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# Anticonvulsant Activity and Plasma Level of 2,3-Benzodiazepin-4-ones (CFMs) in Genetically Epilepsy-Prone Rats

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DE SARRO G., M. RIZZO, C. SPAGNOLO, R. GITTO, A. DE SARRO, G. SCOTTO, M. ZAPPALA AND A. CHIMIRRI. *Anticonvulsant activity and plasma level of 2,3-benzodiazepin-4-ones (CFMs) in genetically epilepsy-prone rats.* PHARMACOL BIOCHEM BEHAV **63** (4), 621–627, 1999.—Anticonvulsant properties of some 2,3-benzodiazepine derivatives acting as α-amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid (AMPA) antagonists have been examined in vivo in the genetically epilepsy-prone rats using an audiogenic seizures assay. 2,3-Benzodiazepin-4-ones (CFMs) are nonselective AMPA antagonists that have been found to be potent anticonvulsant compound is in acute models of epilepsy. Because very little is known about their actions in a chronic model of epilepsy, and no correlations exist between anticonvulsant potency and plasma levels of these derivatives, we planned to investigate such a relationship. Maximal anticonvulsant protection occurred 15–60 min after the IP administration of GYKI 52466, 30–90 min after CFM-2, and 45–120 min after CFM-3. In addition, maximal anticonvulsant effect was observed 60–120 min after the IP administration of CFM-4 and at 90 min after CFM-5. The therapeutic index revealed that GYKI 52466 was slightly more toxic than CFM-2 and CFM-3. The time course of plasma levels of rats treated showed that peak plasma concentration was observed 45 min after IP administration of CFM-2 and CFM-3 and 75 min after CFM-4 and CFM-5. Following IP administration of CFM-3 two curves were detected, one is referred to the injected compound, and the other to its demethylated metabolite, which corresponds to CFM-2. Also, for the nitroderivative CFM-4 two curves were detected: one of an injected compound and the second due to its reduced metabolite (CFM-2). Finally, three different metabolites were detected in rat plasma after IP administration of CFM-5. The present study demonstrated that CFMs showed a significant protection against auditory stimulation during the period of peak plasma concentrations, suggesting a marked inhibition of those brain structures involved in the initiation and/or spreading of the audiogenic seizures. © 1999 Elsevier Science Inc.

AMPA antagonists 2,3-Benzodiazepines Genetically epilepsy-prone rats Plasma level

GLUTAMATERGIC synaptic transmission in the central nervous (CNS) is mediated by different types of glutamate receptors and primarily by α-amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid (AMPA) receptor type (1). The discovery of AMPA receptor antagonists such as 6-nitro-7-sulfamoyl-benzo[f]quinoxaline-2,3-dione (NBQX) and 1-(4′-aminophenyl)-4-methyl-7,8-methylenedioxy-5H-2,3-benzodiazepine (GYKI 52466) have facilitated extensive studies of the physiological role of AMPA receptors in CNS. Several actions of selective AMPA receptor antagonists, for example, neuroprotective actions against ischemia (2,15,23,24,26), glutamate-induced neurotoxicity (30), and anti-Parkinson actions (18,29) have been reported. Systemic administration of 2,3-benzodiazepines was able to attenuate in a dose-dependent manner the audiogenic seizures in DBA/2 mice (4,5). Furthermore, 2,3-benzodiazepines antagonized in DBA/2 mice seizures induced by

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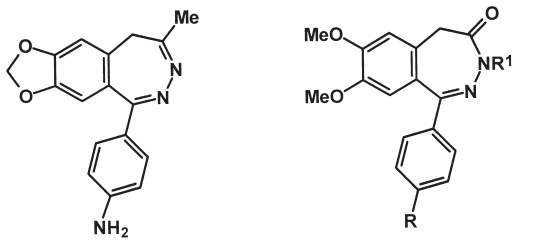


GYKI 52466

**CFM-2** R= $NH_2$ $R^1$ = H
**CFM-3** R= $NH_2$ $R^1$ = Me
**CFM-4** R= $NO_2$ $R^1$ = H
**CFM-5** R= $NO_2$ $R^1$ = Me

FIG. 1. Chemical structures of compounds studied.

AMPA, but not those induced by NMDA (3,6). Although the neurological activity of these AMPA receptor antagonists have been studied in acute models of epilepsy and neurodegenerative diseases in vivo and/or in vitro (3,5,10,16, 21,25,31), very little is known about their action in a chronic model of generalized epilepsy. In addition, no correlations exist between anticonvulsant potency and plasma levels of these 2,3-benzodiazepine derivatives.

The present study was aimed to determine the anticonvulsant activity profile of 1-(4′-aminophenyl)-3,5-dihydro-7,8-dimeth–oxy-4H-2,3-benzodiazepin-4-one (CFM-2) and its analogues CFM-3, CFM-4, CFM-5 (Fig. 1) in genetically epilepsy-prone rats, a strain widely used to demonstrate the anticonvulsant and proconvulsant properties of novel and conventional compounds (7–9,11–13,20,25). Moreover, we performed a HPLC study to explore a possible metabolic pathway of tested compounds and also to correlate plasma concentrations of these compounds with their anticonvulsant activity.

## METHOD

### *Animals*

Genetically epilepsy-prone rats (GEPR-9s), a strain derived from Sprague–Dawley rats were generously supplied by our breeding stock (Institute of Pharmacology, University of Messina) from a colony originally instituted at the Louisiana State University at Shreveport, LA, by Dr. P. C. Jobe, and obtained from Prof. B. S. Meldrum (University of London). Progenitors of this latter were raised at the University of Arizona (17), and named [UAZ: AGS (SD)]. The rats were housed three or four per cage in stable conditions of humidity (60 ± 5%) and temperature (22 ± 2°C), and allowed free access to food and water until the time of the experiments. Animals were maintained on a 12L:12D cycle (lights on 70.00–19.00 h, off 19.00–07.00 h). GEPR-9s were tested three times at weekly intervals between 6 and 8 weeks of their life, and only animals that showed an audiogenic seizure in all three exposures to sound stimulation were used for these experiments. The experimental protocol and all the procedures involving animals and their care were conducted in conformity with the

TABLE 1

EFFECTS OF GYKI 52466, CFM-2, CFM-3, CFM-4, AND CFM-5 ON SOUND-INDUCED SEIZURES IN GENETICALLY EPILEPSY-PRONE RATS

| Compound | Pretreatment Time (min) | Clonus (μmol/kg) | Tonus (μmol/kg) |
|---|---|---|---|
| GYKI 52466 | 15 | 42 (18–98) | 18 (10–32) |
| | 30 | 31 (16–60) | 16 (9–28) |
| | 60 | 65 (33–128) | 33 (15–73) |
| | 120 | ND | ND |
| CFM-2 | 15 | ND | ND |
| | 30 | 17.4 (8.6–35.2) | 6.5 (4.8–8.8) |
| | 60 | 13.8 (6.5–29.3) | 8.5 (4.7–15.4) |
| | 90 | 17.5 (8.5–36.03) | 10.4 (5.3 –20.4) |
| | 120 | 26.2 (14.3–48.1) | 15.3 (7.6–30.8) |
| CFM-3 | 15 | ND | ND |
| | 30 | 50.8 (28.2–91.4) | 26.5 (17.4–40.3) |
| | 60 | 33.8 (22.1–51.7) | 17.7 (10.8–29) |
| | 90 | 43.3 (20–53.2) | 27.8 (13–42.9) |
| | 120 | 50.8 (28.2–91.4) | 25.9 (14.1–47.6) |
| CFM-4 | 30 | ND | ND |
| | 60 | 75.8 (46.5–123.6) | 47.6 (34.7–65.3) |
| | 90 | 52.5 (34.6–79.66) | 31.2 (25.9–37.6) |
| | 120 | 76.2 (44.3–131.1) | 49.3 (37.6–64.6) |
| CFM-5 | 30 | ND | ND |
| | 60 | 93.6 (66.9–130.96) | 58.5 (41.6–82.3) |
| | 90 | 56.8 (39.3–82.1) | 38.4 (25.3–58.3) |
| | 120 | 82.6 (58.6–116.4) | 57.5 (43.6–75.8) |

Groups of 8–10 rats received drugs and the percentage of animals displaying clonic or tonic seizures were calculated. The table shows the $ED_{50}$ values (with 95% confidence limits) representing the dose of the compound that protected 50% of the rats from clonic or tonic component of the audiogenic seizures. All data are calculated according to the method of Litchfield and Wilcoxon (19), and are expressed as μmol/kg IP.

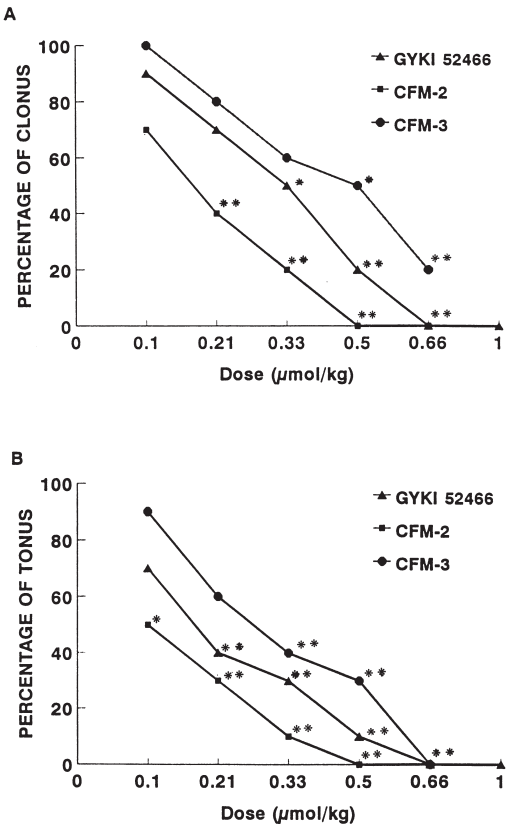


FIG. 2. Dose–response curves of the anticonvulsant activity of GYKI 52466, CFM-2, and CFM-3, against audiogenic seizures in genetically epilepsy-prone rats observed 30 min after drug administration. Ordinate shows seizure score, abscissa shows the dose expressed as μmol/kg IP. For the determination of each point six to eight animals were used. Significant differences are denoted as $*p < 0.05$ and $**p < 0.01$.

institutional guidelines and the European Council Directive of laws and policies.

*Anticonvulsant Activity*

Seizures were induced in GEPR-9s, 180–260 g, 12–18 weeks old, male ($n = 160$) by exposing them to a mixed frequency sound of 12–16 kHz, 109-dB intensity under a hemispheric Plexiglas dome (58-cm diameter). Individual animals were initially tested 10 min before sound stimulation for assessment of locomotor activity and then placed into the home cage for habituation and assessment of anticonvulsant activity. Auditory stimulation was applied for 60 s or until the onset of convulsions occurred. A full-seizure response (S.R.) consisted of one or two running phases, followed by a convulsion (clonus of forelimbs, hindlimbs, head, pinnae, vibrissae, and tail) and tonic extension to give a score of 9. In particular, the audiogenic seizure response was assessed on the following scale previously reported (7): 0 = no response; 1 = running only; 2 = one running phases, followed by a clonic convulsion (clonus of forelimbs, hindlimbs, head, pinnae, vibrissae, and tail); 3 = two running phases, followed by a clonic convulsion (clonus of forelimbs, hindlimbs, head, pinnae, vibrissae, and tail); 4 = two running phases followed by tonus of neck, trunk, and forelimb and hindlimb clonus; 5 = one running phase followed by tonus of neck, trunk, and forelimb and

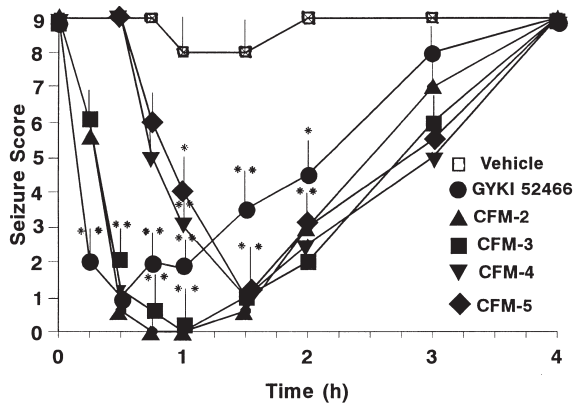


FIG. 3. Anticonvulsant effects observed after IP administration of GYKI 52466 (33 μmol/kg), CFM-2 (33 μmol/kg), CFM-3 (33 μmol/kg), CFM-4 (100 μmol/kg), and CFM-5 (100 μmol/kg) against audiogenic seizures in genetically epilepsy-prone rats. Ordinate shows seizure score, abscissa shows the time after intraperitolneal administration of drug in hours. For the determination of each point six to eight animals were used. Each point represents median seizure score and deviation of the median. Significant differences are denoted as $*p < 0.05$ and $**p < 0.01$.

hindlimb clonus; 6 = two running phases followed by nearly complete tonic extension except hindfeet; 7 = one running phase followed by nearly complete tonic extension except hindfeet; 8 = two running phases followed by complete tonic extension; 9 = one running phase followed by complete tonic extension. The maximum response was recorded for each animal. Behavioral changes were observed during the period between drug administration and auditory testing.

*Effects on Motor Movement*

GEPR-9s were trained just before anticonvulsant testing to do coordinated motor movements, continuously for 5 min on a rotarod 4 cm in diameter, at 4.5 rpm (U. Basile, Comerio, Varese, Italy). Impairment of coordinated motor movements was defined as the inability of the animals to remain on the rotarod for a test period of 5 min, according to Dunham and Miya (14).

*Determination of Plasma Levels of 2,3-Benzodiazepines*

2,3-Benzodiazepines plasma levels were analyzed by a high-performance liquid chromatography (HPLC) method as recently described by Rizzo et al. (22), and suitable modified. Briefly, the method included a single-step extraction and the use of internal standard (I.S.) for quantitation. The HPLC system consisted of a Beckman, System Gold 125 solvent module with a 20-μl loop injection valve and a variable wavelength ultraviolet 166 Detector set a 240 nm, and a Epson Endeavoir 4D×2/50 L integrator. A Partisil 10 ODS (25 cm × 4.6 mm i.d.) reverse-phase column was used with a ODS guard (4.5 cm × 4.6 mm). The column was eluted with $CH_3COONa$ 0.01 M/$CH_3CN$ (65:35 v/v) at the flow rate of 2 ml/min, at room temperature. Stock solution (1 mg/ml) of 2,3-benzodiazepines (GYKI 52466, CFM-2, CFM-3, CFM-4, and CFM-5) were done in acetonitrile. Working solutions were made by appropriate dilution with methanol, and used to prepare blood and aqueous standards. Under 4% chloral hydrate anesthesia, rats were decapitated, blood cells were removed by centrifugation, and the separated plasma was stored at −20°C until use. An aliquot of 0.5 ml of plasma was mixed

TABLE 2

EFFECTS OF GYKI 52466, CFM-2, CFM-3, CFM-4, AND CFM-5 ON ROTAROD PERFORMANCE IN GENETICALLY EPILEPSY-PRONE RATS

| Compound | Pretreatment Time (min) | $TD_{50}$ Locomotor Deficit (μmol/kg) | $TD_{50}/ED_{50}$ |
|---|---|---|---|
| GYKI 52466 | 15 | 97 (77–122) | 2.3 |
| | 30 | 68 (46–100) | 2.2 |
| | 60 | 149 (101–220) | 2.3 |
| CFM-2 | 30 | 57.4 (48–68.7) | 3.3 |
| | 60 | 46.9 (39.1–56.4) | 3.4 |
| | 120 | 94.3 (76.2–116.7) | 3.6 |
| CFM-3 | 30 | 157.5 (112–221.4) | 3.1 |
| | 60 | 108.2 (86–136.1) | 3.2 |
| | 120 | 167.6 (136.1–206.4) | 3.3 |
| CFM-4 | 60 | 214.9 (142.2–324.3) | 2.8 |
| | 90 | 141.7 (98.2–204.5) | 2.7 |
| | 120 | 205.6 (138.6–305.2) | 2.7 |
| CFM-5 | 60 | ND | ND |
| | 90 | 157.5 (114.03–217.6) | 2.8 |
| | 120 | ND | ND |

Groups of 8–10 rats received drugs and were tested on the rotarod for assessment of possible motor impairment. $TD_{50}$ (with 95% confidence limits) were calculated according to the method of Litchfield and Wilcoxon (19). TI, therapeutic index represents the ratio between $TD_{50}$ and $ED_{50}$ (from the clonic phase of the audiogenic seizures).

with 0.1 ml of NaOH 2 M and 0.1 ml of 3,5-dihydro-7,8-dimethoxy-3-methyl-1-phenyl-4H-2,3-benzodiazepin-4-one (22) (0.01 mg/ml,) as the internal standard was added. The sample was applied to Extrelut I (Merck, Germany), a prepacked glass column. After 10 min ethyl acetate (6 ml) was added to the column. The eluate was collected and evaporated to dryness under a stream of nitrogen. The residue was dissolved in 0.1 ml of the mobile phase, and was injected into the chromatographic system. The lower limit of detection was 15 ng/ml for GYKI 52466, 10 ng/ml for CFM-2, 12 ng/ml for CFM-3, 14 ng/ml for CFM-4, and 17 ng/ml for CFM-5. The sensitivity of the method allowed for easy quantitation of 20 ng/ml of these drugs in a 0.5-ml plasma sample.

### *Drugs*

CFM-2 [1-(4′-aminophenyl)-3,5-dihydro-7,8-dimethoxy-4H-2,3-benzodiazepin-4-one] now available by Tocris Cookson, CFM-3 [1-(4′-aminophenyl)-3,5-dihydro-7,8-dimethoxy-3-methyl-4H-2,3-benzodiazepin-4-one], CFM-4 [3,5-dihydro-7,8-dimethoxy-l-(4′-nitrophenyl)-4H-2,3-benzodiazepin-4-one], and CFM-5 [3,5-dihydro-7,8-dimethoxy-3-methyl-1-(4′-nitrophenyl)-4H-2,3-benzodiazepin-4-one] were synthesized in our laboratory as previously described (4), and dissolved in a solution containing 50% of dimethylsulfoxide and 50% of sterile saline. GYKI 52466 (Research Biomedicals, Natick, MA) was dissolved in sterile saline. For systemic administration, all compounds were administered intraperitoneally (IP) (0.4 ml/100 g of body weight of the rat), as a freshly ultrasonicated solution. At least six animals were used once for each dose level studied.

### *Statistical Analysis*

The effects of treatment were analyzed statistically, using nonparametric methods. A Kruskall–Wallis analysis of variance was first carried out, and if this was significant, a Mann–Whitney *U*-test was used to compare control and drug-treated animals. The percentage of animals exhibiting tonic extension (seizure response = 4–5) or clonic phase (seizure response = 2–3) of the audiogenic seizure was determined for each dose of compound administered, and these values were plotted against corresponding doses by a computer construction of the dose–effect curves for calculation of the $ED_{50}$ (with 95% confidence limits). Median neurotoxic dose ($TD_{50}$ with 95% confidence limits), the dose that made 50% of animals fall from the rotarod, was calculated as the $ED_{50}$. The $ED_{50}$ and $TD_{50}$ values for each compound were determined, using the method of Litchfield and Wilcoxon (19). At least 32 animals were used to calculate each $ED_{50}$ and $TD_{50}$ value. The values of blood samples determined by HPLC are expressed as means ± SEM.

## RESULTS

### *Anticonvulsant Properties of GYKI 52466*

We have previously described the anticonvulsant activity of GYKI 52466 in genetically epilepsy-prone rats (10). Briefly, this compound reduced the occurrence and the severity of audiogenic seizure phases in a dose-dependent manner. In particular, GYKI 52466 (33, 50, and 66 μmol/kg IP) was able to significantly ($Hs = 8.16$, $U = 38.5$, $p < 0.01$) reduce the median seizure scores and the incidence of clonic and tonic component of the audiogenic seizures (Table 1 and Fig. 2). The time-course studies revealed that GYKI 52466 (33 μmol/kg IP) showed the maximum activity from 15 to 60 min (Fig. 3). Lower doses of GYKI 52466 (10 and 25 μmol/kg IP) had a weak and not significant anticonvulsant effect. The $ED_{50}$ values (with 95% confidence limits) for suppression of clonic and tonic phases of the audiogenic seizures in genetically epilepsy-prone rats are reported in Table 1. The group of GEPR-9s receiving vehicle did not affect any component of the audiogenic seizures ($Hs < 0.124$, $p > 0.13$) (Fig. 3).

### *Anticonvulsant Properties of CFM-2 and CFM-3*

CFM-2 (10–100 μmol/kg IP) and its 3-methyl derivative CFM-3 (10–100 μmol/kg IP) dose dependently reduced the

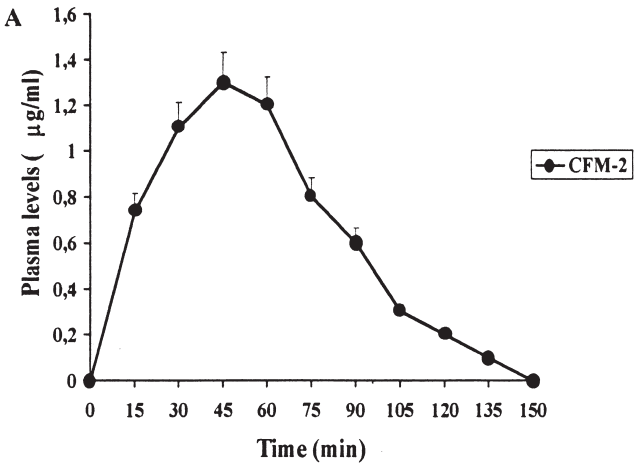


FIG. 4. Time course of plasma levels of CFM-2 (A) and CFM-3 (B) in rats. Ordinate shows the plasma level; abscissa shows the time after intraperitoneal administration of CFM-2 and CFM-3.

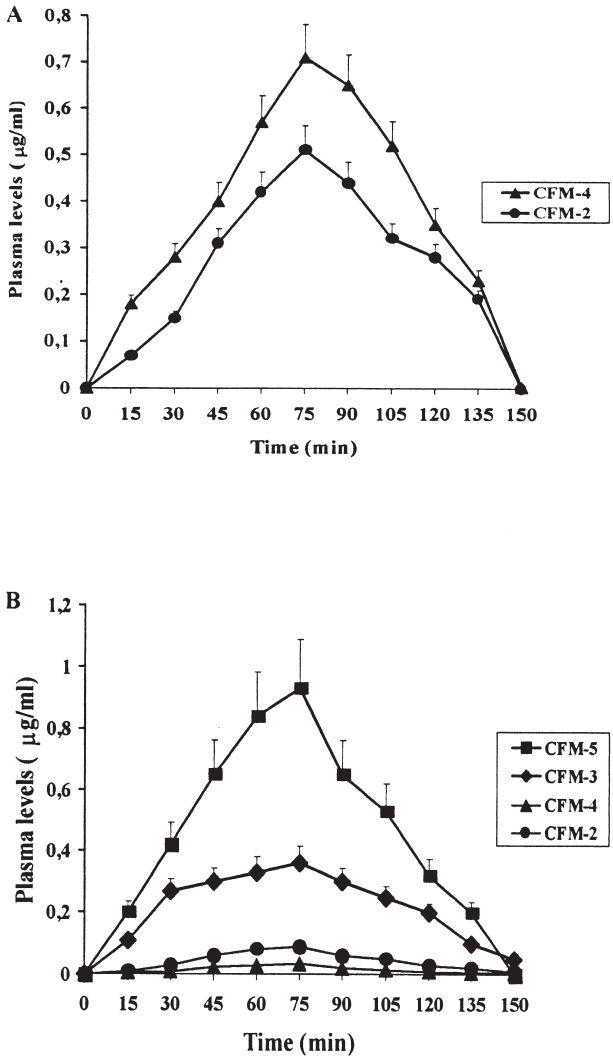


FIG. 5. Time course of plasma levels of CFM-4 (A) and CFM-5 (B) in rats. Ordinate shows the level; abscissa shows the time after intraperitoneal administration of CFM-4 and CFM-5.

median seizure scores and the incidence of clonic and tonic component of audiogenic seizures in genetically epilepsy-prone rats (Table 1 and Fig. 2). In particular, CFM-2 (21, 33, 50, 66, and 100 μmol/kg IP) and CFM-3 (33, 50, 66, and 100 μmol/kg IP) were able to significantly ($Hs = 8.93$, $U = 40.5$, 0.01 and $Hs = 7.67$, $U = 35.5$, $p < 0.01$, respectively) reduce the tonic and clonic component of the audiogenic seizures. A lower dose of CFM-2 (10 μmol/kg IP) had a weak anticonvulsant activity that sometimes appeared significant against the tonic component of the audiogenic seizures, whereas lower doses of CFM-3 (10 and 21 μmol/kg IP) had a weak and non-significant anticonvulsant activity. The time-course studies revealed that CFM-2 (33 μmol/kg IP) showed the maximum activity from 30 to 90 min, while CFM-3 (33 μmol kg IP) had the maximum activity from 45 to 120 min (Fig. 3). The $ED_{50}$ values (with 95% confidence limits) for suppression of clonic and tonic phases of the audiogenic seizures in genetically epilepsy-prone rats are reported in Table 1. The data reported in Fig. 3 clearly indicate that after various pretreatments the anticonvulsant properties of CFM-2 become evident before those of CMF-3, and that this latter compound demonstrated a longer lasting anticonvulsant. activity than the CFM-2.

*Anticonvulsant Properties of CFM-4 and CFM-5*

Compounds CFM-4 (33–200 μmol/kg IP) and CFM-5 (33–200 μmol/kg IP) dose dependently reduced the median seizure scores of audiogenic seizures in genetically epilepsy-prone rats when administered 90 min before test (Table 1), while both compounds showed weak activity when their anticonvulsant properties were assessed 30 min after drug administration. Derivatives CFM-4 and CFM-5 (66, 100, and 200 μmol/kg IP) were able to significantly ($Hs = 7.34$, $U = 35.5$, $p < 0.01$, and $Hs = 7.12$, $U = 35.5$, $p < 0.01$, respectively) reduce the tonic and clonic component of the audiogenic seizures.

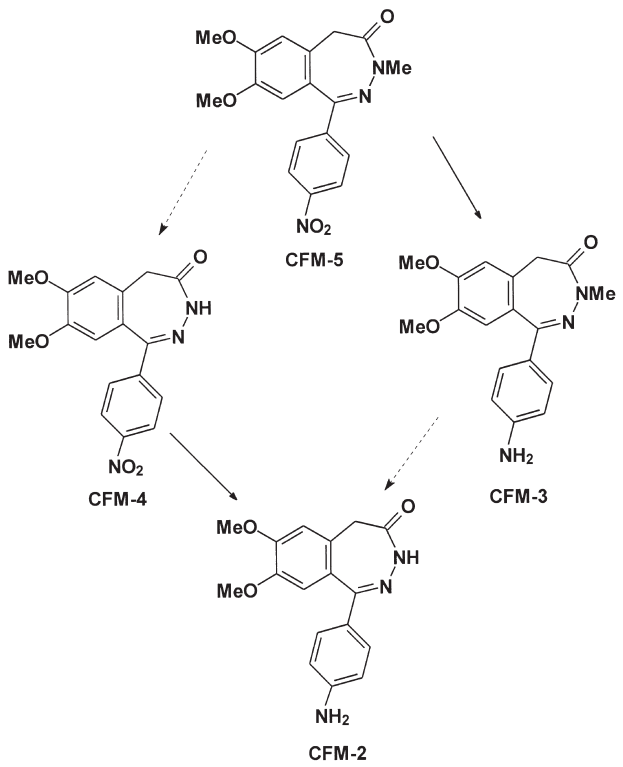


FIG. 6. The metabolic pathway of the studied 2,3-benzodiazepines in GEPR-9s.

Lower doses of these compounds (33 and 50 μmol/kg IP) had a weak and not significant anticonvulsant effect.

The time-course studies revealed that CFM-4 (100 μmol/kg IP) showed the maximum activity from 60 to 120 min, while its methyl derivative CFM-5 (100 μmol kg IP) had the maximum activity at 90 min and appeared less potent than CFM-4 (Fig. 3). The $ED_{50}$ values (with 95% confidence limits) for suppression of clonic and tonic phases of the audiogenic seizures in genetically epilepsy-prone rats are shown in Table 1. The data reported in Fig. 3 clearly indicated that, after various time pretreatments, the anticonvulsant properties of CFM-2 and CFM-3 become evident before those of CFM-4 and CFM-5. The latter compounds also demonstrated a weaker anticonvulsant activity when compared with CFM-2 and CFM-3 (Table 1).

*Effects on Motor Impairments*

In rats, no adverse side effects were observed with doses of CFMs ranging from 10 to 50 μmol/kg IP. Higher doses (66 and/or 100 μmol/kg IP) of all tested compounds induced reduction of locomotor activity and sedation. An impairment of locomotor performance was observed in some rats from 15 to 120 min following the higher doses (100 or 200 μmol/kg IP) of compounds studied. The $TD_{50}/ED_{50}$ ratio of the 2,3-benzodiazepine derivatives is reported in Table 2. No loss of righting reflex or other neurological adverse effects were evident except at a dose level of 200 μmol/kg IP for CFM-4 and CFM-5. In particular, following the highest dose of the latter compounds (200 μmol/kg IP) two out of eight rats for each group displayed loss of the righting reflex.

*Time Course of Plasma Concentrations*

Figure 4A–B reports the time course of plasma concentrations of rats treated with CFM-2 and CFM-3. Peak plasma concentration of both compounds was achieved after 45 min from IP administration. The time course of plasma concentrations of rats treated with CFM-3 showed two curves (Fig. 4B); the first is referred to the IP-injected compound, and the second to its demethylated metabolite, the CFM-2.

In Fig. 5A–B the time course of plasma levels of CFM-4 and CFM-5 is reported. Peak plasma concentration of both compounds was achieved 75 min after IP administration. Moreover, in Fig. 5A it is possible to observe another curve due to the detection of a metabolite, i.e., CFM-2, obtained by nitroreduction. Owing to this possibility, both *N*-dealkylation and nitroreduction (Fig. 6), in the plasma of rats treated with CFM-5 three metabolites, namely CFM-2, CFM-3, and CFM-4, have been detected (Fig. 5B).

## DISCUSSION

Previous studies demonstrated that GYKI 52466 inhibits spinal reflexes in cats but does not potentiate the inhibitory action of GABA, and acts as an AMPA receptors antagonist (27,28) showing antiepileptic properties in various seizures models (3,4,5,24,25,31). Analogously, a series of 2,3-benzodiazepines synthesized in our laboratories, showed to be potent anticonvulsant agents, acting as AMPA receptor antagonists by an allosteric blocking mechanism (4,5).

The present study demonstrated that 2,3-benzodiazepines CFMs also showed anticonvulsant activity in genetically epilepsy-prone rats. The novel 2,3-benzodiazepines, similar to GYKI 52466, produce a marked inhibition of those brain structures involved in the initiation and/or spreading of the audiogenic seizures.

In particular, CFM-2 and CFM-3 have a potency higher or comparable to that of GYKI 52466, whereas CFM-4 and CFM-5 were less active. Furthermore, these 2,3-benzodiazepines have a longer-lasting activity than that of GYKI 52466.

A comparison between the time-course of anticonvulsant activity (Fig. 3) and that of plasma levels (Figs. 4 and 5) put in evidence that the compounds under study showed a significant protection against audiogenic seizures during the period of peak plasma concentrations.

From the point of view of the metabolism of these derivatives (CFMs), all characterized by the presence of a substituent at 4′-position of the phenyl ring at C-1, this analytical study demonstrated that the degree of *N*-dealkylation is not significant (Figs: 4B and 5B), contrary to our previous findings regarding analogous 2,3-benzodiazepines in which an unsubstituted phenyl ring at C-1 was present (22). It is interesting to note that for compounds CFM-4 and CFM-5, the main metabolic pathway is characterized by a nitroreduction, which afford CFM-2 and CFM-3, respectively (Fig. 6). This bioactivation is responsible for the observed anticonvulsant activity of CFM-4 and CFM-5, which was evident after a long latency.

In conclusion, the data reported in this study suggest that the pharmacological profile of CFMs is highly dependent on the substitution pattern both at the phenyl ring at C-1 and the nitrogen atom N-3.

## ACKNOWLEDGEMENTS

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