Communications to the Editor

3-Phenyl-4-hydroxyquinolin-2(1*H*)-ones: Potent and Selective Antagonists at the Strychnine-Insensitive Glycine Site on the *N*-Methyl-D-aspartate Receptor Complex

The *N*-methyl-D-aspartic acid (NMDA) ion channel complex has been implicated in a number of excitotoxic events leading to neuronal degeneration.[1] The characterization of a number of distinct sites[2] by which the complex may be regulated allows for a variety of mechanistic approaches for intervention. Of particular interest to us was the finding that glycine acts as a coagonist at this receptor and is required for channel opening.[3] Consequently, antagonists of the glycine site offer a promising approach for the suppression of glutamate excitotoxicity expressed through the NMDA receptor.

A number of partial agonists and antagonists of the strychnine-insensitive glycine site on the NMDA receptor complex have been reported. HA-966, **1**, is a partial agonist with limited efficacy and can block a number of NMDA-induced responses by action at the glycine site.[4] Antagonists include a number of quinoxalinediones, **2a–c**,[5,6] 6,7-dichloroquinoxalic acid **3**,[6] and 2-carboxyindoles **4a**[7] and **4b**.[8] We and others have reported that 5,7-dichlorokynurenic acid (**5a**) is a potent and selective glycine antagonist.[9,10] Recent modifications include 4-substituted kynurenates[11] and a series of 2-carboxytetrahydroquinolines typified by **6**.[12] We wish to report on a new

Chart I

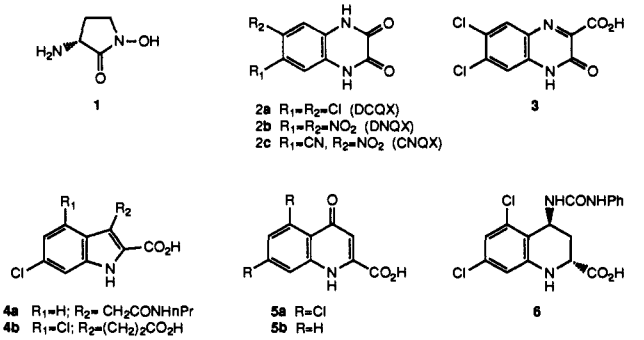
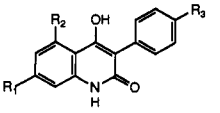


Table I. 3-Phenyl-4-hydroxyquinolin-2(1*H*)-ones Displacement of [^{3}H]Glycine Binding[a] and Inhibition of [^{3}H]MK-801 Binding[b]



no.	R_1	R_2	R_3	[^{3}H]glycine K_i (μM)	[^{3}H]MK-801 IC_{50} (μM)
7a	H	H	H	4.5 ± 1.7	7.29 ± 0.21
7b	Cl	Cl	H	0.057 ± 0.015	0.22 ± 0.01
7c	Cl	Cl	CH_3	0.30 ± 0.021	0.55 ± 0.05
7d	Cl	Cl	CH_3O	0.067 ± 0.013	1.23 ± 0.23
7e	Cl	Cl	NO_2	0.86 ± 0.14	
7f	Cl	Cl	OH	0.013 ± 0.001	0.11 ± 0.05
7g	Cl	Cl	NH_2	0.018 ± 0.004	0.21 ± 0.13
5a	5,7-Cl_2-kynurenic acid			0.04 ± 0.04	0.86 ± 0.19
5b	kynurenic acid			5.4 ± 0.05	40.2 ± 16.4

[a] [^{3}H]Glycine binding was performed on rat cortical membranes prepared by the freeze/thaw Triton extraction procedure developed for GABA-receptor binding with minor modifications.[20] Samples were incubated in the presence of 10 mM [^{3}H]glycine and 25 μg of membrane fragments on ice for 1 h and terminated by rapid filtration through Whatman GF/B filters. Nonspecific binding was determined in the presence of 100 μM D-serine. [b] [^{3}H]MK-801 binding was performed with well washed rat cortical membranes[15] with an added freeze/thaw procedure. The effect of compounds on [^{3}H]MK-801 binding (2.5 nM) was determined in the presence of glutamate (1 μM) and glycine (0.2 μM). Samples were incubated for 2 h at 27 °C and terminated by filtration. Nonspecific [^{3}H]MK-801 binding was determined in the presence of 0.5 μM MK-801.

(1) Meldrum, B.; Garthwaite, J. Excitatory amino acid neurotoxicity and neurodegenerative disease. *Trends Pharmacol. Sci.* **1990**, *11*, 379–387.

(2) Wong, E. H. F.; Kemp, J. A. Sites for antagonism on the N-methyl-D-aspartate receptor channel complex. *Annu. Rev. Pharmacol. Toxicol.* **1991**, *31*, 401–425.

(3) (a) Johnston, J. W.; Ascher, P. Glycine potentiates the NMDA response in cultured mouse brain neurons. *Nature* **1987**, *325*, 529–531. (b) Kleckner, N. W.; Dingledine, R. Requirement for glycine in activation of NMDA-receptors expressed in *Xenopus* oocytes. *Science* **1988**, *241*, 835–837.

(4) (a) Fletcher, E. J.; Lodge, D. Glycine reverses antagonism of N-methyl-D-aspartate (NMDA) by 1-hydroxy-3-aminopyrrolidone-2 (HA-966) but not by D-2-amino-5-phosphonovalerate (D-AP5) on rat cortical slices. *Eur. J. Pharmacol.* **1988**, *151*, 161–162. (b) Foster, A. C.; Kemp, J. A. HA-966 antagonizes N-methyl-D-aspartate receptors through a selective interaction with the glycine modulatory site. *J. Neurosci.* **1989**, *9*, 2191–2196.

(5) Birch, P. J.; Grossman, C. J.; Hayes, A. G. 6,7-Dinitro-quinoxaline-2,3-dion and 6-nitro,7-cyano-quinoxaline-2,3-dion antagonise responses to NMDA in the rat spinal cord via an action at the strychnine-insensitive glycine receptor. *Eur. J. Pharmacol.* **1988**, *156*, 177–180.

(6) Kleckner, N. W.; Dingledine, R. Selectivity of quinoxalines and kynurenines as antagonists of the glycine site on N-methyl-D-aspartate receptors. *Mol. Pharmacol.* **1989**, *36*, 430–436.

(7) Gray, N. M.; Dappen, M. S.; Cheng, B. K.; Cordi, A. A.; Biesterfeldt, J. P.; Hood, W. F.; Monahan, J. B. Novel Indole-2-carboxylates as Ligands for the Strychnine-Insensitive N-Methyl-D-aspartate-Linked Glycine Receptor. *J. Med. Chem.* **1991**, *34*, 1283–1292.

(8) Salituro, F. G.; Harrison, B. L.; Baron, B. M.; Nyce, P. L.; Stewart, K. T.; McDonald, I. A. 3-(2-Carboxyindol-3-yl)-propionic Acid Derivatives: Antagonists of the Strychnine-Insensitive Glycine Receptor Associated with the N-Methyl-D-aspartate Receptor Complex. *J. Med. Chem.* **1990**, *33*, 2944–2946.

(9) (a) Leeson, P. D.; Baker, R.; Carling, R. W.; Curtis, N. R.; Moore, K. W.; Williams, B. J.; Foster, A. C.; Donald, A. E.; Kemp, J. A.; Marshall, G. R. Kynurenic Acid Derivatives. Structure-Activity Relationships for Excitatory Amino Acid Antagonism and Identification of Potent and Selective Antagonists at the Glycine Site on the N-Methyl-D-aspartate Receptor. *J. Med. Chem.* **1991**, *34*, 1243–1252. (b) McNamara, D.; Smith, E. C. R.; Calligaro, D. O.; O'Malley, P. J.; McQuaid, L. A.; Dingledine, R. 5,7-Dichlorokynurenic acid, a potent and selective competitive antagonist of the glycine site on NMDA receptors. *Neuroscience Lett.* **1990**, *120*, 17–20. (c) Baron, B. M.; Harrison, B. L.; Miller, F. P.; McDonald, I. A.; Salituro, F. G.; Schmidt, C. J.; Sorenson, S. M.; White, H. S.; Palfreyman, M. G. Activity of 5,7-Dichlorokynurenic Acid, a Potent antagonist at the N-methyl-D-aspartate Receptor-Associated Glycine Binding Site. *Mol. Pharmacol.* **1990**, *38*, 554–561.

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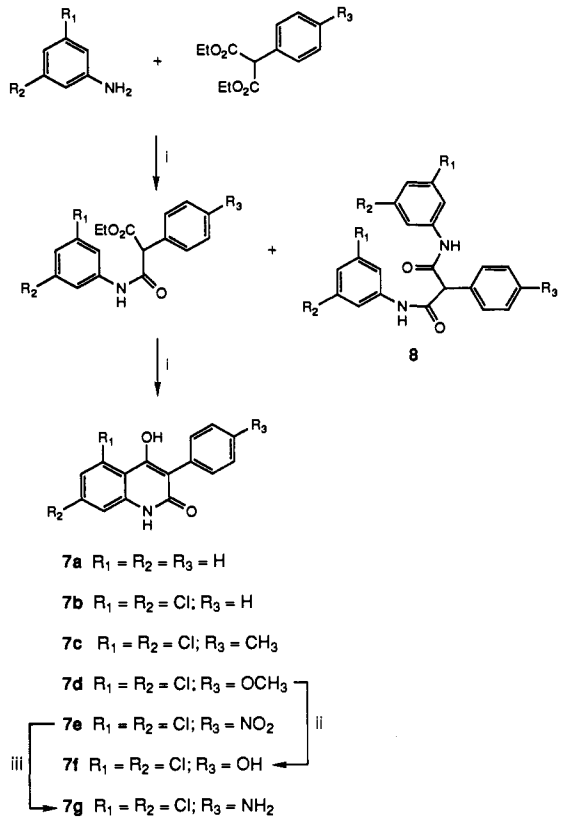
Scheme I. Synthesis of 3-Phenyl-4-hydroxyquinolin-2(1*H*)-ones[a]



7a $R_1 = R_2 = R_3 = H$

7b $R_1 = R_2 = Cl$; $R_3 = H$

7c $R_1 = R_2 = Cl$; $R_3 = CH_3$

7d $R_1 = R_2 = Cl$; $R_3 = OCH_3$

7e $R_1 = R_2 = Cl$; $R_3 = NO_2$

7f $R_1 = R_2 = Cl$; $R_3 = OH$

7g $R_1 = R_2 = Cl$; $R_3 = NH_2$

[a] (i) Ph_2O, 260 °C; (ii) HBr, AcOH; (iii) H_2, Pt/sulfide C, DMF.

series of 3-phenyl-4-hydroxyquinolin-2(1*H*)-ones **7a–g** which show excellent inhibition of binding to the glycine site on the NMDA receptor complex.

Preparation of the 3-phenyl-4-hydroxyquinolin-2-(1*H*)-ones **7a–g** is illustrated in Scheme I. Condensation of either aniline or 3,5-dichloroaniline with a variety of phenyl malonates in diphenyl ether followed by thermal cyclization afforded the desired quinolinones **7a–e**.[13] We found it convenient to isolate the product as a precipitate from the cooled reaction mixture and purify it by recrystallization from DMF/H_2O. In some cases, the formation of diamide **8** made the purification of product difficult. The 4′-methoxy derivative **7d** was converted to the 4′-hydroxy compound **7f** by treatment with hydrogen bromide in acetic acid. The 4′-nitro derivative **7e** was hydrogenated over 5% platinum on sulfide carbon to afford the corresponding 4′-amino compound **7g**. All final products exhibited NMR, mass spectral, and microanalytical data consistent with the assigned structures.

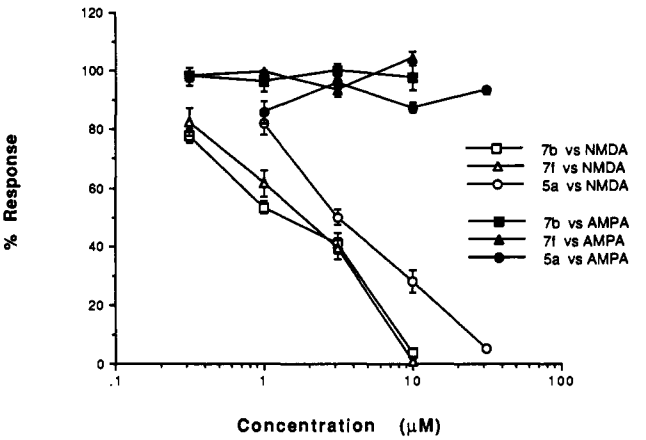


Figure 1. Dose–response curves for the antagonism of the response to 40 μM NMDA (open symbols) and to 40 μM AMPA (closed symbols) by **7b**, **7f**, and 5,7-dichlorokynurenic acid (**5a**) in neonatal rat spinal cord.[10]

Quinolinones **7a–g** were evaluated for their ability to displace strychnine-insensitive [^{3}H]glycine binding to rat cortical membranes (Table I). The unsubstituted analog **7a** was found to possess a K_i of 4.5 μM for the glycine site which is very similar to that found for kynurenic acid, **5b** (K_i = 5.4 μM). As one might expect on the basis of the kynurenic acid SAR, the 5,7-dichloroquinolinone **7b** was significantly more potent (K_i = 57 nM). Holding the 5,7-dichloro substitution pattern constant, we next examined the effect of para substitution in the 3-phenyl ring. The 4′-hydroxy analog **7f** was found to be the most potent analog in this series with a K_i of 13 nM for the glycine site. The order of potency was OH > NH_2 > H = OCH_3 ≫ CH_3 > NO_2.

Functional antagonism of the NMDA receptor-ion channel complex was demonstrated by the ability of the 3-phenyl-4-hydroxyquinolin-2(1*H*)-ones to inhibit the binding of the channel blocking agent [^{3}H]MK-801[14] in a glycine-sensitive rat cortical membrane preparation[15] (Table I). As was observed previously in the [^{3}H]glycine displacement assay, the 4′-hydroxy analog **7f** was the most potent compound tested with an IC_{50} of 0.11 μM. By comparison, 5,7-dichlorokynurenic acid (**5a**) exhibited an IC_{50} of 0.86 μM for inhibition of [^{3}H]MK-801 binding.

Selectivity of the series was examined using a neonatal rat spinal cord preparation.[5,10] Quinolinones **7b** and **7f** had IC_{50} values of 2.0 ± 0.03 and 2.1 ± 0.03 μM, respectively, against the responses induced by 40 μM NMDA (Figure 1). In contrast, **7b** and **7f** had no effect on the response induced by 40 μM AMPA. Antagonism of the NMDA response by **7b** and **7f** was reversed by the addition of 100 μM D-serine, an agonist at the glycine site. 5,7-Dichloro-

(10) Pralong, E.; Millar, J. D.; Lodge, D. Specificity and potency of N-methyl-D-aspartate glycine site antagonists and of mephenesin on the rat spinal cord in vitro. *Neuroscience Lett.* **1992**, *136*, 56–58.

(11) Harrison, B. L.; Baron, B. M.; Cousino, D. M.; McDonald, I. A. 4-[(Carboxymethyl)oxy]- and 4-[(Carboxymethyl)amino]-5,7-dichloroquinoline-2-carboxylic Acid: New antagonist of the Strychnine-Insensitive Glycine Binding Site on the N-methyl-D-aspartate Receptor Complex. *J. Med. Chem.* **1990**, *33*, 3130–3132.

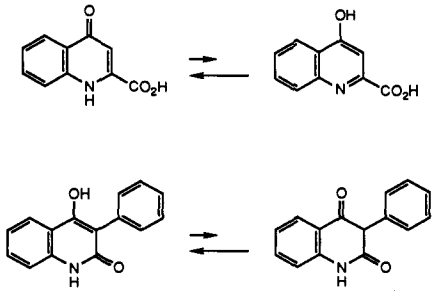
(12) (a) Leeson, P. D.; Carling, R. W.; Smith, J. D.; Baker, R.; Foshter, A. C.; Kemp, J. A. *trans*-2-Carboxy 4-Substituted Tetrahydroquinolines. Potent Glycine-site NMDA Receptor Antagonists. *Med. Chem. Res.* **1991**, *1*, 64–73. (b) Carling, R. W.; Leeson, P. D.; Moseley, A. M.; Baker, R.; Foster, A. C.; Grimwood, S.; Kemp, J. A.; Marshall, G. R. 2-Carboxytetrahydroquinolines. Conformational and Stereochemical Requirements for Antagonism of the Glycine Site on the NMDA Receptor. *J. Med. Chem.* **1992**, *35*, 1942–1953. (c) Leeson, P. D.; Carling, R. W.; Moore, K. W.; Moseley, A. M.; Smith, J. D.; Stevenson, G.; Chan, T.; Baker, R.; Foster, A. C.; Grimwood, S.; Kemp, J. A.; Marshall, G. R.; Hoogsteen, K. 4-Amido-2-carboxytetrahydroquinolines. Structure-Activity Relationships for Antagonism at the Glycine Site of the NMDA Receptor. *J. Med. Chem.* **1992**, *35*, 1954–1968.

(13) Yamada, M.; Kawase, Y. The Synthesis of Benzofuroquinolines. III. Two Dihydroxybenzofuroquinolinones. *J. Heterocycl. Chem.* **1984**, *21*, 737–739.

(14) Wong, E. H. F., Kemp, J. A.; Priestley, T.; Knight, A. R.; Woodruff, G. N.; Iverson, L. L. The anti-convulsant MK-801 is a potent N-methyl-D-aspartate antagonist. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 7104–7108.

(15) Wong, E. H. F.; Knight, A. R.; Ranson, R. Glycine modulates [^{3}H]MK-801 binding to the NMDA receptor in rat brain. *Eur. J. Pharmacol.* **1987**, *142*, 487–488.

Scheme II. Keto–Enol Tautomers



kynurenic acid (**5a**) has an IC_{50} of 4 ± 0.4 μM versus NMDA under identical conditions.

A quantitative structure activity relationship (QSAR) analysis of the series **7b–g** was conducted using regression analysis[16] between the individual 4′-substituent parameters[17] and the log of the reciprocal of the observed inhibitory constant [log $(1/K_i)$]. A meaningful relationship (r = 0.84) between the electronic parameter σ and activity was observed. The negative coefficient for the σ parameter suggests a positive effect of electron-donating substituents on binding affinity for the glycine site. In the QSAR equation, the numbers in parentheses are the 95% confidence intervals, n is the number of observations, r is the correlation coefficient, and F is the Fisher test for significance of the equation.

$$\log (1/K_i) = 0.95\ (\pm 0.18) - 1.26\ (\pm 0.41)\sigma p \qquad (1)$$

$$n = 6 \qquad r = 0.84 \qquad F = 9.43 \qquad p = 0.04$$

The 3-phenyl-4-hydroxyquinolinones represent a novel class of glycine antagonists which combine the 4-hydroxy group of the enol tautomer of the kynurenic acid series with the quinolin-2-one moiety of the quinoxalinediones. It has been shown that the keto tautomer of the kynurenic series predominates in solution,[9a,18] although there is some evidence that a small portion of the enol tautomer is present[18] (Scheme II). This finding led to the proposal that the keto tautomer is the active form in the kynurenic acid series. In contrast, the 3-phenyl-4-hydroxyquinolinones exist as the enol form in solution as determined by ^1H and ^{13}C NMR analysis.[19] It should be noted that the preferred tautomer of both series possesses the 1-NH form and a substituent on the 4-position which can act as an H-bond acceptor[12c] in accordance with a recent model of the glycine site on the NMDA receptor.

In conclusion, we have shown that 5,7-dichloro-3-phenyl-4-hydroxyquinolinones are potent and selective antagonists of the glycine site on the NMDA receptor ion channel complex. The 4′-hydroxy analog **7f** has greater affinity for the glycine site than 5,7-dichlorokynurenic acid and is a more potent antagonist of NMDA-induced depolarizations in the neonatal rat spinal cord. An expanded SAR of this novel series will be the subject of future reports from our laboratories.

Acknowledgment. We would like to thank Dr. James A. Monn for helpful discussions during the preparation of this communication.

(16) Regression analysis was performed using JMP, Statistical Visulization Software, Version 2.0.2, SAS Institute Inc., Cary, NC.

(17) (a) Hansch, C.; Leo, A. *Substituents Constants for Correlation Analysis in Chemistry and Biology*; Wiley: New York, 1979. (b) Verloop, A.; Hoogenstraaten, W.; Tipker, J. *Drug Design*; Ariens, E. J., Ed.; Academic Press: New York, 1976; Vol. 7, pp 165–207.

(18) Huber, E. W.; Stemerick, D. M.; Cousino, D. M.; Harrison, B. L. ^1H, ^{19}F, and ^{13}C NMR assignments of 5,7-Dihalogenated Kynurenic acid Derivatives. *Magn. Reson. Chem.* **1991**, *29*, 859–860.

(19) Chemical shifts in ppm for **7a** (DMSO-d_6): ^{13}C NMR δ 113.1, 115.3, 115.9, 121.5, 123.5, 127.3, 128.1 (2 C), 131.0, 131.6 (2 C), 133.8, 138.5, 157.7 (C4), 163.1 (C2); ^1H NMR δ 11.43 (s, 1 H, OH), 10.03 (br s, 1 H, NH), 7.91 (d, J = 8.0 Hz, 1 H), 7.47 (t, J = 7.6 Hz, 1 H), 7.24–7.39 (m, 6 H), 7.14 (t, J = 7.6 Hz, 1 H).

(20) Zukin, S. R.; Young, A. B.; Snyder, S. H. Gamma-aminobutyric acid binding to receptor sites in the rat central nervous system. *Proc. Natl. Acad. Sci. U.S.A.* **1974**, *71*, 4802–4807.

† Royal Veterinary College.
‡ Current address: Lilly Research Centre Ltd., Erl Wood Manor, Windlesham, Surrey GU20, UK.

Loretta A. McQuaid,* Edward C. R. Smith
David Lodge,†,‡ Etienne Pralong,† James H. Wikel
David O. Calligaro, Patrick J. O'Malley
Lilly Research Laboratories
A Division of Eli Lilly and Company
Corporate Center
Indianapolis, Indiana 46285
Royal Veterinary College
London, NW10TU, UK.
Received June 12, 1992