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1-Aryl-6,7-methylenedioxy-3*H*-quinazolin-4-ones as Anticonvulsant Agents

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Abstract—A set of novel 1-aryl-6,7-methylenedioxy-3*H*-quinazolin-4-(thi)ones (**3a**–**f**) has been designed and screened as anticonvulsant agents in DBA/2 mice. The new compounds are provided with anticonvulsant properties comparable to those of GYKI 52466. To clarify the mode of action, their affinity for the quinazolinone/2,3-benzodiazepine site of the AMPA receptor complex has been assayed.

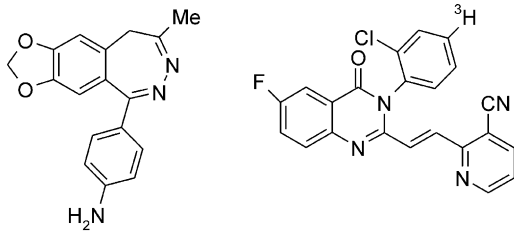
In the human brain more than fifty percent of all synapses use glutamate (Glu) as a transmitter. The postsynaptic activity of Glu is mediated by both ionotropic receptors (iGluRs), ligand-gated ion channels, and metabotropic receptors (mGluRs) which are coupled to G-proteins. The iGluRs are constituted by different subunits and classified into the following three heterogeneous types based on the acronym of specific agonists: NMDA (*N*-methyl-D-aspartic acid), AMPA [(*R,S*)-2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionic acid] and KA (kainic acid) receptors.[1,2]

It is now widely accepted that in ischemic or hypoxic conditions such as stroke, trauma and epilepsy an excessive amount of endogenous Glu accumulates extracellularly, resulting in overstimulation of iGluRs causing neuronal death.[3,4]

In the last few years there has been considerable interest in noncompetitive AMPA receptor antagonists since prototype compounds, that is, GYKI 52466 (Fig. 1), a 2,3-benzodiazepine derivative, have demonstrated significant anticonvulsant[5,6] and neuroprotective[7,8] action, thus providing the basis for an extensive research in this area.

Recently, one of us has pharmacologically identified the binding site for GYKI 52466 using the radioligand [^{3}H]CP-526,427 (Fig. 1).[9] This radioligand is a member of a set of new quinazolin-4-ones that, like GYKI 52466, are highly specific antagonists of the noncompetitive AMPA receptors binding site.

In our ongoing studies aimed at identifying novel anticonvulsants based on the noncompetitive AMPA receptor antagonist template, we synthesized a number of 7,8-methylenedioxy-4*H*-2,3-benzodiazepin-4-ones, for example, **1e**, as well as their 6,7-methylenedioxyphthalazin-1(2*H*)-one analogues, for example, **2e**. (Fig. 2) These compounds have been shown to possess remarkable anticonvulsant properties.[10–13] In this context, we



GYKI 52466 [^{3}H]CP526,427

Figure 1.

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demonstrated that compound **1e** interacts with the same allosteric AMPA receptor binding site of GYKI 52466. Surprisingly, however, derivative **2e** does not bind at either the GYKI 52466- or AMPA-receptor binding sites.[14]

Thus, we designed and screened for anticonvulsant activity new 1-aryl-6,7-methylenedioxy-3*H*-quinazolin-4-ones (**3a–3f**), envisaged as structural hybrids between derivatives **1**, **2** and CP-526,427 (Fig. 3).

To clarify their mode of action, the affinity of the compounds for the quinazolinone/2,3-benzodiazepine site on the AMPA receptor complex has been also assayed.

Target compounds **3a–f** were obtained according to the reaction sequences reported in Scheme 1. 4,5-Methylenedioxy-2-aminobenzamide (**6**) was prepared from commercially available 3,4-methylenedioxy-benzonitrile (**4**). by standard reactions (Scheme 1). Condensation of intermediate **6** with the appropriate aromatic aldehyde, in the presence of a catalytic amount of *p*-toluenesulfonic acid, yielded 2-aryl-6,7-methylenedioxy-3*H*-quinazolin-4-ones (**3a–d**). It is worth pointing out that the intermediate 1,2-dihydro derivatives could not be isolated due to a spontaneous oxidation to the final derivatives. On the contrary, the related reaction with 4-nitrobenzaldehyde allowed the isolation of 1,2-dihydro derivative **7**. Surprising enough, the subsequent reduction of its nitro group was accompanied by a concomitant spontaneous oxidation of the heterocycle to give derivative **3e** as the sole isolated product. Compound **3e** was also fruitfully obtained by condensing nitroamide **5** with 4-nitrobenzoyl chloride, in the presence of 4-dimethylaminopyridine (DMAP), to yield *N*-(4-nitro-benzoyl)-4,5-methylenedioxy-2-nitrobenzamide (**8**). The subsequent reduction of the nitro groups of **8**, carried out with Raney-Ni/ammonium formate, brought about the ring closure to derivative **3e**. Compound **3f** was obtained by refluxing a dioxane solution of **3e** with the Lawesson's reagent. Both analytical and ^{1}H NMR spectral data of all the synthesized compounds are in full agreement with the proposed structures.[15]

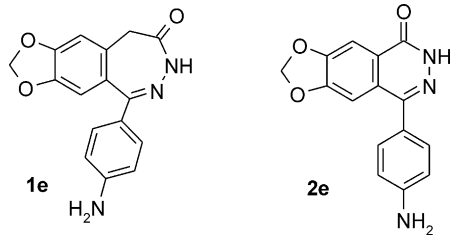


Figure 2.

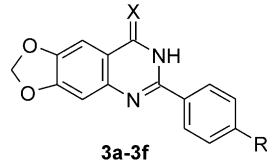


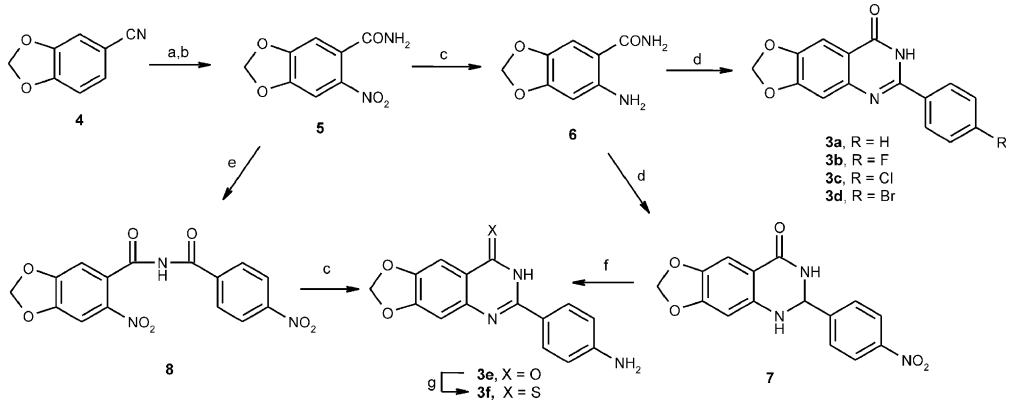
Figure 3.

The anticonvulsant activity of derivatives **3a–f** against audiogenic seizures was evaluated 30 min after intraperitoneal administration at DBA/2 mice, a strain genetically susceptible to sound-induced seizures. This test has been considered an excellent animal model for generalized epilepsy and for screening new anticonvulsant drugs.[16] The results are compared with those previously reported for derivatives **1** and **2** as well as for GYKI 52466, the reference compound (Table 1).[13]

As shown in Table 1, some of the new compounds (**3b**, **3c**, **3e**, **3f**) possess anticonvulsant properties comparable to those of GYKI 52466. Nevertheless their activity is lower than that displayed by structure analogues **1e** and **2e**.

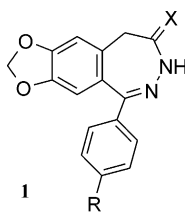
The unsubstituted phenyl derivative **3a**, similarly to **1a** and **2a**, is less active than GYKI 52466; the insertion the the 4-amino moiety enhanced significantly its anticonvulsant activity. This result parallels those previously noticed in the 2,3-benzodiazepine and phthalazine series.

The insertion of an halogen in the 4-position of the phenyl ring (**3b–d**) seems also fruitful. It is worth noting that the anticonvulsant activity is dependent upon the kind of the halogen. As a matter of fact, whereas the

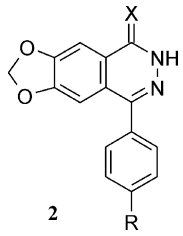


Scheme 1. (a) HNO_3, H_2SO_4, AcOH, Δ; (b) Percarbamide, Na_2CO_3/Me_2CO–H_2O, rt, 18 h; (c) Ni/Raney, $HCOONH_4$/MeOH, Δ, 2 h; (d) ArCHO, *p*-TsOH, C_6H_6 Δ, 3–4 h; (e) 4-nitrobenzoyl chloride, DMAP/benzene, Δ, 24 h; (f) Zn, NH_4Cl, EtOH/H_2O, rt, 1 h; (g) Lawesson's reagent, dioxane, Δ, 24 h.

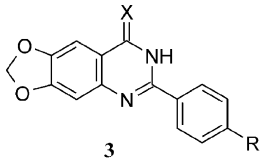
Table 1. Anticonvulsant activity against audiogenic seizures in DBA/2 mice[a]



1



2



3

Compd	R	X	ED_{50} μmol/kg[b]		ED_{50} μmol/kg[b]		ED_{50} μmol/kg	
			Clonus	Tonus	Clonus	Tonus	Clonus	Tonus
a	H	O	43.3 (34.4–54.6)	40.6 (30.1–54.9)	36.7 (24.4–55.1)	31.3 (19.4–50.6)	59.5 (31.4–113)	46.1 (21.7–97.8)
b	F	O	89.8 (69.1–105)	63.8 (45.1–90.2)			32.7 (21.3–50.2)	27.8 (18.8–41.0)
c	Cl	O					36.5 (23.6–56.5)	21.6 (11.4–40.8)
d	Br	O					68.3 (47.8–97.5)	36.6 (14.9–89.8)
e	NH_2	O	15.4 (10.1–23.5)	10.9 (4.60–24.6)	21.2 (9.04–49.8)	7.56 (2.47–23.1)	27.8 (15.3–51.1)	17.7 (4.64–67.4)
f	NH_2	S	11.8 (6.14–22.5)	5.09 (2.14–12.1)	15.2 (6.52–35.4)	8.94 (4.02–19.9)	37.8 (17.6–81.3)	17.9 (7.82–40.8)
f[c]	NH_2	S	5.9 (4.70–7.41)				79.4 (42.2–149)	
GYKI 52466			35.8 (24.4–52.4)	25.3 (16.0–40.0)				

[a]All compounds were given ip (at doses spanning the range 3.3–200 μmol/kg) 30 min before auditory stimulation. All data were calculated according to the method of Litchfield and Wilcoxon;[18] 95% confidence limits are given in parentheses. At least 32 animals were used to calculate each ED_{50} value.
[b]Taken from references 11–13.
[c]ED_{50} (clonus) at 15 min after ip administration.

4-F substituted derivative (**3b**) is the most potent member of the series, followed by the 4-Cl analogue (**3c**), and both are more potent than unsubstituted derivative **3a**, the 4-Br derivative (**3d**) is the least potent of the set and is even less potent than **3a**. The different contribution of the halogens to the anticonvulsant activity could be due to electronic effects, that is, a different ionic radius, and/or to a difference in lipophilicity.

The replacement of the carbonyl group with the more lipophylic thiocarbonyl moiety (**3f**) gave a decrease in potency, at variance with trend previously observed in the series of derivatives **1** and **2**. Furthermore, such a bioisosteric modification does not produce the short-time activity noticed in derivative **1f** and attributed to a more favorable diffusion across the blood–brain barrier due to an increased lipophilicity.[12] As shown in Table 1, the audiogenic seizure test, performed at 15 min rather than at 30 min after ip administration, gave for derivative **3f** a minor anticonvulsant activity.

Compounds **3a–c** and **3e** were also examined for their ability to displace [^{3}H]CP-526,427 from the corresponding binding site of the AMPA receptor complex. The inhibition of [^{3}H]CP-526,427 specific binding (3 nM) to rat forebrain membranes was evaluated as previously described.[9,17] In this assay, the quinazolinone CP-465,022 was used as a positive control; its IC_{50} values spanned the range of 20–40 nM. Analogously to derivative **2e**, compounds **3a–c** and **3e** turned out to be totally inactive ($IC_{50} > 100$ μM), differently to what was observed for compound **1e** which showed $IC_{50} = 32$ μM, similar to that reported for GYKI 52466 ($IC_{50} = 12.6$ μM).

Compounds **3a–c** and **3e** were also tested for their ability to inhibit the kainate-induced increase in [Ca^{2+}]i in rat cerebellar granule neurons in primary culture. The results of such an investigation confirmed the data of the binding experiments. Compounds **3a–c** and **3e**, as well as phthalazine derivative **2e**, showed no concentration-dependent inhibition up to 100 μM, whereas dervative **1e** was slightly more potent than GYKI 52466 (IC_{50} 13 μM for **1e** vs IC_{50} 22 μM for GYKI 52466).

In summary, 2-aryl-6,7-methylenedioxy-3*H*-quinazolin-4-ones **3** possess an anticonvulsant activity comparable to that of GYKI 52466 and, similarly to phthalazine derivatives **2**, do not interact with the quinazolinone/2,3-benzodiazepine site on the AMPA receptor complex. Thus, at present their mode of action is unknown. A comparison of the biological data of derivatives **3** with those of the phthalazine derivatives **2** puts in evidence that the inversion of the azomethine moiety marginally affects the anticonvulsant activity. Further investigations are needed to account for the anticonvulsant activity of derivatives **3**, as well as of phthalazine derivatives **2**, observed in the in vivo test performed on DBA/2 mice.

Acknowledgements

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15. Selected data for compound **3e**: mp $<300\,^{\circ}C$. ^{1}H NMR (300 MHz, $CDCl_3$) 4.07 (bs, 2H, NH_2), 6.12 (s, 2H, CH_2), 6.79 (d, 2H, $J=8.2$, H-3′,5′), 7.16 (s, 1H, H-5), 7.29 (s, 1H, H-8), 7.83 (d, 2H, $J=8.2$, H-2′,6′), 9.63 (bs, 1H, NH).
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