PYRIDAZINO [4,5-B]-QUINOLINE 5-OXIDE DERIVATIVES, THEIR PREPARATION AND THEIR USE AS GLYCINE ANTAGONISTS

FIELD OF INVENTION

New chemical compounds which are pyrido-phtalazin diones, pharmaceutical compositions containing the same, and their use against neurological disorders associated with excitotoxicity and malfunctioning of glutamatergic neurotransmission.

BACKGROUND OF THE INVENTION AND PRIOR ART

Glutamate is probably the major excitatory transmitter in the central nervous system but is also likely to be involved in many pathological and excitotoxic processes. As such there is a great deal of interest in the development of glutamate antagonists for therapeutic use (see Danysz et al., 1995 for review). Glutamate activates three major types of ionotropic receptor, namely α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), kainate and N-methyl-D-aspartate (NMDA) and several types of metabotropic receptors. Antagonism of NMDA receptors potentially has a wide range of therapeutic applications. Functional inhibition of NMDA receptors can be achieved through actions at different recognition sites such as the primary transmitter site, strychnine-insensitive glycine site (glycine$_B$), polyamine site, and phencyclidine site located inside the cation channel.

Receptor desensitisation may represent a physiological process serving as an endogenous control mechanism to prevent long term neurotoxic activation of glutamate receptors but allow their transient physiological activation. In the case of the NMDA receptor, the co-agonist glycine is an endogenous ligand inhibiting such desensitisation via activation of the glycine$_B$ site. Interestingly, ischaemia increases not only the concentration of extracellular glutamate but also that of glycine and, although this latter effect is less pronounced, it actually persists for much longer. Hence, some full glycine$_B$ antagonists could restore normal synaptic transmission under such conditions by increasing

CONFIRMATION COPY

NMDA receptor desensitisation to its physiological level. Indeed, it has been suggested on the basis of central administration in laboratory animals that glycine$_B$ antagonists may offer a better therapeutic window than agents acting at other recognition sites of NMDA receptor complex. Unfortunately, poor pharmacokinetic properties of most glycine$_B$ antagonists have, until very recently, excluded clear verification of this suggestion after systemic administration. However, some glycine$_B$ antagonists have been reported to have very good therapeutic indices following systemic administration in models of hyperalgesia and as anxiolytics.

THE PRESENT INVENTION

We have now developed a series of tricyclic ''pyrido-phtalazin diones''. Compounds of class I are structurally related to patented glycine$_B$ antagonists of Zeneca (ICI, EPA 0 516 297 A1, 02.12.92). Class II compounds are N-oxide derivatives of these compounds and are not disclosed or suggested in the Zeneca patent. The class II compounds are also potent glycine$_B$ antagonists *in vitro* and show a much better *in vivo* systemic availability and / or penetration of the blood brain barrier than class I compounds. Moreover, salt derivatives of these compounds, made for example by additon of choline and 4-tetramethylammonium (4-NH_3), improve bioavailability further.

The novel compounds of the present invention have predictable utility in the treatment of the following disorders. 1. Acute excitotoxicity such as ischaemia during stroke, trauma, hypoxia, hypoglycaemia and hepatic encephalopathy. 2. Chronic neurodegenerative diseases such as Alzheimer´s disease, vascular dementia, Parkinson´s disease, Huntington´s disease, multiple sclerosis, amyotrophic lateral sclerosis, AIDS-neurodegeneration, olivopontocerebellar atrophy, Tourette´s syndrome, motor neurone disease, mitochondrial dysfunction, Korsakoff syndrome, and Creutzfeldt-Jakob disease. 3. Other disorders related to long term plastic changes in the central nervous system such as chronic pain, drug tolerance, dependence and addiction (e.g. opioids, cocaine, benzodiazepines, and alcohol), and tardive dyskinesia. 4. Epilepsy (generalised and partial

complex seizures), schizophrenia, anxiety, depression, acute pain, spasticity, and tinnitus.

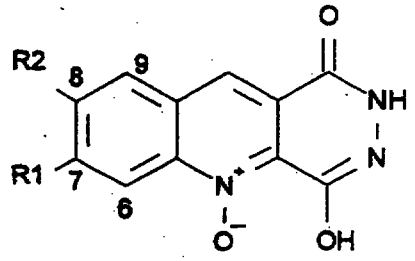
OBJECTS OF THE INVENTION

It is an object of the invention to provide new and more effective pyrido-phtalazin dione compounds, pharmaceutical compositions thereof, and method of treating neurological disorders associated with excitotoxicity and malfunctioning of glutamatergic neurotransmission therewith. It is a further object of the invention to provide such novel compounds, compositions, and method which fulfill the foregoing theoretical requirements. Additonal objects will become apparent hereinafter, and still other objects of the invention will be apparent to one skilled in the art.

SUMMARY OF THE INVENTION

The invention, then, comprises the following aspects, inter alia, singly or in combination:

A compound selected from those pyridyl-phtalazin diones having the following formula:



wherein R1 and R2 are selected from the group consisting of hydrogen, halogen, and methoxy or wherein R1 and R2 together form methylenedioxy, and pharmaceutically-acceptable salts thereof; such

a compound wherein the salt is selected from a choline and a 4-tetramethyl ammonium salt thereof; such

a compound which is selected from

the group consisting of

4-hydroxy-1-oxo-1,2-dihydro-pyridazino[4,5-b]-quinoline 5-oxide,

8-chloro-4-hydroxy-1-oxo-1,2-dihydropyridazino[4,5-b]-quinoline 5-oxide,

8-bromo-4-hydroxy-1-oxo-1,2-dihydropyridazino[4,5-b]-quinoline 5-oxide,

8-fluoro-4-hydroxy-1-oxo-1,2-dihydropyridazino[4,5-b]-quinoline 5-oxide,

7,8-dichloro-4-hydroxy-1-oxo-1,2-dihydropyridazino-

[4,5-b]-quinoline 5-oxide,

7-bromo-8-chloro-4-hydroxy-1-oxo-1,2-dihydropyridazino-[4,5-b]-quinoline 5-oxide, and

7-chloro-8-bromo-4-hydroxy-1-oxo-1,2-dihydropyridazino-[4,5-b]-quinoline 5-oxide, and a pharmaceutically-acceptable salt of any of the foregoing; and such

a compound selected from the group consisting of

4-hydroxy-1-oxo-1,2-dihydro-pyridazino[4,5-b]-quinoline 5-oxide choline salt,

8-chloro-4-hydroxy-1-oxo-1,2-dihydropyridazino[4,5-b]-quinoline 5-oxide choline salt,

8-bromo-4-hydroxy-1-oxo-1,2-dihydropyridazino[4,5-b]-quinoline 5-oxide choline salt,

8-fluoro-4-hydroxy-1-oxo-1,2-dihydropyridazino[4,5-b]-quinoline 5-oxide choline salt,

7,8-dichloro-4-hydroxy-1-oxo-1,2-dihydropyridazino-

[4,5-b]-quinoline 5-oxide choline salt,

7-bromo-8-chloro-4-hydroxy-1-oxo-1,2-dihydropyridazino-[4,5-b]-quinoline 5-oxide choline salt, and

7-chloro-8-bromo-4-hydroxy-1-oxo-1,2-dihydropyridazino-[4,5-b]-quinoline 5-oxide choline salt.

Moreover, a pharmaceutical composition containing as active ingredient an effective glycine$_B$ antagonistic amount of such a compound; such

a pharmaceutical composition containing as active ingredient an effective glycine$_B$ antagonistic amount of such a compound in the form of a choline salt thereof; such

a pharmaceutical composition containing as active ingredient an effective glycine$_B$ antagonistic amount of a compound selected from the group consisting of

4-hydroxy-1-oxo-1,2-dihydro-pyridazino[4,5-b]-quinoline 5-oxide,

8-chloro-4-hydroxy-1-oxo-1,2-dihydropyridazino[4,5-b]-quinoline 5-oxide,

8-bromo-4-hydroxy-1-oxo-1,2-dihydropyridazino[4,5-b]-quinoline 5-oxide,

8-fluoro-4-hydroxy-1-oxo-1,2-dihydropyridazino[4,5-b]-quinoline 5-oxide,

7,8-dichloro-4-hydroxy-1-oxo-1,2-dihydropyridazino-

[4,5-b]-quinoline 5-oxide,

7-bromo-8-chloro-4-hydroxy-1-oxo-1,2-dihydropyridazino-[4,5-b]-quinoline 5-oxide, and

7-chloro-8-bromo-4-hydroxy-1-oxo-1,2-dihydropyridazino-[4,5-b]-quinoline 5-oxide, or a pharmaceutically-acceptable salt of any of the foregoing; and such

a pharmaceutical composition containing as active ingredient an effective glycine$_B$ antagonistic amount of a compound selected from the group consisting of

4-hydroxy-1-oxo-1,2-dihydro-pyridazino[4,5-b]-quinoline 5-oxide choline salt;

8-chloro-4-hydroxy-1-oxo-1,2-dihydropyridazino[4,5-b]-quinoline 5-oxide choline salt,

8-bromo-4-hydroxy-1-oxo-1,2-dihydropyridazino[4,5-b]-quinoline 5-oxide choline salt,

8-fluoro-4-hydroxy-1-oxo-1,2-dihydropyridazino[4,5-b]-quinoline 5-oxide choline salt,

7,8-dichloro-4-hydroxy-1-oxo-1,2-dihydropyridazino-

[4,5-b]-quinoline 5-oxide choline salt,

7-bromo-8-chloro-4-hydroxy-1-oxo-1,2-dihydropyridazino-[4,5-b]-quinoline 5-oxide choline salt, and

7-chloro-8-bromo-4-hydroxy-1-oxo-1,2-dihydropyridazino-[4,5-b]-quinoline 5-oxide choline salt.

Further, a method of combatting neurological disorders associated with excitotoxicity and malfunctioning of glutamatergic neurotransmission in a living animal

comprising the step of administering to a living animal in need thereof an effective glycine$_B$ antagonistic amount of such a compound or pharmaceutical composition; such

a method wherein the compound is in the form of a choline salt thereof; such a

a method wherein the compound is selected from the group consisting of

4-hydroxy-1-oxo-1,2-dihydro-pyridazino[4,5-b]-quinoline 5-oxide,

8-chloro-4-hydroxy-1-oxo-1,2-dihydropyridazino[4,5-b]-quinoline 5-oxide,

8-bromo-4-hydroxy-1-oxo-1,2-dihydropyridazino[4,5-b]-quinoline 5-oxide,

8-fluoro-4-hydroxy-1-oxo-1,2-dihydropyridazino[4,5-b]-quinoline 5-oxide,

7,8-dichloro-4-hydroxy-1-oxo-1,2-dihydropyridazino-

[4,5-b]-quinoline 5-oxide,

7-bromo-8-chloro-4-hydroxy-1-oxo-1,2-dihydropyridazino-[4,5-b]-quinoline 5-oxide, and

7-chloro-8-bromo-4-hydroxy-1-oxo-1,2-dihydropyridazino-[4,5-b]-quinoline 5-oxide, or a pharmaceutically-acceptable salt of any of the foregoing; and such

a method of combatting neurological disorders associated with excitotoxicity and malfunctioning of glutamatergic neurotransmission in a living animal comprising the step of administering to a living animal in need thereof an effective glycine$_B$ antagonistic amount of a compound selected from the group consisting of

4-hydroxy-1-oxo-1,2-dihydro-pyridazino[4,5-b]-quinoline 5-oxide choline salt,

8-chloro-4-hydroxy-1-oxo-1,2-dihydropyridazino[4,5-b]-quinoline 5-oxide choline salt,

8-bromo-4-hydroxy-1-oxo-1,2-dihydropyridazino[4,5-b]-quinoline 5-oxide choline salt,

8-fluoro-4-hydroxy-1-oxo-1,2-dihydropyridazino[4,5-b]-quinoline 5-oxide choline salt,

7,8-dichloro-4-hydroxy-1-oxo-1,2-dihydropyridazino-

[4,5-b]-quinoline 5-oxide choline salt,

7-bromo-8-chloro-4-hydroxy-1-oxo-1,2-dihydropyridazino-[4,5-b]-quinoline 5-oxide choline salt, and

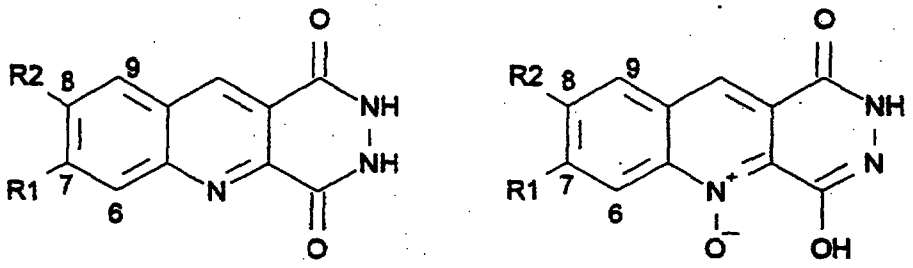
7-chloro-8-bromo-4-hydroxy-1-oxo-1,2-dihydropyridazino-[4,5-b]-quinoline 5-oxide choline salt.

DETAILED DESCRIPTION OF THE INVENTION

The following Discussion, Examples, and Pharmacology are given to illustrate the present invention, but are not to be construed as limiting.

METHODS AND RESULTS

Basic structure of class I and II tricyclic ''pyrido-phtalazin diones''



R1 /R2 = H and / or Halogen

R1 /R2 = H and / or O-CH_3

R1 /R2 = H and / or Methylendioxy

CHEMISTRY

General procedure for preparation of dimethyl quinoline-2,3-dicarboxylate 1-oxides (3).

A cold (ice bath) solution of 2-nitrobenzaldehyde 1 (25 mM) and sodium (27 mM) in anhydrous methanol (40 ml) was treated during 30 min with a solution of dimethyl (diethoxyphosphinyl) succinate 2 (30 mM, prepared as described by S. Linke et al., Lieb. Ann. Chem.,1980(4), 542) in anhydrous methanol (10 ml). The resulting dark solution was stirred at 0-5°C for 1.5 h, the solvent was evaporated under reduced pressure and the residue

partitioned between ethyl acetate and water. The ethyl acetate was dried over sodium sulphate and then evaporated under reduced pressure. The residue was recrystallized from isopropanol to provide the title dimethyl quinoline-2,3-dicarboxylate 1-oxide 3 as an off white (or light yellow) powder.

Physical properties and ^{1}H-NMR spectral data of the compounds 3 are given in Tables 1 and 2

a. 5-Bromo-4-chloro-2-nitrobenzaldehyde (1f).

To a mixture of sulphuric acid (40 ml) and sodium nitrate (2.66 g, 31.3 mM) at 0-5°C was added 3-bromo-4-chlorobenzaldehyde (6.25 g, 28.5 mM). The resulting mixture was stirred at room temperature for 7 h and then diluted with ice water (300 ml). The precipitated solids were filtered, washed with water and dried to give a powder. Recrystallization of this material from the mixture of isopropanol and water (2:1) provided the title 2-nitrobenzaldehyde 1f (3.6 g, 51.5%) as a light yellow powder, m.p. 81- 82 °C.

Analysis for $C_7H_3BrClNO_3$:

Calculated (%):	C 31.79	H 1.14	N 5.30
Found (%):	C 31.55	H 0.98	N 5.09

^{1}H-NMR ($CDCl_3$), δ: 8.22 (s,1H), 8.23 s,1H),10.39 (s,1H).

b. 4-Bromo-5-chloro-2-nitrobenzaldehyde (1g).

Using a procedure (a) except starting with 4-bromo-3-chlorobenzaldehyde (2.97 g, 13.5 mM) the title compound 1g was obtained (1.9 g, 53.0%) as a light yellow powder, m.p. 95 - 98°C.

Analysis for $C_7H_3BrClNO_3$:

Calculated (%):	C 31.79	H 1.14	N 5.30
Found (%):	C 31.60	H 1. 01	N 5.1 1

^{1}H-NMR ($CDCl_3$), δ: 8.02 (s,1H), 8.43 (s,1H), 10.39 (s,1H).

General procedure for preparation of dimethyl quinoline-2,3-dicarboxylates (7).

A solution of N-oxide 3 (10 mM) and phosphorus trichloride (30 mM) in anhydrous chloroform (100 ml) was refluxed for 7 h. Solvent was removed under reduced pressure and the residue was partitioned between ethyl acetate and water. The organic layer was dried over sodium sulphate and then evaporated under reduced pressure. The residue was recrystallized from isopropanol to provide the title dimethyl quinoline -2,3-dicarboxylate 7 as an off white (or light yellow) powder.

Physical properties and ^{1}H-NMR spectral data of the compounds 7 are given in Tables 3 and 4.

General procedure for preparation of 4-hydroxy-1-oxo-1,2-dihydropyridazino[4,5-b]-quinoline 5-oxides (5).

To a stirred solution (or suspension) of dimethyl quinoline-2,3-dicarboxylate 1-oxide 3 (5 mM) in boiling ethanol (25 ml) under an argon atmosphere was added hydrazine hydrate (15 mM) and the mixture was refluxed for 3 h during which time a dark precipitate formed. After cooling to room temperature the reaction mixture was filtered and the collected solids were washed with ethanol and ether and dried to provide the hydrazine salt 4. This material was stirred at 70 -110°C for 3 h in acetic acid (15 ml) and, after cooling to room temperature, the mixture was diluted with water (45 ml) and then filtered to collect the solids. The collected solids were washed with ethanol and dried to provide a dark-yellow solid. Several recrystallizations of this material from dimethylformamide provided the title pyridazino[4,5-b]quinoline 5-oxide 5 as an orange powder.

Physical properties and ^{1}H-NMR spectral data of the compounds 5 are given in Tables 5 and 6.

General procedure for preparation of 1,4-dioxo-1,2,3,4-tetrahydropyridazino[4,5-b]-quinolines (9).

To a stirred solution (or suspension) of dimethyl quinoline -2,3-dicarboxylate 7 (5 mM) in boiling ethanol (25 ml) was added hydrazine hydrate (30mM) and the mixture was refluxed for 8 h during which time a precipitate formed. After cooling to room temperature the reaction mixture was filtered and the collected solids were washed with ethanol and ether and dried to provide the hydrazine salt 8. This material was stirred at 70-100° C for 3 h in acetic acid (15 ml) and, after cooling to room temperature, the mixture was diluted with water (45 ml) and then filtered to collect the solids. The collected solids were washed with ethanol and ether and dried to provide the title pyridazino[4,5-b]quinoline 9 as a yellow powder.

Physical properties and ^{1}H-NMR spectral data of the compounds 9 are given in Tables 7 and 8.

General procedure for preparation of 4-hydroxy-1-oxo-1,2-dihydropyridazino[4,5-b]-quinoline 5-oxide choline salts (6) and 1,4-dioxo-1,2,3,4-tetrahydropyridazino[4,5-b]-quinoline choline salts (10).

To a stirred suspension of pyridazino[4,5-b]quinoline 9 or N-oxide 5 (10 mM) in methanol (50 ml) was added choline hydroxide (10.5 mM, 45 wt. % solution in methanol). The resulting solution was concentrated using a rotary evaporator and the solid residue was recrystallized from ethanol to provide the title choline salt 10 or 6 as a hygroscopic orange (or red) powder.

Physical properties and ^{1}H-NMR spectral data of the compounds 6 and 10 are given in Tables 9, 10 and 11, 12 respectively.

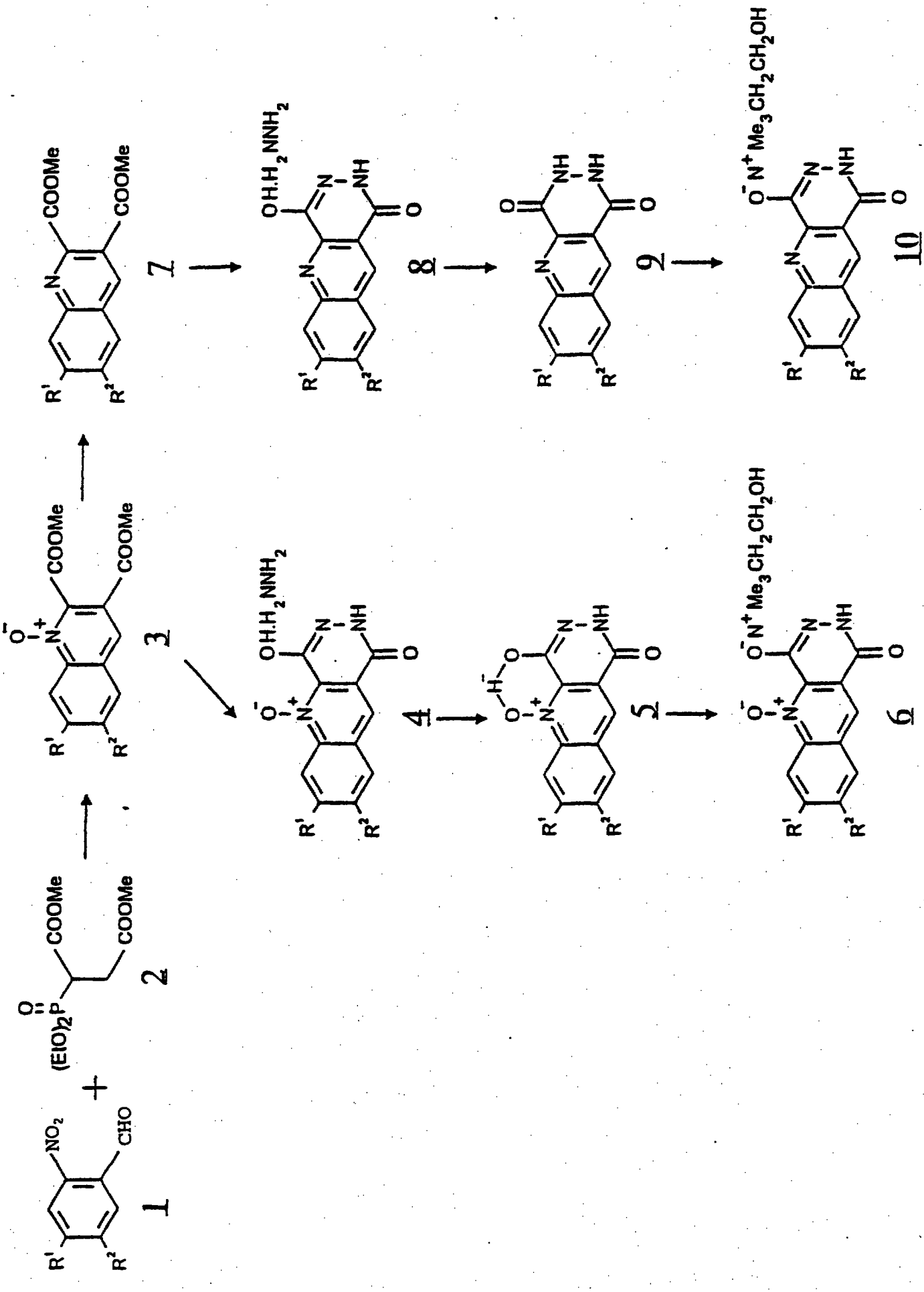


Table 1. Dimethyl quinoline-2,3-dicarboxylate 1-oxides 3 prepared

Compd	R^1	R^2	Formula (mw)	Elemental analysis						mp (°C)	Yield (%)
				Calculated (%)			Found (%)				
				C	H	N	C	H	N		
3a	H	H	$C_{13}H_{11}NO_5$ (261.2)	59.77	4.24	5.36	59.84	4.11	5.31	175-176	61.5
3b	H	Cl	$C_{13}H_{10}ClNO_5$ (295.7)	52.81	3.41	4.74	52.80	3.32	4.78	126-127	49.0
3c	H	Br	$C_{13}H_{10}BrNO_5$ (340.2)	45.89	2.96	4.11	45.57	2.75	4.00	168-170	72.0
3d	H	F	$C_{13}H_{10}FNO_5$ (279.2)	55.86	3.60	5.01	55.19	3.38	4.95	194-196	49.0
3e	Cl	Cl	$C_{13}H_9Cl_2NO_5$ (330.1)	47.30	2.75	4.24	47.18	2.62	4.14	183-186	41.0
3f	Cl	Br	$C_{13}H_9BrClNO_5$ (374.6)	41.69	2.42	3.74	41.39	2.13	3.65	171-173	60.0
3g	Br	Cl	$C_{13}H_9BrClNO_5$(374.6)	41.69	2.42	3.74	41.68	2.25	3.75	206-208	62.5

Table 2. ^{1}H-NMR ($CDCl_3$) spectral data of compounds 3

Compd	δ (ppm), J (Hz)
3a	3.98(s, 3H), 4.11, (s, 3H), 7.66-8.05 (m, 3H), 8.43 (s, 1H), 8.75 (dd, J_1 = 8.5, J_2 = 2.0, 1H)
3b	3.98 (s, 3H), 4.11 (s, 3H), 7.71 (dd, J_1 =8.5, J_2 = 2.5, 1H), 7.91 (d, J =8.5, 1H), 8.38 (s, 1H), 8.74 (d, J =2.5, 1H)
3c	3.91 (s, 3H), 4.07 (s, 3H), 7.13 (dd, J_1 =9.5, J_2 = 2.0, 1H), 7.44 (d, J = 2.0, 1H), 8.22 (s, 1H), 8.58 (d, J =9.5, 1H)
3d	3.98 (s, 3H), 4.11 (s, 3H), 7.48-7.72 (m, 2H), 8.31 (s, 1H), 8.73 (dd, J_1 = 10.0, J_2 = 5.0, 1H)
3e	3.97 (s, 3H), 4.10 (s, 3H), 8.08(s, 1H), 8.28 (s, 1H), 8.83 (s, 1H)
3f	3.97 (s, 3H), 4.09 (s, 3H), 8.26 (s, 2H), 8.82 (s, 1H)
3g	3.97 (s, 3H), 4.09 (s, 3H), 8.06 (s, 1H), 8.27 (s, 1H), (02 9s, 1H)

Table 3. Dimethyl quinoline-2,3-dicarboxylates 7 prepared

Compd	R^1	R^2	Formula (mw)	Elemental analysis						mp (°C)	Yield(%)
				Calculated (%)			Found (%)				
				C	H	N	C	H	N		
7a	H	H	$C_{13}H_{11}NO_4$ (245.2)	63.67	4.52	5.71	63.48	4.52	5.63	104-106	88.0
7b	H	Cl	$C_{13}H_{10}ClNO_4$ (279.7)	55.83	3.60	5.01	55.74	3.59	5.00	152-154	90.0
7c	H	Br	$C_{13}H_{10}BrNO_4$ (324.1)	48.17	3.11	4.32	48.09	3.05	4.26	155-157	81.5
7d	H	F	$C_{13}H_{10}FNO_4$ (263.2)	59.32	3.83	5.32	59.23	3.79	5.26	119-121	85.0
7e	Cl	Cl	$C_{13}H_9Cl_2NO_4$ (314.1)	49.71	2.89	4.46	49.56	2.85	4.41	113-115	96.0
7f	Cl	Br	$C_{13}H_9BrClO_4$ (348.6)	43.55	2.53	3.91	43.60	2.48	3.88	128-130	95.0
7g	Br	Cl	$C_{13}H_9BrClO_4$ (348.6)	43.55	2.53	3.91	43.47	2.51	3.87	142-144	67.0

Table 4. *^{1}H-NMR ($CDCl_3$) spectral data of compounds 7*

Compd	δ (ppm), J (Hz)
7a	3.98 (s, 3H), 4.06 (s, 3H), 7.58-8.00 (m, 3H), 8.21 (dd, J_1 = 9.5, J_2 = 2.0, 1H), 8.77 (m, 2H), 8.77 (s, 1H)
7b	3.97 (s, 3H), 4.06 (s, 3H), 7.76 (dd, J_1 = 9.5, J_2 = 2.0, 1H), 7.90 (d, J = 2.0, 1H), 8.67 (s, 1H)
7c	3.97 (s, 3H), 4.07 (s, 3H), 7.90 (dd, J_1 = 9.5, J_2 = 2.0, 1H), 8.09 (m, 2H), 8.66 (s, 1H)
7d	3.98 (s, 3H), 4.07 (s, 3H), 7.49-7.72 (m, 2H), 8.20 (dd, J_1 = 10, J_2 = 5.0, 1H), 8.69 (s, 1H)
7e	3.97 (s, 3H), 4.04 (s, 3H), 8.02 (s, 1H), 8.31 (s, 1H), 8.64 (s, 1H)
7f	3.98 (s, 3H), 4.06 (s, 3H), 8.24 (s, 1H), 8.33 (s, 1H), 8.68 (s, 1H)
7g	3.96 (s, 3H), 4.04 (s, 3H), 8.03 (s, 1H), 8.53 (s, 1H), 8.62 (s, 1H)

Table 5. 4-Hydroxy-1-oxo-1,2-dihydropyridazino[4,5-b]quinoline 5-oxides 5 prepared

Mrz 2/	Compd	R^1	R^2	Formula (mw)	Elemental analysis						mp (°C)	Yield(%)
					Calculated (%)			Found (%)				
					C	H	N	C	H	N		
499	5a	H	H	$C_{11}H_7N_3O_3$ (229.2)	57.65	3.08	18.33	57.56	2.93	18.22	>300	44.5
502	5b	H	Cl	$C_{11}H_6ClN_3O_3$ (263.6)	50.11	2.29	15.94	49.34	2.29	15.40	>300	88.0
514	5c	H	Br	$C_{11}H_6BrN_3O_3$ (308.1)	42.88	1.96	13.63	42.57	1.91	13.49	>300	78.0
516	5d	H	F	$C_{11}H_6FN_3O_3$ (247.2)	55.44	2.44	16.99	53.44	2.35	16.90	297-298	37.0
518	5e	Cl	Cl	$C_{11}H_5Cl_2N_3O_3$ (298.1)	44.32	1.69	14.10	44.17	1.91	14.34	>300	16.0
551	5f	Cl	Br	$C_{11}H_5BrClN_3O_3$ (342.5)	38.57	1.47	12.27	37.93	1.33	11.94	>300	15.0
568	5g	Br	Cl	$C_{11}H_5BrClN_3O_3$ (342.5)	38.57	1.47	12.27	38.17	1.31	12.00	>300	17.0

Table 6. ^{1}H-NMR (DMSO-d_6) spectral data of compounds 5

Compd	δ (ppm), J (Hz)	
	aromatic protons (and OCH_3)	NH, OH (exchangeable)
5a	7.88-8.28 (m, 2H), 8.46-8.79 (m, 2H), 9.07 (s, 1H)	10.65 (br. s, 1H), 12.00 (br. s, 1H)
5b	8.07 (dd, J_1 = 9.0, J_2 = 2.5, 1H),8.59 (d, J = 9.0, 1H), 8.69 (d, J = 2.5, 1H), 9.11 (s, 1H)	12.05 (br. s, 1H), 14.60 (br. s, 1H)
5c	8.27 (dd, J_1 = 9.0, J_2 = 2.0, 1H), 8.60 (d, J = 9.0, 1H), 8.82 (d, J = 2.0, 1H), 9.00 (s, 1H)	11.00 (br. s, 1H), 12.00 (br. s, 1H)
5d	8.07 (ddd, J_1 = 9.5, J_2 = 8.5, J_3 = 2.5, 1H), 8.36 (dd, J_1 = 9.5, J_2 = 2.5, 1H), 8.75 (dd, J_1 = 9.5, J_2 = 5.0, 1H), 9.02 (s, 1H)	10.92 (br. s, 1H), 12.00 (br. s, 1H)
5e	8.83 (s, 1H), 8.90 (s, 1H), 9.06 (s, 1H)	11.25 (br. s, 1H), 12.05 (br. s, 1H)
5f	8.80 (s, 1H), 9.00 (s, 1H), 9.01 (s, 1H)	12.06 (br. s, 1H), 14.28 (br. s, 1H)
5g	8.90 (s, 1H), 9.01 (s, 1H), 9.04 (s, 1H)	12.13 (br. s, 1H), 14.32 (br. s, 1H)

Table 7. 1,4-Dioxo-1,2,3,4-tetrahydropyridazino[4,5-b]quinolines 9 prepared

Mrz 2/	Compd	R^1	R^2	Formula (mw)	Elemental analysis						mp (°C)	Yield(%)
					Calculated (%)			Found (%)				
					C	H	N	C	H	N		
585	9a	H	H	$C_{11}H_7N_3O_2$ (213.2)	61.97	3.31	19.71	61.43	3.45	19.16	>300	86.0
501	9b	H	Cl	$C_{11}H_6ClN_3O_2$ (247.6)	53.35	2.44	16.97	32.89	2.28	16.68	>300	88.5
503	9c	H	Br	$C_{11}H_6BrN_3O_2$ (292.1)	45.23	2.07	14.39	44.74	2.11	14.09	>300	82.0
519	9d	H	F	$C_{11}H_6FN_3O_2$ (231.2)	57.14	2.60	18.18	56.73	2.47	17.99	>300	84.0
515	9e	Cl	Cl	$C_{11}H_5Cl_2N_3O_2$ (282.1)	46.84	1.79	14.90	46.44	1.70	14.87	>300	82.5
539	9f	Cl	Br	$C_{11}H_5BrClN_3O_2$ (326.5)	40.46	1.54	12.87	40.16	1.42	12.88	>300	69.5
538	9g	Br	Cl	$C_{11}H_5BrClN_3O_2$ (326.5)	40.46	1.54	12.87	40.23	1.40	12.98	>300	88.0

Table 8. ^{1}H-NMR (DMSO-d_6) spectral data of compounds 9

Compd	δ (ppm), J (Hz)	
	aromatic protons	NH (exchangeable)
9a	7.76-8.16 (m, 2H), 8.22-8.47 (m, 2H), 9.30 (s, 1H)	11.60 (br. s, 2H)
9b	8.02 (dd, J_1 = 9.0, J_2 = 2.5, 1H), 8.28 (d, J = 9.0, 1H), 8.52 (d, J = 2.5, 1H), 9.26 (s, 1H)	11.60 (br. s, 2H)
9c	8.16 (m, 2H), 8.60 (br. s, 1H), 9.25 (s, 1H)	11.55 (br. s, 2H)
9d	7.93 (ddd, J_1 = 9.5, $J_{2(H,F)}$ =9.0, J_3 = 2.5, 1H), 8.18 (dd, $J_{1(H,F)}$ = 9.5, J_2 = 2.5, 1H), 8.36 (dd, J_1= 9.5, $J_{2(H,F)}$ = 5.5, 1H), 9.24 (s, 1H)	11.90 (br. s, 2H)
9e	8.47 (s, 1H), 8.67 (s, 1H), 9.22 (s, 1H)	11.60 (br. s, 2H)
9f	8.52 (s, 1H), 8.91 (s, 1H), 9.28 (s, 1H)	11.65(br. s, 2H)
9g	8.68 (s, 1H), 8.71 (s, 1H), 9.26 (s, 1H)	11.70 (br. s, 2H)

Table 9. *4-Hydroxy-1-oxo-1,2-dihydropyridazino[4,5-b]quinoline 5-oxide choline salts 6 prepared*

Mrz 2/	Compd	R^1	R^2	Formula (mw)	Elemental analysis						mp (°C)	Yield(%)
					Calculated for 6·x H_2O* (%)			Found (%)				
					C	H	N	C	H	N		
577	6a	H	H	$C_{16}H_{20}N_4O_4$ (332.4)	54.84	6.32	15.99	54.76	6.32	15.86	179-180	52.5
576	6b	H	Cl	$C_{16}H_{19}ClN_4O_4$ (366.8)	49.93	5.50	14.55	49.31	5.47	14.24	185-188	87.5
570	6c	H	Br	$C_{16}H_{19}BrN_4O_4$ (411.4)	44.75	4.92	13.04	44.85	4.93	12.86	191-193	71.5
571	6d	H	F	$C_{16}H_{19}FN_4O_4$ (366.4)	51.19	5.63	14.92	51.74	5.73	14.95	201-203	27.0
574	6e	Cl	Cl									
	6f	Cl	Br									
	6g	Br	Cl									

* x = 1.0 (a, b, c)
0.5 (d)

Table 10. ^{1}H-NMR (CD_3OD) spectral data of choline salts 6

Compd	δ (ppm), J (Hz)	
	choline protons	aromatic protons
6a	3.20 (s, 9H), 3.47 (m, 2H), 3.98 (m, 2H)	7.69-8.00 (m, 2H), 8.18 (d, J = 8.0, 1H), 8.59 (s, 1H), 8.76 (d, J = 8.5, 1H)
6b	3.22 (s, 9H), 3.50 (m, 2H), 4.01 (m, 2H)	7.88 (dd, J_1 = 9.0, J_2 = 2.5, 1H), 8.27 (d, J = 2.5, 1H), 8.52 (s, 1H), 8.76 (d, J = 9.0, 1H)
6c	3.20(s, 9H), 3.48 (m, 2H), 3.99 (m, 2H)	7.99 (dd, J_1 = 9.5, J_2 = 2.0, 1H), 8.41 (d, J = 2.0, 1H), 8.53 (s, 1H), 8.64 (d, J = 9.5, 1H)
6d	3.22 (s, 9H), 3.51 (m, 2H), 4.02 (m, 2H)	7.64-7.98 (m, 2H), 8.62 (s, 1H), 8.87 (dd, J_1 = 10.0, J_2 = 5.0, 1H)
6e		
6f		
6g		

Table 11. *1, 4-Dioxo-1,2,3, 4-tetrahydropyridazino[4,5-b]quinoline choline salts 10 prepared*

Mrz 2/	Compd	R^1	R^2	Formula (mw)	Elemental analysis						mp (°C)	Yield(%)
					Calculated for 10·x H_2O* (%)			Found (%)				
					C	H	N	C	H	N		
604	10a	H	H	$C_{16}H_{20}N_4O_3$ (316.36)	54.26	7.59	14.06	54.23	7.39	14.25	102-110	82.0
596	10b	H	Cl	$C_{16}H_{19}ClN_4O_3$ (350.8)	54.08	5.53	15.76	54.10	5.55	15.61	189-191	84.0
586	10c	H	Br	$C_{16}H_{19}BrN_4O_3$ (395.3)	43.64	5.49	12.72	44.07	5.14	12.73	234-236	61.0
572	10d	H	F	$C_{16}H_{19}FN_4O_3$ (334.4)	52.16	15.20	5.74	52.08	6.23	15.19	229-230	95.0
574	10e	Cl	Cl	$C_{16}H_{18}Cl_2N_4O_3$ (385.3)	47.65	4.99	13.89	47.24	5.03	13.60	205-208	83.0
598	10f	Cl	Br	$C_{16}H_{18}BrClN_4O_3$ (429.7)	42.92	4.5	12.51	42.78	4.60	12.44	207-209	92.0
597	10g	Br	Cl	$C_{16}H_{18}BrClN_4O_3$ (429.7)	42.92	4.5	12.51	42.66	4.57	12.37	201-203	95.0

* x = 0.25 (d)
1.0 (d, c)
2.5 (c)

Table 12. *^{1}H-NMR (CD_3OD) spectral data of choline salts 10*

Compd	δ (ppm), J (Hz)	
	choline protons	aromatic protons
10a	3.21 (s, 9H), 3.51 (m, 2H), 4.01 (m, 2H)	7.64-8.57 (m, 4H), 9.17 (s, 1H)
10b	3.25 (s, 9H), 3.52 (m, 2H), 4.02 (m, 2H)	7.89 (dd, J_1 = 9.0, J_2 = 2.5, 1H), 8.23 (d, J = 2.5, 1H), 8.34 (d, J = 9.0, 1H), 9.13 (s, 1H)
10c	3.22 (s, 9H), 3.47 (m, 2H), 3.97 (m, 2H)	7.99 (dd, J_1 = 9.0, J_2 = 2.0, 1H), 8.26 (d, J = 9.0, 1H), 8.41 (d, J = 2.0, 1H), 9.09 (s, 1H)
10d	3.20(s, 9H), 3.49 (m, 2H), 3.98 (m, 2H)	7.64-7.81 (m, 2H), 8.39 (dd, J_1 = 9.5, J_2 = 5.0, 1H), 9.12 (s, 1H)
10e	3.22 (s, 9H), 3.51 (m, 2H), 4.02 (m, 2H)	8.46 (s, 1H), 8.55 (s, 1H), 9.14 (s, 1H)
10f	3.22 (s, 9H), 3.50 (m, 2H), 4.00 (m, 2H)	8.53 (s, 1H), 8.64 (s, 1H), 9.13 (s, 1H)
10g	3.22 (s, 9H), 3.50 (m, 2H), 4.02 (m, 2H)	8.43 (s, 1H), 8.73 (s, 1H), 9.13 (s, 1H)

PHARMACOLOGY

In vitro

Receptor Binding Studies

Membrane preparation and protein determination

Tissue preparation was performed according to Foster and Wong (1987). Male Sprague-Dawley rats (200-250g) were decapitated and their brains were removed rapidly. The cortex was dissected and homogenised in 20 volumes of ice-cold 0.32 M sucrose using a glass-Teflon homogenizer. The homogenate was centrifuged at 1000 x g for 10 min. The pellet was discarded and the supernatant centrifuged at 20,000 x g for 20 min. The resulting pellet was re-suspended in 20 volumes of distilled water and centrifuged for 20 min at 8000 x g. Then the supernatant and the buffy coat were centrifuged three times (48,000 x g for 20 min) in the presence of 5 mM Tris-HCl, pH 7.4. All centrifugation steps were carried out at 4°C. After resuspension in 5 volumes of 5 mM Tris-HCl, pH 7.4 the membrane suspension was frozen rapidly at -80°C until the day of assay. On the day of assay the membranes were thawed and washed four times by resuspension in 5 mM Tris-HCl, pH 7.4 and centrifugation at 48,000 x g for 20 min. The final pellet was suspended in assay buffer.

The amount of protein in the final membrane preparation was determined according to the method of Lowry (1951) with some modifications (Hartfree, 1972). 50µl of Protein samples (in triplicates) were diluted to 1ml with distilled water and treated with 0.9ml of a solution containing 2g potassium sodium tartrate and 100g Na_2CO_3 in 500ml 1N NaOH and 500ml water. Blank and standard (with bovine serum albumin) were set up in the same way. The tubes were placed in a water bath at 50°C for 10 min. and cooled at room temperature. 100µl of a solution containing 2 g potassium sodium tartrate and 1 g $CuSO_4x$ 5 H_2O in 90ml water and 10ml 1N NaOH were added. The samples were left at room temperature for at least 10 min., then 3ml of Folin-Ciocalteu reagent (1ml of reagent diluted with 15ml water) was added rapidly during mixing. The tubes were again heated at 50°C for 10 min. and cooled to room temperature. Absorbencies were then read in 1 cm

cuvets at 650 nm. The final protein concentration used for our studies was between 100 and 250 µg/ml.

Incubation in both binding assays was terminated using a Millipore filter system. The samples, all in triplicate, were rinsed three times with 2.5ml ice cold assay buffer over glass fibre filters obtained from Schleicher & Schuell under a constant vacuum. Following separation and rinse the filters were placed into scintillation liquid (5 ml; Ultima Gold) and radioactivity retained on the filters was determined by using conventional liquid scintillation counter (Hewlett Packard, Liquid Scintillation Analyser). 'Total binding' was the absolute amount of radioligand bound in the absence of any additives whereas 'non-specific' binding was determined in the presence of a high concentration of competitor.

[^{3}H]5,7-DCKA binding assay

Experiments were performed according to the methods modified from previous groups (Canton et al., 1992; Yoneda et al., 1993). Membranes were suspended and incubated in 10 mM Tris-HCl, pH 7.4. Incubation time was 45 min at 4°C. Non-specific binding of [^{3}H]5,7-DCKA was defined by the addition of unlabeled glycine at 0.1 mM. The stop-solution contained 10 mM Tris-HCl and 10 mM magnesium sulphate, pH 7.4. Filtration was performed as rapidly as possible. Displacement experiments were performed with a fixed [^{3}H]5,7-DCKA concentration of 10 nM. Test compounds were diluted in water or DMSO and added in at least 5 different concentrations.

[^{3}H]glycine binding assay

[^{3}H]glycine binding assays were performed according to the method described by Kessler and co-workers (1989). Rat cortical membranes were prepared as previously described and the final pellet was suspended in 50 mM Tris-acetate, pH 7.4. At least 5 different concentrations of test compounds were incubated with 20nM [^{3}H]glycine for 30 min at 4°C in the presence of 100µM strychnine. All compounds were dissolved in water or DMSO, respectively. Non-specific binding was determined by including 100µM glycine in the incubation mixture. The incubation was terminated by diluting the samples with 2ml of stop solution (50 mM Tris-HCl including 10 mM magnesium sulphate, pH 7.4, cooled to < 2°C) followed by

a further rinse with 2.5ml buffer. Filtration was performed as rapidly as possible.

Results

Eight of the tested compounds had IC_{50}s in the [^{3}H]-DCKA assay ≤ 1μM (see table 13). The potency of six selected compounds in the [^{3}H]-glycine assay appears at first sight to be greater but this is not reflected in large differences in Kds (not shown). Of the pairs of compounds of particular interest, class II compounds had a greater affinity than class I compounds in the [^{3}H]-DCKA assay. This difference was not so evident in the [^{3}H]-glycine assay.

Table 13a

Mrz 2/	Substance	[^{3}H]DCKA IC_{50} μM	[^{3}H]Glycine IC_{50} μM
499	II	16.0	
501	8-Cl-I	0.120	0.080
502	8-Cl-II	0.020	0.013
503	8-Br-I	0.250	0.013
514	8-Br-II	0.010	0.004
519	8-F-I	1.100	0.015
516	8-F-II	0.300	0.017
515	7,8-DiCl-I	0.530	
518	7,8-DiCl-II	0.650	

Table 13b

Mrz 2/	Substance	[^{3}H]DCKA IC_{50} µM
572	8-F-I (Chol)	1.14
571	8-F-II (Chol)	0.32
569	8-Cl-I (Chol)	0.97
576	8-Cl-II (Chol)	0.45

Patch clamp

Methods

Superior colliculi were obtained from rat embryos (E20 to E21) and were then transferred to calcium and magnesium free Hank's buffered salt solution (Gibco) on ice. Cells were mechanically dissociated in 0.05% DNAase / 0.3% ovomucoid (Sigma) following a 15 minute pre-incubation with 0.66% trypsin / 0.1% DNAase (Sigma). The dissociated cells were then centrifuged at 18G for 10 minutes, re-suspended in minimum essential medium (Gibco) and plated at a density of 200,000 cells cm^{-2} onto poly-L-lysine (Sigma)-precoated plastic petri dishes (Falcon). The cells were nourished with $NaHCO_3$/HEPES-buffered minimum essential medium supplemented with 5% foetal calf serum and 5% horse serum (Gibco) and incubated at 37°C with 5%CO_2 at 95% humidity. The medium was exchanged completely following inhibition of further glial mitosis with cytosine-β-D-arabinofuranoside (20µM Sigma) after about 7 days *in vitro*. Thereafter the medium was exchanged partially twice weekly. The superior colliculus culture was chosen for these experiments as it provides very stable recording conditions which are an absolute prerequisite for voltage-dependency and kinetic experiments. Moreover, the relatively small neurones (soma 15-20 µm Ø) are ideally suited to minimise problems of buffered diffusion for concentration clamp experiments.

Patch clamp recordings were made from these neurones with polished glass electrodes (4-6 mΩ) in the whole cell mode at room temperature (20-22°C) with the aid of an EPC-7 amplifier (List). Test substances were applied by switching channels of a custom made fast superfusion

system with a common outflow (10-20 ms exchange times). The contents of the intracellular solution were as follows (mM): CsCl (120), TEACl (20), EGTA (10), $MgCl_2$ (1), $CaCl_2$ (0.2), glucose (10), ATP (2), cAMP (0.25); pH was adjusted to 7.3 with CsOH or HCl. The extracellular solutions had the following basic composition (mM): NaCl (140), KCl (3), $CaCl_2$ (0.2), glucose (10), HEPES (10), sucrose (4.5), tetrodotoxin (TTX 3*10-4). For most experiments glycine (1µM) was present in all solutions. Experiments to test the glycine-dependence of the tricyclic ''pyrido-phtalazin diones'' were performed in the continuous presence of increasing concentrations of glycine (1-10µM).

Results

Five pairs of tricyclic ''pyrido-phtalazin diones'' had IC_{50}s against inward currents to NMDA (200µM) in the low µM range and class II compounds were generally about 2-3 times more potent than class I compounds (table 14a). The most potent of these were Mrz 2/502 and Mrz 2/514. This effect was mediated at the $glycine_B$ site as evidenced by the parallel shift in the concentration-response curves in the presence of increasing glycine concentrations. Thus the Kbs of Mrz 2/502 as assessed according to the Cheng-Prusoff relationship were similar in glycine 1, 3 and 10µM (80, 124 and 118nM respectively). Furthermore, the effects of Mrz 2/501 and 2/502 were not voltage-dependent. All compounds tested were about three to ten times more potent against steady-state currents than against peak currents. Choline derivatives had similar potencies to the free acids in vitro (Table14b).

In contrast, three of these potent $glycine_B$ antagonists were only very weak antagonists of inward currents to AMPA (100µM). Mrz 2/502, 2/514 and 2/516 had IC_{50}s against peak AMPA induced currents of 25, 73 and 18 µM respectively but were essentially inactive against plateau currents all IC_{50}s > 100µM (table 14a). This profile of action, although very weak, is typical for competitive AMPA receptor antagonists which preferentially block the peak non-desensitised state, low affinity state of the receptor (see Parsons et al., 1994).

Table 14a

Mrz 2/	Substance	Peak NMDA IC_{50} µM	Plateau NMDA IC_{50} µM	Peak AMPA IC_{50} µM	Plateau AMPA IC_{50} µM
585	I	65.9	19.1		
499	II	51.2	13.8		
501	8-Cl-I	2.3	0.7		
502	8-Cl-II	0.8	0.3	25.0	150.0
503	8-Br-I	1.7	0.6		
514	8-Br-II	0.5	0.2	72.7	307.0
519	8-F-I	18.0	5.8		
516	8-F-II	6.3	1.6	17.6	>100
515	7,8-DiCl-I	3.7	0.9		
518	7,8-DiCl-II	3.8	0.8		
539	7-Cl,8-Br-I	5.3	0.7		
551	7-Cl,8-Br-II	2.4	0.6		
538	7-Br,8-Cl-I	93.9	2.5		
568	7-Br,8-Cl-II	10.0	1.5		
554	8-O-CH_3-I	170	36.2		

Table 14b

Mrz 2/	Substance	Peak NMDA IC_{50} µM	Plateau NMDA IC_{50} µM
569	8-Cl-I (Chol)	2.0	0.5
576	8-Cl-II (Chol)	1.1	0.5
586	8-Br-I (Chol)	2.2	0.6
570	8-Br-II (Chol)	0.6	0.1
572	8-F-I (Chol)	12.4	3.5
571	8-F-II (Chol)	4.9	1.0
578	8-O-CH_3-II(Chol)	101	7.7
575	7-O-CH_3-I (Chol)	94.0	14.5

Excitotoxicity in vitro

Methods

Isolation of cortical neurones was similar to that described for patch clamp recordings except for the use of foetal rats at 17-19 days gestation. Neurones were plated in 24-multiwell (Greiner) at a density of 300,000 cells / well coated with poly-D-lysine 0.025mg/ml. Cells were cultured in Dulbecco's modified essential medium (DMEM, GIBCO) supplemented with 10% heat inactivated foetal calf serum (GIBCO). Cultures were maintained at 37°C at 5% CO_2. The medium was changed first after one week and then every 3 days by replacing half of the medium with fresh medium. Cultures aged 17 days were used for experiments.

Exposure to EAA was performed in serum-free MEM-N2 medium (Bottenstein 1979) containing 0.5mM NMDA/1µM glycine and the drug to be tested. Cells were pre-incubated with drugs and 1µM glycine for 15 minutes before addition of NMDA. After 24h the cytotoxic effect was morphologically examined under a phase contrast microscope and biochemically quantified by measuring the efflux of LDH.

The activity of LDH was determined in the supernatant after 24h according to the method of Wroblewski and La Due (1955). Briefly, 0.1ml of the supernatant was added to 0.9ml sodium-phosphate buffer (pH=7.5) containing sodium pyruvate (22.7 mM) and NADH (0.8mg/10ml) at room

temperature. The conversion of pyruvate to lactate was recorded at 340nm over 10 minutes in a Kontron Spectrophotometer.

Results

Full concentration-response curves are not yet available. However, low µM concentrations of Mrz 2/501 and Mrz 2/502 were effective neuroprotectants *in vitro*, with Mrz 2/502 seeming to be more potent in this regard (see table 15).

Table 15

Mrz 2/	Substance	Cytotoxicity *in vitro* IC_{50} µM
501	8-Cl-I	<5
502	8-Cl-II	<<5
503	8-Br-I	>20

In vivo

Anticonvulsive activity

Aim

To assess NMDA receptor antagonistic properties of the tested agents by assessing anticonvulsive effects. Additionally the role of organic acid transporters in the elimination from the brain of tested agents was assessed by using an inhibitor, Probenicid, on the duration of anticonvulsive activity.

Methods

Male albino Swiss mice (19-21 g) housed 10-15 per cage were used for the NMDA lethality test (Leander et al., 1988). For pentylenetetrazol (PTZ)-induced convulsions male albino Swiss mice (25-34 g.) housed 40 per cage (58x38x20 cm) were used while in the maximal electroshock (MES) and motor impairment tests NMR female mice (18-28 g) housed 5 per cage were employed. All animals were kept with water and food *ad libitum* under a 12-h light-dark

cycle (light on at 6 a.m.), and at a controlled temperature (20 ±0.5°C). All experiments were performed between 10 a.m. and 5 p.m. Tested agents were injected 15 min. i.p before the induction of convulsions if not stated otherwise (see below). Mrz 2/502 was dissolved in saline added with NaOH. Most other agents were dissolved in the following solution: 0.606g Tris; 5.0g. glucose; 0.5g. Tween 80; and 95 ml water. The choline and tetramethylammonium salts were dissolved in distilled water.

In the NMDA-induced convulsions test in mice, a dose-response relationship for NMDA was first performed to determine the ED_{97} dose which was then used for testing of antagonistic properties. After injection of the ED_{97} dose of NMDA the animals were placed in a small cage (20x28x14 cm) and observed for 20 min. Death preceded by clonic convulsions and by tonic seizures was the pharmacological end-point.

Pentylenetetrazol was injected at a dose of 90 mg/kg (i.p). The presence of general tonic convulsions was then scored for 30 min as this parameter is more sensitive to NMDA receptor antagonists than clonic convulsions. The pharmacological end-point was taken as the presence of tonus in the hind limbs with stretching.

MES (100 Hz, 0.5 sec shock duration, 50 mA shock intensity, 0.9 ms impulse duration, Ugo Basile) was applied through corneal electrodes. The presence of tonic convulsions was scored (tonic extension of hind paws with minimum angle to the body of 90°). In an additional experiment mice were injected with Probenicid (200 mg/kg) 30 min before administration of the tested agents to assess the role of organic acid transport in elimination (duration of action). The aim was to obtain ED_{50}s for all parameters scored using the Litchfield Wilcoxon (1949) test for quantal dose responses.

Results

Of the compounds tested, only four compounds, all class II, were effective when given i.p. in the M.E.S. test (Mrz 2/499, Mrz 2/502, Mrz 2/516 and Mrz 2/514 see table 16a). The associated class I compounds were inactive. All four compounds apparently had very short half lives in

vivo. The PTZ test seemed to be a more sensitive model for activity of glycine$_B$ antagonists given i.p. and, indeed, the same class II compounds were active at 2-4 fold lower doses whereas class I compounds remained inactive (table 16a).

The choline salts of these same N-oxide derivatives (structures II) had clear anticonvulsive activity in all three models, while their non-N-oxide derivatives were either inactive, or weak (Table 16b). Moreover it seems that choline salts have a longer duration of action. Probenecid injection prolonged considerably the duration of anticonvulsive action of all agents tested. For example the half lives of 2/514 and 2/570 were around 40 and 80 minutes respectively in the absence of probenicid. In the presence of probenicid the half lives were prolonged to around 180 and 210 minutes respectively. Thus, it seems that organic acids transport in the choroid plexus out of the brain plays an important role in the short duration of action of the compounds tested.. Probenecid at the dose used (200 mg/kg) has no independent effect on MES-induced convulsions per se.

Table 16a

Mrz 2/	Substance	MES i.p. (ID_{50} mg/kg)	NMDA i.p. (ID_{50} mg/kg)	PTZ i.p. (ID_{50} mg/kg)
585	I	>100.0	58.9	59.0
499	II	87.0		18.6
501	8-Cl-I	>100.0	>100.0	>40.0
502	8-Cl-II	47.6	26.0	8.3
503	8-Br-I	>100.0	> 100.0	>100.0
514	8-Br-II	20.2	99.0	12.8
519	8-F-I	>60.0	>100.0	>100.0
516	8-F-II	16.6	40.0	7.9
515	7,8-DiCl-I	>100.0	98.0	>100.0
518	7,8-DiCl-II	>60.0	>100.0	
539	7-Cl,8-Br-I	>60.0	>100.0	>100.0
538	7-Br,8-Cl-I	>60.0	106.0	>100.0
554	8-O-CH_3-I	>100.0		

Table 16b

Mrz 2/	Substance	MES i.p. (ID_{50} mg/kg)
577	II (Chol)	23.7
569	8-Cl-I (Chol)	>50
576	8-Cl-II (Chol)	7.7
586	8-Br-I (Chol)	>50
570	8-Br-II (Chol)	12.8
572	8-F-I (Chol)	>100
571	8-F-II (Chol)	15.5
574	7,8-DiCl-I (Chol)	>100
578	8-O-CH_3-II(Chol)	>100.0
575	7-O-CH_3-I (Chol)	>100.0

Microelectrophoretic application of EAA agonists to spinal neurones in vivo

The ability of these glycine$_B$ antagonists to act as NMDA receptor antagonists *in vivo* was assessed using i.v. administration against responses of single neurones in the rat spinal cord to microelectrophoretic application of AMPA and NMDA. The class II compounds Mrz 2/502 and Mrz 2/516 were potent NMDA receptor antagonists *in vivo* with ID_{50}s of 1.2 and 1.8 mg/kg i.v. respectively whereas the parent class I compounds were completely inactive at up to 16 mg/kg i.v. Three to four fold higher doses also antagonised responses to AMPA, although this apparent lack of selectivity contrasts with the *in vitro* assays (Table 17a).

Table 17a

Mrz 2/	Substance	Microelectrophoretic NMDA (ID_{50} mg/kg i.v.)	Microelectrophoretic AMPA (ID_{50} mg/kg i.v.)
501	8-Cl-I	>16.0	> 16.0
502	8-Cl-II	1.2	4.9
519	8-F-I	>16.0	>16.0
516	8-F-II	1.8	3.6

The choline salts were about equipotent as the free acids in this model following i.v. administration but were somewhat more selective for NMDA versus AMPA (Table 17b). Once again, the non-N-oxide derivatives (class I compounds) were inactive.

Table 17b

Mrz 2/	Substance	Microelectrophoretic NMDA (ID_{50} mg/kg i.v.)	Microelectrophoretic AMPA (ID_{50} mg/kg i.v.)
577	II (Chol)	34.0	>32.0
569	8-Cl-I (Chol)	>16.0	> 16.0
576	8-Cl-II (Chol)	2.8	> 16.0
586	8-Br-I (Chol)	>16.0	> 16.0
570	8-Br-II (Chol)	4.5	> 16.0
572	8-F-I (Chol)	>16.0	>16.0
571	8-F-II (Chol)	4.7	9.2

DISCUSSION

The four class II compounds Mrz 2/499, 2/501, 2/514 and 2/516 are glycine$_B$ antagonists *in vitro* and have a much better *in vivo* systemic and / or CNS availability than their associated parent class I compounds (Mrz 2/585, 2/501, 2/503 and 2/519). Access to the CNS is a major problem for almost all glycine$_B$ antagonists developed to date, but this new class of compounds has overcome this major hindrance and are accordingly therapeutically relevant glycine$_B$ antagonists.

ADDITION SALTS

By using methods as outlined hereinbefore for compounds 5, 6, 7, 8, 9, and 10, addition salts are prepared with quaternary amines (e.g., 4-tetramethylammonium, 4-tetraethylammonium), quaternary aminoalcohols (e.g., choline), or. quaternary aminoacids (e.g., N,N,N-trimethylserine). Choline and 4-tetramethylammonium (4-

NH_3) salts improve bioavailability substantially and are preferred.

PHARMACEUTICAL COMPOSITIONS

The compounds according to the present invention may be processed into pharmaceutical compositions comprising a pharmaceutically-acceptable carrier or diluent in addition to the active compound of the present invention. Such compositions can be administered to a living animal, especially a living human, by the oral or the parenteral route. For example, solid preparations or pharmaceutical compositions for oral administration may take the form of capsules, tablets, pills, powders, or granulates. In such solid pharmaceutical formulations, the active substance or a prodrug therefor is mixed with at least one pharmaceutically-acceptable diluent or carrier such as cane sugar, lactose, starch, talc, or synthetic or natural gums, a binder such as gelatin, a lubricant such as sodium stearate, and/or a disintegrant such as sodium bicarbonate. To enable a sustained-release effect, a substance such as a hydrocolloid or other polymer may be incorporated into the pharmaceutical composition. Additional substances such as lubricants or buffers may also be added, as is conventional in the art. The tablets, pills, or granulates may be subjected to enteric coating, if desired. Liquids for oral application may be in the form of liposomes, emulsions, solutions, or suspensions, containing commonly-used inert diluents such as water. Additionally, such liquid pharmaceutical compositions may also contain wetting, emulsifying, dispersing, or generally surface-active agents as well as sweetening, flavoring, or fragrance-imparting substances.

Suitable preparations for parenteral application may be, among others, sterile aqueous or non-aqueous solutions, suspensions, liposomes, or emulsions. Additional substances, of which there are many, already known for this form of presentation of a pharmaceutical composition, may be employed as pharmaceutically-acceptable diluent or carrier material.

Depending upon the intended mode of application and duration of treatment, the exact dosage of the active compounds in the preparations of the invention may be varied, especially as deemed appropriate by the attending physician or veterinarian. The active agents of the

present invention may obviously be combined for administration with other pharmacologically-active agents.

In the compositions of the present invention, the proportions of the active agent or agents in the composition may be varied widely, it being necessary only that the active ingredient of the invention or a prodrug therefor constitute or provide an effective amount, i.e., such that a suitable effective dose will be obtained consistent with the dosage form employed. Obviously several dosage forms as well as several individual active compounds may be administered at or about the same time or even in the same pharmaceutical composition or formulation.

METHOD-OF-TREATING

As previously indicated, the compounds of the present invention are suitable, especially in the form of pharmaceutical compositions or formulations thereof, for oral or parenteral administration, the exact individual dosages as well as daily dosages in a particular case of course being determined according to well-established medical and/or veterinarian principles in accord with the directions of the physician or veterinarian in charge.

In addition to oral and parenteral administration, rectal and/or intravenous administration may be employed, the dosages generally being considerably reduced where parenteral administration is involved, although oral administration is preferred. An amount of approximately one to three grams per day in the form of repeated or divided dosages is suitable. Broader ranges of about 0.5 to about 10 grams per day may also be employed, depending upon the circumstances of an individual case. Although 500 mg of active principle has been found especially suitable for use in tablets, individual dosages may vary from about 200 to 1,000 mg, and the 500 mg suggested for use in tablets may of course be administered orally, for example, from one to three times a day. It goes without saying that more than one tablet may be administered in a single dose, as would be required to attain the above-identified suggested daily oral administration amounts of one to three grams per day.

As already stated, a compound of the invention or a prodrug therefor may be administered to the living animal including a living human in any one of numerous ways, for example, orally as in capsules or tablets, parenterally in the form of sterile solutions or suspensions, or by pellet implantation, and in some cases intravenously in the form of sterile solutions. Other obvious modes of administration are cutaneously, subcutaneously, bucally, intramuscularly, and intraperitoneally, and the particular mode of administration will as usual be selected by the physician or veterinarian in charge.

It is thus seen that the present invention provides novel pyrido-phtalazin dione compounds and pharmaceutical compositions thereof, as well as a method of combating neurological disorders associated with excitotoxicity and malfunctioning of glutamatergic neurotransmission therewith, these collectively providing a long-awaited solution to a previously-existing problem not adequately solved by the prior art.

It is to be understood that the present invention is not to be limited to the exact compounds, compositions, methods, or procedures disclosed, as numerous modifications and changes therein will immediately become apparent to one skilled in the art to which this invention pertains, wherefore the present invention is to be understood as limited only by the full scope which can be legally accorded to the appended claims.

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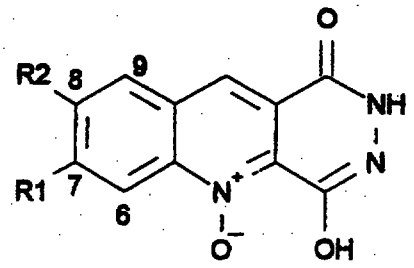
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WE CLAIM:

1. A compound selected from those pyridyl-phtalazin diones having the following formula:



wherein R1 and R2 are selected from the group consisting of hydrogen, halogen, and methoxy or wherein R1 and R2 together form methylenedioxy, or a pharmaceutically-acceptable salt thereof.

2. A compound of Claim 1 wherein the salt is selected from a choline and a 4-tetramethyl ammonium salt thereof.

3. A compound of Claim 1 which is selected from the group consisting of

4-hydroxy-1-oxo-1,2-dihydro-pyridazino[4,5-b]-quinoline 5-oxide,

8-chloro-4-hydroxy-1-oxo-1,2-dihydropyridazino[4,5-b]-quinoline 5-oxide,

8-bromo-4-hydroxy-1-oxo-1,2-dihydropyridazino[4,5-b]-quinoline 5-oxide,

8-fluoro-4-hydroxy-1-oxo-1,2-dihydropyridazino[4,5-b]-quinoline 5-oxide,

7,8-dichloro-4-hydroxy-1-oxo-1,2-dihydropyridazino-[4,5-b]-quinoline 5-oxide,

7-bromo-8-chloro-4-hydroxy-1-oxo-1,2-dihydropyridazino-[4,5-b]-quinoline 5-oxide, and

7-chloro-8-bromo-4-hydroxy-1-oxo-1,2-dihydropyridazino-[4,5-b]-quinoline 5-oxide, and a pharmaceutically-acceptable salt of any of the foregoing.

4. A compound of Claim 2 selected from the group consisting of

4-hydroxy-1-oxo-1,2-dihydro-pyridazino[4,5-b]-quinoline 5-oxide choline salt,

8-chloro-4-hydroxy-1-oxo-1,2-dihydropyridazino[4,5-b]-quinoline 5-oxide choline salt,

8-bromo-4-hydroxy-1-oxo-1,2-dihydropyridazino[4,5-b]-quinoline 5-oxide choline salt,

8-fluoro-4-hydroxy-1-oxo-1,2-dihydropyridazino[4,5-b]-quinoline 5-oxide choline salt,

7,8-dichloro-4-hydroxy-1-oxo-1,2-dihydropyridazino-

[4,5-b]-quinoline 5-oxide choline salt,

7-bromo-8-chloro-4-hydroxy-1-oxo-1,2-dihydropyridazino-[4,5-b]-quinoline 5-oxide choline salt, and

7-chloro-8-bromo-4-hydroxy-1-oxo-1,2-dihydropyridazino-[4,5-b]-quinoline 5-oxide choline salt.

5. A pharmaceutical composition containing as active ingredient an effective glycine$_B$ antagonistic amount of a compound of Claim 1 together with a pharmaceutically-acceptable carrier or diluent.

6. A pharmaceutical composition containing as active ingredient an effective glycine$_B$ antagonistic amount of a compound of Claim 1 in the form of a choline salt thereof together with a pharmaceutically-acceptable carrier or diluent.

7. A pharmaceutical composition containing as active ingredient an effective glycine$_B$ antagonistic amount of a compound selected from the group consisting of

4-hydroxy-1-oxo-1,2-dihydro-pyridazino[4,5-b]-quinoline 5-oxide,

8-chloro-4-hydroxy-1-oxo-1,2-dihydropyridazino[4,5-b]-quinoline 5-oxide,

8-bromo-4-hydroxy-1-oxo-1,2-dihydropyridazino[4,5-b]-quinoline 5-oxide,

8-fluoro-4-hydroxy-1-oxo-1,2-dihydropyridazino[4,5-b]-quinoline 5-oxide,

7,8-dichloro-4-hydroxy-1-oxo-1,2-dihydropyridazino-

[4,5-b]-quinoline 5-oxide,

7-bromo-8-chloro-4-hydroxy-1-oxo-1,2-dihydropyridazino-[4,5-b]-quinoline 5-oxide, and

7-chloro-8-bromo-4-hydroxy-1-oxo-1,2-dihydropyridazino-[4,5-b]-quinoline 5-oxide, or a pharmaceutically-acceptable salt of any of the foregoing.

8. A pharmaceutical composition containing as active ingredient an effective glycine$_B$ antagonistic amount of a compound selected from the group consisting of

4-hydroxy-1-oxo-1,2-dihydro-pyridazino[4,5-b]-quinoline 5-oxide choline salt;

8-chloro-4-hydroxy-1-oxo-1,2-dihydropyridazino[4,5-b]-quinoline 5-oxide choline salt,

8-bromo-4-hydroxy-1-oxo-1,2-dihydropyridazino[4,5-b]-quinoline 5-oxide choline salt,

8-fluoro-4-hydroxy-1-oxo-1,2-dihydropyridazino[4,5-b]-quinoline 5-oxide choline salt,

7,8-dichloro-4-hydroxy-1-oxo-1,2-dihydropyridazino-

[4,5-b]-quinoline 5-oxide choline salt,

7-bromo-8-chloro-4-hydroxy-1-oxo-1,2-dihydropyridazino-[4,5-b]-quinoline 5-oxide choline salt, and

7-chloro-8-bromo-4-hydroxy-1-oxo-1,2-dihydropyridazino-[4,5-b]-quinoline 5-oxide choline salt.

9. A method of combatting neurological disorders associated with excitotoxicity and malfunctioning of glutamatergic neurotransmission in a living animal comprising the step of administering to a living animal in need thereof an effective glycine$_B$ antagonistic amount of a compound of Claim 1.

10. A method of combatting neurological disorders associated with excitotoxicity and malfunctioning of glutamatergic neurotransmission in a living animal comprising the step of administering to a living animal in need thereof an effective glycine$_B$ antagonistic amount of a compound of Claim 1 in the form of a choline salt thereof.

11. A method of combatting neurological disorders associated with excitotoxicity and malfunctioning of glutamatergic neurotransmission in a living animal comprising the step of administering to a living animal in need thereof an effective glycine, antagonistic amount of a compound selected from the group consisting of

4-hydroxy-1-oxo-1,2-dihydro-pyridazino[4,5-b]-quinoline 5-oxide,

8-chloro-4-hydroxy-1-oxo-1,2-dihydropyridazino[4,5-b]-quinoline 5-oxide,

8-bromo-4-hydroxy-1-oxo-1,2-dihydropyridazino[4,5-b]-quinoline 5-oxide,

8-fluoro-4-hydroxy-1-oxo-1,2-dihydropyridazino[4,5-b]-quinoline 5-oxide,

7,8-dichloro-4-hydroxy-1-oxo-1,2-dihydropyridazino-

[4,5-b]-quinoline 5-oxide,

7-bromo-8-chloro-4-hydroxy-1-oxo-1,2-dihydropyridazino-[4,5-b]-quinoline 5-oxide, and

7-chloro-8-bromo-4-hydroxy-1-oxo-1,2-dihydropyridazino-[4,5-b]-quinoline 5-oxide, or a pharmaceutically-acceptable salt of any of the foregoing.

12. A method of combatting neurological disorders associated with excitotoxicity and malfunctioning of glutamatergic neurotransmission in a living animal comprising the step of administering to a living animal in need thereof an effective glycine, antagonistic amount of a compound selected from the group consisting of

4-hydroxy-1-oxo-1,2-dihydro-pyridazino[4,5-b]-quinoline 5-oxide choline salt,

8-chloro-4-hydroxy-1-oxo-1,2-dihydropyridazino[4,5-b]-quinoline 5-oxide choline salt,

8-bromo-4-hydroxy-1-oxo-1,2-dihydropyridazino[4,5-b]-quinoline 5-oxide choline salt,

8-fluoro-4-hydroxy-1-oxo-1,2-dihydropyridazino[4,5-b]-quinoline 5-oxide choline salt,

7,8-dichloro-4-hydroxy-1-oxo-1,2-dihydropyridazino-

[4,5-b]-quinoline 5-oxide choline salt,

7-bromo-8-chloro-4-hydroxy-1-oxo-1,2-dihydropyridazino-[4,5-b]-quinoline 5-oxide choline salt, and

7-chloro-8-bromo-4-hydroxy-1-oxo-1,2-dihydropyridazino-[4,5-b]-quinoline 5-oxide choline salt.

13. A process for the preparation of a 4-hydroxy-1-oxo-1,2-dihydropyridazino[4,5-b]-quinoline 5-oxide (5) comprising the step of converting a dimethyl quinoline-2,3-dicarboxylate 1-oxide (3) to the hydrazine salt (4) by reaction with hydrazine hydrate and hydrolyzing the resulting hydrazine salt (4) to produce the desired 4-hydroxy-1-oxo-1,2-dihydropyridazino[4,5-b]-quinoline 5-oxide (5).

14. The process of Claim 13 wherein the 4-hydroxy-1-oxo-1,2-dihydropyridazino[4,5-b]-quinoline 5-oxide (5) thus produced is converted to its choline salt (6) by reaction with choline hydroxide.