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Quinazolinedione sulfonamides: A novel class of competitive AMPA receptor antagonists with oral activity

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ABSTRACT

Quinazoline-2,4-diones with a sulfonamide group attached to the N(3) ring atom constitute a novel class of competitive AMPA receptor antagonists. One of the synthesized compounds, **28**, shows nanomolar receptor affinity, whereas other examples of the series display oral anticonvulsant activity in animal models.

The ionotropic glutamate receptors (iGluRs) are a family of ion channels that are divided into the three subtypes *N*-methyl-D-aspartate (NMDA), (*S*)-2-amino-3-(3-hydroxy-5-methyl-4-isoxazolyl)propionic acid (AMPA), and kainate receptors. The channels are permeable for Na^+, K^+ and Ca^{2+} and mediate excitatory synaptic transmission. Over-stimulation of these receptors causes an uncontrolled Ca^{2+}-influx into the cells resulting in excitotoxicity and possible cell death. Several disorders are—at least in part—linked to over-activity of iGluRs, such as epilepsy, chronic pain or neuropathology ensuing from cerebral ischemia or cardiac arrest.[1] Different types of antagonists acting at various sites of these receptors were shown to have anticonvulsant, neuroprotective or antinociceptive effects in a range of animal models. Therefore, iGluRs are considered as interesting drug targets, and in particular AMPA receptors may offer the opportunity for therapeutic intervention[2] without the side effects associated with inhibition of NMDA receptors.[3]

AMPA receptors are composed of four different subunits, GluA1-4, which likely assemble to functional tetrameric heteromers.[4] Each subunit consists of three membrane spanning segments, a reentrant loop, an intracellular carboxy terminal domain and an extracellular glutamate binding domain. Since many of the diseases assumed to be related to defects of AMPA receptors are chronic, oral activity will be an important advantage for a drug blocking AMPA receptors. In this Letter we present a novel series of competitive AMPA receptor antagonists of which several examples show oral activity in animal models for anticonvulsant activity.

Since the early 1990s a number of heterocyclic compounds have been known to be competitive AMPA receptor antagonists, bearing, as a common structural feature, an acidic group attached to a heterocyclic ring system. Examples are the broadband antagonist kynurenic acid(I)[5], 3-oxo-3,4-dihydro-quinoxaline-2-carboxylic acid(II)[6], or the potent and selective quinoxalinediones CNQX(III), YM90K(IV)[7] and AMP397(V).[8] At physiological pH, quinoxalinediones are deprotonated with the negative charge located on the oxygen atom[9] (VIb).

In search for novel scaffolds endorsing AMPA receptor antagonism, we designed cyclic hydroxamic acids(VII) with a hydroxyl group in position 3, that is, similar to the position of the negatively charged oxygen atom of (VIb). The N–OH functionality of (VII) has a pK_a value of 7–7.5, consistent with a partial deprotonation at physiological pH. Several compounds of type VII with various substituents on the benzene ring were prepared and some were found to be competitive antagonists at AMPA receptors.[10] However, the compounds were not active after oral administration in animal models testing for anticonvulsant effects; at best, activity after intraperitoneal administration was found. In general, these

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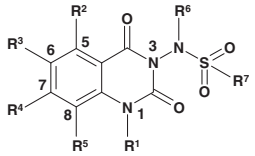
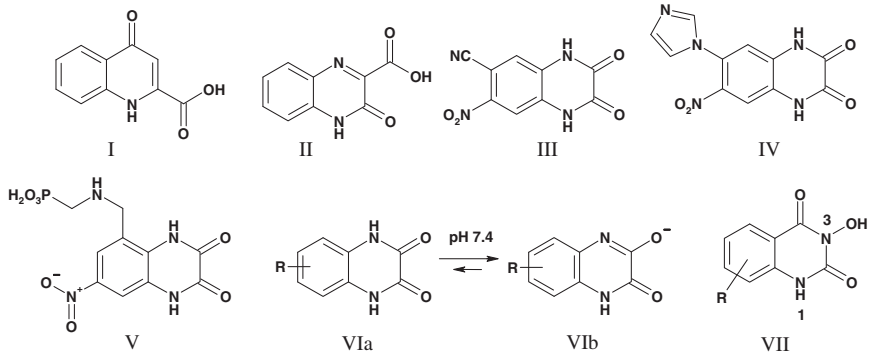
Table 1
Binding affinity[a] of variously substituted quinazolinedione sulfonamides at AMPA receptors and at the NMDA-receptor associated glycine site



Cmpd.	R^1	R^2	R^3	R^4	R^5	R^6	R^7	AMPA IC_{50} (μM) or conc. (μM)/%inhibition	NMDA/Gly
1	H	H	H	H	H	H	Me	57	100/48%
2	H	Cl	H	H	H	H	Me	29	100/34%
3	H	H	Cl	H	H	H	Me	34	100/50%
4	H	H	H	Cl	H	H	Me	2.4 ± 0.8	10/32%
5	H	H	H	H	Cl	H	Me	>100	100/37%
6	Me	H	H	Cl	H	H	Me	100/45%	100/36%
7	H	H	H	Cl	H	Me	Me	100/12%	100/18%
8	H	H	H	CN	H	H	Me	0.75	10/28%
9	H	H	H	CF_3	H	H	Me	0.92 ± 0.08	17.8 ± 2.7
10	H	H	H	NO_2	H	H	Me	1.2	10/16%
11	H	H	H	Br	H	H	Me	2.2	10/49%
12	H	H	H	MeO	H	H	Me	3.7	10/22%
13	H	H	H	F	H	H	Me	8.2	10/17%
14	H	H	H	$MeSO_2$	H	H	Me	10/42%	>100
15	H	H	H	COOH	H	H	Me	10/0%	n.t.
16	H	H	H	CH_3	H	H	Me	9.7	10/13%
17	H	H	H	Ethyl	H	H	Me	3.0	n.t.
18	H	H	H	Vinyl	H	H	Me	1.9	>10
19	H	H	H	*t*-Butyl	H	H	Me	4.9	>100
20	H	H	H	Cyclopentyl	H	H	Me	10/25%	n.t.
21	H	H	H	Phenyl	H	H	Me	10/36%	100/36%
22	H	H	H	Phenethyl	H	H	Me	1.1	10/26%
23	H	H	H	Cl	H	H	Ethyl	2.3	35.0 ± 13.7
24	H	H	H	Cl	H	H	Phenyl	4.9	9.3
25	H	H	H	Cl	H	H	Benzyl	2.9	0.21 ± 0.06
26	H	H	H	Cl	H	H	*N*-Methyl-(4)imidazolyl	1.6 ± 0.3	n.t.
27	H	H	1-Imidazolyl	Cl	H	H	Me	0.52 ± 0.16	17.2 ± 2.2
28	H	H	1-Imidazolyl	NO_2	H	H	Me	0.082 ± 0.001	10/24%

n.t. = not tested.
[a] Values are single determinations performed in triplicate or means ± s.e.m. of 2–4 independent determinations performed in triplicate.

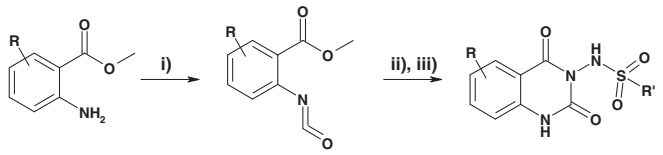


Scheme 1. Known scaffolds for competitive AMPA receptor antagonism.

compounds are highly polar, have high melting points (>300 °C) and low solubility at pH 7.4, properties not favoring absorption or brain penetration.

This prompted us to replace the hydroxyl group of VII by a bioisosteric group of similarly weak acidity but possibly more extended charge distribution after deprotonation. One concept to attain this is the replacement of an electronegative atom by an atom of lower electronegativity but substituted with an electron-withdrawing group, thereby restoring the electron attracting power of the original electronegative atom. This concept was realized with the quinazolinedione N(3)-sulfonamide **4** (see Table 1), where the *N*-hydroxy-group of VII is replaced by the NH–methylsulfone group.[11] Compound **4**, with a pK_a value of 6.9, was found to inhibit [^{3}H]CNQX-binding at the AMPA receptor with an IC_{50}-value of 2.4 μM. In addition, **4** was orally active in the E-shock test in mice where it inhibited convulsions with an ED_{50}-value of 23 mg/kg after a pretreatment time of 1 h. With this encouraging result we started a derivatization program to search for compounds with improved receptor affinity and possibly better oral activity.

The quinazolinedione sulfonamides[12] were synthesized as outlined in Scheme 2. The substituted anthranilic esters were treated with phosgene and the obtained isocyanates were further reacted



Scheme 2. General access to the quinazolinedione sulfonamides **1**–**5**, **7**–**26**. (i) Phosgene gas/phosgene in toluene 20%, reflux, 80–100%; (ii) H_2NNHSO_2R', THF, rt [for **7** $H_2NN(CH_3)SO_2CH_3$ was used]; (iii) addition of 1 M NaOH aq, rt, 42–85% (two steps).

with the corresponding sulfonyl hydrazide in THF. Ring closure was performed by adding aqueous sodium hydroxide to the reaction mixture.

The N(1)-methylated compound **6** was obtained by N(1)-methylation of 3-amino-7-chloro-1H-quinazoline-2,4-dione followed by mesylation of the hydrazide group (Scheme 3).

The imidazole group of compounds **27** and **28** was introduced in two different ways (Scheme 4): For **27** the 6-nitro derivative was reduced and the imidazole ring built up from the 6-amino group by using slightly acidic conditions. Direct replacement of the fluorine by imidazole in the 7-nitro derivative gave **28** in moderate yield.

Binding experiments were performed with rat brain homogenates, based on procedures described in the literature and using the radioligand [^{3}H]CNQX for the AMPA recepor[13] and [^{3}H]MDL105519 for the glycine site on the NMDA receptor (NMDAR1).[14]

The unsubstituted compound **1** displays only weak binding affinity at the AMPA receptor (Table 1). To identify the most sensitive position for substitution to possibly improve affinity, we compared the four chloro-derivatives **2**–**5** and found the best activity with compound **4**, bearing the chlorine atom in position 7. Both H-bond donors are mandatory for affinity, as illustrated by the loss of activity of the *N*-methyl derivatives **6** and **7**.

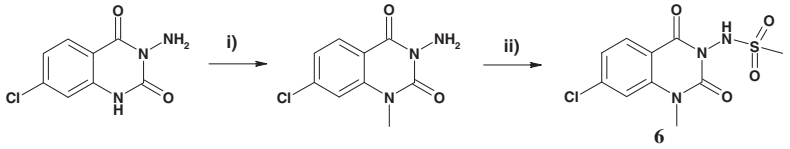
The chlorine atom in position 7 can be replaced without loss of affinity by other small groups such as nitrile, trifluoromethyl, nitro, bromo, methoxy or fluoro (**8**–**13**), whereas methylsulfone, **14**, and carboxylate, **15**, are less tolerated. Alkyl groups in position 7 lead to compounds with affinities at the AMPA receptor in the low micromolar range (**16**–**19**); however, the cyclopentyl derivative **20** displayed low affinity. Phenyl substitution, as in **21**, has little influence on affinity (compared to the unsubstituted **1**), whereas a phenyl group separated by an ethyl spacer in position 7 (phenethyl, **22**) improves affinity. Furthermore, it was of interest to examine the space neighboring the methyl sulfonamide side chain by attaching larger substituents to the sulfone group. To this end, the methyl group in compound **4** was replaced by ethyl, phenyl, benzyl and *N*-methyl-(4)imidazolyl (**23**–**26**). All four derivatives have about the same affinity as compound **4**, indicating that no marked interaction with the receptor takes place in this position.

Several quinoxalinediones substituted in position 6 with an 1-imidazolyl group are strong AMPA receptor antagonists (such as IV, Scheme 1).[7,15] We speculated that analogous substitution in the quinazolinedione sulfonamide series would have a beneficial effect and therefore prepared the compounds **27** and **28**. Both derivatives have considerably higher receptor affinity compared to their mono-substituted counterparts, with an improvement by a factor of 5 for **27** (vs **4**) and of 15 for **28** (vs **10**). The low IC_{50}-value (82 nM) for **28** demonstrates that nanomolar binding affinity can be achieved within this new class of compounds as AMPA receptor antagonists.[16]

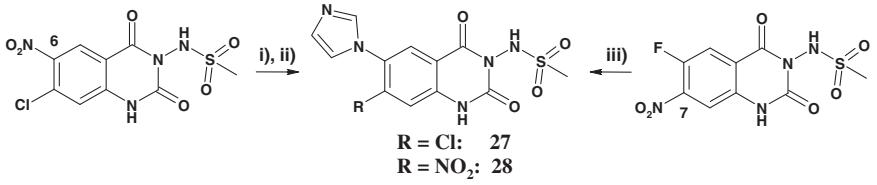
Previously, Armstrong and Gouaux[17] explored the binding mode of quinoxalinediones at the AMPA receptor by co-crystallizing the potent antagonist 6,7-dinitro-1,4-dihydro-quinoxaline-2,3-dione (DNQX) with a construct of the GluA2 flop ligand binding core of the rat receptor. By using a similar methodology we prepared a construct of the human receptor hGluA2 and successfully co-crystallized it with the antagonist **28**.[18] An X-ray structure with a resolution of 2.1 Å was obtained and demonstrated the analogy of the binding mode of the two chemotypes (Fig 1).

Compound **28** is oriented such that a favorable π-stacking with Tyr450 (for ease of comparison, the numbering of hGluA2 was modified to the numbering of mature rat GluA2) is possible, and a network of hydrogen bonds is formed. The sulfonamide nitrogen interacts with H_3N^+–Arg485, indicating that this nitrogen is negatively charged, which enables a strong Coulomb interaction. The ring HN(1)-group donates a hydrogen bond to the carbonyl group of Pro478 which may explain the drop of activity of compound **6**. The imidazole ring forms a hydrogen bond to the side chain hydroxyl group of Thr686, and the adjacent nitro group makes water mediated contacts to Tyr405 and Thr707.

The ligand binding domain of the AMPA receptor shows a high degree of homology to that of the glycine site on the NMDA receptor.[19] We, therefore, tested the binding affinity of the quinazolinedione sulfonamides also to this site and found considerable selectivity for the AMPA receptor with most derivatives (see data in Table 1). It is noteworthy that by introducing an imidazole group in position 6 not only the affinity but also the selectivity to the AMPA receptor is enhanced: whereas the affinity to the glycine site remains essentially unchanged in the imidazolyl-substituted compounds **27** (compared to **4**) and **28** (compared to **10**), the affinity to the AMPA receptor is considerably increased and,



Scheme 3. Synthesis of **6**. (i) MeI, NaH, DMF, rt, 77%; (ii) $MeSO_2Cl$ (neat), 115 °C, 90%.



Scheme 4. Syntheses of **27** and **28**. (i) Fe, NH_4Cl, MeOH, H_2O, reflux, 62%; (ii) formaldehyde (37% in water), glyoxal (40% in water), AcOH, NH_4OAc, 70 °C, 22%; (iii) imidazole, DMF, 140 °C, 2 h, 29%.

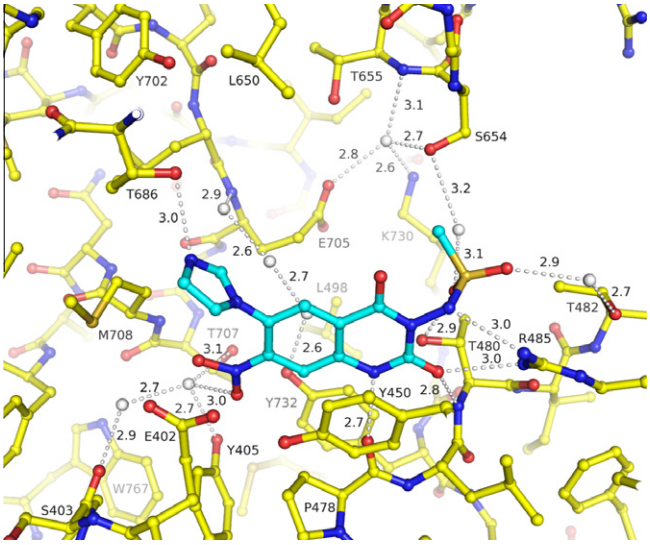


Figure 1. X-ray structure at 2.1 Å resolution of the ligand binding domain of a hGluA2 construct (carbons in yellow, nitrogens in blue, oxygens in red and sulfurs in brown) bound to **28** (carbons in cyan). Selected interactions (distances in Å) and water molecules are shown in white.

Table 2
Anticonvulsant effects against E-shock induced seizures after oral administration in mice (pretreatment time 60 min); $N = 5$

Compd	ED_{50} [mg/kg]
16	9
17	16
4	23
19	29.5
9	54
12	60%[a]
28	40%[b]

[a] Inhibition after oral administration of 30 mg/kg.
[b] Inhibition after intraperitoneal administration of 30 mg/kg (28 is orally inactive against sound induced seizures in DBA/2 mice).

therefore, selectivity is improved. On the other hand, the benzyl-substituted sulfonamide **25** shows good selectivity for the glycine receptor (about 14-fold), demonstrating that quinazolinedione sulfonamides could be modified towards glycine site selective ligands.

The promising oral activity found with **4** in the mouse E-shock test led to further in vivo examination of the series. Several compounds turned out to be orally active in the audiogenic seizure paradigm in DBA/2 mice[20], and a selection of those, together with the orally inactive compound **28**, was then tested in the E-shock assay in OF1 mice[21] (Table 2). All compounds of the selection inhibited E-shock induced seizures after oral administration, and only **28** was devoid of oral activity.

The orally active compounds shown display IC_{50}-values of 0.9–9.7 μM for the AMPA receptor. Within these narrow boundaries, the oral ED_{50}-values are not directly correlated to the affinities of the compounds. For example, the weakest ligand **16** has the lowest ED_{50}-value, whereas **9**, with an IC_{50}-value slightly below 1 μM, requires the highest dose for anticonvulsant effects. On the other hand, the nanomolar compound **28** is devoid of oral activity in DBA/2 mice and requires high intraperitoneal doses to inhibit E-shock induced convulsions in mice. This disappointing result may—at least in part—be explained by the higher polar surface area (PSA) of **28** (164 $Å^2$) compared to the PSA of the orally active compounds (101–110 $Å^2$). Given the strongly polar character of the glutamate binding cavity of the AMPA receptor, such an increase in PSA may strengthen the receptor interactions but also hamper absorption/distribution, and in particular the brain penetration of the compounds.[22]

In conclusion, the quinazolinedione sulfonamides represent a novel class of competitive AMPA receptor antagonists, displaying nanomolar affinities and providing examples—albeit of lower affinity—with oral activity in animal models for anticonvulsant effects. Whether these two properties—high receptor affinity and oral in vivo activity—can be combined within a single compound, will be the topic of further investigations.

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