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Synthesis and anticonvulsant activity of new 2,3-benzodiazepines as AMPA receptor antagonists

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Abstract

Novel 1-aryl-3,5-dihydro-7,8-methylenedioxy-4*H*-2,3-benzodiazepin-4-ones (**12a–j**) were prepared and their anticonvulsant effects were evaluated by using various models of experimental epilepsy. The seizures were evoked both by means of auditory stimulation in DBA/2 mice and by pentylenetetrazole or maximal electroshock in Swiss mice. Some of these compounds possess marked anticonvulsant properties in all tests employed. Compounds **12** antagonise seizures induced by AMPA in analogy to the structurally-related 1-(4′-aminophenyl)-4-methyl-7,8-methylenedioxy-5*H*-2,3-benzodiazepine (**1**) (GYKI 52466), a well-known non-competitive AMPA-receptor antagonist. On the other hand, these novel 2,3-benzodiazepines exhibit anticonvulsant properties that are not affected by flumazenil, but are reversed by aniracetam. In addition, when compared to model compound **1**, compounds **12** show a longer-lasting anticonvulsant activity and a lower toxicity. A structure–activity relationship study carried out on compounds **12** as well as analogous 7,8-dimethoxy derivatives **2** offers an approach for designing more potent agents.
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Keywords: 7,8-Methylenedioxy-4*H*-2,3-benzodiazepin-4-ones; Anticonvulsant activity; AMPA-receptor antagonists

1. Introduction

(*S*)-Glutamic acid (Glu), the most abundant excitatory neurotransmitter of the central nervous system (CNS), plays a key role in the physiological function of the CNS [1]. Two main classes of Glu receptors have been characterised: the metabotropic (mGluRs) and ionotropic (iGluRs) receptors. The mGluRs regulate the activity of ionic channels or enzymes by producing second messengers via GTP-coupled proteins [2]. The iGluRs are Glu-gated cationic channels directly responsible for the fast depolarisation of postsynaptic cells. They are constituted of different subunits and classified into three heterogeneous types based on their pharmacology and functional properties: the *N*-methyl-D-aspartic acid (NMDA) receptor and two non-NMDA receptors named (*R*,*S*)-2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionic acid (AMPA) and kainic acid (KA) receptors [3,4]. It is now widely accepted that in ischemic or hypoxic conditions such as stroke, trauma and epilepsy both NMDA and non-NMDA receptors are overstimulated by an increased amount of endogenous Glu [5,6]. Therefore, the identification of competitive and non-competitive NMDA as well as non-NMDA receptor antagonists, which prevent the opening of the ion channels associated with these iGluRs, could be of potential clinical value both in acute (stroke and trauma) and chronic (Alzheimer's disease, epilepsy) neurological disorders [7]. During the past 15 years numerous NMDA antagonists have been the subject of an intense research program [8,9] but preliminary results were not encouraging since strong side-effects e.g. psychotomimetic action [10,11] and impairment of learning and memory [12] hampered their clinical development.

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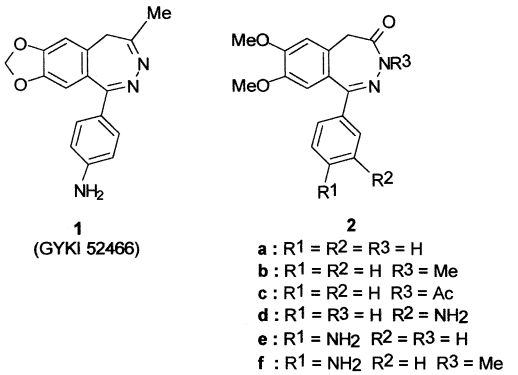
The general interest seems now to be focused on agents acting selectively on AMPA receptors because of their relevance in the treatment of epilepsy [13,14] and cerebral ischaemia [15,16]. At this time, 6-nitro-7-sulfamoylbenzo[*f*]quinoxaline-2,3-dione (NBQX) [15] and 6-(1*H*-imidazol-1-yl)-7-nitro-2,3(1*H*,4*H*)-quinoxalinedione (YM90K) [17], both belonging to the class of the quinoxaline-2,3-diones, are among the most potent and selective competitive AMPA-antagonists.

1-(4′-Aminophenyl)-4-methyl-7,8-methylenedioxy-5*H*-2,3-benzodiazepine (**1**) (GYKI 52466) (Fig. 1) has been identified as a potent and selective non-competitive AMPA-receptor antagonist that appears to act via a novel allosteric site on the receptor complex [18,19]. It shows anticonvulsant properties [20–23] and behaves as a neuroprotective agent in focal and global ischaemia [24,25]. Compound **1**, in spite of its chemical similarity to 1,4-benzodiazepines, does not bind to the benzodiazepine receptor (BZR) complex and, therefore, it is devoid of any sedative–hypnotic effect [26].

As part of a program aimed at identifying potent and selective AMPA receptor antagonists, we have recently reported on the synthesis of a series of 1-aryl-3,5-dihydro-7,8-dimethoxy-4*H*-2,3-benzodiazepin-4-ones (**2**) (Fig. 1) [27,28]. Some of these compounds proved to be more potent than **1** as anticonvulsants in various seizure models. Furthermore, when compared to the model compound **1**, they showed a longer-lasting activity and a lower toxicity. Electrophysiological assays suggest that the above-mentioned 2,3-benzodiazepines **2**, in analogy to the lead compound **1**, act via a non-competitive blocking mechanism of the AMPA receptor complex [28].

If we compare the structure of 2,3-benzodiazepines **1** and **2**, we note two structural modifications, i.e. the replacement of the dioxole nucleus with two methoxy groups on the fused aromatic ring and the introduction of a carbonyl moiety in position 4 of the heptatomic nucleus. In order to test the role of the two methoxy groups of derivatives **2** on the biological activity, we prepared and tested some dioxole analogues as anticonvulsants.

This paper reports the synthesis of novel 1-aryl-3,5-dihydro-7,8-methylenedioxy-4*H*-2,3-benzodiazepin-4-ones (**12a–j**) and the evaluation of their anticonvulsant properties in DBA/2 mice, a strain genetically susceptible to sound-induced seizures, which has been considered an excellent animal model for generalised epilepsy and for screening new anticonvulsant drugs [29,30]. Some derivatives were also examined in Swiss mice both against pentylenetetrazole (PTZ) and maximal electroshock (MES)-induced seizures and the time course of anticonvulsant activity was also studied. In addition, we attempted to correlate the anticonvulsant properties of the new 2,3-benzodiazepines **12** with their affinity for the BZR or AMPA receptor. The affinity of



1
(GYKI 52466)

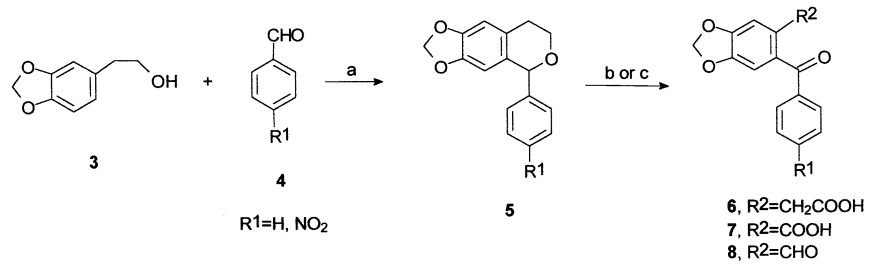
2
a : R1 = R2 = R3 = H
b : R1 = R2 = H R3 = Me
c : R1 = R2 = H R3 = Ac
d : R1 = R3 = H R2 = NH_2
e : R1 = NH_2 R2 = R3 = H
f : R1 = NH_2 R2 = H R3 = Me

Fig. 1. Structures of compounds **1** (GYKI 52466) and **2a–f**.

compounds **12** for the benzodiazepine receptor was evaluated through the inhibition of [^{3}H]flumazenil (Ro 15-1788) binding in a cortical membrane preparation. In addition the potency of compounds **12** for the BZR was assessed in vivo by the ability of flumazenil to reverse their anticonvulsant activity. The activity of **12a** and **12e** on the AMPA-receptor complex was evaluated through their ability to inhibit the AMPA-induced seizures. Furthermore, aniracetam (Ro 13-5057), a compound which potentiates the effects of AMPA [31], antagonises the anticonvulsant properties of compounds **12a** and **12e**. Finally, we assessed the propensity of title compounds to induce neurological impairment by using the rotarod test. A preliminary account of the present results has been reported [32].

2. Chemistry

At first, we tackled the synthesis of compounds **12** through the procedure already successfully applied to the preparation of derivatives **2** [28]. This strategy requires a Jones oxidation of 1-aryl-6,7-methylenedioxyisochromans **5** to yield ketoacids **6** followed by a cyclocondensation with hydrazine (Scheme 1). This procedure proved to be unsatisfactory when applied to isochromans **5** because the condensation of 3,4-methylenedioxyphenethyl alcohol (**3**) with an aromatic aldehyde **4** gave the expected products **5** in poor yields. Furthermore, the oxidation of compounds **5** with Jones reagent (method b) led to 2-aroyl-4,5-methylenedioxybenzoic acid (**7**) as the only isolable product, whereas, a mixture of 2-aroyl-4,5-methylenedioxyphenylacetaldehyde (**8**), the main product, and a small amount of acid **7** was obtained by using ruthenium dioxide/sodium periodate (method c) [33]. In both cases the expected 2-aroyl-4,5-methylenedioxyphenylacetic acid (**6**) was never detected. Therefore, we developed an alternative strategy as shown in Scheme 2. Ketoesters **11** were



Scheme 1. Reagents: (a) $HCl_{(g)}$ saturated dioxane, reflux, 1 h; (b) 35% H_2SO_4, CrO_3, acetone; (c) $NaIO_4$, RuO_2, $CCl_4/CH_3CN/H_2O$, room temperature, 20 h.

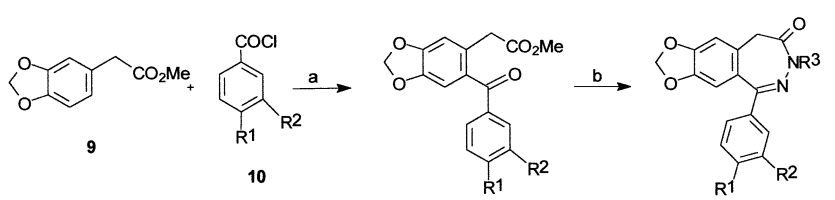
easily prepared via Friedel–Craft acylation of methyl 3,4-methylenedioxyphenylacetate (**9**) with the appropriate aroyl chloride **10** by using tin(IV) chloride as the catalyst. The subsequent treatment of ketoesters **11** with hydrazine or *N*-methylhydrazine gave 1-aryl-3,5-dihydro-7,8-methylenedioxy-4*H*-2,3-benzodiazepin-4-ones (**12**) in good yields. When an amino or hydroxy group is present on the phenyl ring, i.e. **12d–g**, the Friedel–Craft acylation step was carried out on trifluoroacetamido- (**10d–e**) or acetoxy- (**10f**) benzoyl chlorides, respectively. The treatment of the resulting ketoesters **11d–f** with an excess of hydrazine or *N*-methylhydrazine gave both cyclisation and complete deprotection of the amino and hydroxy functionalities to yield directly final derivatives **12d–g**. *N*-Acetyl derivative **12c** was obtained by reacting **12a** with an excess of acetic anhydride in the presence of triethylamine. Physical and spectral data (^{1}H NMR) of the synthesised compounds are in agreement with the proposed structures.

3. Results and discussion

The novel 7,8-methylenedioxy-4*H*-2,3-benzodiazepines (**12a–j**) were tested for anticonvulsant activity against audiogenic seizures in DBA/2 mice and the results were compared with those previously reported [27,28] for 7,8-dimethoxy-4*H*-2,3-benzodiazepine analogues (**2a–f**) and for GYKI 52466 (**1**), chosen as the reference compound. The compounds were intraperitoneally (i.p.) injected in various doses (dose range: 3.3–200 μmol/kg) in DBA/2 mice and their anticonvulsant properties were evaluated 30 min after administration; the measured ED_{50} values are reported in Table 1.

Among the synthesised compounds, derivatives **12d** and **12e** are the most active ones showing anticonvulsant potency comparable to **2d** and **2e** and higher than that of GYKI 52466.

A survey of the present results put in evidence that the replacement of the two methoxy groups on the benzene fused ring with a dioxole nucleus seems to have



Scheme 2. Reagents: (a) $SnCl_4/CH_2Cl_2$, 0–5°C, 1 h; room temperature, 12 h; (b) $NH_2NH_2 \cdot H_2O$ or $MeNHNH_2$, EtOH, reflux, 6–7 h; (c) Ac_2O, Et_3N, $CHCl_3$, room temperature, 1–2 h.

Table 1
Anticonvulsant activity of compounds **1**, **2a–f**[a] and **12a–j** against audiogenic seizures in DBA/2 mice and TD50 values on locomotion assessed by rotarod test

Comp.	ED_{50}[b] (μmol/kg)		TD_{50} (μmol/kg)	TI[c]
	Clonic phase	Tonic phase	Locomotor deficit	
2a	33.9 (26.0–44.2)	31.8 (24.8–40.6)	142 (87.3–231)	4.2
12a	43.3 (34.4–54.6)	40.6 (30.1–54.9)	159 (82.6–306)	3.7
2b	37.8 (23.7–60.1)	26.7 (14.7–48.2)	154 (104–227)	4.1
12b	82.5 (58.6–116)	70.4 (58.8–84.3)	>200	N.D.[d]
2c	101 (52.0–194)	72.1 (47.6–109)	181 (106–309)	1.8
12c	36.6 (28.5–47.1)	32.6 (25.5–42.1)	110 (79.3–151)	3.0
2d	19.3 (16.9–22.0)	18.3 (16.0–20.8)	51.5 (34.1–77.8)	2.7
12d	18.0 (10.0–32.5)	12.7 (6.13–26.2)	101 (52.0–194)	5.6
2e	15.0 (9.01–24.0)	12.6 (8.01–19.0)	56.8 (39.3–82.1)	3.8
12e	21.8 (13.2–36.0)	10.9 (4.60–26.4)	99.1 (72.4–135)	4.5
2f	50.2 (34.6–73.0)	43.7 (31.3–61.0)	159 (95.0–268)	3.2
12f	41.1 (30.8–55.0)	29.5 (18.0–48.5)	80.1 (55.3–116)	2.0
12g	52.3 (44.5–61.4)	49.6 (41.0–60.1)	151 (103–221)	2.9
12h	>120	>120	>200	N.D.
12i	89.8 (55.2–146)	63.8 (45.4–89.7)	>200	N.D.
12j	>120	>120	>200	N.D.
1	35.8 (24.4–52.4)	25.3 (16–40.0)	76.1 (47.5–122)	2.1

[a] Data taken from Ref. [28].
[b] ED_{50} values with 95% confidence limits were calculated according to the method of Litchfield and Wilcoxon [46].
[c] TI = ratio between TD_{50} and ED_{50} (from the clonic phase of the audiogenic seizures).
[d] N.D., not determined.

little influence on anticonvulsant activity. As a matter of fact, 7,8-methylenedioxy derivatives **12a**, **12d** and **12e** display a potency comparable to that of the corresponding compounds **2a**, **2d** and **2e**.

When compared to the unsubstituted compound **12a**, the introduction of a fluorine atom, a methoxy or a trifluoromethyl group at C-4′ drastically reduces or suppresses the activity. On the contrary, the presence of an amino group, in position 3′ or 4′, increases the anticonvulsant activity as previously observed [28] for derivatives **2d** and **2e**. An increase in activity was also obtained by the introduction of a hydroxy group at C-4′ even if its effect is minor than that of the amino group.

As a matter of fact, derivative **12e**, the most active term of the series, is roughly three times more potent than GYKI 52466 (**1**), i.e. ED_{50} 10.9 μmol/kg for **12e** versus 31.8 μmol/kg for **1**. Since the structures of **1** and **12e** differ solely in the replacement of the azomethine group of **1** with a lactam moiety, it can be deduced that such a modification is fruitful and allows a better fitting of the new structure with the complementary receptor subsites of the AMPA-receptor complex.

The introduction of a methyl group at N-3 decreases the activity i.e. $ED_{50} = 29.5$ μmol/kg for **12f** versus $ED_{50} = 10.9$ μmol/kg for **12e**, whereas an opposite effect was observed with the acetyl group i.e. $ED_{50} = 32.6$ μmol/kg for **12c** versus $ED_{50} = 40.6$ μmol/kg for **12a** in contrast to what was previously observed for derivatives **2** (Table 1). Further work is in progress in order to clarify the role played by the substituent at N-3.

The time course of anticonvulsant activity was also studied in order to discover new compounds with a longer time course than **1**. Following i.p. administration of some active compounds such as **2a** and **12a** (50 μmol/kg), maximum protection was observed after 45–90 min with subsequent return to control seizure response at 180 min. In the same experimental conditions, GYKI 52466 displayed the maximum protection in 5–15 min followed by a gradual return to control seizure response in 30–90 min (Fig. 2).

Derivatives **12d** and **12e**, the most active ones of the series, as well as the unsubstituted parent compound **12a** were selected for further evaluation against MES- and PTZ-induced seizures in Swiss mice. As shown in Table 2, the tonic extension and the clonic phase of the seizures induced by MES and PTZ, respectively were significantly reduced at 45 min after i.p. administration of the tested compounds.

In order to correlate the anticonvulsant activity of novel compounds **12** with affinity for AMPA receptor, an additional test against AMPA-induced seizures in DBA/2 mice was performed (Table 2). As shown in Fig. 3, the clonic and tonic phases of the seizures induced by intracerebroventricular (i.c.v.) administration of AMPA were significantly reduced 30 min after i.p. administration of **12a**, **12d**, **12e** and **1**, analogously to what has already been reported [28] for **2a**, and **2e**. In the present study we also demonstrated that aniracetam, a potentiator of the AMPA effects [31], markedly antagonised the anticonvulsant effects of **12e** in DBA/2 mice (Table 2) and shifted to the right the dose–response curves (Fig. 4) with a pattern of activity similar to that of **1** and **2e** [28]. On the basis of electrophysiological experi-

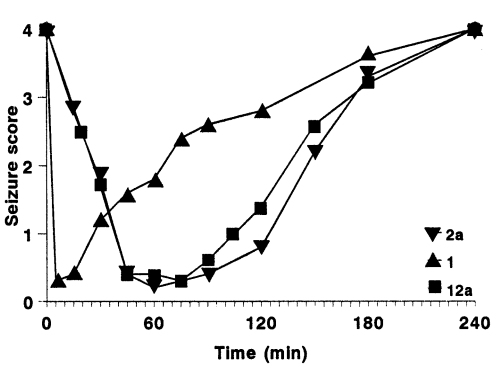


Fig. 2. Anticonvulsant effects of **2a**, **12a**, and **1** (50 μmol/kg i.p.) against audiogenic seizures in DBA/2 mice. The ordinate shows seizure score, the abscissa shows the time after i.p. administration of drug in min. Ten animals were used for the determination of each point.

Table 2
Anticonvulsant activity of some benzodiazepines against the MES-, PTZ- and AMPA-induced seizures and against audiogenic seizures after pretreatment with aniracetam

Comp.	ED_{50}[a] (μmol/kg)					
	MES (Swiss mice)	PTZ (Swiss mice)	AMPA[b] (DBA/2 mice)		Pretreatment with aniracetam[c] (DBA/2 mice)	
	Tonic phase	Clonic phase	Clonic phase	Tonic phase	Clonic phase	Tonic phase
2a[d]	35.8 (28.6–44.7)	68.2 (54.6–85.2)	66.0 (45.9–94.9)	42.6 (26.4–68.8)	215 (142–324)*	157 (114–217)*
12a	42.6 (26.4–68.8)	78.0 (46.0–132)	70.3 (58.7–84.1)	56.9 (40.9–79.2)	N.D.[e]	N.D.
12d	19.3 (16.9–22.0)	40.5 (22.9–71.7)	29.2 (16.9–50.4)	23.8 (14.2–39.9)	N.D.	N.D.
2e[d]	15.9 (7.3–33.5)	22.6 (11.7–43.8)	32.1 (23.2–44.3)	25.0 (16.5–30.0)	65.4 (44.5–96.2)*	58.2 (43.4–77.9)*
12e	32.1 (23.2–44.3)	71.8 (53.2–96.9)	37.9 (27.3–52.8)	28.5 (20.6–39.4)	62.6 (44.7–87.7)	39.6 (22.9–68.7)
1	35.7 (29.3–43.4)	68.3 (56.2–83.1)	57.5 (43.5–76.0)	40.5 (26.3–60.8)	134 (88.8–203)*	100 (63.4–158)*

[a] ED_{50} values with 95% confidence limits were calculated according to the method of Litchfield and Wilcoxon [46].
[b] AMPA was administered i.c.v. at the CD_{97} for either clonus (9.7 nmol) or forelimb tonic extension (11.7 nmol) 30 min after injection of each compound.
[c] Significant differences between ED_{50} values of group treated with aniracetam + 2,3-benzodiazepine and group treated with 2,3-benzodiazepine alone (Table 1) are denoted: * $P < 0.01$.
[d] Data taken from Ref. [28].
[e] N.D., not determined.

ments carried out on derivatives **1** and **2e** [28], along with the observation that the anticonvulsant properties of **12e** were significantly reduced (from 2.9 to 3.6 times) by aniracetam, a positive allosteric modulator of AMPA receptors [31], we suggest that the present compounds **12** antagonise the AMPA receptor-mediated responses via an allosteric blocking mechanism.

To rule out a possible involvement of BZR on pharmacological effects of the present compounds, derivatives **1**, **12a**, and **12e** were administered concomitantly with flumazenil, but no significant modification of the antiseizure effects of these derivatives was observed (Table 3). On the other hand, compounds **12** were assessed for the ability to displace [^{3}H]flumazenil from membranes of cortex. No inhibition was observed up to concentration of 10 μM ($IC_{50} > 10$ μM).

Furthermore, by means of crude cortical synaptic membranes, derivatives **12a** and **12e** (100 μM) failed to displace [^{3}H]spiperone from dopamine and 5-HT_1 receptors; [^{3}H]ketanserin from 5-HT_2 receptors; [^{125}I]-pindolol from β-adrenergic receptors; [^{3}H](*R*,*S*)-3-(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid ([^{3}H]-CPP) and [^{3}H]dizocilpine from NMDA receptors; [^{3}H]-5,7-dichlorokynurenic acid ([^{3}H]5,7-DCKA) from glycine site on NMDA receptors; [^{3}H]AMPA and [^{3}H]6-cyano-7-nitroquinoxaline-2,3-dione ([^{3}H]CNQX) from AMPA/kainate receptors; mixture of [^{3}H](1S,3R)ACPD and [^{3}H](1R,3S)ACPD from metabotropic glutamate receptors. Despite the lack of activity of the present 2,3-benzodiazepines at dopamine, serotonin, noradrenaline, NMDA, glycine site on NMDA and metabotropic glutamate binding sites an interaction at other sites involved in the generation or expression of seizures cannot presently be ruled out.

The anticonvulsant activity of the title compounds **12** was evident at doses which generally did not cause sedation and ataxia. It has long been known that

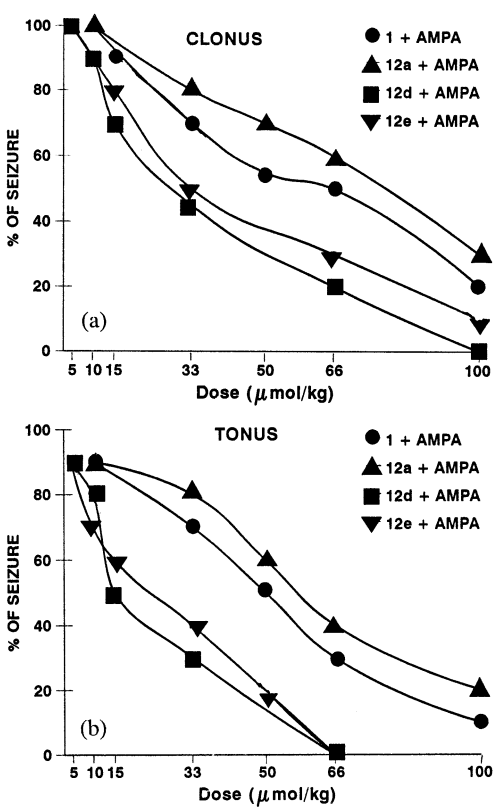


Fig. 3. Anticonvulsant effects of **12a**, **12d**, **12e** and **1** against seizures induced by AMPA in DBA/2 mice. The ordinate shows % of response of clonic (a) or tonic (b) seizures, abscissa shows the dose in μmol/kg i.p. For the determination of each point ten animals were used.

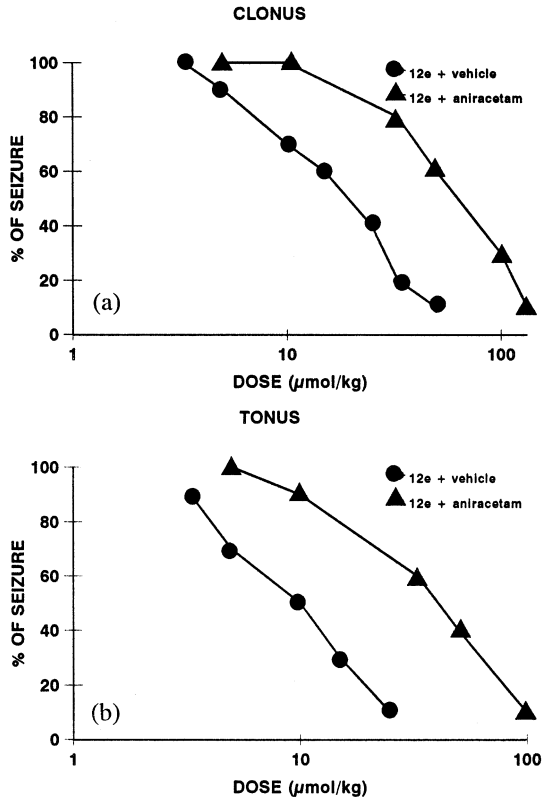


Fig. 4. Antagonism by aniracetam (50 nmol i.c.v.) of the anticonvulsant effects of **12e** against audiogenic seizures in DBA/2 mice. The ordinate shows % of response of clonic (a) or tonic (b) seizures. The abscissa shows the dose in μmol/kg i.p. For the determination of each point ten animals were used.

NMDA antagonists, especially those which block ion channels (e.g. dizocilpine), can induce cognitive deficits and a variety of other neurological and behavioural side effects [19,34–36]. There are, however, studies showing that potent antagonists at the AMPA/kainate receptor possess anticonvulsant effects at doses below those impairing learning and behaviour [37–39]. Table 1 shows the doses which induced motor toxicity in 50% of mice (TD_{50} values) obtained 30 min following the i.p. administration of 2,3-benzodiazepines **12a–j**. It is noteworthy that the present compounds **12** cause significantly less motor impairment in the rotarod test with respect to **1** and some of them have therapeutic index (TI) values approximately twice that of **1** (Table 1).

Table 3
Anticonvulsant activity against audiogenic seizures after concomitant treatment with flumazenil in DBA/2 mice

Comp.	Flumazenil (μmol)	ED_{50}[a] (μmol/kg)	
		Clonic phase	Tonic phase
2a[b]	8.24	38.2 (28.5–51.2)	35.8 (26.2–49.0)
	24.72	50.9 (36.9–70.1)	41.3 (28.0–60.7)
12a	8.24	41.2 (36.0–47.2)	40.6 (27.4–60.2)
	24.72	36.8 (28.3–47.7)	37.6 (31.2–45.3)
2e[b]	8.24	14.1 (10.1–19.9)	12.0 (6.82–21.0)
	24.72	12.0 (6.82–21.0)	10.2 (7.61–13.7)
12e	8.24	21.0 (16.2–27.2)	10.2 (7.61–13.7)
	24.72	19.5 (16.7–22.8)	9.1 (5.70–14.5)
1	8.24	37.8 (23.7–60.1)	26.7 (14.7–48.2)
	24.72	39.5 (29.6–53.7)	29.7 (20.9–42.1)

[a] ED_{50} values with 95% confidence limits were calculated according to the method of Litchfield and Wilcoxon [46].

[b] Data taken from Ref. [28].

4. Conclusions

The novel 7,8-methylenedioxy-4*H*-2,3-benzodiazepin-4-ones (**12**) reported in this study, analogously to 7,8-dimethoxy-4*H*-2,3-benzodiazepin-4-ones (**2**), possess a marked anticonvulsant activity both in the audiogenic seizure test in DBA/2 mice and against MES and PTZ induced seizures in Swiss mice. In particular, derivatives **12a** and **12c–f** show a potency comparable to or higher than that of **1**, the reference compound. They also exhibit longer-lasting anticonvulsant action and lower toxicity than compound **1**. Moreover, they display anticonvulsant effects against seizures induced by AMPA in agreement with an involvement of AMPA receptors. The structure–activity relationships in this series were examined by three types of structural changes: (i) a number of substituents at the C-4′ position; (ii) the insertion of an amino group in different positions on the phenyl ring at C-1; (iii) the introduction of a methyl or an acetyl group at N-3. In the light of these findings, we can conclude that two methoxy groups or a dioxole nucleus on the benzene ring play a similar role in determining anticonvulsant effectiveness of these compounds, whereas the replacement of the azomethine moiety of GYKI 52466 with the lactam moiety plays a role of utmost importance in the interaction with the AMPA-receptor complex. Such a structural modification is responsible for the improvement in the pharmacological profile observed in this new class of 2,3-benzodiazepines which promise to become useful tools in the mapping of the AMPA receptors.

5. Experimental

5.1. Chemistry

Melting points were determined on a Kofler hot stage apparatus and are uncorrected. Elemental analyses were carried out on a Carlo Erba 1106 elemental analyzer for C, H, and N, and the results are within $\pm 0.40\%$ of the theoretical values. Merck silica gel 60 F_{254} plates were used for analytical TLC; column chro-

Table 4
Physical and spectral data of compounds **11 and 12**

Comp.	M.p. (°C)[a]	Yield (%)	^{1}H NMR δ (ppm, J [Hz])[b]
11a	80–82 (A)	81	3.59 (s, 3H, CH_3), 3.80 (s, 2H, CH_2), 5.98 (s, 2H, O–CH_2–O), 6.82 and 6.87 (2s, 2H, H-3 and H-6), 7.42–7.78 (m, 5H, Ar)
11d	150–152 (B)	72	3.62 (s, 3H, CH_3), 3.78 (s, 2H, CH_2), 6.02 (s, 2H, O–CH_2–O), 6.79 and 6.82 (2s, 2H, H-3 and H-6), 7.66 and 7.76 (2d, 4H, $J = 8.4$, Ar), 8.76 (br s, 1H, NH)
11e	107–109 (B)	59	3.62 (s, 3H, CH_3), 3.82 (s, 2H, CH_2), 6.06 (s, 2H, O–CH_2–O), 6.83 and 6.87 (2s, 2H, H-3 and H-6), 7.51 (dd, 1H, $J = 7.9$ and 8.1, H-5′), 7.64 (d, 1H, $J = 7.9$, H-6′), 7.77 (s, 1H, H-2′), 8.00 (d, 1H, $J = 8.1$, H-4′), 8.18 (br s, 1H, NH)
11g	150–152 (B)	83	2.31 (s, 3H, CH_3CO), 3.60 (s, 3H, CH_3), 3.78 (s, 2H, CH_2), 6.03 (s, 2H, O–CH_2–O), 6.84 and 6.87 (2s, 2H, H-3 and H-6), 7.14 and 7.82 (2d, 4H, $J = 8.8$, Ar)
11h	99–101 (C)	89	3.60 (s, 3H, CH_3), 3.76 (s, 2H, CH_2), 3.88 (s, 3H, CH_3O-4′), 6.04 (s, 2H, O–CH_2–O), 6.84 and 6.87 (2s, 2H, H-3 and H-6), 6.94 and 7.79 (2d, 4H, $J = 8.9$, Ar)
11i	129–131 (C)	50	3.61 (s, 3H, CH_3), 3.79 (s, 2H, CH_2), 6.04 (s, 2H, O–CH_2–O), 6.83 and 6.85 (2s, 2H, H-3 and H-6), 7.13 (dd, 2H, $J = 8.8$ and $J_{H-F} = 8.5$, H-3′,5′), 7.81 (dd, 2H, $J = 8.8$ and $J_{H-F} = 5.5$, H-2′,6′)
11j	157–159 (C)	53	3.62 (s, 3H, CH_3), 3.85 (s, 2H, CH_2), 6.05 (s, 2H, O–CH_2–O), 6.83 and 6.84 (2s, 2H, H-3 and H-6), 7.73 and 7.88 (2d, 4H, $J = 8.0$, Ar)
12a	184–186 (A)	74	3.46 (s, 2H, CH_2-5), 6.03 (s, 2H, OCH_2O), 6.63 (s, 1H, H-9), 6.85 (s, 1H, H-6), 7.38–7.61 (m, 5H, Ar), 8.56 (br s, 1H, NH)
12b	170–172 (A)	58	3.44 (s, 5H, CH_2-5 and CH_3), 6.03 (s, 2H, OCH_2O), 6.63 (s, 1H, H-9), 6.86 (s, 1H, H-6), 7.42–7.64 (m, 5H, Ar)
12c	188–190 (A)	46	2.58 (s, 3H, CH_3), 3.56 (s, 2H, CH_2-5), 6.06 (s, 2H, OCH_2O), 6.67 (s, 1H, H-9), 6.90 (s, 1H, H-6), 7.42–7.71 (m, 5H, Ar)
12d	246–248 (B)	42	3.45 (s, 2H, CH_2-5), 3.78 (br s, 2H, NH_2), 6.03 (s, 2H, OCH_2O), 6.68 (s, 1H, H-9), 6.78 (dd, 1H, $J = 7.6$ and 1.5, H-6′), 6.82 (s, 1H, H-6), 6.89 (dd, 1H, $J = 7.6$ and 1.5, H-4′), 6.96 (t, 1H, $J = 1.9$, H-2′), 7.19 (t, 1H, $J = 7.6$, H-5′), 8.50 (br s, 1H, NH)
12e	228–230 (B)	51	3.42 (s, 2H, CH_2-5), 3.82 (bs s, 2H, NH_2), 6.01 (s, 2H, OCH_2O), 6.70 (s, 1H, H-9), 6.81 (s, 1H, H-6), 6.67 and 7.40 (2d, 4H, $J = 8.5$, Ar), 8.57 (br s, 1H, NH)
12f	236–238 (B)	38	3.37 (s, 5H, CH_2-5 and CH_3), 3.88 (br s, 2H, NH_2), 6.03 (s, 2H, OCH_2O), 6.70 (s, 1H, H-9), 6.83 (s, 1H, H-6), 6.68 and 7.43 (2d, 4H, $J = 8.7$, Ar)
12g	308–310 (B)	64	3.40 (s, 2H, CH_2-5), 6.09 (s, 2H, OCH_2O), 6.60 (s, 1H, H-9), 7.06 (s, 1H, H-6), 6.81 and 7.36 (2d, 4H, $J = 8.2$, Ar), 9.88 (br s, 1H, NH), 10.74 (br s, 1H, OH)
12h	205–207 (C)	68	3.45 (s, 2H, CH_2-5), 3.87 (s, 3H, OCH_3), 6.04 (s, 2H, OCH_2O), 6.66 (s, 1H, H-9), 6.83 (s, 1H, H-6), 6.94 and 7.55 (2d, 4H, $J = 8.9$, Ar), 8.32 (br s, 1H, NH)
12i	206–208 (C)	56	3.46 (s, 2H, CH_2-5), 6.04 (s, 2H, OCH_2O), 6.61 (s, 1H, H-9), 6.84 (s, 1H, H-6), 7.11 (dd, 2H, $J = 8.8$ and $J_{H-F} = 8.5$, H-3′,5′), 7.60 (dd, 2H, $J = 8.8$ and $J_{H-F} = 5.5$, H-2′,6′), 8.48 (br s, 1H, NH)
12j	265–267 (C)	53	3.48 (s, 2H, CH_2-5), 6.05 (s, 2H, OCH_2O), 6.58 (s, 1H, H-9), 6.85 (s, 1H, H-6), 7.68 and 7.74 (2d, 4H, $J = 8.3$, Ar), 8.60 (br s, 1H, NH)

[a] Crystallisation solvent: A, ethanol; B, ethyl acetate; C, methanol.
[b] ^{1}H NMR spectra were recorded in $CDCl_3$, and in DMSO-d_6 for **12g**.

matography was performed on Merck silica gel 60 (70–230 mesh or 230–240 mesh for compounds **11d–e** and **12d–f**). ^{1}H NMR spectra were recorded using a Varian Gemini-300 spectrometer in the indicated solvents. Chemical shifts were expressed in ppm and coupling constants (J) in Hz. All exchangeable protons were confirmed by addition of D_2O. The data are collected in Table 4. The preparation of 3′- and 4′-trifluoroacetamidobenzoic acid was accomplished following a reported procedure [40]. The starting acyl chlorides **10a** and **10h–j** are commercially available whereas **10d**, **10e**, and **10g** were prepared as follows.

*5.1.1. General procedure for acyl chlorides **10d–e**, **10g***

The appropriate benzoic acid (13 mmol) was heated with thionyl chloride (11 ml, 150 mmol) under reflux for 2–3 h. Excess $SOCl_2$ was evaporated under reduced pressure and the residue was dissolved in dry CH_2Cl_2 (20 ml) and used immediately in the following step.

*5.1.2. General procedure for methyl 2-aroyl-4,5-methylenedioxyphenylacetate (**11a**, **11d–e**, **11g–j**)*

To a cooled (0–5°C) and stirred solution of methyl 3,4-methylenedioxyphenylacetate (**9**) (1.94 g, 10 mmol) in CH_2Cl_2 (20 ml) were added 16.5 ml of 0.1 M tin(IV) chloride (16.5 mmol) in CH_2Cl_2 and the appropriate acyl chloride **10** (13 mmol) in the same solvent (20 ml). The ice-bath was removed and the mixture was stirred at 20°C overnight, then poured into water and the product was isolated in ether and dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue was subjected to chromatography eluting with diethyl ether–light petroleum (6:4).

*5.1.3. General procedure for 1-aryl-3,5-dihydro-7,8-methylenedioxy-4H-2,3-benzodiazepin-4-ones (**12a–b**, **12d–j**)*

The appropriate methyl 2-aroyl-4,5-methylenedioxyphenylacetate (**11**) (3 mmol) was refluxed for 6–7 h with hydrazine hydrate (10 or 30 mmol for **12d**, **12e**, and **12g**) in EtOH (50 ml) or monomethylhydrazine (12 or 36 mmol for **12f**) in dry toluene (50 ml). After cooling, the solvent was removed under reduced pressure and the residue purified by column chromatography using EtOAc–light petroleum (70:30) for **12d–g** and diethyl ether/light petroleum (80/20) for **12a**,**b** and **12h–j** as eluant.

*5.1.4. 3-Acetyl-3,5-dihydro-7,8-methylenedioxy-1-phenyl-4H-2,3-benzodiazepin-4-one (**12c**)*

To a solution of **12a** (0.28 g, 1 mmol) in $CHCl_3$ (30 ml) and Et_3N (5 ml) which was cooled while stirring, Ac_2O (5 ml) was added dropwise. The mixture reaction was stirred at room temperature for 1–2 h, then poured into water, extracted with $CHCl_3$, and dried over Na_2SO_4. Evaporation of the solvent under reduced pressure afforded a crude product, which was purified by treatment with EtOH–light petroleum.

5.2. Pharmacology

5.2.1. Test of anticonvulsant activity against audiogenic seizures in DBA/2 mice

Experiments were performed with DBA/2 mice which are genetically susceptible to sound-induced seizures [41]. DBA/2 mice (8–12 g, 22–25 days old) were purchased from Charles River (Calco, Como, Italy). Groups of ten mice of either sex were exposed to auditory stimulation 30 min following administration of vehicle or drugs. The compounds were given i.p. (0.1 ml/10 g body weight of the mouse) as a freshly prepared solution in 50% DMSO and 50% sterile saline (0.9% NaCl). Individual mice were placed under a hemispheric perspex dome (diameter 58 cm) and 60 s was 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, and induced a sequential seizure response in control DBA/2 mice, consisting of an early wild running phase, followed by generalised myoclonus and tonic flexion and extension sometimes followed by respiratory arrest. The control (vehicle-treated) and drug-treated mice were scored for latency to and incidence of the different phases of the seizures [27]. The time course of the anticonvulsant action of **2a**, **12a**, and **1** was determined following the administration of 50 μmol/kg of 2,3-benzodiazepines to groups of 10 mice for each time. The animals were tested for sound-induced seizure responses at 5 to 240 min after drug administration.

5.2.2. Maximal electroshock seizure test in Swiss mice

Electrical stimuli were applied via ear-clip electrodes to Swiss mice (rectangular constant current impulses, amplitude 50 mA, width 20 ms, frequency 35 Hz, duration 400 ms) according to the method of Swinyard et al. [42]. Abolition of tonic hindlimb extension after drug treatment was considered as the endpoint of protection. In general, the dose–response curves were estimated by testing four to five doses using eight to ten mice for each dose.

5.2.3. PTZ-induced seizures test in Swiss mice

Male Swiss mice (20–26 g, 42–48 days old) were purchased from Charles River (Calco, Como, Italy) and were pretreated with vehicle or drug 45 min before the subcutaneous (s.c.) administration of pentylenetetrazole. For systemic injections, all 2,3-benzodiazepines were given i.p. (0.1 ml/10 g of body weight of the mouse) as a freshly prepared solution in 50% DMSO and 50% sterile saline (0.9% NaCl). The convulsive dose 97 (CD_{97}) of pentylenetetrazole (85 mg/kg) was applied and the animals observed for 30 min. A threshold convulsion was an episode of clonic spasms lasting for at least 5 s. Absence of this threshold convulsion over 30 min indicated that the tested substance had the ability to elevate pentylenetetrazole seizure threshold [43].

5.2.4. AMPA-induced seizures in DBA/2 mice

Seizures were also induced by i.c.v. injection of AMPA. 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. The CD_{97} (the dose which induced convulsions in 97% of the mice) values of 9.7 nmol for all-limb clonic seizures (latency 1.3 ± 0.3 min) and 11.7 nmol for forelimb tonic seizures (latency 1.9 ± 0.4 min), respectively were used to assess the effects of some 2,3-benzodiazepines on the convulsant properties of AMPA. For i.c.v. injection, mice were anaesthetised with diethyl 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 [44]. Injections of drugs by this procedure led to an uniform distribution throughout the ventricular system within 10 min. The animals were placed singly in a $30 \times 30 \times 30$ cm box and the observation time was 30 min after the administration of AMPA.

5.2.5. Pretreatment with aniracetam

The i.c.v. microinjection of aniracetam was performed according to experimental procedures previously described for AMPA microinjection [44]. The dose of aniracetam (50 nmol i.c.v.) was administered 60

min before auditory stimulation or 30 min before 2,3-benzodiazepines in DBA/2 mice.

5.2.6. Treatment with flumazenil

As previously demonstrated [27], flumazenil, administered i.p. at 8.24 or 24.72 μmol/kg, is not itself convulsant and did not significantly modify the phases of the audiogenic seizure response in DBA/2 mice, but antagonised the anticonvulsant action of classical 1,4-benzodiazepines. Flumazenil was administered i.p. concomitantly to 2,3-benzodiazepine derivatives **12a**, **12e** and **1**.

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

The binding affinities were obtained by the methods previously described by us [28].

5.2.8. Effects on motor movements

Male Swiss mice (20–26 g, 48–54 days old) were purchased from Charles River (Calco, Como, Italy). Groups of ten mice were trained to do coordinated motor movements continuously for 2 min on a rotarod, 3 cm diameter, at 8 r.p.m. (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 [45]. The ability of the mice to remain on the rotarod was tested 30 min after administration of various 2,3-benzodiazepines.

5.2.9. 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 ANOVA followed by post-hoc Dunnett's *t*-test (rectal temperatures). The ED_{50} values of each phase of the audiogenic seizure or seizures induced by MES, PTZ or AMPA were determined for each dose of compound administered and dose–response curves were fitted using a computer program by the method of Litchfield and Wilcoxon [46]. The TD_{50} values were estimated using the method of Litchfield and Wilcoxon [46]. For the binding experiments IC_{50} values for the [^{3}H]flumazenil or other ligands displacement were determined by the non-linear curve-fitting program based on LIGAND [47].

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