Pergamon

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Bioorganic & Medicinal Chemistry Letters 13 (2003) 443–446

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Characterization of the Mechanism of Anticonvulsant Activity for a Selected Set of Putative AMPA Receptor Antagonists

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Received 19 July 2002; accepted 29 October 2002

Abstract—A selected set of 1-aryl-7,8-methylenedioxy-2,3-benzodiazepin-4-ones and their analogues were evaluated for their ability to bind the competitive and noncompetitive sites of the AMPA receptors complex as well as to the glycine site of the NMDA receptors. The results put in evidence that most of the test compounds, despite a close structural similarity with GYKI 52466, possess a significantly different pharmacological profile.

Evidence suggests that L-glutamate is the major fast excitatory neurotransmitter in the mammalian central nervous system (CNS). Glutamate plays an essential role in many physiological CNS functions through the activation of three major types of postsynaptic ionotropic glutamate receptors designated according to the substances that selectively activate them: *N*-methyl-d-aspartic acid (NMDA), 2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionic acid (AMPA), and kainic acid (KA).[1] The excessive release of glutamate is thought to participate in the pathophysiological cascade of a variety of neurological disorders, including cerebral ischemia, thromboembolic or hemorrhagic stroke, epilepsy, and cerebral or spinal cord trauma.[2,3] Glutamate might also contribute to the progression of chronic neurodegenerative diseases such as Alzheimer's and Parkinson's diseases.[4] Consequently, competitive and noncompetitive antagonists of glutamate receptors are at present attractive therapeutic targets.[5,6]

Although the initial focus was on NMDA receptor antagonists, in the last few years there has been considerable interest in selective AMPA/KA receptor antagonists since prototype compounds have demonstrated significant anticonvulsant and neuroprotective action.[7,8] However, from a structural, pharmacological, and mechanistic point of view, the class of AMPA/KA-receptor antagonists is rather heterogeneous. Thus, characterization of differences in selectivity for AMPA versus KA receptors and in the mechanisms of inhibition may allow for identification of pharmacological features within this broad class that affords particular therapeutic efficacies and/or reduced side effect burden.

As part of a program aimed at identifying potent and selective AMPA receptor antagonists, we previously reported[9,10] an investigation of the anticonvulsant activity of a series of 1-aryl-3,5-dihydro-7,8-methylenedioxy-4*H*-2,3-benzodiazepin-4-ones (Fig. 1, Table 1). These compounds are structurally related to GYKI 52466 (Fig. 2) a noncompetitive antagonist with a high degree of specificity for AMPA over kainate receptors.[11] Within our series, where the iminohydrazone portion of GYKI 52466 was replaced by the iminohydrazide moiety, derivative **2** emerged as the most potent anticonvulsant agent. It was roughly 2-fold more potent than GYKI 52466 and it was provided with a better protective index. Furthermore, by taking into account modifications previously made by other Authors on analogues of GYKI 52466,[12] we prepared a number of 3-*N*-alkylcarbamoyl-derivatives.[13] Introduction of a methyl-carbamoyl group at *N*-3, that is, **3**, further increased the anticonvulsant potency. Worth noting, derivative **3** was also provided with a longer-lasting anticonvulsant activity.

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PII: S0960-894X(02)00955-1

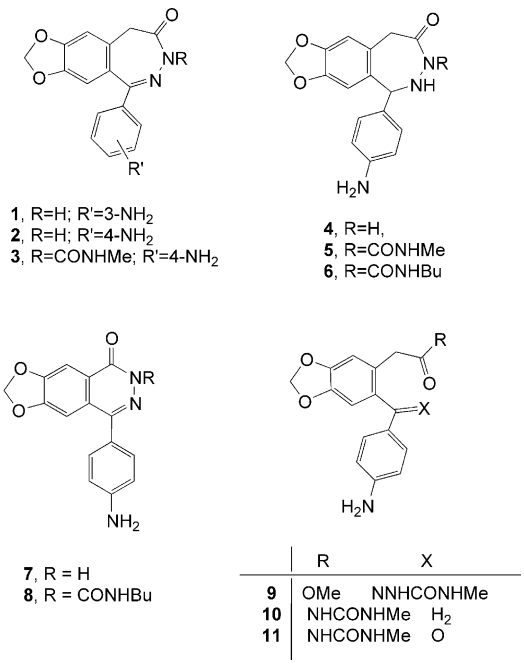

Figure 1.

Table 1. Anticonvulsant activity against audiogenic seizures in DBA/2 mice[a]

Compd	ED_{50}, μmol/kg	
	Clonic phase	Tonic phase
1	18.0 (10.0–32.5)	12.7 (6.13–26.2)
2	15.4 (10.1–23.5)	10.9 (4.60–24.6)
3	12.4 (6.44–23.8)	8.70 (4.61–16.4)
4	11.4 (5.93–22.1)	6.47 (4.20–9.98)
5	14.7 (8.56–25.3)	12.4 (7.93–19.5)
6	39.2 (24.9–61.7)	32.7 (21.3–50.2)
7	21.2 (9.04–49.8)	7.56 (2.47–23.1)
8	3.25 (1.61–6.56)	2.23 (1.32–3.79)
9	7.87 (4.68–13.2)	4.62 (2.47–8.61)
10	9.28 (4.82–17.8)	7.65 (3.79–46.3)
11	39.5 (29.4–53.0)	28.9 (17.9–46.3)
GYKI 52466	35.8 (24.4–52.4)	25.3 (16.0–40.0)

[a]Data taken from refs 15–17.

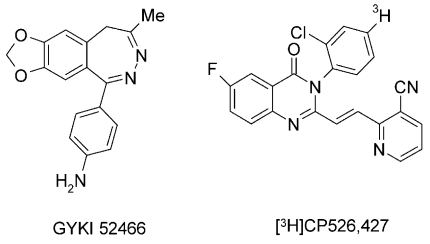

Figure 2.

In our ongoing studies on the structure–activity relationship (SAR) of 2,3-benzodiazepine derivatives,[14] we have recently prepared a number of 1,2,3,5-tetrahydro-2,3-benzodiazepines, for example, **4–6**,[15] as well as a series of novel phthalazin-1(2*H*)-ones, e.g., **7–8**,[16] in order to check the influence of both the 1,2-azomethine moiety of the diazepine nucleus and the size of the heterocyclic ring on the anticonvulsant activity. The results showed that some tetrahydroderivatives, for example, **4**, were provided with a noteworthy anticonvulsant activity. Such derivatives were characterized by an induction time and a longer-lasting activity if compared to the corresponding unsaturated analogues, for example, **2**. On the other hand, the activity of the phthalazino derivatives was strictly dependent upon the size of the alkyl group of the carbamoyl moiety appended at *N*-2; the peak value was reached with the *n*-butyl group (**8**). Based on these results, we designed and tested a series of 4,5-methylendioxyphenyl acetic acid derivatives bearing an *N*-alkyl-semicarbazono moiety at position 1 or 2, for example, **9–11**, that may be envisaged as 'open models' of reference compound **3**.[17] Compound **9** possessed an anticonvulsant activity slightly higher than that of its parent derivative **3** and, similarly to **3**, was provided with a longer-lasting activity when compared to GYKI 52466.

Given that some of the compounds in our series displayed improved anticonvulsant efficacy and reduced side effect burden compared to GYKI 52466, it was of utmost importance to ascertain if these heterogeneous compounds display their biological activities through an identical mode of action. Therefore, we selected the most representative derivatives among the different classes of compounds and evaluated their affinity for the different binding sites on AMPA and NMDA receptors. In particular it was of interest to determine whether these compounds interact with the AMPA receptor in a manner analogous to GYKI 52466. Recently, the binding site for GYKI 52466, the prototype of a series of 2,3-benzodiazepines, has been pharmacologically identified using the radioligand [^{3}H]CP-526,427[18] (Fig. 2). The latter compound is one of a class of newly identified quinazolin-4-ones that, like GYKI 52466, are highly specific noncompetitive AMPA receptor antagonists. [^{3}H]CP-526,427 binds with high affinity ($Kd = 3.3$ nM) to a single site in rat forebrain membranes. Affinity of several quinazolinedione analogues for the [^{3}H]CP-526,427 binding site correlates closely with the ability of these compounds to inhibit AMPA receptor mediated responses.[18] Furthermore, it has been demonstrated that the [^{3}H]CP-526,427 binding site overlaps with that of GYKI 52466 and is apparently independent of both the agonist-binding site and the site(s) involved in receptor desensitization. Worth noting, such a radioligand was not displaced by the dye Evans blue, thus suggesting the existence of at least two sites through which AMPA receptor activity can be allosterically inhibited: the quinazolinone/2,3-benzodiazepine site and the Evans blue site.[18]

Thus, compounds **1–11** were examined for their ability to displace [^{3}H]CP-526,427 and [^{3}H]AMPA from the corresponding binding sites of the AMPA receptors complex and [^{3}H]glycine from NMDA receptors (Table 2). The active compounds were also tested in a functional assay, inhibition of KA-induced increase in [Ca^{2+}]i in rat cerebellar granule neurons (Table 2).

The inhibition of [^{3}H]CP-526,427 specific binding (3 nM) to rat forebrain membranes caused by compounds **1–11**

Table 2. Pharmacological data of compounds **1–11**

Compd	[^{3}H]CP-526,427[a]		KA-[Ca^{2+}]i	[^{3}H]AMPA	[^{3}H]glycine
	IC_{50} (μM)	I%[b]	IC_{50} (μM)	IC_{50} (μM)[c] or I%[d]	IC_{50} (μM)[c] or I%[d]
1	> 320	31	> 320	18%	0%
2	32	74	13	24%	0%
3	12	59	11	21%	0%
4		10	> 100	0%	9%
5		0	> 100	3%	12%
6		0	> 100	0%	13%
7		13	> 100	3%	12%
8		0	> 100	0%	13%
9		16		0%	2%
10		0		15%	0%
11		3		0%	11%
GYKI 52466	12.6		22	0%	18%
NBQX	—		—	0.07	3%
DCKA	—		—	5%	0.09

[a]Applied (3 nM) to rat forebrain membranes.
[b]Percent inhibition (I%) of specific binding at 32 μM.
[c]Means of three separate determinations in triplicate.
[d]Percent inhibition (I%) of specific binding at 100 μM was based on two separate assays in triplicate.

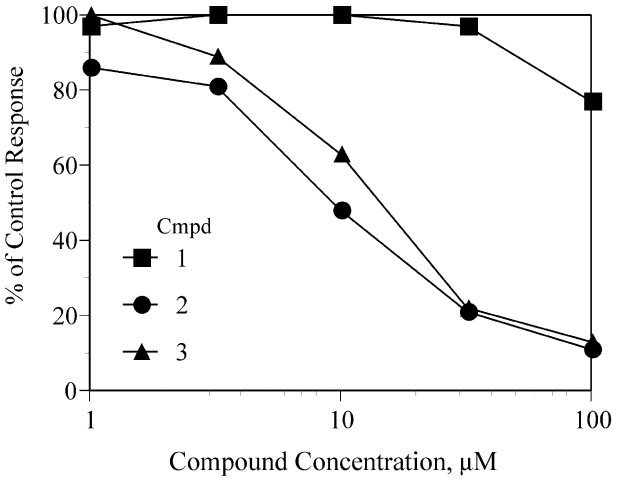

Figure 4. Data are expressed as the % of control response; control response is the increase in [Ca^{2+}]i caused by kainate in the absence of added competitor. Each data point is the mean of triplicates from a single assay.

was evaluated as previously described.[18,19] In this assay, the quinazolindione CP-465,022 was used as a positive control; its IC_{50} values spanned the range 20–40 nM, consistent with previous reported data.[18] Submitted to such a protocol, compound **1**, and **4–11** turned out to be either marginally active (**1**: IC_{50} > 320 μM) or totally inactive whereas compounds **2** and **3** inhibited [^{3}H]CP-526,427 specific binding in the 10–30 μM range in a dose related fashion (Fig. 3). The IC_{50} values found for **2** and **3** were 32 and 12 μM, respectively; values similar to that reported for GYKI 52466 (IC_{50} equal to 12.6 μM). Compounds **1–8** were then tested for their ability to inhibit the kainate-induced increase in $[Ca^{2+}]_i$ in rat cerebellar granule neurons in primary culture (Table 2, Fig. 4).

The results of such an investigation confirmed the data of the binding experiments. Compound **1** turned out to be marginally active; derivatives **2** and **3** were slightly more potent than GYKI 52466, the reference compound (IC_{50} 13 and 11 μM for **2** and **3** versus 22 μM for GYKI 52466), whereas **4–8** showed no concentration-dependent inhibition up to 100 μM. As a consequence, the data of the binding assay correlate very well with the ability of the compounds to inhibit the functional response in the cerebellar granule neurons.

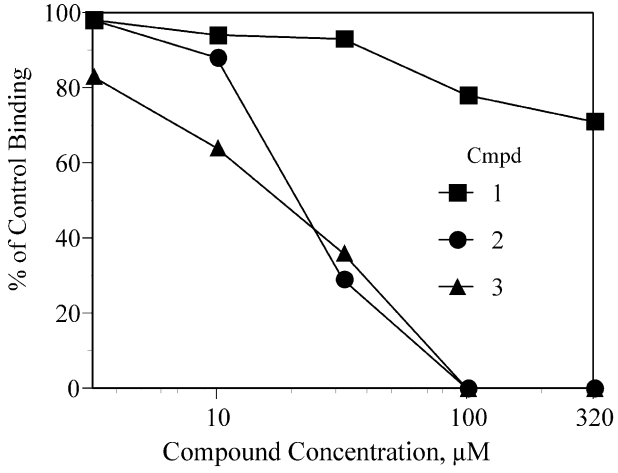

Figure 3. Data are expressed as the % of control binding; control binding is the specific binding in the absence of added competitor. Each data point is the mean of duplicates from a single assay.

On the other hand, the data collected in the present investigation do not correlate with the anticonvulsant activity of derivatives **1–11** (Table 1) obtained in an in vivo test on DBA/2 mice,[15–17] since almost all the compounds are more potent than GYKI 52466. For such a reason we performed further pharmacological investigations.

We assayed our compounds as competitive antagonists through the evaluation of their ability to displace [^{3}H]AMPA from its binding site (Table 2). Compounds **1–11** and reference compound NBQX (2,3-dihydroxy-6-nitro-7-sulfamoylbenzo[*f*]quinoxaline) were tested in a binding assay performed on rat cortical membranes.[20,21] The results indicate that compounds **1–3** and **10** possess a sizeable binding affinity for the AMPA recognition site at variance with derivatives **4–9**, **11**, and GYKI 52466[11] that are devoid of any affinity for such a receptor site.

Furthermore, we evaluated the effectiveness of compounds **1–11** to displace [^{3}H]glycine from its binding site and the results are herewith compared with that of DCKA (5,7-dichlorokynurenic acid), the reference compound. As shown in Table 2, derivatives **4–8** and **11** possess a marginal affinity for the glycine-binding site of the NMDA receptors, in analogy to GYKI 52466, whereas the remaining compounds are unable to displace [^{3}H]glycine from its binding site.

To rule out the possibility that the anticonvulsant effect of compounds **1** and **4–11** could be mediated by the activation of the $GABA_A$-receptor, compounds **7–9**

were selected and tested on GABA-evoked currents. They not only were devoid of any positive modulatory activity on GABA-currents but showed a feeble decrease of the current (**7**: $-13\pm7\%$, $n=8$, **8**: -8 ± 4, $n=9$, **9**: -7 ± 11, $n=8$), which could be ascribed to the solvent (DMSO) since it produced a similar effect ($-8\pm5\%$, $n=3$) when administered by itself.

In conclusion, among the set of the examined derivatives, compounds **2** and **3**, the closest analogues of GYKI 52466, display their anticonvulsant activity mainly by blocking the allosteric AMPA-receptor binding site. Derivatives **1**–**3**, along with **10**, behave also as weak competitive antagonists at the AMPA binding site at variance with the structurally related GYKI 52466. The remaining compounds do not interact with either the GYKI 52466- and AMPA-receptor binding sites. Furthermore, a marginal or no interaction was noticed with the glycine-binding site of the NMDA receptors as well with the inhibitory $GABA_A$-receptor complex. As a consequence further investigations are needed to account for the anticonvulsant activity of derivatives **1**, and **4**–**11** observed in the in vivo test performed on DBA/2 mice.

At the moment we can assess that the remarkable anticonvulsant activity of compounds **1**–**11** derives from a different mode of action and, consequently, no general structure–activity relationship can be drawn. It is noteworthy that inhibition of AMPA or NMDA receptors, in addition to providing anticonvulsant efficacy is also associated with considerable side effects. Thus, elucidating the mechanism of action of these compounds may pave the way for identification of a new therapeutic target for the treatment of epilepsy that avoids the side effect liability of the currently available therapies.

Acknowledgements

This work was financially supported by the Ministero dell'Istruzione, dell'Università e della Ricerca (MIUR-COFIN2001), Rome, Italy. We thank Martin J. Pagnozzi, Mary A. Collins and John T. Lazzaro of Pfizer Inc. for excellent technical assistance.

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