ELSEVIER

European Journal of Pharmacology 294 (1995) 411–422

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GYKI 52466 and related 2,3-benzodiazepines as anticonvulsant agents in DBA/2 mice

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Received 13 April 1995; revised 27 July 1995; accepted 1 September 1995

Abstract

The behavioural and anticonvulsant effects of several 1-aryl-3,5-dihydro-4*H*-2,3-benzodiazepin-4-ones (2,3-BZs) and of 11*b*-aryl-7,11-dihydro-3-phenyl[1,2,4]oxadiazolo[5,4-*a*][2,3]benzodiazepin-6-ones (2,3-OBZs) were studied after intraperitoneal (i.p.) administration in DBA/2 mice, a strain genetically susceptible to sound-induced seizures. The seizures were evoked by means of auditory stimulation (109 dB, 12–16 kHz) in animals placed singly under a hemispheric Perspex dome. The 2,3-benzodiazepines studied after 30 min pretreatment were generally less potent than the related derivative 1-(4-aminophenyl)-4-methyl-7,8-methylenedioxy-5*H*-2,3-benzodiazepine hydrochloride (GYKI 52466) except 3,5-dihydro-7,8-dimethoxy-1-phenyl-4*H*-2,3-benzodiazepin-4-one (2,3-BZ-2) and 2,3-BZ-2M (3-methyl derivative of 2,3-BZ-2) which showed comparable activity. Thirty minutes after i.p. administration of 2,3-benzodiazepines, the rank order of potency for anticonvulsant activity against clonus was 2,3-BZ-2 > GYKI 52466 > 2,3-BZ-2M > 2,3-BZ-1 > 2,3-BZ-3 > 2,3-OBZ-1 > 2,3-OBZ-2 > 2,3-OBZ-3. The intracerebroventricular (i.c.v.) injection of aniracetam on it own (12.5–100 nmol/mouse) had no convulsant activity, but it reversed the anticonvulsant effects of some 2,3-benzodiazepines. In particular, the pharmacological actions of GYKI 52466, 2,3-BZ-2 and 2,3-BZ-2M, which proved to be the most potent 2,3-benzodiazepine derivatives as anticonvulsants, were significantly reduced by an i.c.v. pretreatment with aniracetam (50 nmol/mouse). Concomitant treatment with aniracetam (50 nmol/mouse) shifted to the right the dose-response curves and significantly increased the ED_{50} values for GYKI 52466, 2,3-BZ-2 and 2,3-BZ-2M. After 30 min pretreatment 2,3-BZ-2 showed a similar potency to GYKI 52466 in antagonizing seizures induced by i.c.v. administration of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), thus suggesting a clear involvement of AMPA receptors in the anticonvulsant activity of these compounds. In addition, 2,3-BZ-2 and 2,3-BZ-2M showed anticonvulsant properties longer lasting than GYKI 52466.

Keywords: 2,3-Benzodiazepine; GYKI 52466; Aniracetam; AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid); Anticonvulsant drug; Epilepsy; Audiogenic seizure; (DBA/2 mouse)

1. Introduction

Benzodiazepines were synthesized over 30 years ago (Bell et al., 1962; Sternbach et al., 1968) and are widely used as therapeutic agents active in a variety of neurological and psychiatric disorders such as epilepsy, anxiety, insomnia, spasticity and depression (Sternbach et al., 1968; Harvey, 1985; Villar et al., 1991). Significant efforts have been made to separate these therapeutic effects of benzodiazepines.

The 2,3-benzodiazepine, tofisopam, has proved to be an anxioselective drug without sedative-hypnotic, anticonvulsant and muscle-relaxant properties of the 1,4- and 1,5-benzodiazepines (Goldberg and Finnerty, 1979).

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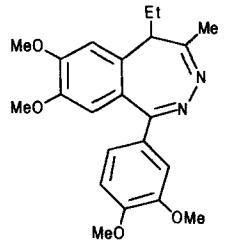
SSDI 0014-2999(95)00561-7

1-(4-Aminophenyl)-4-methyl-7,8-methylenedioxy-5*H*-2,3-benzodiazepine hydrochloride (GYKI 52466) possesses some chemical similarities with tofisopam but displays a different pharmacological profile. Tarnawa et al. (1989,1990) have shown that GYKI 52466 inhibits spinal reflex in cats but does not potentiate the inhibitory action of GABA and acts as a non-NMDA antagonist. In particular, a concentration of 10–50 μM of GYKI 52466 considerably attenuates the quisqualate- but not the NMDA-induced neuronal depolarization in rat somatosensory cortical slices (Tarnawa et al., 1990). In addition, GYKI 52466 showed a selective anticonvulsant activity against seizures induced by intracerebroventricular (i.c.v.) administration of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and kainate in mice (Smith and Meldrum, 1990; Chapman et al., 1991; Yamaguchi et al., 1993). GYKI 52466 has also demonstrated to possess anticonvulsant properties in two genetic models of audiogenic seizures, the DBA/2 mouse and the genetically epilepsy-prone rat as well as in the photosensitive baboon *Papio papio* (Chapman et al., 1991; Smith et al., 1991). The time course for the anticonvulsant effects of GYKI 52466 in mice, rats and baboons was very short (from 5 to 60 min after intraperitoneal or intravenous administration) (Chapman et al., 1991; Smith et al., 1991).



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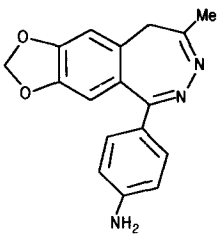
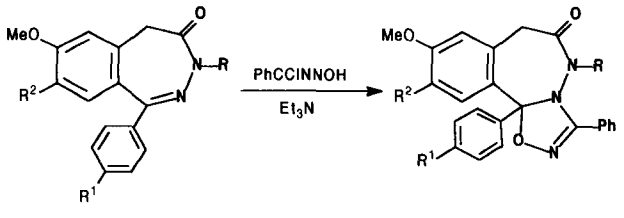


GYKI 52466



2,3-BZ-series 2,3-OBZ-series

	R	R^1	R^2	
2,3-BZ-1	H	H	H	2,3-OBZ-1
2,3-BZ-2	H	H	OMe	2,3-OBZ-2
2,3-BZ-2M	Me	H	OMe	
2,3-BZ-3	H	Cl	OMe	2,3-OBZ-3

Fig. 1. Chemical structures of the 2,3-benzodiazepine derivatives studied.

The present study was designed to determine whether a series of 2,3-benzodiazepines, chemically similar to GYKI 52466, synthesized in our laboratories (Fig. 1), have anticonvulsant effects in DBA/2 mice. This strain of mice is genetically susceptible to sound-induced seizures and has been considered an excellent animal model for the study of certain kinds of human epilepsy and for testing new anticonvulsant drugs (Chapman et al., 1984; Seyfried and Glaser, 1985; Engstrom and Woodbury, 1988). The time course was also studied for some of them in order to discover new compounds with a longer time course than GYKI 52466.

In addition, in order to correlate their anticonvulsant activity with the different affinity for benzodiazepine or AMPA receptors, 2,3-benzodiazepines were evaluated against seizures induced by AMPA, an agonist at the non-NMDA receptor complex (Honoré et al., 1988) and against a concomitant treatment with aniracetam, a compound which potentiates the effects of quisqualate and AMPA in *Xenopus* oocytes (Ito et al., 1990), or against a concomitant treatment with flumazenil, a 'neutral' benzodiazepine receptor antagonist.

2. Materials and methods

2.1. Chemical synthesis and nuclear magnetic resonance (NMR) data of 2,3-benzodiazepine derivatives

Melting points (m.p.) were determined on a Kofler hot stage apparatus and are uncorrected. Elemental analyses were made on a Carlo Erba 1106 analyzer; for C,H,N the results agreed to within $\pm 0.4\%$ of theoretical values. ^{1}H-NMR and ^{13}C-NMR spectra were recorded in $CDCl_3$ on a Varian Gemini-300 spectrometer. Chemical shifts were expressed in δ (ppm) and the coupling constants J in Hz.

General procedure for synthesis of 2,3-benzodiazepines

The synthesis of 3,5-dihydro-7-methoxy-1-phenyl-4*H*-2,3-benzodiazepin-4-one (2,3-BZ-1), 3,5-dihydro-7,8-dimethoxy-1-phenyl-4*H*-2,3-benzodiazepin-4-one (2,3-BZ-2), 3,5-dihydro-7,8-dimethoxy-3-methyl-1-phenyl-4*H*-2,3-benzodiazepin-4-one (2,3-BZ-2M) and 1-(4-chlorophenyl)-3,5-dihydro-7,8-dimethoxy-4*H*-2,3-benzodiazepin-4-one (2,3-BZ-3) was carried out by condensation of hydrazine or methylhydrazine with 2-aroylphenylacetic acids, according to a procedure described by Gatta et al. (1985).

General procedure for synthesis of oxadiazolo-2,3-benzodiazepines

To a stirred solution of the appropriate 2,3-benzodiazepine derivatives (10 mmol) and triethylamine (15

mmol) in methylene chloride (30 ml) a solution of the benzohydroxamic acid chloride (15 mmol) in the same solvent was added dropwise over a few minutes. The reaction mixture was kept under stirring at room temperature for 20–24 h. After the removal of the solvent at reduced pressure, diethyl ether was added to the residue and the triethylamine chloride was filtered. The solvent was then evaporated off and the residue subjected to column chromatography with diethyl ether/light petroleum 1:1.

7,11-Dihydro-3,11b-diphenyl-9-methoxy[1,2,4]oxadiazolo[5,4-a][2,3]benzodiazepin-6-one (2,3-OBZ-1):. M.p. 196–198°C, yield 58%. Anal. calcd. for $C_{23}H_{19}N_3O_3$: C, 71.69; H, 4.93; N, 10.90. Found: C, 72.07; H, 4.57; N, 11.19. ^{1}H-NMR (δ): 3.37 and 4.44 (dd, $J = -13.8$, 2H, CH_2), 3.81(s, 3H, OCH_3), 6.74–7.67 (m, 13H, ArH), 7.07 (bs, 1H, NH). ^{13}C-NMR (δ): 42.01 (C-7), 55.37 (OCH_3), 100.15 (C-11b), 113.26 and 116.03 (C-8 and C-11), 123.13 and 123.39 (C-7a and C-11a), 126.78, 127.99, 128.43, 128.92, 129.07, 131.40, 132.85 (aromatic CH), 132.20 (C-1″), 140.67 (C-1′), 160.35 (C-9), 154.00 (C-3), 175.11 (C-6).

7,11-Dihydro-9,10-dimethoxy-3,11b-diphenyl[1,2,4]oxadiazolo[5,4-a][2,3]benzo-diazepin-6-one (2,3-OBZ-2):. M.p. 205–207°C, yield 45%. Anal. calcd. for $C_{24}H_{21}N_3O_4$: C, 69.40; H, 4.82; N, 10.12. Found: C, 69.01; H, 4.28; N, 10.19. ^{1}H-NMR (δ): 3.35 and 4.44 (dd, $J = -14.01$, 2H, CH_2), 3.66 (s, 3H, OCH_3), 3.92 (s, 3H, OCH_3), 6.49 and 6.73 (2s, 2H, H-8 and H-11), 7.01 (bs, 1H, NH), 7.39–7.67 (m, 10H, ArH). ^{13}C-NMR (δ): 41.71 (C-7), 56.04 (OCH_3), 100.15 (C-11b), 113.29 and 113.50 (C-8 and C-11), 123.13 and 123.39 (C-7a and C-11a), 127.08, 127.95, 128.62. 128.97, 129.39, 131.46 (aromatic CH), 128.76 (C-1″), 139.90 (C-1′), 148.54 and 149.62 (C-9 and C-10), 153.62 (C-3), 175.14 (C-6).

11b-(4-Chlorophenyl)-7,11-dihydro-9,10-dimethoxy-3-phenyl[1,2,4]oxadiazolo-[5,4-a][2,3]benzodiazepin-6-one (2,3-OBZ-3):. M.p. 113–115°C, yield 52%. Anal. calcd. for $C_{24}H_{20}ClN_3O_4$: C, 64.14; H, 4.45; N, 9.35. Found: C, 64.42; H, 4.07; N, 9.26. ^{1}H-NMR (δ): 3.34 and 4.40 (dd, $J = -13.08$, 2H, CH_2), 3.68 (s, 3H, OCH_3), 3.92 (s, 3H, OCH_3), 6.44 and 6.73 (2s, 2H, H-8 and H-11), 7.23 (bs, 1H, NH), 7.37–7.65 (m, 10H, ArH). ^{13}C-NMR (δ): 41.45 (C-7), 55.97 (OCH_3), 99.63 (C-11b), 113.30 (C-8 and C-11), 123.10 (C-1), 123.32 (C-1), 126.95, 128.93, 129.23, 130.89, 131.57, (aromatic CH), 128.02 (C-7a), 135.36 (C-Cl), 138.59 (C-11a), 148.51 and 149.72 (C-9 and C-10), 153.64 (C-3), 175.26 (C-6).

2.2. Lipophilicity measurements

The relative lipophilicity of the compounds was measured by reversed-phase thin-layer chromatography according to the method previously described (Chimirri et al., 1989). Briefly, silanized silica gel plates Merck 60 F_{254} were used as nonpolar stationary phase. The plates were dried at 105°C for 1 h before use. The polar mobile phase was a 2:1 v/v mixture of acetone and water. Each compound was dissolved in chloroform (3 mg/ml), and 5 μl of solution was applied onto the plate. The experiments were repeated 5 times with different disposition of the compounds on the plate. The R_f values were expressed as the mean values of the five determinations. The R_m values were calculated from the experimental R_f values according to the formula $R_m = \log[(1/R_f) - 1]$. Higher R_m values indicate higher lipophilicity.

2.3. Testing of anticonvulsant activity

All experiments were performed with DBA/2 mice, an inbred strain, the weanlings of which are genetically susceptible to audiogenic seizures (Collins, 1972; Hall, 1947). Exposure to a loud sound within a specific frequency range induces a sequential seizure response in these animals, consisting of an early wild running phase (WR), followed by generalised myoclonus and tonic flexion and extension sometimes followed by respiratory arrest. Later phases in the seizure sequence do not occur in the absence of earlier phases. Groups of 10 mice, of mixed sexes, weight 8–12 g and 22–25 days of age, were studied at each dose. DBA/2 mice were purchased from Charles River (Calco, Como, Italy) and were exposed to auditory stimulation, 30 min following intraperitoneal (i.p.) administration of vehicle or drugs. Individual mice were placed under a hemispheric Perspex dome (diameter 58 cm) and 60 s allowed for habituation and assessment of locomotor activity. Auditory stimulation (12–16 kHz, 109 dB) was applied for 60 s or until tonic extension occurred. Seizure response (SR) as previously reported (De Sarro et al., 1984) was assessed on the following scale: 0 = no response, 1 = wild running, 2 = clonus, 3 = tonus, 4 = respiratory arrest. 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. Behavioural changes were observed during the period between drug administration and auditory testing. A second group of DBA/2 mice (10 for each group) was used for the time course of the anticonvulsant effects of GYKI 52466, 2,3-BZ-2 and 2,3-BZ-2M. In particular, animals were pretreated at a dose of 33 μmol/kg from 5 min to 180 min and then exposed to auditory stimulation.

Seizures were also induced by intracerebroventricular (i.c.v.) injection of AMPA. For i.c.v. injection, mice were anaesthetized 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; injections of drugs by this procedure led to an uniform distribution throughout the ventricular system within 10 min (De Sarro et al., 1994a). The animals were placed singly in a 30 × 30 × 30 cm box and the observation time was 30 min after the administration of AMPA.

2.4. Effects on motor movements

Groups of male Swiss mice (20–26 g, 48–54 days old) were purchased from Charles River (Calco, Como, Italy) and were trained to do coordinated motor movements continuously for 2 min on a rotarod 3 cm diameter 8 rpm (U. Basile, Comerio, Varese, Italy). Impairment of coordinated motor movements was defined as inability of the mice to remain on the rotarod for a 2 min test period (Dunham and Miya, 1957). The ability of the mice to remain on the rotarod was tested 30 min after administration of various 2,3-benzodiazepines.

2.5. Membrane preparation and [^{3}H]flumazenil binding studies

Male SD/Rij rats (FRAR, S. Pietro al Natisone, UD, Italy) weighing 200–250 g were decapitated and different brain areas were rapidly dissected on ice. Brain regions were homogenized in 20 ml of ice-cold 0.32 M sucrose pH 7.4 by using a glass homogenizer with a Teflon pestle (10 up and down strokes). The homogenate was centrifuged at 1000 × *g* at 4°C for 10 min, the P_1 pellet was discarded, and the supernatant was collected and recentrifuged at 20 000 × *g* at 4°C for 20 min. The resulting crude mitochondrial pellet (P_2) was resuspended in 20 ml of ice-cold distilled water and homogenised. The homogenate was centrifuged at 8000 × *g* at 4°C for 20 min, the supernatant was collected and recentrifuged at 48 000 × *g* at 4°C for 20 min, and the final crude microsomal pellet (P_3) was frozen for at least 24 h. The pellet was resuspended in 10 ml of 50 mM Tris-HCl pH 7.4, centrifuged at 48 000 × *g* at 4°C for 20 min and then resuspended in 10 vols. of the same buffer for the standard binding assay.

Aliquots of membrane suspensions (100 μl, or 0.15 mg of protein) were added to incubation medium containing 1 nM of [^{3}H]flumazenil (specific activity 72.4 Ci/mmol) in a final volume of 1 ml of Tris-HCl 50 mM, NaCl 120 mM and KCl 5 mM, pH 7.4. All BDZs were dissolved in dimethyl sulfoxide (DMSO) at the final concentration of 1%. Incubations were carried out for 60 min at 4°C in triplicate and non-specific binding measured in the presence of 10 μM of diazepam. Reactions were stopped by the addition of 5 ml ice-cold Tris-HCl followed by rapid filtration through Whatman GF/C glass fiber filters (Whatman, Clifton, NJ, USA) and two additional washes. The radioactivity trapped on the filters was counted after the addition of 8 ml of Filter Count (Packard), by liquid scintillation spectrometry. The experiments were run in triplicate with eight different concentrations of competing ligand $IC_{50} \pm$ S.D. values.

2.6. Statistical analysis

Statistical comparisons between groups of control and drug-treated animals were made using Fisher's exact probability test (incidence of the seizure phases) or the analysis of variance (ANOVA) followed by post-hoc Dunnett's *t*-test (rectal temperatures). The percentage incidence of each phase of the audiogenic seizure was determined for each dose of compound administered and dose-response curves were fitted using linear regression analysis of percentage response. The seizures score (SR) was statistically analyzed using the non-parametric methods of the Mann-Whitney *U*-test (median seizure score ± interquartile range). ED_{50} values (with 95% confidence limits) for each compound and each phase of seizure response were estimated using a computer program of the method of Litchfield and Wilcoxon (1949); the relative anticonvulsant activities were determined by comparison of respective ED_{50} values. For the binding experiments ID_{50} values for the statistical package for the [^{3}H]flumazenil displacement were determined by the nonlinear curve-fitting program based on LIGAND (McPherson, 1987).

2.7. Drugs

The sources of the drugs were: 2,3-benzodiazepines synthesized in our laboratories (vide infra); GYKI 52466 obtained from Dr. I. Tarnawa (Inst. Drug Research, Hungary); α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) purchased from Tocris (Buckhurst Hill, UK); aniracetam (1-*p*-anisoyl-2-pyrrolidinone) was obtained from Menarini (Florence, Italy); flumazenil (Ro 15-1788 or ethyl 5,6-dihydro-8-fluoro-5-methyl-6-oxo-4*H*-imidazo[1,5-*a*][1,4]benzodiazepine-3-carboxylate) obtained from Hoffmann-La Roche (Basel, Switzerland); [^{3}H]flumazenil was obtained from New England Nuclear (Boston, MA, USA).

For systemic injections all compounds were given intraperitoneally (0.10 ml/10 g of body weight of the mouse) as freshly prepared solution. 2,3-Benzodiazepines were dissolved in 50% DMSO and 50% sterile saline (0.9% NaCl) whilst flumazenil was suspended in 45% hydroxypropyl-β-cyclodextrin and 55% sterile saline. Doses and time of administration are reported in the tables and figures. Previous experiments have shown that the vehicle used to dissolve 2,3-benzodi-

azepines or flumazenil, when administered i.p., does not affect either behaviour or response to auditory stimulation of DBA/2 mice. All drugs administered i.c.v. (aniracetam or AMPA) were dissolved in sodium phosphate buffer 67 mM, microinjected in a volume of 10 μl per mouse. In order to avoid the light sensitivity of some compounds, weighing and handling were carried out under sodium vapour lamps and the substances were protected from light during the experiments.

3. Results

3.1. Synthesis and chemical characterization of oxadiazolo-2,3-benzodiazepines

The reaction of 2,3-benzodiazepines (2,3-BZ-1–3) with a slight excess of the benzonitriloxide (PhCNO), generated in situ from benzohydroxamoyl chloride (PhCClNNOH) and triethylamine (Et_3N), was performed in methylene chloride at room temperature; the oxadiazolo-2,3-benzodiazepines (2,3-OBZ-1-3) were obtained in good yields (Fig. 1). The structure of the synthesized compounds was assessed by ^1H- and ^{13}C-NMR spectroscopy. The results are in full agreement with the expected structures.

3.2. Physico-chemical parameters

The relative lipophilicity (R_m) and molecular weight (MW) of the 2,3-benzodiazepines studied are summarized in Table 1. The most lipophilic compounds, oxadiazolo-2,3-benzodiazepine derivatives in which highly hydrophobic groups are present, showed a lower activity than 2,3-benzodiazepine precursors.

3.3. Anticonvulsant activity of 2,3-benzodiazepines

As shown in Table 2, the clonic and tonic phases of the audiogenic seizure response were significantly reduced 30 min after i.p. administration of GYKI 52466 (33, 50 and 66 μmol/kg), 2,3-BZ-1 (100 and 200 μmol/kg), 2,3-BZ-2 (33, 50, 66 and 100 μmol/kg), 2,3-BZ-2M (66 and 100 μmol/kg), 2,3-BZ-3 (200 μmol/kg), 2,3-0BZ-1 (100 and 200 μmol/kg), 2,3-OBZ-2 (200 and 300 μmol/kg) and 2,3-OBZ-3 (200 μmol/kg). The wild running phase was significantly reduced after i.p. administration of most of the 2,3-benzodiazepines at the highest doses tested with the exception of 2,3-OBZ-1 and 2,3-OBZ-3 (Table 2). The relative ED_{50} values (with 95% confidence limits) are reported in Table 3. Following i.p. administration of 2,3-BZ-2 and 2,3-BZ-2M, 33 μmol/kg, maximum protection was observed from 45 to 90 min with subsequent return to control seizure response at 180 min, whilst following the i.p. injection of GYKI 52466, 33 μmol/kg, the maximum protection was observed from 5 to 15 min with subsequent return to control seizure response at 90 min (Fig. 2). All the compounds tested did not significantly affect body temperature.

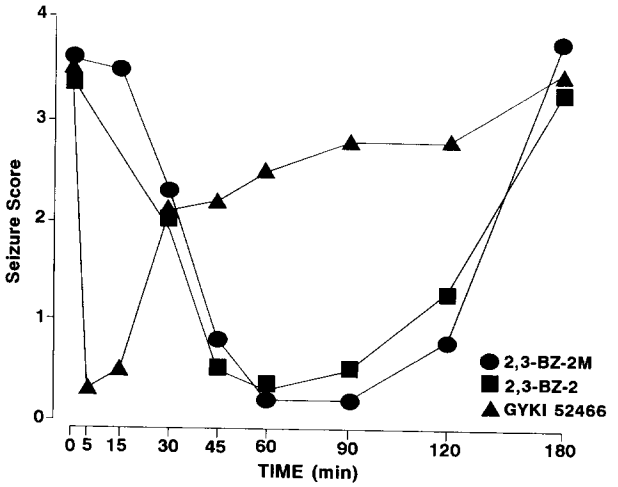


Fig. 2. Anticonvulsant effects of GYKI 52466 (33 μmol/kg i.p.), 2,3-BZ-2 (33 μmol/kg i.p.) and 2,3-BZ-2M (33 μmol/kg i.p.) against audiogenic seizures in DBA/2 mice. Ordinate shows seizure score, abscissa shows the time after intraperitoneal administration of drug in hours. For the determination of each point 10 animals were used.

Table 1
Molecular weight (MW) and relative lipophilicity (R_m) of 2,3-benzodiazepine derivatives

Compound	MW	R_m
GYKI 52466	329.79	−0.393
2,3-BZ-1	266.12	−0.181
2,3-BZ-2	296.15	−0.312
2,3-BZ-2M	310.17	−0.178
2,3-BZ-3	330.59	−0.156
2,3-OBZ-1	385.38	0.028
2,3-OBZ-2	415.41	−0.084
2,3-OBZ-3	449.85	0.061

All data are expressed as R_m values and were calculated according to the method previously described (Chimirri et al., 1989).

3.4. Anticonvulsant activity of 2,3-benzodiazepines against AMPA-induced seizures

Intracerebroventricular injections of AMPA (1–10 nmol/mouse) induced generalized seizures. In particular, hypermotility and circling preceded the first clonic episode (facial clonus, twitching of vibrissae and forelimb clonus) consisting of wild running, jumping and loss of righting; its latency was 1–3 min after the treatment with 5, 8 and 10 nmol and it could be longer for AMPA 1 and 3 nmol (up to 5 min). The tonic component of the seizures occurred at the highest doses tested and in some occasion was followed by death. AMPA exhibited a prolonged sequence of

seizures accompanied by rigid and splayed forelimbs or forelimb clonus or several episodes of rearing associated with forelimb clonus and falling down (limbic seizure). The CD_{50} of AMPA for clonus was 1.76 (1.06–3.07) whilst that for tonus was 2.90 (1.83–4.58) nmol/mouse.

As shown in Table 4 and Fig. 3, GYKI 52466, 2,3-BZ-2 and 2,3-BZ-2M reduced the incidence of both all-limb clonus and forelimb tonic extension seizures induced by the i.c.v administration of the respective CD_{97} of AMPA for clonus (9.72 nmol/10 μl) or forelimb tonic extension (11.73 nmol/10 μl). In particular, the clonic and tonic phases of the seizure induced by AMPA were significantly reduced 30 min after i.p. administration of GYKI 52466 (66 and 100 μmol/kg), 2,3-BZ-2 (100 and 200 μmol/kg) and 2,3-BZ-2M (100 and 200 μmol/kg). The relative ED_{50} values (with 95% confidence limits) are reported in Table 4. The highest doses of 2,3-benzodiazepines studied (i.e. GYKI 52466, 66 and 100 μmol/kg, 2,3-BZ-2, 200 μmol/kg, and 2,3-BZ-2M, 200 μmol/kg) induced in some animals ataxia, splayed hind limbs and tremor during the period of maximal anticonvulsant activity.

Table 2
The effect of some 2,3-benzodiazepine derivatives, GYKI 52446, 2,3-BZ-1, 2,3-BZ-2, 2,3-BZ-2M, 2,3-BZ-3, 2,3-OBZ-1, 2,3-OBZ-2 and 2,3-OBZ-3 on audiogenic seizures in DBA/2 mice

Compound	Dose (μmol/kg)	% Response				MSR ± IR
		WR	Clonus	Tonus	RA	
Vehicle		100	100	100	60	3 ± 1
GYKI 52446	3.3	100	100	100	60	3 ± 1
	10	100	90	80	40	3 ± 1
	21.5	80	80	60	20 [b]	2 ± 1
	33	60	50 [a]	50 [a]	0 [b]	1 ± 1 [a]
	50	40 [a]	40 [b]	30 [b]	0 [b]	1 ± 1 [a]
	66	30 [b]	20 [b]	0 [b]	0 [b]	0.5 ± 1 [b]
Vehicle		100	100	100	60	3 ± 1
2,3-BZ-1	3.3	100	80	80	20	3 ± 1
	66	80	70	70	40	3 ± 1
	100	40 [b]	40 [b]	20 [b]	0 [b]	1 ± 1 [a]
	200	0 [b]	0 [b]	0 [b]	0 [b]	0 ± 0 [b]
Vehicle		100	100	80	80	3 ± 1
2,3-BZ-2	10	100	100	100	20 [b]	3 ± 1
	20	90	90	80	20 [b]	3 ± 1
	33	80	50 [a]	40 [b]	20 [b]	2 ± 1
	50	50 [a]	20 [b]	20 [b]	20 [b]	1 ± 1 [a]
	66	40 [b]	10 [b]	10 [b]	20 [b]	1 ± 1 [a]
	100	0 [b]	0 [b]	0 [b]	0 [b]	0 ± 0 [b]
Vehicle		100	100	100	50	3 ± 1
2,3-BZ-2M	10	100	100	90	40	3 ± 1
	33	100	60	50 [a]	20 [b]	2 ± 1
	66	80	20 [b]	10 [b]	0 [b]	1 ± 1 [a]
	100	40 [b]	0 [b]	0 [b]	0 [b]	0.5 ± 1 [b]
Vehicle		100	100	100	60	3 ± 1
2,3-BZ-3	33	100	100	100	0 [b]	3 ± 0
	66	100	80	60	0 [b]	2 ± 1
	100	100	60	20 [b]	0 [b]	2 ± 1
	200	0 [b]	0 [b]	0 [b]	0 [b]	0 ± 0 [b]
Vehicle		100	100	100	50	3 ± 1
2,3-OBZ-1	10	100	100	100	60	3 ± 1
	33	100	100	100	60	3 ± 1
	66	100	60	60	20 [b]	2 ± 1
	100	80	50 [a]	40 [b]	20 [b]	2 ± 1
	200	70	40 [b]	30 [b]	0 [b]	1 ± 1 [a]
Vehicle		100	100	100	50	3 ± 1
2,3-OBZ-2	33	100	100	100	40	3 ± 1
	66	100	90	80	20 [b]	3 ± 1
	100	90	70	50 [a]	0 [b]	2 ± 1
	200	40 [b]	30 [b]	20 [b]	0 [b]	1 ± 1 [a]
	300	10 [b]	10 [b]	0 [b]	0 [b]	0.5 ± 1 [b]
Vehicle		100	100	100	50	3 ± 1
2,3-OBZ-3	10	100	100	100	60	3 ± 1
	33	100	100	80	60	3 ± 1
	66	100	70	60	10 [b]	2 ± 1
	100	90	60	50 [a]	10 [b]	2 ± 1
	200	80	50 [a]	40 [b]	10 [b]	2 ± 1

Table 3
ED_{50} values (with 95% confidence limits) of various 2,3-benzodiazepines, GYKI 52446, 2,3-BZ-1, 2,3-BZ-2, 2,3-BZ-2M, 2,3-BZ-3, 2,3-OBZ-1, 2,3-OBZ-2 and 2,3-OBZ-3 against the clonic and tonic phases of the audiogenic seizures after 30 min pretreatment

Compound	Clonic phase	Tonic phase
GYKI 52466	35.8 (24.4– 52.4)	25.3 (16.0– 40.0)
2,3-BZ-1	75.5 (47.5–120.1)	64.8 (45.0– 93.3)
2,3-BZ-2	33.9 (26.0– 44.2)	31.8 (24.8– 40.6)
2,3-BZ-2M	37.8 (23.7– 60.1)	26.7 (14.7– 48.2)
2,3-BZ-3	102.3 (76.1–137.4)	75.3 (60.7– 93.2)
2,3-OBZ-1	119.7 (68.2–210.0)	100.2 (63.4–158.3)
2,3-OBZ-2	141.5 (100.8–198.6)	112.3 (83.8–150.5)
2,3-OBZ-3	161.3 (88.9–292.8)	111.4 (63.4–195.9)

All data are expressed as μmol/kg and were calculated according to the method of Litchfield and Wilcoxon (1949).

3.5. Pretreatment with aniracetam

The intracerebroventricular (i.c.v.) injection of aniracetam on it own (12.5–100 nmol/mouse) had no convulsant activity, but aniracetam (50 nmol/mouse i.c.v.), 60 min before testing, reversed the anticonvulsant effects of some 2,3-benzodiazepine derivatives in DBA/2 mice. As reported in Table 5, the previous (30 min before) administration of aniracetam (50

Notes to Table 2
Groups of DBA/2 mice ($n = 10$/dose) were injected intraperitoneally with the stated doses of the drugs or vehicle (50% dimethyl sulphoxide and 50% saline) and exposed to auditory stimulation 30 min after drug injection. Incidence of each seizure phase is expressed as the percentage of mice in each group displaying that phase. Significant differences in the incidence of seizure phases between concurrent control and drug-treated group are denoted by [a] $P < 0.05$; [b] $P < 0.01$. WR = wild running; RA = respiratory arrest; MSR ± IR = median seizure score ± interquartile range (see Materials and methods for grading). Significant differences of MSR ± IR from the concurrent group treated with vehicle or drug were analysed using the Mann-Whitney U-test and denoted by [a] $P < 0.05$; [b] $P < 0.01$.

Table 4
ED_{50} (95% confidence limits) of GYKI 52466, 2,3-BZ-2 and 2,3-BZ-2M against seizures induced by i.c.v. injection of AMPA in DBA/2 mice

Treatment	Range dose (μmol/kg)	Clonic seizures	Tonic extension
GYKI 52466 + AMPA	10–100	57.5 (43.5– 76.0)	40.5 (26.3– 60.8)
2,3-BZ-2 + AMPA	10–200	66.0 (45.9– 94.9)	42.6 (26.4– 68.8)
2,3-BZ-2M + AMPA	10–200	76.1 (47.5–122.1)	69.6 (44.1–110.1)

AMPA was administered i.c.v. at the CD_{97} for either clonus or forelimb tonic extension 30 min after compound injection. All data were calculated according to the method of Litchfield and Wilcoxon (1949).

Table 5
The effect of aniracetam + GYKI 52446, 2,3-BZ-2 or 2,3-BZ-2M, on audiogenic seizures in DBA/2 mice

Treatment	Dose (μmol/kg)	% Response				MSR ± IR
		WR	Clonus	Tonus	RA	
Vehicle		100	100	100	60	3 ± 1
Aniracetam + vehicle		100	100	100	60	3 ± 1
Aniracetam + GYKI 52466	10	100	100	100	60	3 ± 1
	33	100	100 [a]	100 [a]	60	3 ± 1
	66	100 [b]	80 [a]	60 [b]	20	2 ± 1 [a]
	100	80	50	40	20	2 ± 1
	200	70	40	30	0	2 ± 1
Aniracetam + 2,3-BZ-2	33	100	100 [a]	100 [b]	60	3 ± 1
	66	100 [b]	100 [b]	100 [b]	60	3 ± 1
	100	90 [b]	80 [b]	70 [b]	40	3 ± 1 [a]
	200	80	60	40	20	2 ± 1
	300	50	30	10	0	1 ± 1
Aniracetam + 2,3-BZ-2M	33	100	100	100 [c]	40	3 ± 1
	66	100	100 [b]	100 [b]	40	3 ± 1
	100	100 [b]	90 [b]	80 [b]	20	3 ± 1
	200	70	70	60	10	2 ± 1 [a]
	300	50	40	20	0	1 ± 1

Groups of DBA/2 mice ($n = 10$/dose) were injected i.c.v. with aniracetam (50 nmol/mouse) and 30 min later with the stated doses of the 2,3-benzodiazepines and exposed to auditory stimulation 30 min after the last drug injection. Incidence of each seizure phase is expressed as the percentage of mice in each group displaing that phase. Significant differences in the incidence of seizure phases between concurrent control (see Table 2) and drug-treated group are denoted by [a] $P < 0.05$; [b] $P < 0.01$. WR = wild running; RA = respiratory arrest; MSR ± IR = median seizure score ± interquartile range (see Materials and methods for grading). Significant differences of MSR ± IR from the concurrent group treated with 2,3-benzodiazepines alone or aniracetam + 2,3-benzodiazepines were analysed using the Mann-Whitney *U*-test and denoted by [a] $P < 0.05$; [b] $P < 0.01$.

nmol/mouse i.c.v.) reduced the anticonvulsant properties of GYKI 52466, 2,3-BZ-2 and 2,3-BZ-2M. Pretreatment with aniracetam shifted the dose-response curve for 2,3-benzodiazepines to the right (Fig. 4). The ED_{50} values (with 95% confidence limits) for the combined aniracetam + 2,3-benzodiazepines treatment in-

Table 6
ED_{50} values (with 95% confidence limits) of some 2,3-benzodiazepines, GYKI 52446, 2,3-BZ-2 and 2,3-BZ-2M against the clonic and tonic phases of the audiogenic seizures after a pretreatment with aniracetam or flumazenil

Compound	Clonic phase	Tonic phase
GYKI 52466	35.8 (24.4– 52.4)	25.3 (16.0 – 40.0)
2,3-BZ-2	33.9 (25.9– 44.2)	31.8 (24.84– 40.6)
2,3-BZ-2M	37.8 (23.7– 60.1)	26.7 (14.7 – 48.2)
Aniracetam + GYKI 52466	134.4 (88.8–203.6) [a]	100.2 (63.4 –158.3) [a]
Aniracetam + 2,3-BZ-2	214.8 (142.2–324.3) [a]	157.5 (114.0 –217.6) [a]
Aniracetam + 2,3-BZ-2M	258.8 (176.0–380.5) [a]	196.9 (137.9 –281.3) [a]
Flumazenil (8.24 μmol) + GYKI 52466	37.8 (23.7– 60.1)	26.7 (14.7 – 48.2)
Flumazenil (24.72 μmol) + GYKI 52466	39.5 (29.6– 53.7)	29.7 (20.9 – 42.1)
Flumazenil (8.24 μmol) + 2,3-BZ-2	38.2 (28.5– 51.2)	35.8 (26.2 – 49.0)
Flumazenil (24.72 μmol) + 2,3-BZ-2	50.9 (36.9– 70.1)	41.3 (28.0 – 60.7)

All data are expressed as μmol/kg and were calculated according to the method of Litchfield and Wilcoxon (1949). Significant differences between ED_{50} values of group treated with aniracetam + 2,3-benzodiazepine derivatives and group treated with 2,3-benzodiazepine derivatives alone are denoted: [a] $P < 0.01$.

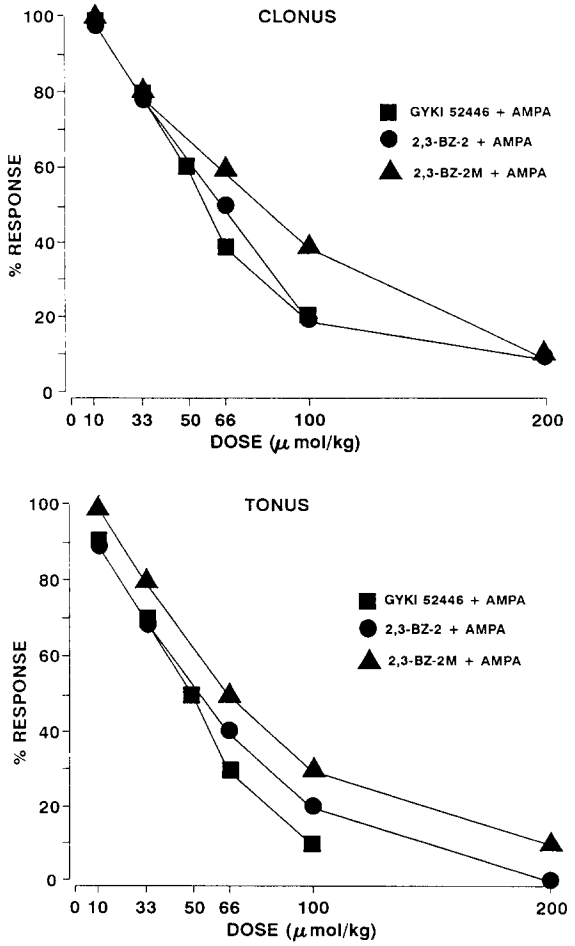


Fig. 3. Anticonvulsant effects of GYKI 52466, 2,3-BZ-2 and 2,3-BZ-2M against seizures induced by AMPA in DBA/2 mice. Ordinate shows percentage of response of clonic (top) or tonic (bottom) seizures, abscissa shows the dose in μmol/kg i.p. For the determination of each point 10 animals were used.

creased from 5.1 to 6.6 times (Table 6). The highest doses of 2,3-benzodiazepines studied (i.e. GYKI 52466, 66, 100 and 200 μmol/kg, 2,3-BZ-2, 200 and 300 μmol/kg, and 2,3-BZ-2M, 200 and 300 μmol/kg) induced in some animals ataxia, splayed hind limbs and tremor during the period of maximal anticonvulsant activity.

3.6. Treatment with flumazenil

As previously demonstrated flumazenil, administered i.p. at 8.24 or 24.72 μmol/kg, is not in itself convulsant (Chapman et al., 1987a; De Sarro et al., 1987). In particular, flumazenil (8.24 or 24.72 μmol/kg i.p.) did not significantly modify the phases of the audiogenic seizure response in DBA/2 mice. In order to ascertain the possible involvement of benzodiazepine receptors in the antiseizure activity of 2,3-benzodiazepines, the most active compounds (i.e. GYKI 52466 and 2,3-BZ-2) were administered concomitantly with flumazenil. In particular, the administration of flumazenil (8.24 or 24.72 μmol/kg i.p.) was unable to significantly suppress the antiseizure effects of compounds GYKI 52466 and 2,3-BZ-2; the ED_{50} values against the various phases of audiogenic seizures are reported in Table 6.

3.7. Effects on motor movements

Table 7 shows the TD_{50} values (with 95% confidence limits) obtained 30 min following i.p. administration of various 2,3-benzodiazepines. As can be seen, the therapeutic indices (TI) of the present compounds were similar to that of GYKI 52466 with the exception of 2,3-BZ-2 and 2,3-BZ-2M which were approximately twice less potent than GYKI 52466 in affecting both the rotarod test and the TI (Table 7).

3.8. Inhibition of [^{3}H]flumazenil binding

The potency of various 2,3-benzodiazepine and oxadiazolo-2,3-benzodiazepine derivatives as inhibitors of

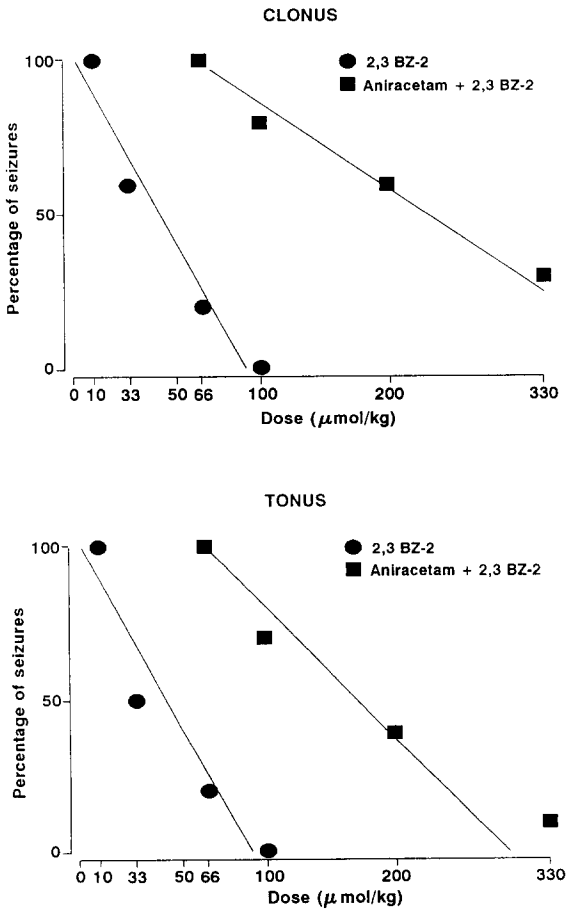


Fig. 4. Antagonism by aniracetam (50 nmol/mouse i.c.v.) of the anticonvulsant effects of 2,3-BZ-2 against audiogenic seizures in DBA/2 mice. Ordinate shows percentage of response. Abscissa shows the dose in μmol/kg i.p. For the determination of each point 10 animals were used.

Table 7
ED_{50} and TD_{50} values (with 95% confidence limits) of various 2,3-benzodiazepines, GYKI 52446, 2,3-BZ-1, 2,3-BZ-2, 2,3-BZ-2M, 2,3-BZ-3, 2,3-OBZ-1, 2,3-OBZ-2 and 2,3-OBZ-3 against the clonic phase of the audiogenic seizures and on motor movements obtained with the rotarod after 30 min pretreatment

Compound	ED_{50} Clonic phase	TD_{50} Rotarod	TI = TD_{50}/ED_{50}
GYKI 52466	35.8 (24.4– 52.4)	76.1 (47.5–122)	2.1
2,3-BZ-1	75.5 (47.5–120.1)	197 (138 –281)	2.6
2,3-BZ-2	33.9 (26.0– 44.2)	142 (87.3–231)	4.2
2,3-BZ-2M	37.8 (23.7– 60.1)	154 (104 –227)	4.1
2,3-BZ-3	102.3 (76.1–137.4)	240 (102 –564)	2.3
2,3-OBZ-1	119.7 (68.2–210.0)	271 (157 –469)	2.3
2,3-OBZ-2	141.5 (100.8–198.6)	289 (186 –448)	2.0
2,3-OBZ-3	161.3 (88.9–292.8)	291 (238 –356)	1.8

All data are expressed as μmol/kg and were calculated according to the method of Litchfield and Wilcoxon (1949). TI = therapeutic index represents the ratio between TD_{50} and ED_{50} (from the clonic phase of the audiogenic seizures).

specific [^{3}H]flumazenil binding to membranes from cortex was evaluated and no inhibition was observed ($IC_{50} > 10000$ nM).

4. Discussion

Tofisopam and GYKI 52466 (Fig. 1) are 2,3-benzodiazepines which differ pharmacologically from conventional 1,4-benzodiazepines, such as diazepam, in that they lack sedative-hypnotic activity, and do not bind to benzodiazepine receptors (Pellow and File, 1986; Tarnawa et al., 1989, 1990). In addition, GYKI 52466 showed anticonvulsant and muscle-relaxant properties and proved to be a highly selective AMPA/kainate receptor antagonist; it does not affect NMDA, metabolotropic glutamate or $GABA_A$ receptor-mediated responses (Tarnawa et al., 1989, 1990; Ouardouz and Durand, 1991; Donevan and Rogawski, 1993; Ornstein et al., 1993; Rogawski, 1993; Yamaguchi et al., 1993; Zorumski et al., 1993).

The recent availability of centrally active antagonists of non-NMDA (AMPA/kainate) receptors has made possible to evaluate the potential of such compounds as antiepileptic agents in animal seizure models. Several previous studies have indicated that the systemic administration of AMPA receptor antagonists to experimental animals produces anticonvulsant activity (Bisaga et al., 1993; Chapman et al., 1991,1993; Honoré et al., 1988,1989; Yamaguchi and Rogawski, 1992), and the present results confirm these effects. In addition, the audiogenic seizure test with DBA/2 mice has been found to be very sensitive and workable in order to distinguish between compounds acting as antagonists of AMPA or other excitatory amino acid receptors (Chapman and Meldrum, 1987,1989; Chapman et al., 1987b, 1990, 1993; De Sarro et al., 1994a,b). The present results show that 2,3-benzodiazepine devivatives have marked anticonvulsant activity but are generally less potent than the GYKI 52466, except derivatives 2,3-BZ-2, the most active compound of our new series, and 2,3-BZ-2M, which showed comparable activity (Table 3).

The structure-activity relationships in this series were examined by four types of structural changes to test the influence of physico-chemical parameters on anticonvulsant activity. One or two methoxy groups were introduced on the benzene fused ring; compounds with no substituents or with a chlorine atom on the phenyl ring at C-1 were considered; NH at 3 position was substituted with a methyl group and finally an oxadiazole nucleus was added to C=N moiety of 2,3-benzodiazepine precursors.

The effects of the changes introduced in modifying the anticonvulsant potency of the studied compounds are shown in Tables 2 and 3.

The presence of two methoxy groups on the benzene fused ring increases the anticonvulsant activity of the compounds under study: in fact, 2,3-BZ-2 is more active than 2,3-BZ-1 and shows an anticonvulsant activity comparable to that of GYKI 52466.

The introduction of a chlorine atom on the phenyl ring at C-1 (2,3-BZ-3) negatively influences the activity which appreciably decreases also when an oxadiazole nucleus (2,3-OBZ-1, 2,3-OBZ-2, and 2,3-OBZ-3) was added to the heptatomic ring.

The variant degree of anticonvulsant activity exhibited by these compounds could be partially correlated to their relative lipophilicity (Table 1). In fact, 2,3-BZ-2 with lipophilicity similar to GYKI 52466 is the most active compound of the series, whereas all the other derivatives, more lipophilic than 2,3-BZ-2, are less active.

Unexpectedly, the presence of a methyl group at N-3 present in 2,3-BZ-2M, which increases the lipophilicity with respect to 2,3-BZ-2, but does not significatively influence the anticonvulsant activity which is comparable with that of 2,3-BZ-2 and GYKI 52466. This behaviour can be explained considering 2,3-BZ-2M as a pro-drug which undergoes biotransformation in 2,3-BZ-2 by loss of the methyl group.

This hypothesis was confirmed when the time course of 2,3-BZ-2M, 2,3-BZ-2 and GYKI 52466 was studied (Fig. 2). The present results show that the anticonvulsant potency of 2,3-BZ-2M is similar to that of 2,3-BZ-2. When anticonvulsant activity was assessed 15 min after treatment with GYKI 52466 (Chapman et al., 1991), 2,3-BZ-2 and 2,3-BZ-2M (unpublished results), GYKI 52466 demonstrated a higher anticonvulsant potency perhaps due to higher capacity to cross the blood-brain barrier or to interact with AMPA receptors. It is interesting to note that 2,3-BZ-2 and 2,3-BZ-2M show a longer time course than GYKI 52466.

The minor anticonvulsant potency of 2,3-OBZ-2 in comparison to 2,3-BZ-2 (Table 3) is principally due to the presence of a new fused heterocyclic nucleus which perhaps reduces the active interaction with anticonvulsant receptors as previously reported by De Sarro et al. (1992). The time course of the anticonvulsant activity of 2,3-OBZ-2 demonstrated that this compound showed the maximal activity at 30 min and 60 min but even at these times it was much less active than the related 2,3-BZ-2 (De Sarro et al., unpublished results).

Therefore, we may consider that the different anticonvulsant potency of 2,3-benzodiazepines showing a similar structure could be due to a different transport by diffusion through the blood-brain barrier that provides an easier access to the central nervous system for some more active compounds or their metabolites.

The present data support the hypothesis that some 2,3-benzodiazepines interact with non-NMDA receptors. Specifically, the drugs have been found to afford effective protection against seizures induced by AMPA, a chemical agent which stimulates the AMPA/kainate receptor complex. Recent studies demonstrate that convulsions induced directly by AMPA itself are potently prevented by 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo(*f*)quinoxaline (NBQX) and GYKI 52466 (Chapman et al., 1991; De Sarro et al., 1994a). Interestingly, both drugs were able to prevent tonic extension seizures and clonus with a pattern of activity similar to that shown by NBQX, a specific antagonist of the AMPA/kainate receptors (Chapman et al., 1991; Bisaga et al., 1993; Yamaguchi et al., 1993; De Sarro et al., 1994a). In the present study we also demonstrated that aniracetam markedly antagonized the anticonvulsant effects of 2,3-BZ-2 and 2,3-BZ-2M in DBA/2 mice with a pattern of activity similar to GYKI 52466 and NBQX (Chapman et al., 1993; De Sarro et al., 1994a). However, the mechanism by which 2,3-benzodiazepines interact functionally with the AMPA/kainate receptor remains unclarified. Since NBQX is considered a competitive AMPA/kainate receptor antagonist (Honoré et al., 1988,1989; Chapman et al., 1991, 1993; Smith et al., 1991) we may suggest that 2,3-benzodiazepines act as antagonists of non-NMDA receptors. It has been shown that aniracetam is a positive allosteric modulator of AMPA/kainate-selective glutamate receptors by reducing the rate of rapid receptor autodesensitization (Ito et al., 1990) and it reverses the anticonvulsant properties of some 2,3-benzodiazepines; therefore we may suggest that GYKI 52466 and the present 2,3-benzodiazepines seem to act as non-competitive antagonists and to antagonize the AMPA/kainate receptor-mediated responses by an allosteric blocking mechanism as previously suggested (Donevan and Rogawski, 1993; Rogawski, 1993; Donevan et al., 1994).

The anticonvulsant activity of these compounds was evident at dose levels which usually did not affect sedation, ataxia and fall of the body temperature. It has long been known that antagonists of the excitatory amino acid receptors, expecially those which block ion channels, e.g. MK-801, may induce cognitive deficits and a variety of other neurological and behavioural side effects (Chapman and Meldrum, 1987, 1989; McEntee and Crook, 1993; Rogawski, 1993; De Sarro and De Sarro, 1992, 1993). There are, however, studies showing that potent antagonists at the AMPA/kainate receptor have anticonvulsant effects at doses below those impairing behaviour (Chiamulera et al., 1990; Löscher et al., 1993). This profile of 2,3-benzodiazepines is an interesting case for the research effort which is being made to discover new drugs interacting with excitatory amino acid receptors and possessing therapeutical potential with lower side effects (Meldrum and Garthwaite, 1990; Rogawski, 1992, 1993). The therapeutic indices (TI) of the present compounds were similar to that of GYKI 52466 with the exception of 2,3-BZ-2 and 2,3-BZ-2M which were approximately twice less potent than GYKI 52466 in affecting both the rotarod test and the TI (Table 7). Since the anticonvulsant effects of 2,3 BZ-2 and GYKI 52466 were not reversed by flumazenil under an identical dose regimen, it seems likely that the actions of these derivatives are not mediated by benzodiazepine receptor sites. The latter consideration derives also from the following observations: (a) the amounts of 2,3-benzodiazepines used in this study have no significant effects on GABA/benzodiazepine receptor complex; (b) 2,3-benzodiazepines showed a good anticonvulsant activity against AMPA; (c) the anticonvulsant effects are reversed by aniracetam but not by flumazenil. Since AMPA/kainate receptor antagonists have been claimed to be more effective than NMDA receptor antagonists in reducing damage in models of global ischaemia (Buchan et al., 1993), the present data would support the suggestion that the neuroprotective effects of 2,3-benzodiazepines are more closely related to its interactions with the AMPA/kainate receptors.

Despite their lack of activity at dopamine, serotonin and noradrenaline binding sites (data not shown) an interaction at other sites involved in the generation or expression of seizures may not be ruled out.

In conclusion, the anticonvulsant activity of the 2,3-benzodiazepines in this genetic model of epilepsy suggests that these compounds and their metabolites merit further study.

Acknowledgements

Financial support from the Italian Ministry of University and Research Techonology (MURST, Rome) is gratefully acknowledged.

References

Bell, S.C., T.S. Sulkowski, C. Gochman and S.J. Childress, 1962, 1,3-Dihydro-2*H*-1,4-benzodiazepine-2-ones and their 4-oxides, J. Org. Chem. 27, 562.

Bisaga, A., P. Krzascik, E. Jankowska, W. Palejko, W. Kostowski and W. Danysz, 1993, Effect of glutamate antagonists on NMDA- and AMPA-induced convulsant effects in mice and rats, Eur. J. Pharmacol. 242, 231.

Buchan, A.M., H. Lesiuk, K.A. Barnes, H. Li, Z-G. Huang, K.E. Smith and D. Xue, 1993, AMPA antagonists: do they hold more promise for clinical stroke trials than NMDA antagonists?, Stroke 24 (Suppl. 1), 148.

Chapman, A.G. and B.S. Meldrum, 1987, Epilepsy prone mice: genetically determined sound-induced seizures, in: Neurotransmitters and Epilepsy, eds. P.C. Jobe and H.E. Laird (The Humana Press, Clifton, NJ) p. 197.

Chapman, A.G. and B.S. Meldrum, 1989, Non-competitive *N*-methyl-D-aspartate antagonists protect against sound-induced seizures in DBA/2 mice, Eur. J. Pharmacol. 166, 201.

Chapman, A.G., M.J. Croucher and B.S. Meldrum, 1984, Evaluation of anticonvulsant drugs in DBA/2 mice with sound-induced seizures, Arzneim.-Forsch. 34, 1261.

Chapman, A.G., G.B. De Sarro, M. Premachandra and B.S. Meldrum, 1987a, Bidirectional effects of β-carbolines in reflex epilepsy, Brain Res. Bull. 19, 337.

Chapman, A.G., B.S. Meldrum, N. Nanji and J.C. Watkins, 1987b, Anticonvulsant action and biochemical effects in DBA/2 mice of CPP (3-((+)-2-carboxypiperazin-4-yl)propyl-1-phosphonate), a novel *N*-methyl-D-aspartate antagonist, Eur. J. Pharmacol. 139, 91.

Chapman, A.G., J.L. Graham and B.S. Meldrum, 1990, Potent oral anticonvulsant action of CPP and CPPene in DBA/2 mice, Eur. J. Pharmacol. 178, 97.

Chapman, A.G., S.E. Smith and B.S. Meldrum, 1991, The anticonvulsant effect of the non-NMDA antagonists, NBQX and GYKI 52466 in mice, Epilepsy Res. 9, 92.

Chapman, A.G., Z. Al-Zubaidy and B.S. Meldrum, 1993, Aniracetam reverses the anticonvulsant action of NBQX and GYKI 52466 in DBA/2 mice, Eur. J. Pharmacol. 231, 301.

Chiamulera, C., S. Costa and A. Reggiani, 1990, Effect of NMDA- and strychnine-insensitive glycine site antagonists on NMDA-mediated convulsions and learning, Psychopharmacology 102, 551.

Chimirri, A., A. De Sarro, G.B. De Sarro, S. Grasso, G.R. Trimarchi and M. Zappalà, 1989, Synthesis and anticonvulsant properties of 2,3,3*a*,4-tetrahydro-1*H*-pyrrolo-[1,2-*a*]benzimidazol-1-ones, J. Med. Chem. 32, 93.

Collins, R.L., 1972, Audiogenic seizures, in: Experimental Models of Epilepsy, eds. P. Purpura, J.K Penry, D. Tower, D.M. Woodbury and R. Walter (Raven Press, New York) p. 347.

De Sarro, G.B. and A. De Sarro, 1992, Anticonvulsant activity of competitive antagonists of NMDA receptor in genetically epilepsy prone rats, Eur. J. Pharmacol. 215, 221.

De Sarro, G.B. and A. De Sarro, 1993, Anticonvulsant properties of non-competitive antagonists of NMDA receptor in genetically epilepsy prone rats. Comparison with CPPene, Neuropharmacology 32, 51.

De Sarro, G.B., M.J. Croucher and B.S. Meldrum, 1984, Anticonvulsant actions of DS 103-282: pharmacological studies in rodents and the baboon *Papio papio*, Neuropharmacology 23, 526.

De Sarro, G.B., V. Libri, C. Ascioti, R. Testa and G. Nisticò, 1987, Anticonvulsant effect of denzimol in DBA/2 mice, Neuropharmacology 26, 1425.

De Sarro, G.B., M. Zappalà, S. Grasso, A. Chimirri, C. Spagnolo and A. De Sarro, 1992, Molecular requirement for anticonvulsant activity in a series of 1,5-benzodiazepines and (1,2,4)oxadiazolo(4,5-*a*)(1,5)benzodiazepine derivatives, Mol. Neuropharmacol. 1, 195.

De Sarro, G.B., E. Ongini, R. Bertorelli, U. Aguglia and A. De Sarro, 1994a, 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. 262, 11.

De Sarro, G.B., S. Renne, F. Nava and A. De Sarro, 1994b, Fenbufen pretreatment potentiates the anticonvulsant activity of CPPene and NBQX in DBA/2 mice, J. Pharmac. Pharmacol. 46, 1017.

Donevan, S.D. and M.A. Rogawski, 1993, GYKI 52466, a 2,3-benzodiazepine, is a highly selective, noncompetitive antagonist of AMPA/kainate receptor responses, Neuron 10, 51.

Donevan, S.D., S.I. Yamaguchi and M.A. Rogawski, 1994, Non-*N*-methyl-D-aspartate receptor antagonism by 3-*N*-substituted 2,3-benzodiazepines: relationship to anticonvusant activity, J. Pharmacol. Exp. Ther. 271, 25.

Dunham, N.W. and T.S. Miya, 1957, A note on a simple apparatus for detecting neurological deficit in rats and mice, J. Am. Pharm. Ass. 46, 208.

Engstrom, F.L. and D.M. Woodbury, 1988, Seizure susceptibility in DBA/2 and C57 mice: the effects of various convulsants, Epilepsia 29, 389.

Gatta, F., D. Piazza, M.R. Del Giudice and M. Massotti, 1985, Derivatives of 2,3-benzodiazepines, Il Farmaco Ed. Sc. 40, 542.

Goldberg, H.L and R.J. Finnerty, 1979, Comparative efficacy of tofisopam and placebo, Am. J. Psychiatry 136, 196.

Hall, C.S., 1947, Genetic differences in fatal audiogenic seizures between two inbred strains of house mice, J. Hered. 38, 2.

Harvey, S.C., 1985, Hypnotic and sedatives, in: The Pharmacological Basis of Therapeutics, eds. A.G. Gilman, L.S. Goodman, T.W. Rall and F. Murod (MacMillan, New York) p. 339.

Honoré, T., S.N. Davies, J. Drejer, E.J. Fletcher, P. Jacobsen, D. Lodge and F.E. Nielsen, 1988, Quinoxalinediones: potent competitive non-NMDA glutamate receptor antagonists, Science 241, 701.

Honoré, T., J. Drejer, F.E. Nielsen and M. Nielsen, 1989, Non-NMDA glutamate receptor antagonist 3H-CQX binds with equal affinity to two agonist states of quisqualate receptor, Biochem. Pharmacol. 38, 3207.

Ito, I., S. Tanabe, A. Kohda and H. Sugiyama, 1990, Allosteric potentiation of quisqualate receptors by nootropic drug aniracetam, J. Physiol. (London) 424, 533.

Litchfield, J.T. and F. Wilcoxon, 1949, A simplified method of evaluating dose-effects experiments, J. Pharmacol. Exp. Ther. 96, 99.

Löscher, W.R., R. Annies and D. Honack, 1993, Comparison of competitive and uncompetitive NMDA receptor antagonist with regard to monoaminergic neuronal activity and behavioural effects in rats, Eur. J. Pharmacol. 242, 263.

McEntee, W.C. and T.H. Crook, 1993, Glutamate, its role in learning, memory, and the aging brain, Psychoparmacology 111, 391.

McPherson, G.I., 1987, LIGAND (release 2.0) (Elsevier Biosoft, Cambridge).

Meldrum, B. and J. Garthwaite, 1990, Excitatory amino acid neurotoxicity and neurodegenerative disease, Trends Pharmacol. Sci. 11, 379.

Ornstein, P.L., M.B. Arnold, N.K. Augustein, D. Lodge, J.D. Leander and D.D. Schoepp, 1993, (3*SR*,4a*RS*,6*RS*,8*aRS*-6-(2-(1*H*-Tetrazol-5-yl)ethyl)-decahydro-isoquinoline-3-carboxylic acid: a structurally novel, systemically active, competitive AMPA receptor antagonist, J. Med. Chem. 36, 2046.

Ouardouz, M. and J. Durand, 1991, GYKI 52466 antagonizes glutamate responses but not NMDA and kainate responses in rat abducens motorneurones, Neurosci. Lett. 125, 5.

Pellow, S. and S.E. File, 1986, Is tofisopam an atypical anxiolytic?, Neurosci. Biobehav. Rev. 10, 221.

Rogawski, M.A., 1992, The NMDA receptor, NMDA antagonists and epilepsy therapy, Drugs 44, 279.

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

Seyfried, T.N. and G.H. Glaser, 1985, A review of mouse mutans as genetic models of epilepsy, Epilepsia 26, 143.

Smith, S.E. and B.S. Meldrum, 1990, Receptor site specificity for the acute effects of beta-*N*-methylamino-alanine in mice, Eur. J. Pharmacol. 187, 131.

Smith, S.E., N. Durmuller and B.S. Meldrum, 1991, The non-*N*-methyl-D-aspartate receptor antagonists, GYKI 52466 and NBQX are anticonvulsant in two animal models of reflex epilepsy, Eur. J. Pharmacol. 201, 179.

Sternbach, L.H., L.O. Randall, R. Banzinger and H. Lehr, 1968, Structure-activity relationships in the 1,4-benzodiazepine series, in: Drugs Affecting the Central Nervous System, ed. A. Burger (Marcel Dekker, New York) p. 237.

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

Tarnawa, I., I. Engberg and J.A. Flatman, 1990, GYKI 52466, an inhibitor of spinal reflexes is a potent quisqualate antagonist, in: Amino Acids: Chemistry, Biology and Medicine, eds. G. Lubec and G.A. Rosenthal (Escom, Leiden) p. 538.

Villar, H.O., M.F. Davies, G.H. Loew and P.A. Maguire, 1991, Molecular models for recognition and activation at the benzodiazepine receptor: a review, Life Sci. 48, 593.

Yamaguchi, S. and M. Rogawski, 1992, Effects of anticonvulsant drugs on 4-aminopyridine-induced seizures in mice, Epilepsy Res. 11, 9.

Yamaguchi, S., S.D. Donevan and M.A. Rogawski, 1993, Anticonvulsant activity of AMPA/kainate antagonists – comparison of GYKI 52466 and NBQX in maximal electroshock and chemoconvulsant seizure models, Epilepsy Res. 15, 179.

Zorumski, C.F., K.A. Yamada, M.T. Price and J.M. Olney, 1993, A benzodiazepine recognition site associated with the non-NMDA glutamate receptor, Neuron 10, 61.