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Comparative anticonvulsant activity of *N*-acetyl-1-aryl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline derivatives in rodents

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Abstract

The anticonvulsant activity of competitive 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo (F)-quinoxaline (NBQX) and noncompetitive 2,3-benzodiazepines and tetrahydroisoquinolines (THIQs) AMPA/kainate receptor antagonists, was tested in different experimental seizure models and compared with diazepam, a conventional antiepileptic drug acting on GABAergic neurotransmission. In particular, the compounds were evaluated against audiogenic and maximal electroshock seizures (MES) test and pentetrazol (PTZ) seizures model, and all of them showed protective action.

In addition, NBQX, 2,3-benzodiazepines and THIQs, but not diazepam, were also protective against clonic and tonic seizures and lethality induced by kainate, AMPA and ATPA, but were ineffective against NMDA-induced seizures. Only 2,3-benzodiazepines and some THIQs were able to affect 4-aminopyridine- and mercaptopropionic-acid-induced seizures.

The duration of anticonvulsant action of 33 μmol/kg of some 2,3-benzodiazepines and THIQs was also investigated in DBA/2 mice, a strain genetically susceptible to audiogenic seizures, and it was observed that the derivative THIQ-10c, possessing an acetyl group at the N-2 and a chlorine atom on the C-1 phenyl ring, showed higher anticonvulsant activity and longer-lasting protective effects.

Keywords: Epilepsy; AMPA/kainate receptor antagonists; NBQX; GYKI 52466; GYKI 53655; GYKI 53773; CFM-2; 2,3-Benzodiazepines; *N*-acetyl-1-aryl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolines; Audiogenic seizures; DBA/2 mice; Maximal electroshock; Chemoconvulsions

1. Introduction

The antagonists of 2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionic acid (AMPA)/kainate receptor possess some advantages compared to *N*-methyl-D-aspartate (NMDA) receptor antagonists, including higher neuroprotective potency after ischaemic attacks, higher anticonvulsant potency in temporal lobe epilepsy and reduced side effects (Buchan et al., 1993; Rogawski, 1993; Löscher and Honack, 1992; Löscher and Schmidt, 1994; Chimirri et al., 1997, 1999; De Sarro et al., 1995, 1998, 1999a,b, 2003; Zappalà et al., 2000). Therefore, AMPA/kainate receptor antagonists could be of interest in the treatment of neurodegenerative disorders (Buchan et al., 1993; Rogawski, 1993; De Sarro et al., 1998, 1999a,b, 2003). In various models of epileptic seizures, both 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo (F)-quinoxaline (NBQX) and the prototype noncompetitive AMPA/kainate receptor antagonist 1-(4-aminophenyl)-4-methyl-7,8-methylendioxy-5*H*-2,3-benzodiazepine (GYKI 52466) were unable to exert anticonvulsant effects at doses below those inducing sedation and motor impairment (Scheme 1). This indicates that the therapeutic index of the current generation of AMPA/kainate receptor antagonists may be lower than initially thought (Yamaguchi et al., 1993; Löscher and Honack, 1994).

Ionotropic AMPA/kainate receptors can be divided into two distinct receptor complexes: AMPA receptors and kainate receptors (Bettler and Mulle, 1995). Because of the lack of potent and selective agonists and antagonists for kainate receptors, the physiological role of kainate

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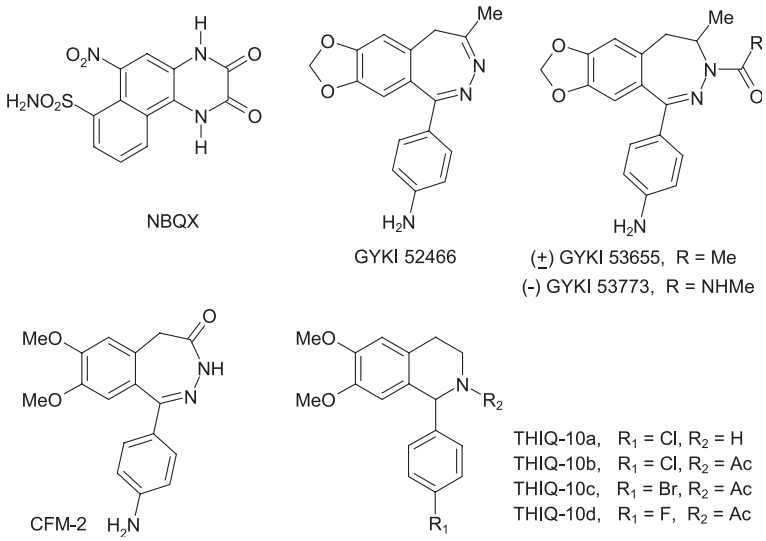

Scheme 1.

receptors has so far remained obscure (Bettler and Mulle, 1995; Jorgensen et al., 1995; Lerma et al., 1997). To date, most AMPA/kainate receptor antagonists show a preference for AMPA receptors although one compound, 5-nitro-6,7,8,9-tetrahydrobenzo (G)indole-2,3-dione-3-oxime (NS-102), has a moderate sevenfold preference for the low-affinity kainate receptor (Barreca et al., 2003).

AMPA receptor types are composed of combinations of four (GluR1–4) subunits, existing as "flip and flop" splice variants, which mediate fast excitatory potentials by the flux of Na^+ and Ca^{2+} (Sutcliffe et al., 1996). The AMPA receptor complex has at least three distinct binding sites at which antagonists can act: (a) the glutamate (Glu) binding sites for competitive antagonists, (b) an allosteric site at which noncompetitive receptor antagonists can bind and (c) a polyamine site within the ion channel (Chimirri et al., 1999). In addition, on these receptor proteins, five proteins (GluR5–7, KA1 and KA2) have a ligand-binding site at which kainate is far more potent than AMPA (Bettler and Mulle, 1995; Jorgensen et al., 1995). Since most currently available AMPA/kainate receptor antagonists have little specificity for the various subunits or combinations of subunits, new and more selective compounds should be developed. These drugs may not only be of great value in discriminating the function of kainate and AMPA receptors and their subunits, but may also have a potential as novel therapeutic agents with less adverse effects than the currently available AMPA/kainate antagonists.

The main classes of noncompetitive AMPA receptor antagonists are 2,3-benzodiazepines (Rogawski, 1993; De Sarro et al., 1995, 1998, 1999b; Chimirri et al., 1997; Gitto et al., 2003b; Zappalà et al., 2000), phthalazines (Pelletier et al., 1996; Parsons et al., 1997; Pei et al., 1999; Gitto et al., 2000; Grasso et al., 2000) and quinazolin-4-ones recently described by Pfizer researchers (Welch et al., 2001), whose lead compound, CP-465,022 seems characterized by excellent pharmacological properties (Lazzaro et al., 2002).

Our research group has recently generated a ligand-based pharmacophore model of negative allosteric modulators of AMPA receptors (Barreca et al., 2003), which led to the discovery of a new class of AMPA ligands and in particular of the *N*-acetyl-1-(4-chlorophenyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (THIQ-10c) showing an anticonvulsant activity more evident than GYKI 52466 and CFM-2 in DBA/2 mice (Gitto et al., 2003a). In addition, various AMPA/kainate agonists have been identified such as ATPA, a *tert*-butyl analog of AMPA (Clarke et al., 1997), which proved to be a potent agonist of recombinant homomeric and heteromeric GluR5 kainate receptors (EC_{50} values 0.6–2 μM) (Cui and Mayer, 1999; Stensbøl et al., 1999), but a weak, partial agonist at AMPA and GluR6/KA2 kainate receptors (Paternain et al., 2000; Clarke and Collingridge, 2002).

Decahydroisoquinolines, such as (3S,4aR,6R,8aR)-6-{2-[1 (2)*H*-tetrazol-5-yl]ethyl}-decahydroisoquinoline-3-carboxylic acid (LY293558) and (3S,4aR,6S,8aR)-6-(4-carboxyphenyl)methyl-1,2,3,4,4a,5,6,7,8,8a-decahydroiquinoline-3-carboxylic acid (LY382884) were able to discriminate the pharmacological responses specifically mediated by GluR5 kainate receptors (Bleakman et al., 1996; Clarke et al., 1997; Li and Rogawski, 1998; O'Neill et al., 1998).

In the present study, we evaluate the anticonvulsant activity of four 1-aryl-6,7-dimetoxy-1,2,3,4-tetrahydroisoquinolines (THIQs) and compare their pharmacological properties with those of other four noncompetitive AMPA/kainate receptor antagonists that were selected from a series of 2,3-benzodiazepines (GYKI52466, CFM-2, GYKI 53655 and GYKI 53773) (Scheme 1). The basis of selection was high potency and selectivity for AMPA and/or kainate receptors, high in vivo potency after systemic

administration and an acceptable ratio between neuroprotective or anticonvulsant properties and adverse effects, i.e., doses exerting neuroprotective and anticonvulsant effects being clearly below those inducing "neurotoxic" effects, e.g., motor impairment or sedation. Pharmacological characteristics of these novel AMPA receptor antagonists are described in this study with special emphasis on their effects in genetic or chemical models of (convulsive) epilepsy.

In addition, a K^+ channel antagonist 4-aminopyridine is able to elicit convulsion in different animal species including humans (Spyker et al., 1980; Yamaguchi and Rogawski, 1992); it produces seizurelike events and interictal epileptiform discharges in the entorhinal and temporal neocortex and short recurrent discharges in the hippocampus. Epileptiform discharges in the hippocampus are considered a model of drug-resistant epilepsy and are sensitive to retigabine (Yonekawa et al., 1995a; Armand et al., 1999) but not to conventional anticonvulsants such as carbamazepine, phenytoin and valproic acid (Dreier and Heinemann, 1990; Zhang et al., 1991; Yonekawa et al., 1995b). For this reason, 2,3-benzodiazepines, tetrahydroisoquinolines, NBQX and diazepam, were also evaluated in the 4-aminopyridine model. Diazepam, commonly used in acute treatment in status epilepticus, has been chosen as a conventional antiepileptic drug being considered a very active compound against seizures induced by convulsant agents impairing the GABAergic neurotransmission.

2. Materials and methods

2.1. Animals

Male DBA/2 mice weighing 6–12 g (22–26 days old) and ICR CD-1 mice weighing 20–30 g (42–48 days old) were used in the present study (Harlan Italy Correzzana, Milano, Italy). The animals were housed in groups of 8–10 under a 12-h light/dark cycle (lights on at 7:00 a.m.) with food and water available ad libitum. The experimental protocol was approved by the University of Catanzaro Ethical Committee. All procedures are in compliance with the National Institutes of Health *Guide for Care and Use of Laboratory Animals* (Publication No. 85-23, revised 1985) and European Communities Council Directive of November 24, 1986 (86/609 EEC).

2.2. Audiogenic seizures in DBA/2 mice

The experiments were performed according to the method previously described by De Sarro et al. (1984). Test compounds or vehicle (controls) were given intraperitoneally (ip) to groups of 10 mice per dose. Thirty minutes later each mouse was placed under a hemispheric perspex dome (58 cm in diameter) challenged with a 12- to 16-kHz sinusoidal tone at 109 dB in a covered plexiglass cylinder. Seizure response was assessed by two independent observers. The sound-evoked behaviour was coded using the following scale: 0 = no response, 1 = wild running, 2 = clonus, 3 = tonic flexor and/or extensor, 4 = respiratory arrest, on the basis of the concordant opinion of the observers. Sound stimulus was applied for 60 s, but it was interrupted earlier, when the observed animal showed tonic extensor seizure. The maximum response was recorded for each animal. Rectal temperature was recorded immediately prior to auditory testing using an Elektrolaboratoriet thermometer type T.E.3. Behavioral changes were observed during the period between drug administration and auditory testing.

In audiogenic seizure test the duration of the anticonvulsant action of 33 μmol/kg GYKI 52466, CFM-2, THIQ-a, THIQ-10b, THIQ-10c, THIQ-10d, GYKI 53655 and GYKI 53773 (LY300164, talampanel) were also examined. The percentage of mice showing clonic or tonic phase and the duration of anticonvulsant activity were compared to the control group and statistically analyzed. In these experiments, separate vehicle-treated and drug-treated groups were used at each pretreatment time (15–240 min).

2.3. Anticonvulsant effects in the maximal electroshock seizure (MES) test

The tested compounds were administered intraperitoneally to groups of 10 male ICR CD-1 mice (body weight 20–26 g) per dose in a volume of 0.1 ml/10 g. Tonic seizures were induced by electroshock via ear electrodes 30 min (NBQX: 5 min) after drug administration. The stimulus was applied for 0.2 s and consisted of rectangular impulses of 4.64-ms duration and 14.7-mA amplitude at a frequency of 100 Hz. The incidence of tonic (hind limb) seizures was counted.

2.4. Pentetrazol (PTZ)-induced seizures in ICR CD-1 mice

Male ICR CD-1 mice (20–26 g, 42–48 days old) were pretreated with vehicle or drug 45 min (groups of 10 mice per dose) before the subcutaneous administration of PTZ. For systemic injections, all tested compounds were given intraperitoneally (0.1 ml/10 g of mouse body weight). The convulsive dose 97 (CD_{97}) of PTZ (85 mg/kg) was applied, and the animals were observed for 30 min. A threshold convulsion was an episode of clonic spasms lasting for at least 5 s. The absence of this threshold convulsion over 30 min indicated that the tested substance had the ability to elevate the PTZ seizure threshold (Swinyard and Woodhead, 1982).

2.5. Seizures induced by administration of kainate

Kainate was administered subcutaneously at a dose of 32 mg/kg (previously determined CD_{97} value) 15 min after

intraperitoneal administration of 2,3-benzodiazepine derivatives. ICR CD-1 (22–30 g, 42–48 days old) mice showing 5 s or more of clonic activity were scored as nonprotected according to Donevan et al. (1994). The period of observation was 60 min.

2.6. Seizures induced by administration of 4-aminopyridine

4-Aminopyridine was administered subcutaneously to ICR CD-1 (22–30 g, 42–48 days old) mice (groups of 10 mice per dose) at a dose of 13.3 mg/kg (previously determined CD_{97} value) 15 min after intraperitoneal administration of 2,3-benzodiazepine derivatives. Animals showing tonic extension or death were scored as nonprotected according to Yamaguchi and Rogawski (1992). The period of observation was 60 min.

2.7. Seizures induced by administration of 3-mercaptopropionic acid

3-Mercaptopropionic acid was administered intravenously at a dose of 110 mg/kg (previously determined CD_{97} value) 15 min after intraperitoneal administration of 2,3-benzodiazepine derivatives (groups of 10 mice per dose). ICR CD-1 (22–30 g, 42–48 days old) mice showing clonic or tonic activity or death were scored as nonprotected according to Horton and Meldrum (1973). The period of observation was 60 min.

2.8. Seizures induced by intracerebroventricular administration of NMDA, ATPA or AMPA

Seizures were induced by intracerebroventricular injections of NMDA, ATPA or AMPA. In particular, animals (groups of 10 mice per dose) were pretreated intraperitoneally with AMPA/kainate receptor antagonists and later for intracerebroventricular injections, mice were anesthetized with ether and injections were made in the left or right lateral ventricle (coordinates 1 mm posterior and 1 mm lateral to the bregma; depth 2.4 mm) using a 10-μl Hamilton microsyringe (type 701N) fitted with a nylon cuff on the needle as previously described (De Sarro et al., 1994; Grasso et al., 2000). Injections of drugs by this procedure led to a uniform distribution throughout the ventricular system within 10 min (De Sarro et al., 1988). The animals were placed singly in a 30 × 30 × 30 cm box; the observation time was 20 min after NBQX injection and 30 min after the administration of AMPA, ATPA or NMDA. The occurrence of clonic and tonic seizure signs and their latency were recorded. For statistical analysis, clonus, tonus or death within the 30-min observation period was used as a parameter, and inhibition of seizure phases or death as a criterion of drug effect. The relationship between the doses (μmol/kg) and the relative rate of protection compared to the vehicle controls (nearly 100% mortality) was assessed.

2.9. Statistical analysis

Statistical comparison between groups of control and drug-treated DBA/2 or ICR CD-1 mice was made using Fisher's exact probability test (incidence of the seizure phases) or analysis of variance (ANOVA) with Dunnett's *t* test (rectal temperatures). The percentage incidence of each phase of the audiogenic seizure was determined for each drug. These values were plotted against the corresponding doses by a computer construction of the dose–effect curves for calculation of ED_{50} (with 95% confidence limits). The ED_{50} values for each compound were calculated using a computer program (SPSS for windows, SPSS, Chicago, IL, USA) of the method of Litchfield and Wilcoxon (1949). At least 32 animals were used to calculate each ED_{50} value. Statistical evaluation for the time course of anticonvulsant effects was carried out using one-way ANOVA followed by Bonferroni's multiple comparison when appropriate.

2.10. Drugs

NBQX (molecular weight, MW = 336.3) was kindly supplied by Novo Nordisk (Malov, Denmark). AMPA and ATPA were purchased from Tocris Cookson (Bristol, UK), NMDA, kainate, 4-aminopyridine and 3-mercaptopropionic acid from Sigma (St. Louis, MO, USA.). GYKI 52466 dihydrochloride (MW = 366.25) was purchased from Tocris Cookson. Talampanel (GYKI 53773, LY 300164; MW = 337.4) was kindly supplied by Lilly Research Labs (Indianapolis, IN, USA) and GYKI 53655 (MW = 333.4) was kindly supplied by EGIS Pharmaceuticals (Budapest, Hungary). The 2,3-benzodiazepine CFM-2 (MW = 311.3) and all tetrahydroisoquinoline derivatives (THIQ-10a, MW = 303.79; THIQ-10b, MW = 390.28; THIQ-10c, MW = 345.83; THIQ-10d, MW = 329.37) studied were synthesized in our laboratories.

For systemic injections, all compounds were given intraperitoneally (0.1 ml/10 g of body weight of the mouse) as a freshly prepared solution in 50% dimethyl sulfoxide and 50% sterile saline (0.9% NaCl). Doses and time of administration are reported in the tables. Previous experiments have shown that this vehicle, when administered intraperitoneally, does not affect either behavior or response to auditory stimulation of DBA/2 mice or seizures induced by the chemoconvulsants used in the present study in ICR CD-1 mice.

All drugs administered intracerebroventricularly were dissolved in sodium phosphate buffer 67 mM, microinjected in a volume of 5 μl per mouse. The drugs were infused at a constant rate of 2 μl/min and the injector left in situ for a further 1 min. Doses and times of administration are reported in the tables. NBQX was dissolved in a minimum quantity of 1 N NaOH; the final volume was made up with sodium phosphate buffer. When necessary, pH was adjusted to 7.3–7.4 by adding 0.2 N HCl. In order to avoid the light sensitivity of some compounds,

weighing and handling were carried out under sodium vapor lamps and the substances were protected from light during the experiments.

3. Results

3.1. Anticonvulsant activity in DBA/2 mice

All 2,3-benzodiazepine and THIQ derivatives, NBQX and diazepam administered 30 min before auditory stimulation were able to protect against sound-induced clonic and tonic seizures in a dose-dependent manner. Tonic fit and death were completely prevented by THIQ-10c at doses over 3.3 μmol/kg, GYKI 53655 and GYKI 53773 at doses over 10 μmol/kg, CFM-2, THIQ-10a, THIQ-10b, THIQ-10d and NBQX at doses over 21 μmol/kg, respectively, while the corresponding value for GYKI 52466 was 33 μmol/kg (Fig. 1). Clonic seizures induced by auditory stimuli were antagonized by higher doses of all compounds tested (Table 1). The ED_{50} values for the inhibition of clonic and tonic seizures are reported in Table 1. Diazepam was able to antagonize the clonic and tonic phases of the seizures in DBA/2 mice at doses over 0.30 μmol/kg. All AMPA/kainate receptor antagonists were much less potent than diazepam (Table 1).

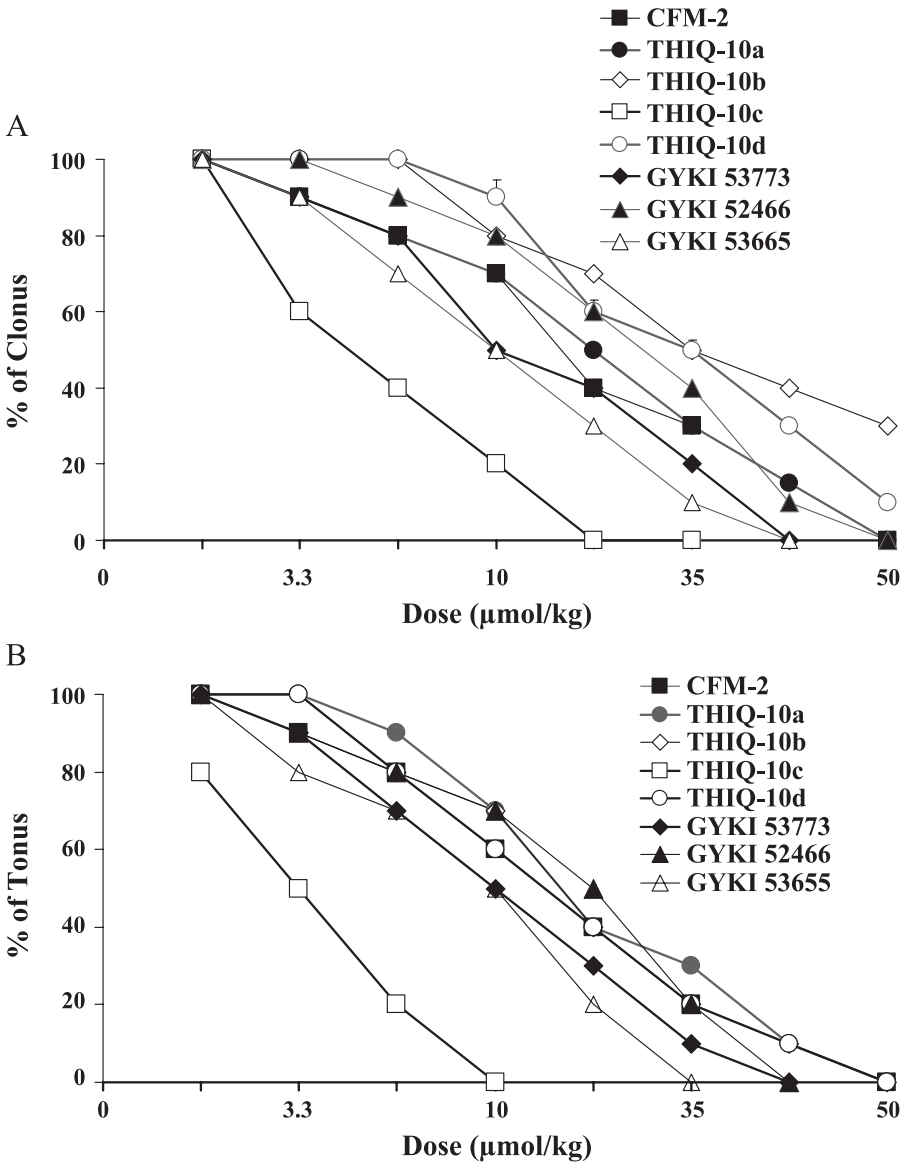

Fig. 1. Dose–response curves of the anticonvulsant effects of some AMPA/kainate receptor antagonists administered intraperitoneally (1–100 μmol/kg at 30 min): CFM-2 (■- -■), THIQ-10a (●- -●), THIQ-10b (◇- -◇), THIQ-10c (□- -□), THIQ-10d (○- -○), GYKI 53655 (△- -△), GYKI 52466 (▲- -▲) and GYKI 53773 (◆- -◆). The abscissa shows the doses; the ordinate shows (A) % of clonic seizures and (B) % of tonic seizures. Ten animals were used for the determination of each point. $n = 40–60$ mice for each compound tested.

Table 1
Anticonvulsant effect in audiogenic sensible DBA/2 mice, in the maximal electroshock and PTZ seizure test in ICR CD-1 mice

Compound	ED_{50} (μmol/kg ip)			
	Audiogenic seizures		MES	PTZ
	Clonus	Tonus		
GYKI 52466	35.8 (24.4–52.4)	25.3 (16–40)	35.7 (29.3–43.4)	68.3 (56.2–83.1)
CFM-2	15 (9–24)	12.6 (8–19)	15.9 (7.3–33.5)	22.6 (11.7–43.8)
THIQ-10a	20.1 (12.3–32.7)	19.3 (11.8–31.5)	29.4 (19.6–44.1)	51.4 (38.5–68.6)
THIQ-10b	43.1 (21.9–84.6)	16.5 (7.8–34.9)	50.2 (32.0–78.9)	100.5 (60.9–165.8)
THIQ-10c	4.2 (2.23–7.84)	2.4 (1.3–4.4)	5.17 (3.0–8.8)	9.2 (5.6–15.3)
THIQ-10d	36.8 (20.6–65.8)	18.1 (7.8–41.8)	52.9 (37.8–74.0)	64.4 (46.7–88.8)
NBQX	18.3 (9.45–35.3)	11.9 (6.1–22.5)	39.6 (26.9–58.3)	85.9 (71.5–103.3)
Diazepam	0.28 (0.20–0.39)	0.24 (0.15–0.39)	>30	0.43 (0.27–0.68)
GYKI 53655	10.3 (7.4–14.3)	8.3 (6.1–11.3)	15.5 (11.5–20.9)	42.5 (25.8–70.1)
GYKI 53773	13.4 (10.1–17.8)	9.7 (7.0–13.4)	28.8 (23.5–35.3)	56.3 (34.2–92.7)

Data are expressed in μmol/kg and were calculated according to the method of Litchfield and Wilcoxon (1949).

3.2. Anticonvulsant effects in the MES test

All AMPA/kainate receptor antagonists, including NBQX, blocked generalized tonic seizures in the MES test after intraperitoneal administration in mice (Table 1). Similarly to earlier reports (Chimirri et al., 1997; Donevan et al., 1994; Vizi et al., 1996) the compounds protected mice against tonic extension seizures in the MES test in a dose-dependent fashion. The ED_{50} values (± 95% confidence limits) of studied compounds are shown in Table 1.

The most potent compound was THIQ-10c (ED_{50}, 5.17 μmol/kg), followed by GYKI 53655 (ED_{50} 15.5 μmol/kg) and CFM-2 (ED_{50}, 15.9 μmol/kg), whereas all other 2,3-benzodiazepines and THIQ-10a had ED_{50} values between 18.5 and 35.7 μmol/kg. NBQX was active at an ED_{50} dose of 39.6 μmol/kg, THIQ-10b had an ED_{50} value of 50.2 μmol/kg and THIQ-10d was the least potent compound with an ED_{50} dose of 52.9 μmol/kg.

3.3. Anticonvulsant effects against PTZ-induced seizures

All 2,3-benzodiazepines studied demonstrated anticonvulsant activity against PTZ-induced seizure at doses double than those able to possess anticonvulsant activity against audiogenic seizures or maximal electroshock test. The compounds showed the following rank order of potency: THIQ-10c>CFM-2>GYKI 53665>THIQ-10a>GYKI 53773>THIQ-10d>GYKI 52466>THIQ-10b. The anticonvulsant activity of NBQX against PTZ-induced seizures was observed following doses 4.69 times larger than those able to exert anticonvulsant activity against the clonic phase of audiogenic seizures (Table 1).

3.4. Anticonvulsant effects against NMDA-, ATPA- and AMPA-induced seizures

All compounds tested were ineffective against NMDA-induced seizures, whereas all drugs, with the exception of diazepam, were able to protect against seizures induced by AMPA or ATPA (Table 2). NBQX was the most potent compound against tonus induced by intracerebroventricular administration of AMPA. The AMPA receptor antagonists studied showed the following rank order of potency against clonus induced by intracerebroventricular administration of AMPA: THIQ-10c>NBQX>GYKI 53655>CFM-2>GYKI 53773>THIQ-10a>GYKI

Table 2
Effects of compounds studied against NMDA-, AMPA- or ATPA-induced seizures in DBA/2 mice

Compound	ED50 (μmol/kg ip) for prevention of seizures induced by					
	NMDA		AMPA		ATPA	
	Tonus	Clonus	Tonus	Clonus	Tonus	Clonus
GYKI 52466	NA	NA	40.5 (26.3–60.8)	57.5 (43.5–76.0)	43.1 (32.4–57.3)	63.8 (45.4–59.7)
CFM-2	NA	NA	25 (16.5–30.0)	32 (23.2–44.3)	26.8 (19.1–37.6)	34.2 (26.3–44.47)
THIQ-10a	NA	NA	30.7 (19.6–48.1)	48.2 (33.4–69.6)	29.1 (22.1–38.3)	38.6 (25.8–57.7)
THIQ-10b	NA	NA	62.1 (41.5–92.9)	83.8 (54.9–127.9)	46.8 (32.3–67.8)	72.4 (58.8–89.1)
THIQ-10c	NA	NA	7.9 (4.7–13.4)	14.1 (7.8–25.5)	6.2 (4.9–7.8)	7.5 (6.2–8.97)
THIQ-10d	NA	NA	51.7 (37.1–72.1)	64.1 (42.5–96.6)	45.2 (29.6–69.02)	56.4 (39.5–80.5)
NBQX	NA	NA	7.4 (4.7–11.6)	16.5 (10.8–25.2)	8.5 (5.9–12.3)b	18.98 (13.4–26.8)
Diazepam	NA	NA	NA	NA	NA	NA
GYKI 53655	NA	NA	16.7 (12.8–21.8)	25.9 (18.1–37.1)	15.6 (11.4–21.3)	22.6 (16.7–30.6)
GYKI 53773	NA	NA	29.1 (22.6–37.5)	NA	44.2 (34.6–56.5)	NA

Data are expressed in μmol/kg and were calculated according to the method of Litchfield and Wilcoxon (1949). NA = not active until 100 μmol/kg.

Table 3
Anticonvulsant effect against 4-aminopyridine-, kainate- or 3-mercaptopropionic acid-induced seizure in ICR CD-1 mice

Compound	4-Aminopyridine	Kainate	3-Mercaptopropionic acid
GYKI 52466	143 (129–169)	27.8 (18.8–40.9)	47 (32.9–67.2)
CFM-2	27.1 (19.7–37.3)	23.06 (19.6–27.13)	28.3 (18.2–44.1)
THIQ-10a	40.3 (26.9–60.4)	31.4 (22.6–43.6)	45.8 (33.2–63.2)
THIQ-10b	NA	78.6 (47.8–129.2)	87.8 (52.3–147.4)
THIQ-10c	12.11 (9.0–13.4)	9.2 (6.72–12.6)	14.8 (12.2–17.9)
THIQ-10d	NA	62.4 (42.7–91.2)	72.6 (46.2–114.1)
Diazepam	NA	NA	0.90 (0.63–1.28)
NBQX	NA	35.6 (24.3–52.1)	NA
GYKI 53655	28.2 (18.8–42.3)	15.4 (9.7–24.5)	57.1 (39.7–82.1)
GYKI 53773	28.8 (23.5–35.3)	15.3 (12.3–19)	53.4 (36.9–77.3)

Data are expressed in μmol/kg and were calculated according to the method of Litchfield and Wilcoxon (1949). NA = not active until 100 μmol/kg.

52466>THIQ-10d>THIQ-10b. The compounds studied showed the following rank order of potency against clonus and tonus induced by intracerebroventricular administration of ATPA: THIQ-10c>NBQX>GYKI 53655>CFM-2>GYKI 52466>THIQ-10d>THIQ-10b.

3.5. Anticonvulsant activity against 4-aminopyridine, kainate or 3-mercaptopropionic-acid-induced seizures

All 2,3-benzodiazepines studied were able to protect against seizures induced by 4-aminopyridine, kainate or 3-

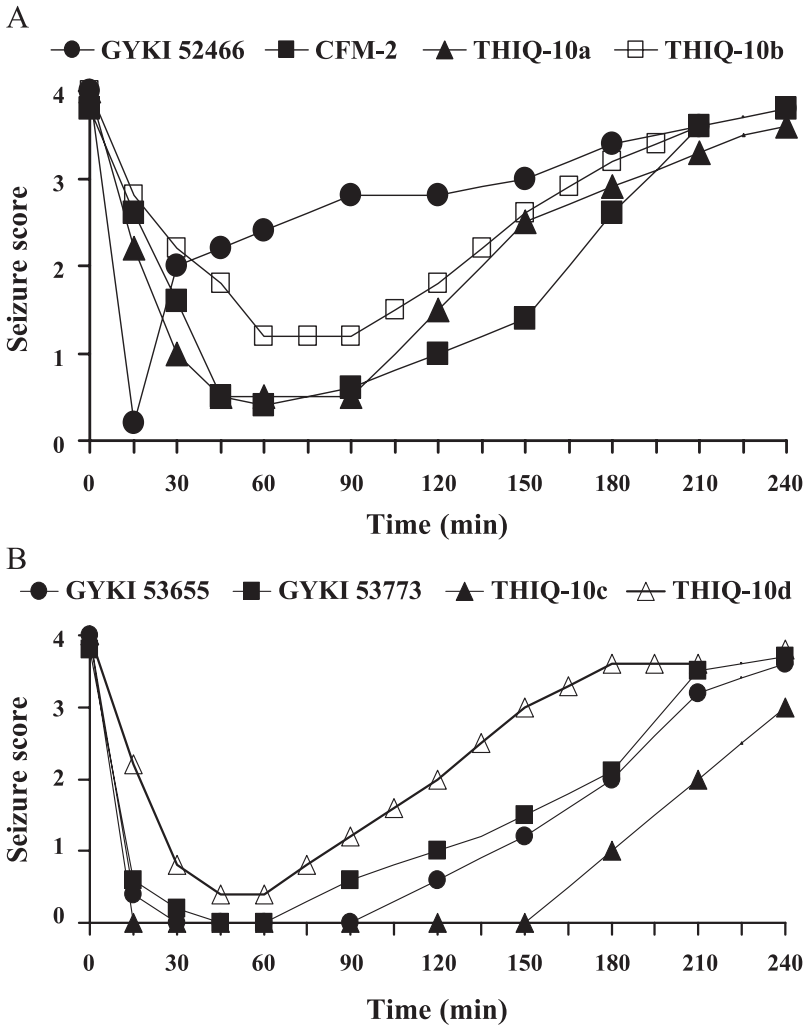

Fig. 2. Anticonvulsant effects of GYKI 52466 (●- -●), CFM-2 (■- -■), THIQ-10a (▲- -▲), THIQ-10b (□- -□) (A), THIQ-10c (▲- -▲), THIQ-10d (△- -△), GYKI 53655 (●- -●) and GYKI 53773 (■- -■) (B) (33 μmol/kg ip) against audiogenic seizures in DBA/2 mice. The ordinate shows seizure score; the abscissa shows the time after intraperitoneal administration of the drug in minutes. Ten animals were used for the determination of each point.

mercaptopropionic acid whereas diazepam was ineffective against 4-aminopyridine and kainate; NBQX, THIQ-10b and THIQ-10d were ineffective against 4-aminopyridine and NBQX was ineffective against 3-mercaptopropionic acid (Table 3). The compounds showed the following rank order of potency against kainate-induced seizures: THIQ-10c>GYKI 53773>GYKI 53655>CFM-2>GYKI 52466>THIQ-10a>NBQX>THIQ-10d>THIQ-10b. The compounds showed the following rank in order of potency against 4-aminopyridine-induced seizures: THIQ-10c>CFM-2>GYKI 53655>GYKI 53773>THIQ-10a>GYKI 52466. In addition, the compounds studied showed the following rank in order against 3-mercaptopropionic-acid-induced seizures: Diazepam>THIQ-10c>CFM-2>THIQ-10a>GYKI 52466>GYKI 53773>GYKI 53655>THIQ-10b.

3.6. Time course of anticonvulsant activity in the audiogenic sensible DBA/2 mice

As for the time course of anticonvulsant activity (Fig. 2) the effect of GYKI 52466 decreased to 50% after approximately 90 min, whereas the other compounds showed more than 70% seizure suppression even after 120 min. The strong anticonvulsive effect of THIQ-10c and GYKI 53733 gradually disappeared from 180 to 240 min, while the effect of CFM-2 and GYKI 53655 rapidly decreased between 150 and 180 min and then slowly disappeared. The antiseizure effects of THIQ-10a, THIQ-10b rapidly decreased between 90 and 120 min, and those of THIQ-10c between 180 and 210 min. However, after 240 min, all compounds investigated were ineffective.

We observed that (Fig. 2) GYKI 52466, THIQ-10b and THIQ-10d were completely protective for only 30 min, whereas the anticonvulsant effect of CFM-2, GYKI 53655 and GYKI 53733 lasted between 90 and 105 min, and those of THIQ-10c and lasted approximately 150 min (Fig. 2).

4. Discussion

The present study demonstrated the anticonvulsant efficacy of some noncompetitive AMPA receptor antagonists in various seizure models of experimental epilepsy. GYKI 52466 and related compounds were found to protect against audiogenic seizures, maximal electroshock, PTZ-, AMPA-, ATPA-, kainate-, 4-aminopyridine- and mercaptopropionic acid-induced seizures. It is very difficult to extrapolate a possible more selective action of one of these compounds on AMPA, ATPA or kainate subtype receptors, and a conclusion could be purely speculative. Reduction in locomotor activity, sedation, muscle relaxant activity and mild ataxia as side effects of these derivatives have been reported by several authors (Honorè et al., 1988; Smith et al., 1991; Turski et al., 1992; Yamaguchi et al., 1993; De Sarro et al., 1995, 1998, 1999a,b, 2003) and might be important factors in the therapeutic window.

In particular, the muscle-relaxant effects of AMPA/kainate receptor antagonists are due to an action on spinal reflexes; GYKI 52466 blocks both mono- and polysynaptic spinal reflexes in cats and rats (Block and Schwarz, 1994; Tarnawa et al., 1989; Abraham et al., 2001).

The AMPA receptor antagonists studied showed excellent, broad-spectrum anticonvulsant properties against seizures evoked by physical (sound and electroshock) or chemoconvulsive agents, indicating for some of them an antiepileptic efficacy superior to NBQX and diazepam against MES test and 4-aminopyridine-induced seizures (Tables 1 and 3).

In particular, as can be seen in Tables 1 and 3, THIQ-10c was the most active compound within the series of compounds antagonists studied, probably due to its physicochemical characteristics. It was also more effective than competitive AMPA/kainate receptor antagonist NBQX both in preventing MES and against some chemoconvulsant-induced seizures (Tables 1 and 3).

Table 2 shows that no compound studied proved to prevent seizures induced by NMDA, whereas all 2,3-benzodiazepine and THIQ derivatives and NBQX generally showed interesting activity against AMPA- or ATPA-induced seizures. If we compare the rank order of potency against AMPA-, ATPA- or kainate-induced seizures, THIQ-10c, GYKI 53773, GYKI 53655 and CFM-2 appear the most active compounds.

It is also interesting to note that THIQ-10c shows the longest time course with respect to the other studied compounds (Fig. 2). This behaviour can be explained by considering its capacity to cross the blood–brain barrier and to interact with AMPA/kainate receptors as such, but it could also be due to its biotransformation to other active metabolites. This possibility is now under investigation. The compounds studied showed a therapeutic index (TI) between 2.1 and 4.3 with the exception of THIQ-10c that possesses a TI of 5.9 (De Sarro et al., data not shown).

In conclusion, THIQ-10c, which possesses an acetyl group at N-2 and a chlorine atom at the 4 position of the C-1 phenyl ring of tetrahydroisoquinoline structure proved to be the most potent and longer lasting of the tested AMPA/kainate receptor antagonists in all seizure models of experimental epilepsy; only diazepam showed more marked effects against audiogenic seizures and PTZ- and mercaptopropionic-acid-induced convulsions. The latter result is obvious since diazepam is the most active compound in enhancing GABAergic neurotransmission among drugs tested in the present study. The fact that some compounds studied showed a broader spectrum of anticonvulsant efficacy when compared with NBQX might suggest that they act on some specific AMPA/kainate receptor subtypes that are not affected by NBQX. It is also possible that 2,3-benzodiazepines and THIQs, but neither NBQX nor diazepam, are able to interfere with

convulsant phenomena elicited by 4-aminopyridine. Previous findings demonstrated that kainate receptors, containing the GluR5 subtype of kainate receptor, regulate synaptic inhibition in the hippocampus and could provide a selective and new target for antiepileptic therapy (Clarke et al., 1997; Löscher et al., 1999; Smolders et al., 2002; Rogawski et al., 2003) and that GluR5-deficient mice appear to be less sensitive to kainate-induced seizures (Mulle et al., 1998; Sailer et al., 1999).

The present data showing that THIQ-10c is 10-fold more potent than GYKI 52466 suggest that THIQ-10c and some related compounds acting on GluR5 subtype of kainate receptors could be evaluated in some form of temporal epilepsy.

References

Abraham KE, McGinty JF, Brewer KL. The role of kainic acid/AMPA and metabotropic glutamate receptors in the regulation of opioid mRNA expression and the onset of pain-related behavior following excitotoxic spinal cord injury. Neuroscience 2001;104:863–74.

Armand V, Rundfeldt C, Heinemann U. Effects of retigabine (D-23129) on different patterns of epileptiform activity induced by 4-aminopyridine in rat entorhinal cortex hippocampal slices. Naunyn-Schmiedeberg's Arch Pharmacol 1999;359:33–9.

Barreca ML, Gitto R, Quartarone S, De Luca L, De Sarro G, Chimirri A. Pharmacophore modeling as an efficient tool in the discovery of novel noncompetitive AMPA receptor antagonists. J Chem Inf Comput Sci 2003;43:651–5.

Bettler B, Mulle C. Review: neurotransmitter receptors II: AMPA and kainate receptors. Neuropharmacology 1995;34:123–39.

Bleakman R, Schoepp DD, Ballyk B, Bufton H, Sharpe EF, Thomas K, et al. Pharmacological discrimination of GluR5 and GluR6 kainate receptor subtypes by (3S,4aR,6R,8aR)-6-[2-(1 (2)*H*-tetrazole-5-yl)ethyl]decahydroisdoquinoline-3 carboxylic-acid. Mol Pharmacol 1996; 49:581–5.

Block F, Schwarz M. The depressant effect of GYKI 52466 on spinal reflex transmission in rats is mediated via non-NMDA and benzodiazepine receptors. Eur J Pharmacol 1994;256:149–53.

Buchan AM, Lesiuk H, Barnes KA, Li H, Huang ZG, Smith KE, et al. AMPA antagonists: do they hold more promise for clinical stroke trials than NMDA antagonists? Stroke 1993;24:I148–52.

Chimirri A, De Sarro GB, De Sarro A, Gitto R, Grasso S, Quartarone S, et al. 1-Aryl-3,5-dihydro-4*H*-2,3-benzodiazepin-4-ones: novel AMPA receptor antagonists. J Med Chem 1997;40:1258–69.

Chimirri A, Gitto R, Zappalà M. AMPA receptor antagonists. Expert Opin Ther Pat 1999;9:557–70.

Clarke VR, Collingridge GL. Characterisation of the effects of, ATPA, a GLU(K5) receptor selective agonist, on excitatory synaptic transmission in area CA1 of rat hippocampal slices. Neuropharmacology 2002;42: 889–902.

Clarke VR, Ballyk BA, Hoo KH, Mandelzys A, Pellizzari A, Bath CP, et al. A hippocampal GluR5 kainate receptor regulating inhibitory synaptic transmission. Nature 1997;389:599–603.

Cui C, Mayer ML. Heteromeric kainate receptors formed by the coassembly of GluR5, GluR6, and GluR7. J Neurosci 1999;19:8281–91.

De Sarro GB, Croucher MJ, Meldrum BS. Anticonvulsant actions of DS 103–282. Neuropharmacology 1984;23:525–30.

De Sarro GB, Meldrum BS, Nisticò G. Anticonvulsant effects of some calcium entry blockers in DBA/2 mice. Br J Pharmacol 1988;93: 247–56.

De Sarro GB, Ongini E, Bertorelli R, Aguglia U, De Sarro A. Excitatory amino acid neurotransmission through both NMDA and non-NMDA receptors is involved in the anticonvulsant activity of felbamate in DBA/2 mice. Eur J Pharmacol 1994;262:11–9.

De Sarro GB, Chimirri A, De Sarro A, Gitto R, Grasso S, Giusti P, et al. GYKI 52466 and related 2,3-benzodiazepines as anticonvulsant agents in DBA/2 mice. Eur J Pharmacol 1995;294:411–22.

De Sarro GB, Donato Di Paola E, Gareri P, Gallelli L, Scotto G, De Sarro A. Effects of some AMPA receptor antagonists on the development of tolerance in epilepsy-prone rats and in pentylenetetrazole kindled rats. Eur J Pharmacol 1998;368:149–59.

De Sarro A, De Sarro GB, Gitto R, Grasso S, Micale N, Zappalà M. Synthesis and anticonvulsant activity of new 2,3-benzodiazepines as AMPA receptor antagonists. Farmaco 1999a;54:178–87.

De Sarro GB, Rizzo M, Spagnolo C, Gitto R, De Sarro A, Scotto G, et al. Anticonvulsant activity and plasma level of 2,3-benzodiazepin-4-ones (CFMs) in genetically epilepsy-prone rats. Pharmacol Biochem Behav 1999b;4:621–7.

De Sarro G, Ferreri G, Gareri P, Russo E, De Sarro A, Gitto R, et al. Comparative anticonvulsant activity of some 2,3-benzodiazepine derivatives in rodents. Pharmacol Biochem Behav 2003;74:595–602.

Donevan SD, Yamaguchi SI, Rogawski MA. Non-*N*-methyl-D-aspartate receptor antagonism by 3-*N*-substituted 2,3-benzodiazepines: relationship to anticonvulsant activity. J Pharmacol Exp Ther 1994; 271:25–9.

Dreier JP, Heinemann U. Late low magnesium-induced epileptiform activity in rat entorhinal cortex slices becomes insensitive to the anticonvulsant valproic acid. Neurosci Lett 1990;119:68–70.

Gitto R, Chimirri A, Zappalà M, De Sarro GB, De Sarro A. Synthesis and pharmacological evaluation of 4-aryl-6,7-dimethoxyphtalazines as anticonvulsant agents. Med Chem Res 2000;10:1–10.

Gitto R, Barreca ML, De Luca L, De Sarro G, Ferreri G, Quartarone S, et al. Discovery of a novel and highly potent noncompetitive AMPA receptor antagonist. J Med Chem 2003a;46:197–200.

Gitto R, Orlando V, Quartarone S, De Sarro G, De Sarro A, Russo E, et al. Synthesis and evaluation of pharmacological properties of novel annelated 2,3-benzodiazepine derivatives. J Med Chem 2003b;46: 3758–61.

Grasso S, De Sarro G, De Sarro A, Micale N, Zappala M, Puja G, et al. Synthesis and anticonvulsant activity of novel and potent 6,7-methylenedioxyphthalazin-1 (2H)-ones. J Med Chem 2000;43:2851–9.

Honorè T, Davies SN, Drejer J, Fletcher EJ, Jacobsen P, Lodge D, et al. Quinoxalinediones: potent competitive non-NMDA glutamate receptor antagonists. Science 1988;241:701–3.

Horton RW, Meldrum BS. Seizures induced by allylglicyne, 3-mercaptopropionic acid and 4-deoxy-pyridoxine in mice and photosensitive baboons, and different modes of inhibition of cerebral glutamic acid decarboxylase. Br J Pharmacol 1973;49:52–63.

Jorgensen M, Tygesen CK, Andersen PH. Ionotropic glutamate receptors—focus on non-NMDA receptors. Pharmacol Toxicol 1995;76:312–9.

Lazzaro JT, Paternain AV, Lerma J, Chenard BL, Ewing FE, Huang J, et al. Functional characterization of CP-465,022, a selective, noncompetitive AMPA receptor antagonist. Neuropharmacology 2002;42:143–53.

Lerma J, Morales M, Vicente MA, Herreras O. Glutamate receptors of the kainate type and synaptic transmission. Trends Neurosci 1997;20:9–12.

Li H, Rogawski MA. GluR5 kainate receptor mediated synaptic transmission in rat basolateral amygdala in vitro. Neuropharmacology 1998;37: 1279–86.

Litchfield JT, Wilcoxon F. A simplified method of evaluating dose–effect experiments. J Pharmacol Exp Ther 1949;96:99–113.

Löscher W, Honack D. The behavioural effects of MK-801 in rats: involvement of dopaminergic, serotonergic and noradrenergic systems. Eur J Pharmacol 1992;215:199–208.

Löscher W, Honack D. Over-additive anticonvulsant effect of memantine and NBQX in kindled rats. Eur J Pharmacol 1994;259:R3–5.

Löscher W, Schmidt D. Strategies in antiepileptic drug development: is rational drug design superior to random screening and structural variation? Epilepsy Res 1994;17:95–134.

Löscher W, Lehmann H, Behl B, Seemann D, Teschendorf HJ, Hofmann HP,

et al. A new pyrrolyl-quinoxalinedione series of non-NMDA glutamate receptor antagonists: pharmacological characterization and comparison with NBQX and valproate in the kindling model of epilepsy. Eur J Neurosci 1999;11:250–62.

Mulle C, Sailer A, Perez-Otano I, Dickinson-Anson H, Castillo PE, Bureau I, et al. Altered synaptic physiology and reduced susceptibility to kainate-induced seizures in GluR6-deficient mice. Nature 1998;392:601–5.

O'Neill MJ, Bond A, Ornstein PL, Ward MA, Hicks CA, Hoo K, et al. Decahydroisoquinolines: novel competitive AMPA/kainate antagonists with neuroprotective effects in global cerebral ischaemia. Neuropharmacology 1998;37:1211–22.

Parsons CG, Danysz W, Quack G, Hartmann S, Lorenz B, Wollenburg C, et al. Novel systemically active antagonists of the glycine site of the *N*-methyl-D-aspartate receptor: electrophysiological, biochemical and behavioral characterization. J Pharmacol Exp Ther 1997;283:1264–75.

Paternain AV, Herrera MT, Nieto MA, Lerma J. GluR5 and GluR6 kainate receptor subunits coexist in hippocampal neurons and coassemble to form functional receptors. J Neurosci 2000;20:196–205.

Pei X, Sturgess MA, Valenzuela CF, Maccecchini ML. Allosteric modulators of AMPA receptor: novel 6-substituted dihydrophthalazines. Bioorg Med Chem Lett 1999;9:539–42.

Pelletier JC, Hesson DP, Jones KA, Costa AM. Substituted 1,2-dihydrophthalazines: potent, selective, and noncompetitive inhibitors of the AMPA receptor. J Med Chem 1996;39:343–6.

Rogawski MA. Therapeutic potential of excitatory amino acid antagonists: channel blockers and 2,3-benzodiazepines. Trends Pharmacol Sci 1993;14:325–31.

Rogawski MA, Gryder D, Castaneda D, Yonekawa W, Banks MK, Lia H. GluR5 kainate receptors, seizures, and the amygdala. Ann N Y Acad Sci 2003;985:150–62.

Sailer A, Swanson GT, Perez-Otano I, O'Leary L, Malkmus SA, Dyck RH, et al. Generation and analysis of GluR5 (Q636R) kainate receptor mutant mice. J Neurosci 1999;19:8757–64.

Smith SE, Durmuller N, Meldrum BS. The non-*N*-methyl-D-aspartate receptor antagonist, GYKI 52466 and NBQX are anticonvulsant in two animal models of reflex epilepsy. Eur J Pharmacol 1991;201:179–83.

Smolders I, Bortolotto ZA, Clarke VR, Warre R, Khan GM, O'Neill MJ, et al. Antagonists of GLU (K5)-containing kainate receptors prevent pilocarpine-induced limbic seizures. Nat Neurosci 2002;5:796–804.

Spyker DA, Lynch C, Shabanowitz J, Sinn JA. Poisoning with 4-aminopyridine: report of three cases. Clin Toxicol 1980;16:487–97.

Stensbøl TB, Borre L, Johansen TN, Egebjerg J, Madsen U, Ebert B, et al. Resolution, absolute stereochemistry and molecular pharmacology of the enantiomers of ATPA. Eur J Pharmacol 1999;380:153–62.

Sutcliffe MJ, Wo ZG, Oswald RE. Three-dimensional models of non-NMDA glutamate receptors. Biophys J 1996;70:1575–89.

Swinyard EA, Woodhead JM. Experimental detection, quantification and evaluation of anticonvulsants. In: Woodbury DM, Penry JK, Pippenger CE, editors. Epileptic drugs. New York: Raven Press, 1982. pp. 111–26.

Tarnawa I, Farkas S, Berzsenyi P, Pataki A, Andrasi F. Electrophysiological studies with a 2,3-benzodiazepine muscle relaxant: GYKI 52466. Eur J Pharmacol 1989;167:193–9.

Turski L, Jacobsen P, Honore T, Stephens DN. Relief of experimental spasticity and anxiolytic/anticonvulsant actions of the alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate antagonist 2,3-dihydroxy-6-nitro-7-sulfamoyl benzo (F) quinoxaline. J Pharmacol Exp Ther 1992;260:742–7.

Vizi ES, Mike A, Tarnawa I. 2,3-Benzodiazepines (GYKI 52466 analogues): negative allosteric modulators of AMPA receptors. CNS Drug Rev 1996;2:91–126.

Welch WM, Ewing FE, Huang J, Menniti FS, Pagnozzi MJ, Kelly K, et al. Atropisomeric quinazolin-4-one derivatives are potent noncompetitive alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor antagonists. Bioorg Med Chem Lett 2001;11:177–81.

Yamaguchi S, Rogawski MA. Effects of anticonvulsant drugs on 4-aminopyridine-induced seizure in mice. Epilepsy Res 1992;11:9–16.

Yamaguchi S, Donevan SD, Rogawski MA. Anticonvulsant activity of AMPA/kainate antagonists: comparison of GYKI 52466 and NBQX in maximal electroshock and chemoconvulsant seizure models. Epilepsy Res 1993;15:179–84.

Yonekawa WD, Kapetanovic IM, Kupferberg HJ. The effects of D-23129 (ADD230001) and standard anticonvulsant on the paired-pulse paradigm in rat hippocampus in vitro (abstract). Soc Neurosci Abstr 1995a;21:R2117.

Yonekawa WD, Kapetanovic IM, Kupferberg HJ. The effects of anticonvulsant agents on 4-aminopyridine-induced epileptiform activity in rat hippocampus in vitro. Epilepsy Res 1995b;20:137–50.

Zappalà M, Gitto R, Bevacqua F, Quartarone S, Chimirri A, Rizzo M, et al. Synthesis and evaluation of pharmacological and pharmacokinetic properties of 11*H*-(1,2,4)triazolo (4,5-c) (2,3)benzodiazepin-3 (2*H*)-ones. J Med Chem 2000;43:4834–9.

Zhang CL, Dreier JP, Heinemann U. Low Mg^{2+} induced epileptiform activity in rat temporal cortex slices: development of pharmacoresistant epileptiform activity (abstract). Pflugers Archiv 1991;418:R10.