Pyridazino[4,5-*b*]quinolinediones: Novel Glycine/*N*-Methyl-D-aspartate Antagonists for the Treatment of Stroke

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Introduction.

After heart disease and cancer, strokes are the third largest cause of death and a major cause of adult disability in the United States [1]. There are approximately 730,000 strokes per year in the United States [2] and survivors of stroke may be left with varying degrees of mental and physical impairments or disabilities. Because it is estimated that about $18-30 billion is spent annually in the U.S. alone on the acute and chronic care of stroke victims [3], stroke is one of the most costly events in our health-care system. Clearly, there is a significant unmet medical need in this area.

A stroke is, quite simply, an interruption in the blood supply to part of the brain. Deprived of the blood's essential supply of oxygen and nutrients, brain cells in the affected area begin to die, with the time to death depending on the magnitude and duration of blood flow deficits. There are two main classifications of stroke: ischemic and hemorrhagic [4]. Ischemic strokes are the most common and cause an area of cerebral damage by blockade of a blood vessel leading to the brain. Hemorrhagic strokes are caused by rupture of blood vessel in or on the surface of the brain and cause injury not only by depletion of blood supply to part of the brain but also by the accumulated blood increasing pressure on surrounding brain tissue [5]. The incidences of ischemic and hemorrhagic strokes are 80% and 20%, respectively [6].

When we initiated our Neurological Disorders/Stroke project at Zeneca in 1987, there was no proven treatment for stroke. However, advances in the understanding of the pathophysiology of stroke lead researchers to investigate several approaches to halt chemical and physiological events that were hypothesized to occur and to cause ischemic stroke-related brain injury [7,8]. Many of these approaches relied on the concept of an area in the brain called the ischemic penumbra [9] which surrounds the severely ischemic core caused by blockade of a blood vessel. In the core region, where the supply of blood is essentially non existent, only relatively rapid removal of the clot can salvage the brain cells from death. However, cells in the ischemic penumbra, while not fully viable, still receive some blood from nonoccluded vessels and, consequently, may be able to be rescued by therapeutic intervention [6].

Role of Excitatory Amino Acids in Stroke.

During ischemia, cells in the penumbral region are damaged by a complicated interwoven cascade of events which include the release of abnormally high amounts of the excitatory amino acid L-glutamate, the major excitatory neurotransmitter in the mammalian central nervous system (CNS) [10,11]. The presence of excessive glutamate causes prolonged stimulation of excitatory amino acid receptors and causes calcium dependent [8,12] neuronal damage and eventually, cell death - giving rise to the concept of excitotoxicity [13]. Very importantly, it has been known for many years that antagonists of excitatory amino acid receptors protect against excitotoxicity and prevent glutamate-mediated cell death [12,14].

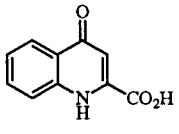
There are four major types of excitatory amino acid receptors that have been identified. Three of these (the *N*-methyl-D-aspartate, α-amino-3-hydroxy-5-methyl-4-isoxazolylpropionic acid and kainate receptors) are ionotropic receptors and have been named for selective agents that activate them [15]. The fourth receptor subtype, the metabotropic glutamate receptor, is G-protein-coupled and, until recently [16], was less well understood compared to the ionotropic receptors. Of the several types of excitatory amino acid receptors, we focused our efforts on the *N*-methyl-D-aspartate (NMDA) receptor because it had been proposed to be pivotal in the initiation of calcium dependent cell death [17].

The *N*-Methyl-D-aspartate Receptor.

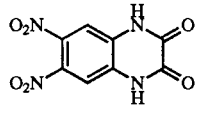
Functionally, the *N*-methyl-D-aspartate receptor consists of an ion channel which is ligand controlled and, when activated, allows the entry of calcium and sodium ions into the cell [18]. Prolonged stimulation of the *N*-methyl-D-aspartate receptor during ischemia caused by abnormally high glutamate levels allows entry of excess sodium, which causes acute cell swelling, and excess calcium, which promotes delayed cell death through a variety of mechanisms such as free radical generation, lipid peroxidation, proteolysis and arachidonic acid accumulation [19,20]. These neurodegenerative processes take place in the penumbral region of the stroke [9] and may occur over a 1-3 day period.

Several regulatory sites are associated with the *N*-methyl-D-aspartate receptor complex [18]. These sites consist of the neurotransmitter recognition or competitive binding

site which binds L-glutamate and the synthetic agonist *N*-methyl-D-aspartate. A noncompetitive or channel blocking site for compounds such as phencyclidine [1-(1-phenylcyclohexyl)piperidine] ("angel dust", an illicit street drug) and dizocilpine (10,11-dihydro-5-methyl-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine) [MK-801] is present within the ion channel along with a recognition site for magnesium +2. Another divalent cation site preferring zinc +2 and a polyamine modulatory site are also present on the receptor. Finally, there is a strychnine-insensitive glycine site. We decided to focus our efforts on discovering antagonists for the glycine site since there was evidence that such agents, in contrast to compounds acting at the competitive and channel blocking (noncompetitive) sites [21], were not associated with hallucinogenic or phencyclidine-like side effects [22,23]. Additionally, the discovery in 1987 that both the competitive and glycine sites must be occupied by agonists (coagonism) for *N*-methyl-D-aspartate receptor activation [24,25] to occur was unique and represented a new approach to *N*-methyl-D-aspartate antagonism. Also, some interesting lead compounds, *i.e.* kynurenic acid (**1**) [26] and 6,7-dinitroquinoxalinedione (**2**) [27], with an affinity for the glycine/*N*-methyl-D-aspartate site were already available; however, these leads did have major drawbacks in that they were not selective for the glycine/*N*-methyl-D-aspartate site and had little (kynurenic acid) or modest 6,7-dinitroquinoxalinedione systemic activity, presumably because of poor brain penetration [28,29].



1



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Once deciding on the glycine/*N*-methyl-D-aspartate recognition site as the target, the formal goal set for the Zeneca stroke team was to discover a selective glycine/-*N*-methyl-D-aspartate antagonist which could be administered intravenously after the occurrence of an ischemic stroke to limit brain cell neurodegeneration.

Biological Assays.

The screening cascade for evaluation of compounds synthesized for our stroke program consisted of sequential screening in a number of *in vitro* and *in vivo* assays. First, compounds were tested *in vitro* for their ability to interact at the glycine site of the *N*-methyl-D-aspartate receptor by using [^{3}H]glycine ([^{3}H]Gly) and rat cortical membranes [30]. Affinities of the test compounds for the receptor site were determined and expressed as IC_{50}'s (the concentration of the test compound necessary to displace 50% of the radioligand from its binding site). To address the receptor selectivity issue, IC_{50}'s were also determined for the competitive *N*-methyl-D-aspartate site and the α-amino-3-hydroxy-5-methyl-4-isoxazolylpropionic acid receptor using [^{3}H]D,L-(*E*)-2-amino-4-propyl-5-phosphono-3-pentenoic acid ([^{3}H]CGP 39653) [31] and [^{3}H]α-amino-3-hydroxy-5-methyl-4-isoxazolylpropionic acid ([^{3}H]AMPA) [28], respectively. Finally, displacement of [^{3}H]-1-[(2-thienyl)cyclohexyl]piperidine from the open *N*-methyl-D-aspartate channel site and reversal of the displacement with glycine confirmed functional glycine/*N*-methyl-D-aspartate antagonist activity [32].

Antagonism of receptor function was determined *in vivo* by using the rat red nucleus assay [33,34]. In this test, *N*-methyl-D-aspartate was iontophoretically applied to red nucleus cells in the rat brain to stimulate cell firing. The test compound was then administered intravenously in the tail vein until cell firing was completely inhibited. An ID_{50}, or a dose that inhibits cell firing by 50%, was determined for each compound. Reversal of the inhibition by the glycine agonist D-serine verified glycine antagonism.

A middle cerebral artery occlusion (MCA) model of focal ischemia in spontaneously hypertensive rats was employed to assess neuroprotectant efficacy in an animal model of stroke [35,36]. Immediately after permanent occlusion (*via* cauterization) of the middle cerebral artery, the test compound was administered as an intravenous (iv) bolus in the jugular vein at a particular dose followed by an infusion of the same dose per hour for 4 hours. Twenty-four hours after induction of the occlusion, the animal was sacrificed, the brain removed and the per cent reduction in infarct volume of treated *versus* control animals determined using 2,3,5-triphenyl-2*H*-tetrazolium chloride staining techniques [37,38] to determine the lesioned area. Zeneca was fortunate in being able to establish this labor intensive assay requiring microsurgical techniques in-house at an early stage in our stroke program; the relatively high throughput (2-3 compounds/week) achieved by our bioscientists allowed us to determine structure activity relationships for our compounds in an assay that we felt had more clinical relevance to stroke than the anticonvulsant or seizure models used by other organizations to determine systemic activity [29].

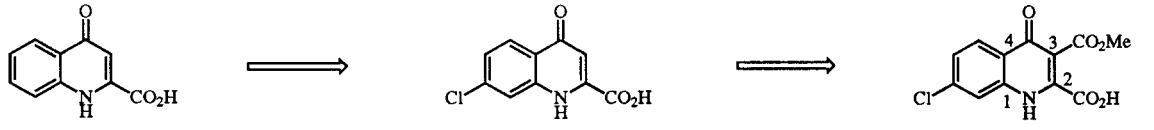
Discovery of the Pyridazino[4,5-*b*]quinolinediones as Potent Glycine/*N*-Methyl-D-aspartate Antagonists.

As was mentioned previously, we decided to focus our initial synthetic program on the lead compounds-kynurenic acid **1** [26] and 6,7-dinitroquinoxalinedione **2** [27]. Both of these agents are relatively modest glycine antagonists and not selective as indicated by comparable binding affinities at the competitive *N*-methyl-D-aspartate

receptor site and the α-amino-3-hydroxy-5-methyl-4-isoxazolylpropionic acid receptor (Figures 1 and 2). However, shortly after we initiated our chemistry program around kynurenic acid, workers at Merck reported that 7-chlorokynurenic acid **3** was a potent and selective glycine/*N*-methyl-D-aspartate antagonist (Figure 1) [28]. Unfortunately, **3** had weak *in vivo* activity, most likely because of poor brain penetration. Our synthetic modifications of kynurenic acid focused mostly on the pyridone ring and demonstrated, among other things, that introduction of simple substituents such as methyl or bromo into the 3-position caused a loss of glycine binding affinity. An exception to this generalization was the 3-carbomethoxykynurenic acid analog **4** (Figure 1) which retained modest affinity for the glycine site. In contrast to the 3-bromo or 3-methyl groups, the 3-carbomethoxy group evidently possessed structural features that were somewhat tolerated by the glycine binding site.

bomethoxy groups. Since the 3-carbomethoxy group is obviously not coplanar with the kynurenic acid ring system, we were especially curious whether a coplanar arrangement of this group might be beneficial in enhancing interaction with the receptor; a similar coplanar arrangement had previously been observed to be essential for optimal receptor interaction in a series of unrelated CNS agents with which we had experience [40]. Extensive studies by the Merck group demonstrated that size-limited hydrophobic groups on the 5- and 7-positions of the kynurenic acid benzene ring were required for optimal interaction with the binding site [39].

During our structure activity studies of kynurenic acid, a simultaneous program of selected screening of acidic bi- and tri-cyclic heterocycles related to kynurenic acid and 6,7-dinitroquinoxalinedione from the Zeneca compound collection lead to the discovery of a pyridazinedione **5** (Figure 2) with weak affinity for the glycine/*N*-methyl-D-



1

[^{3}H]Gly IC_{50} = 33 μ*M*
[^{3}H]AMPA IC_{50} = 100 μ*M*
[^{3}H]CGP 39653 IC_{50} = 49 μ*M*

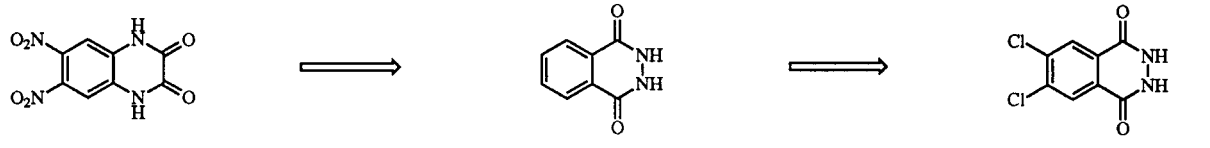
3

[^{3}H]Gly IC_{50} = 0.6 μ*M*
[^{3}H]AMPA IC_{50} = >100 μ*M*
[^{3}H]CGP 39653 IC_{50} = 88 μ*M*

4

[^{3}H]Gly IC_{50} = 6.5 μ*M*
[^{3}H]AMPA IC_{50} = >100 μ*M*

Figure 1.



2

[^{3}H]Gly IC_{50} = 1.3 μ*M*
[^{3}H]AMPA IC_{50} = 0.5 μ*M*
[^{3}H]CGP 39653 IC_{50} = 31 μ*M*

5

Weak affinity for glycine/*N*-methyl-D-aspartate receptor

6

[^{3}H]Gly IC_{50} = 21 μ*M*
[^{3}H]AMPA IC_{50} = >100 μ*M*
[^{3}H]CGP 39653 IC_{50} = 93 μ*M*

Figure 2.

Structural modifications of kynurenic acid in our and other laboratories allowed us and others [39] to make initial proposals on features of the kynurenic acid molecule that were necessary to interact with the glycine/*N*-methyl-D-aspartate receptor site. As illustrated for the 3-carbomethoxykynurenic acid derivative **4** (Figure 3), we proposed that key roles were played by hydrogen bond donation of the 1-NH group of the pyridone ring, an electrostatic interaction of the 2-carboxylate anion and by hydrogen bond acceptor properties of the 4-oxo and/or the 3-car-

aspartate site. A limited synthetic program to explore this series showed that the 6,7-dichloro pyridazinedione **6** possessed modest affinity ([^{3}H]Glycine IC_{50} = 21 μ*M*) for the glycine recognition site. In light of the proposed glycine/*N*-methyl-D-aspartate receptor model, it is not surprising that **6** did not possess a high affinity for the binding site since it is missing several key structural features present in our 3-carbomethoxykynurenic acid analog **4** (Figure 3), namely a hydrogen bond donor corresponding to the pyridone NH of **4**, one of the hydrogen bond accep-

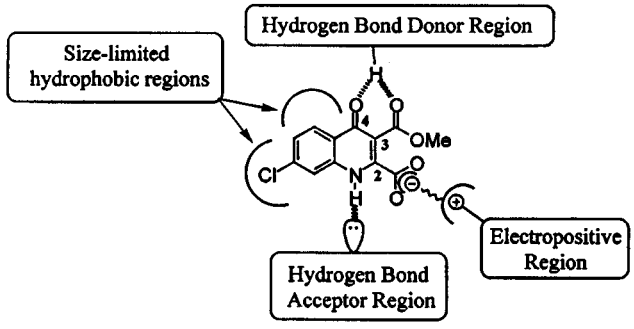


Figure 3. Proposed Glycine/*N*-Methyl-D-aspartate.

tors corresponding to the 4-oxo group of **4**, and an additional aromatic ring corresponding to the benzene ring of **4**. Other features deemed necessary for binding, namely an acidic pyridazinedione ring corresponding to the 2-carboxy group of **4** and an oxo group corresponding to the 3-carbomethoxy group of **4** were present. Very importantly, this series suggested the possibility of using the pyridazinedione ring as a mimic for both the 2-carboxy and 3-carbomethoxy groups of **4**; the pyridazinedione ring also had the additional quality of forcing the 3-carbonyl group into a coplanar arrangement with the ring system. The resulting structural hybrid (Figure 4) of **4** and **6** was synthesized and provided the first compound **7** in a series of pyridazino[4,5-*b*]quinolinediones with potent affinity ([^{3}H]Glycine IC_{50} = 0.075 μM) and greater than 1000-fold selectivity for the glycine site *versus* the competitive site and the α-amino-3-hydroxy-5-methyl-4-isoxazolylpropionic acid receptor.

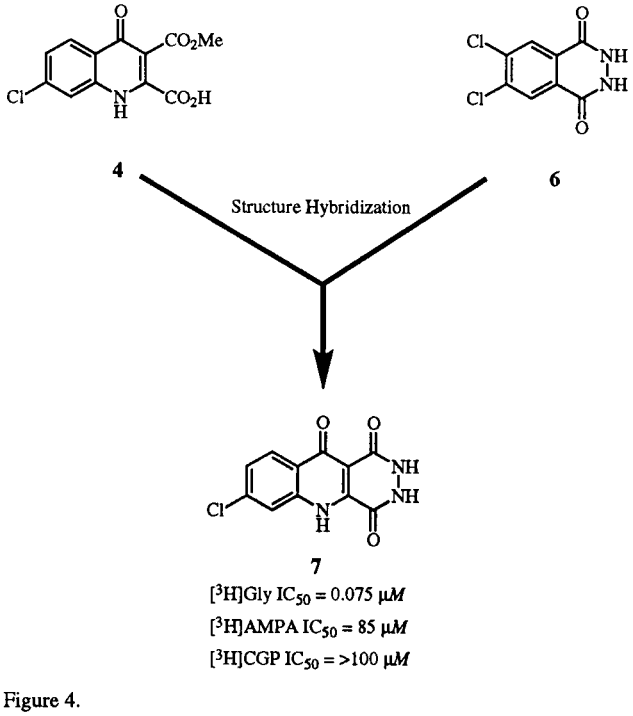


Figure 4.

A proposed glycine site receptor map for the pyridazino[4,5-*b*]quinolinediones is shown in Figure 5. All of the key structural features that were proposed for interaction of our 3-carbomethoxykynurenic acid analog **4** with the receptor are present, including, additionally, the coplanar arrangement of the two potential hydrogen bond accepting 1- and 10-oxo groups. With this novel basic active series now in hand, we set out to investigate the structure activity relationships and biological properties of these compounds.

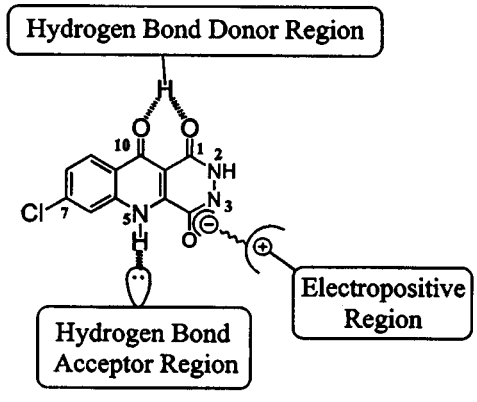


Figure 5. Pyridazino[4,5-*b*]quinolinedione Receptor Map.

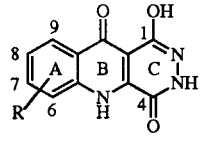
Structure Activity Relationships of the Pyridazino[4,5-*b*]-quinolinediones.

Since the pyridazino[4,5-*b*]quinolinediones were based in part on kynurenic acid, we were particularly interested in comparing the effects of substituents on the pyridazino[4,5-*b*]quinolinedione benzene "A" ring with the structure activity relationship of the corresponding kynurenic acid benzene ring (Table 1) [39]. Like kynurenic acid **1**, the unsubstituted pyridazino[4,5-*b*]quinolinedione **8** was relatively weak in interacting at the glycine/*N*-methyl-D-aspartate site ([^{3}H]Glycine IC_{50} = 14 μM) and was not selective, showing comparable affinities for the competitive site ([^{3}H]D,L-(*E*)-2-amino-4-propyl-5-phosphono-3-pentenoic acid IC_{50} = 34 μM) and α-amino-3-hydroxy-5-methyl-4-isoxazolylpropionic acid receptor ([^{3}H]α-amino-3-hydroxy-5-methyl-4-isoxazolylpropionic acid IC_{50} = 37 μM). Introduction of a chlorine at the 7-position of both the pyridazino[4,5-*b*]quinolinedione and kynurenic acid series (**7** and **3**, respectively) significantly enhanced glycine affinity and selectivity (Figure 4, Table 1). Other mono-chloro substitutions on the pyridazino[4,5-*b*]quinolinedione seemed to follow the structure activity relationship of the kynurenic acid series in that, compared to the parent, glycine affinity modestly increased with substitution at the 9-position (**11** compared to it's kynurenic acid

analog **27**) and decreased with substitution at the 6- and 8-positions of the pyridazino[4,5-*b*]quinolinedione (**9** and **10**, respectively). In contrast to kynurenic acid where 5,7-dichloro substitution had a synergistic effect on glycine affinity and provided a highly potent binder (**28**), no such effect was observed with comparable 7,9-dichlorination of the pyridazino[4,5-*b*]quinolinedione system (**12**). Other interesting features of the pyridazino[4,5-*b*]quinolinedione system included a rapid decrease in glycine affinity with increase in size of alkyl substituents at the 9-position as illustrated in going from methyl to ethyl to *n*-propyl substitution (**20**, **22** and **23**, respectively), a significant loss of affinity by replacement of the 7-chloro substituent (**7**) with methyl (**19**) or methoxy (**17**) and the superior affinity of the 7-chloropyridazino[4,5-*b*]quinolinedione (**7**) for the glycine site when compared with other 7-halo substituted compounds (**14**, **15**, and **16**). Even though the 7-methylpyridazino[4,5-*b*]quinolinedione (**19**) was essentially inactive in binding to the glycine site, introduction of an additional methyl substituent at the 9-position provided a disubstituted compound (**21**) with modest affinity and greater potency than the 9-methylpyridazino[4,5-*b*]-quinolinedione (**20**). The compounds with the most potent affinity for the glycine site included the 7-chloro (**7**), 7,9-dichloro (**12**) and 7-chloro-9-methyl (**24**) pyridazino[4,5-*b*]-quinolinediones. The remaining structure activity relationship studies were carried out with **7** as the base system, primarily because of it's easy synthetic accessibility.

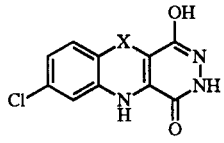
Modification of the 10-position of the pyridone "B" ring of 7-chloropyridazino[4,5-*b*]quinolinedione **7** demonstrated that several changes were fairly well tolerated by the receptor (Table 2). Four- and five-fold losses in glycine/*N*-methyl-D-aspartate binding potency were obtained by substitution of the 10-carbonyl group with thiocarbonyl (**30**) and imino (**29**) groups, respectively. Other substitutions and replacements at the 10-position caused lesser decreases in binding affinity compared to **7**. Especially noteworthy in this regard was replacement of the 10-carbonyl group with sulfur in various oxidation states (**31**, **32**, **33**) and with saturated carbon (**34**) which resulted in relatively minor changes in affinity for the binding site. Removal of the carbonyl group and contraction of the pyridone "B" ring to an indole analog (**35**) also caused only a moderate decrease in glycine affinity. These results suggest that the pyridazine ring 1-oxygen substituent, and not the 10-oxo group, of the pyridazino[4,5-*b*]-quinolinedione system may be of primary importance for optimal interaction with the glycine/*N*-methyl-D-aspartate binding site. Indeed, removal of the 1-oxo substituent from the pyridazinedione "C" ring of the pyridazino[4,5-*b*]-quinolinedione (Table 3, **38** and **39**) caused a loss of binding affinity. Taken in conjunction with the above data from Table 2, these results verify that for the pyridazino[4,5-*b*]-quinolinediones, the 1-oxo group is essential for glycine/-*N*-methyl-D-aspartate receptor interaction.

Table 1



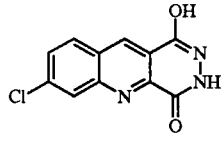
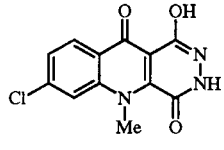
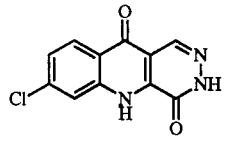
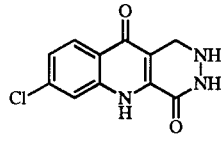
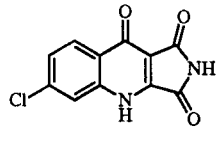
Compound Number	R	[^{3}H]Gly IC_{50}, μM	Compound Number	R	[^{3}H]Gly IC_{50}, μM
8	H	14	**19**	7-Me	>100
9	6-Cl	>50	**20**	9-Me	1.2
7	7-Cl	0.075	**21**	7,9-di-Me	0.57
10	8-Cl	55	**22**	9-Et	6.2
11	9-Cl	2.4	**23**	9-*n*-Pr	>500
12	7,9-di-Cl	0.094	**24**	7-Cl, 9-Me	0.08
13	7,8,9-tri-Cl	3.2	**25**	7-Me, 9-Cl	0.22
14	7-F	0.47	**26**	7-NO_2	8.6
15	7-Br	0.12	**1**	Kynurenic Acid	33
16	7-I	0.62	**27**	5-Chlorokynurenic Acid	2.3
17	7-OMe	>100	**3**	7-Chlorokynurenic Acid	0.63
18	7-CF_3	6.1	**28**	5,7-Dichlorokynurenic Acid	0.25

Table 2



Compound Number	X	[^{3}H]Gly IC_{50}, μM
7	C=O	0.075
29	C=NH	0.4
30	C=S	0.3
31	S	0.2
32	SO	0.2
33	SO_2	0.2
34	CH_2	0.1
35	Chemical bond (indole)	0.3

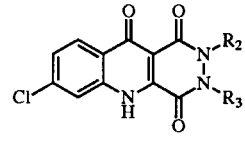
Table 3

Compound Number	Structure	[^{3}H]Gly IC_{50}, μM
36		10
37		>100
38		>100
39		>10
40		>100

The importance of the 5-hydrogen for optimal glycine binding of the pyridazino[4,5-*b*]quinolinedione system was verified by the weak binding activity of analogs obtained by its removal through aromatization of the "B" ring (**36**) and by replacement of the 5-H with methyl (**37**) as shown in Table 3. Contraction of the 6-membered pyridazinedione "C" ring to the pyrrolodione **40** (Table 3) also resulted in a loss of activity.

Substitution of the 2- and 3-nitrogens of the 7-chloropyridazino[4,5-*b*]quinolinedione **7** with various alkyl and heteroalky substituents generally lead to modest decreases in glycine binding potency for the 2-substituted compounds and to significant decreases for the 3-substituted compounds (Table 4). This was exemplified by the N-2 and N-3 methyl substituted pyridazino[4,5-*b*]quinolinediones **41** and **42**, respectively. The 2-methyl compound **41** was about 4 times less potent than the parent compound while the 3-methyl analog was more than 500 times weaker. A variety of 2-alkyl and 2-heteroalkyl substituents shown in Table 4 illustrated that the glycine receptor appeared to have a significant degree of tolerance for substituents in the northeast quadrant of the pyridazino[4,5-*b*]quinolinedione molecule. Alkyl branching (**45**) and linear substituents, including both alkyl (**44**) and heteroalkyl (**46**), up to 8 atoms long still retained modest affinity for the receptor. Note that the acetylenic substituent (**47**) provided a pyridazino[4,5-*b*]quinolinedione that was twice as potent as the unsubstituted parent 7-chloropyridazino[4,5-*b*]quinolinedione **7**.

Table 4

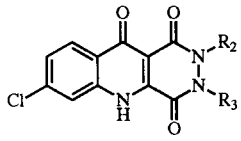


Compound Number	R_2	R_3	[^{3}H]Gly IC_{50}, μM
7	H	H	0.075
41	Me	H	0.34
42	H	Me	41
43	$(CH_2)_2OCOMe$	H	0.26
44	$(CH_2)_5Me$	H	0.23
45	$CHMe(CH_2)_2Me$	H	0.42
46	$(CH_2)_2O(CH_2)_2OEt$	H	0.42
47	$CH_2C{\equiv}CMe$	H	0.041

Introduction of aromatic containing substituents to the pyridazino[4,5-*b*]quinolinedione 2- and 3-positions also demonstrated tolerance at the 2- and not the 3-position (Table 5). Notice that with a simple phenyl substituent, the 2-substituted compound **48** was more than twice as potent as the parent compound **7** while the 3-phenyl compound **49** was greater than 1000 times weaker. Insertion

of up to 4 methylene spacers between the pyridazino[4,5-*b*]-quinolinedione 2-nitrogen and the aromatic ring provided compounds (**50**, **53**) with potent affinity for the receptor. Alpha branching of the spacer (**52**) appeared to be detrimental to binding while insertion of certain heteroatoms (**51**) and amides (**54**) was tolerated without loss of potency. Again, one of the most potent compounds contained an acetylenic group (**55**) in the spacer. Generally speaking, the region of the receptor corresponding to the northeast quadrant of the pyridazino[4,5-*b*]quinolinedione was remarkably tolerant to a wide range of substituents with certain acetylenic and aryl containing substituents demonstrating highest affinities for the glycine recognition site.

Table 5

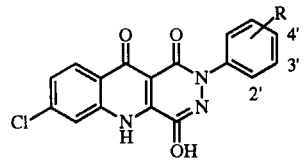


Compound Number	R_2	R_3	[^{3}H]Gly IC_{50}, μM
7	H	H	0.075
48	Ph	H	0.031
49	H	Ph	100
50	CH_2Ph	H	0.088
51	$(CH_2)_3OPh$	H	0.081
52	$CHMeCH_2Ph$	H	0.33
53	$(CH_2)_4Ph$	H	0.073
54	$CH_2CONHPh$	H	0.089
55	$CH_2C{\equiv}CPh$	H	0.016

For reasons that will become apparent shortly, our synthetic efforts became focused on 2-aryl substituted pyridazino[4,5-*b*]quinolinedione analogs. Table 6 shows that monosubstitution at the 2-, 3-, and 4-postions of a pendant 2-phenyl ring with a variety of electron donating and withdrawing substituents provided compounds with excellent to good glycine/*N*-methyl-D-aspartate binding affinity. Several compounds (**56**, **57**, **60**, **65** and **66**) had IC_{50} values of 11 n*M* or better with the 2-hydroxyphenyl analog (**65**) being the most potent with a 6 n*M* affinity. Generally, most simple monosubstituted 2-aryl pyridazino-[4,5-*b*]quinolinediones that were prepared had glycine IC_{50} values of 100 n*M* or better.

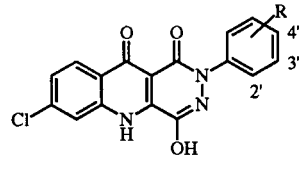
Similarly, disubstitution of the pyridazino[4,5-*b*]quinolinedione 2-aryl ring with a variety of simple substituents provided compounds demonstrating excellent to good glycine/*N*-methyl-D-aspartate binding affinity with most compounds having IC_{50}s of 200 n*M* or better. A sampling of 2,4- and 3,4-disubstitued compounds is shown in Table 7 as representative examples of disubstituted pyridazino-[4,5-*b*]quinolinediones. Both Tables 6 and 7 indicate that potent receptor affinity may be obtained when the pendant 2-phenyl ring is mono or di-substituted by a variety of simple substituents with varied electronic properties.

Table 6



Compound Number	R	[^{3}H]Gly IC_{50}, μM
56	2'-Cl	0.010
57	3'-Cl	0.011
58	4'-Cl	0.054
59	2'-Me	0.028
60	3'-Me	0.011
61	4'-Me	0.028
62	2'-OMe	0.068
63	3'-OMe	0.032
64	4'-OMe	0.020
65	2'-OH	0.006
66	3'-OH	0.011
67	4'-OH	0.069
68	3'-NO_2	0.024
69	4'-NO_2	0.11

Table 7



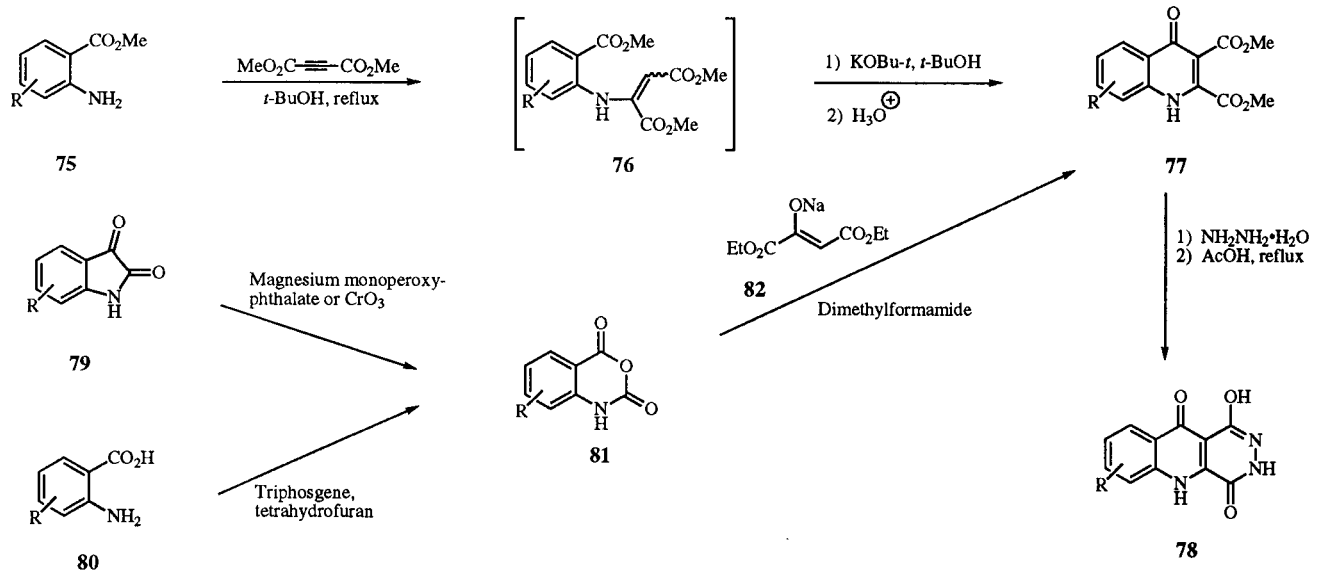
Compound Number	R	[^{3}H]Gly IC_{50}, μM
70	3'-Cl, 4'-OMe	0.028
71	3'-Cl, 4'-OH	0.008
72	2',4'-di-Me	0.035
73	2'-Me, 4'-OMe	0.075
74	3', 4'-di-OH-Me	0.058

Chemistry.

The parent pyridazino[4,5-*b*]quinolinediones were synthesized (Scheme 1) [41] from anthranilic esters (**75**) or isatoic anhydrides (**81**). The requisite anthranilic esters were heated with dimethyl acetylenedicarboxylate to provide an intermediate enaminodiester (**76**) which was not isolated, but treated with potassium *t*-butoxide to give quinolone-2,3-dicarboxylic acid esters (**77**) [41,42]. With certain anthranilic esters containing electron withdrawing substituents, the initial condensation with dimethyl acetylenedicarboxylate was unsuccessful and an alternate route to the quinolone diester **77** involving reaction of isatoic anhydrides **81** with the sodium salt of 2-oxosuccinate diester (**82**) was used [43]. Treatment of the key quinolone diesters **77** with an excess of hydrazine hydrate in refluxing ethanol [41,44] provided the hydrazine salt of the desired pyridazino[4,5-*b*]quinolinediones **78**. These salts were then converted to the free acid form by refluxing with glacial acetic acid.

Scheme 1

Synthesis of the Parent Pyridazino[4,5-*b*]quinolinediones



The initial synthetic route [45] to 2-arylpyridazino[4,5-*b*]-quinolinediones first involved selective hydrolysis [42] of the more reactive 2-carbomethoxy ester of the quinolone diesters **77** (Scheme 2). Treatment of the resulting acid ester **83** with thionyl chloride provided the acid chloride **84** which was reacted with an aryl hydrazine **88** to form an intermediate acyl hydrazide **85**. Heating **85** in methanol containing methanesulfonic acid provided a mixture of the desired 2- and 3-aryl pyridazino[4,5-*b*]quinolinediones (**86** and **87**, respectively). The 3-isomers **87** were generally extremely insoluble and were quantitatively removed from the cooled methanesulfonic acid/methanol reaction mixture by simple filtration at the completion of the reaction. Concentration of the filtrate and recrystallization of the residue provided modest to good yields of the desired 2-aryl isomer **86**.

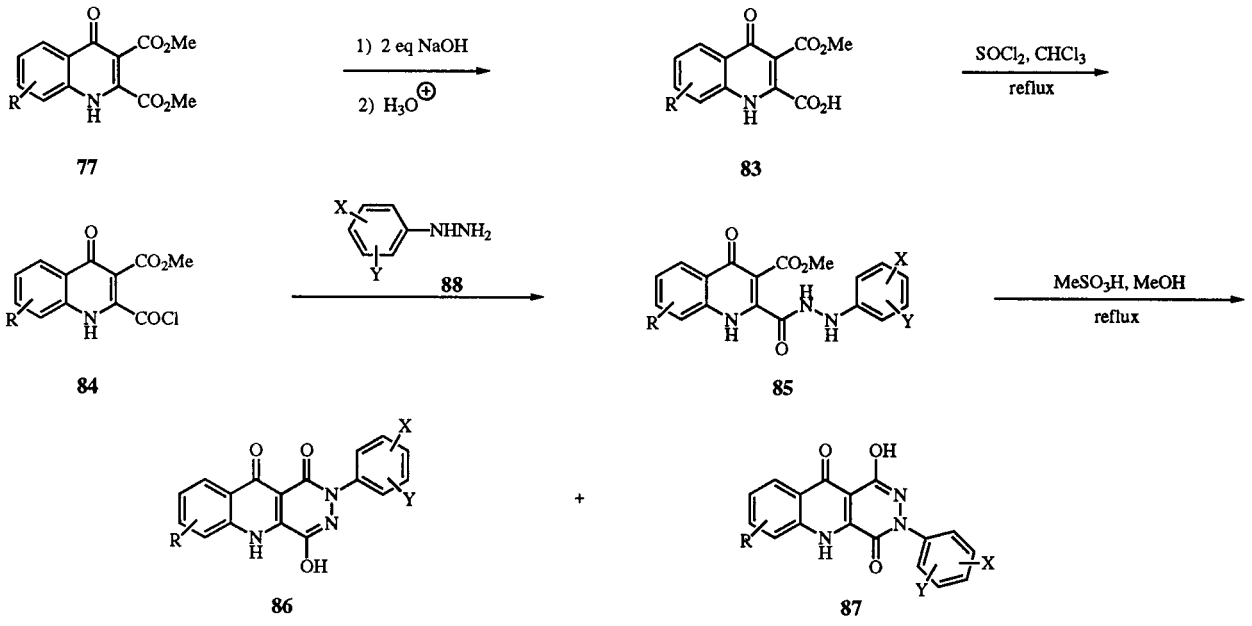
As the stroke project progressed and it was discovered that the 2-substituted pyridazino[4,5-*b*]quinolinediones were of interest because of their biological activity, more efficient regiospecific methods of preparing the desired 2-substituted pyridazino[4,5-*b*]quinolinedione isomers were developed [45]. For example (Scheme 3), condensation of aryl hydrazine **88** with benzaldehyde provided the aldimine derivative **89** which was reduced to the benzyl hydrazine **90** using borane and hydrogen chloride gas [46]. Reaction of **90** with acid chloride **84** provided the benzyl protected acyl hydrazide **91** which was cyclized to the 3-benzyl-2-arylpyridazino[4,5-*b*]quinolinedione **92** by treatment with choline hydroxide. Refluxing **92** with methanesulfonic acid and methanol gave moderate overall yields of the desired 2-arylpyridazino[4,5-*b*]quinolinediones **86**. A more general efficient regiospecific synthesis [45] of both 2-aryl and 2-alkylpyridazino[4,5-*b*]quinolinediones involved the formation of quinolone pyrrolidine amide acids **93** (Scheme 4) by treatment of acid chloride **84** with pyrrolidine and selective hydrolysis of the resulting ester amide to the acid amide **93**. Coupling of **93** with BOC-protected hydrazines **94** using dicyclohexylcarbodiimide or other (water soluble) carbodiimides generally provided moderate to good yields of BOC-protected acylhydrazides **95**. Refluxing **95** with methanesulfonic acid in methanol gave exclusively the desired 2-substituted pyridazino[4,5-*b*]quinolinediones **96**.

In Vivo and Physical Properties.

During the course of our glycine/*N*-methyl-D-aspartate project, many compounds with selective high affinity for the glycine site were reported in the literature [29] and, with very few exceptions, all were weak or inactive in *in vivo* testing - presumably because of poor penetration across the blood brain barrier. The pyridazino[4,5-*b*]quinolinediones did not appear to be plagued with this problem and many demonstrated excellent *in vivo* activity in both our functional rat red nucleus assay and in our focal model of cerebral ischemia. Table 8 shows *in vivo* data for some representative pyridazino[4,5-*b*]quinolinediones. The rat red nucleus test demonstrated that the pyridazino[4,5-*b*]quinolinediones rapidly (inhibition of *N*-methyl-D-aspartate-induced cell firing was generally observed within several minutes after initiation of the compound's

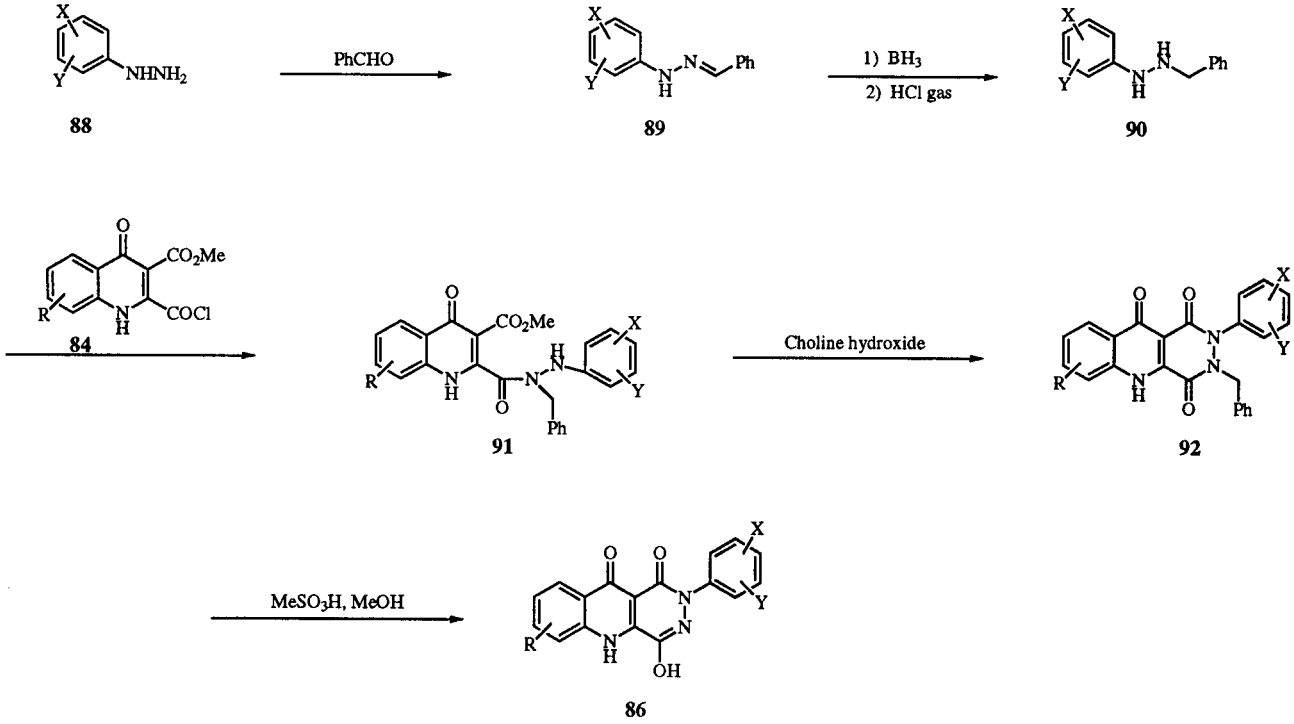
Scheme 2

Initial Synthesis of 2-Arylpyridazino[4,5-*b*]quinolinediones



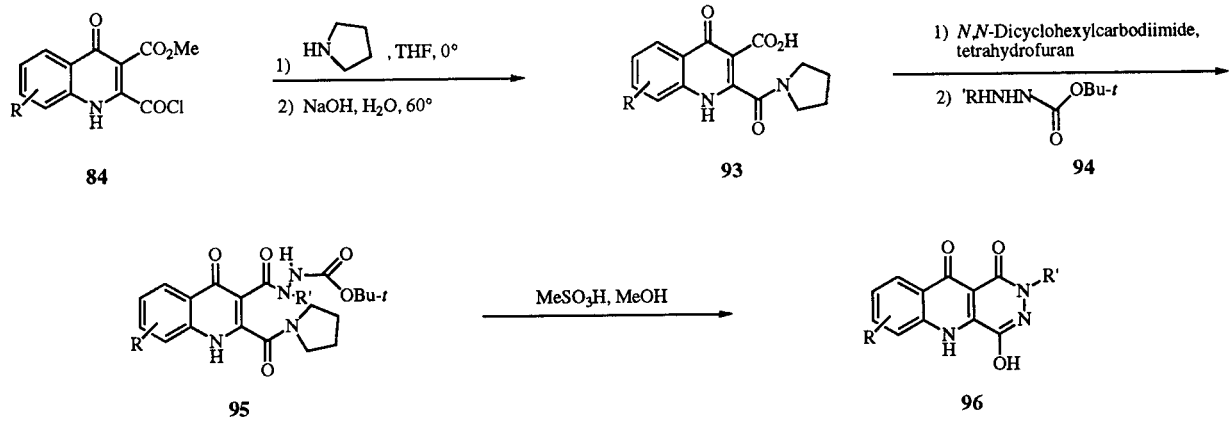
Scheme 3

Regiospecific Synthesis of 2-Arylpyridazino[4,5-*b*]quinolinediones



Scheme 4

Regiospecific Synthesis of 2-Aryl and 2-Alkylpyridazino[4,5-*b*]quinolinediones

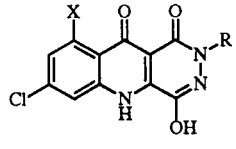


84 93 94 95 96

R = H, single or multiple substituents
R' = Alkyl, aryl, aralkyl, heteroaralkyl

iv infusion) penetrated into the brain and the rat middle cerebral artery occlusion model of focal ischemia showed that the pyridazino[4,5-*b*]quinolinediones significantly reduced the size of infarct volume compared to controls. Consequently, in contrast to most glycine antagonists developed by others which had poor systemic activity, the major challenge with the pyridazino[4,5-*b*]quinolinediones was devising a way to rationally select a compound for development from a relatively large pool of compounds with good *in vivo* activity.

Table 8



Compound Number	X	R	Rat Red Nucleus, ID_{50}, mg/kg, iv	Rat MCA Bolus + 4 hours infusion (mg/kg, iv): % infarct reduction
7	H	H	5.5	3 + 3: 25% [a]
97	Cl	2,4-di-MePh	6.5	10 + 10: 22% [a]
98	H	$(CH_2)_3Me$	2.1	20 + 20: 19% [a]
61	H	4-MePh	6.1	20 + 20: 59% [a]
99	H	$(CH_2)Ph$	4.2	20 + 20: 23% [a]

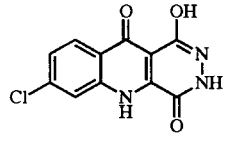
[a] Statistically significant infarct volume reduction.

One of the essential attributes required for development of our glycine/*N*-methyl-D-aspartate antagonist for stroke was that it had to be soluble in vehicles suitable for iv administration. The major obstacle that had to be overcome with the pyridazino[4,5-*b*]quinolinediones was their generally unfavorable physical chemical properties; in particular, poor solubility of the pyridazino[4,5-*b*]quinolinediones resulted in, initially, intravenous dosing being difficult or impossible. Other series of glycine and α-amino-3-hydroxy-5-methyl-4-isoxazolylpropionic acid antagonists reported in the literature also appeared to be associated with solubility problems and there were reports of certain of these compounds precipitating in the urine or causing toxicity because of crystallization in the kidneys [47] after administration to animals or humans.

Table 9 illustrates some of the physical properties of our first synthesized pyridazino[4,5-*b*]quinolinedione (**7**). This material did not melt up to 400° and was generally insoluble in all common organic solvents; pyridazino[4,5-*b*]-quinolinedione **7** could be recrystallized from hot dimethyl sulfoxide. Even though it is acidic, **7** was very poorly soluble in aqueous solutions of inorganic bases and in buffer at the physiological *p*H of 7.4. However, it was soon discovered that **7** could be dissolved in aqueous solutions of certain organic amines, such as meglumine (an amino-sugar) which is a suitable, though not ideal, vehicle for iv administration. Also, by making choline salts [41] of **7** and other pyridazino[4,5-*b*]quinolinediones, dramatic

increases of solubility in water or aqueous meglumine could be achieved with some pyridazino[4,5-*b*]quinolinedione concentrations reaching 75 mg/ml. By using these dissolution techniques, it was possible to screen the great majority of our compounds using iv administration.

Table 9
Physical Properties of Pyridazino[4,5-*b*]quinolinedione **7**



Color: Yellow crystalline solid

Melting point: >400°

p*K*a: 6.1, 10.1

Log D: 0.45
Log P: 1.75

Solubility:

- Insoluble in all common solvents; recrystallized from dimethyl sulfoxide
- Dissolves at 0.012 mg/ml in *p*H 7.4 buffer
- Dissolves at 0.32 mg/ml in *p*H 8.9 buffer

Stability:

- Thermal - Stable ($t_{1/2}$>240 hours @ 90°) at *p*H 4-10
- Photo - Stable

Salts:

- Alkali metal salts - Low solubility in aqueous media
- Organic amines

⇒ Soluble (10 mg/ml) in 0.1*M* meglumine

⇒ Slightly soluble (1.4 mg/ml) in 0.1*M* arginine

⇒ Choline salt - soluble (>25 mg/ml) in water, 5% dextrose, or 0.3*M* meglumine

With methods now available to solubilize the majority of pyridazino[4,5-*b*]quinolinediones, we were concerned that, once administered, the compounds might precipitate on mixing with blood and cause toxicity. Indeed, when solutions of **7** in various organic amines were mixed with saline and inorganic buffers, a voluminous precipitate (the pyridazino[4,5-*b*]quinolinedione alkali metal salt) formed. To address solubility and potential precipitation concerns, a qualitative solubility/saline compatibility test (Figure 6) was devised to screen compounds that were active in our glycine binding assay. This solubility assay simply involved attempting to dissolve pyridazino[4,5-*b*]quinolinediones with good affinity for the glycine receptor in 0.3*M* aqueous meglumine. If soluble, the resulting solution was diluted with a 10-fold volume of isotonic saline to determine whether or not a precipitate formed. Those compounds that did not form a precipitate on dilution with saline were deemed to be suitably soluble and carried forward for additional biological testing.

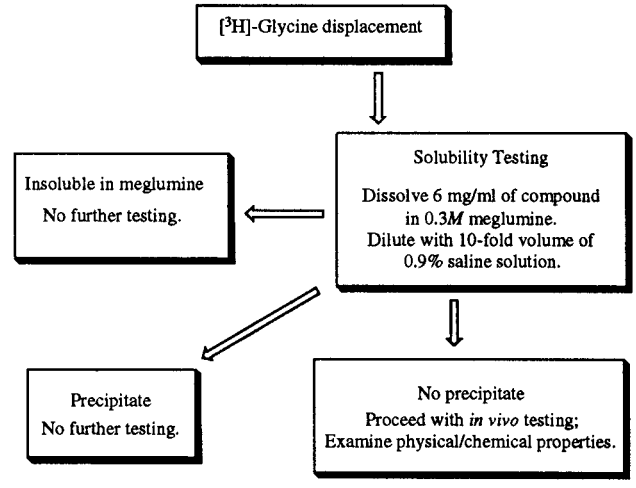


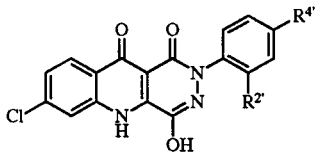
Figure 6. Solubility Screening Cascade.

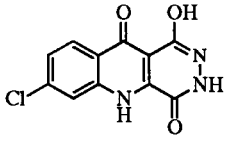
With regard to the above solubility assay, we felt that lack of precipitation provided two valuable pieces of information. First, we reasoned that dilution with saline might mimic in a crude fashion the situation that occurs when a pyridazino[4,5-*b*]quinolinedione solution is injected into the blood stream; the results could provide an indication of whether *in vivo* precipitation of the pyridazino[4,5-*b*]quinolinedione might occur. Second, a lack of precipitation might also indicate that a sodium or potassium salt of the compound would probably be soluble and compatible with isotonic saline/dextrose, a common intravenous vehicle that we preferred to use for development of our stroke agent.

Our qualitative solubility tests correlated quite nicely with quantitative solubility testing in *p*H 7.4 buffer. Several important and interesting trends in solubility of the pyridazino[4,5-*b*]quinolinediones were observed (Table 10). In particular, 2-aryl substitution, in contrast to most alkyl and aryl alkyl substitution, dramatically increased solubility compared to the corresponding unsubstituted 7-chloropyridazino[4,5-*b*]quinolinedione **7**. For example, the 2-phenylpyridazino[4,5-*b*]quinolinedione **48** is 50 times more soluble than the corresponding unsubstituted compound **7**. Substitution of the 2-phenyl ring at the *para*-position (**61**, **64** and **58**) caused decreases in solubility compared to the unsubsitituted 2-phenyl analog **48**. *Meta*-substituted compounds were even more

insoluble than the *para* analogs. Very interestingly, *ortho*-substituted phenyl analogs (**59**, **62** and **56**) resulted in marked increases in solubilities compared to the unsubstituted parent and comparably substituted *meta*- and *para*-analogs. Finally, as shown by the bottom three compounds (**72**, **73** and **100**) in Table 10, it was determined that pyridazino[4,5-*b*]quinolinediones substituted with an *ortho*-methyl substituent combined with a *para* substituent provided compounds with optimal solubilities that were 300-600 times greater than the original parent compound.

Table 10



Compound Number	$R^{2'}$	$R^{4'}$	Solubility, mg/ml *p*H 7.4, phosphate buffer
7			0.012
48	H	H	0.59
61	H	Me	0.17
64	H	OMe	0.37
58	H	Cl	0.16
59	Me	H	2.64
62	OMe	H	1.24
56	Cl	H	0.75
72	Me	Me	4.28
73	Me	OMe	7.40
100	Me	Cl	3.77

An evaluation of *in vitro* and *in vivo* activities along with solubility properties lead to a short list of four pyridazino[4,5-*b*]quinolinedione candidates (Table 11) possessing potentially suitable profiles (selective nanomolar affinity for the glycine/*N*-methyl-D-aspartate receptor site; activity in the rat red nucleus and/or middle cerebral artery assays; passed qualitative solubility assay) for development. Notice that three (**100**, **72**, **73**) out of four these potential development candidates have the 2,4-disubstitution pattern on the 2-phenyl substituent of the pyridazino[4,5-*b*]quinolinedione. Additional head to head biological testing and comparison of physical chemical properties of these compounds eventually lead to the emergence of **ZD9379** (Figure 7, the sodium salt of **73**) as our development candidate for the treatment of ischemic stroke.

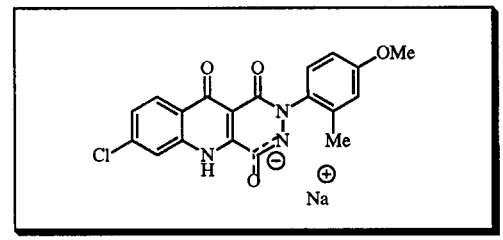


Figure 7. Sodium 7-Chloro-2-(4'-methoxy-2'-methylphenyl)-1,2,5,10-tetrahydropyridazino[4,5-*b*]quinoline-1,10-dion-4-enolate (**ZD9379**).

ZD9379 - Biological and Physical Properties.

In vitro Properties.

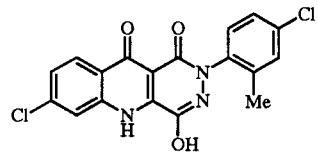
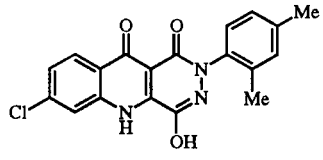
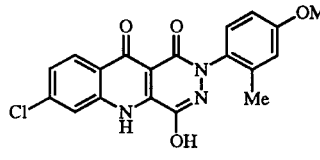
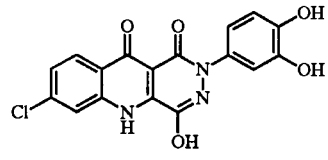
Sodium 7-chloro-(4'-methoxy-2'-methylphenyl)-1,2,5,10-tetrahydropyridazino[4,5-*b*]quinoline-1,1,0-dion-4-enolate (**ZD9379**) is a selective glycine/*N*-methyl-D-aspartate antagonist. It potently binds to both rat and human brain glycine/*N*-methyl-D-aspartate ([^{3}H]Glycine IC_{50}s = 0.075 μ*M* and 0.088 μ*M*, respectively) recognition sites and has weak affinities for the competitive *N*-methyl-D-aspartate site ([^{3}H]D,L-(*E*)-2-amino-4-propyl-5-phosphono-3-pentenoic acid IC_{50} = NA @ 10 μ*M*) and the α-amino-3-hydroxy-5-methyl-4-isoxazolylpropionic acid receptor ([^{3}H]α-amino-3-hydroxy-5-methyl-4-isoxazolylpropionic acid IC_{50} = 40 μ*M*). **ZD9379** antagonizes *N*-methyl-D-aspartate receptor function, inhibiting tritiated 1-[(2-thienyl)cyclohexyl]piperidine binding to the open channel of the receptor. This inhibition was overcome with additional glycine, consistent with **ZD9379** acting as an antagonist at the glycine site of the *N*-methyl-D-aspartate receptor [32]. No additional binding activity was observed in a broad screen of binding and functional assays carried out by Panlabs, Inc. [48].

In vivo Activity.

In the *in vivo* rat red nucleus assay, intravenously administered **ZD9379** rapidly (inhibitory effects began 2-3 minutes after initiation of **ZD9379** infusion) crosses the blood brain barrier to inhibit *N*-methyl-D-aspartate-induced rat red nucleus cell firing with an ID_{50} of 5.8 mg/kg. An appropriate dose of **ZD9379** administered by bolus or infusion completely inhibits *N*-methyl-D-aspartate-induced neuronal firing within 10-20 minutes. Iontophoretic application of the glycine agonist, D-serine, reverses red nucleus cell firing inhibition caused by **ZD9379**, consistent with the compound being a glycine/*N*-methyl-D-aspartate antagonist [34].

ZD9379 is neuroprotective in several different animal models of focal cerebral ischemia. In the Zeneca middle cerebral artery model in spontaneously hypertensive rats, a severe test of neuroprotectant activity [6], **ZD9379** significantly reduced cerebral infarct volume by 31% compared to controls (Table 12). The compound was dosed

Table 11

Compound Number	Structure	[^{3}H]Gly IC_{50}, μM	Red Nucleus ID_{50}, mg/kg, iv	MCA Bolus + 4 hours infusion (mg/kg, iv): % infarct reduction
100		0.028	>16	10 + 10: 28% [a]
72		0.035	7.8	10 + 10: 17% 15 + 15: 31% [a] 40 bolus: 38% [a]
73		0.075	5.8	10 + 10: 31% [a] 40 bolus: 42% [a]
74		0.058	14	10 + 10: 22% [a]

[a] Statistically significant infarct volume reduction.

intravenously in isotonic saline/dextrose as a 10 mg/kg bolus immediately after induction of the occlusion followed by a 10 mg/kg/hour infusion for 4 hours. For the sake of comparison, the glycine/*N*-methyl-D-aspartate antagonist 6,7-dichloro-5-nitro-1,2,3,4-tetrahydroquinoxaline-2,3-dione (ACEA 1021) [49] was tested in this middle cerebral artery test. Using the same bolus/infusion dosing regimen, 6,7-dichloro-5-nitro-1,2,3,4-tetrahydroquinoxaline-2,3-dione had to be administered at twice the dose (*i.e.*, a 20 mg/kg bolus followed by a 4 hour infusion of 20 mg/kg/hour for 4 hours) used for **ZD9379** to obtain a significant reduction (42%) in infarct volume. Also, note (Table 12) that a single 40 mg/kg dose of **ZD9379** administered immediately after the occlusion caused 42% reduction in infarct volume.

ZD9379 was also neuroprotective (Table 12) in the normotensive rat permanent filament middle cerebral artery occlusion model of focal ischemia when evaluated by Dr. Marc Fisher of the Medical Center of Central Massachusetts [50]. This model employs a filament to occlude the middle cerebral artery and is not as invasive as the Zeneca model. In this test, intravenous dosing of **ZD9379**

Table 12
ZD9379 - Activity in Focal Ischemia Models

Focal Ischemia Model	Dose (iv) Bolus + Infusion (mg/kg)	Infarct Reduction (% Change from Control)
Zeneca SH Rat Permanent MCA Occlusion	10 + 10 40 bolus	31% [a] 42% [a]
Permanent Filament MCA in Rat [50]	5 + 5 (30 minutes) 2.5 + 2.5 (30 minutes) 1 + 1 (30 minutes)	51% [a] 43% [a] 20%
Permanent MCA Occlusion - MRI Study [51]	10 + 2.5	41% [a]
Temporary Filament MCA in Rat [52]	10 + 10 (60 minutes) 40 bolus (150 minutes)	39% [a] 40% [a]

[a] Statistically significant infarct volume reduction.

was delayed for 30 minutes after the occlusion and **ZD9379** still demonstrated highly significant infarct reductions of 51% and 43% at doses of 5 and 2.5 mg/kg, respectively, using our normal bolus/infusion dosing regimen. Even the 1 mg/kg dosing regimen caused a 20% non significant trend towards infarct reduction.

ZD9379 also reduced (Table 12) the stroke volume in a rat permanent middle cerebral artery occlusion model using three dimensional diffusion weighted magnetic resonance imaging (MRI) techniques to determine lesion size; these studies were carried out by Dr. Alan Johnson's group at Duke University Medical Center [51]. In this test **ZD9379** was administered immediately after the occlusion as a 10 mg/kg iv bolus followed by a 4 hour infusion of 2.5 mg/kg/hour. Six hours after the occlusion, a 41% diffusion weighted reduction in stroke volume was observed. No significant changes in blood pressure, heart rate and body temperature were observed during the course of this experiment.

Finally, the effect of delays in dosing **ZD9379** after induction of the occlusion was studied (Table 12) in a temporary rat filament model of cerebral ischemia by Dr. Chung Hsu of Washington University (St. Louis, MO) [52]. A significant reduction (39%) in infarct volume was observed when **ZD9379** was administered 60 minutes after the onset of ischemia using the 10 mg/kg bolus/10 mg/kg/hour infusion (4 hours) dosing paradigm; even more impressive was a significant reduction (40%) in infarct volume obtained when **ZD9379** was administered as a 40 mg/kg bolus 2.5 hours after induction of ischemia. The therapeutic window observed in this assay suggests that **ZD9379** might be an effective neuroprotectant when administered to stroke victims after significant time has elapsed between occurrence of the stroke and treatment initiation.

Since electroencephalogram records synchronous brain activity, drugs with similar brain effects should produce similar electroencephalogram effects. Both competitive and noncompetitive *N*-methyl-D-aspartate antagonists are known to increase electroencephalogram activity and to produce distinctive electroencephalogram spectral power changes [53] characteristic of psychotomimetic agents. In the rat, **ZD9379** (20 mg/kg bolus followed by 4 hour infusion of 20 mg/kg/hour) reduced electroencephalogram activity and produced similar broad changes in electroencephalogram band power observed with another glycine antagonist 6,7-dichloro-5-nitro-1,2,3,4-tetrahydroquinoxaline-2,3-dione (40 mg/kg bolus followed by 4 hour infusion of 40 mg/kg/hr). In contrast, increases in electroencephalogram activity were observed with the competitive antagonist Selfotel and the noncompetitive antagonist Cerestat (each given as a 10 mg/kg bolus followed by a 4 hour infusion of 10 mg/kg/hour), both of which have produced psychotomimetic-like effects when administered to humans [54,55]; the alterations in electroencephalogram activity observed with both Selfotel and Cerestat occurred concomitantly with stereotypical behaviors (forepaw treading, head weaving and circling) characteristic of psychotomimetic agents. No such stereotypical behaviors were observed with **ZD9379** and 6,7-dichloro-5-nitro-1,2,3,4-tetrahydroquinoxaline-2,3-dione during the electroencephalogram experiments.

In mice, **ZD9379** produced transient sedation as measured by locomotor activity and ataxia after intravenous administration; the sedation was transient with locomotor activity being resumed within 30 minutes. No circling behavior was observed at any dose of **ZD9379** while both competitive and noncompetitive antagonists displayed this behavior.

ZD9379 does not produce phencyclidine- or dizocilpine-like transient reversible vacuolizations [56] in the cingulate cortex of rats at 50 mg/kg, iv. These vacuolizations may be associated with the production of hallucinations [57].

Thus, the lack of stereotypical behaviors in both rats and mice, the lack of phencyclidine-like increases in electroencephalogram activity and the lack of vacuolization formation suggest that **ZD9379** will not have a propensity to produce hallucinogenic side effects in man as seen in the clinic with both Selftel (CGS 19755) which is *cis*-4-(phosphonomethyl)-2-piperidine [54] and Cerestat (CNS 1102) which is *N*-(1-naphthyl)-*N'*-(3-ethylphenyl)-*N'*-methylguanidine hydrochloride [55].

Finally, **ZD9379** (administered at 50 mg/kg, iv) has been demonstrated not to increase the severity of bleeding in a collagenase induced hemorrhagic stroke model. In the clinic and especially in the field, it can be difficult to rule out intracerebral hemorrhage as a cause of stroke symptoms and it is important that **ZD9379** not be contraindicated in hemorrhagic stroke.

The physical properties of **ZD9379** are shown in Table 13. The compound is a water soluble (>40 mg/ml) sodium salt (recrystallized form methanol/water) and is directly soluble in isotonic saline/dextrose with a maximum solubility of 17 mg/ml. The *p*H of the resulting saline/dextrose solutions is about 6.7 which appears to be ideal for iv administration. Solutions of **ZD9379** in saline/dextrose are compatible with rat and dog blood with no evidence of precipitation or morphological changes in the blood. **ZD9379** is extremely stable in solution at *p*H ranges from 4-10 and is stable towards light.

ZD9379 has a terminal elimination plasma half-life of ~34 hours in the rat and ~15 hours in the dog and is ~65% orally bioavailable in the rat.

Table 13
ZD9379 - Physical/Chemical Properties

- Crystalline sodium salt:
  Recrystallized from methanol/water
- p*K*a = 4.5
- Log D = 0.73 (*p*H 7.4)
- Solubility:
  Water: >40 mg/ml
  Isotonic saline/dextrose: 10-17 mg/ml (*p*H 6.7)
  Phosphate buffer (*p*H 7.4): 16.5 mg/ml
  Blood: dextrose/saline solutions compatible with rat and dog blood
- Stability:
  Solution: half-life at *p*H 4-10 >18 years
  Photo: Stable

Based on it's excellent pharmacological profile and favorable physical chemical properties, **ZD9379** was placed into development for the prevention/limitation of brain neurodegeneration caused by stroke.

In conclusion, we have discovered a series of novel selective glycine/*N*-methyl-D-aspartate antagonists, the pyridazino[4,5-*b*]quinolinediones. Structure activity relationships of the pyridazino[4,5-*b*]quinolinediones *versus* the glycine/*N*-methyl-D-aspartate receptor site were determined and *in vitro* and *in vivo* activity as well as physical chemical properties were optimized for the 2-arylpyridazino[4,5-*b*]quinolinediones. As a result of these efforts, **ZD9379** was selected as a development compound for the treatment of stroke.

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