ChemPubSoc Europe

DOI: 10.1002/cmdc.201300547

GluN2B-Selective *N*-Methyl-D-aspartate (NMDA) Receptor Antagonists Derived from 3-Benzazepines: Synthesis and Pharmacological Evaluation of Benzo[7]annulen-7-amines

Andre Benner,[a] Alessandro Bonifazi,[b] Chikako Shirataki,[c] Louisa Temme,[a] Dirk Schepmann,[a] Wilma Quaglia,[b] Osami Shoji,[c] Yoshihito Watanabe,[d] Constantin Daniliuc,[e] and Bernhard Wünsch*[a]

Given their high neuroprotective potential, ligands that block GluN2B-containing *N*-methyl-D-aspartate (NMDA) receptors by interacting with the ifenprodil binding site located on the GluN2B subunit are of great interest for the treatment of various neuronal disorders. In this study, a novel class of GluN2B-selective NMDA receptor antagonists with the benzo[7]annulene scaffold was prepared and pharmacologically evaluated. The key intermediate, *N*-(2-*m*ethoxy-5-oxo-6,7,8,9-tetrahydro-5*H*-benzo[7]annulen-7-yl)acetamide (**11**), was obtained by cyclization of 3-acetamido-5-(3-methoxyphenyl)pentanoic acid (**10b**). The final reaction steps comprise hydrolysis of the amide, reduction of the ketone, and reductive alkylation, leading to *cis*- and *trans*-configured 7-(ω-phenylalkylamino)benzo[7]annulen-5-ols. High GluN2B affinity was observed with *cis*-configured γ-amino alcohols substituted with a 3-phenylpropyl moiety at the amino group. Removal of the benzylic hydroxy moiety led to the most potent GluN2B antagonists of this series: 2-methoxy-*N*-(3-phenylpropyl)-6,7,8,9-tetrahydro-5*H*-benzo[7]annulen-7-amine (**20a**, $K_i = 10$ nM) and 2-methoxy-*N*-methyl-*N*-(3-phenylpropyl)-6,7,8,9-tetrahydro-5*H*-benzo[7]annulen-7-amine (**23a**, $K_i = 7.9$ nM). The selectivity over related receptors (phencyclidine binding site of the NMDA receptor, σ_1 and σ_2 receptors) was recorded. In a functional assay measuring the cytoprotective activity of the benzo[7]annulenamines, all tested compounds showed potent NMDA receptor antagonistic activity. Cytotoxicity induced via GluN2A subunit-containing NMDA receptors was not inhibited by the new ligands.

Introduction

The excitatory amino acid neurotransmitter (*S*)-glutamate mediates its effects by activation of metabotropic (mGlu1–8) and ionotropic (*N*-methyl-D-aspartate [NMDA], 2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionic acid [AMPA], kainate) glutamate receptors. In contrast to AMPA and kainate receptors, the NMDA receptor allows penetration of Ca^{2+} ions into the neuron, which has to be carefully controlled. The influx of Ca^{2+} ions, the voltage-dependent Mg^{2+} blocking, and the requirement of two agonists for activation, (*S*)-glutamate and glycine, render the NMDA receptor a challenging target for drugs.[1–3]

The NMDA receptor is formed by four subunits.[4] Today, seven different NMDA receptor-forming subunits encoded by different genes are known, which are classified into three types: one GluN1 subunit with eight splice variants termed GluN1a–h, four GluN2 subunits termed GluN2a–d, and two GluN3 subunits termed GluN3a–b. A functional NMDA receptor protein contains at least one GluN1 and one GluN2 subunit bearing the binding sites for the agonists glycine and (*S*)-glutamate, respectively.[5,6]

The heteromeric assembly of four proteins offers different binding sites for ligands to modulate the opening state of the ligand-gated ion channel. In addition to the binding sites for the two agonists (*S*)-glutamate and glycine, there exist binding sites for phencyclidine within the channel pore and NO, H^+, Zn^{2+}, polyamines, and ifenprodil (**1**) at the amino terminus. We are particularly interested in ligands interacting with the ifenprodil binding site of the NMDA receptor, which is only found at the amino terminal end of the GluN2B subunit. Ligands blocking the NMDA receptor by interaction with the ifenprodil binding site address only NMDA receptors containing the GluN2B subunit. Expression of the GluN2B subunit is restricted to only some regions of the central nervous system (CNS), for example, the cortex, hippocampus, and striatum. Therefore,

[a] *Dr. A. Benner, L. Temme, Dr. D. Schepmann, Prof. Dr. B. Wünsch*
Institut für Pharmazeutische und Medizinische Chemie der Universität Münster, Corrensstraße 48, 48149 Münster (Germany)
E-mail: wuensch@uni-muenster.de

[b] *A. Bonifazi, Prof. W. Quaglia*
Scuola di Scienze del Farmaco e dei Prodotti della Salute
Università di Camerino, Via S. Agostino 1, 62032 Camerino (Italy)

[c] *C. Shirataki, Prof. O. Shoji*
Department of Chemistry, Graduate School of Science
Nagoya University, Furo-cho, Chikusa-ku, Nagoya, 464-8602 (Japan)

[d] *Prof. Y. Watanabe*
Research Center for Materials Science
Nagoya University, Furo-cho, Chikusa-ku, 464-8602 (Japan)

[e] *Dr. C. Daniliuc*
Organisch-chemisches Institut der Westfälischen Wilhelms-Universität Münster, Corrensstraße 40, 48149 Münster (Germany)

GluN2B-selective antagonists can only block NMDA receptors in those regions of the CNS. NMDA receptors in other parts of the CNS, for example, the cerebellum, which does not express the GluN2B subunit, are not affected by this type of antagonist. This mode of action leads to a decreased side effect profile.[5–9]

Due to their high neuroprotective potential,[10] compounds blocking GluN2B-containing NMDA receptors by selective interaction with the ifenprodil binding site of the GluN2B subunit can be used for the treatment of traumatic brain injury,[11] stroke (cerebral ischemia),[12] neuropathic pain,[7] and Parkinson's disease.[13] Migraine,[14] depression,[10] and alcohol withdrawal symptoms[15] represent further indications of GluN2B-selective antagonists.

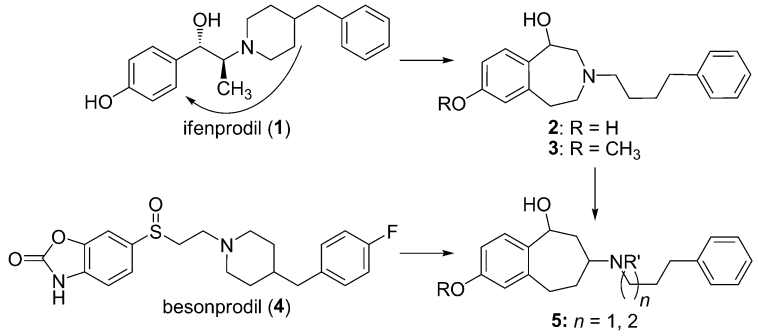


Figure 1. Development of GluN2B-selective NMDA receptor antagonists with a benzo[7]annulen-7-amine scaffold.

Very recently, X-ray crystal structures of an artificial dimer of the amino terminal parts of the GluN1b and the GluN2B subunits, together with ifenprodil (**1**) and Ro 25-6981, have shown that these GluN2B-selective antagonists bind at the interface between the two subunits. However, the GluN2B subunit forms crucial polar interactions with the antagonists, that is, two hydrogen bonds between Gln 110 and the protonated piperidine and the aliphatic hydroxy moiety, as well as a hydrogen bond between Glu 236 and the phenolic hydroxy moiety.[16]

The piperidinopropanol derivative ifenprodil (Vadilex®, **1**),[17,18] which was originally developed as an α_1-receptor antagonist, represents the first NMDA receptor antagonist addressing the ifenprodil binding site of the GluN2B subunit (IC_{50} = 13.3 nM).[19] (Figure 1) However, low bioavailability caused by fast biotransformation[20] and undesired side effects (e.g., psychotomimetic effects, memory deficits, hypertension) resulting from low receptor selectivity (5-HT_{1A}, 5-HT_2, α_1, σ_1, and σ_2 receptors were affected as well) prevented further development of ifenprodil.

Despite these unfavorable properties, ifenprodil served as a lead compound for the development of potent, selective, and bioavailable GluN2B antagonists. Formal opening of the piperidine ring and reconnection of the free end of the chain to the phenol of ifenprodil led to the tetrahydro-3-benzazepines **2** and **3** with low nanomolar affinity toward GluN2B-containing NMDA receptors (K_i(**2**) = 14 nM, K_i(**3**) = 5.4 nM). Whereas phenol **2** revealed lower GluN2B affinity than methyl ether **3**, its NMDA receptor antagonistic activity was considerably higher (IC_{50} = 18.4 nM) than that of **3** (IC_{50} = 360 nM).[21,22]

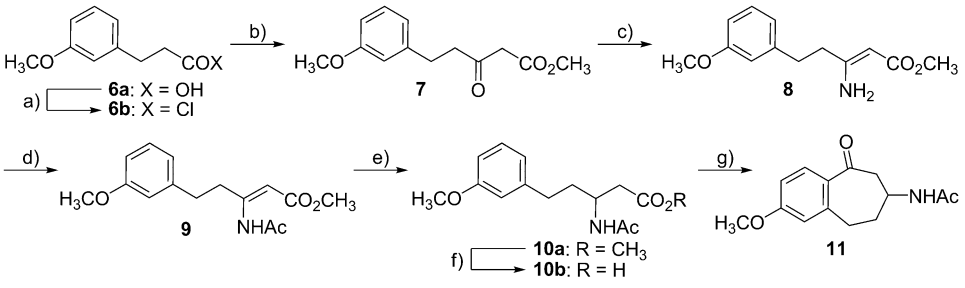
Due to the promising GluN2B affinity and NMDA receptor antagonistic activity of 3-benzazepines **2** and **3**, we planned to expand this type of GluN2B-selective NMDA antagonistic activity to benzo[7]annulenamines of type **5**. In the benzo[7]annulenamines **5**, the basic amino moiety of 3-benzazepines **2** and **3** was shifted to the side chain. Thus, benzo[7]annulen-7-amines **5** represent regioisomers of 3-benzazepines **2** and **3**, with an increased distance of four bond lengths between the left benzene ring and the basic amino moiety. An analogously increased distance between the basic amino moiety and the benzene moiety of the benzoxazolone system was also realized in the potent GluN2B antagonist besonprodil (**4**).[8,9] Different phenylalkyl residues (n = 1, 2) were considered in this project, due to their similarity to the nitrogen residues of **2**–**4**.

Synthesis

The synthesis of the benzo[7]annulene scaffold started with phenylpropionic acid **6a**, which was converted with oxalyl chloride into acid chloride **6b**. Reaction of acid chloride **6b** with Meldrum's acid and subsequent methanolysis led to β-keto ester **7** in 71% yield (Scheme 1).

After transformation of β-keto ester **7** into enamido ester **9** upon condensation with NH_4OAc and subsequent acetylation, hydrogenation provided amido ester **10a**. Hydrolysis of **10a** with KOH yielded acid **10b**, which represented the key compound for the construction of the benzo[7]annulene scaffold.

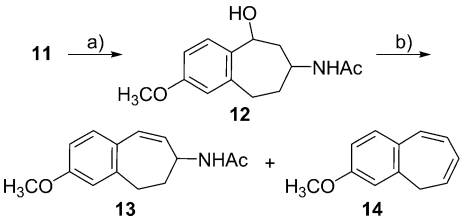
Several reaction conditions (e.g., PPA, P_4O_{10} in CH_2Cl_2, $SOCl_2$/$AlCl_3$, cyanuric chloride/$AlCl_3$, or cyanuric chloride/$SnCl_4$) were



Scheme 1. Synthesis of benzo[7]annulen-5-one **11**. *Reagents and conditions*: a) 1. oxalyl chloride, CH_2Cl_2, DMF, RT, 21 h, 94%; b) Meldrum's acid, CH_2Cl_2, pyridine, 0 °C, 30 min, RT, 1 h, then CH_3OH, reflux, 3 h, 71%; c) NH_4OAc, CH_3OH, 35 °C, 16 h, 100%; d) Ac_2O, pyridine, THF, reflux, 48 h, 100%; e) H_2 (5 bar), Pd/C, CH_3OH, RT, 48 h, 76%; f) KOH, CH_3OH, RT, 3 h, 93%; g) P_4O_{10}, CH_3SO_3H, CH_2Cl_2, −10 °C, 30 min, RT, 2 h, 65%.

tested for the intramolecular Friedel–Crafts acylation of acid **10b**. Finally, a solution of P_4O_{10} in methanesulfonic acid[23] gave the highest yield (65%) of cyclic ketone **11**. During this cyclization, small amounts (<15%) of the regioisomeric benzo[7]annulene with the methoxy moiety in position 1 were formed, which were separated by flash chromatography.

Ketone **11** was reduced with $NaBH_4$ to yield amido alcohol **12** as a 6:4 mixture of diastereomers, which were not separated by flash chromatography (Scheme 2). However, all attempts



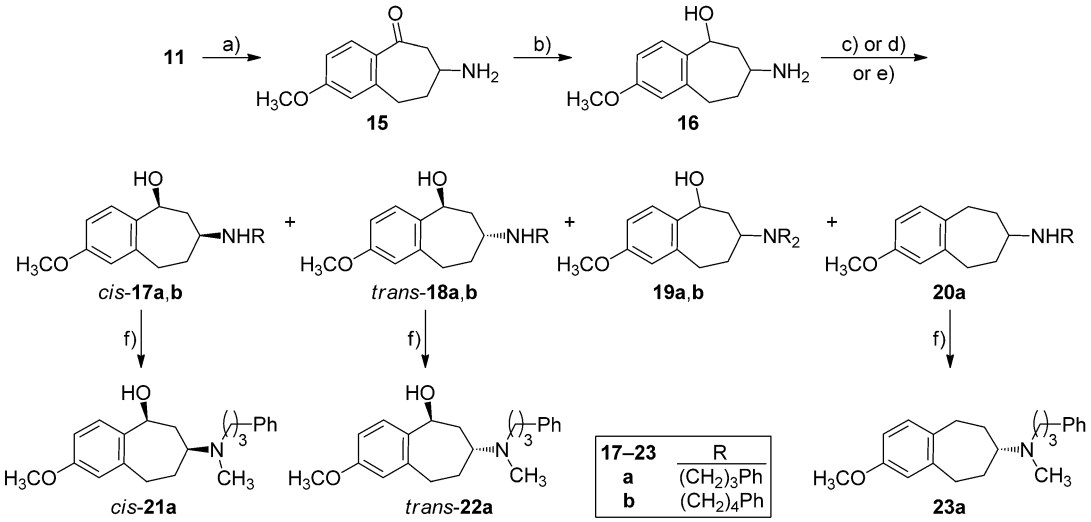
Scheme 2. Elimination reactions of hydroxyamide **12**. *Reagents and conditions*: a) $NaBH_4$, CH_3OH, RT, 16 h, 56%; b) HCl, 5 M, DMF, 60 °C, 24 h, 12% (**13**), 8% (**14**).

to hydrolyze the amide to obtain the primary amine failed. Either no reaction occurred, or undesired transformations took place. For example, elimination products **13** and **14** were isolated after treatment of amido alcohol **12** with 5 M HCl at 60 °C for 24 h. Therefore, the reaction sequence was changed.

Hydrolysis of acetamide **11** with 2 M HCl at 95 °C led to primary amine **15** in 85% yield after careful optimization (Scheme 3). Reduction of amino ketone **15** with $LiBH_4$ provided amino alcohol **16**, which was obtained in 85% yield as a 1:1 mixture of diastereomers. The mixture of diastereomeric amino alcohols **16** was reacted with 3-phenylpropanal and $NaBH(OAc)_3$[24] to give a mixture of four products, which were separated by flash chromatography. In addition to the desired *cis*- and *trans*-configured phenylpropylamines (*cis*-**17a** and *trans*-**18a**), tertiary amine **19a** was isolated. During the reductive amination, an unexpected hydrogenolytic cleavage of the benzylic hydroxy moiety also occurred, leading to phenylpropylamine **20a**. The homologous phenylbutylamines *cis*-**17b** and *trans*-**18b** were obtained by reductive alkylation of amino alcohol **16** with phenylbutanal. Alternatively, alkylation of **16** with 1-chloro-4-phenylbutane and tetrabutylammonium iodide in acetonitrile at reflux led to a mixture of diastereomeric phenylbutylamines *cis*-**17b**/*trans*-**18b** and *N*,*N*-bis(4-phenylbutyl)amine **19b** as the main product.

In addition to the secondary amines *cis*-**17**, *trans*-**18**, and **20a**, we thought it interesting to test the corresponding tertiary amines as well. For this purpose, the secondary amines *cis*-**17a**, *trans*-**18a**, and **20a** were reductively methylated with formaldehyde and $NaBH(OAc)_3$ to yield tertiary amines *cis*-**21a**, *trans*-**22a**, and **23a** with a small third substituent.

It was very difficult to unequivocally assign the relative configuration of the amino and hydroxy moiety in the seven-membered ring of **17**, **18**, **21** and **22** by analyzing the coupling constants in the corresponding ^{1}H NMR spectra. Therefore, amino alcohol *cis*-**17a** was recrystallized with ethyl acetate and diisopropyl ether (3:1) to yield crystals that were suitable for X-ray crystal structure analysis. In Figure 2, only one of the two independent enantiomers of *cis*-**17a** in the asymmetric unit is shown. The figure shows that the hydroxy moiety and the phenylpropylamino moiety are on the same side of the benzo[7]annulene ring system, indicating *cis*-configuration. The relative configurations of *cis*-**17b**, *trans*-**18a**,**b**, *cis*-**21a**, and *trans*-**22a** were defined by comparing their NMR spectra with the NMR spectrum of *cis*-**17a**.



Scheme 3. Synthesis of various benzo[7]annulen-7-amines. *Reagents and conditions*: a) HCl (2 M), 95 °C, 16 h, 85%; b) $LiBH_4$, THF, −78 °C, 30 min, 85%; c) $PhCH_2CH_2CH{=}O$, CH_2CH_2, RT, 20 min, then $NaBH(OAc)_3$, RT, 4 h, 4% (*cis*-**17a**), 2%, (*trans*-**18a**), 13% (*trans*-**19a**), 5% (**20a**); d) $Ph(CH_2)_3CH{=}O$, $MgSO_4$, CH_2Cl_2, RT, 16 h, then $NaBH(OAc)_3$, RT, 4 h, 2% (*cis*-**17b**: 2%), 2% (*trans*-**18b**); e) $Ph(CH_2)_4Cl$, Bu_4NI, K_2CO_3, CH_3CN, 85 °C, 24 h, 23% (*cis*-**17b**+*trans*-**18b**), 59% (**19b**); f) $CH_2{=}O$, H_2O, CH_2Cl_2, $NaBH(OAc)_3$, RT, 12–16 h, 71–75% (only phenylpropylamines of series **a** were reductively methylated).

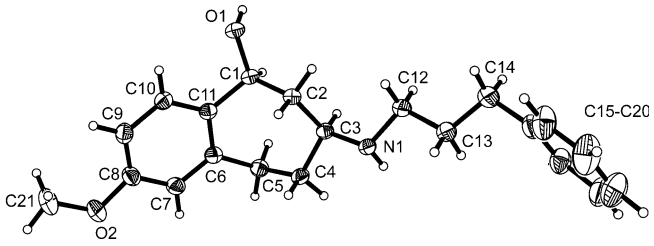


Figure 2. X-ray crystal structure analysis of *cis*-**17a**. Only one of the two independent enantiomers in the asymmetric unit is shown. Thermal ellipsoids are shown at 30% probability.

Biological Activity

Affinity toward the ifenprodil binding site of GluN2B--containing NMDA receptors

The affinity of the diastereomeric benzo[7]annulen-7-amines toward GluN2B-containing NMDA receptors was investigated using the competitive receptor binding assay recently developed by our group.[19] Membrane preparations obtained by ultrasonic irradiation of L(tk−) cells stably expressing recombinant human GluN1a and GluN2B subunits were employed as receptor materials in this assay. The synthesis of functional NMDA receptors was induced by addition of dexamethasone to the growth medium of these L(tk−) cells. To protect against cell death, ketamine was added to the medium, which blocks the NMDA receptor by interaction with the phencyclidine binding site within the channel pore. Tritium-labeled [^{3}H]ifenprodil (**1**) served as a radioligand. Although the selectivity of the radioligand was rather low, this assay is selective for the ifenprodil binding site of GluN2B-containing NMDA receptors, due to the high number of receptors in this cell line.

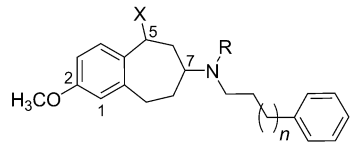
In Table 1, the GluN2B affinity of the test compounds is summarized. Generally, the GluN2B affinity of *cis*-configured amino alcohols is higher than the GluN2B affinity of the corresponding *trans*-configured diastereomers (e.g., *cis*-**17a**/*trans*-**18a**). Elongation of the distance between the basic amine and the phenyl moiety in the side chain led to lower GluN2B affinity (e.g., *trans*-**18a**/*trans*-**18b**). Introduction of an additional methyl group to the basic amino moiety did not affect the GluN2B affinity considerably. The secondary amine (*cis*-**17a**, K_i = 16 nM) and the tertiary amine (*cis*-**21a**, K_i = 15 nM) exhibit almost identical GluN2B affinity. Reductive elimination of the hydroxy moiety in the benzylic position resulted in benzo[7]annulenamines with increased GluN2B affinity. The GluN2B affinities of deoxygenated benzo[7]annulenamines **20a** (K_i = 10 nM) and **23a** (K_i = 7.9 nM) are slightly higher than the GluN2B affinities of corresponding hydroxy derivatives *cis*-**17a** and *cis*-**21a**. The high GluN2B affinity of deoxygenated benzo[7]annulenamines **20a** and **23a** was unexpected, as most of the lead compounds contain a benzylic hydroxy moiety or a bioisosteric replacement (e.g., **1**–**4**, traxoprodil, eliprodil, Ro 25-6981). In conclusion, deoxygenated benzo[7]annulenamines **20a** and **23a** show the highest GluN2B affinity within this series of ligands.

Selectivity over related receptors

To prove the selectivity of the GluN2B ligands, the affinity toward a number of related receptors was recorded in competitive radioligand receptor binding studies. First, affinity toward the phencyclidine (PCP, 1-(1-phenylcyclohexyl)piperidine) binding site within the channel pore was determined using the radioligand [^{3}H](+)-MK-801.[25,26] The benzo[7]annulenamines did not show considerable interaction with the PCP binding site, indicating high selectivity over this binding site. (Table 1)

As ifenprodil exhibits considerable affinity toward both the σ_1 and σ_2 receptors, the affinity of the new ligands toward these receptor systems was recorded employing the radioligands [^{3}H](+)-pentazocine and [^{3}H]di-*o*-tolylguanidine ([^{3}H]DTG), respectively.[27–29] (Table 1). The $K_i(\sigma_1)$ values for benzo[7]annulenamines **17** and **18** are higher than 125 nM, indicating considerable selectivity over the σ_1 receptor. However, removal of the benzylic hydroxy moiety and methylation of the secondary amines led to increased σ_1 affinity, with $K_i(\sigma_1)$ values in the range of 21–81 nM. Deoxygenated compounds **20a** and **23a**, which represent the GluN2B ligands with the highest affinity, also show the highest σ_1 affinity, which leads to a decreased GluN2B/σ_1 selectivity of 2.7. With respect to GluN2B affinity and GluN2B/σ_1 selectivity, *cis*-**17a** represents a promising ligand.

Table 1. Affinities of benzo[7]annulenes toward the ifenprodil binding site of GluN2B-containing NMDA receptors, the PCP binding site of the NMDA receptor, and σ_1 and σ_2 receptors.



Compd	R	X	*n*	K_i [nM][a]			
				GluN2B[b]	PCP	σ_1[b]	σ_2[b]
2[22]	H	–	–	14 ± 1.5	35%	194	> 10 μM
3[21]	CH_3	–	–	5.4 ± 0.4	22%	182	554
cis-**17a**	H	OH	3	16 ± 3	12%	132	9.2 ± 2.1
cis-**17b**	H	OH	4	24 ± 10	0%	489	209
trans-**18a**	H	OH	3	48 ± 11	4%	167	1100
trans-**18b**	H	OH	4	127 ± 16	5%	197	1400
20a	H	H	3	10 ± 4	0%	27 ± 7	15 ± 5
cis-**21a**	CH_3	OH	3	15 ± 2	0%	81 ± 11	33 ± 4
trans-**22a**	CH_3	OH	3	39 ± 3	32%	42 ± 1	600
23a	CH_3	H	3	7.9 ± 4	1%	21 ± 7	137
ifenprodil (**1**)				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				–	–	89 ± 29	57.5 ± 18

[a] For low-affinity compounds, only the inhibition of the radioligand binding at a test compound concentration of 1 μM is given. [b] K_i values are the mean ± SEM of three independent experiments performed in triplicate.

Trans-configured benzo[7]annulenamines (*trans*-**18a,b**, *trans*-**22a**) and phenylbutylamines (*cis*-**17b**, *trans*-**18b**) show rather low σ_2 affinity, indicating high selectivity. The highest σ_2 affinity was found for the *cis*-configured phenylpropylamine *cis*-**17a**. Its σ_2 affinity ($K_i = 9.2$ nM) even exceeds the GluN2B affinity ($K_i = 16$ nM). Removal of the benzylic hydroxy moiety (**20a**: $K_i =$ 15 nM), N-methylation (*cis*-**21a**: $K_i = 33$ nM), and both modifications (**23a**: $K_i = 137$ nM) led to a progressive decrease in σ_2 affinity. With the exception of *cis*-**17a**, the highest affinity GluN2B ligands (**20a**, *cis*-**21a**, and **23a**) display a 1.5–17-fold preference for the GluN2B receptor over the σ_2 receptor.

Functional activity

The antagonistic activity of benzo[7]annulenamines *cis*-**17a**, **20a**, *cis*-**21a**, and **23a** was investigated by inhibition of the excitotoxicity induced by activation of NMDA receptors with (*S*)-glutamate and glycine. In the assays, L(tk−) cells stably expressing the NMDA receptor subunits GluN1a and GluN2B (GluN2B assay) or GluN1a and GluN2A (GluN2A assay) were employed. Expression of the functional NMDA receptors was induced by addition of dexamethasone. At the same time, (*S*)-glutamate, glycine, and different concentrations of the test compounds were added. After an incubation period of 12 h, the amount of released lactate dehydrogenase (LDH) was recorded by measuring the amount of formed formazan dye.[30]

Table 2 displays the cytoprotective effect of benzo[7]annulenamines. In the GluN2B assay, all tested compounds inhibited (*S*)-glutamate/glycine-induced cytotoxicity in the low-to-medium nanomolar range, which confirms that the new benzo[7]annulene-based GluN2B-selective ligands represent potent NMDA receptor antagonists. The inhibition of cytotoxicity correlates nicely with the GluN2B affinity of these compounds. Whereas *cis*-**21a**, with the lowest GluN2B affinity, has the lowest cytoprotective effect ($IC_{50} = 55$ nM), deoxygenated benzo[7]annulenamine **23a** shows the highest GluN2B binding and cytoprotective activity ($IC_{50} = 9.7$ nM). Thus, the benzo[7]annulenamines reported herein show similar cytoprotective potential as channel blockers like phencyclidine and ketamine.

Table 2. Inhibition of cell death by GluN2B-selective NMDA receptor antagonists.

Compd	R	X	*N*	K_i [nM][a]	IC_{50} [nM][a]	
				GluN2B	GluN2B	GluN2A
cis-**17a**	H	OH	3	16±3	12±0.4	515±64
20a	H	H	3	10±4	29±1	1700±400
cis-**21a**	CH_3	OH	3	15±2	55±24	882±109
23a	CH_3	H	3	7.9±4	9.7±3.9	450±163

[a] K_i and IC_{50} values are the mean±SEM of three independent experiments performed in triplicate.

To show the selectivity over GluN2A-containing NMDA receptors, the same assay was conducted with GluN2A subunit-producing cells. It can be seen in Table 2 that the benzo[7]annulenamines interacting with high affinity with the ifenprodil binding site of the GluN2B subunit did not inhibit (*S*)-glutamate/glycine-induced damage of cells expressing GluN2A-containing NMDA receptors. This result proves unequivocally the high GluN2B selectivity of this novel class of benzo[7]annulenamines.

Conclusions

cis- and *trans*-Configured benzo[7]annulenes with a 5-hydroxy moiety and various phenylalkylamino moieties in the 7-position were prepared in 10–11 synthetic steps. High GluN2B affinity was observed for *cis*-configured 3-phenylpropylamines *cis*-**17a** and cis-**21a**. Unexpectedly, the GluN2B affinity of *cis*-**17a** and cis-**21a** was exceeded by that of deoxygenated benzo[7]annulenamines **20a** and **23a**. With respect to selectivity over the σ receptors **20a**, *cis*-**21a**, and **23a** represent the most promising GluN2B antagonists. In the functional GluN2B assay recording cytoprotective effects, the *cis*-configured phenylpropylamines *cis*-**17a** and *cis*-**21a** and the deoxygenated phenylpropylamines **20a** and **23a** show high antagonistic activity. Due to their high selectivity over GluN2A-containing NMDA receptors, the benzo[7]annulenamines represent novel GluN2B-selective antagonists with a promising therapeutic potential and side effect profile.

Experimental Section

Chemistry

General: Unless otherwise noted, moisture-sensitive reactions were conducted under dry nitrogen. THF was dried with sodium/benzophenone and was freshly distilled before use. Thin layer chromatography (TLC): silica gel 60 F254 plates (Merck); flash chromatography (FC): silica gel 60, 40–64 μm (Merck); parentheses include diameter of the column, eluent, fraction size, and R_f value; melting point. Melting points were taken using an SMP 3 melting point apparatus (Stuart Scientific) and are uncorrected. For microwave reactions, a Discover microwave apparatus (CEM GmbH) was used. Mass spectrometry (MS) measurements were taken using a MAT GCQ (Thermo-Finnigan). IR spectrum were collected using a 480Plus FT-ATR-IR spectrophotometer (Jasco); optical rotation was determined using a Polarimeter 341 (PerkinElmer) at a wavelength of 589 nm, path length (*l*): 1 dm, *T*+20 °C; unit [deg mL^{-1} dm^{-1} g^{-1}] is omitted with concentration [mg mL^{-1}] and solvent given in brackets. ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were collected using a Unity Mercury Plus 400 spectrometer (Varian), with δ in ppm related to tetramethylsilane; coupling constants are given with 0.5 Hz resolution. HPLC was performed using Merck Hitachi Equipment: L-7400 UV detector, L-7200 autosampler, L-7100 pump, L-7614 degasser, column: LiChrospher 60 RP-select B (5 μm), LiChroCART 250–4 mm cartridge, flow rate: 1.0 mL min^{-1}, injection volume: 5.0 μL, detection at $\lambda =$ 210 nm, solvents: A) water with 0.05% (*v*/*v*) trifluoroacetic acid; B) CH_3CN with 0.05% (*v*/*v*) trifluoroacetic acid, with gradient elution (A%): 0–4 min: 90%, 4–29 min: 90 → 0%, 29–31 min: 0%, 31–31.5 min: 0 → 90%, 31.5–40 min: 90%. Elemental analyses were performed using a CHN-Rapid Analysator (Fons-Heraeus).

3-(3-Methoxyphenyl)propanoyl chloride (6b): Oxalyl chloride (42.8 g, 337.2 mmol) was added under N_2 to a solution of 3-(3-me-

thoxyphenyl)propanoic acid (**6a**, 30.5 g, 168.6 mmol) in CH_2Cl_2 (60 mL). After the mixture was stirred for 1 h at room temperature, dimethylformamide (DMF; 0.5 mL) was added, and the mixture was stirred for 20 h at room temperature. The solvent and excess oxalyl chloride were removed by distillation under atmospheric pressure, and the crude product was purified by distillation under vacuum to yield **6b** as a pale yellow oil (31.4 g, 94%): bp_{13} 145 °C; ^{1}H NMR ($CDCl_3$): δ = 2.99 (t, J = 7.6 Hz, 2H, Ar-CH_2-CH_2), 3.21 (t, J = 7.6 Hz, 2H, -CH_2-COCl), 3.80 (s, 3H, Ar-OCH_3), 6.73 (t, J = 2.0 Hz, 1H, 2-H_{arom}), 6.78 (dd, J = 7.5/2.0 Hz, 2H, 4-H_{arom}, 6-H_{arom}), and 7.23 ppm (t, J = 7.5 Hz, 1H, 5-H_{arom}); $C_{10}H_{11}ClO_2$, M_r = 198.6 Da.

Methyl 5-(3-methoxyphenyl)-3-oxopentanoate (7): Meldrum's acid (20.5 g, 142.2 mmol) was dissolved in CH_2Cl_2 (34 mL) and cooled to 0 °C. Pyridine (25.5 mL) was added dropwise, then a solution of **6b** (31.4 g, 158.0 mmol) in CH_2Cl_2 (57 mL) was added at 0 °C over 60 min. The mixture was stirred for 30 min at 0 °C and 60 min at room temperature. The mixture was diluted with CH_2Cl_2 (100 mL), washed with 2 M HCl (100 mL) and brine (100 mL), and the organic layer was dried (Na_2SO_4) and concentrated in vacuum. The residue (43.5 g) was dissolved in CH_3OH (50 mL) and stirred at reflux for 3 h. The solvent was evaporated under vacuum, and the product was divided into three parts for FC purification (d = 8 cm, l = 25 cm, V = 65 mL, cyclohexane/EtOAc, 90:10, R_f = 0.21) to yield **7** as a colorless oil (26.4 g, 71%): ^{1}H NMR ($CDCl_3$): δ = 2.84–2.93 (m, 4H, Ar-CH_2-CH_2), 3.43 (s, 2H, CO-CH_2-CO_2CH_3), 3.71 (s, 3H, CO_2CH_3), 3.78 (s, 3H, Ar-OCH_3), 6.71–6.80 (m, 3H, 2-H_{arom}, 4-H_{arom}, 6-H_{arom}), and 7.17–7.22 ppm (m, 1H, 5-H_{arom}); purity by HPLC: 90% (t_r = 19.82 min); MS (ESI): m/z (%) = 254 [M+NH_4^+, 100], 495 [2M+Na, 95], 219 [M−H_2O, 47]; FTIR: $\tilde{\nu}$ = 3004 (s, ν-C−H_{arom}), 2953 (C−H_{aliph}), 2837 (C−H_{OCH3}), 1744 (C=O_{ester}), 1714 (C=O_{ketone}), and 776 cm^{-1} (C=$C_{1,3\text{-disubst arom}}$); $C_{13}H_{16}O_4$, M_r = 236.3 Da;

Methyl 3-amino-5-(3-methoxyphenyl)pent-2-enoate (8): β-Keto ester **7** (8.0 g, 33.8 mmol) was dissolved in MeOH (50 mL) under N_2. Dry ammonium acetate (10.5 g, 172.0 mmol) was added, and the mixture was stirred at 35 °C for 16 h. The MeOH was removed under vacuum, and the residue was suspended in EtOAc and filtered. The residue in the filter was washed with EtOAc (4×). The organic layer was dried (Na_2SO_4), filtered, and concentrated under vacuum. The crude product, **8**, was used in the next reaction without purification as a colorless oil (8.12 g, 100%): ^{1}H NMR ($CDCl_3$): δ = 2.42 (t, J = 8.0 Hz, 2H, Ar-CH_2-CH_2), 2.84 (t, J = 8.0 Hz, 2H, Ar-CH_2-CH_2), 3.65 (s, 3H, -CO_2CH_3), 3.79 (s, 3H, Ar-OCH_3), 4.59 (s, 1H, =CH-CO_2CH_3), 6.73–6.80 (m, 3H, 2-H_{arom}, 4-H_{arom}, 6-H_{arom}), and 7.20–7.24 ppm (m, 1H, 5-H_{arom}), signals for the NH_2 group are not observed in the spectrum; purity by HPLC: 88.5% (t_r = 17.49 min); MS (ESI): m/z (%) = 135 [M−C_4H_6NO, 100], 236 [M+H, 38]; IR (neat): $\tilde{\nu}$ = 3453 (N−H), 3333 (N−H), 3028 (C−H_{arom}), 2947 (C−H_{aliph}), 1740 (C=O), 787 cm^{-1} (C=$C_{1,3\text{-disubst arom}}$); $C_{13}H_{17}NO_3$, M_r = 235.3 Da.

Methyl 3-acetamido-5-(3-methoxyphenyl)pent-2-enoate (9): Enamine **8** (7.5 g, 31.8 mmol) was dissolved in THF (40 mL) under N_2. Pyridine (5.3 g, 66.8 mmol) and acetic anhydride (19.9 g, 195.2 mmol) were added, and the reaction mixture was stirred at reflux for 48 h. The THF was removed under vacuum, the residue was dissolved in EtOAc, and the organic layer was washed with H_2O, 2 M HCl, sat. $NaHCO_3$, and brine. The organic layer was dried (Na_2SO_4), filtered, and concentrated under vacuum. Crude product **9**, a colorless oil, was used in the next reaction without purification (yield: 8.95 g, 100%): ^{1}H NMR ($CDCl_3$): δ = 1.86 (s, 3H, NHCO-CH_3), 2.87 (t, J = 7.4 Hz, 2H, Ar-CH_2-CH_2), 3.00 (t, J = 7.7 Hz, 2H, Ar-CH_2-CH_2), 3.69 (s, 3H, CO_2CH_3), 3.79 (s, 3H, Ar-OCH_3), 6.45 (s, 1H, NH), 6.76 (ddd, J = 8.1/2.6/0.9 Hz, 1H, 4-H_{arom}), 6.78–6.80 (m, 1H, 2-H_{arom}), 6.80 (s, 1H, =C$H$$CO_2CH_3$), 6.84 (ddd, J = 7.6/1.4/0.9 Hz, 1H, 6-H_{arom}), and 7.22 ppm (t, J = 7.9 Hz, 1H, 5-H_{arom}); ^{13}C NMR ($CDCl_3$): δ = 25.0 (1C, NHCO-CH_3), 34.3 (1C, Ar-CH_2-CH_2), 35.1 (1C, Ar-CH_2-CH_2), 51.2 (1C, Ar-OCH_3), 55.4 (1C, -CH-CO_2CH_3), 102.3 (1C, $CHCO_2CH_3$), 112.0 (1C, 4-C_{arom}), 114.4 (1C, 2-C_{arom}), 121.0 (1C, 6-C_{arom}), 129.9 (1C, 5-C_{arom}), 142.6 (1C, 1-H_{arom}), 152.3 (1C, -C-NHCOCH$_3$), 160.1 (1C, 3-C_{arom}), 168.6 (1C, -NHCO-CH$_3$), and 169.2 ppm (1C, -CO_2CH_3); purity by HPLC: 97.5% (t_r = 22.36 min); MS (ESI): m/z (%) = 218 [M−$C_2H_3O_2$, 100], 234 [M−C_2H_3O, 39], 278 [M+H, 15]; IR (neat): $\tilde{\nu}$ = 3267 (N−H), 3221 (N−H), 3028 (C−H_{arom}), 2951 (C−H_{aliph}), 1744 (C=O_{ester}), 1628 (C=O_{amide}), 779 cm^{-1} (C=$C_{1,3\text{-disubst arom}}$); $C_{15}H_{19}NO_4$, M_r = 277.1 Da.

Methyl 3-acetamido-5-(3-methoxyphenyl)pentanoate (10a): Enamide **9** (8.85 g, 31.8 mmol) was dissolved in abs. MeOH (40 mL), and 10% Pd/C (0.71 g) was added. The mixture was shaken for 48 h at room temperature under H_2 atmosphere (5 bar). The catalyst was removed by filtration, and the MeOH was removed under reduced pressure. The residue was purified by FC (d = 8 cm, l = 25 cm, V = 65 mL, cyclohexane/EtOAc, 4:6, R_f = 0.18) to yield **10a** as a colorless oil (6.7 g, 76%): ^{1}H NMR ($CDCl_3$): δ = 1.76–1.85 (m, 1H, Ar-CH_2-CH_2), 1.86–1.94 (m, 1H, Ar-CH_2-CH_2), 1.96 (s, 3H, NHCO-CH_3), 2.52 (dd, J = 16.1/5.0 Hz, 1H, -CH_2-CO_2CH_3) 2.59 (dd, J = 16.1/5.0 Hz, 1H, -CH_2-CO_2CH_3), 2.63 (t, J = 8.2 Hz, 2H, Ar-CH_2-), 3.67 (s, 3H, CO_2CH_3), 3.78 (s, 3H, Ar-OCH_3), 4.25–4.34 (m, 1H, -CH-NHCOCH$_3$), 6.07 (s broad, 1H, NH), 6.72–6.77 (m, 3H, 2-H_{arom}, 4-H_{arom}, 6-H_{arom}), and 7.17–7.21 ppm (m, 1H, 5-H_{arom}); purity by HPLC: 93.4% (t_r = 21.33 min); MS (ESI): m/z (%) = 279 [M, 10], 278 [M−H, 100]; IR (neat): $\tilde{\nu}$ = 3305 (N−H), 2916 (C−H_{aliph}), 1728 (C=O_{ester}), 1610 (C=$O_{amide\ I}$), 1574 (C=$O_{amide\ II}$), 783 cm^{-1} (C=$C_{1,3\text{-disubst arom}}$); $C_{15}H_{21}NO_4$, M_r = 279.1 Da.

3-Acetamido-5-(3-methoxyphenyl)pentanoic acid (10b): Ester **10a** (8.5 g, 30.4 mmol) was dissolved in MeOH (20 mL) and cooled to 0 °C. Next,1 M KOH (100 mL) was added, and the mixture was stirred for 3 h at room temperature. The mixture was acidified with 5 M HCl and extracted with EtOAc (4×). The organic layer was washed with brine, dried (Na_2SO_4), filtered, and concentrated under vacuum. Crude product **10b**, a colorless oil, was used in the next reaction step without purification (6.5 g, 93%): ^{1}H NMR ($CDCl_3$): δ = 1.77–1.91 (m, 2H, Ar-CH_2-CH_2), 1.94 (s, 3H, NHCO-CH_3), 2.48 (dd, J = 15.9/5.4 Hz, 1H, -CH_2-CO_2H) 2.56 (dd, J = 15.9/5.4 Hz, 1H, -CH_2-CO_2H), 2.61 (t, J = 8.2 Hz, 2H, Ar-CH_2-), 3.77 (s, 3H, Ar-OCH_3), 4.21–4.27 (m, 1H, -CH-NHCOCH$_3$), 6.46–6.49 (m, 1H, NH), 6.70–6.75 (m, 3H, 2-H_{arom}, 4-H_{arom}, 6-H_{arom}), and 7.14–7.18 ppm (m, 1H, 5-H_{arom}), a signal for the CO_2H proton is not observed in the spectrum; purity by HPLC: 96.8% (t_r = 18.68 min); MS (ESI): m/z (%) = 265 [M, 80], 122 [M−$C_6H_{10}NO_3$, 100]; IR (neat): $\tilde{\nu}$ = 3267 (N−H), 3221 (N−H), 3028 (C−H_{arom}), 2951 (C−H_{aliph}), 1744 (C=O), 1628 (C=O_{amide}), 779 cm^{-1} (C=$C_{1,3\text{-disubst arom}}$); $C_{14}H_{19}NO_4$, M_r = 265.3 Da.

***N*-(2-Methoxy-5-oxo-6,7,8,9-tetrahydro-5*H*-benzo[7]annulen-7-yl)acetamide (11)**: Acid **10b** (6.5 g, 24.5 mmol) was dissolved in abs. CH_2Cl_2 (100 mL) under N_2, and the mixture was cooled to −10 °C. A solution of P_4O_{10} (11 g, 38.7 mmol) in methanesulfonic acid (55 mL) was added slowly, and the mixture was stirred for 30 min at −10 °C and 2 h at room temperature. The mixture was then cooled to 0 °C, and water (10 mL) and NaOH were added (pH 10). The aqueous layer was extracted with CH_2Cl_2 (4×). The combined organic layers were washed with brine and dried (Na_2SO_4), the solvent was removed under reduced pressure, and the residue was purified by crystallization (EtOAc/iPr$_2$O/CH_3OH, 8:2:1) to yield **11** as a colorless solid (3.9 g, 65%): mp: 158–160 °C, ^{1}H NMR ($CDCl_3$): δ = 1.53 (dddd, J = 14.7/9.3/6.7/5.5 Hz 1H, Ar-CH_2-CH_2), 1.94 (s, 3H, NHCO-CH_3), 2.48 (dddd, J = 14.0/7.5/6.1/4.9 Hz 1H, Ar-CH_2-CH_2), 2.78 (dd, J = 13.7/5.6 Hz, 1H, Ar-CO-CH_2), 2.95 (t, J =

5.8 Hz, 2H, Ar-CH_2-), 3.11 (dd, J=13.7 Hz/5.1, 1H, Ar-CO-CH_2), 3.78 (s, 3H, Ar-OCH_3), 4.42–4.51 (m, 1H, -C*H*-NHCOCH$_3$), 5.67–5.68 (m, 1H, N*H*), 6.71 (d, J=2.5 Hz, 1H, 1-H$_{arom}$), 6.80 (dd, J=8.7/2.5 Hz, 1H, 3-H$_{arom}$), and 7.77 ppm (d, J=8.7 Hz, 1H, 4-H$_{arom}$); ^{13}C NMR ($CDCl_3$): δ=23.6 (1C, -NC=O-*C*H$_3$), 32.1 (1C, Ar -*C*H$_2$-CH$_2$), 33.5 (1C, Ar-CH$_2$-*C*H$_2$-), 45.3 (1C, -*C*H-NHAc), 47.2 (1C, -CH=O-C*H*$_2$-), 55.6 (1C, Ar-O*C*H$_3$), 112.2 (1C, 3-C$_{arom}$), 115.5 (1C, 1-*C*$_{arom}$), 131.4 (1C, 4-*C*$_{arom}$), 131.5 (1C$_q$, 11-C$_{arom}$), 145.7 (1C$_q$, 10-C$_{arom}$), 162.8 (1C$_q$, 2-C$_{arom}$), 169.7 (1 C$_q$, -NC=O-CH$_3$), and 200.3 ppm (1C$_q$, -*C*=O-CH$_2$-); purity by HPLC: 95.5% (t_r=13.93 min); MS (HRMS): m/z (%)=calcd for $C_{14}H_{18}NO_3^+$: 248.1281, found: 248.1297; IR (neat): $\tilde{\nu}$=3289 (N–H), 3060 (C–H$_{arom}$), 2944 (C–H$_{aliph}$), 1702 (C=O$_{ketone}$), 1634 (C=O$_{amide\ I}$), 1599 (C=O$_{amide\ II}$), 819 cm^{-1} (C=C$_{1,3,4\text{-trisubst arom}}$); $C_{14}H_{17}NO_3$, M_r= 247.3 Da.

***cis*- and *trans*-*N*-(5-Hydroxy-2-methoxy-6,7,8,9-tetrahydro-5*H*-benzo[7]annulen-7-yl)acetamide (*cis*-12 and *trans*-12)**: $NaBH_4$ (14.0 mg, 0.4 mmol) was added to a solution of amido ketone **11** (92 mg, 0.4 mmol) in CH_3OH (5 mL) at 0 °C. After stirring overnight at room temperature, the solvent was removed under vacuum. The residue was treated with 1 M HCl (5 mL), and the aqueous layer was extracted with EtOAc (3×). The combined organic layers were washed with brine and dried (Na_2SO_4), the solvent was removed under reduced pressure, and the residue (55 mg) was purified by FC (d=2 cm, l=18 cm, V=30 mL, cyclohexane/EtOAc, 1:9, R_f=0.11) to yield the target compound mixture as a colorless oil (52 mg, 56%): ^{1}H NMR (CD_3OD): δ=1.40–1.66 (m, 2H, Ar-CH$_2$-C*H*$_2$), 1.91 (s, 3×0.6H, NHCO-C*H*$_3$), 1.92 (s, 3×0.4H, NHCO-C*H*$_3$), 2.03–2.08 (m, 2×0.6H, -CHOH-C*H*$_2$-), 2.17–2.20 (m, 2×0.4H, -CHOH-C*H*$_2$-), 2.62–2.79 (m, 2H, Ar-C*H*$_2$-), 3.75 (s, 3×0.4H, Ar-OC*H*$_3$), 3.76 (s, 3×0.6H, Ar-OC*H*$_3$), 4.81 (d, J=10.1 Hz, 0.6H, -C*H*OH-), 4.07–4.16 (m, 0.6H, -C*H*-NH-), 4.39–4.47 (m, 0.4H, -C*H*-NH-), 4.86 (d, J=10.1 Hz, 0.4H, -C*H*OH-), 6.65–6.68 (m, 1H, 1-H$_{arom}$), 6.73–6.76 (m, 1H, 3-H$_{arom}$), 7.12–7.13 (m, 0.4H, 4-H$_{arom}$), and 7.39–7.41 ppm (m, 0.6H, 4-H$_{arom}$), signals for the NH and OH groups are not observed in the spectrum; MS (ESI): m/z (%)=248 [M−H, 100; $C_{14}H_{19}NO_3$, M_r= 249.3 Da.

***N*-(3-Methoxy-6,7,-dihydro-5*H*-benzo[7]annulen-7-yl)acetamide (13) and 3-Methoxy-5*H*-benzo[7]annulene (14)**: Amido alcohol **12** (100 mg, 0.4 mmol) was dissolved in 5 M HCl (8 mL) and *N*,*N*-dimethylformamide. The mixture was stirred at 60 °C for 24 h. After cooling to room temperature, the mixture was washed with Et_2O (3×). Next, NaOH was added (pH>12), and the mixture was extracted with EtOAc (3×). The organic layer was washed with brine, dried (Na_2SO_4), concentrated under vacuum, and the residue (34 mg) was purified by FC (d=4 cm, l=6 cm, V=10 mL, EtOAc/cyclohexane, 8:2) to yield **13** and **14** as colorless oils. Compound **13** (yield 11 mg, 12%): R_f=0.15; ^{1}H NMR ($CDCl_3$): δ=2.00 (s, 3H, -NHCOC*H*$_3$), 1.94–2.10 (m, 2H, 6-H), 2.77 (ddd, J=15.4/9.4/1.9 Hz, 1H, 5-H), 2.84 (ddd, J=15.4/8.6/2.06 Hz, 1H, 5-H), 3.80 (s, 3H, Ar-OCH$_3$), 4.78–4.85 (m, 1H, 7-H), 5.56 (dd, J=12.3/4.2 Hz, 1H, 8-H), 5.60–5.67 (m, 1H, N*H*), 6.41 (dd, J=12.3/1.9 Hz, 1H, 9-H), 6.68 (d, J=2.7 Hz, 1H, 4-H), 6.70 (dd, J=8.3/2.7 Hz, 1H, 2-H), 7.10 ppm (d, J=8.3 Hz, 1H, 1-H); $C_{14}H_{17}NO_2$, M_r=231.3 Da. Compound **14** (yield: 5 mg, 8%): R_f=0.44; ^{1}H NMR ($CDCl_3$): δ=2.97 (d, J=6.8 Hz, 2H, 5-H), 3.75 (s, 3H, Ar-OCH$_3$), 5.66 (dt, J=9.8/6.8 Hz 1H, 6-H), 5.99 (dd, J=9.8/5.4 Hz, 1H, 7-H), 6.29 (dd, J=11.4/5.4 Hz, 1H, 8-H), 6.63 (d, J=2.6 Hz, 1H, 4-H), 6.72 (dd, J=8.5/2.6 Hz, 1H, 2-H), 6.95 (d, J=11.5 Hz, 1H, 9-H), and 7.17 ppm (d, J=8.6 Hz, 1H, 1-H); $C_{12}H_{12}O$, M_r=172.2 Da.

7-Amino-2-methoxy-6,7,8,9-tetrahydrobenzo[7]annulen-5-one (15): Amido ketone **11** (1.3 g, 5.3 mmol) was suspended in 2 M HCl (20 mL) and heated at 95 °C for 16 h. After cooling to room temperature, the mixture was washed with CH_2Cl_2 (2×). Next, NaOH was added (pH>12), and the mixture was extracted with CH_2Cl_2 (4×). The organic layer was washed with brine, and dried (Na_2SO_4), filtered, and concentrated under vacuum. The residue was purified by FC (d=8 cm, l=18 cm, V=65 mL, CH_2Cl_2/CH_3OH, 9:1, R_f=0.28) to yield **15** as a pale yellow oil (1.1 g, 85%): ^{1}H NMR ($CDCl_3$): δ= 1.61 (dtd, J=15.4/7.8/2.8 Hz, 1H, Ar-CH$_2$-C*H*$_2$), 2.24 (dddd, J=16.6/9.6/6.7/2.8 Hz 1H, Ar-CH$_2$-C*H*$_2$), 2.77 (dd, J=13.4/7.6 Hz, 1H, Ar-CO-C*H*$_2$), 2.87 (ddd, J=16.0/8.0/2.5 Hz, 1H, Ar-C*H*$_2$), 2.98 (dd, J=13.4/4.5 Hz, 1H, Ar-CO-C*H*$_2$), 2.87 (ddd, J=15.8/9.9/2.7 Hz, 1H, Ar-C*H*$_2$), 3.42–3.48 (m, 1H, -C*H*-NHCOCH$_3$), 3.84 (s, 3H, Ar-OCH$_3$), 6.72 (d, J= 2.5 Hz, 1H, 1-H$_{arom}$), 6.80 (dd, J=8.7/2.5 Hz, 1H, 3-H$_{arom}$), and 7.80 ppm (d, J=8.7 Hz, 1H, 4-H$_{arom}$), a signal for the NH_2 group is not observed in the spectrum; purity by HPLC: 94.7% (t_r= 9.70 min); MS (HRMS): m/z (%)=calcd for $C_{12}H_{13}O_2^+$: 189.0910, found: 189.0921 [M−NH$_2$, 100]; $C_{12}H_{15}NO_2$, M_r=205.1 Da.

***cis*- and *trans*-7-Amino-2-methoxy-6,7,8,9-tetrahydro-5*H*-benzo[7]annulen-5-ol (*cis*-16 and *trans*-16)**: $LiBH_4$ (2 M in THF, 5.3 mL) was added over 60 min to amino ketone **15** (1.1 g, 5.3 mmol) in abs. THF (15 mL) at −78 °C under N_2. After 30 min, the mixture was warmed to room temperature, and H_2O (1 mL) and HCl (2 M, 6 mL) were added. The suspension was dissolved by addition of NaOH (2 M) up to pH 8. The solvents were removed under vacuum, and the residue was dissolved in EtOAc (10 mL) and H_2O (10 mL). The aqueous layer was separated and extracted with EtOAc (3×15 mL). The combined organic layers were washed with brine and dried (Na_2SO_4), and the solvent was removed under reduced pressure. The crude residue, a 50:50 ratio of diastereomers, was used in the next reaction step without purification (colorless oil, yield: 0.9 g, 85%): ^{1}H NMR ($CDCl_3$): δ=1.02–1.24 (m, 1H, 8-CH$_2$), 1.25–1.40 (m, 0.5H, 6-CH$_2$), 1.41–1.54 (m, 0.5H, 8-CH$_2$), 1.81–1.90 (m, 0.5H, 8-CH$_2$), 1.99–2.18 (m, 1H, 6-CH$_2$), 2.55–2.77 (m, 3×0.5H, 9-CH$_2$, 6-CH$_2$), 2.85–2.98 (m, 1H, 9-CH$_2$), 3.10–3.25 (m, 0.5H, 7-CH), 3.31 (tt, J=8.9/3.4 Hz, 0.5H, 7-CH), 3.75 (s, 3×0.5H, Ar-OC*H*$_3$), 3.76 (s, 3×0.5H, Ar-OC*H*$_3$), 4.77 (d, J=8.0 Hz, 0.5H, *cis*-5-H), 4.99 (d, J=7.7 Hz, 0.5H, *trans*-5-H), 6.62 (dd, J=8.2/2.8 Hz, 0.5H, *trans*-3-H$_{arom}$), 6.66–6.69 (m, 1H, 1-H$_{arom}$),6.72 (dd, J=8.4/2.8 Hz, 0.5H, *cis*-3-H$_{arom}$), 7.15 (d, J=8.3 Hz, 0.5H, *trans*-4-H$_{arom}$), and 7.32 ppm (d, J=8.3 Hz, 0.5H, *cis*-4-H$_{arom}$), signals for the OH and NH_2 protons are not observed in the spectrum; $C_{12}H_{17}NO_2$, M_r=207.3 Da.

***cis*-2-Methoxy-7-[*N*-(3-phenylpropyl)amino]-6,7,8,9-tetrahydro-5*H*-benzo[7]annulen-5-ol (*cis*-17a), *trans*-2-methoxy-7-[*N*-(3-phenylpropyl)amino]-6,7,8,9-tetrahydro-5*H*-benzo[7]annulen-5-ol (*trans*-18a), *trans*-7-[*N*,*N*-Bis(3-phenylpropyl)amino]-2-methoxy-6,7,8,9-tetrahydro-5*H*-benzo[7]annulen-5-ol (*trans*-19a), and 2-methoxy-*N*-(3-phenylpropyl)-6,7,8,9-tetrahydro-5*H*-benzo[7]annulen-7-amine (20a)**: Amino ketone **15** (915 mg, 4.5 mmol) was reduced with $LiBH_4$ as described above. After workup, the crude amino alcohol **16** and 3-phenylpropionaldehyde (**86**) (510 mg, 3.8 mmol) were dissolved in abs. CH_2Cl_2 (20 mL). After the mixture was stirred for 30 min at room temperature under N_2, $NaBH(OAc)_3$ (1.1 g, 5.3 mmol) was added. The reaction was stopped after 4 h by addition of a sat. aqueous $NaHCO_3$ solution (25 mL). The aqueous layer was separated and extracted with CH_2Cl_2 (3×20 mL). The combined organic layers were washed with brine and dried (Na_2SO_4), the solvent was removed under reduced pressure, and the residue (1.4 g) was purified by FC (d=3 cm, l=17 cm, V= 30 mL, CH_2Cl_2/CH_3OH, 95:5).

cis-**17a**: The crude product (200 mg) was purified by recrystallization (EtOAc/diisopropyl ether, 3:1) to yield a colorless solid (yield: 57 mg, 4%): R_f=0.25; ^{1}H NMR ($CDCl_3$): δ=1.87–2.04 (m, 3H, -CHOH-C*H*$_2$-, Ar-CH$_2$-C*H*$_2$-), 1.99 (quint, J=7.5 Hz, 2H, -C*H*$_2$-CH$_2$-

C_6H_5), 2.22–2.36 (m, 1H, -CHOH-CH_2-), 2.49 (td, J=9.9/4.9 Hz, 1H, Ar-CH_2-), 2.68 (t, J=7.5 Hz, 2H, -CH_2-CH_2-C_6H_5), 2.78 (dt, J=11.6/7.5 Hz, 1H, -NH-CH_2-CH_2-CH_2-C_6H_5), 2.85 (dt, J=11.7/7.5 Hz, 1H, -NH-CH_2-CH_2-CH_2-C_6H_5), 3.19–3.37 (m, 2H, -CH-NH-, Ar-CH_2-), 3.77 (s, 3H, Ar-OCH_3), 4.84 (d, J=7.5 Hz, 1H, -CH-OH), 6.64 (d, J=2.4 Hz, 1H, 1-H_{arom}), 6.67 (dd, J=8.2/2.4 Hz, 1H, 3-H_{arom}), and 7.17–7.31 ppm (m, 6H, -C_6H_5, 4-H_{arom}), signals for the OH and NH groups are not observed in the spectrum; ^{13}C NMR ($CDCl_3$): δ=30.0 (1C, Ar-CH_2-), 30.3 (1C, -CH_2-CH_2-C_6H_5), 32.0 (1C, Ar-CH_2-CH_2-), 33.5 (1C, -CH_2-C_6H_5), 46.1 (1C, -NH-CH_2-CH_2-), 55.4 (1C, Ar-OCH_3), 58.1 (1C, -CH-NH-), 74.5 (1C, -CH-OH), 110.6 (1C, 3-C_{arom}), 116.3 (1C, 1-C_{arom}), 126.3 (1C, p-C_6H_5), 128.6 (2C, -C_6H_5), 128.7 (2C, -C_6H_5), 136.1 (1C, 4-C_{arom}), 141.0 (1C_q, 11-C_{arom}), 142.0 (1C_q, -C_6H_5), 146.1 (1C_q, 10-C_{arom}), and 158.8 ppm (1C_q, 2-C_{arom}), the signal for C-6 (CHOH-CH_2) is not observed in the spectrum; purity by HPLC: 99.3% (t_r=16.43 min); MS (HRMS): m/z (%)=calcd for $C_{21}H_{28}NO_2^+$: 326.2115, found: 326.2155; $C_{21}H_{27}NO_2$, M_r=325.4 Da

trans-**18a**: The crude product (118 mg) was purified by additional FC (d=2 cm, l=25 cm, V=10 mL, CH_2Cl_2/CH_3OH, 18:1+0.84% NH_3 (25% aq), R_f=0.50) to yield a colorless oil (yield: 21 mg, 2%); ^{1}H NMR ($CDCl_3$): δ (ppm)=1.37–1.45 (m, 1H, Ar-CH_2-CH_2-), 1.61 (dd, J=13.2/10.0 Hz, 1H, -CHOH-CH_2-), 1.84 (quint, J=7.4 Hz, 2H, -CH_2-CH_2-C_6H_5), 1.99–2.05 (m, 1H, Ar-CH_2-CH_2-), 2.20–2.29 (m, 1H, -CHOH-CH_2-), 2.67 (t, J=7.7 Hz, 1H, -CH_2-CH_2-C_6H_5), 2.68–2.77 (m, 3H, Ar-CH_2-, -NH-CH_2-CH_2-CH_2-C_6H_5), 2.99 (t, J=12.4 Hz, 1H, Ar-CH_2-), 3.18 (tt, J=9.5/3.3 Hz, 1H, -CH-NH-), 3.79 (s, 3H, Ar-OCH_3), 4.99 (d, J=7.6 Hz, 1H, -CH-OH), 6.67 (dd, J=8.5/2.7 Hz, 1H, 3-H_{arom}), 6.64 (d, J=2.4 Hz, 1H, 1-H_{arom}), 7.15 (d, J=8.5 Hz, 1H, 4-H_{arom}), and 7.17–7.32 (m, 5H, -C_6H_5), signals for the OH and NH groups are not observed in the spectrum; ^{13}C NMR ($CDCl_3$): δ=31.7 (1C, Ar-CH_2-), 32.2 (1C, -CH_2-CH_2-C_6H_5), 34.0 (1C, -CH_2-C_6H_5), 34.5 (1C, Ar-CH_2-CH_2-), 41.5 (1C, -CHOH-CH_2-), 47.0 (1C, -NH-CH_2-CH_2-), 55.1 (1C, Ar-OCH_3), 55.4 (1C, -CH-NH-), 72.3 (1C, -CH-OH), 110.3 (1C, 3-C_{arom}), 116.3 (1C, 1-C_{arom}), 126.0 (1C, p-C_6H_5), 128.5 (2C, -C_6H_5), 128.6 (2C, -C_6H_5), 128.9 (1C, 4-C_{arom}), 135.6 (1C_q, 11-C_{arom}), 142.3 (1C_q, -C_6H_5), 143.4 (1C_q, 10-C_{arom}), and 159.1 ppm (1C_q, 2-C_{arom}); purity by HPLC: 98.0% (t_r=16.88 min); $C_{21}H_{27}NO_2$, M_r=325.4 Da.

trans-**19a**: The crude product (255 mg) was purified by additional FC (d=2.5 cm, l=15 cm, V=10 mL, EtOAc/cyclohexane, 8:2, R_f 0.28) to yield a colorless oil (yield: 173 mg, 13%); ^{1}H NMR ($CDCl_3$): δ=1.42 (q, J=11.9 Hz, 1H, Ar-CH_2-CH_2), 1.61 (m, 1H, Ar-CH_2-CH_2), 1.78 (quint, J=7.3 Hz, 4H, -CH_2-CH_2-C_6H_5), 2.06–2.14 (m, 1H, -CHOH-CH_2-), 2.29–2.36 (m, 1H, -CHOH-CH_2-), 2.49 (t, J=7.2 Hz, 4H, -CH_2-CH_2-C_6H_5), 2.62 (m, 1H, Ar-CH_2), 2.63 (t, J=7.5 Hz, 4H, -CH_2-CH_2-CH_2-C_6H_5), 3.15 (t, J=12.8 Hz, 1H, Ar-CH_2), 3.37–3.44 (m, 1H, CH-N-), 3.80 (s, 3H, Ar-OCH_3), 4.96 (d, J=6.0 Hz, 1H, -CH-OH), 6.66 (dd, J=8.1/2.6 Hz, 1H, 3-H_{arom}), 6.71 (d, J=2.6 Hz, 1H, 1-H_{arom}), 7.10 (d, J=8.2 Hz, 1H, 4-H_{arom}), and 7.14–7.31 ppm (m, 10H, -C_6H_5), a signal for the OH group is not observed in the spectrum; $C_{30}H_{37}NO_2$, M_r=443.6 Da.

20a: The crude product (183 mg) was further purified by FC (d=2.0 cm, l=17 cm, V=10 mL, EtOAc/cyclohexane, 9:1, R_f=0.29) to yield a colorless oil (yield: 70 mg, 5%): ^{1}H NMR ($CDCl_3$): δ=1.20–1.41 (m, 4H, Ar-(CH_2-CH_2-)$_2$CH-), 1.82 (quint, J=6.66 Hz, 2H, -CH_2-CH_2-C_6H_5), 2.60–2.79 (m, 9H, CH-N-, 2×Ar-CH_2, -NH-CH_2-CH_2-CH_2-C_6H_5), 3.78 (s, 3H, Ar-OCH_3), 6.63 (dd, J=8.1/2.7 Hz, 1H, 3-H_{arom}), 6.68 (d, J=2.7 Hz, 1H, 1-H_{arom}), 7.01 (d, J=8.1 Hz, 1H, 4-H_{arom}), and 7.19–7.31 ppm (m, 5H, -C_6H_5), a signal for the NH group is not observed in the spectrum; ^{13}C NMR ($CDCl_3$): δ=31.6 (1C, Ar-(CH_2-)$_2$), 32.4 (1C, -CH_2-CH_2-C_6H_5), 32.8 (1C, Ar-(CH_2-)$_2$), 34.0 (1C, -CH_2-C_6H_5), 34.8 (1C, Ar-(CH_2-CH_2-)$_2$), 35.2 (1C, Ar-(CH_2-CH_2-)$_2$), 46.8 (1C, -NH-CH_2-CH_2-), 55.4 (1C, Ar-OCH_3), 61.6 (1C, -CH-NH-), 110.7 (1C, 3-C_{arom}), 115.2 (1C, 1-C_{arom}), 128.9 (1C, p-C_6H_5), 128.5 (2C, -C_6H_5), 128.6 (2C, -C_6H_5), 129.9 (1C, 4-C_{arom}), 135.0 (1C_q, 11-C_{arom}), 142.4 (1C_q, -C_6H_5), 144.1 (1C_q, 10-C_{arom}), and 159.1 ppm (1C_q, 2-C_{arom}); purity by HPLC: 97.1% (t_r=18.37 min); MS (HRMS): m/z (%)=calcd for $C_{21}H_{28}NO^+$: 310.2165, found: 310.2131; $C_{21}H_{27}NO$, M_r=309.4 Da.

***cis*-2-Methoxy-7-[*N*-(4-phenylbutyl)amino]-6,7,8,9-tetrahydro-5*H*-benzo[7]annulen-5-ol (*cis*-17b) and *trans*-2-methoxy-7-[*N*-(4-phenylbutyl)amino]-6,7,8,9-tetrahydro-5*H*-benzo[7]annulen-5-ol (*trans*-18b)**: Amino ketone **15** (912 mg, 4.4 mmol) was reduced with $LiBH_4$ as described above. After workup, the crude amino alcohol **16**, 4-phenylbutanal (204 mg, 1.38 mmol), and $MgSO_4$ (159 mg, 1.32 mmol) were dissolved and suspended in abs. CH_2Cl_2 (25 mL), and the mixture was stirred at room temperature for 16 h. $NaBH(OAc)_3$ (426 mg, 2.01 mmol) was added, and the mixture was stirred for 4 h at room temperature. The reaction was stopped by addition of HCl (2 M, pH 1). The mixture was diluted with H_2O (10 mL) and CH_2Cl_2 (10 mL) and alkalized with NaOH (2 M, pH 8). The aqueous layer was extracted with CH_2Cl_2 (2×15 mL). The combined organic layers were washed with brine and dried (Na_2SO_4), the solvent was removed under reduced pressure, and the residue was purified by FC (d=2 cm, l=17 cm, V=10 mL, CH_2Cl_2/CH_3OH, 18:1+0.84% NH_3 (25% aq)).

cis-**17b** was isolated as a colorless oil (yield: 9 mg, 2%): R_f=0.43; ^{1}H NMR ($CDCl_3$): δ=1.54–1.60 (m, 2H, -NH-CH_2-CH_2-), 1.70 (quint, J=8.0 Hz, 2H, -CH_2-CH_2-C_6H_5), 1.72–1.81 (m, 1H, -CHOH-CH_2-), 1.84–2.02 (m, 2H, Ar-CH_2-CH_2-), 2.25–2.38 (m, 1H, -CHOH-CH_2-), 2.46–2.52 (m, 1H, Ar-CH_2-), 2.65 (t, J=7.5 Hz, 2H, -CH_2-CH_2-C_6H_5), 2.68 (dt, J=11.3/7.1 Hz, 1H, N-CH_2-CH_2-), 2.83 (dt, J=11.2/7.1 Hz, 1H, N-CH_2-CH_2-), 3.21–3.27 (m, 1H, Ar-CH_2-), 3.37–3.52 (m, 1H, -CH-NH-), 3.78 (s, 3H, Ar-OCH_3), 4.84 (d, J=6.5 Hz, 1H, -CH-OH), 6.65 (dd, J=8.1/2.6 Hz, 1H, 3-H_{arom}), 6.68 (d, J=2.6 Hz, 1H, 1-H_{arom}), 7.12 (d, J=8.2 Hz, 1H, 4-H_{arom}), and 7.15–7.30 ppm (m, 5H, -C_6H_5), signals for the OH and NH groups are not observed in the spectrum; ^{13}C NMR ($CDCl_3$): δ=29.5 (1C, -CH_2-CH_2-C_6H_5), 30.3 (1C, -NH-CH_2-CH_2-), 31.7 (1C, Ar-CH_2-), 34.5 (1C, Ar-CH_2-CH_2-), 36.1 (1C, -CH_2-C_6H_5), 41.4 (1C, -CHOH-CH_2), 47.3 (1C, -NH-CH_2-CH_2-), 55.1 (1C, -CH-NCH_3-), 55.4 (1C, Ar-OCH_3), 72.3 (1C, -CH-OH), 110.3 (1C, 3-C_{arom}), 116.3 (1C, 1-C_{arom}), 125.9 (1C, p-C_6H_5), 128.5 (2C, -C_6H_5), 128.6 (2C, -C_6H_5), 128.9 (1C, 4-C_{arom}), 135.6 (1C_q, 11-C_{arom}), 142.7 (1C_q, -C_6H_5), 143.4 (1C_q, 10-C_{arom}), and 159.1 ppm (1C_q, 2-C_{arom}); purity by HPLC: 98.9% (t_r=17.86 min); MS (HRMS): m/z (%)=calcd for $C_{22}H_{30}NO_2^+$: 340.2271, found: 340.2245; $C_{22}H_{29}NO_2$, M_r=339.5 Da.

trans-**18b** was isolated as a colorless oil (yield: 15 mg, 2%): R_f=0.35; ^{1}H NMR ($CDCl_3$): δ=1.36–1.44 (m, 1H, Ar-CH_2-CH_2-), 1.49–1.70 (m, 5H, -CHOH-CH_2-, -CH_2-CH_2-CH_2-C_6H_5), 1.99–2.04 (m, 1H, Ar-CH_2-CH_2-), 2.21–2.27 (m, 1H, -CHOH-CH_2-), 2.63 (t, J=7.5 Hz, 2H, -NH-CH_2-CH_2-), 2.68 (dd, J=7.1/3.5 Hz, 1H, -CH_2-CH_2-C_6H_5), 2.70 (dd, J=7.4/3.6 Hz, 1H, -CH_2-CH_2-C_6H_5), 2.71–2.77 (m, 1H, Ar-CH_2-), 2.93–3.04 (m, 1H, Ar-CH_2-), 3.16 (tt, J=9.5/3.3 Hz, 1H, -CH-NH-), 3.78 (s, 3H, Ar-OCH_3), 4.99 (d, J=7.5 Hz, 1H, -CH-OH), 6.65 (dd, J=7.7/2.7 Hz, 1H, 3-H_{arom}), 6.68 (d, J=2.7 Hz, 1H, 1-H_{arom}), and 7.12–7.30 ppm (m, 6H, 4-H_{arom}, -C_6H_5), signals for the OH and NH groups are not observed in the spectrum; ^{13}C NMR ($CDCl_3$): δ=29.5 (1C, -CH_2-CH_2-C_6H_5), 30.3 (1C, -NH-CH_2-CH_2-), 31.7 (1C, Ar-CH_2-), 34.5 (1C, Ar-CH_2-CH_2-), 36.1 (1C, -CH_2-C_6H_5), 41.4 (1C, -CHOH-CH_2), 47.3 (1C, -NH-CH_2-CH_2-), 55.1 (1C, -CH-NCH_3-), 55.4 (1C, Ar-OCH_3), 72.3 (1C, -CH-OH), 110.3 (1C, 3-C_{arom}), 116.3 (1C, 1-C_{arom}), 125.9 (1C, p-C_6H_5), 128.5 (2C, -C_6H_5), 128.6 (2C, -C_6H_5), 128.9 (1C, 4-C_{arom}), 135.6 (1C_q, 11-C_{arom}), 142.7 (1C_q, -C_6H_5), 143.4 (1C_q, 10-C_{arom}), and 159.1 ppm (1C_q, 2-C_{arom}); purity by HPLC: 95.1% (t_r=17.81 min); $C_{22}H_{29}NO_2$, M_r=339.5 Da.

***cis*- and *trans*-2-Methoxy-7-[*N*-(4-phenylbutyl)amino]-6,7,8,9-tetrahydro-5*H*-benzo[7]annulen-5-ol (*cis*-17b/*trans*-18b) and *cis*- and *trans*-7-[*N*,*N*-Bis(4-phenylbutyl)amino]-2-methoxy-6,7,8,9-tetrahydro-5*H*-benzo[7]annulen-5-ol (19b)**: A suspension of amino alcohol **16** (60 mg, 0.3 mmol), 1-chloro-4-phenylbutane (108 mg, 0.6 mmol), Bu_4NI (236 mg, 0.6 mmol) and K_2CO_3 (240 mg, 1.7 mmol) in CH_3CN (6 mL) was stirred at 85 °C for 24 h. The mixture was filtered, and the solvent was removed under reduced pressure. The residue was purified by FC (d = 2.5 cm, l = 14 cm, V = 10 mL, EtOAc/cyclohexane, 95:5+2% *N*,*N*-dimethylethylamine).

cis-**17b**/*trans*-**18b** was isolated as a colorless oil (yield: 23 mg, 23%): R_f = 0.28; ^{1}H NMR ($CDCl_3$): δ = 1.43–1.55 (m, 2H, -NH-CH_2-CH_2-), 1.56–1.74 (m, 3H, -CH_2-CH_2-C_6H_5, -CHOH-CH_2-), 1.75–1.90 (m, 2H, Ar-CH_2-CH_2-), 2.13–2.26 (m, 1H, -CHOH-CH_2-), 2.38–2.46 (m, 1H, Ar-CH_2-), 2.53–2.63 (m, 3H, -CH_2-CH_2-C_6H_5, N-CH_2-CH_2-), 2.71–2.78 (m, 1H, N-CH_2-CH_2-), 2.87–2.96 (m, 1H, Ar-CH_2-), 3.13–3.18 (m, 1H, -CH-NH-), 3.71 (s, 3H, Ar-OCH_3), 4.74 (d, J = 6.7 Hz, 0.7×1H, -CH-OH), 4.92 (d, J = 7.0 Hz, 0.3×1H, -CH-OH), 6.58 (dd, J = 8.2/2.7 Hz, 1H, 3-H_{arom}), 6.60 (d, J = 2.7 Hz, 1H, 1-H_{arom}), 7.05 (d, J = 8.1 Hz, 1H, 4-H_{arom}), and 7.08–7.23 ppm (m, 5H, -C_6H_5), signals for the OH and NH groups are not observed in the spectrum; $C_{22}H_{29}NO_2$, M_r = 339.5 Da.

19b was isolated as a colorless oil (yield: 81 mg, 59%): R_f = 0.36; ^{1}H NMR ($CDCl_3$): δ = 1.28–1.42 (m, 5H, -NH-CH_2-CH_2-, Ar-CH_2-CH_2-), 1.46–1.58 (m, 5H, -CH_2-CH_2-C_6H_5, -CHOH-CH_2-), 1.94–2.02 (m, 1H, Ar-CH_2-CH_2-), 2.16–2.24 (m, 1H, -CHOH-CH_2-), 2.32 (t, J = 6.9 Hz, 4H, N-CH_2-CH_2-), 2.52 (t, J = 7.7 Hz, 4H, -CH_2-CH_2-C_6H_5), 2.52–2.59 (m, 1H, Ar-CH_2-), 3.00–3.10 (m, 1H, Ar-CH_2-), 3.20–3.28 (m, 1H, -CH-NH-), 3.72 (s, 3H, Ar-OCH_3), 4.69 (d, J = 9.8 Hz, 0.4×1H, -CH-OH), 4.87 (d, J = 6.3 Hz, 0.6×1H, -CH-OH), 6.57 (dd, J = 8.2/2.6 Hz, 1H, 3-H_{arom}), 6.62 (d, J = 2.6 Hz, 1H, 1-H_{arom}), 7.01 (d, J = 8.2 Hz, 1H, 4-H_{arom}), and 7.06–7.23 ppm (m, 10H, -C_6H_5), a signal for the OH group is not observed in the spectrum; $C_{32}H_{41}NO_2$, M_r = 471.7 Da.

***cis*-2-Methoxy-7-[*N*-methyl-*N*-(3-phenylpropyl)amino]-6,7,8,9-tetrahydro-5*H*-benzo[7]annulen-5-ol (*cis*-21a)**: *cis*-**17a** (35 mg, 0.1 mmol) and formaldehyde (37%, 179 μL, 2.2 mmol) were dissolved in CH_2Cl_2 (3 mL). $NaBH(OAc)_3$ (46 mg, 0.22 mmol) was added, and the mixture was stirred at room temperature for 16 h. The reaction was stopped by addition of H_2O and HCl (2 M, pH 1). The mixture was alkalized with NaOH (2 M, pH 8) and extracted with CH_2Cl_2 (4×10 mL). The organic layer was washed with brine, dried (Na_2SO_4), and concentrated under vacuum, and the residue was purified by FC (d = 2 cm, l = 14 cm, V = 10 mL, CH_2Cl_2/CH_3OH, 9:1, R_f = 0.33) to yield a colorless oil (24 mg, 72%): ^{1}H NMR ($CDCl_3$): δ = 1.50–1.66 (m, 1H, Ar-CH_2-CH_2-), 1.83 (quint, J = 7.6 Hz, 2H, -CH_2-CH_2-C_6H_5), 1.88–2.08 (m, 3H, Ar-CH_2-CH_2-, -CHOH-CH_2-), 2.28 (s, 3H, -N-CH_3), 2.46–2.58 (m, 3H, -CH_2-CH_2-C_6H_5, Ar-CH_2-), 2.63 (t, J = 7.6 Hz, 2H, -NH-CH_2-CH_2-), 2.86–2.98 (m, 2H, Ar-CH_2-, -CH-N-), 3.79 (s, 3H, Ar-OCH_3), 4.79 (d, J = 10.4 Hz, 1H, -CH-OH), 6.67 (d, J = 2.7 Hz, 1H, 1-H_{arom}), 6.73 (dd, J = 8.4/2.7 Hz, 1H, 3-H_{arom}), 7.16–7.30 (m, 5H, -C_6H_5), and 7.36 ppm (d, J = 8.4 Hz, 1H, 4-H_{arom}), a signal for the OH group is not observed in the spectrum; ^{13}C NMR ($CDCl_3$): δ = 29.3 (1C, -CH_2-CH_2-C_6H_5), 30.0 (1C, Ar-CH_2-CH_2-), 31.8 (1C, Ar-CH_2-), 33.8 (1C, -CH_2-C_6H_5), 38.2 (1C, -NCH_3), 53.5 (1C, -NCH_3-CH_2-CH_2-), 55.4 (1C, Ar-OCH_3), 64.0 (1C, -CH-NCH_3-), 72.1 (1C, -CH-OH), 110.6 (1C, 3-C_{arom}), 115.8 (1C, 1-C_{arom}), 126.0 (1C, *p*-C_6H_5), 128.5 (2C, -C_6H_5), 128.6 (2C, -C_6H_5), 136.7 (1C, 4-C_{arom}), 141.3 (1C_q, 11-C_{arom}), 142.2 (1C_q, -C_6H_5), 146.1 (1C_q, 10-C_{arom}), and 158.6 ppm (1C_q, 2-C_{arom}), the signal for C-6 (CHOH-CH_2) is not observed in the spectrum; purity by HPLC: 99.2% (t_r = 16.93 min); MS (HRMS): m/z (%) = calcd for $C_{22}H_{30}NO_2^+$: 340.2271, found: 340.2307; $C_{22}H_{29}NO_2$, M_r = 339.5 Da.

***trans*-2-Methoxy-7-[*N*-methyl-*N*-(3-phenylpropyl)amino]-6,7,8,9-tetrahydro-5*H*-benzo[7]annulen-5-ol (*trans*-22a)**: *trans*-**18a** (46 mg, 0.14 mmol) and formaldehyde (37%, 228 μL, 2.8 mmol) were dissolved in CH_2Cl_2 (5 mL). $NaBH(OAc)_3$ (42 mg, 0.2 mmol) was added, and the mixture was stirred for 12 h at room temperature. The reaction was stopped by the addition of H_2O and HCl (2 M, pH 1). The mixture was alkalized with NaOH (2 M, pH 8) and extracted with CH_2Cl_2 (4×10 mL). The organic layers were washed with brine, dried (Na_2SO_4), and concentrated under vacuum, and the residue was purified by FC (d = 2 cm, l = 20 cm, V = 5 mL, CH_2Cl_2/CH_3OH, 18:1+0.84% NH_3 (25% aq), R_f = 0.23) to yield a colorless oil (25 mg, 74%): ^{1}H NMR ($CDCl_3$): δ = 1.45 (m, 1H, Ar-CH_2-CH_2-), 1.65 (dd, J = 11.9/1.4 Hz, 1H, -CHOH-CH_2-), 1.81 (quint, J = 7.5 Hz, 2H, -CH_2-CH_2-C_6H_5), 2.03–2.12 (m, 1H, Ar-CH_2-CH_2-), 2.23 (s, 3H, -NCH_3), 2.27–2.33 (m, 1H, -CHOH-CH_2-), 2.47 (t, J = 7.4 Hz, 2H, -NCH_3-CH_2-CH_2-), 2.63 (t, J = 7.5 Hz, 2H, -CH_2-CH_2-C_6H_5), 2.63–2.69 (m, 1H, Ar-CH_2-), 3.13 (t, J = 12.8 Hz, 1H, Ar-CH_2-), 3.31 (tt, J = 11.7/2.7 Hz, 1H, -CH-N-), 3.78 (s, 3H, Ar-OCH_3), 4.97 (d, J = 6.8 Hz, 1H, -CH-OH), 6.64 (dd, J = 8.2/2.6 Hz, 1H, 3-H_{arom}), 6.69 (d, J = 2.6 Hz, 1H, 1-H_{arom}), 7.09 (d, J = 8.4 Hz, 1H, 4-H_{arom}), and 7.16–7.30 ppm (m, 5H, -C_6H_5), a signal for the OH group is not observed in the spectrum; ^{13}C NMR ($CDCl_3$): δ = 30.2 (1C, -CH_2-CH_2-C_6H_5), 30.5 (1C, Ar-CH_2-CH_2-), 33.0 (1C, Ar-CH_2-), 33.9 (1C, -CH_2-C_6H_5), 36.1 (1C, -CHOH-CH_2-), 37.6 (1C, -NCH_3), 53.4 (1C, -NCH_3-CH_2-CH_2-), 55.4 (1C, Ar-OCH_3), 59.8 (1C, -CH-NCH_3-), 74.1 (1C, -CH-OH), 110.3 (1C, 3-C_{arom}), 116.6 (1C, 1-C_{arom}), 125.9 (1C, *p*-C_6H_5), 128.5 (2C, -C_6H_5), 128.6 (2C, -C_6H_5), 130.2 (1C, 4-C_{arom}), 135.1 (1C_q, 11-C_{arom}), 142.6 (1C_q, -C_6H_5), 144.1 (1C_q, 10-C_{arom}), and 159.4 ppm (1C_q, 2-C_{arom}); purity by HPLC: 95.2% (t_r = 17.35 min); MS (HRMS): m/z (%) = calcd for $C_{22}H_{30}NO_2^+$: 340.2271, found: 340.2297; $C_{22}H_{29}NO_2$, M_r = 339.5 Da.

2-Methoxy-*N*-methyl-*N*-(3-phenylpropyl)-6,7,8,9-tetrahydro-5*H*-benzo[7]annulen-7-amine (23a): Secondary amine **20a** (31 mg, 0.1 mmol) and formaldehyde (37%, 162 μL, 2 mmol) were dissolved in CH_2Cl_2 (3 mL). $NaBH(OAc)_3$ (44 mg, 0.21 mmol) was added, and the mixture was stirred for 12 h at room temperature. The reaction was stopped by addition of H_2O and HCl (2 M, pH 1), then the mixture was alkalized with NaOH (2 M, pH 8) and extracted with CH_2Cl_2 (4×10 mL). The organic layer was washed with brine, dried (Na_2SO_4), and concentrated under vacuum, and the residue was purified by FC (d = 1.5 cm, l = 15 cm, V = 5 mL, CH_2Cl_2/CH_3OH, 95:5, R_f = 0.36) to yield a colorless oil (23 mg, 71%): ^{1}H NMR ($CDCl_3$): δ = 1.30–1.43 (m, 2H, Ar-CH_2-CH_2-), 1.93 (quint, J = 7.5 Hz, 2H, -CH_2-CH_2-C_6H_5), 2.14–2.21 (m, 2H, Ar-CH_2-CH_2-), 2.33 (s, 3H, -N-CH_3), 2.58 (t, J = 7.6 Hz, 2H, -CH_2-CH_2-C_6H_5), 2.65 (t, J = 7.5 Hz, 2H, -NH-CH_2-CH_2-), 2.65–2.71 (m, 2H, Ar-CH_2-), 2.73–2.80 (m, 2H, Ar-CH_2-), 2.88–2.93 (m, 1H, -CH-N-), 3.78 (s, 3H, Ar-OCH_3), 6.65 (dd, J = 8.1/2.7 Hz, 1H, 3-H_{arom}), 6.69 (d, J = 2.7 Hz, 1H, 1-H_{arom}), 7.02 (d, J = 8.1 Hz, 1H, 4-H_{arom}), and 7.16–7.30 ppm (m, 5H, -C_6H_5); ^{13}C NMR ($CDCl_3$): δ = 29.1 (1C, -CH_2-CH_2-C_6H_5), 29.5 (1C, Ar(-CH_2-CH_2-)$_2$), 29.7 (1C, Ar(-CH_2-CH_2-)$_2$), 32.4 (1C, Ar(-CH_2-)$_2$), 33.6 (1C, Ar(-CH_2-)$_2$), 33.7 (1C, -CH_2-C_6H_5), 37.5 (1C, -NCH_3), 53.3 (1C, -NCH_3-CH_2-CH_2-), 55.5 (1C, Ar-OCH_3), 68.0 (1C, -CH-NH-), 111.0 (1C, 3-C_{arom}), 115.2 (1C, 1-C_{arom}), 126.2 (1C, *p*-C_6H_5), 128.5 (2C, -C_6H_5), 128.6 (2C, -C_6H_5), 130.1 (1C, 4-C_{arom}), 134.4 (1C_q, 11-C_{arom}), 141.8 (1C_q, -C_6H_5), 143.5 (1C_q, 10-C_{arom}), and 158.3 ppm (1C_q, 2-C_{arom}); purity by HPLC: 96.9% (t_r = 19.14 min); MS (HRMS): m/z (%) = calcd for $C_{22}H_{30}NO^+$: 324.2322, found: 324.2298; $C_{22}H_{29}NO$, M_r = 323.5 Da.

X-ray crystal structure analysis of cis-*17a*

Data sets were collected with a Nonius KappaCCD diffractometer using COLLECT (R. W. W. Hooft, Bruker AXS, 2008, Delft, The Netherlands) for data collection, Denzo-SMN[31] for data reduction,

Denzo[32] for absorption correction, SHELXS-97[33] for structure solution, SHELXL-97[34] for structure refinement, and XP (BrukerAXS, 2000) for graphics. Thermals ellipsoids are shown with 30% probability, *R*-values are given for observed reflections, and wR^2 values are given for all reflections.

Formula $C_{21}H_{27}NO_2$, $M=325.44$, colorless crystal, $0.30\times0.13\times0.10$ mm, $a=9.9223(2)$, $b=35.8022(7)$, $c=10.7061(2)$ Å, $\beta=105.745(1)^\circ$, $V=3660.5(1)$ Å^3, $\rho_{calc}=1.181$ g cm^{-3}, $\mu=0.587$ mm^{-1}, empirical absorption correction ($0.843\leq T\leq0.943$), $Z=8$, monoclinic, space group $P2_1/n$ (No. 14), $\lambda=1.54178$ Å, $T=223(2)$ K, ω and ϕ scans, 23476 reflections collected ($\pm h$, $\pm k$, $\pm l$), $[(\sin\theta)/\lambda]=0.60$ Å^{-1}, 6030 independent ($R_{int}=0.054$) and 5093 observed reflections [$I>2\sigma(I)$], 443 refined parameters, $R=0.058$, $wR^2=0.170$, max. (min.) residual electron density 0.24 (−0.25) e Å^{-3}, the hydrogens at nitrogen atoms were refined freely, but with fixed U-values; others were calculated and refined as riding atoms. CCDC 976582 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Pharmacology

Materials and general procedures: A high-speed cooling Sorvall RC-5C plus centrifuge (Thermo Finnigan) was used, with a printed Filtermat Type B filter (PerkinElmer), presoaked in 0.5% aqueous polyethylenimine for 2 h at room temperature before use. 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 GluN2B assay:[19] In the assay, mouse L(tk−) cells stably transfected with the dexamethasone-inducible eukaryotic expression vectors pMSG NR1a and pMSG NR2B were used in a 1:5 ratio. 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 reached ~90% confluency. For 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 buffered saline (PBS, pH 7.4), and the number of cells was determined using an improved Neubauer's counting chamber (VWR, Darmstadt, Germany). Subsequently, the cells were lysed by sonication (4 °C, 6×10 s cycles with breaks of 10 s, using a Soniprep 150, MSE, London, UK). The resulting cell fragments were centrifuged with a high performance 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 per 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.

Performing of the GluN2B binding assay:[19] The competitive binding assay was performed with the radioligand [^{3}H]ifenprodil (60 Ci mmol^{-1}; PerkinElmer) using standard 96-well multiplates (Diagonal, Münster, 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 presoaked filtermats using a cell harvester. After washing each well five times with 300 μL of water, the filtermats were dried at 95 °C. Subsequently, the solid scintillator was placed on the filtermat and melted at 95 °C. After 5 min, the solid scintillator was allowed to solidify at room temperature. The bound radioactivity trapped on the filters was counted in the scintillation analyzer. Nonspecific binding was determined with 10 μM unlabeled ifenprodil. The K_d value of ifenprodil is 10 nM.[19]

Protocol for functional GluN2A and GluN2B assay: The test compound solutions were prepared by dissolving ~10 μmol (usually 2–4 mg) of test compound in DMSO so that a 10 mM stock solution was obtained. To obtain the required test solutions for the assay, the DMSO stock solution was diluted with phosphate buffered saline solution (PBS).

Mouse L(tk−) cells stably transfected with the dexamethasone-inducible eukaryotic expression vectors pMSG NR1-1a and pMSG NR2A (1:5 ratio) for the GluN2A assay or pMSG and NR2B (1:5 ratio) for the GluN2B assay, grown in sterile 96-well plates in Dulbecco Modified Earl's Medium (DMEM) containing 10% of standardized fetal calf serum (FCS, Biochrom AG, Berlin, Germany). When the monolayer of the adherent growing cells almost covered the bottom area of each well, the medium was removed, and the cells in each well were rinsed with 200 μL PBS.

Subsequently, the cells were incubated with 100 μL dexamethasone solution (8 μM in growth medium), 100 μL CSS reagent (120 mM NaCl, 25 mM HEPES, 50 mM KCl, 4 mM EDTA, 1.8 mM $CaCl_2$, 15 mM glucose, 100 μM glycine and 100 μM Na-glutamate), and 50 μL of the respective test compound in six different concentrations (40 μM, 4 μM, 400 nM, 40 nM, 4 nM and 0.4 nM). Each concentration of the test compound was incubated at least in triplicate for 12 h at 37 °C.

Fifty microliters of the supernatant were transferred into a second 96-well plate and incubated with 50 μL of lactate dehydrogenase (LDH) assay mixture (18 U/mL diaphorase, 1% Na-lactate, 0.1% NAD^+, 0.08% BSA, 0.4% iodonitrotetrazolium chloride in 75 mM PBS) at room temperature. Previously, the 96-well plate was blocked with 1% BSA solution for 12 h. The reaction was terminated after 30 min by the addition of 100 μL of acetic acid (1 M), and UV absorption was measured at $\lambda=490$ nm in a plate reader (TECAN, Crailsheim, Germany). Total LDH activity was determined with buffer instead of ligand solution. A solution of 0.1% Triton-X served as a positive control. Data were analyzed using Sigma Plot by nonlinear regression analysis (sigmoidal dose response).

Affinity toward σ_1 and σ_2 receptors and the PCP binding site of the NMDA receptor: The affinity toward the PCP binding site of the NMDA receptor[25,26] and the affinity toward σ_1 and σ_2 receptors[27–29] were recorded as previously described.

Abbreviations

2-Amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionic acid (AMPA); bovine serum albumin (BSA); central nervous system (CNS); di-*o*-tolylguanidine (DTG); 1-(1-phenylcyclohexyl)piperidine (PCP); *N*-methyl-D-aspartate (NMDA).

Acknowledgements

The authors are grateful to Prof. Dr. D. Steinhilber (Department of Pharmacy, University of Frankfurt) for donating L(tk−) cells stably expressing GluN1a/GluN2A and GluN1a/GluN2B receptor proteins. Funding by the Deutsche Forschungsgemeinschaft is gratefully acknowledged.

Keywords: benzo[7]annulenamines · cytoprotective activity · GluN2B · NDMA receptors · selectivity · structure–affinity relationships

[1] M. B. Kennedy, *Science* **2000**, *290*, 750 – 754.
[2] H. Bräuner-Osborne, J. Egebjerg, E. O. Nielsen, U. Madsen, P. Krogsgaard-Larsen, *J. Med. Chem.* **2000**, *43*, 2609 – 2644.
[3] H. Stark, S. Graßmann, U. Reichert, *Pharm. Unserer Zeit* **2000**, *29*, 228 – 236.
[4] H. Furukawa, S. K. Singh, R. Mancusco, E. Gouaux, *Nature* **2005**, *438*, 185 – 192.
[5] R. Paoletti, J. Neyton, *Curr. Opin. Pharmacol.* **2007**, *7*, 39 – 47.
[6] D. J. Goebel, M. S. Poosch, *Mol. Brain Res.* **1999**, *69*, 164 – 170.
[7] S. Boyce, A. Wyatt, J. K. Webb, R. O′Donnell, G. Mason, M. Rigby, D. Sirinathsinghji, R. G. Hill, N. M. J. Rupniak, *Neuropharmacology* **1999**, *38*, 611 – 623.
[8] M. E. Layton, M. J. Kelly III, K. J. Rodzinak, *Curr. Top. Med. Chem.* **2006**, *6*, 697 – 709.
[9] I. Borza, G. Domány, *Curr. Top. Med. Chem.* **2006**, *6*, 687 – 695.
[10] L. Mony, J. N. C. Kew, M. J. Gunthorpe, P. Paoletti, *Br. J. Pharmacol.* **2009**, *157*, 1301 – 1317.
[11] L. Yurkewicz, J. Weaver, M. R. Bullock, L. F. Marshall, *J. Neurotrauma* **2005**, *22*, 1428 – 1443.
[12] X. Di, R. Bullock, J. Watson, P. Fatouros, B. Chenard, F. White, F. Corwin, *Stroke* **1997**, *28*, 2244 – 2251.
[13] R. H. Wessell, S. M. Ahmed, F. S. Menniti, G. L. Dunbar, T. N. Chase, J. D. Oh, *Neuropharmacology* **2004**, *47*, 184 – 194.
[14] S. F. Menniti, J. M. Pagnozzi, P. Butler, B. L. Chenard, S. S. Jaw-Tasi, W. F. White, *Neuropharmacology* **2000**, *39*, 1147 – 1155.
[15] J. Nagy, C. Horvath, S. Farkas, S. Kolok, Z. Szombathelyi, *Neurochem. Int.* **2004**, *44*, 17 – 23.
[16] E. Karakas, N. Simorowski, H. Furukawa, *Nature* **2011**, *475*, 249 – 253.
[17] I. J. Reynolds, R. J. Miller, *Mol. Pharmacol.* **1989**, *36*, 758 – 765.
[18] K. Williams, *Curr. Drug Targets* **2001**, *2*, 285 – 298.
[19] D. Schepmann, B. Frehland, K. Lehmkuhl, B. Tewes, B. Wünsch, *J. Pharm. Biomed. Anal.* **2010**, *53*, 603 – 608.
[20] E. Falck, F. Begrow, E. Verspohl, B. Wünsch, *J. Pharm. Biomed. Anal.* **2014**, *88*, 96 – 105.
[21] B. Tewes, B. Frehland, D. Schepmann, K.-U. Schmidtke, T. Winckler, B. Wünsch, *ChemMedChem* **2010**, *5*, 687 – 695.
[22] B. Tewes, B. Frehland, D. Schepmann, K.-U. Schmidtke, T. Winckler, B. Wünsch, *Bioorg. Med. Chem.* **2010**, *18*, 8005 – 8015.
[23] P. E. Eaton, G. R. Carlson, J. T. Lee, *J. Org. Chem.* **1973**, *38*, 4071 – 4073.
[24] A. F. Abdel-Magid, S. J. Mehrmann, *Org. Process Res. Dev.* **2006**, *10*, 971 – 1031.
[25] T. Utech, J. Köhler, B. Wünsch, *Eur. J. Med. Chem.* **2011**, *46*, 2157 – 2169.
[26] U. Wirt, D. Schepmann, B. Wünsch, *Eur. J. Org. Chem.* **2007**, 462 – 475.
[27] C. A. Maier, B. Wünsch, *J. Med. Chem.* **2002**, *45*, 438 – 448.
[28] C. A. Maier, B. Wünsch, *J. Med. Chem.* **2002**, *45*, 4923 – 4930.
[29] C. Meyer, B. Neue, D. Schepmann, S. Yanagisawa, J. Yamaguchi, E.-U. Würthwein, K. Itami, B. Wünsch, *Bioorg. Med. Chem.* **2013**, *21*, 1844 – 1856.
[30] Y. Rook, K.-U. Schmidtke, F. Gaube, D. Schepmann, B. Wünsch, J. Heilmann, J. Lehmann, T. Winckler, *J. Med. Chem.* **2010**, *53*, 3611 – 3617.
[31] Z. Otwinowski, W. Minor, *Methods in Enzymology*, Vol. 276: Macromolecular Crystallography, Part A (Eds.: C. W. Carter, Jr., R. M. Sweet), Academic Press, New York, **1997**, pp. 307 – 326.
[32] Z. Otwinowski, D. Borek, W. Majewski, W. Minor, *Acta Crystallogr. Sect. A* **2003**, *59*, 228 – 234.
[33] G. M. Sheldrick, *Acta Crystallogr. Sect. A* **1990**, *46*, 467 – 473.
[34] G. M. Sheldrick, *Acta Crystallogr. Sect. A* **2008**, *64*, 112 – 122.

Received: December 20, 2013
Published online on February 23, 2014