Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 6, No. 5, pp. 499-504, 1996

Published by Elsevier Science Ltd
Printed in Great Britain
0960-894X/96 $15.00 + 0.00

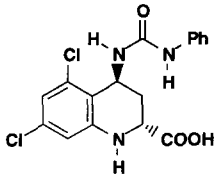
S0960-894X(96)00031-5

3-HYDROXY-QUINOLIN-2-ONES: INHIBITORS OF [^{3}H]-GLYCINE BINDING TO THE SITE ASSOCIATED WITH THE NMDA RECEPTOR

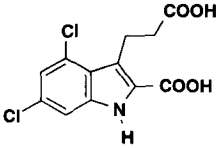
Sing-Yuen Sit, * Frederick J. Ehrgott, Jinnian Gao, and Nicholas A. Meanwell
Division of Chemistry, Bristol-Myers Squibb Pharmaceutical Research Institute
5 Research Parkway, Wallingford, CT 06492-7660

Abstract: A series of substituted 3-hydroxy-quinolin-2-one derivatives **6** was synthesized and evaluated as inhibitors of [^{3}H]-glycine and [^{3}H]-AMPA binding to rat cortical membranes. These compounds were generally found to be more potent ligands for the NMDA-associated glycine binding site than the AMPA receptor. Affinity for the glycine site was found to be influenced by both the electronic and steric properties associated with the C-4 substituent and the nature and pattern of substitution of the aromatic ring. The most active compound in this series, **6y**, displaces [^{3}H]-glycine with an IC_{50} of 29 nM.

Excitatory amino acids have been implicated in the pathophysiology of acute neurodegenerative disorders including stroke, head trauma, and spinal cord injury, as well as the more chronic processes associated with Alzheimer's and Huntington's diseases.[1] The elucidation of the spectrum of excitatory amino acid receptor subtypes and their inherent complexity, particularly with respect to the NMDA receptor, has characterized several distinct sites that provide targets for biochemical intervention and for which effective and selective antagonists have been identified and evaluated as potential neuroprotective agents.[2] Known inhibitors of glycine binding to the allosteric site of the NMDA receptor can be broadly categorized into two pharmacophoric classes.[3] Kynurenic acid and its structural homologues, typified by L-689560,[4] (**1**) and indole-2-carboxylic acid derivatives, of which MDL-29951[5] (**2**) is representative, form one class which exhibit a high degree of specificity for the NMDA glycine site.[3] In contrast, quinoxaline-dione derivatives, which do not contain a carboxylic acid moiety, are recognized by both glycine and AMPA receptors with selectivity influenced by both the nature and pattern of substitution.[3,6,7] For example, NBQX[6] (**3**) and the acid **4b**[7a] exhibit selectivity for AMPA receptors whilst **4a** is a more balanced AMPA/glycine antagonist[3,4]

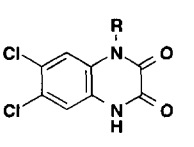


1 (L-689,560)



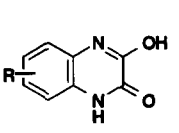
2 (MDL-29951)

3 (NBQX)

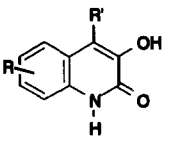


4a, R = H
4b, R = CH_2CO_2H

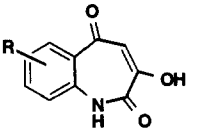
The mono-hydroxy imine tautomer **5**, in which the amide and enol hydroxy moieties mimic a protonated glycine, has been postulated as the species recognized and bound by these receptors.[4] In order to further explore this hypothesis, we have synthesized and evaluated a series of 3-hydroxyquinolin-2-one derivatives[8] **6** that are formally isosteric with the tautomer **5**, but offer the advantage of providing an additional site at C-4 suitable for structural elaboration. The concept of using an enol to mimic the hydroxy imine moiety of **5** has recently been examined[9] in the context of the benzazepine ring system **7**.



5



6



7

Chemistry

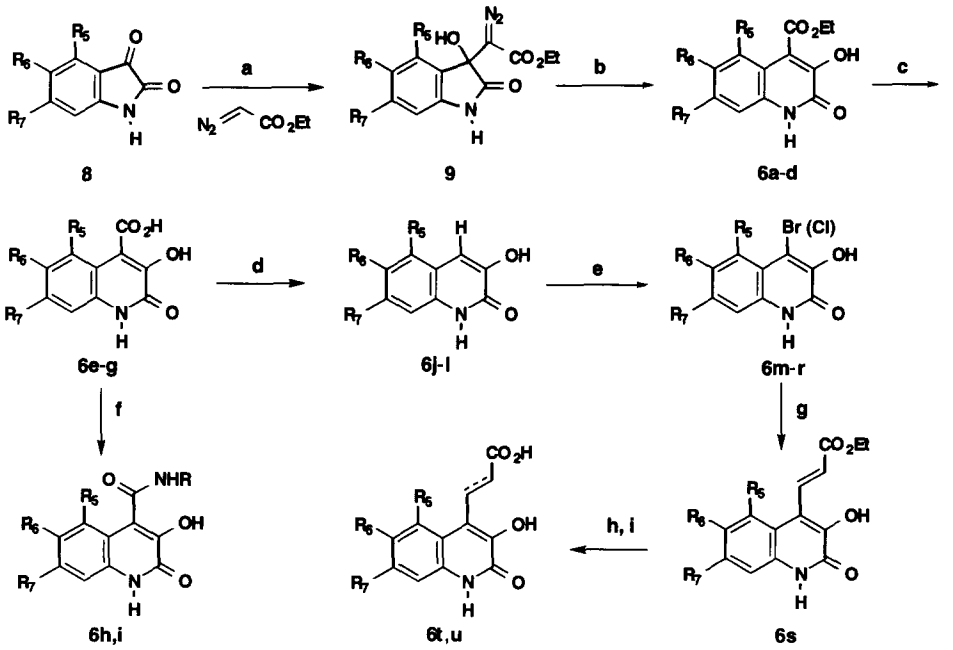
The 4-ethoxycarbonyl-3-hydroxyquinolin-2-one[10] derivatives 6a-d were identified as versatile synthetic precursors that were prepared from isatins **8** by treating with ethyl diazoacetate[11] in the presence of Et_2NH as the base and then exposing the crude adducts **9** to dilute aqueous HCl to effect an Eistert ring expansion, as depicted in Scheme 1. This process afforded the 3-hydroxy-4-substituted quinolin-2-one isomer **6** exclusively with no detectable trace of the regioisomeric 4-hydroxy-3-substituted compounds. Saponification of the unsubstituted ester **6a** gave the corresponding carboxylic acid **6e**, which was converted to amides **6h** and **6i** via the acid chloride. The acids of **6b-d** were thermally unstable and readily decarboxylated to the 4-unsubstituted quinoline derivatives **6j-l** upon heating. Halogenation of **6j-l**, by exposing to NCS or NBS in DMF, afforded the 4-halo derivatives **6m-r**, of which **6m** was exploited as a useful synthetic precursor to additional 4-substituted compounds. Thus, Pd-catalyzed functionalization of **6m** with ethyl acrylate under Heck reaction conditions[12] afforded the unsaturated ester **6s**, which was sequentially saponified to the carboxylic acid **6t** and reduced by catalytic hydrogenation to saturated acid **6u**.

As summarized in Scheme 2, an analogous sequence of reactions using ethyl 3-diazo-2-oxopropionate[13] afforded the pyruvate ester derivatives **6v**, **w**, **y**, **z** of which **6v** and **6y** were hydrolyzed to carboxylic acids **6x** and **6aa**, respectively. Since attempts to convert the acid **6aa** to amides were unsuccessful, the preparation of **6ab** and **6ac** necessitated amide bond formation as an early synthetic step. To this end, methyl oxalyl chloride was coupled with the appropriate amine, the ester saponified and exposed to diazomethane to provide the requisite substituted diazo ketone. Base-mediated reaction with 4,6-dichloroisatin and subsequent rearrangement afforded the quinolines **6ab** and **6ac**.

The O-methyl derivatives **6ad-6af** were obtained from **6a**, **6b** and **6d**, respectively, by treatment with ethereal diazomethane.

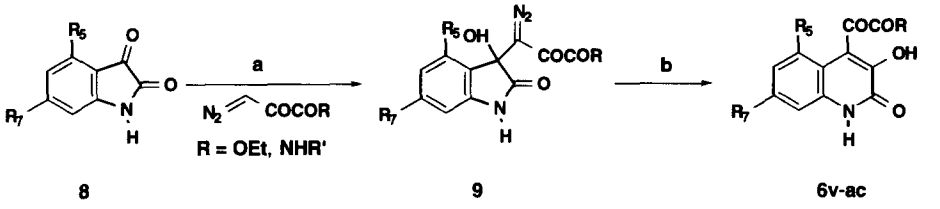
The compounds that comprise this study are compiled in the Table shown below.

Scheme 1



Reagents: (a) Et_2NH/EtOH/RT; (b) Dil. HCl/H_2O, 70-80% over 2 steps; (c) $NaOH/H_2O$/reflux then careful addition of 2N HCl, 85-95% for 2 steps; (d) Toluene/reflux, 100%; (e) NBS (or NCS)/DMF, 70-90%; (f) first step: $SOCl_2$, second step: RNH_2; (g) ethyl acrylate/$(Ph_3P)_4Pd$; (h) NaOH/THF; (i) H_2/Pd on C

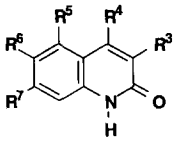
Scheme 2



Reagents: (a) Et_2NH/EtOH/RT; (b) Dil. HCl/H_2O, 70-80% in two steps.

Table

Displacement of [^{3}H]-glycine and [^{3}H]-AMPA from rat cortical membranes by 3-hydroxyquinolin-2-one derivatives.



Cmpd #	R^3	R^4	R^5	R^6	R^7	[^{3}H]-glycine IC_{50} (μM) [slope]$^\diamond$ or (% Inh. at 10 μM)	[^{3}H]-AMPA (% Inh. at 10 μM)
6a	OH	CO_2Et	H	H	H	(50)	
6b	OH	CO_2Et	Cl	H	Cl	0.062 [-0.63]	
6c	OH	CO_2Et	Cl	Cl	H	(38)	
6d	OH	CO_2Et	H	Cl	Cl	(43)	
6e	OH	CO_2H	H	H	H	(60)	
6f	OH	CO_2H	Cl	H	Cl	Not Tested$^\Delta$	
6g	OH	CO_2H	Cl	Cl	H	Not Tested	
6h	OH	$CONHCH_2Ph$	H	H	H	(40)	
6i	OH	CONH(3,4-DMP)†	H	H	H	(0)	
6j	OH	H	H	H	H	(29)	
6k	OH	H	Cl	H	Cl	(29)	
6l	OH	H	Cl	Cl	H	(0)	
6m	OH	Br	H	H	H	(59)	
6n	OH	Cl	H	H	H	1.4 [-0.46]	
6o	OH	Br	Cl	H	Cl	2.8 [-0.55]	(41)
6p	OH	Cl	Cl	H	Cl	4.5 [-0.46]	(48)
6q	OH	Br	Cl	Cl	H	(23)	
6r	OH	Cl	Cl	Cl	H	(20)	
6s	OH	(E)CH=$CHCO_2Et$	H	H	H	(11)	
6t	OH	(E)CH=$CHCO_2H$	H	H	H	(43)	
6u	OH	$CH_2CH_2CO_2H$	H	H	H	(25)	
6v	OH	$COCO_2Et$	H	H	H	12 [-0.59]	
6w	OH	$COCO_2Me$	H	H	H	3.0 [-0.59]	(2.6)
6x	OH	$COCO_2H$	H	H	H	1.8 [-0.65]	(31.9)
6y	OH	$COCO_2Et$	Cl	H	Cl	0.029 [-0.54]	(56)
6z	OH	$COCO_2Me$	Cl	H	Cl	0.15 [-0.41]	(28)
6aa	OH	$COCO_2H$	Cl	H	Cl	0.52 [-0.79]	(63)
6ab	OH	COCONHMe	Cl	H	Cl	3.8 [-0.64]	(15)
6ac	OH	COCONHPh	Cl	H	Cl	0.76 [-0.59]	(40)
6ad	OCH_3	CO_2Et	H	H	H	(0)	
6ae	OCH_3	CO_2Et	Cl	H	Cl	(5)	
6af	OCH_3	CO_2Et	H	Cl	Cl	(0)	

$^\Delta$Thermal instability of **6f** and **6g** precluded any attempt to purify and isolate the free acids for assay.

†3,4-DMP = 3,4-dimethoxyphenyl

$^\diamond$In all cases, displacement of radiolabelled glycine was practically 100% at high concentrations.

Results and Discussion

The target compounds were evaluated for their ability to displace [^{3}H]-glycine[14] or [^{3}H]-AMPA[15] from rat cortical membranes. For reference purposes, 5,7-dichlorokynurenic acid was used as the standard ligand for the glycine site where it exhibited a K_i of 0.50 μM[16] and quisqualic acid was employed as the reference AMPA ligand, K_i= 0.010 μM. IC_{50}'s were generally determined for those synthetic compounds that demonstrated greater than 60% displacement of the radio ligand at 10 μM. and in all cases the displacement of labelled glycine by the test compound was complete at high (10^{-3} M) concentrations.

It is apparent from the data presented in the Table that the 3-hydroxyquinolin-2-one heterocycle represents an effective structural element for glycine ligands and several structure-activity trends are readily discerned. The presence of an electron withdrawing substituent at C-4 of the heterocycle invariably leads to ligands with higher affinity for the glycine site, an effect that probably relates to the acidity of the 3-hydroxy moiety. This physical chemical property was explicitly examined for the 4-chloro derivative 6n, where the pKa of the 3-hydroxyl proton was determined to be 6.06, and the more potent glycine ligands 6y and 6z, which exhibited pKa's of 5.00 and 4.95, respectively. That a free 3-hydroxyl group is essential for effective receptor recognition is clearly emphasized by the inactivity of the three methyl ethers **6ad-6af** that were prepared. However, the presence of an acidic 3-hydroxyl does not uniquely determine the affinity of a compound for the glycine site and the values measured are the result of a subtle combination of both electronic and steric factors associated not only with the C-4 substituent, but also the nature and pattern of substitution in the aromatic ring. In general, it was found that small uncharged electron withdrawing substituents at C-4 are preferred, with the pyruvate ester moiety found in **6v**, **6w**, **6y** and **6z** providing an optimal combination of physical chemical properties.

The 5,7-dichloro pattern of substitution of the aromatic ring portion of the heterocycle is superior to both the 5,6- and 6,7-dichloro isomeric alternatives, a topology that closely mimics the optimal substitution pattern found in derivatives of kynurenic acid that exhibit high affinity for NMDA-associated glycine receptors.[3,4] More specifically, for the pyruvate esters **6v** and **6w**, the introduction of chlorine substituents at the C-5 and C-7 positions of the heterocycle results in a substantial increase in affinity and **6y** and **6z** are the most potent glycine ligands to be identified from this survey of structure-activity relationships.

Some of the more potent glycine ligands were assessed for their ability to displace [^{3}H]-AMPA from rat cortical membranes. However, those 3-hydroxy-quinoline-2-ones examined were generally found to be considerably less effective ligands for the AMPA receptor. For example, the optimal glycine site ligands **6y** and **6z** bound to the AMPA receptor with potency at least 2 orders of magnitude lower. Interestingly, from the limited SAR presented in the Table it would appear that for unsubstituted compounds the AMPA receptor displays some preference for an acidic substituent at C-4, since **6x** is superior to **6w**, a structure-activity relationship similar to that observed for a recently synthesized series of substituted quinoxaline-dione derivatives.[7a] However, this is preference is less pronounced in the background of 5,7-dichloro-substitution (**6y-6ac**) where increased bulk appears to correlate with enhanced binding to the AMPA receptor, although these compounds are weak ligands for this receptor.

In summary, we have described the synthesis and structure-binding relationships of a series of 3-hydroxy-quinolin-2-one derivatives that represent a new structural class of ligand for the glycine site associated with the NMDA subtype of glutamate receptor. That these compounds demonstrate high affinity for the glycine site is consistent with the hypothesis that the hydroxy imine tautomer of quinoxaline diones is that which is recognized by the receptor.

References

(1) Meldrum, B. *Cerebrovascular and Brain Metabolism Reviews*, **1990**, *2*, 27.

(2) (a) Cunningham, M. D.; Ferkany, J. W.; Enna, S.J. *Life Sci.* **1994**, *54*, 135.
(b) Danysz, W.; Parsons, C. G.; Bresink, I.; Quack, G. *Drug News Persp.* **1995**, *8*, 261.

(3) (a) Leeson, P. D.; Iversen, L. L. *J. Med. Chem.* **1994**, *37*, 4053. (b) Carter, A. J. *Drugs Fut.* **1992**, *17*, 595.

(4) 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. *J. Med. Chem.* **1991**, *34*, 1243.

(5) Salituro, F. G.; Harrison, B. L.; Baron, B. M.; Nyce, P. L.; Stewart, K. T.; Kehne, J. H.; White, H. S.; McDonald, I. A. *J. Med. Chem.* **1992**, *35*, 1791.

(6) Sheardown, M. J.; Nielsen, E. O.; Hansen, A. J.; Jacobsen, P.; Honore, T. *Science*, **1990**, *247*, 571.

(7) (a) Epperson, J. R.; Hewawasam, P.; Meanwell, N. A.; Boissard, C. G.; Gribkoff, V. K.; Post-Munson, D. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 2801.
(b) Woodward, R. M.; Huettner, J. E.; Guastella, J.; Keana, J. F. W. and Weber, E. *Mol. Pharmacol.* **1995**, *47*, 568-581;

(8) The isomeric 3-substituted-4-hydroxy quinoline derivatives have recently been described as potent glycine antagonists: Kulagowski, J. J.; Baker, R.; Curtis, N. R.; Leeson, P. D.; Mawer, I. M.; Moseley, A. M.; Ridgill, M. P.; Rowley, M.; Stansfield, I.; Foster, A. C.; Grimwood, S.; Hill, R. G.; Kemp,J. A.; Marshall, G. R.; Saywell, K. L. and Tricklebank, M. D. *J. Med. Chem.* **1994**, *37*, 1402-1405

(9) Swartz, K. J.; Koroshetz, W.J .; Rees, A. H.; Huettner, J. E. *Mol. Pharmacol.* **1992**, *41*, 1130;

(10) (a) Eistert, B.; Borggrefe, G. *Annalen*, **1968**, *718*, 143.
(b) Jones, G. "Quinolines", Part 1; Jones, G. Ed.: John Wiley & Sons, New York, 1977.

(11) Ethyl diazoacetate was obtained from Aldrich and used as received.

(12) (a) Heck, R. F. *Acct. Chem. Res.* **1979**, *12*, 146.
(b) Heck, R. F. *Org. React.* **1982**, *27*, 345.

(13) Ethyl 3-diazo-2-oxopropionate was prepared as described by Gallucci, R. R; Jones, M., Jr. *J. Org. Chem.* **1985**, *50*, 4404.

(14) Snell, L. D.; Marter, R. S.; Johnson, K. M. *Eur. J. Pharm.* **1988**, *156*, 105.

(15) Murphy, D. E.; Snowhill, E. W.; Williams, M. *Neurochem. Res.* **1987**, *12*, 775.

(16) Most IC_{50} and K_i values were obtained from 7- or 14-point concentration-response curves, and were averaged from 2-7 experiments.

(*Received in USA* 27 *November* 1995; *accepted* 12 *January* 1996)