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N-SULFONYL DERIVATIVES OF 6,7-DICHLORO 3,4-DIHYDRO-3-OXO-QUINOXALINECARBOXYLATE AS GLYCINE-SITE NMDA AND AMPA ANTAGONISTS

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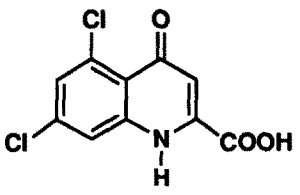
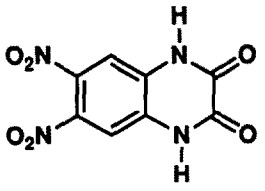
Abstract: N-Phenylsulfonyl and N-methylsulfonyl derivatives of 6,7-dichloro-3,4-dihydro-3-oxo-2-quinoxalinecarboxamide have been synthesized. Both compounds have been characterized as antagonists, at the glycine-site of the NMDA receptor and AMPA receptor.

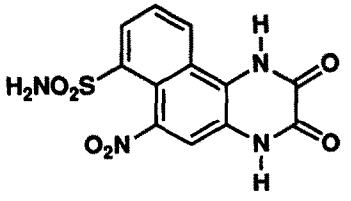
The N-methyl-D-aspartate (NMDA) receptor, a subtype of glutamate receptor, possesses several allosteric binding sites which can affect cellular responses. In particular, glycine acting at a strychnine-insensitive site has been shown to act as a coagonist along with glutamate and is a necessary requirement for receptor activation.[2,3] The NMDA receptor complex has been implicated in the pathophysiology of several neurodegenerative disorders[4] and may play a role in human epilepsy.[5] Therefore, a receptor antagonist, such as the prototypic 5,7-dichlorokynurenic acid (5,7-DCKA),[6,7] may have clinical potential as an antiischemic or anticonvulsant agent. Glutamate receptors for non-NMDA sites such as that named for (+/-)-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) may also be a part of the etiology of various neurological disorders.[8] Recently, it has been demonstrated that 6,7-dinitro-2,3-quinoxalinedione (DNQX)[9] and 6-nitro-2,3-dioxo- benzo(f)quinoxaline-7-sulfonamide (NBQX)[10] displace tritiated AMPA in receptor binding studies. Additionally, NBQX, given postischemia, has resulted in a significant reduction of neuronal damage in an experimental model of global ischemia, where NMDA antagonists showed no protection.[8,10,11] It has been suggested that in fact a dual inhibitor of both the NMDA receptor as well as a non-NMDA receptor may be necessary for maximal protection from cell death during an ischemic event.[12] As a consequence, a compound which demonstrates activity at both the glycine site of the NMDA receptor and the AMPA receptor may be more efficacious as a neuroprotective agent than a compound which exhibits selectivity for only one receptor.



5,7-DCKA



DNQX

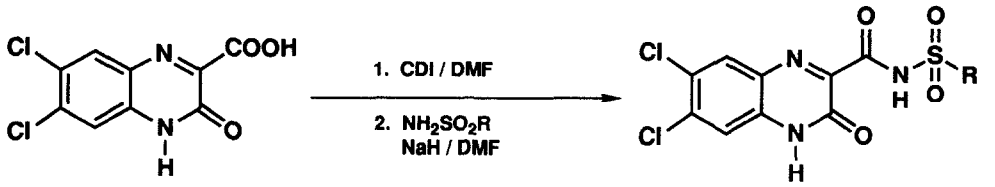


NBQX

6,7-Dichloro-3,4-dihydro-3-oxo-2-quinoxalinecarboxylic acid (DCHQC) exhibits good affinity for both glycine-site NMDA and AMPA receptor sites.[13] DCHQC antagonized functional responses mediated at both NMDA and non-NMDA receptors.[14] In an effort to mimic the pKa of the carboxylate moiety of DCHQC and to increase the compound's lipophilicity, the acylsulfonamide derivatives **1** and **2** have been synthesized and biologically evaluated.

Compounds **1** and **2** were prepared by activation of DCHQC with two equivalents of carbonyldiimidazole (CDI) at 80°C in dimethylformamide (DMF). Displacement of imidazole, using at least two equivalents of the preformed sodium salt of the appropriate sulfonamide, resulted in 60-85% yields of the targeted compounds after recrystallization from dimethylformamide and water as shown in Scheme 1.

Scheme 1



DCHQC

1 R = CH_3
2 R = Ph

The acylsulfonamide derivatives **1** and **2** have retained or shown improved glycine-site NMDA affinity while maintaining the parent compound's good affinity for the AMPA receptor (see Table 1). The log P of compound **1** was roughly equivalent to DCHQC, but the log P of analog **2** has been increased by more than one log unit relative to DCHQC. Increased lipophilicity is important for improved bioavailability and in vivo activity, however lipophilicity alone cannot account for the improved affinity at the glycine-site since modification of DCHQC to simple amide or imide derivatives resulted in decreased affinity for the receptors (results not shown). It may be that the ability of the acylsulfonamide moiety (pKa ~ 4-5)[15] to mimic the acidity of the carboxylate is the requisite feature accounting for the retention of binding affinity. Affinity for the non-NMDA kainate site is lower for DCHQC and its derivatives than for the other glutamate receptor sites.

Table 1. Affinity of agents for excitatory amino acid receptors.

	IC_{50}(μM) ± S.E.M.			
	[^{3}H] Gly[16] (N=3)	[^{3}H] AMPA[17] (N=3)	[^{3}H] Kainate[18] (N=3)	log P[19]
5,7-DCKA	0.068 ± 0.019	>100	>100	-0.56
NBQX	>100	0.2	8.0	—
DNQX	0.84 ± 0.25	0.2	2.9 ± 0.5	0.09
DCHQC	0.46 ± 0.19	6.7 ± 2.5	32.6 ± 4.6	-1.0
1	0.36 ± 0.18	14.0 ± 3.6	103 ± 24	-1.1
2	0.12 ± 0.04	4.3 ± 0.4	19.7 ± 4.2	0.15

Compounds **1** and **2** were examined for their ability to suppress the spontaneous epileptiform discharge rate (SED) in a cortical wedge model[20] (see Figure 1A). The activity of 5,7-DCKA (IC_{50} = 5 μM) versus **1** (IC_{50} = 11 μM) and **2** (IC_{50} = 11 μM) was roughly consistent with the compounds' relative affinity for the glycine site. This contrasts with the results in the cortical wedge where DNQX, **1** and **2** inhibited the responses to AMPA approximately equally (see Figure1B) despite the fact that DNQX possessed an order of magnitude increased affinity in tritiated AMPA binding.

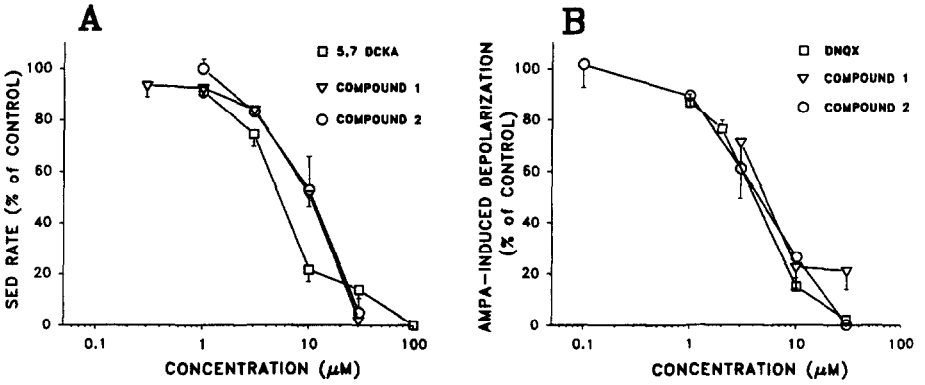


Figure 1. Actions of compounds 1 and 2 compared to reference agents in the rat cortical wedge model. In (**A**), the rate of spontaneous epileptiform discharges (SED) in Mg^{++}-free buffer was measured 30 min after addition of varying concentrations of drug and expressed as a percent of the mean of three control readings. In (**B**), tissues were repeatedly challenged with 10 μM AMPA in the presence of 0.5 μM tetrodotoxin. Data were collected 30 min after adding drug and are expressed as a percent of the mean of two control depolarizations to AMPA (n = 3-8 tissues per point ± S.E.M.).

The same compounds were tested in vivo in models of epileptic seizures and glutamate-induced neurodegeneration. Rat pups (postnatal day 7) were injected with the agonist NMDA into the corpus striatum,[21,22] a treatment that causes necrosis of tissues at the injection site which can be prevented by systemic administration of NMDA antagonists. Co-injection of analog **2** into the striatum together with NMDA, significantly reduced damage (Figure 2). However, with intraperitoneal administration of either **1** or **2**, no activity was seen against damage from striatal NMDA in rat pups and no anticonvulsant activity was seen with intravenous administration against seizures from low-intensity electroshock[22-24] in mice. These results suggest that despite increased lipophilicity, compound **2** still does not adequately penetrate the blood-brain barrier. Other factors may also contribute to the inability of these compounds to act in the brain after systemic administration. 5,7-DCKA appears to be a substrate for acidic transporter systems in the central nervous system and recent evidence suggests that 5,7-DCKA is transported by a saturable process out of the central nervous system.[25] Despite, the steric bulk attached to the acidic functionality of **2**, this acylsulfonamide may also be a substrate for active transport out of the brain.

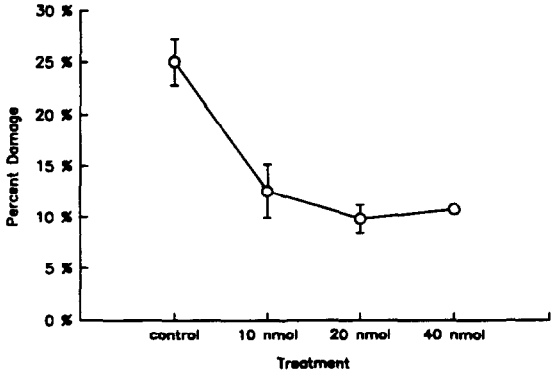


Figure 2. Injection of 15 nmol of NMDA into the striatum of neonatal rat pups decreased hemispheric weight by 25% due to cellular necrosis from activation of NMDA-type glutamate receptors (control). Coinjection of **2** (10 to 40 nmol) along with NMDA decreased damage by approximately 50% (n = 5 to 7 rat pups per data point, S.E.M. shown by bars).

In conclusion, we have described the synthesis and biological evaluation of the novel N-methyl and N-phenylsulfonyl derivatives of 6,7-dichloro-3,4-dihydro-3-oxo-2-quinoxalinecarboxamide. Compounds **1** and **2** show good affinity for both glycine-site NMDA and AMPA receptors and were functional antagonists at both these sites. Neither compound, however, demonstrated in vivo activity. Therefore, additional work is needed to discover glycine-site NMDA antagonists or AMPA antagonists that have significant activity after systemic administration and that are structurally related to the compounds described here.

References and Notes:

1. Neurosearch, 26 Smedeland, DK-2600 Glostrup, Denmark.
2. Kleckner, N. W.; Dingledine, R. *Science* **1989**, *241*, 835.
3. Kemp, J. A.; Foster, A. C.; Leeson, P. D.; Priestly, T.; Tridgett, R.; Iversen, L. L. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 6547.
4. Robinson, M. B.; Coyle, J. T. *FASEB J.* **1987**, *1*, 446.
5. Meldrum, B. *Excitatory Amino Acid Transmission*; Hicks, T.P.; Lodge, D.; and McLennan, H. Eds.: Alan R. Liss, Inc.; New York, 1987; pp 189-196.
6. McNamara, D.; Smith, E. C. R.; Calligaro, D. O.; O'Malley, P. J.; McQuaid, L. A.; Dingledine, R. *Neurosci. Lett.* **1990**, *120*, 17.
7. Baron, B. M.; Harrison, B. L.; Miller, F.P.; McDonald, I. A.; Salituro, F. G.; Schmidt, C. J. ; Sorensen, S. M.; White, H. S.; Palfreyman, M. G. *Mol. Pharmacol.* **1990**, *38*, 554.
8. Nellgard, B.; Wieloch, T. *J. Cereb. Blood Flow Metab.* **1992**, *12*, 2.
9. Honore, T.; Davies, S. N.; Drejer, J.; Fletcher, E. J.; Jacobsen, P.; Lodge, D.; Nielsen, F. E. *Science* **1988**, *241*, 701.
10. Sheardown, M.J.; Nielsen, E.O.; Hansen, A.J.; Jacobsen, P.; Honore, T. *Science* **1990**, *247*, 571.
11. Peillet, E. L.; Arvin, B.; Moncada, C.; Meldrum, B. S. *Brain Research* **1992**, *571*, 115.
12. Siesjo, B. K.; Memezawa, H.; Smith, M. L. *Fundam. Clin. Pharmacol.* **1991**, *5*, 755.
13. Pellegrini-Giampietro, D. E.; Galli, A.; Alesiani, M.; Cherici, G.; Moroni, F. *Br. J. Pharmacol.* **1989**, *98*, 1281.
14. Birch, P. J.; Grossman, C. J.; Hayes, A. G. *Eur. J. Pharmacol.* **1989**, *163*, 127.
15. The pKa's of **1** and **2** were determined in 67% DMF/water as described in Vandenbelt, J.M.; Spurlock, C.H.; Giffels, M.; Eash, M.W. *Science* **1955**, *121*, 646. Each compound had two pKa's: 4.3 and 8.4 for compound **1** and 5.6 and 9.6 for compound **2**. Measured pKa's run in the DMF/water media may be as much as 2.5 units higher than values obtained in water alone. These compounds were insoluble in water until a pH > 9.5 was attained.
16. Kishimoto, H.; Simon, J.R.; Aprison, M.H. *J. Neurochem.***1981**, *37*, 1015.
17. Honore, T.; Nielsen, M. *Neurosci. Lett.* **1985**, *54*, 27.
18. Honore, T.; Drejer, J.; Nielsen, M. *Neurosci. Lett.* **1986**, *65*, 47.
19. Log P was measured using a shake flask method with octanol/buffer at physiological pH.
20. Harrison, N.L.; Simmonds, M. A. *Br. J. Pharmacol.* **1985**, *84*, 381.
21. McDonald, J.W.; Roeser, N.F.; Silverstein, F.S.; Johnston, M.V. *Exp. Neurol.* **1989**, *106*, 289.
22. Vartanian, M.G.; Taylor, C.P. *Neurosci. Lett.* **1991**, *133*, 109.
23. Piredda, S.G.: Woodhead, J.H.; Swinyard, D.A. *J. Pharmacol. Exp. Ther.* **1985**, *232*, 741.
24. Compound **2** was inactive against low-intensity electroshock seizures in mice following IV doses of 10, 30 or 100 mg/kg. Likewise, it was inactive in the rat pup model after an IP dose of 100 mg/kg. 5,7-DCKA was active in only a small fraction of mice (low-intensity electroshock) after IV or IP administration of 100 mg/kg and was inactive in the rat pup model at IP doses up to 30 mg/kg.
25. Welty, D. F. Unpublished results. Preliminary studies suggest that tritiated 5,7-DCKA leaves the brain following intracerebral administration by a process that saturates at high doses, most likely a transporter system.