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7,8-Methylenedioxy-4*H*-2,3-benzodiazepin-4-ones as Novel AMPA Receptor Antagonists[§]

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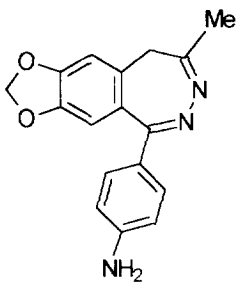
Abstract: The synthesis and anticonvulsant activity of novel 7,8-methylenedioxy-4H-2,3-benzodiazepin-4-ones **3a-e,** structurally-related to GYKI 52466 **1**, a well-known noncompetitive AMPA-receptor antagonist, are reported. The new compounds possess marked anticonvulsant properties and, in analogy to **1**, antagonize seizures induced by AMPA. In addition, when compared to the model compound **1**, compounds **3** show a longer-lasting anticonvulsant activity and a lower toxicity.

Keywords: 7,8-Methylenedioxy-4*H*-2,3-benzodiazepin-4-ones; Anticonvulsant activity; AMPA-receptor antagonists.

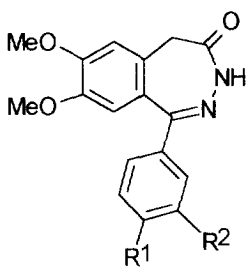
There is a growing interest focused on agents acting selectively on AMPA receptors because of their relevance in the treatment of epilepsy[1-3] and cerebral ischemia.[4,5]

7,8-Methylenedioxy-5*H*-2,3-benzodiazepine GYKI 52466 (**1**, Figure 1) has been identified as a potent and selective noncompetitive AMPA-receptor antagonist that appears to act *via* a novel

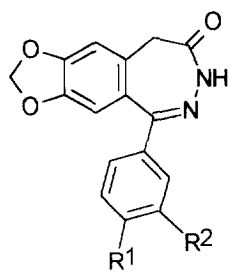
Figure 1



1 (GYKI 52466)



2a-c



3a-e

a, $R^1=R^2=H$
b, $R^1=H$, $R^2=NH_2$
c, $R^1=NH_2$, $R^2=H$
d, $R^1=OH$, $R^2=H$
e, $R^1=MeO$, R^2H

[§] A preliminary account of this work was presented at the First Italian-Swiss Meeting on Medicinal Chemistry, Torino, September 23-26, 1997, Abstract A51 p.105.

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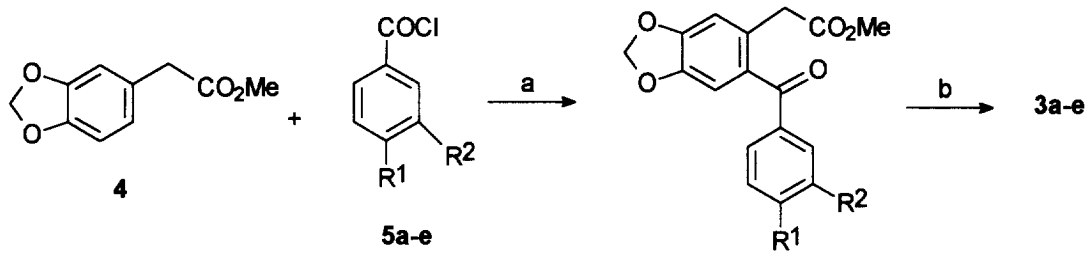
allosteric site on the receptor complex.[6,7] It shows anticonvulsant properties[8-11] and behaves as a neuroprotective agent in focal and global ischemia.[12,13]

We have recently reported the synthesis of a series of 7,8-dimethoxy-4*H*-2,3-benzodiazepin-4-ones (**2**, Figure 1).[14,15] Some of these compounds proved to be more potent than **1** as anticonvulsants in various seizure models. Furthermore, when compared to the reference compound **1**, they showed a longer-lasting activity and a lower toxicity. Electrophysiological assays suggest that compounds **2** act at the AMPA receptor complex *via* a noncompetitive blocking mechanism.[15]

If we compare structures **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 novel 1-aryl-3,5-dihydro-7,8-methylenedioxy-4*H*-2,3-benzodiazepin-4-ones (**3a-e**, Figure 1) as anticonvulsants.

Target compounds were prepared along the reaction sequence reported in Scheme 1. Ketoesters **6** were easily prepared *via* Friedel-Craft acylation of methyl 3,4-methylenedioxyphenylacetate **4** with the appropriate aroyl chloride **5** by using tin(IV) chloride as the catalyst. The subsequent treatment of ketoesters **6** with hydrazine gave **3** in good overall yields (34-70%). When an amino or hydroxy group is present on the phenyl ring, i.e. **3b-d**, the Friedel-Craft acylation step was carried out on trifluoroacetamido- (**5b-c**) or acetoxy-benzoyl (**5d**) chlorides respectively. The subsequent treatment of ketoesters **6b-d** with an excess of hydrazine gave both cyclization and complete deprotection of the amino and hydroxy functionalities to yield directly the final derivatives **3b-d**. The analytical and ^{1}H NMR spectral data of **3a-e** are in full agreement with the proposed structures.

Scheme 1



5a, **6a**: R^1=R^2=H
5b, **6b**: R^1=H, R^2=$NHCOCF_3$
5c, **6c**: R^1=$NHCOCF_3$, R^2=H
5d, **6d**: R^1=OCOMe, R^2=H
5e, **6e**: R^1=MeO, R^2H

a: $SnCl_4/CH_2Cl_2$, 0-5°C, 1h; room temperature, 12h;
b: $NH_2NH_2 \cdot H_2O$, EtOH, reflux, 6-7h.

Derivatives **3a-e** were tested for anticonvulsant activity against audiogenic seizures in DBA/2 mice following a previously described experimental protocol[14] and the results were compared to those recently reported[15] for **1** and **2a-c** (Table 1).

A survey of the results brings about the following considerations: the replacement of the two methoxy groups on the benzene fused ring with a dioxole nucleus seems to have little influence on anticonvulsant activity. As a matter of fact, 7,8-methylenedioxy derivatives **3a-c** display a potency comparable to that of the corresponding compounds **2a-c**. The introduction of a methoxy group at C-4' drastically reduces the activity (ED_{50} >37.2 for **3e** *versus* ED_{50} 12.1 for **3a**). The presence of an amino group in position 3' or 4' increases the anticonvulsant activity, in agreement with our previous results.[15] The presence of an hydroxy group in *para* position also increases the activity, even if less efficiently than the isostere amino group. Finally, a comparison of the structures of **3c** and **1** reveals that the substitution of the azomethine group of **1** with a lactam moiety leads to a significant enhancement in activity.

Table 1.

Anticonvulsant activity of compounds **1**, **2a-c** and **3a-e** against audiogenic seizures in DBA/2 mice[a] and TD50 values on locomotion assessed by rotarod test.

Compound	ED_{50}[b] (mg/kg)		TD_{50} (mg/kg)	TI[c]
	clonic phase	tonic phase	Locomotor deficit	
2a[d]	10.0 (7.71-13.1)	9.41 (7.34-12.0)	42.1 (25.9-67.9)	4.2
3a	12.1 (9.64-15.3)	11.4 (8.44-15.4)	44.6 (23.1-85.7)	3.7
2b[d]	6.00 (5.25-6.84)	5.69 (4.98-6.47)	15.9 (10.5-24.0)	2.7
3b	5.32 (2.95-9.59)	3.74 (1.81-7.74)	29.8 (15.3-57.3)	5.6
2c[d]	4.66 (2.80-7.46)	3.92 (2.49-5.91)	17.4 (12.1-25.4)	3.8
3c	6.44 (3.89-10.6)	3.22 (1.35-7.79)	29.3 (21.4-39.9)	4.5
3d	15.5 (13.2-18.2)	14.7 (12.1-17.8)	44.7 (30.5-65.5)	2.9
3e	> 37.2	> 37.2	>62.1	N.D.[e]
1	10.5 (7.15-24.7)	7.41 (4.68-11.7)	22.3 (13.4-35.8)	2.1

[a]All compounds were given ip 30 min before auditory stimulation.
[b]ED_{50} values with 95% confidence limits were calculated according to the method of Litchfield and Wilcoxon.[20]
[c]TI = Ratio between TD_{50} and ED_{50} (from the clonic phase of the audiogenic seizures).
[d]Reference 15.
[e]N.D. = not determined.

The time course of anticonvulsant activity was also studied in order to discover new compounds with a longer time course than **1**. Following ip administration of **3a** at 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, **1** displayed the maximum protection in interval 5-15 min followed by a gradual return to control seizure response in 30-90 min.

The most active derivatives **3a-c** were also tested 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 ip administration of the tested compounds.

In order to correlate the anticonvulsant activity of novel compounds **3** with affinity for AMPA receptor, an additional test against AMPA-induced seizures in DBA/2 mice was performed (Table 2). The clonic and tonic phases of the seizures induced by icv administration of AMPA were significantly reduced at 30 min after ip administration of **3a-c** analogously to what already reported for **1**, **2a** and **2c**.

Table 2.

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

Compound	ED_{50}[a] (mg/kg) MES tonic phase (Swiss mice)	ED_{50} (mg/kg) PTZ Clonic phase (Swiss mice)	ED_{50} (mg/kg) AMPA[b] clonic phase (DBA/2 mice)	tonic phase	ED_{50} (mg/kg) pretreatment with aniracetam[c] clonic phase (DBA/2 mice)	tonic phase
2a[d]	10.6 (8.01-12.5)	19.1 (15.3-23.9)	18.5 (12.8-26.6)	11.9 (7.40-19.3)	60.2* (39.8-90.8)	44.0* (31.9-60.8)
3a	12.6 (7.82-20.4)	23.1 (13.6-39.1)	20.8 (17.4-24.9)	16.8 (12.1-23.5)	N.D.[e]	N.D.
3b	5.70 (4.99-6.50)	11.9 (6.76-21.2)	8.62 (4.99-14.9)	7.03 (4.19-11.8)	N.D.	N.D.
2c[f]	4.92 (2.26-10.4)	6.99 (3.62-13.5)	9.93 (7.17-13.7)	7.73 (5.10-9.28)	20.2* (13.7-29.7)	18.0* (13.4-24.1)
3c	9.48 (6.85-13.1)	21.2 (15.7-28.6)	11.2 (8.06-15.6)	8.41 (6.08-11.6)	18.5* (13.2-25.9)	11.7* (6.76-20.3)
1	10.5 (8.59-12.7)	20.0 (16.5-24.4)	16.8 (12.7-22.3)	11.9 (7.71-17.8)	39.3* (26.0-59.5)	29.3* (18.6-46.3)

[a] ED_{50} values with 95% confidence limits were calculated according to the method of Litchfield and Wilcoxon.[20]
[b] AMPA was administered icv 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] Reference 14.
[e] N.D.: not detected.
[f] Reference 15.

In the present study we also demonstrated that aniracetam, a potentiator of AMPA effect,[16] markedly antagonized the anticonvulsant effects of **3c** in DBA/2 mice (Table 2) and shifted to the right the dose-response curves with a pattern of activity similar to **1** and **2c**.[15]

On the basis of electrophysiological experiments carried out with derivatives **1** and **2c**,[15] along with the observation that the anticonvulsant properties of **3c** were significantly reduced (from 2.9 to 3.6 times) by aniracetam, a positive allosteric modulator of AMPA receptors,[16] we suggest that the present compounds **3** antagonize the AMPA receptor-mediated responses *via* an allosteric blocking mechanism.

In order to rule out a possible involvement of benzodiazepine receptor, compounds **3a-e** were assessed for the ability to displace [^{3}H]flumazenil from membranes of cortex, following a previously described experimental protocol.[15] No inhibition was observed at up to a concentration of 10 μM (IC_{50} > 10 μM).

The anticonvulsant activity of the title compounds **3** was evident at doses which generally did not cause sedation and ataxia, in agreement with several studies showing that potent antagonists at the AMPA/kainate receptor have anticonvulsant effects at doses below those impairing learning and behaviour.[17-19] It is noteworthy that the present compounds **3** 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).

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 this class of 2,3-benzodiazepines, whereas the presence of a lactam functionality in the heptatomic ring instead of the azomethine moiety present in **1**, appears to be a structural requirement favourable for activity.

In conclusion, the results of the present study indicate that the novel 7,8-methylenedioxy-4*H*-2,3-benzodiazepin-4-ones **3** possess a marked anticonvulsant activity. In particular, derivatives **3a-c** exhibit a higher potency, a longer-lasting anticonvulsant action and a lower toxicity than compound **1**. Moreover, they display anticonvulsant effects against seizures induced by AMPA in agreement with an involvement of AMPA receptors.

Further investigations on this novel class of AMPA receptor antagonists are in progress and the results will be reported in due course.

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