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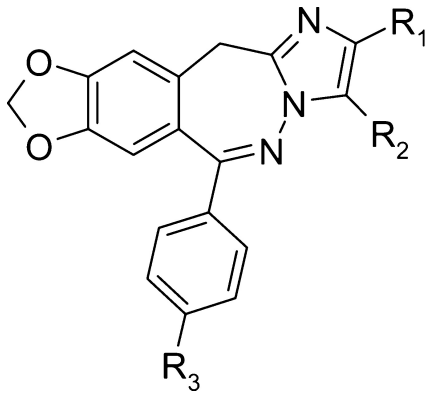

R_1 = H, Br, Cl, Me
R_2 = H, Br, Cl, Me
R_3 = H, F, Cl

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Novel α-Amino-3-hydroxy-5-methyl-4-isoxazole Propionate (AMPA) Receptor Antagonists of 2,3-Benzodiazepine Type: Chemical Synthesis, in Vitro Characterization, and in Vivo Prevention of Acute Neurodegeneration

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Under pathophysiological conditions, α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) receptor activation is considered to play a key role in several disorders of the central nervous system. In the search for AMPA receptor antagonists, the synthesis and pharmacological characterization of a series of novel compounds that are structurally related to GYKI 52466 (**1**), a well-known selective noncompetitive AMPA receptor antagonist, was performed. In vitro, 2,3-dimethyl-6-phenyl-12*H*-[1,3]dioxolo[4,5-*h*]imidazo[1,2-*c*][2,3]benzodiazepine (ZK 187638, **14a**) antagonized the kainate-induced currents in cultured hippocampal neurons with an IC_{50} of 3.4 μM in a noncompetitive fashion. When tested in a clinically predictive rat model of acute ischemic stroke, this noncompetitive AMPA receptor antagonist significantly reduced brain infarction, indicating that it is neuroprotective after permanent focal cerebral ischemia.

Introduction

AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionate) receptors are a group of glutamatergic receptors that are present in various cerebral cell types such as neurons, oligodendrocytes, and microglia. AMPA receptors are implicated as causative in the pathogenesis of numerous acute and chronic neurological disorders.[1−3] In the search for agents to treat these diseases competitive AMPA receptor antagonists have been synthesized. As an example, YM-872 ([2,3-dioxo-7-(1*H*-imidazol-1-yl)-6-nitro-1,2,3,4-tetrahydro-1-quinoxalinyl]acetic acid monohydrate, **2**) is a competitive AMPA receptor antagonist that has shown efficacy in rats after permanent MCA occlusion and that is currently undergoing clinical stroke trials.[4] However, the risk that compounds that inhibit AMPA receptors in a competitive way could fail in clinical development for stroke therapy is potentially high because the development of the compounds 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo[*f*]quinoxaline (NBQX, **3**) and [1,2,3,4-tetrahydro-7-morpholinyl-2,3-dioxo-6-(trifluoromethyl)quinoxalin-1-yl]methylphosphonate (ZK 200775, **4**) has been discontinued because of unwanted side effects. Therefore, the search for neuroprotective agents was extended to chemical structures that inhibit AMPA receptors in a noncompetitive way. Here, we describe the synthesis and pharmacological characterization of 2,3-dimethyl-6-phenyl-12*H*-[1,3]dioxolo[4,5-*h*]imidazo[1,2-*c*][2,3]benzodiazepine (**14a**) and its metabolite (**18**). Compound **14a** emerged as the most potent AMPA receptor inhibitor of a series of chemical structures (Chart 1) that were synthesized as derivatives of GYKI 52466 (**1**), the prototype of noncompetitive AMPA receptor antagonists.[5]

Chemistry

The first step in the chemical synthesis of 2,3-dimethyl-6-phenyl-12*H*-[1,3]dioxolo[4,5-*h*]imidazo[1,2-*c*]-[2,3]benzodiazepine (**14a**) was the reduction of **5** with $LiAlH_4$ to alcohol **6**, which was cyclized with benzaldehyde to isochromane **7a** (Scheme 1). Oxidation of **7a** yielded benzoylphenylacetic acid **8a**. Cyclization via the mixed anhydride and subsequent reaction with hydrazine gave benzo-2,3-diazepine-4-one **9a**, which was converted to thione **7**. Thione **10a** could be cyclized by reaction with aminoketal **12** (prepared by reductive amination of **11**), followed by treatment with acidic EtOH to give **14a** directly. Intermediate **13a** could not be isolated using the described reaction conditions. The syntheses of **14b** and **14c** were accomplished in an analogous manner (Table 1 and Scheme 1).

5-Phenyl-8-methyl-9-hydroxymethyl-11*H*-1,3-dioxolo-[4,5-*h*]imidazo[1,2-*c*][2,3]benzodiazepine (**18**), which is a metabolite of **14a**, could be synthesized via the monomethyl derivative **17** by reaction with aqueous formaldehyde in the presence of sodium acetate and glacial acetic acid as shown in Scheme 2. **17** was synthesized from thione **10a** in a manner analogous to the preparation of **14a**. In this case intermediate **16** could be isolated (Scheme 2).

The central intermediate for the synthesis of compounds **24** and **27** was 5-(4-nitrophenyl)-11*H*-1,3-dioxolo[4,5-*h*]imidazo[1,2-*c*][2,3]benzodiazepine (**21**), which could be obtained by reaction of thione **10d** with aminoacetal **19**. Also in this case, the intermediate, acetal **20**, could be isolated. **24** was synthesized from **21** by reduction to **22**, reductive diazotization to **23**, and subsequent chlorination of the imidazole ring by NCS in acetonitrile. In the case of **27** it was necessary to first brominate **21**−**25** to reduce the nitro group selectively by Fe in acetic acid and finally to eliminate the amino group by reductive diazotization, giving **27** (Scheme 3).

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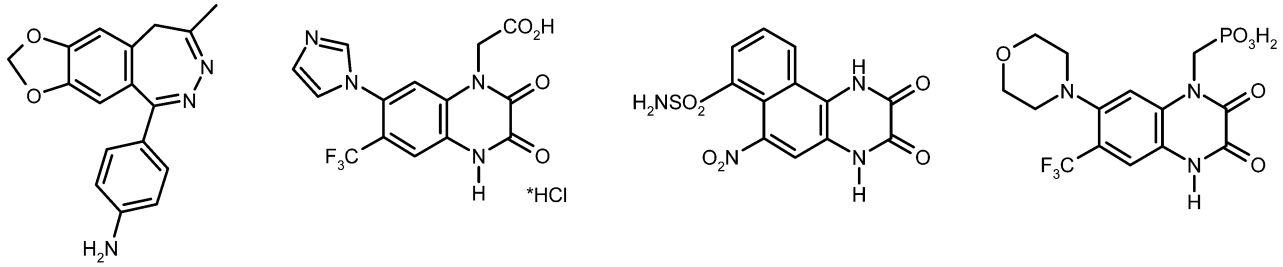
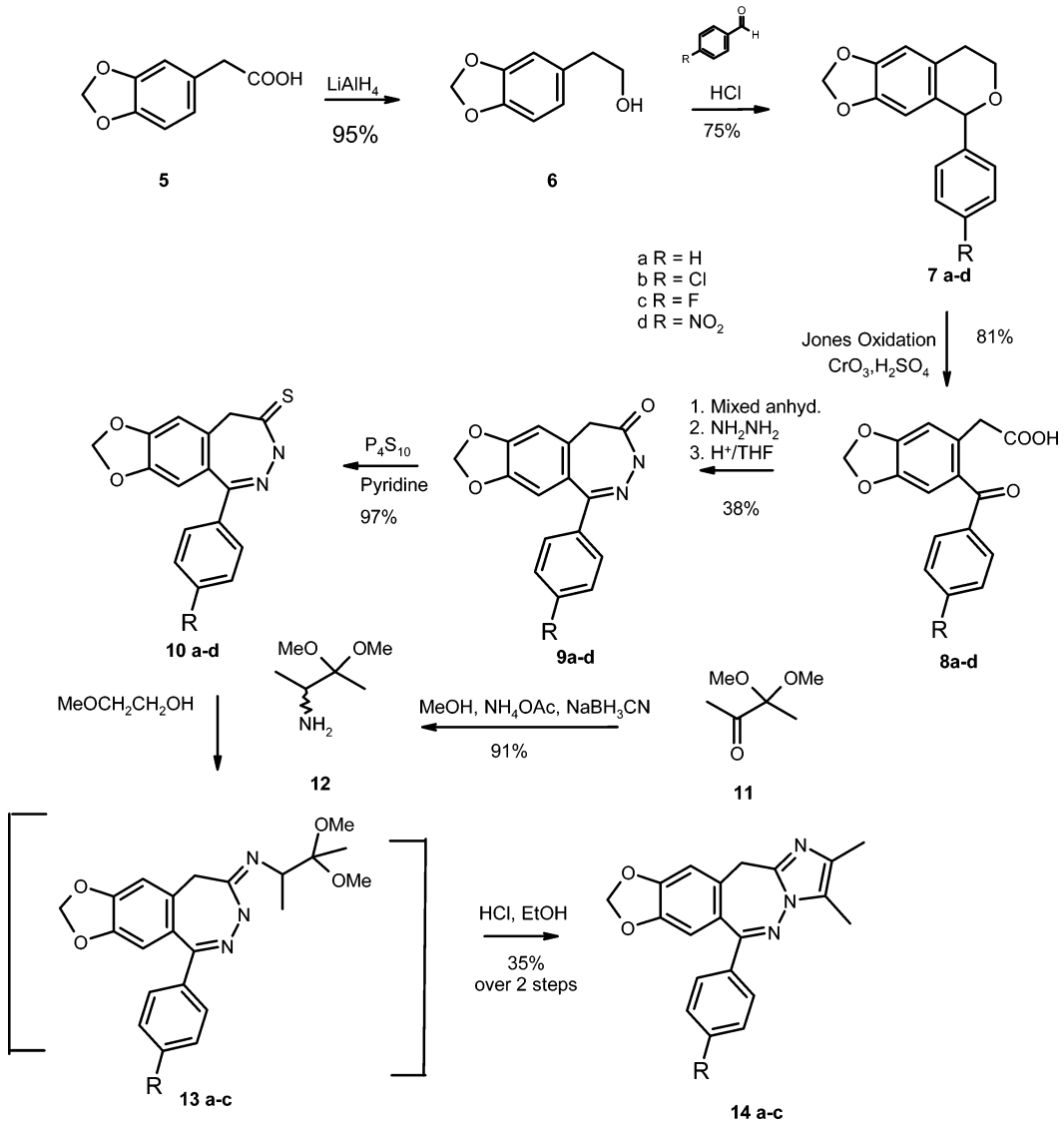
Chart 1. Noncompetitive and Competitive AMPA Receptor Antagonists of 2,3-Benzodiazepine-Type (**1**) and Quinoxalinedione-Type (**2**−**4**), Respectively



GYKI 52466 **1** | YM 872 **2** | NBQX **3** | ZK 200775 **4**

Scheme 1

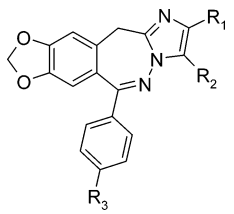


Pharmacology

The ability of the newly synthesized compounds to inhibit AMPA receptors in a noncompetitive fashion was determined in a functional in vitro test measuring kainate-induced currents in primary cultured rat fetal hippocampal neurons.

Selected compounds were screened in vivo for neuroprotective efficacy using a pretreatment paradigm in the mouse model of permanent focal cerebral ischemia.[6] In addition, posttreatment of compound **14a** was studied in the rat model of proximal middle cerebral artery (MCA) occlusion. This animal model has been shown to have a predictive value for stroke in humans because beneficial drug effects after permanent MCA occlusion in rats were reflected in a positive outcome of a phase III clinical trial.[7,8] For appropriate dosing of **14a** in the in vivo studies, the pharmacokinetics properties of the compound were evaluated. Finally, potential hypother-

Table 1. Inhibition of Kainate-Induced Whole-Cell Currents by 2,3-Benzodiazepine Derivatives in Cultured Rat Hippocampal Neurons



compd	substituents			AMPA receptor mediated currents IC_{50} (μmol)
	R_1	R_2	R_3	
14a	Me	Me	H	3.4
18	CH_2OH	Me	H	4.6
23	H	H	H	6.5
24	Cl	Cl	H	>10
27	Br	Br	H	>10
14b	Me	Me	Cl	10
14c	Me	Me	F	10
GYKI 52466 (**1**)			NH_2	9.0

mic activity of **14a** was assessed in rats, which could eventually account for indirect neuroprotective effects.

Results

As mentioned above, derivatives of the selective noncompetitive AMPA receptor antagonist GYKI 52466 (**1**) were synthesized (Chart 1 and Table 1). The basic structural differences of all of the derivatives **14a**−**c**, **23**, **24**, and **27** to GYKI 52466 are (1) the replacement of the 4-methyl substitution by a nitrogen-containing heterocycle attached to the 3,4-position of the 2,3-benzodiazepine ring system and (2) the replacement of the amino group on the phenyl ring system. The inhibitory effects of these compounds on induced AMPA receptor-mediated currents were investigated in primary cultured rat fetal hippocampal neurons. Long-lasting inward currents due to activation of AMPA receptors were induced by the nondesensitizing AMPA receptor ligand kainate. The novel structure (5-phenyl-11*H*-1,3-dioxolo[4,5-*h*]imidazo[1,2-*c*][2,3]benzodiazepine, **23**) with an IC_{50} of 6.5 μM was slightly more potent than **1** even though it lacks the amino group on the phenyl ring (Table 1). However, the activity was lost when halogen atoms were used as substituents on the nitrogen-containing heterocycle (compounds **24** and **27**). On the other hand, the potency of **23** was further enhanced when the nitrogen-containing heterocycle was methylated as in **14a**. The IC_{50} of **14a** for inhibition of kainate-induced currents was 3.4 μM (Figure 1). The IC_{50} for the reference compounds GYKI 52466 (noncompetitive) and NBQX (competitive) in antagonizing kainate-induced currents were 9 and 0.03 μM, respectively, in this experimental setting. When a halogen atom was introduced as a substituent on the phenyl ring of **14a**, the potency for AMPA receptor inhibition was reduced (compounds **14b** and **14c**).

To investigate the mechanism of current inhibition, the effect of compound **14a** on kainate concentration−response curves was studied in a separate set of experiments (Figure 2). The antagonist caused a concentration-dependent reduction of kainate-evoked currents at all kainate concentrations (i.e., a reduction in efficacy) without altering the potency of the agonist, thus behaving as a noncompetitive antagonist. Competitive binding of **14a** and **18** to glutamate receptors, i.e., AMPA, *N*-methyl-D-aspartate (NMDA), the kainate competitive site, and the NMDA/glycine site, was inhibited by less than 30% at 10 μM.

After iv injection of **14a**, the plasma clearance and half-life were calculated to be 54 $mL \cdot min^{-1} \cdot kg^{-1}$ and 1.2 h for the terminal elimination phase. The volume of distribution was 2.7 L/kg. LC/MS/MS analysis revealed a compound in the plasma samples of rats that was prominent besides **14a**. The chemical structure of **18** (Scheme 2) has been attributed to this metabolite of **14a**.

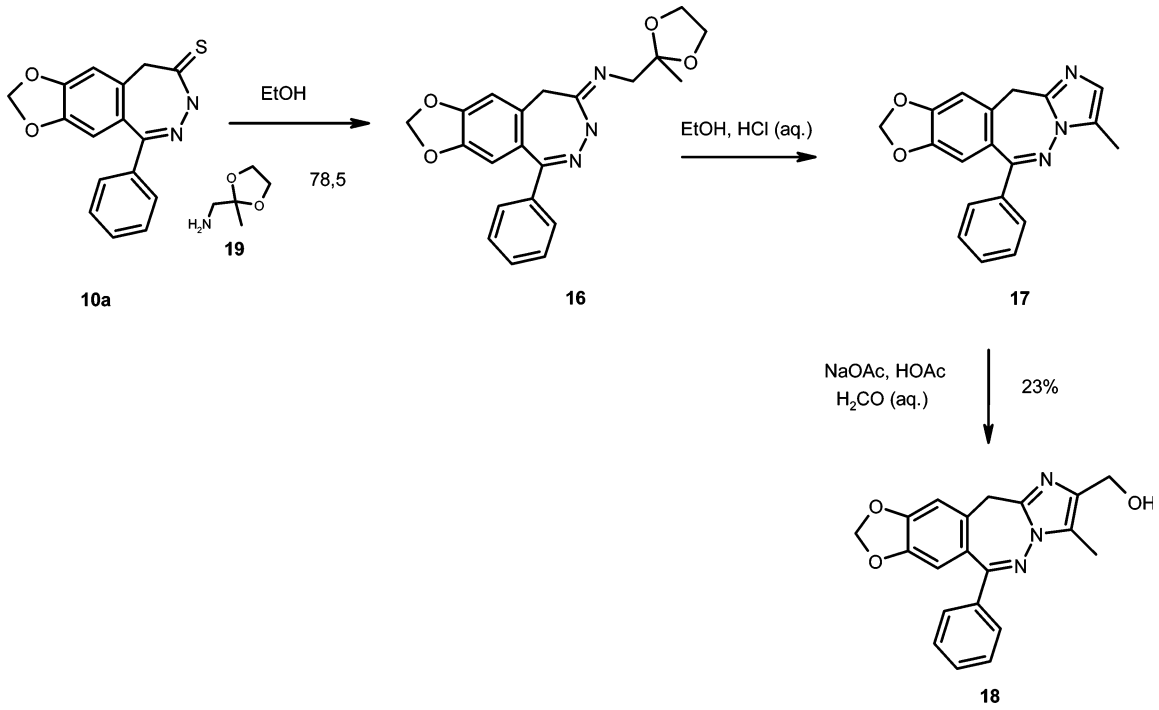
Neuroprotective efficacy of **14a** and **18** was studied after focal cerebral ischemia in mice. Following permanent MCA occlusion in mice, the ischemic territory was strictly ipsilateral (left side) and localized exclusively in the temporoparietal cortex. The animal model was validated for pharmacological testing using the competitive AMPA receptor antagonist NBQX (**3**) as a reference. Total cerebral infarct volume was significantly ($p < 0.05$) reduced by 21% from 23.2 ± 4.8 mm^3 (mean ± SD) in vehicle-treated controls ($n = 9$) to 18.4 ± 5.2 mm^3 in mice ($n = 12$) treated with three ip injections (each 30 mg/kg NBQX) 60, 70, and 85 min after permanent MCA occlusion.

14a was tested as pretreatment in MCA-occluded mice, which consisted of four intraperitoneal injections in total. **14a** was given 1 h before and 0, 1, and 2 h after MCA occlusion. Twenty-four hours after permanent MCA occlusion, total cerebral lesion volumes were significantly ($p < 0.05$) reduced by 34% from 26.0 ± 4.5 mm^3 in vehicle-treated controls ($n = 8$) to 17.1 ± 5.2 mm^3 in mice ($n = 10$) treated with four ip injections of **14a** (each injection with a dose of 10 mg/kg). Compound **18** was studied in MCA-occluded mice using the same treatment paradigm as for **14a**; however, no significant effects on brain lesion volumes were observed (Table 2).

Therapeutic compound effects on infarct volume were investigated 24 h following permanent MCA occlusion in male Fischer-344 rats. Both cerebral cortex and striatum were affected by ischemia on the ipsilateral side. Total infarct volume was significantly ($p < 0.05$) reduced from 190 ± 54 mm^3 in vehicle-treated controls ($n = 15$) to 138 ± 62, 142 ± 51, and 132 ± 59 mm^3 in rats given four ip injections of **14a** in doses of 3 ($n = 12$), 10 ($n = 15$), and 30 ($n = 10$) mg/kg, respectively, starting 1 h after MCA occlusion (Table 3). Lesion reduction was not dose-dependent, suggesting that a maximal drug effect (30.5% reduction) was reached under the given experimental conditions. Investigation of the NMDA receptor antagonist MK-801 as a reference compound yielded effects that were similar in magnitude to those of **14a** in this stroke model. Infarct volume was significantly ($p < 0.05$) reduced by 29% from 166 ± 43 mm^3 in controls (physiological saline, $n = 10$) to 118 ± 25 mm^3 in rats ($n = 6$) given a single ip injection of MK-801 (3 mg/kg) immediately after MCA occlusion.

Core body temperature in conscious rats decreased significantly by 2 °C after injection of the reference compound 8-OH-DPAT. However, no difference in body temperature was found between vehicle-treated controls and rats injected a high dosage (100 mg/kg ip) of **14a** (data not shown), indicating that cerebroprotection after

Scheme 2



permanent focal cerebral ischemia is not mediated by unspecific hypothermia.

Discussion

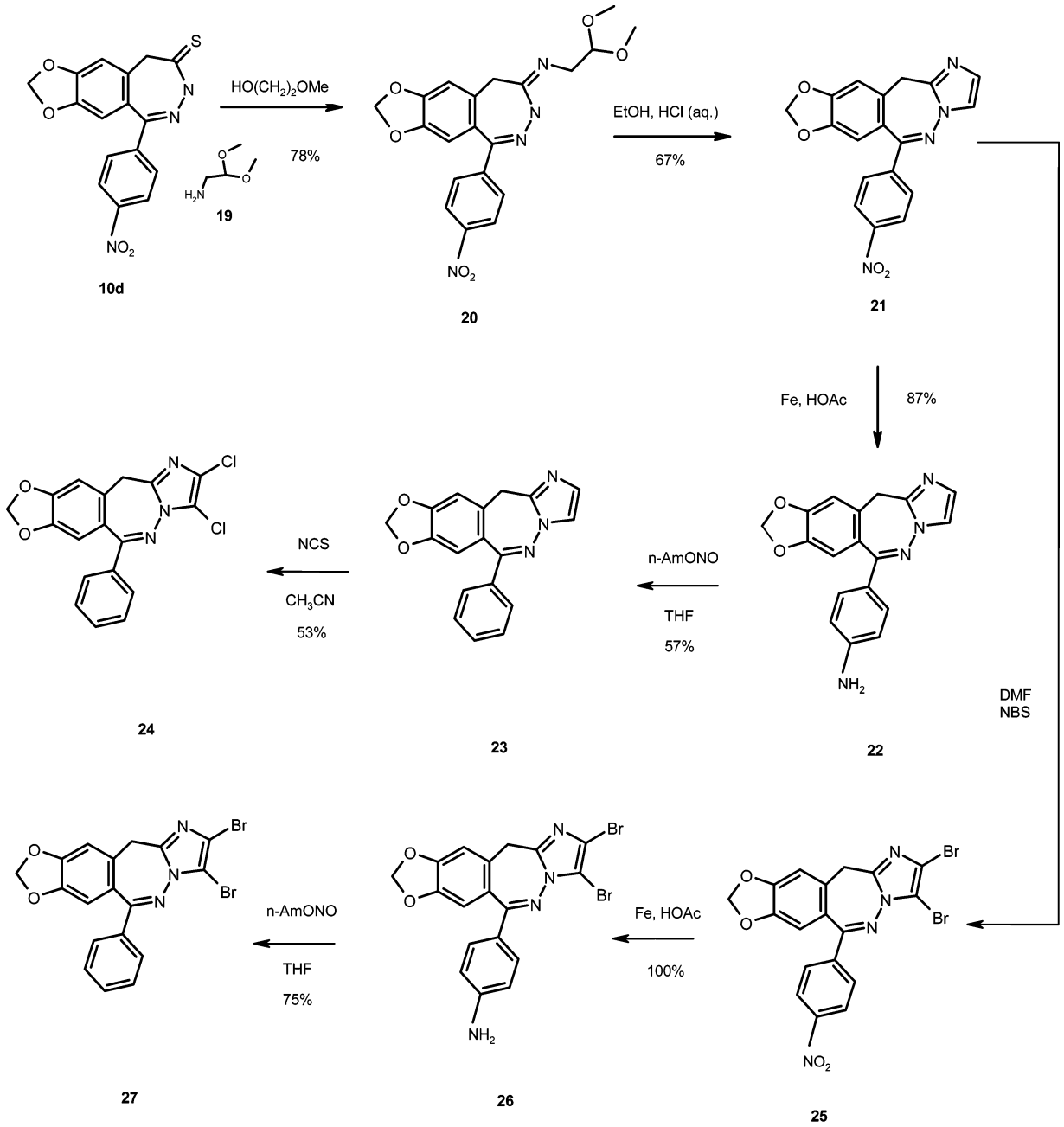
Derivatives of the selective prototype noncompetitive AMPA receptor antagonist GYKI 52466 (**1**) were synthesized, and the new 2,3-dimethyl-6-phenyl-12 *H*-[1,3]-dioxolo[4,5-*h*]imidazo[1,2-*c*][2,3]benzodiazepine (**14a**) was found to be about 3 times more potent than the parent compound in antagonizing AMPA receptor mediated currents in hippocampal neurons. Inhibition studies in hippocampal neurons showed that **14a** acts in a noncompetitive manner at the AMPA receptor. Moreover, our in vitro examinations revealed that the effects of **14a** were non-NMDA antagonistic and that there was no relevant competitive inhibition of the AMPA recognition site, as has also been demonstrated for GYKI-52466.[9,10]

When tested in vivo, **14a** displayed about 30% reduction of total cerebral lesion volume in rats after permanent MCA occlusion. The magnitude of lesion reduction was similar to that of the NMDA receptor antagonist MK-801, which is an established gold standard reference compound in this animal stroke model.[11] The cerebroprotection elicited by **14a** did not result from induction of unspecific systemic hypothermia because administration of even higher dosages of **14a** did not reduce the body temperature of rats.

Similarities of neuroprotective drug effects after permanent MCA occlusion in rats and mice have been repeatedly observed.[6] Likewise, compound **14a** produced comparable infarct reductions after permanent MCA occlusion in rats and mice. In comparison to the well-known competitive AMPA receptor antagonist NBQX, which was tested in MCA-occluded mice at an effective total dose of 90 mg/kg as reported in the literature,[12,13] our noncompetitive inhibitor **14a** achieved significant infarct reductions at a total dose of 40 mg/kg, although NBQX is about 100-fold more potent in antagonizing AMPA receptor mediated currents in cultured hippocampal neurons. These findings support the notion that noncompetitive AMPA receptor antagonists may have an advantage under conditions in which high glutamate levels may render the competitive antagonists relatively ineffective.[5]

The main difference in the chemical structures of **14a** and **23** compared to those of the other well-known noncompetitive AMPA receptor antagonists GYKI 52466 and GYKI 53773 (LY300164, talampanel)[9] is the replacement of the 4-methyl substitution by a nitrogen-containing heterocycle attached to the 3,4-position of the 2,3-benzodiazepine ring system. The electrophysiological results of the present study indicate that the potency for AMPA receptor inhibition is maintained despite this modification, which is in line with structure−activity relationship investigations by others.[9] In contrast to the noncompetitive AMPA receptor antagonist GYKI 52466, **14a** and **23** do not possess an amino group on the phenyl moiety. Nevertheless, both compounds displayed higher in vitro activity than GYKI 52466. This result confirms the general conclusion from previous SAR studies with 2,3-benzodiazepine derivatives related to **1** that the amino-substituent on the phenyl ring is not essential for AMPA receptor antagonistic efficacy.[9] In recently published SAR studies on annelated 2,3-benzodiazepine derivatives, it has been suggested that electron-withdrawing substituents on the phenyl ring might be useful for optimizing the pharmacological properties of AMPA receptor antagonists.[14] However, electrophysiological tests of **14b** and **14c** in the present investigation revealed that inhibition of AMPA receptor mediated currents in cultured neurons is reduced in azolo-condensed 2,3-benzodiazepines carrying a halogen atom on the phenyl ring compared to **14a**. Halogen atoms as substituents on the nitrogen-containing heterocycle also led to significantly decreased activities of compounds

Scheme 3



24 and **27**. Furthermore, our findings with **18** in MCA-occluded mice show that the in vivo efficacy of **14a** is lost when the methyl group of the nitrogen-containing heterocycle is hydroxylated.

Conclusions

From a series of azolo-condensed 2,3-benzodiazepines, **14a** emerged as a potent noncompetitive AMPA receptor antagonist that reduces ischemic brain lesions after permanent middle cerebral artery occlusion in both mice and rats. The compound is efficacious when treatment is started before or 1 h after MCA occlusion. Since the animal models were validated by use of reference compounds that are considered as "gold standard", these studies indicate that the noncompetitive AMPA receptor antagonist **14a** has potential for therapy of acute stroke.

Experimental Section

^{1}H NMR spectra were measured with a Bruker Aspekt 3000 (300 MHz) unless stated otherwise in the text. Chemical shifts are expressed in δ (ppm), and coupling constants (J) are in hertz. The purity of the compounds was checked by HPLC on a YMC Pro 18RS 5 μm column (150 mm $\times$ 4.6 mm), with a gradient of 95% to 5% water (containing 0.1% ammonia) in acetonitrile (1 mL/min) and detection at 254 nm. Reanalyses of compounds by a different HPLC method was performed as described in detail in the Supporting Information. The high-resolution mass spectra were measured on a AutoSpec Q, V6-Micromass at 70 eV.

Compounds **5** and **11** are commercially available.

3,4-Methylenedioxyphenethyl Alcohol (6). To a stirred ice cold suspension of lithium aluminum hydride (58 g, 1500 mmol) in Et_2O (2.5 L, 0−10 °C internal temperature) under argon was added a solution of 3,4-methylenedioxyphenylacetic acid (191 g, 0.347 mol) in dry THF (579 mL) dropwise within 45 min. After being stirred at ambient temperature for 8 h, the mixture was cooled to 0 °C and dilute aqeuous sodium hydroxide (4 M, 58 mL) was added in such a manner to maintain the temperature at 5−10 °C. Afterward, an amount of 250 mL of water was added and the mixture was stirred overnight at room temperature. The precipitate was removed

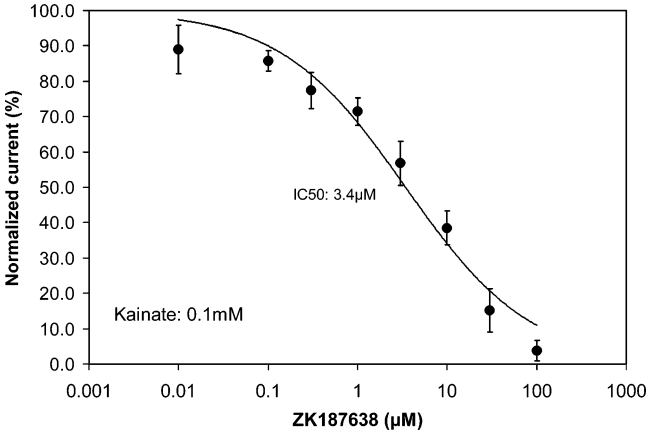


Figure 1. Concentration-dependent inhibition of kainate-induced whole-cell currents in hippocampal neurons. Data points represent the mean ± SD of five to six independent measurements (cells) normalized to the current values recorded in the absence of antagonist. Compound **14a** (ZK 187638) and kainate were applied by continuous-bath perfusion. Antagonist effects were measured after 90 s of preincubation. **14a** was dissolved in pure DMSO and diluted in recording solution to a final DMSO concentration of 0.3%. DMSO was present in all solutions at the same concentration.

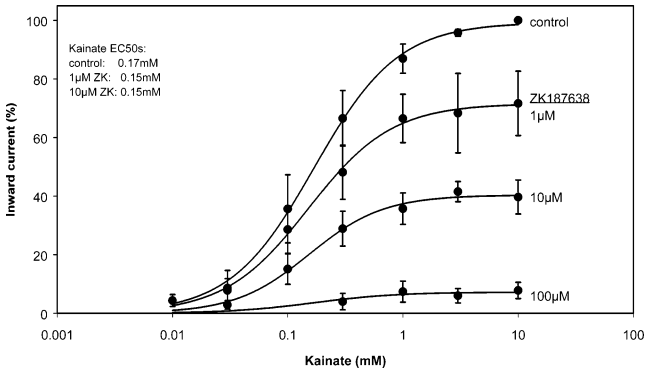


Figure 2. Effect of **14a** (ZK 187638) on kainate concentration−response curves. Data points represent the mean ± SD of 4−10 independent measurements (cells) normalized to the inward current obtained with 10 mM kainate in the absence of antagonist.

Table 2. Effects of NBQX (**3**) and Compound **14a** and Its Metabolite **18** on Brain Lesion Measured 24 h after Permanent MCA Occlusion in Mice[a]

treatment	dose (mg/kg)	total infarct volume[b] (mm^3)
vehicle		23.2 ± 4.8 (9)
NBQX	3 × 30	18.4 ± 5.2 (12)[c]
vehicle		26.0 ± 4.5 (8)
14a	4 × 10	17.1 ± 5.2 (10)[c]
vehicle		23.7 ± 7.2 (11)
18	4 × 10	20.6 ± 6.7 (9)
18	4 × 30	23.0 ± 8.9 (8)

[a] NBQX was injected ip 60, 70, and 80 min after MCA occlusion. Compounds **14a** and **18** were given ip 1 h before and again 0, 1, and 2 h after MCA occlusion. [b] Data are the mean ± SD with numbers of animals in parentheses. [c] $p < 0.05$ vs corresponding vehicle-treated group.

by suction filtration and washed twice with methyl *tert*-butyl ether, and the combined filtrates were extracted three times with methyl *tert*-butyl ether. The combined organic layers were washed with brine, dried, and concentrated in vacuo to obtain 3,4-methylenedioxyphenethyl alcohol[15,16] (171 g, 95%) as an oil. ^{1}H NMR (DMSO), δ: 2.65 (t, J = 7.5 Hz; 2H), 3.55 (dd, J = 7.5, 5 Hz, 2H), 4.6 (t, J = 5 Hz, 1H), 5.95 (s, 2H), 6.7 (d, J = 7.5 Hz; 1H), 6.8 (m, 2H).

Table 3. Effect of MK-801 and Compound **14a** on Total Infarct Volume Measured 24 h after Permanent MCA Occlusion in Rats[a]

treatment	dose (mg/kg)	total infarct volume[b] (mm^3)
vehicle		166 ± 43 (10)
MK-801	3	118 ± 25 (6)[c]
vehicle		190 ± 54 (15)
14a	4 × 3	138 ± 62 (12)[c]
14a	4 × 10	142 ± 51 (15)[c]
14a	4 × 30	132 ± 59 (10)[c]

[a] MK-801 was injected ip immediately after MCA occlusion, and compound **14a** was given ip 1, 2, 3, and 4 h after MCA occlusion. [b] Data are the mean ± SD with numbers of animals in parentheses. [c] $p < 0.05$ vs corresponding vehicle-treated group.

5-Phenyl-1,3-dioxolo[4,5-*h*]-isochromane (7a). To a stirred solution of 3,4-methylenedioxyphenethyl alcohol (191 g, 0.347 mol) in toluene (300 mL) at room temperature were added sequentially benzaldehyde (14 g, 13.5 mL, 132 mmol) and concentrated HCl (11.3 mL). Stirring was continued for 2 h before quenching with water (130 mL). The mixture was extracted with EtOAc (3 × 150 mL), and the combined organic layers were washed with water, dried, and concentrated in vacuo. Trituration with hexane (400 mL) followed by suction filtration gave 5-phenyl-1,3-dioxolo[4,5-*h*]isochromane (22.4 g, 75%) as light-ochre crystals (mp 102 °C). ^{1}H NMR (DMSO), δ: 2.7 (m, 1H), 2.9 (m, 1H), 3.8 (m, 1H), 5.6 (s, 1H), 5.9 (s, 1H), 5.95 (s, 1H), 6.27 (s, 1H), 6.8 (s, 1H), 7.3 (m, 5H).

4,5-Methylenedioxy-2-benzoylphenylacetic Acid (8a). Jones reagent was prepared by dissolving chromium(VI) oxide (26.7 g) in concentrated sulfuric acid (23 mL) and adding water to make up to a final volume of 100 mL. To a stirred solution of 5-phenyl-1,3-dioxolo[4,5-*h*]-isochromane (**7a**, 18.6 g, 73.14 mmol) in acetone (1.17 L, pa) was added Jones reagent (110 mL, 2.66 M, 292.6 mmol) dropwise in such a manner that the internal temperature did not exceed 15 °C. The reaction mixture was warmed to room temperature, and stirring continued for 30 min before recooling to 0 °C and quenching with 2-propanol (22 mL). Stirring was continued for 5 min, and the chromium salts were removed by suction filtration and washed with acetone. The filtrate was evaporated to dryness, treated with 100 mL of water, and filtered off to obtain 4,5-methylenedioxy-2-benzoylphenylacetic acid (**8a**, 16.98 g, 81%) as ochre crystals (mp 181.2 °C; lit.[17] 182−184 °C). ^{1}H NMR (DMSO), δ: 3.7 (s, 2H), 6.15 (s, 2H), 6.9 (s, 1H), 7.1 (t, J = 7.5 Hz, 2H), 7.7 (m, 3H).

5-Phenyl-7*H*-1,3-dioxolo[4,5-*h*][2,3]benzodiazepine-8(9*H*)-one (9a). To a stirred solution of 4,5-methylenedioxy-2-benzoylphenylacetic acid (**8a**, 12 g, 42 mmol) in dry THF (600 mL) under nitrogen at −20 °C (internal temperature) was added sequentially triethylamine (4.3 g, 5.9 mL, 42 mmol) and isobutylchloroformate (5.8 g, 5.5 mL, 42 mmol) in such a manner that the internal temperature did not exceed −15 °C. After being stirred for 30 min at −20 °C, the reaction mixture was cooled to −30 °C, treated dropwise with hydrazine (100%, 7 mL) (instead of 100% hydrazine; hydrazine hydrate (98%, 10 mL, 210 mmol) can be used), warmed to room temperature, and then stirred for a further 1.5 h. The reaction mixture was filtered. The filtrate was concentrated to ca. 200 mL, concentrated hydrochloric acid (18 mL) was added, and the mixture was stirred for 1 h. Water (200 mL) was then added, tetrahydrofuran was removed in vacuo, and the residue was extracted three times with EtOAc. The combined organic layers were washed with water, dried, and evaporated to dryness to obtain 5-phenyl-7*H*-1,3-dioxolo[4,5-*h*][2,3]benzodiazepine-8(9*H*)-one (**9a**, 4.56 g, 38%) as ochre crystals (mp 182.9 °C; lit. 182−184,[18] 184−186 °C [19]). ^{1}H NMR (DMSO), δ: 3.4 (s, 2H), 6.1 (s, 2H), 6.6 (s, 1H), 7.1 (s, 1H), 7.5 (m, 5H), 10.9 (broad s, 1H).

5-Phenyl-7*H*-1,3-dioxolo[4,5-*h*][2,3]benzodiazepine-8(9*H*)-thione (10a). A stirred solution of 5-phenyl-7*H*-1,3-dioxolo[4,5-*h*][2,3]benzodiazepin-8(9*H*)-one (**9a**, 20.5 g, 73.14 mmol) in dry pyridine (360 mL) under argon was heated to 110 °C (bath temperature) before treating with phosphorus pentasulfide (12.2 g, 54.9 mmol). Stirring was continued at

this temperature for a further 1.5 h before cooling to room temperature. The mixture was concentrated in vacuo to ca. 30 mL. To this well-stirred residue, water (800 mL) was added. The resulting precipitate was isolated by suction filtration and washed successively with water (150 mL), ethanol (150 mL), and hexane (150 mL) to obtain after drying 5-phenyl-7*H*-1,3-dioxolo[4,5-*h*][2,3]benzodiazepine-8(9*H*)-thione (**10a**, 21.1 g, 97.3%) as a reddish brown powder (mp 203.6 °C; lit. 201−203 °C[20]). [If yields exceed 100%, then the product contains amounts of sulfur or phosphosulfur impurities. These impurities disturb the next reaction by the necessity of much higher amounts of aminoketal (**12**).] ^{1}H NMR (DMSO), δ: 3.8 (broad d, 2H), 6.2 (s, 2H), 6.6 (s, 1H), 7.1 (s, 1H), 7.5 (m, 5H), 12.7 (s, 1H).

2-Amino-3,3-dimethoxybutane (12).[21] To a stirred solution of 3,3-dimethoxybutan-2-one (**11**, 50 g, 378 mmol) in methanol (1250 mL) under nitrogen were added sequentially molecular sieves (3Å, 60 mL), ammonium acetate (290 g, 3.75 mol), and sodium cyanoborohydride (25 g, 0.41 mol). Stirring was continued overnight. The mixture was filtered through Celite, and the filtrate was concentrated in vacuo (bath temperature of 30 °C) to ca. 300 mL. The residue was diluted with dichloromethane (1.5 L) and washed successively with dilute aqueous sodium hydroxyde solution (2 M, 2 × 300 mL), water (2 × 200 mL), and brine. The organic layer was dried, filtered, and evaporated to dryness to obtain 2-amino-3,3-dimethoxybutane (**12**, 45.9 g, 91%).

5-Phenyl-8,9-dimethyl-11*H*-1,3-dioxolo[4,5-*h*]imidazo-[1,2-*c*][2,3]benzodiazepine (14a). 5-Phenyl-7*H*-1,3-dioxolo-[4,5-*h*][2,3]benzodiazepine-8(9*H*)-thione (**10a**, 6 g, 20.5 mmol) [in the scale-up of the reaction, the yield dropped] was taken up in ethylene glycol monomethyl ether (10 mL) and concentrated in vacuo (bath temperature of 50 °C, 20−30 mbar) almost to dryness (ca. 3−5 mL). 2-Amino-3,3-dimethoxybutane (**12**, 4 mL) was then added. The reaction mixture was heated at 110 °C while a very slow stream of nitrogen was passed continuously through it. The reaction mixture was cooled, and further cycles of 2-amino-3,3-dimethoxybutane (**12**) addition and heating were carried out according to a specified time schedule. Finally, the reaction mixture was evaporated to dryness and the residue was purified by chromatography on silica gel (eluant: 800 mL of 50% EtOAc in hexane, then 50% acetone in hexane). Further purification by trituration with 25% EtOAc in hexane gave pure 5-phenyl-8,9-dimethyl-11*H*-1,3-dioxolo[4,5-*h*]imidazo[1,2-*c*][2,3]benzodiazepine (**14a**, 2.3 g, 35%) as cream-white crystals (mp 176.6 °C). ^{1}H NMR (DMSO), δ: 2.0 (s, 3H), 2.2 (s, 3H), 3.8 (s, 2H), 6.1 (s, 2H), 6.6 (s, 1H), 7.2 (s, 1H), 7.5 (m, 3H), 7.7 (m, 2H). Anal. Calcd: C= 72.49%; H= 5.17%; N= 12.68%; O=9.66%. Obsd: C= 72.30%; H= 5.43%; O= 9.82%; N= 12.51%. HPLC: t_R = 9.03 min; purity, 99.78%. HR MS: Molpeak, $C_{20}H_{17}N_3O_2$; calcd, 331.132 077; found 331.131 844. A second crop of slightly impure **14a** (1.2 g) was obtained, which could be purified by chromatography.

Compounds **14b** and **14c** were synthesized in the same manner as **14a** (Scheme 1).

14b. ^{1}H NMR (DMSO), δ: 2.0 (s, 3H), 2.2 (s, 3H), 3.85 (s, 2H), 6.1 (s, 2H), 6.6 (s, 1H), 7.2 (s, 1H), 7.6 (d, J = 7.5 Hz, 2H), 7.75 (d, J = 7.5 Hz, 2H). HPLC: t_R = 10.18 min; purity, 99.44%. HR MS: Molpeak, $C_{20}H_{16}N_3O_2{}^{37}Cl$; calcd, 367.090 155; found, 367.089 925.

14c. HPLC: t_R = 9.05 min; purity, 95.75%. HR MS: Molpeak, $C_{20}H_{16}N_3O_2F$; calcd, 349.122 655; found, 349.121 815.

(2-Methyl[1,3]dioxolan-2-ylmethyl)-(5-phenyl-7,9-dihydro-1,3-dioxa-6,7-diazacyclohepta[*f*]inden-8-ylidene)amine (16). A stirred solution of 5-phenyl-7*H*-1,3-dioxolo[4,5-*h*][2,3]benzodiazepine-8(9*H*)-thione (**10a**, 400 mg, 1.35 mmol) in ethanol (1 mL) was treated with *C*-(2-methyl[1,3]dioxolan-2-yl)methylamine[22] (1 mL) while a very slow stream of argon was passed continuously through it. The mixture was then refluxed for 3.5 h. After addition of diisopropyl ether the precipitate was filtered off to obtain (2-methyl[1,3]dioxolan-2-ylmethyl)-(5-phenyl-7,9-dihydro-1,3-dioxa-6,7-diaza-cyclohepta-[*f*]inden-8-ylidene)amine (**16**, 402 mg, 78.5%) as a dark powder (mp >300 °C). ^{1}H NMR (DMSO), δ: 1.1, s, 3H), 2.8 (d, J = 12 Hz, 1H), 3.25 (d, J = 12 Hz, 1H), 3.9 (m, 4H), 6.05 (s, 1H), 6.1 (s, 1H), 6.5 (t, J = 6 Hz, 1H), 6.6 (s, 1H), 6.85 (s, 1H), 7.4 (m, 3H), 7.55 (m, 2H).

5-Phenyl-8-methyl-11*H*-1,3-dioxolo[4,5-*h*]imidazo[1,2-*c*][2,3]benzodiazepine (17). A stirred solution of (2-methyl-[1,3]dioxolan-2-ylmethyl)-(5-phenyl-7,9-dihydro-1,3-dioxa-6,7-diazacyclohepta[*f*]inden-8-ylidene)amine (**16**, 402 mg, 1.06 mmol) in ethanol (8 mL) was treated with concentrated hydrochloric acid (8 mL) and heated to 100 °C for 2.5 h. After dilution with 20 mL of water the ethanol was distilled off. The aqueous layer was brought to pH 11 by addition of 5 M NaOH and extracted three times with EtOAc. The organic layer was washed, dried, filtered, and concentrated to dryness. The residue was triturated with a mixture of ethyl acetate and hexane to obtain 5-phenyl-8-methyl-11*H*-1,3-dioxolo[4,5-*h*]-imidazo[1,2-*c*][2,3]benzodiazepine (**17**, 258 mg, 77% in 2 batches (mp 214.3 °C). ^{1}H NMR (DMSO), δ: 2.3 (s, 3H), 3.9 (s, 2H), 6.1 (s, 2H), 6.6 (s, 1H), 6.65 (s, 1H), 7.2 (s, 1H), 7.5 (m, 3H), 7.7 (m, 2H).

5-Phenyl-8-methyl-9-hydroxymethyl-11*H*-1,3-dioxolo[4,5-*h*]-imidazo[1,2-*c*][2,3]benzodiazepine (18). 5-Phenyl-8-methyl-11*H*-1,3-dioxolo[4,5-*h*]imidazo[1,2-*c*][2,3]benzodiazepine (**17**, 2.7 g, 8.54 mmol) was treated with acetic acid (3.6 mL), sodium acetate (3.4 g, 41.9 mmol, dry), and formalin (28.6 mL, 37% in water, 94.1 mmol). This mixture was heated for 5 h to 110 °C. After diluting with water to 100 mL, it was made alkaline by addition of potassium carbonate and extracted three times with ethyl acetate. The organic layer was washed, dried, filtered, and evaporated to dryness. The residue was purified by chromatography (silica gel, eluent CH_2Cl_2/EtOH = 10:1). Further purification by trituration with EtOAc gave 5-phenyl-8-methyl-9-hydroxymethyl-11*H*-1,3-dioxolo[4,5-*h*]imidazo[1,2-*c*][2,3]benzodiazepine (**18**, 695 mg, 23%, mp 229.4 °C). ^{1}H NMR (DMSO), δ: 2.3 (s, 3H), 3.9 (s, 2H), 4.25 (d, J = 5 Hz, 2H), 4.7 (t, J = 5 Hz, 1H), 6.1 (s, 2H), 6.55 (s, 1H), 6.65 (s, 1H), 7.2 (s, 1H), 7.5 (m, 3H), 7.7 (m, 2H). HPLC: t_R = 6.80 min; purity, 98.75%; HR MS: Molpeak, $C_{20}H_{17}N_3O_3$; calcd, 347.126 992; found, 347.125 081.

2,2-Dimethoxyethyl-5-phenyl-7,9-dihydro-1,3-dioxa-6,7-diazacyclohepta[*f*]inden-8-ylidene)amine (20). A mixture of 5-(4-nitrophenyl-7*H*-1,3-dioxolo[4,5-*h*][2,3]benzodiazepine-8(9*H*)-thione (3 g, 8.8 mmol) and 2,2-dimethoxyethylamine in 2-methoxyethanol (82 mL) was heated at 110 °C for 3 h, while a slow stream of argon was passed through it. When the mixture was cooled and evaporated to dryness, the residue was triturated by a mixture of EtOAc and hexane to give 2,2-dimethoxyethyl-5-phenyl-7,9-dihydro-1,3dioxa-6,7diazacyclohepta[*f*]inden-8-ylidene)amine (**20**, 2.6 g, 72%).

5-(4-Nitrophenyl)-11*H*-1,3-dioxolo[4,5-*h*][1,2-*c*][2,3]benzodiazepine (21). A mixture of 2,2-dimethoxyethyl-5-phenyl-7,9-dihydro-1,3dioxa-6,7-diazacyclohepta[*f*]inden-8-ylidene)amine (**20**,1.6 g, 3.8 mmol), ethanol (50 mL), and concentrated hydrochloric acid (50 mL) was refluxed for 3 h. On cooling, the mixture was concentrated in vacuo and the residue was treated at 0 °C with 3 M NaOH and extracted three times with EtOAc. The organic layer was washed with brine and evaporated to dryness. The residue was purified by chromatography on silica gel (eluant CH_2Cl_2/ethanol = 95:5) to obtain 5-(4-nitrophenyl)-11*H*-1,3-dioxolo[4,5-*h*][1,2-*c*][2,3]benzodiazepine (**21**, 1.04 g, 80%). ^{1}H NMR (DMSO), δ: 4.1 (s, 2H), 6.1 (s, 2H), 6.65 (s, 1H), 6.9 (d, J = 1.5 Hz, 1H), 7.2 (s, 1H), 7.5 (d, J = 1.5 Hz, s, 1H), 7.9 (d, J = 10 Hz, 2H), 8.4 (d, J = 10 Hz, 2H).

5-(4-Aminophenyl)-11*H*-1,3-dioxolo[4,5-*h*][1,2-*c*][2,3]-benzodiazepine (22). 5-(4-Nitrophenyl)-11*H*-1,3-dioxolo[4,5-*h*][1,2-*c*][2,3]benzodiazepine (**21**, 1.28 g, 3.7 mmol) was treated under argon with HOAc (50 mL) and iron powder (3.6 g, 64.8 mmol). This mixture was heated for 15 min in an oil bath, which was preheated at 90 °C. After filtration of the hot mixture through Celite and subsequent washing with HOAc, the filtrate was concentrated to dryness. The residue was treated with 2 M NaOH and extracted three times with EtOAc. The organic layer was washed with brine and evaporated to dryness. The residue was purified by chromatography on silica

gel (eluant CH_2Cl_2/ethanol = 95:5) to obtain 5-(4-aminophenyl)-11*H*-1,3-dioxolo[4,5-*h*][1,2-*c*][2,3]benzodiazepine (**22**, 787 mg, 67%).

5-Phenyl-11*H*-1,3-dioxolo[4,5-*h*][1,2-*c*][2,3]benzodiazepine (23). 5-(4-Aminophenyl)-11*H*-1,3-dioxolo[4,5-*h*][1,2-*c*]-[2,3]benzodiazepine (**22**, 820 mg, 2.6 mmol) in THF (35 mL) was treated under argon with 5.7 mL (43.4 mmol) *n*-pentyl nitrite. This mixture was refluxed for 2.5 h. On cooling, the mixture was concentrated in vacuo and the residue was purified by chromatography on silica gel (eluant CH_2Cl_2/ethanol = 95:5) to obtain 5-phenyl-11*H*-1,3-dioxolo[4,5-*h*][1,2-*c*][2,3]benzodiazepine (**23**, 500 mg, 57%). ^{1}H NMR (Bruker Avance 300 (300 MHz), DMSO), δ: 3.9 (s, 2H), 5.95 (s, 2H), 6.5 (s, 1H), 6.9 (d, J = 2.5 Hz, 1H), 7.2 (s, 1H), 7.45 (d, J = 2.5 Hz, s, 1H), 7.5 (m, 3H), 7.7 (dd, J = 2.5 Hz, 7.5 Hz, 2H); HPLC: t_R = 7.88 min; purity, 99.26%. HR MS: Molpeak, $C_{18}H_{13}N_3O_2$; calcd, 303.100 777; found, 303.099 342.

5-Phenyl-8,9-dichloro-11*H*-1,3-dioxolo[4,5-*h*][1,2-*c*][2,3]-benzodiazepine (24). A mixture of 5-phenyl-11*H*-1,3-dioxolo-[4,5-*h*][1,2-*c*][2,3]benzodiazepine (**23**, 100 mg, 0.3 mmol) and *N*-chlorosuccinimide (85 mg, 0.64 mmol) in acetonitrile (4 mL) was refluxed under argon for 2 h. On cooling, the mixture was diluted with water and extracted with EtOAc. The organic layer was washed with brine and evaporated to dryness. The residue was purified by chromatography on silica gel (eluant CH_2Cl_2/ethanol = 95:5) to obtain 5-phenyl-8,9-dichloro-11*H*-1,3-dioxolo[4,5-*h*][1,2-*c*][2,3]benzodiazepine (**24**, 63 mg, 53%). ^{1}H NMR (Bruker Avance 300 (300 MHz), DMSO), δ: 4.0 (s, 2H), 5.9 (s, 2H), 6.6 (s, 1H), 7.2 (s, 1H), 7.55 (m, 3H), 7.5 (d, J = 1.5 Hz, s, 1H), 7.7 (dd, J = 2.5 Hz, J = 7.5 Hz, 2H); HPLC: t_R = 11.56 min; purity, 99.44%. HR MS: Molpeak, $C_{18}H_{11}N_3O_2{}^{37}Cl_2$; calcd, 375.016 932; found, 375.018 669.

5-(4-Nitrophenyl)-8,9-dibromo-11*H*-1,3-dioxolo[4,5-*h*]-[1,2-*c*][2,3]benzodiazepine (25). A mixture of 5-(4-nitrophenyl)-11*H*-1,3-dioxolo[4,5-*h*][1,2-*c*][2,3]benzodiazepine (**21**, 420 mg, 1.2 mmol) and *N*-bromosuccinimide (475 mg, 2.64 mmol) in dry DMF (10 mL) was stirred for 2 h at ambient temperature. The mixture was partitioned between EtOAc and water. The organic layer was washed with brine and evaporated to dryness. The residue was purified by chromatography on silica gel (eluant EtOAc/hexane = 1:1) to obtain 5-(4-nitrophenyl)-8,9-dibromo-11*H*-1,3-dioxolo[4,5-*h*][1,2-*c*][2,3]benzodiazepine (**25**, 432 mg, 82%). ^{1}H NMR (DMSO), δ: 4.1 (s, 2H), 6.15 (s, 2H), 6.7 (s, 1H), 7.3 (s, 1H), 8.0 (d, J = 10 Hz, 1H), 8.4 (d, J = 10 Hz, 2H).

5-(4-Aminophenyl)-8,9-dibromo-11*H*-1,3-dioxolo[4,5-*h*]-[1,2-*c*][2,3]benzodiazepine (26). In a manner analogous to that for 5-(4-nitrophenyl)-8,9-dibromo-11*H*-1,3-dioxolo[4,5-*h*]-[1,2-*c*][2,3]benzodiazepine (**25**, 1.16 g, 2.3 mmol) was obtained 5-(4-nitrophenyl)-8,9-dibromo-11*H*-1,3-dioxolo[4,5-*h*][1,2-*c*][2,3]-benzodiazepine (**26**, 930 mg, 85%).

5-Phenyl-8,9-dibromo-11*H*-1,3-dioxolo[4,5-*h*][1,2-*c*][2,3]-benzodiazepine (27). In a manner analogous to that for 5-(4-aminophenyl)-8,9-dibromo-11*H*-1,3-dioxolo[4,5-*h*][1,2-*c*][2,3]-benzodizepine (**26**, 490 mg, 1 mmol) was obtained 5-phenyl-8,9-dibromo-11*H*-1,3-dioxolo[4,5-*h*][1,2-*c*][2,3]benzodiazepine (**27**, 316 mg, 75%). ^{1}H NMR (DMSO), δ: 4.05 (s, 2H), 6.1 (s, 2H), 6.6 (s, 1H), 7.2 (s, 1H), 7.6 (m, 3H), 7.75 (dd, J = 7.5 Hz, J = 1.5 Hz, 2H), 8.4 (d, J = 10 Hz, 2H). HR MS: Molpeak, $C_{18}H_{11}N_3O_2{}^{81}Br_2$; calcd, 462.917 707; found, 462.918 205. HPLC: t_R = 10.98 min; purity, 93.5%.

In Vitro Studies: Functional Inhibition of AMPA Receptors. Electrophysiological studies were carried out on primary cultured hippocampal neurons. Hippocampi were isolated from fetal Wistar rats (E19), dissected into small pieces (about 1 mm^2), and incubated in Mg/Ca-free Hank's balanced salt solution containing 0.25% trypsin at 37 °C for 15 min. Cells were dissociated by trituration and transferred into medium (Neurobasal/B27, GIBCO) with 5% fetal calf serum, then plated on poly-L-lysine-coated dishes (35 mm i.d., 7.5 × 10^5 cells/dish). Dishes were stored in a humidified incubator at 37 °C, 5% CO_2. Medium was changed on days 3 and 6. On day 6 the medium was supplemented with 10 μM cytosine-arabinoside.

Membrane currents were recorded in the whole-cell configuration of the patch clamp technique from cells cultured for 6−14 days. The external recording medium contained the following (mM): NaCl 150; KCl 5; $CaCl_2$ 1.8; $MgCl_2$ 2; HEPES 10; pH adjusted to 7.4 with NaOH. The patch-pipet solution contained the following (mM): CsCl 150; $MgCl_2$ 1; EGTA 10; HEPES 10; Mg-ATP 2; pH adjusted to 7.4 with NaOH. For recording sodium currents, the external medium and pipet solution contained 10 mM TEA-Cl, and 0.1 mM $CdCl_2$ was added to the external medium. Compounds were dissolved in pure DMSO and diluted in the recording medium to a final concentration of 0.3% DMSO. The nondesensitizing AMPA receptor agonist kainate was dissolved in recording medium. Kainate and **14a** were applied by bath perfusion. If not otherwise indicated in the text, the holding potential was set to −60 mV. Inhibition curves were fitted to the data according to the logistic equation

$$I = \frac{100}{1 + \left(\frac{IC_{50}}{[\mathbf{14a}]}\right)^n}$$

Kainate concentration−response curves were fitted according to the logistic equation

$$I = \frac{I_{max}}{1 + \left(\frac{EC_{50}}{[\text{kainate}]}\right)^n}$$

except for 100 μM **14a**, where the EC_{50} was forced to 0.15 mM (I is the normalized inward current (%), I_{max} is the maximal inward current obtained at 10 mM kainate, and n is the slope).

Ionotropic Glutamate Receptor Binding Studies. Competitive binding studies were performed at 10 μM in rat forebrain membranes using the specific ligands [^{3}H]AMPA, [^{3}H]CGP 39653, [^{3}H]kainic acid, [^{3}H]MDL-105,519.[23−26]

In Vivo Experiments. Male rats and male mice were maintained under standard conditions (12 h day/night cycle, 22 °C, 50% humidity) with free access to food and tap water. Before use for the experiments, the animals were allowed to recover from transportation for about 1 week. All experiments were performed in accordance to the requirements of the German laws for the protection of animals (Deutsches Tierschutzgesetz) and in compliance with the European Community Directive 86/609.

Pharmacokinetics of 14a. Rat plasma was taken at nine time points (5 min to 7 h) after iv injection of **14a** (2.5 mg/kg). Blood samples were collected in heparinized tubes and centrifuged to separate the plasma, which was stored at −20 °C until analysis. Plasma was precipitated with acetonitrile (1:5), and the supernatant was taken directly for LC/MS/MS quantification, using calibration curves in the matrix and a related compound as internal standard. The separation was performed on a XTerra MS C18 column (10 cm × 2.1 mm i.d., 3.5 μm particle size, precolumn 1 cm × 2.1 mm) at 25 °C. The mobile phase was A (H_2O + 0.1% acetic acid) and B (acetonitrile + 0.1 acetic acid): flow rate 0.3 mL/min, gradient 75% A to 5% A in 5 min. The retention time for **14a** was 3.2 min, and that for the internal standard was 3.1 min. Mass spectrometric detection was by TIS Pos MS.

Permanent MCA Occlusion in Rats. Male Fischer-344 rats weighing 250−300 g were anesthetized with 4% halothane for induction and 1.5% halothane for maintenance in 30% O_2/70% N_2O via a face mask. The left proximal middle cerebral artery was occluded by a slight modification of the technique of Tamura et al.[27] Briefly, a vertical 2 cm skin incision was made between the left eye and the left ear. Under an operating microscope (Wild AG, Heerbrugg, Switzerland) with low-power magnification, the temporalis muscle was divided and retracted in order to expose the zygoma and the squamosal bone. Parts of the temporalis muscle were removed, but the zygomatic bone was left intact. The left middle cerebral artery was exposed through a burr hole craniectomy (2 mm diameter)

drilled close to the foramen ovale. After the dura was opened, the proximal portion of the middle cerebral artery was electrocauterized with bipolar forceps (Erbotom T130, Erbe, Elektromedizin GmbH, Tübingen, Germany) and cut through with scissors. The burr hole was covered with Lyostypt (Braun Dexon), and the skin was sutured. During surgery, the rectal temperature of the anesthetised animals was maintained at 37 ± 0.5 °C by a feedback-regulated heating pad.

Permanent MCA Occlusion in Mice. Male mice (NMRI strain) weighing 23−30 g were anesthetized with Avertin (tribromoethanol dissolved in 10% cremophor), 240 mg/kg ip in a volume of 10 mL/kg. Under an operating microscope (Wild AG, Heerbrugg, Switzerland) with low-power magnification, a 5−10 mm incision of the skin was made vertically on the left side between the ear and the orbit. The parotid gland and surrounding soft tissue were removed by electrocoagulation. Parts of the underlying temporal muscle were removed until the MCA became visible through the surface of the skull. A small burr hole (1 mm) was made using a high-speed microdrill through the outer surface of the semitranslucent skull just over the visibly identified MCA. The inner layer of the skull was removed with fine forceps. The MCA was electrocoagulated with a bipolar diathermy above the level of the olfactory tract; thus, lenticulostriate arteries were left intact. Afterward, the wound was sutured. The animals did not sustain blood loss, and all surgeries were completed within 7−10 min.

Body Temperature. The core body temperature was determined in conscious rats using a rectal probe (eight animals/group). Rats were placed separately in Perspex boxes. The temperature was repeatedly measured up to 3 h after drug or vehicle injection. The method was validated using the 5-HT_{1A} receptor agonist 8-OH-DPAT (1 mg/kg sc), which is known to reduce body temperature in rats.[28]

Measurement of Lesion Volume Using 2,3,5-Triphenyltetrazolium Chloride (TTC). Twenty-four hours after MCA occlusion, animals were anesthetized using Nembutal. The chests were opened, and the brains were perfused by intracardiac infusion of 1 mL of a saline solution containing 10% TTC. The brains were left in situ for 10−15 min, removed, and stored in fixative (4% paraformaldehyde and 2% sucrose in 0.1 M sodium phosphate) for 3 days. The brains were sliced into 1 mm coronal sections with a matrix (Harvard Bioscience). The slices were captured using a color camera combined with a Macintosh computer (Apple, Cupertino, CA). The areas of the infarcts were measured using the NIH Image software package (freeware), and the infarct volumes were calculated according to the Cavalieri principle.[29] This procedure has been shown to be reliable for distinction between ischemic and nonischemic tissue.[30]

Drugs. MCA-occluded rats and mice received four ip injections of a solution of **14a** containing 10% cremophor EL (Sigma). The injections were spaced by 1 h intervals. Pretreatment, starting 1 h before MCA occlusion was used in mice, and posttreatment beginning 1 h after MCA occlusion were used in rats. The NMDA receptor antagonist MK-801 (RBI) and the AMPA receptor antagonist NBQX (Novo Nordisk) were dissolved in physiological saline (0.9% NaCl) and studied as reference compounds using treatment paradigms that have been efficacious in animal models of global as well as focal cerebral ischemia.[11−13] MK-801 (3 mg/kg) was administered ip immediately after MCA occlusion, and NBQX (30 mg/kg) was injected ip 60, 70, and 85 min after MCA occlusion.

Statistical Analyses. All results are given as the mean and standard deviation (SD). For each experimental group the deviation from the Gaussian distribution was tested by the Kolmogorov−Smirnov test. All the data passed the normality test. Statistical comparisons between control and treatment groups were made using analysis of variance followed by post-hoc Dunnett's multiple comparison test.

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Supporting Information Available: Table on cycles of 2-amino-3,3-dimethoxybutane (**12**) addition and heating in the synthesis of **14a**, yields, mass spectra analyses, and HPLC reanalyses. This material is available free of charge via the Internet at http://pubs.acs.org.

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