losing selectivity against σ receptors. Compound **25** with the rather flexible 4-phenylbutyl side chain is the only potent NR2B ligand of this series showing an excellent selectivity over the σ_2 receptor.

Taking NR2B affinity and selectivity over related receptor systems into account, the phenylbutyl derivative **25** (K_i = 14 nM) represents the most promising ligand of this study. Therefore, further pharmacological properties of **25** were investigated.

At first a receptor binding profile of **25** was performed. In competition experiments with radioligands the interaction of **25** with more than 100 relevant receptors, transporters and enzymes including α_1, D_1, D_2, κ-opioid, μ-opioid, δ-opioid, 5-HT_{1A}, 5-HT_2, σ_1, and σ_2 receptors, glutamate, glycine and PCP bindings sites of the NMDA receptor, histamine and noradrenalin transporter, cyclooxygenase, phosphodiesterase and some kinases was investigated. The complete list of considered targets and corresponding radioligands is given in Supplementary data. At a concentration of 1–3 μM **25** did not compete significantly with the employed radioligands. The only exception is a 56% inhibition of radioligand binding to the calcitonine gene-related peptide $CGRP_1$ at a compound concentration of 3 μM and, furthermore, an IC_{50}-value of 560 nM at hERG channel[34,35] was estimated for **25**. Altogether, this screening revealed very high selectivity of **25** for the ifenprodil binding site of NR2B containing NMDA receptors over the investigated systems.

These results are of particular interest, since the low receptor selectivity is one of the major drawbacks of the lead compound **1** (see Section 1). Obviously, the reduced conformational flexibility of the ethylene spacer in the 3-benzazepine system of **25** significantly reduces interaction with competing α_1 (IC_{50} = 5.3 μM), σ_1 (K_i = 194 nM), σ_2 (K_i > 10 μM), and PCP (no affinity) receptors. A similar result had been obtained with the methyl ether **2a**.[23]

4. Functional activity

In order to verify the antagonistic activity of the potent NR2B selective ligands **25** and *cis*-**32** the inhibition of the excitotoxicity induced by overactivation of NMDA receptors was investigated. In this assay L(tk-)-cells stably expressing NR1a/NR2B receptors were employed. After addition of (*S*)-glutamate and glycine the cell damage (excitotoxicity) was determined by measuring the amount of lactate dehydrogenase (LDH) released from the cells into the culture supernatant. Different concentrations of the test compounds **25** and *cis*-**32** were added 30 min before addition of (*S*)-glutamate and glycine and the released amount of LDH was measured.[36]

In Figure 2 the inhibition of glutamate/glycine induced excitotoxicity at different concentrations of phenols **25**, *cis*-**32** and methyl ether **2a** is compared. The phenylbutyl derivative **25** reduced the excitotoxicity with an IC_{50}-value of 18.4 nM. This result clearly shows that **25** is not only binding at the ifenprodil binding site of NR2B containing NMDA receptors but is also blocking the NMDA associated ion channel and producing antagonistic effects. We speculate that the H-bond donor properties of the phenolic OH-moiety of **25** are responsible for the ion channel blockade due to conformational changes of the receptor protein. The corresponding methyl ether **2a**, which is even more potent in the binding assay, showed an IC_{50}-value of only 360 nM in the excitotoxicity assay. This result indicates that the methyl ether **2a** is well recognized by the receptor protein, presumably by a slightly different binding mode, but the ion channel blocking activity is considerably reduced due to the missing H-bond donor group.

Surprisingly, *cis*-**32**, which represents the most potent ligand in the competition experiments, was considerably less potent in the functional assay (IC_{50} = 12.1 μM). However, the structure of the curve and the dose/activity relationship proves the antagonistic activity of *cis*-**32** as well.

The same experiment was performed using L12-G10 cells stably expressing NR1a/NR2A receptors. The inhibition of excitotoxicity was lower than 10%, when a concentration of 10 μM of **25**, *cis*-**32**, and **2a** was used. Obviously, **25**, *cis*-**32**, and **2a** did not block NMDA receptors containing NR2A subunits instead of NR2B subunits.

5. Metabolic stability

Studies on metabolic stability were carried out using microsomes prepared from mouse, rat and human livers.[37] The half-life of phenol **25** was 28 min (mouse), <3 min (rat) and 21 min (human), respectively. The same set-up resulted in half-life of ifenprodil (**1**) of <5 min (mouse), <1 min (rat), and 5 min (human), respectively. Obviously the metabolic degradation of **25** by CYP enzymes is reduced compared to the lead compound **1** indicating a higher metabolic stability of **25**.

6. Animal test

At first the tolerability of phenol **25** was investigated in the Irwin test with mice.[38] In this assay increasing doses of **25** (1–100 mg/kg body weight) were injected intraperitoneally and then the behavior of the mice was observed for 24 h. The mice did not show unusual reactions after ip application of the phenol **25**. Only diarrhea was observed in some animals 30 min after ip injection of the highest dose of 100 mg/kg body weight. The phenol **25** seems to be well tolerated by the mice.

Next, the potential of **25** for the treatment of neuropathic pain was investigated with the formalin test.[39] In this assay different doses (10, 30, and 100 mg/kg) of the NR2B antagonist **25** were injected intraperitoneally to mice. After 15 min, an inflammatory process was induced upon injection of formalin into the hind paw. The animal tried to reduce the acute pain (0–5 min) by licking of the paw. The number of licking activities per second was taken as a correlate for pain intensity. After 5 min the acute pain phase decreased and a phase of neuropathic pain developed. In this situation pain stimuli were set by pressing a von Frey filament on the inflamed paw of the mice. The reactions of the mice were recorded for 10–35 min after formalin injection.

The phenol **25** was virtually inactive in the acute early pain phase at all tested doses. However in the late neuropathic pain phase a dose dependent analgesic activity starting at 30 mg/kg of **25** was observed. At a dose of 10 mg/kg body weight **25** did not show any analgesic effects. The lowest active dose of 30 mg/kg is relatively high considering the high in vitro potency of **25**, but the formalin model might not be ideal for NR2B antagonists.

7. Conclusion

Receptor binding studies with [^{3}H]ifenprodil have provided five phenolic 3-benzazepines interacting with low nanomolar affinity with NR2B containing NMDA receptors. Four of these compounds revealed very low selectivity over σ_2 receptors. Therefore, only the 4-phenylbutyl substituted derivative **25** was considered for further pharmacological characterization. In addition to high selectivity against σ_1 and σ_2 receptors **25** did not interact with more than 100 further relevant target systems. Its antagonistic activity (IC_{50} = 18.4 nM) was proved in the excitotoxicity assay using L(tk-)-cells stably expressing NR1a/NR2B receptors. Despite the

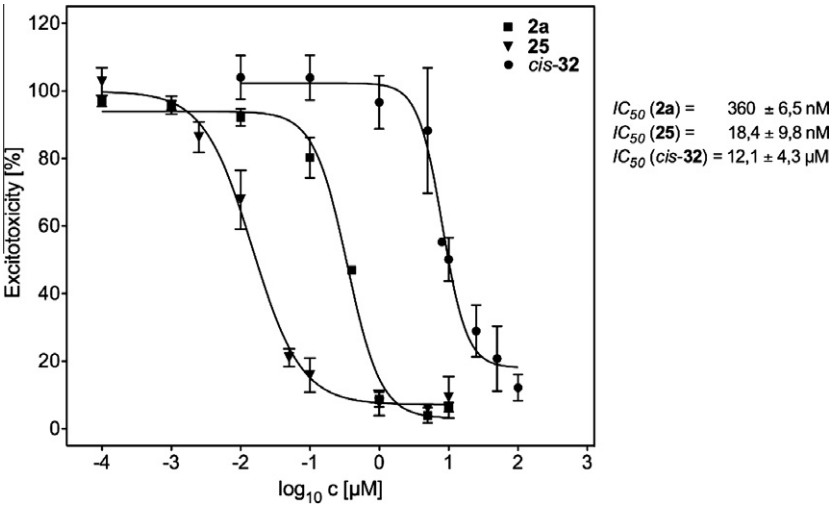


Figure 2. Inhibition of NMDA receptor-mediated cell toxicity by compounds **2a**, **25**, and *cis*-**32**. Different concentrations of the potent and selective ligands **2a**, **25**, and *cis*-**32** were applied to L13-E6 cells and NMDA receptors were activated by addition of (*S*)-glutamate and glycine. The excitotoxicity was determined by the amount of released lactate dehydrogenase (LDH). Compounds **2a**, **25**, and *cis*-**32** have IC_{50} values of 360 nM, 18.4 nM, and 12.1 μM.

corresponding methyl ether **2a** and the conformationally restricted 4-phenylcyclohexyl derivative *cis*-**32** showed similar receptor affinities, their antagonistic activities were considerably reduced. Incubation with microsome preparations indicated a higher metabolic stability of **25** compared with **1** and in the first animal studies **25** was well tolerated by mice. In the formalin assay **25** showed dose dependent analgesic activity starting at a relatively high dose of 30 mg/kg. Altogether these results render **25** a very promising starting point for the development of potent and selective NR2B receptor antagonists with improved metabolic stability and tolerability.

8. Experimental section

8.1. Synthesis general

Unless otherwise noted, moisture sensitive reactions were conducted under dry nitrogen. 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 related to tetramethylsilane; coupling constants are given with 0.5 Hz resolution. According to HPLC analysis the purity of all test compounds is greater than 95%. Elemental analyses: Vario EL (Elementaranalysesysteme GmbH). In addition to the HPLC analysis the purity of key compounds was proved by elemental analysis; all values are within ±0.4%.

8.1.1. 7-Hydroxy-3-(4-tosyl)-2,3,4,5-tetrahydro-3-benzazepin-1-one (5)

$AlCl_3$ (4.91 g, 36.8 mmol) was added to a solution of methyl ether **4**[23] (1.06 g, 3.07 mmol) in CH_2Cl_2 (50 mL). The suspension was heated to reflux for 23 h. After addition of water (60 mL) under ice-cooling the mixture was stirred for 1 h. The aqueous layer was separated and extracted with a mixture of CH_2Cl_2 and CH_3OH ((8:2) 3 × 30 mL). The combined organic layers were dried (Na_2SO_4) and concentrated in vacuum. The residue was purified by fc (3 cm, *n*-hexane/ethyl acetate 7:3, 30 mL, R_f = 0.14) to afford **5** (0.69 g, 68%) as colorless solid, mp 198 °C (decomposition). $C_{17}H_{17}NO_4S$ (331.4). ^{1}H NMR (CD_3OD): δ (ppm) = 2.26 (s, 3H, Ph-CH_3), 2.88 (t, *J* = 6.6 Hz, 2H, 5-H), 3.54 (t, *J* = 6.7 Hz, 2H, 4-H), 4.15 (s, 2H, 2-H), 6.47 (d, *J* = 2.4 Hz, 1H, 6-H), 6.51 (dd, *J* = 8.4/2.4 Hz, 1H, 8-H), 7.06 (d, *J* = 7.9 Hz, 2H, 3-H tosyl and 5-H tosyl), 7.19 (d, *J* = 8.4 Hz, 1H, 9-H), 7.31 (d, *J* = 8.3 Hz, 2H, 2-H tosyl and 6-H tosyl). A signal for the OH-proton is not seen.

8.1.2. 7-Benzyloxy-3-(4-tosyl)-2,3,4,5-tetrahydro-3-benzazepin-1-one (8)

A mixture of **5** (0.61 g, 1.83 mmol), K_2CO_3 (1.01 g, 7.32 mmol), benzyl bromide (0.38 g, 2.20 mmol) and acetone (60 mL) was heated to reflux for 4 h. After removal of K_2CO_3 by filtration the solution was concentrated in vacuum. The residue was purified by fc (5.5 cm, *n*-hexane/ethyl acetate 7:3, 65 mL, R_f = 0.31) to afford **8** (0.66 g, 85%) as colorless solid, mp 136 °C. $C_{24}H_{23}NO_4S$ (421.1). ^{1}H NMR ($CDCl_3$): δ (ppm) = 2.33 (s, 3H, Ph-CH_3), 2.94 (t, *J* = 6.9 Hz, 2H, 5-H), 3.67 (t, *J* = 6.7 Hz, 2H, 4-H), 4.17 (s, 2H, 2-H), 5.10 (s, 2H, O–CH_2-Ph), 6.71 (d, *J* = 2.5 Hz, 1H, 6-H), 6.80 (dd, *J* = 8.6/2.5 Hz, 1H, 8-H), 7.08 (d, *J* = 8.1 Hz, 2H, 3-H tosyl and 5-H tosyl), 7.36–7.45 (m, 8H, 9-H, 2-H tosyl, 6-H tosyl, 2-H phenyl, 3-H phenyl, 4-H phenyl, 5-H phenyl and 6-H phenyl).

8.1.3. (±)-7-Benzyloxy-3-(4-tosyl)-2,3,4,5-tetrahydro-1*H*-3-benzazepin-1-ol (9)

$NaBH_4$ (0.21 g, 5.64 mmol) was added in several portions to a suspension of **8** (0.40 g, 0.94 mmol) in CH_3OH (25 mL) and the mixture was stirred for 6 h at rt. Then H_2O (30 mL) was added 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 vacuum. The residue was purified by fc (3 cm, *n*-hexane/ethyl acetate 5:5, 10 mL, R_f = 0.59) to afford **9** (0.39 g, 98%) as colorless solid, mp 132 °C. $C_{24}H_{25}NO_4S$ (423.1). ^{1}H NMR ($CDCl_3$): δ (ppm) = 2.32 (s, 3H, Ph-CH_3), 2.71–2.77 (m, 2H, OH and 5-H), 3.00–3.06 (m, 1H, 4-H), 3.16–3.21 (m, 2H, 2-H and 4-H), 3.47–3.60 (m, 2H, 2-H and 5-H), 4.75 (br t, *J* = 5.6 Hz, 1H, 1-H), 4.94 (s, 2H, O–CH_2-Ph), 6.63 (d, *J* = 2.6 Hz, 1H, 6-H), 6.67 (dd, *J* = 8.3/2.6 Hz, 1H, 8-H), 7.14 (d, *J* = 8.4 Hz, 1H, 9-H), 7.20 (d, *J* = 8.4 Hz, 2H, 3-H tosyl and 5-H tosyl), 7.31–7.33 (m, 5H, 2-H phenyl, 3-H phenyl, 4-H phenyl, 5-H phenyl and 6-H phenyl), 7.58 (d, *J* = 8.3 Hz, 2H, 2-H tosyl and 6-H tosyl).

8.1.4. (±)-7-Benzyloxy-2,3,4,5-tetrahydro-1*H*-3-benzazepin-1-ol (10)

Mg (144.2 mg, 5.93 mmol) was added to a solution of **9** (114 mg, 0.27 mmol) in CH_3OH (10 mL) and the mixture was heated to reflux for 5 h. Then concentrated H_2SO_4 (0.33 mL) was added under

ice-cooling and the mixture was filtered. The pH value was adjusted to pH 9 by addition of NaOH. The aqueous layer was extracted with CH_2Cl_2 (5 × 15 mL), the organic layer was dried (Na_2SO_4) and concentrated in vacuum. The residue was purified by fc (2 cm, CH_2Cl_2/CH_3OH 9.5:0.5 and 2% NH_3, 10 mL, R_f = 0.11) to afford **10** (72.7 mg, 83%) as colorless solid, mp 137 °C. $C_{17}H_{19}NO_2$ (269.3). ^{1}H NMR ($CDCl_3$): δ (ppm) = 2.64 (dd, *J* = 15.5/5.8 Hz, 1H, 5-H), 2.77 (br t, *J* = 11.4 Hz, 1H, 4-H), 2.86 (d, *J* = 12.6 Hz, 1H, 2-H), 3.19–3.33 (m, 3H, 2-H, 4-H, 5-H), 4.58 (d, *J* = 6.1 Hz, 1H, 1-H), 5.08 (s, 2H, Ph-CH_2–O), 6.70–6.74 (m, 2H, 6-H and 8-H), 7.12 (d, *J* = 8.0 Hz, 1H, 9-H), 7.30–7.43 (m, 5H, 2-H benzyl, 3-H benzyl, 4-H benzyl, 5-H benzyl and 6-H benzyl). Signals for the OH- and NH-protons are not visible. Anal. ($C_{17}H_{19}NO_2$) C, H, N.

8.1.5. (±)-7-Benzyloxy-3-(4-phenylbutyl)-2,3,4,5-tetrahydro-1*H*-3-benzazepin-1-ol (11)

1-Chloro-4-phenylbutane (39.5 μL, 0.24 mmol) was added to a mixture of **10** (42.9 mg, 0.16 mmol), CH_3CN (5 mL), Bu_4NI (59.1 mg, 0.16 mmol) and K_2CO_3 (176.9 mg, 1.28 mmol) and the reaction mixture was heated to reflux for 72 h. Afterwards the K_2CO_3 was filtered off and the solvent was evaporated in vacuum. The residue was purified by fc (2 cm, *n*-hexane/ethyl acetate 7:3 and 1% *N*,*N*-dimethylethylamine, 10 mL, R_f = 0.30) to afford **11** (44.3 mg, 69%) as colorless oil. $C_{27}H_{31}NO_2$ (401.2). ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.43–1.54 (m, 2H, N–CH_2–CH_2–CH_2–CH_2-Ph), 1.55–1.64 (m, 2H, N–CH_2–CH_2–CH_2–CH_2-Ph), 2.32 (t, *J* = 11.7 Hz, 1H, 4-H), 2.43 (d, *J* = 12.0 Hz, 1H, 2-H), 2.51–2.59 (m, 5H, N–CH_2–CH_2–CH_2–CH_2-Ph and 5-H), 2.92 (ddt, *J* = 12.3/6.2/2.0 Hz, 1H, 4-H), 3.09 (ddd, *J* = 12.0/6.8/1.8 Hz, 1H, 2-H), 3.15–3.22 (m, 1H, 5-H), 4.49 (d, *J* = 6.7 Hz, 1H, 1-H), 4.96 (s, 2H, O–CH_2-Ph), 6.63–6.66 (m, 2H, 6-H and 8-H), 7.02 (d, *J* = 9.0 Hz, 1H, 9-H), 7.10–7.13 (m, 3H, CH phenyl), 7.19–7.36 (m, 7H, CH phenyl). A signal for the OH-proton is not visible.

8.1.6. (±)-*cis*- and (±)-*trans*-7-(Benzyloxy)-3-(4-phenyl cyclohexyl)-2,3,4,5-tetrahydro-1*H*-3-benzazepin-1-ol (*cis*-23 and *trans*-23)

$NaBH(OAc)_3$ (496 mg, 2.34 mmol) was added to a solution of **10** (350 mg, 1.30 mmol), 4-phenylcyclohexanone (272 mg, 1.56 mmol) and CH_3CO_2H (105 μL) in 1,2-dichloroethane (25 mL). The reaction mixture was stirred for 16 h at rt. A saturated $NaHCO_3$ solution (20 mL) and H_2O (20 mL) were added, the layers were separated and the aqueous layer was extracted with CH_2Cl_2 (3 × 40 mL). The combined organic layers were dried (Na_2SO_4) and the solvent was evaporated in vacuum. The residue was purified by fc (3 cm, *n*-hexane/ethyl acetate 9:1 and 1% *N*,*N*-dimethylethylamine, 10 mL, R_f (*trans*-**23**) = 0.30, R_f (*cis*-**23**) = 0.36).*trans*-**23** (R_f = 0.30): Colorless solid, mp 147 °C, yield 80.6 mg (14%). $C_{29}H_{33}NO_2$ (427.6). ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.40–1.55 (m, 4H, CH_2 cyclohexyl), 1.87–1.99 (m, 4H, CH_2 cyclohexyl), 2.40–2.49 (m, 1H, 4-H cyclohexyl), 2.59–2.68 (m, 4H, 2-H, 4-H, 5-H and 1-H cyclohexyl), 3.10 (ddt, *J* = 12.3/5.6/1.9 Hz, 1H, 4-H), 3.21–3.29 (m, 2H, 2-H and 5-H), 4.54 (d, *J* = 6.7 Hz, 1H, 1-H), 5.02 (s, 2H, O–CH_2-Ph), 6.71 (dd, *J* = 8.0/2.3 Hz, 1H, 8-H), 6.72 (d, *J* = 1.9 Hz, 1H, 6-H), 7.09 (d, *J* = 8.0 Hz, 1H, 9-H), 7.15–7.20 (m, 3H, CH aromatic), 7.26–7.33 (m, 3H, CH aromatic), 7.34–7.42 (m, 4H, CH aromatic). A signal for the OH-proton is not visible.*cis*-**23** (R_f = 0.36): Colorless oil, yield 391.6 mg (71%). $C_{29}H_{33}NO_2$ (427.6). ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.57–1.70 (m, 4H, CH_2 cyclohexyl), 1.72–1.82 (m, 2H, CH_2 cyclohexyl), 2.16–2.24 (m, 2H, CH_2 cyclohexyl), 2.43 (t, *J* = 11.8 Hz, 1H, 4-H), 2.47 (d, *J* = 12.2 Hz, 1H, 2-H), 2.60–2.68 (m, 2H, 5-H and 1-H cyclohexyl), 2.92 (quint, *J* = 4.8 Hz, 1H, 4-H cyclohexyl), 3.08 (ddt, *J* = 12.4/5.8/2.1 Hz, 1H, 4-H), 3.17–3.26 (m, 2H, 2-H and 5-H), 4.54 (d, *J* = 6.7 Hz, 1H, 1-H), 5.01 (s, 2H, O–CH_2-Ph), 6.68–6.70 (m, 2H, 6-H and 8-H), 7.06 (d, *J* = 8.9 Hz, 1H, 9-H), 7.16–7.21 (m, 1H, 4-H phenyl), 7.28–7.41 (m, 9H, C–H aromatic). A signal for the OH-proton is not visible.

8.1.7. (±)-3-(4-Phenylbutyl)-2,3,4,5-tetrahydro-1*H*-3-benzazepine-1,7-diol (25, WMS-1410)

A suspension of **11** (39 mg, 0.10 mmol) and Pd/C (37 mg, 10%) in abs. CH_3OH (5 mL) was stirred under H_2-atmosphere (1 bar) for 30 min at rt. The catalyst was removed by filtration and the solvent was evaporated in vacuum. The residue was purified by recrystallization from diisopropyl ether to afford **25** (20.1 mg, 67%) as pale yellow solid, mp 116 °C. $C_{20}H_{25}NO_2$ (333.4). ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.42–1.62 (m, 4H, N–CH_2–CH_2–CH_2–CH_2-Ph), 2.32 (t, *J* = 12.0 Hz, 1H, 4-H), 2.43 (d, *J* = 12.0 Hz, 1H, 2-H), 2.46–2.58 (m, 5H, N–CH_2–CH_2–CH_2–CH_2-Ph and 5-H), 2.85–2.91 (m, 1H, 4-H), 3.03–3.15 (m, 2H, 5-H and 2-H), 4.49 (d, *J* = 6.7 Hz, 1H, 1-H), 7.75 (br s, 1H, 7-OH), 6.43–6.45 (m, 2H, 6-H and 8-H), 8.82 (d, *J* = 6.9 Hz, 1H, 9-H), 7.09–7.13 (m, 3H, 2-H phenyl, 4-H phenyl and 6-H phenyl), 7.18–7.23 (m, 2H, 3-H phenyl and 5-H phenyl). A signal for the OH-proton (C-1) is not visible. Anal. ($C_{20}H_{25}NO_2$) C, H, N.

8.1.8. (±)-*cis*-3-(4-Phenylcyclohexyl)-2,3,4,5-tetrahydro-1*H*-3-benzazepine-1,7-diol (*cis*-32)

A suspension of *cis*-**23** (340 mg, 0.80 mmol) and Pd/C (200 mg, 10%) in abs. CH_3OH (30 mL) was stirred under H_2-atmosphere (1 bar) for 2 h at rt. The catalyst was removed by filtration over Celite 535® and the solvent was evaporated in vacuum. The residue was purified by fc (2 cm, *n*-hexane/ethyl acetate 6:4 and 1% *N*,*N*-dimethylethylamine, 10 mL, R_f = 0.19) to afford *cis*-**32** (199.3 mg, 74%) as colorless solid, mp 67 °C. $C_{22}H_{27}NO_2$ (337.5). ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.56–1.66 (m, 4H, CH_2 cyclohexyl), 1.67–1.76 (m, 2H, CH_2 cyclohexyl), 2.12–2.17 (m, 2H, CH_2, cyclohexyl), 2.40–2.57 (m, 3H, 2-H, 4-H and 5-H), 2.71 (br s, 1H, 1-H cyclohexyl), 2.88 (quint, *J* = 4.6 Hz, 1H, 4-H cyclohexyl), 2.97–3.17 (m, 3H, 2-H, 4-H and 5-H), 4.51 (d, *J* = 6.7 Hz, 1H, 1-H), 6.43 (d, *J* = 2.2 Hz, 1H, 6-H), 6.46 (dd, *J* = 8.0/2.4 Hz, 1H, 8-H), 6.88 (d, *J* = 8.0 Hz, 1H, 9-H), 7.09–7.14 (m, 1H, 4-H phenyl), 7.22–7.27 (m, 4H, 2-H phenyl, 3-H phenyl, 5-H phenyl and 6-H phenyl). Signals for the OH-protons are not visible.

8.2. Pharmacological studies

8.2.1. Materials and general procedures

Centrifuge: High-speed cooling centrifuge model Sorvall RC-5C plus (Thermo Finnigan). Filter: Printed Filtermat Type B (Perkin–Elmer), presoaked in 0.5% aqueous polyethylenimine for 2 h at rt before use. The filtration was carried out with a MicroBeta FilterMate-96 Harvester (Perkin–Elmer). The scintillation analysis was performed using Meltilex (Type A) solid scintillator (Perkin–Elmer). The scintillation was measured using a MicroBeta Trilux scintillation analyzer (Perkin–Elmer). The overall counting efficiency was 20%.

8.2.2. Cell culture and preparation of membrane homogenates for the NR2B assay[28]

In the assay mouse L(tk-)-cells stably transfected with the dexamethasone inducible eukaryotic expression vectors pMSG NR1a, pMSG NR2B in a 1:5 ratio were used. The transformed L(tk-)-cells 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 growth medium containing 4 μM dexamethasone and 4 μM ketamine (final concentration). After 24 h the cells were harvested by scraping and pelleted (10 min, 5000 *g*, Hettich Rotina 35R centrifuge, Tuttlingen, Germany).

For the binding assay, the cell pellet was resuspended in phosphate buffer saline (PBS) buffer and the number of cells was deter-

mined 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, device: Soniprep 150, MSE, London, UK). The resulting cell fragments were centrifuged with a high performance cool centrifuge (20,000 *g*, 4 °C, Sorvall RC-5 plus, Thermo Scientific). The supernatant was discarded and the pellet resuspended in a defined volume of PBS yielding cell fragments of approximately 500,000 cells/mL. 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.

8.2.3. Performing of the NR2B binding assay[28]

The competitive binding assay was performed with the radioligand [^{3}H]ifenprodil (60 Ci/mmol; Perkin Elmer) using standard 96-well-multiplates (Diagonal, Muenster, Germany). The thawed cell membrane preparation (about 20 μg protein) was incubated with 6 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 filtermats by using the 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 **1**. The K_d-value of **1** is 7.6 nM.[28]

8.2.4. Affinity towards σ_1 and σ_2 receptors and the PCP binding site of the NMDA receptor

The receptor binding studies were performed as previously described.[30–33]

8.2.5. Excitotoxicity assay[36]

Glutamate-induced cytotoxicity was determined as described recently.[36] Briefly, L(tk-)-cells stably expressing either NR1-1a/NR2A (L12-G10 cells) or NR1-1a/NR2B (L13-E6 cells) were preincubated with compounds (concentration range 1 nM–10 μM, dissolved in DMSO) for 30 min. Then 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 supernatants. In this assay 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 **25** and *cis*-**32** on LDH activity was excluded by performing the excitotoxicity assay in the presence of test compound as described above, however, all cells were lysed by addition of 1% triton X-100 prior to quantification of LDH activity.[36] No effect of **25** and *cis*-**32** was observed under this condition. Under standard assay conditions **25** and *cis*-**32** had no effect on L(tk-)-untransfected cells.

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Supplementary data

Purity data of all test compounds; physical and spectroscopic data of all new compounds; screening list of all considered receptors, transporters and enzymes; general chemistry methods; details of the pharmacological assays. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.09.026.

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