

CONVENTION

AUSTRALIA

Patents Act 1990

NOTICE OF ENTITLEMENT

We, NOVO NORDISK A/S of Novo Alle, DK-2880 Bagsvaerd, Denmark state the following in connection with Australian Application No. 75767/91:

1. The nominated person is the assignee of the actual inventors.
2. The nominated person is the applicant of the basic application listed in the declaration under Article 8 of the PCT.
3. The basic application is the application first made in a Convention country in respect of the invention.

Dated: 4 September 1992

By PHILLIPS ORMONDE & FITZPATRICK
Patent Attorneys for the Applicant
By:

.....*David B Fitzpatrick*.....

To: The Commissioner of Patents

Our Ref: 303910

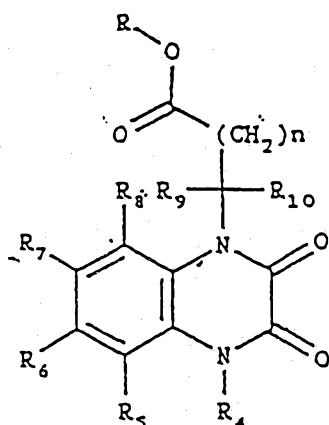
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AU9175767

(12) PATENT ABRIDGMENT (11) Document No. AU-B-75767/91
(19) AUSTRALIAN PATENT OFFICE (10) Acceptance No. 652560

- (54) Title
QUINOXALINE COMPOUNDS AND THEIR PREPARATION AND USE
- International Patent Classification(s)
(51)⁵ C07D 241/44 C07D 241/52 A61K 031/495
- (21) Application No. : 75767/91 (22) Application Date : 15.03.91
- (87) PCT Publication Number : WO91/13878
- (30) Priority Data
- (31) Number (32) Date (33) Country
0697/90 16.03.90 DK DENMARK
- (43) Publication Date : 10.10.91
- (44) Publication Date of Accepted Application : 01.09.94
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- (56) Prior Art Documents
AU 618766 24949/88 C07D 241/44 241/38
AU 624885 46874/89 C07D 241/52
AU 626093 46872/89 C07D 241/52
- (57) Claim
1. 1-carboxyalkylquinoxaline-2,3(1H,4H)-dione compounds or tautomeric forms thereof of the formula



wherein

R represents hydrogen, C₁₋₆-alkyl, including branched chains, or aralkyl and n is an integer from 0 to 5; R⁴ represents hydrogen or hydroxy; R⁵, R⁶, R⁷ and R⁸ independently represent hydrogen, nitro,

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halogen, alkoxy, aryloxy, aralkoxy, C₁₋₆-alkyl including branched chains, or aryl provided that at least one of R⁵, R⁶, R⁷ and R⁸ does not represent hydrogen when R represents hydrogen, -CH₃ or -CH(CH₃)₂ and n is 0 or 1; R⁹ represents hydrogen, lower alkyl, or aryl; R¹⁰ represents hydrogen, or alkyl, and pharmaceutically acceptable salts thereof.

OPI DATE 10/10/91 APPLN. NO 75767 / 91

A0JP DATE 07/11/91 PCT NUMBER PCT/DK91/00078

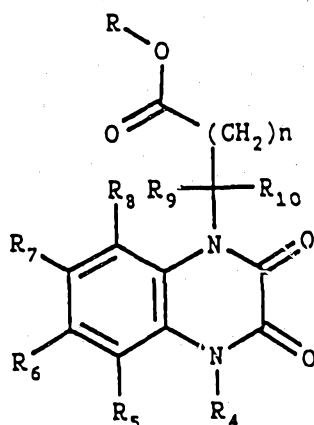
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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁵ : C07D 241/44, 241/52	A1	(11) International Publication Number: WO 91/13878 (43) International Publication Date: 19 September 1991 (19.09.91)
(21) International Application Number: PCT/DK91/00078 (22) International Filing Date: 15 March 1991 (15.03.91) (30) Priority data: 0697/90 15 March 1990 (16.03.90) DK (71) Applicant: NOVO NORDISK A/S [DK/DK]; Novo Alle, DK-2880 Bagsværd (DK). (72) Inventors: JØRGENSEN, Anker, Steen ; Bryggergade 6, DK-2100 Copenhagen Ø (DK). STIDSEN, Carsten, Enggaard ; Haspegårdsvej 63, DK-2880 Bagsværd (DK). FAARUP, Peter ; Skråplanet 15, DK-3500 Værløse (DK). GRØNVALD, Frederik, Christian ; Dronningengen 19, DK-2950 Vedbæk (DK).		(74) Agent: LEHMANN & REE; Frederiksberg Allé 26, DK-1820 Frederiksberg C (DK). (81) Designated States: AT (European patent), AU, BE (European patent), CA, CH (European patent), DE (European patent), DK (European patent), ES (European patent), FI, FR (European patent), GB (European patent), GR (European patent), HU, IT (European patent), JP, KR, LU (European patent), NL (European patent), NO, PL, SE (European patent). Published With international search report.

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(54) Title: QUINOXALINE COMPOUNDS AND THEIR PREPARATION AND USE



(I)

(57) Abstract

Novel 1-carboxyalkylquinoxaline-2,3(1H,4H)-dione compounds or tautomeric forms thereof of formula (I), wherein R represents hydrogen, C₁₋₆-alkyl, including branched chains, or aralkyl and n represents the number from 0 to 5; R₄ represents hydrogen or hydroxy; R₅, R₆, R₇ and R₈ independently represent hydrogen, nitro, halogen, alkoxy, aryloxy, aralkoxy, C₁₋₆-alkyl including branched chains, or aryl; R₉ represents hydrogen, lower alkyl, or aryl; R₁₀ represents hydrogen, or alkyl. The compounds are useful in the treatment of neurological and psychiatric diseases.

5 Quinoxaline compounds and their Preparation and use.

10 The present invention relates to therapeutically active
1-carboxyalkylquinoxaline-2,3(1H,4H)-dione compounds or
tautomeric forms thereof, a method of preparing the
same, pharmaceutical compositions, ^{including} ~~comprising~~ the com-
pounds, and a method of treating therewith.

15 Interaction with glutamic acid mediated neurotransmission
is considered a useful approach in the treatment of neuro-
logical and psychiatric diseases. Thus, known antagonists
of excitatory amino acids have shown potent antiepileptic
and muscle relaxant properties (A. Jones et al., Neurosci.
Lett. 53, 321 (1985)).

20 It has been suggested that accumulation of extracellular
excitatory and neurotoxic amino acids, followed by hyper-
stimulation of neurons, may explain the neuronal degenera-
tions seen in neurological diseases as Huntingtons chorea,
25 Parkinsonism, epilepsy, senile dementia, and deficiencies
of mental and motoric performance seen after conditions
of brain ischemia, anoxia and hypoglycemia (E.G. McGeer
et al., Nature, 263, 517 (1976) and R. Simon et al., Sci-
ence, 226, 850 (1984)).

30 Excitatory amino acids exert their actions via specific
receptors located postsynaptically or presynaptically.
Such receptors are at present conveniently subdivided in-
to three groups based on electrophysiological and neuro-
35 chemical evidence: 1 the quisqualate receptors, 2 the kai-
nate receptors, and 3 the NMDA (N-methyl-D-aspartate) re-
ceptors. L-glutamic acid and aspartic acid probably acti-



vate all the above types of excitatory amino acid receptors and possibly other types as well.

It was recently found that glycine was able to increase
5 NMDA receptor agonist induced responses in cultured neurons (J.W. Johnson et al., *Nature* 325, 529 (1987)). In contrast to glycine activated chloride conductance in spinal cord neurons, this response was insensitive to strychnine (D.W. Bonhaus et al., *European J. Pharmacol.* 142,
10 489 (1987)).

Glycine is believed to potentiate NMDA action through a high affinity binding (H. Kishimoto et al., *J. Neurochem.* 37, 1015 (1981)) to an allosteric modulatory site located
15 at the NMDA receptor/ionophor complex (T. Honoré et al., *European J. Pharmacol.* 172, 239 (1989)). D-serine and D-alanine exert a strong agonist activity on this site (J.B. Monahan et al., *J. Neurochem.* 53, 370 (1989)), whereas
20 1-amino-cyclopropanecarboxylate (P. Skolnick et al., *Life Sci.* 45, 1647 (1989), V. Nadler et al., *European J. Pharmacol.* 157, 115 (1988), R. Trullas et al., *Pharmacol. Biochem. Behav.*, 34, 313 (1989)) and D-cycloserine (W.F. Hood et al., *Neurosci. Lett.* 98, 91 (1989)) act as partial agonists.

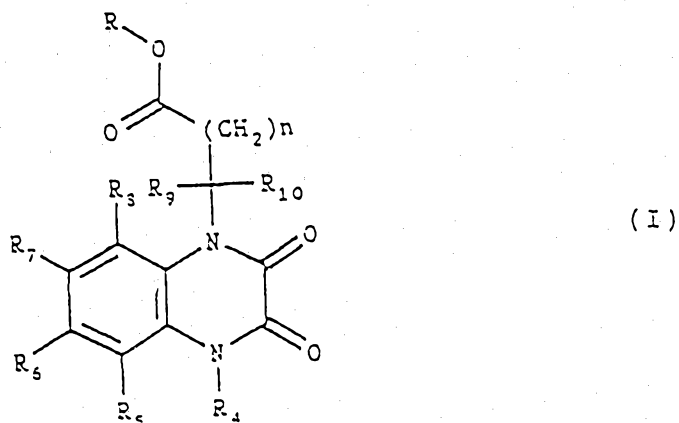
25 1-amino-cyclobutanecarboxylate (W.F. Hood et al., *European J. Pharmacol.* 161, 281 (1989)), 1-amino-cyclopentanecarboxylate (L.D. Snell et al., *European J. Pharmacol.* 151, 165 (1988)), 3-amino-1-hydroxy-2-pyrrolidone (HA-966)
30 (E.J. Fletcher et al., *European J. Pharmacol.* 151, 161 (1988)), 5-chloro-indole-2-carboxylate (J.E. Huettner, *Science* 243, 1611 (1989)) and 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) (R.A.J. Lester et al., *Mol. Pharmacol.* 35, 565 (1989)) are all weak antagonists, whereas 7-chloro-
35 kynurenate (R. Sircar et al., *Brain Res.* 504, 325 (1989)) and 6,7-dichloro-3-hydroxy-quinoxalin-2-carboxylate (M. Kessler et al., *Brain Res.* 489, 377 (1989)) are quite

strong, but non-selective antagonists of glycine at the glycine site.

Additionally, certain compounds closely related to the present invention claimed as non-NMDA receptor antagonists are disclosed in European Patent Publication No. 315959 and a method of preparing a closely related dione by oxidation of 1,2,3,4-tetrahydro-3-oxo-quinoxaline-1-acetic acid is disclosed in Chem. Abstr. Vol. 96, 1982, abstract no. 20066m.

We have now discovered a series of 1-carboxyalkyl-quinoxaline-2,3-dione derivatives acting as potent and selective glycine antagonists at the NMDA receptor-associated glycine site.

The present invention accordingly provides compounds of the formula (I) or tautomeric forms thereof:



wherein

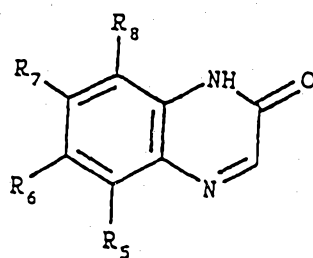
R represents hydrogen, C_{1-6} -alkyl, including branched chains, or aralkyl and n is an integer from 0 to 5; R^4 represents hydrogen or hydroxy; R^5 , R^6 , R^7 and R^8 independently represent hydrogen, nitro, halogen, alkoxy, aryloxy, aralkoxy, C_{1-6} -alkyl including branched chains, or aryl provided that at least one of R^5 , R^6 , R^7 and R^8 does not represent hydrogen when R represents hydrogen, $-CH_3$ or $-CH(CH_3)_2$ and n is 0 or 1; R^9 represents hydrogen, lower alkyl, or aryl; R^{10} represents hydrogen, or alkyl, and pharmaceutically acceptable salts thereof.



More specifically, the invention relates to compounds of the formula (I) or tautomeric forms thereof wherein R^4 is hydrogen and $n = 0$.

The invention also relates to a method of preparing the above mentioned compounds. This method ^{includes} ~~comprises~~:

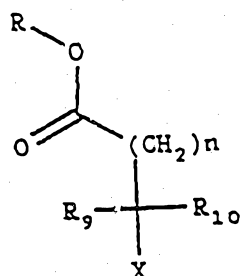
a) alkylating a compound of the formula (II) (methods for preparation of (II) are cited by G.W.H. Cheeseman and R.F. Cookson. In: The Chemistry of Heterocyclic Compounds (A. Weissberger and E.C. Taylor, eds.). John Wiley & Sons, New York, Vol. 35, p. 78-111 (1979))



(II)

20

wherein R^5 - R^8 have the meanings set forth above, with a compound of formula (III)



(III)

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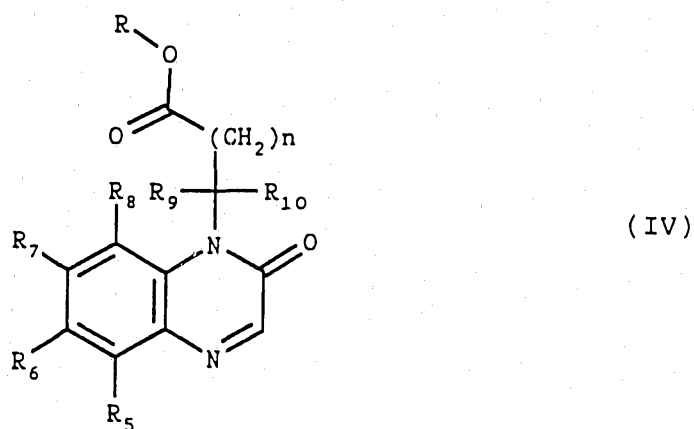
wherein R , n , R^9 and R^{10} are as previously defined, pro-

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vided that R is not hydrogen, and X represents a leaving group, preferably bromine or iodine, to form a compound of formula (IV)

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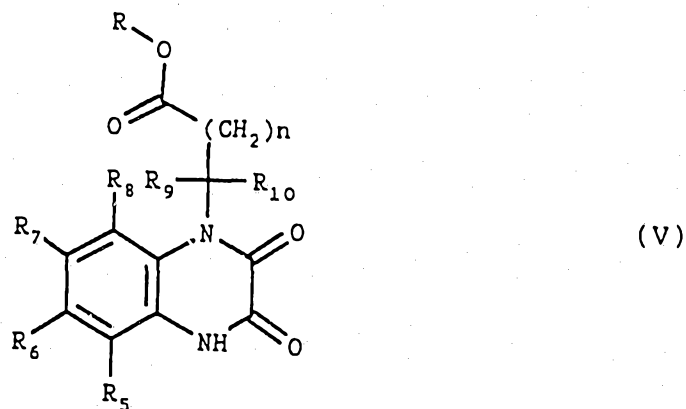
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wherein R, n, R⁵, R⁶, R⁷, R⁸, R⁹ and R¹⁰ have the meanings set forth above, provided that R is not hydrogen.

20 Oxidation of compound (IV) with aqueous hydrogen peroxide in acetic acid to form a compound of formula (V) (for similar type of reaction see, for example, G.H.W. Cheeseman J. Chem. Soc., 1246 (1961))

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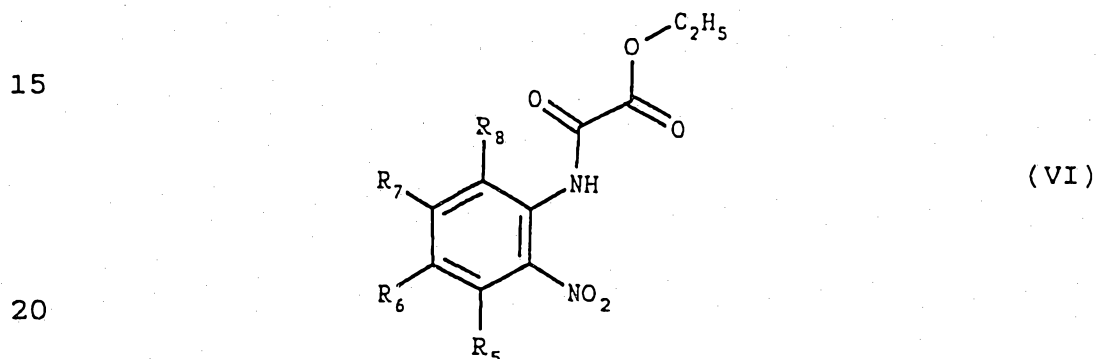
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wherein R, n, R⁵, R⁶, R⁷, R⁸, R⁹ and R¹⁰ have the meanings

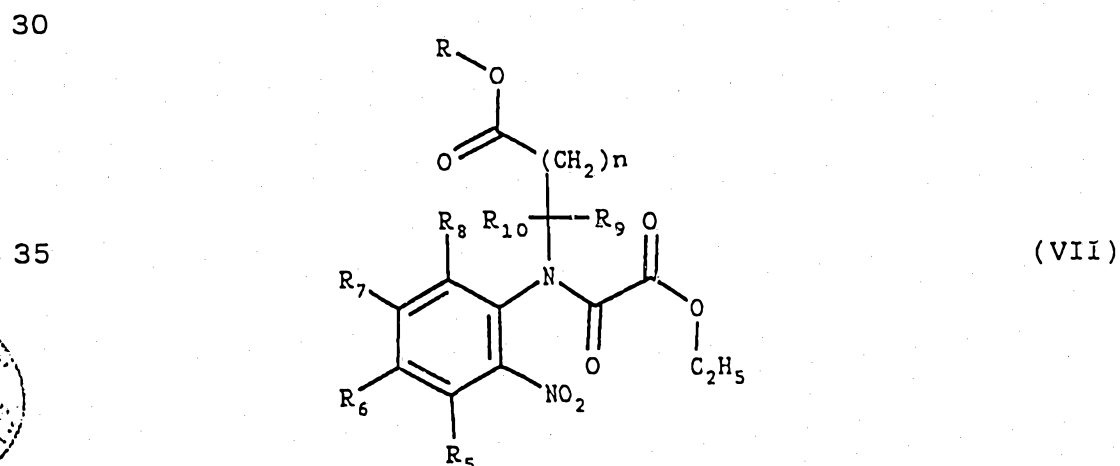
set forth above, provided that R is not hydrogen.

Hydrolysis of compound (V), or reacting a compound of formula (IV) with aqueous $\text{H}_2\text{O}_2/\text{NaOH}$ mixture and precipitation with a mineral acid, preferably hydrochloric acid, to form a compound of formula I wherein R and R^4 are hydrogen and R^5 , R^6 , R^7 , R^8 , R^9 and R^{10} are as previously defined.

b) alkylating a compound of formula (VI) (methods for preparation of (VI), see for example U.S. 3,999,378)

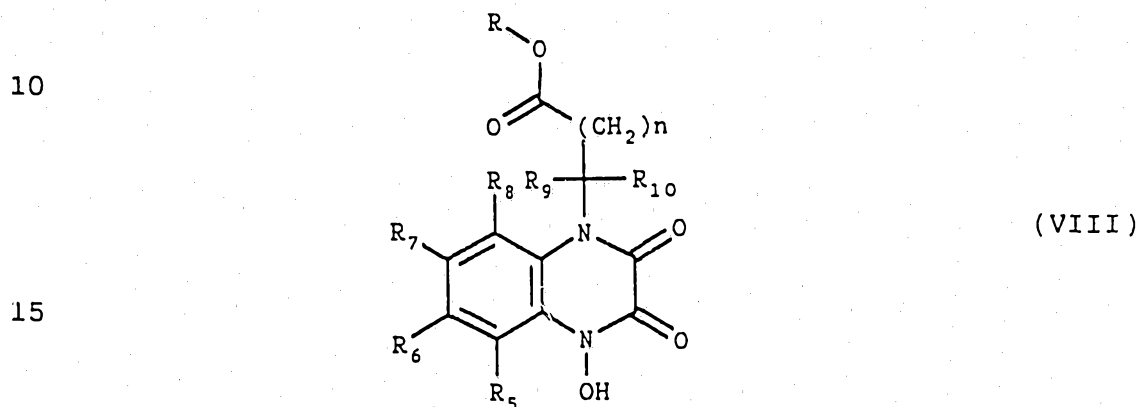


wherein ~~R⁵, R⁶, R⁷ and R⁸~~ ^{and} ~~R⁹ and R¹⁰~~ have the meanings set forth above, provided that R^5 , R^6 , R^7 and R^8 is not NO_2 , with a compound of formula (III), in a suitable solvent, e.g. alcohol or DMF and sodium ethoxide or sodium hydride as base, to form a compound of formula (VII)



wherein R is preferably tert-butyl and n and R⁵, R⁶, R⁷, R⁸, R⁹, R¹⁰ have the meanings defined for formula (I), provided that R⁵, R⁶, R⁷ and R⁸ is not NO₂.

- 5 Reducing a compound of formula (VII) to form a compound of the formula (VIII)

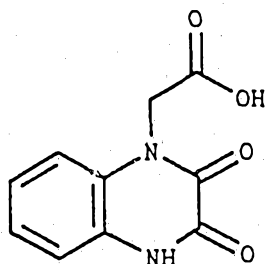


- 20 wherein R is preferably tert-butyl and n and R⁵, R⁶, R⁷, R⁸, R⁹ and R¹⁰ have the meanings defined for formula (I), provided that R⁵, R⁶, R⁷ and R⁸ is not NO₂.

- 25 Reacting a compound of formula (VIII) to form a compound of formula (I) wherein R represents hydrogen.

- c) halogenating or nitrating a compound of formula (IX) by general known methods, (see for example, Jerry March:
30 Advanced Organic Chemistry 3rd ed., McGraw-Hill, New York, 1985, p. 468-70, 476-79)

(IX)



to form a compound of formula (I) wherein $n=0$, and wherein R^4 , R^9 and R^{10} are hydrogen and wherein R^5 , R^6 , R^7 and R^8 are independently hydrogen and halogen or independently hydrogen and NO_2 .

The affinity of a substance for one or more of the different types of excitatory amino acid receptors may be studied in simple radioligand binding experiments. In essence, the method involves incubation of a particular selected radiolabelled ligand and the particular specific substance to be investigated with brain homogenates which contain the receptor. Measurement of receptor occupancy is made by determination of the radioactivity bound to the homogenate and subtraction of nonspecific binding.

The influence of glutamic acid analogues on secondary effects of glutamate receptor interactions, such as on c-GMP formation and on channel opening, may be studied in vitro by using brain slices or homogenates. Such experiments will provide information as to the efficacies (agonist/antagonist) of the test substances.

It has now been found that the heterocyclic compounds of the invention have affinity for the glycine site of the NMDA receptor complex and are antagonists in connection with this type of receptors, which make them useful in the treatment of any of the numerous diseases caused

by hyperactivity of excitatory amino acids, such as epilepsy, ischemia, anxiety states and convulsions.

5 The glycine site binding activity of these compounds of the present invention can be illustrated by determining their capability for displacing radioactively labelled glycine from the glycine site.

10 The displacement activity of the compounds may be shown by determining the IC_{50} value which represents the concentration (μM) which causes a displacement of 50% of the specific binding of [3H]-glycine.

15 The glycine antagonistic properties of the compounds is demonstrated by their capability to antagonize glycine enhanced binding of the non-competitive NMDA antagonist [3H]-MK-801 to brain homogenates. The glycine antagonism is measured by determining the K_i value which represents the dissociation constant (μM) of the
20 receptor-antagonist complex.

The NMDA antagonistic properties of the compounds is illustrated by determining their capability to antagonize NMDA stimulated [3H]-GABA release from cultured mouse cortex
25 neurons. The NMDA antagonistic activity of the compounds may be shown by determining the IC_{50} value, which represents the concentration (μM) which inhibits 50% of NMDA induced [3H]-GABA release.

30 [3H]-glycine binding (Test 1)

1 ml of thawed rat cerebral cortical membrane homogenate in HEPES-Tris (5 mM) and $MgCl_2$ (1 mM) pH 7.1 were incubated at 0°C for 10 min. with 25 μl [3H]-glycine (10 nM
35 final concentration) and the test compound and buffer. Non-specific binding was determined by incubating with D-serine (1 mM final concentration). The binding reaction

was terminated by centrifugation at 15,000 x g at 4°C followed by washing of the pellet with three times 3 ml of ice-cold buffer. Bound radioactivity was measured by scintillation counting. IC₅₀ was determined by Hill analysis of at least four concentrations of test compound.

Antagonism of glycine enhanced [³H]-MK-801 binding (Test 2)

1 ml of thawed and extensively washed rat cerebral membrane homogenate in HEPES-NaOH (20 mM) pH 7.40 were incubated at 23°C for 60 min. with 25 µl [³H]-MK-801 (1 nM final concentration), 25 µl glutamate (300 nM final concentration), test compound in concentrations corresponding to 0, 0.5, 2 and 5 times the IC₅₀ value from the [³H]-glycine binding assay (test 1) for each concentration of glycine (10; 100; 1,000; 10,000 and 100,000 nM final concentration). The binding reaction was terminated by adding 5 ml of ice-cold buffer followed by rapid filtration through Whatman GF/C glass fiber filters and 5 ml wash with ice-cold buffer. Bound radioactivity was measured by scintillation counting. K_i values were determined by Schild analysis of the data using

$$\log(\text{dosis ration} - 1) = \log[\text{Inhibitor}] - \log(K_i).$$

Inhibition of NMDA stimulated [³H]-GABA release from cultured mouse cerebral cortex interneurons (Test 3)

Release experiments are performed using the model described by Drejer et al. (Life Sci. 38, 2077 (1986)). To cerebral cortex interneurons cultured in petri dishes (30 mm) are added 100 µg/ml 3-vinyl-GABA one hour before the experiment in order to inhibit degradation of GABA in the neurons. 30 min. before the experiment 5 µCi [³H]-GABA is added to each culture and after this preloading period the

cells are washed twice with HEPES buffered saline (HBS) containing 10 mM HEPES, 135 mM NaCl, 5 mM KCl, 0.6 mM MgSO₄, 1.0 mM CaCl₂ and 6 mM D-glucose; pH 7 and placed in a superfusion system. This system consists of a peristaltic pump continuously delivering thermostated 37°C superfusion medium from a reservoir to the top of a slightly tilted petri dish. The cell monolayer at the bottom of the dish is covered with a piece of nylon mesh to facilitate dispersion of medium over the cell layer. The medium is continuously collected from the lower part of the dish and delivered to a fraction collector. Initially, the cells are superfused with HBS for 15 min. (flow rate 2 ml/min.). Then cells are stimulated for 30 sec. every 4 min. by changing the superfusion medium from HBS to a corresponding medium containing NMDA and antagonist according to the following scheme:

Stimulation no. 1: 3 µg/ml NMDA
Stimulation no. 2: 3 µg/ml NMDA + 0.3 µg/ml antagonist
20 Stimulation no. 3: 3 µg/ml NMDA + 3.0 µg/ml antagonist

The release of [³H]-GABA in the presence of NMDA is corrected for the mean basal release before and after stimulation. The stimulated release in the presence of antagonist is expressed relative to the stimulated release by NMDA alone and the IC₅₀ value is calculated.

Test results obtained by testing some compounds employed in the present invention will appear from the following table 1.

TABLE 1

	Compound of Example	Test 1 IC ₅₀ μM	Test 2 K _i μM	Test 3 IC ₅₀ μM
	1	0.350	0.162	0.163
	3	0.544	0.127	0.505
10	4	0.500	0.118	0.328
	5	0.554	0.212	0.509
	6	0.520	3.40	0.394
	7	2.000	ND	ND
	8	2.200	0.770	1.882
15	9	5.000	1.90	2.86
	10	8.000	ND	2.62
	11	3.000	0.900	ND
	12	0.849	0.390	0.127
	13	8.000	ND	>3.75
20	15	0.587	0.049	2.64
	16	0.409	0.100	ND
	19	7.000	ND	ND
	22	0.852	ND	ND

25 ND: Not determined

The compound of the invention, together with a conventional
 adjuvant, carrier, or diluent, and if desired in the form of
 a pharmaceutically-acceptable acid addition salt thereof,
 30 may be placed into the form of pharmaceutical compositions
 and unit dosages thereof, and in such form may be employed
 as solids, such as tablets or filled capsules, or liquids,
 such as solutions, suspensions, emulsions, elixirs, or cap-
 sules filled with the same, all for oral use, in the form of
 35 suppositories for rectal administration; or in the form of
 sterile injectable solutions for parenteral (including sub-
 cutaneous) use. Such pharmaceutical compositions and unit

dosage forms thereof may comprise conventional ingredients in conventional proportions, with or without additional active compounds or principles, and such unit dosage forms may contain any suitable effective central nervous system ailment alleviating amount of the active ingredient commensurate with the intended daily dosage range to be employed. Tablets containing one (1) to two hundred (200) milligrams, per tablet, are accordingly suitable representative unit dosage forms.

10

The compounds of this invention can thus be used for the formulation of pharmaceutical preparations, e.g., for oral and parenteral administration to mammals including humans, in accordance with conventional methods of galenic pharmacy.

15

Conventional excipients are such pharmaceutically acceptable organic or inorganic carrier substances suitable for parenteral or oral application which do not deleteriously react with the active compound.

20

Examples of such carriers are water, salt solutions, alcohols, polyethylene glycols, polyhydroxyethoxylated castor oil, gelatin, lactose, amylose, magnesium stearate, talc, silicic acid, fatty acid monoglycerides and diglycerides, pentaerythritol fatty acid esters, hydroxymethylcellulose and polyvinylpyrrolidone.

25

The pharmaceutical preparations can be sterilized and mixed, if desired, with auxiliary agents, such as lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salt for influencing osmotic pressure, buffers and/or coloring substances and the like, which do not deleteriously react with the active compound.

30

For parenteral application, particularly suitable are injectable solutions or suspensions, preferably aqueous solutions with the active compound dissolved in polyhydroxylated cas-

35

tor oil.

Ampoules are convenient unit dosage forms.

- 5 For oral application, particularly suitable are tablets, dragees, or capsules having talc and/or a carbohydrate carrier or binder or the like, the carrier preferably being lactose and/or corn starch and/or potato starch. A syrup, elixir or like can be used when a sweetened vehicle can be employed. Generally, as to broader ranges, the compounds of the invention are dispensed in unit dosage form comprising 0.05-100 mg in a pharmaceutically-acceptable carrier per unit dosage.
- 10
- 15 A typical tablet which may be prepared by conventional tableting techniques contains:

	Active compound	2.0 mg
	Lactosum	67.8 mg Ph.Eur.
20	Avicel®	31.4 mg
	Amberlite® IRP 88	1.0 mg
	Magnesii stearas	0.25 mg Ph.Eur.

- Due to their high degree of effect as glycin antagonists, the compounds of the invention are extremely useful in the treatment of central nervous system ailments or disorders, when administered in an amount effective for the alleviation, amelioration, or elimination thereof. The important CNS activity of the compounds of the invention includes both anticonvulsant, hypnotic, nootropic and anxiolytic activities along with a low toxicity, together presenting a most favorable therapeutic index. The compounds of the invention may accordingly be administered to a subject, e.g., a living mammal body, including a human, in need of the same for the treatment, alleviation, amelioration, or elimination of an indication, associated with the central nervous system and the so-called NMDA receptors, which re-
- 25
- 30
- 35

quires such psychopharmaceutical treatment, e.g., especially convulsion, anxiety epilepsy and ischemia, if desired in the form of a pharmaceutically acceptable acid addition salt thereof (such as the hydrobromide, hydrochloride, or sulfate, in any event prepared in the usual or conventional manner, e.g., evaporation to dryness of the free base in solution together with the acid), ordinarily concurrently, simultaneously, or together with a pharmaceutically acceptable carrier or diluent, especially and preferably in the form of a pharmaceutical composition thereof, whether by oral, rectal, or parenteral (including subcutaneous) route, in an effective psychopharmaceutical central nervous system ailment alleviating amount, e.g., an anticonvulsant and/or anxiolytic amount, and in any event an amount which is effective for the alleviation of such a central nervous system ailment due to their NMDA receptor affinity. Suitable dosage ranges are 1-200 milligrams daily, depending as usual upon the exact mode of administration, form in which administered, the indication toward which the administration is directed, the subject involved and the body weight of the subject involved, and the preference and experience of the physician or veterinarian in charge.

The invention will now be described in further detail with reference to the following examples, which may not be construed as limiting:

EXAMPLE 1

1-Carboxymethyl-6,7-dichloroquinoxaline-2,3(1H,4H)-dione

Under a nitrogen atmosphere Na (0.25 g, 11 mmol) was dissolved in 100 ml of abs. ethanol and 6,7-dichloroquinoxalin-2(1H)-one (2.15 g, 10 mmol) (Liebigs Ann. Chem., (1982), 754) was added. The mixture was refluxed for 30

- min., cooled to room temperature, and ethyl iodoacetate (1.43 ml, 12 mmol) was added. The clear reaction mixture was refluxed for 2 h during which time a precipitate was formed. This was filtered off, washed with abs. ethanol and dried to afford 2.56 g (85%) of 6,7-dichloro-1-ethoxycarbonylmethylquinoxalin-2(1H)-one. $^1\text{H-NMR}$ (DMSO-d_6): δ 1.23 (t, 3H), 4.20 (q, 2H), 5.08 (s, 2H), 8.05 (s, 1H), 8.17 (s, 1H), 8.38 (s, 1H).
- 10 The above ester (1.5 g, 5 mmol) was suspended in 70 ml of 2.5% NaOH and 30% H_2O_2 (1.95 ml) was added. The mixture was warmed at 70-80°C for 5 h, cooled and acidified (pH = 1) with concentrated hydrochloric acid. The precipitate was filtered off and recrystallized from DMF-water to
- 15 afford 1.17 g (81%) of the title compound. M.p. 317-19°C. $^1\text{H-NMR}$ (DMSO-d_6): δ 4.95 (s, 2H), 7.36 (s, 1H), 7.72 (s, 1H), 12.13 (s, 1H), 13.32 (br.s, 1H).
- Analysis: Calculated for $\text{C}_{10}\text{H}_6\text{Cl}_2\text{N}_2\text{O}\cdot\text{H}_2\text{O}$: C, 39.11; H, 2.63; Cl, 23.09; N, 9.12%. Found: C, 39.11; H, 2.69; Cl, 23.21; N, 9.02%.

EXAMPLE 2

- 25 6-Bromo-1-ethoxycarbonylmethylquinoxaline-2,3(1H,4H)-dione

- Under a nitrogen atmosphere 6-bromoquinoxalin-2(1H)-one (2.03 g, 9 mmol) (J. Med. Chem., 24, (1981), 93) was dissolved in 22 ml of dry DMF and sodium hydride (0.44 g, 10.8 mmol (60% mineral oil dispersion)) was added. After stirring for 2 h, ethyl bromoacetate (1.25 ml, 11.3 mmol) was added and the mixture was stirred for 3.5 h. The reaction mixture was poured onto crushed ice and acidified
- 35 (pH = 4.5) by addition of dilute hydrochloric acid. The precipitate was filtered off, washed with water and air dried. The crude product (containing a minor fraction of

O-alkylated product) was triturated with ether (100 ml), the precipitate filtered off, washed with ether and dried to afford 1.95 g (80%) of pure 6-bromo-1-ethoxycarbonylmethylquinoxalin-2(1H)-one. $^1\text{H-NMR}$ (DMSO-d_6): δ 1.22 (t, 3H), 4.17 (q, 2H), 5.08 (s, 2H), 7.56 (d, 1H), 7.83 (dd, 1H), 8.09 (dd, 1H), 8.37 (s, 1H).

The above ester (1.25 g; 4 mmol) was reacted with 30% H_2O_2 (6 ml) in glacial acetic acid (16 ml) at 55°C for 2 h. The mixture was cooled, the precipitate filtered off and recrystallized from dilute acetic acid to afford 1.0 g (77%) of the title compound. M.p. $282-83^\circ\text{C}$. $^1\text{H-NMR}$ (DMSO-d_6): δ 1.22 (t, 3H), 4.17 (q, 2H), 4.92 (s, 1H), 7.33 (m, 3H), 12.24 (br.s, 1H).

EXAMPLE 3

6-Bromo-1-carboxymethylquinoxaline-2,3(1H,4H)-dione

The ester of example 2 (0.33 g, 1 mmol) was reacted with 4% NaOH (9 ml) for 3 h at room temperature. The mixture was cooled in an ice bath and the pH was adjusted to 1 with 4M hydrochloric acid. The precipitate was filtered off, washed with water and recrystallized from acetic acid to afford 0.21 g (70%) of the title compound. M.p. $> 320^\circ\text{C}$. $^1\text{H-NMR}$ (DMSO-d_6): 4.86 (s, 1H), 7.29 (m, 3H), 12.24 (s, 1H), 13.28 (br.s, 1H).

Analysis: Calculated for $\text{C}_{10}\text{H}_7\text{BrN}_2\text{O}_4 \cdot \text{H}_2\text{O}$: C, 40.16; H, 2.36; Br, 26.72; N, 9.36%. Found: C, 40.06; H, 2.37; Br, 26.93; N, 9.22%.

EXAMPLE 41-Carboxymethyl-6,7-difluoroquinoxaline-2,3(1H,4H)-dione

5 4,5-difluoro-2-nitroaniline (2.17 g, 12.5 mmol) was dissolved in 55 ml of ethanol and 250 mg of 10% palladium on carbon was added. The mixture was hydrogenated until the theoretical amount of hydrogen had been absorbed, using a
10 low pressure hydrogenation apparatus. The catalyst was filtered off and the filtrate, containing 4,5-difluoro-o-phenylenediamine, was kept under a nitrogen atmosphere at 0°C followed by addition of a solution of glyoxylic acid hydrate (1.28 g, 13.7 mmol) in water (3 ml). When the addi-
15 tion was complete the reaction temperature was allowed to reach room temperature during 2 h and 30 ml of water was added. The precipitate was filtered off and dissolved in 60 ml of 5% NaOH solution at 50°C followed by addition of activated carbon. The mixture was filtered and the fil-
20 trate was acidified (pH = 1) by addition of concentrated hydrochloric acid. The pinky precipitate was filtered off, washed with water and dried to afford 1.25 g (55%) of 6,7-difluoroquinoxalin-2(1H)-one melting at 284-85°C. ¹H-NMR (DMSO-d₆): 7.27 (m, 1H), 7.95 (m, 1H), 8.20 (s, 1H), 12.55
25 (s, 1H).

6,7-Difluoroquinoxalin-2(1H)-one (1.1 g, 6 mmol) was reacted following the procedure of example 1 to afford the
30 title compound melting with destruction at 315-16°C. ¹H-NMR (DMSO-d₆): δ 4.86 (s, 2H), 7.16 (m, 1H), 7.65 (m, 1H), 12.27 (s, 1H), 13.30 (br.s, 1H).

Analysis: Calculated for C₁₀H₆N₂F₂O₄.H₂O: C, 43.81; H, 2.94; N, 10.22%. Found: C, 43.85; H, 2.94; N 10.22%.

EXAMPLE 51-Carboxymethyl-6,7-dimethylquinoxaline-2,3(1H,4H)-dione

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The title compound was prepared employing the procedure described in example 1 starting from 6,7-dimethylquinoxalin-2(1H)-one (Liebigs Ann.Chem., (1982), 754). M.p. 308-10°C (dec.). ¹H-NMR (DMSO-d₆): δ 2.19 (s, 3H), 2.22 (s, 3H), 10 4.84 (s, 2H), 6.96 (s, 1H), 7.11 (s, 1H), 12.05 (s, 1H), 13.18 (br.s., 1H).

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Analysis: Calculated for C₁₂H₁₂N₂O₄·1½ H₂O: C, 52.36; H, 5.49; N, 10.18%. Found: C, 52.45; H, 5.28; N, 10.14%.

EXAMPLE 61-Carboxymethyl-6-chloroquinoxaline-2,3(1H,4H)-dione

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In accordance with the procedure described in example 1 the title compound was prepared starting from 6-chloroquinoxalin-2(1H)-one (Heterocycles, 23, (1985), 143). M.p. 318-19°C. ¹H-NMR (DMSO-d₆): δ 4.88 (s, 2H), 7.30-7.40 (m, 3H), 12.25 (s, 1H), 13.32 (br.s, 1H). 25

30

Analysis: Calculated for C₁₀H₇N₂ClO₄: C, 47.17; H, 2.77; N, 11.00; Cl, 13.92%. Found: C, 47.12; H, 2.79; N, 10.96; Cl, 13.89%.

EXAMPLE 71-Carboxymethyl-6-nitroquinoxaline-2,3(1H,4H)-dione

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6-nitroquinoxalin-2(1H)-one (J.Chem.Soc. (1961), 1246) was reacted in accordance with the procedure described in

example 2 yielding 1-ethoxycarbonylmethyl-6-nitroquinoxaline-2,3(1H,4H)-dione, which after recrystallization from dilute acetic acid had m.p. 252-54°. ¹H-NMR (DMSO-d₆): δ 1.23 (t, 3H), 4.17 (q, 2H), 5.02 (s, 2H), 7.56 (d, 1H), 8.00 (dd, 1H), 8.01 (d, 1H), 12.48 (s, 1H).

The above ester (0.29 g, 1 mmol) was suspended in 1.25 ml of 2M NaOH and 10 ml of water and stirred for 3 h at 0°C. The clear solution was maintained at 0°C and the pH was adjusted to 1 by addition of 4 M HCl. The precipitate was filtered off, washed with water and recrystallized from dilute acetic acid to afford 0.13 g (50%) of the title compound. M.p. > 320°C. ¹H-NMR (DMSO-d₆): δ 4.96 (s, 2H), 7.56 (d, 1H), 8.02 (dd, 1H), 8.06 (d, 1H), 12.52 (s, 1H), 13.42 (br.s, 1H).

EXAMPLE 8

1-((1-Carboxy)pentyl)-quinoxaline-2,3(1H,4H)-dione

Quinoxalin-2(1H)-one (2.00 g, 13.7 mmol) was suspended in 40 ml of dry DMF under nitrogen. Sodium hydride (0.66 g, 16.4 mmol (60% mineral oil dispersion)) was added and the mixture was stirred for 1 h. Ethyl 2-bromohexanoate (3.87 g, 16.4 mmol) was added, and the mixture was stirred at room temperature for 20 h and at 70°C for 5.5 h, then poured onto crushed ice and acidified by dilute hydrochloric acid. The precipitated oil was extracted with ethyl acetate and purified by column chromatography on silica gel (eluent 15% THF in heptane) to afford 1.89 g of the O-alkylated compound and 0.46 g of the desired N-alkylated product, 1-((1-ethoxycarbonyl)pentyl)quinoxaline-2(1H)-one. ¹H-NMR (DMSO-d₆): δ 0.78 (t, 3H), 1.09 (t, 3H), 1.15-1.36 (m, 4H), 2.04-2.32 (m, 2H), 4.02-4.18 (m, 2H), 5.6-5.9 (m, 1H), 7.42 (t, 1H), 7.67 (t, 1H), 7.65-7.82 (br.s, 1H), 7.90 (d, 1H), 8.28 (s, 1H).

- The above ester (0.40 g, 1.39 mmol) was reacted with 30% H_2O_2 (2.2 ml) in glacial acetic acid (5.8 ml) at 55°C for 20 h. The mixture was poured onto crushed ice and extracted with ethyl acetate (2 x 75 ml). The combined organic
5 extract was dried (Na_2SO_4) and evaporated in vacuo to an oil, which was reacted with 0.5 M NaOH (7 ml) for 4 h at room temperature. The solution was acidified with 1 M HCl to pH 2.0 and the precipitate was filtered off, washed with water and then with ether to afford 166 mg of the title
10 compound. M.p. $253-55^\circ\text{C}$. $^1\text{H-NMR}$ (DMSO-d_6): δ 0.80 (t, 3H), 1.04-1.36 (m, 4H), 1.95-2.30 (m, 2H), 5.1-5.9 (m, 1H), 7.2 (m, 3H), 7.2-7.8 (m, 1H), 12.2 (s, 1H), 12.9 (br.s, 1H).
- 15 Analysis: calculated for $\text{C}_{14}\text{H}_{16}\text{N}_2\text{O}_4$: C, 60.86; H, 5.84; N, 10.14%. Found: C, 60.46; H, 5.86; N, 10.56%.

The compounds mentioned in examples 9, 10 and 11 were prepared using essentially the same procedure outlined in example 8 above, starting from quinoxalin-2(1H)-one.
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EXAMPLE 9

1-((1-Carboxy)butyl)quinoxaline-2,3(1H,4H)-dione
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(The alkylation reaction was performed with ethyl 2-bromopentanoate as alkylating agent and diglyme as solvent by heating to reflux for 2 h). M.p. $272-75^\circ\text{C}$. $^1\text{H-NMR}$ (DMSO-d_6): δ 0.83 (t, 3H), 1.05-1.38 (m, 2H), 1.95-2.20 (m, 2H),
30 δ 5.1-5.9 (br.m, 1H) (by heating the probe to 100°C the band appears as a triplet at 5.5), 7.1-7.25 (m, 3H), 7.2-7.9 (br.m, 1H) (by heating the probe to 100°C the band appears as a sharp multiplet at 7.25), 12.2 (s, 1H), 12.85
35 (br.s, 1H).

EXAMPLE 101-(1-Carboxy-1-phenylmethyl)quinoxaline-2,3(1H,4H)-dione

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(The alkylation reaction was performed with ethyl 2-bromo-2-phenylacetate as alkylating agent, ethanol as solvent, and sodium ethoxide as base by heating to reflux for 72 h). M.p. 242-44°C. ¹H-NMR (DMSO-d₆): δ 6.04 (s, 1H), 7.0-7.55 (m, 9H), 12.23 (s, 1H), 13.1 (br.s, 1H).

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EXAMPLE 111-((1-Carboxy)ethyl)quinoxaline-2,3(1H,4H)-dione

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(The alkylation reaction was performed with ethyl 2-bromopropionate as alkylating agent, DMSO as solvent, potassium carbonate (2 equivalents) as base and potassium iodide (0.1 equivalent) as catalyst by heating to 85°C for 4 h). M.p. 301-303°C (decomp.). ¹H-NMR (DMSO-d₆): δ 1.47 (d, 3H), 5.47 (broad signal, 1H), 7.1-7.5 (m, 4H), 12.13 (s, 1H), 12.8 (br.s, 1H).

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EXAMPLE 121-Carboxymethyl-6,7-dibromoquinoxaline-2,3(1H,4H)-dione

1-Carboxymethylquinoxaline-2,3(1H,4H)-dione (0.50 g, 2.27 mmol) (Indian J. Chem. 20B, (1981), 822) and Ag₂SO₄ (0.744 g, 2.384 mmol) was suspended in 98% H₂SO₄ (2.3 ml). Bromine (0.244 ml, 4.77 mmol) was added and the mixture was stirred at room temperature for 24 h, then tetrachloromethane (2.3 ml) was added and the reaction mixture was stirred at 50°C for 2 h, filtered and the filtrate was poured onto crushed ice (20 ml). The precipitate was fil-

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tered off, washed with water and dried to afford 0.57 g of crude product. Purification was performed by dissolving the crude substance in 10 ml of 1M sodium hydroxide (pH = 12) followed by precipitation with 1M hydrochloric acid (pH = 2) M.p. > 300 (decomp.). $^1\text{H-NMR}$ (DMSO-d_6): δ 4.82 (s, 2H), 7.47 (s, 1H), 7.71 (s, 1H), 12.29 (s, 2H).

EXAMPLE 13

10 1-Carboxymethyl-4-hydroxy-6-methoxyquinoxalin-2,3(1H,4H)-dione

4-Methoxy-2-nitroaniline (16.8 g, 0.1 mol) was dissolved in 300 ml of pyridine under nitrogen and cooled to -10°C , and dimethylaminopyridine (1.22 g, 0.01 mol) was added. The mixture was stirred and a solution of ethyl oxalylchloride (21.4 g, 0.157 mol) in 50 ml of tetrahydrofuran was added over a period of 15 min. After the addition was complete the reaction mixture was kept at -20°C overnight, and poured onto 1 l of ice-water. The separated solid was collected and dried to afford 25.0 g (93%) of ethyl N-(4-methoxy-2-nitrophenyl)oxamate. M.p. $156-7^\circ\text{C}$. $^1\text{H-NMR}$ (CDCl_3): δ 1.41 (t, 3H), 3.85 (s, 3H), 4.42 (q, 2H), 7.20 (dd, 2H), 7.70 (d, 1H), 8.70 (d, 1H), 11.6 (s, 1H).

The above prepared oxamate (5.35 g, 0.02 mol) was dissolved in 50 ml of DMF under N_2 and NaH (0.96 g, 0.024 mol (60% mineral oil dispersion)) was added under stirring at room temperature. After 1 h tert-butyl bromoacetate (4.78 g, 0.024 mol) was added and stirring was continued at room temperature for 3 h. The reaction mixture was poured into water and was extracted with ethyl acetate. The organic phase was dried and evaporated to afford 4.1 g (55%) of ethyl N-(tert-butoxycarbonylmethyl)-N-(4-methoxy-2-nitrophenyl)oxamate. $^1\text{H-NMR}$ (DMSO-d_6): δ 0.90 (t, 3H), 1.42 (s, 9H), 3.89 (s, 3H), 3.95 (q, 2H), 4.35 (dd, 2H), 7.46

(dd, 1H), 7.60 (d, 1H), 7.73 (d, 1H).

1 g of the above prepared compound was dissolved in 150 ml of ethanol and under a nitrogen atmosphere was added
5 0.25 g Pd/C 10%. The hydrogenation was carried out in a Parr apparatus at 50 psi during 3 h. The catalyst was removed by filtration and the filtrate was evaporated to dryness giving raw 1-tert-butoxycarbonylmethyl-4-hydroxy-6-methoxyquinoxaline-3,4(1H,4H)-dione (0.51 g) (55%).
10 ¹H-NMR (DMSO-*d*₆): δ 1.42 (s, 9H), 3.86 (s, 3H), 4.96 (s, 2H), 6.9 (dd, H), 7.35 (d, H), 7.54 (d, H), 11.8 (br.s, H). M.p. 250°C (decomp.).

0.5 g of the above prepared compound was treated with HCl
15 in dioxane (16 ml, 2.5 M) at room temperature for 24 h. During this period a white solid separated. The solid was collected by filtration, washed with cold methanol and dried to afford 1-carboxymethyl-4-hydroxy-6-methoxy-quinoxaline-3,4(1H,4H)-dione (0.220 g) (40%). Purity HPLC
20 98%. ¹H-NMR (DMSO-*d*₆): δ 3.82 (s, 3H), 4.92 (s, 2H), 6.89 (dd, 1H), 7.11 (d, 1H), 7.33 (d, 1H), 12.0 (br.s, 1H) 13.28 (br.s, 1H). M.p. > 275°C.

Analysis: Calculated for C₁₁H₁₀N₂O₆ · 0.25 H₂O: C, 48.80;
25 H, 3.90; N, 10.35%. Found: C, 48.91; H, 3.88; N, 10.04%.

EXAMPLE 14

1-((1-Ethoxycarbonyl)hexyl)-6-bromoquinoxaline-2,3(1H,
30 4H)-dione

Under a nitrogen atmosphere sodium (1.40 g, 61 mmol) was dissolved in 400 ml of abs. ethanol and 6-bromoquinoxalin-
35 2(1H)-one (11.93 g, 53 mmol) (J. Med. Chem., 24, (1981), 93) was added. The mixture was stirred until the compound dissolved and ethyl 2-bromoheptanoate, (12.71 ml, 63.6

mmol) was added. The reaction mixture was refluxed for 72 h, cooled to room temperature, then poured onto a mixture of 200 ml crushed ice, 200 ml 1M hydrochloric acid and 500 ml dichloromethane.

5

The organic phase was separated, washed with 100 ml of 1M hydrochloric acid and 100 ml of water, filtered, dried and evaporated to give an oil, which was purified by column chromatography on silica gel (eluent 3% THF in heptane) to afford 3.61 g of 1-((1-ethoxycarbonyl)hexyl)-6-bromoquinoxaline-2(1H)-one. $^1\text{H-NMR}(\text{DMSO}-d_6)$: δ 0.78(t, 3H), 1.09(t, 3H), 1.11 - 1.35(m, 6H), 2.0 - 2.3(m, 2H), 4.02 - 4.18(m, 2H), 5.54 - 5.86(m, 1H), 7.5-7.8(br.m, 1H), 7.85(dd, 1H), 8.09(d, 1H), 8.33(s, 1H).

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The above ester (3.43 g, 9.0 mmol) was reacted with 30% H_2O_2 (14.9 ml) in glacial acetic acid (40 ml) at 55°C for 20h. The mixture was poured onto crushed ice (250 ml) and extracted with ethyl acetate (2 x 400 ml). The combined organic extract was washed with half saturated sodium hydrogen carbonate solution and water, dried (Na_2SO_4), evaporated to dryness and stripped with ether. The product was triturated with heptane (20 ml) overnight, the precipitate filtered off, washed with heptane and dried to afford 2.86 g of the title compound. M.p. 187-190°C. $^1\text{H-NMR}(\text{DMSO}-d_6)$: δ 0.80(m, 3H), 1.1(t, 3H), 1.12 - 1.4(m, 6H) 1.9 - 2.25(m, 2H), 4.0 - 4.17(m, 2H), 5.2 - 5.7(m, 1H), 7.3 - 7.7(m, 3H), 12.28(s, 1H).

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Analysis: Calculated for $\text{C}_{17}\text{H}_{21}\text{N}_2\text{O}_4\text{Br}$: C, 51.39; H, 5.33; N, 7.05; Br, 20.12% Found: C, 51.49; H, 5.43; N, 7.02; Br, 20.24%.

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EXAMPLE 15

1-((1-Carboxy)hexyl)-6-bromoquinoxaline-2,3(1H, 4H)-dione

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The ester of example 14 (1.40 g, 3.52 mmol) was suspended in 19.21 ml of 0.5 M NaOH and stirred for 20h at room temperature. The reaction mixture was filtered and the pH was adjusted to 2 by addition of 1 M HCl. After cooling
10 in an ice bath the precipitate was filtered off and washed with water to afford 1.21 g of the title compound. M.p. 265 - 267°C. ¹H-NMR(DMSO-d₆): δ 0.79(m, 3H), 1.08 - 1.37(m, 6H), 1.87 - 2.25 (m, 2H), 5.1 - 5.7(m, 1H), 7.25 - 7.8(m, 3H), 12.25(s, 1H), 12.5 - 13.2(br.s, 1H).

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Analysis: Calculated for C₁₅H₁₇N₂O₄Br.1/2H₂O: C, 47.63; H, 4.80; N, 7.41%. Found: C, 47.80; H, 4.65; N, 7.59%.

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EXAMPLE 16

1-((1-carboxy)pentyl)-6,7-dichloroquinoxaline-2,3(1H, 4H)-dione

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Under a nitrogen atmosphere sodium (0.148 g, 6.42 mmol) was dissolved in 50 ml of abs. ethanol and 6,7-dichloroquinoxalin-2(1H)-one (1.20 g, 5.58 mmol) (Liebigs Ann. Chem., (1982), 754) was added. The mixture was stirred
30 until the compound dissolved, and ethyl 2-bromohexanoate (1.30 ml, 7.1 mmol) was added. The reaction mixture was refluxed for 72 h, cooled to room temperature, then poured onto crushed ice and acidified by dilute hydrochloric acid (pH = 2). The precipitated compound was extracted with
35 ethyl acetate and purified by column chromatography on silica gel (eluent 15% THF in heptane) to afford 0.21 g of 1-((1-ethoxycarbonyl)pentyl)-6,7-dichloroquinoxalin-

2(1H)-one $^1\text{H-NMR}(\text{DMSO-d}_6)$: δ 0.78(t, 3H), 1.1(t, 3H), 1.15 - 1.35(m, 4H), 2.0 - 2.25(m, 2H), 4.02 - 4.18(m, 2H), 5.58 - 5.75(m, 1H), 8.02 - 8.36(broad, 1H), 8.18(s, 1H), 8.33(s, 1H).

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The above ester (0.16 g, 0.448 mmol) was suspended in 3 ml of 5% NaOH and 30% H_2O_2 (0.175 ml) was added. The mixture was warmed at 80°C under stirring for 5 h, cooled and acidified (pH = 1) with 5 M hydrochloric acid. The precipitate was filtered off, washed with water and dried to afford 90 mg of the title compound. $^1\text{H-NMR}(\text{DMSO-d}_6)$: δ 0.81(t, 3H), 1.03 - 1.33(m, 4H), 1.82 - 2.23(m, 2H), 5.22 - 5.5(m, 1H), 7.35(s, 1H), 7.5 - 8.2(broad, 1H), 12.3(s, 1H), 12.6 - 13.3(br.s, 1H).

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EXAMPLE 17

1-Phenylmethyloxycarbonylmethyl-6,7-dichloroquinoxaline-2,3(1H, 4H)-dione

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Under a nitrogen atmosphere 6,7-dichloroquinoxalin-2(1H)-one (10.0 g, 46.5 mmol) (Liebigs Ann. Chem, (1982), 754) was suspended in 135 ml of dry DMF and sodium hydride (2.23 g, 55.8 mmol (60% mineral oil dispersion)) was added. After stirring for 2 h, benzyl bromoacetate (9.18 ml, 58.6 mmol) was added and the mixture was stirred for 3.5 h. The reaction mixture was poured onto 300 g of crushed ice and the precipitate was filtered off, washed with water and dried. The product was triturated with ether (500 ml), the precipitate filtered off, washed with ether and dried to afford 13.3 g of 1-benzyloxycarbonylmethyl-6,7-dichloroquinoxaline-2(1H)-one. $^1\text{H-NMR}(\text{DMSO-d}_6)$: δ 5.18(s, 2H), 5.23(s, 2H), 7.3-7.43(m, 5H), 8.05(s, 1H), 8.18(s, 1H), 8.38(s, 1H).

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The above ester (12.0 g, 33 mmol) was reacted with 30%

H₂O₂ (50 ml) in glacial acetic acid (350 ml) at 55°C for 24 h. The mixture was cooled and 600 ml of icecold water was added. The precipitate was filtered off and recrystallized from DMF-water to afford 7.16 g of the
5 title compound. M.p. 286-289°C ¹H-NMR(DMSO-d₆): δ 5.08(s, 2H), 5.22(s, 2H), 7.28 - 7.45(m, 6H), 7.75(s, 1H), 12.35(s, 1H).

Analysis: Calculated for C₁₇H₁₂N₂O₄Cl₂: C, 53.84; H, 3.19; N, 7.39; Cl, 18.70%. Found: C, 53.65; H, 3.26; N, 7.44; Cl, 18.52%.

EXAMPLE 18

15 1-Tert-butoxycarbonylmethyl-6,7-dichloroquinoxaline-2,3(1H, 4H)-dione

The title compound was prepared using essentially the
20 same procedure outlined in example 17. The alkylation reaction was performed with tert-butylbromoacetate as alkylating agent, ethanol as solvent and sodium ethoxide as base by heating to reflux for 20 h. M.p. > 300°C
¹H-NMR(DMSO-d₆): δ 1.43(s, 9H), 4.88(s, 2H), 7.35(s, 1H),
25 7.66(s, 1H), 12.32(s, 1H).

EXAMPLE 19

30 1-Carboxymethyl-7-nitroquinoxaline-2,3(1H, 4H)-dione

Under a nitrogen atmosphere 7-nitroquinoxalin-2(1H)-one (3.06 g, 16 mmol) (J Chem. Soc. (1961), 1246) was dissolved in 40 ml of dry DMF and sodium hydride (0.768
35 g, 19.2 mmol (60% mineral oil dispersion)) was added. After stirring for 1.5 h, ethyl bromoacetate (2.22 ml, 20 mmol) was added and the mixture was stirred for 3 h.

The reaction mixture was poured onto crushed ice and acidified (pH = 4.5) by addition of dilute hydrochloric acid. The precipitate was filtered off, washed with water and air dried. Purification was performed by column chromatography on silica gel (eluent 1-10% THF in heptane). The faster moving fraction (R_F = 0.68, THF/heptane (1:1)) was collected and evaporated to afford 0.78 g (17%) of 2-ethoxycarbonylmethoxy-7-nitroquinoxaline-2(1H)-one. $^1\text{H-NMR}$ (DMSO-d_6): 1.22 (t, 3H), 4.20 (q, 2H), 5.18 (s, 2H), 8.28 (d, 1H), 8.39 (dd, 1H), 8.43 (dd, 1H), 8.97 (s, 1H). The slower moving fraction (R_F = 0.62, THF/heptane (1:1)) was collected and evaporated to afford 1.54 g (35%) of 1-ethoxycarbonylmethyl-7-nitroquinoxaline-2(1H)-one. $^1\text{H-NMR}$ (DMSO-d_6): δ 1.23 (t, 3H), 3.32 (q, 2H), 4.19 (q, 2H), 5.20 (s, 2H), 8.11 (d, 1H), 8.17 (dd, 1H), 8.40 (d, 1H), 8.50 (s, 1H).

The latter compound (1.0 g, 3.6 mmol) was reacted with 30% H_2O_2 (5.4 ml) in glacial acetic acid (15 ml) at 56°C for 16 h. The mixture was cooled, the precipitate filtered off, washed with water and dried to afford 0.8 g (76%) of 1-ethoxycarbonylmethyl-7-nitroquinoxaline-2,3(1H,4H)-dione. $^1\text{H-NMR}$ (DMSO-d_6): δ 1.24 (t, 3H), 4.20 (q, 2H), 5.10 (s, 2H), 7.36 (d, 1H), 8.12 (dd, 1H), 8.17 (dd, 1H), 12.72 (br. s, 1H).

The above ester (0.4 g, 1.36 mmol) was reacted with 2 M NaOH (3.9 ml) for 1 h at room temperature. The mixture was cooled in an ice bath and the pH was adjusted to 1 with 4 M hydrochloric acid. The precipitate was filtered off, washed with water and dried to afford 0.34 g (88%) of the title compound. M.p. $305-306^\circ\text{C}$. $^1\text{H-NMR}$ (DMSO-d_6): δ 5.02 (s, 2H), 7.37 (d, 1H), 8.12 (m, 2H), 12.70 (s, 1H), 13.40 (br. s, 1H).

Analysis: Calculated for $\text{C}_{10}\text{H}_7\text{N}_3\text{O}_6 \cdot \text{H}_2\text{O}$: C, 42.41; H, 3.20; N, 14.83%. Found: C, 42.71; H, 3.35; N, 14.65%.

Alternatively 1-carboxymethylquinoxaline-2,3(1H, 4H)-dione (Indian J. Chem. 20B, (1981), 822) could be nitrated using excess of 75% HNO_3 in a mixture of acetic anhydride and acetic acid at 0°C to give the
5 title compound.

EXAMPLE 20

10 1-Carboxymethyl-7-chloro-4-hydroxyquinoxaline-2,3(1H, 4H)-dione

A solution of 5-chloro-2-nitroaniline (3.0 g, 17 mmol) and 4-dimethylaminopyridine (0.21 g, 1.7 mmol) in 30 ml
15 of pyridine was stirred at -10°C and a solution of ethyl oxalyl chloride (3.5 g, 26 mmol) in 10 ml of tetrahydrofuran was added dropwise. After the addition was complete the ice bath was removed and stirring was continued for 7 h. The reaction mixture was poured onto
20 crushed ice, the precipitate was filtered off, washed with water and dried to afford 4.4 g (97%) of ethyl N-(3-chloro-6-nitrophenyl)oxamate. M.p. $103-4^\circ\text{C}$. $^1\text{H-NMR}$ (DMSO-d_6): δ 1.33 (t, 3H), 4.34 (q, 2H), 7.52 (dd, 1H), 8.19 (d, 1H), 8.24 (d, 1H), 11.46 (br.s, 1H).

25 Under a nitrogen atmosphere ethyl N-(3-chloro-6-nitrophenyl)oxamate (4.09 g, 15 mmol) was dissolved in 35 ml of dry DMF and sodium hydride (0.67 g, 16.8 mmol) was added portionwise. After the evolution of hydrogen had subsided t-butyl bromoacetate (3.5 g, 18 mmol (60% mineral
30 oil dispersion)) was added and the mixture was stirred overnight at room temperature. The reaction mixture was poured onto ice and extracted with dichloromethane (3 x 50 ml). The combined organic extracts was washed with
35 water, dried over Na_2SO_4 and filtered. The solvent was evaporated leaving a slightly yellow oil (4.4 g) having $^1\text{H-NMR}$ (DMSO-d_6): δ 0.96 (t, 3H), 1.44 (s, 9H), 4.00 (q,

2H), 4.42 (dd, 2H), 7.78 (d, 1H), 7.84 (dd, 1H), 8.24 (d, 1H) assigned to ethyl (N-(t-butoxycarbonylmethyl)- N-(3-chloro-6-nitrophenyl)oxamate.

- 5 A mixture of the latter crude oil (1.93 g, 5 mmol) and 0.19 g of 10% Pd/C in 65 ml of ethanol was hydrogenated at 50 psi for 5h using a Parr apparatus. The catalyst was removed by filtration and the filtrate was evaporated in vacuo to afford 1.1 g (70%) of 1-(t-butoxycarbonylmethyl)-7-chloro-4-hydroxyquinoxaline-2,3(1H,4H)-dione.
- 10 ¹H-NMR (CDCl₃): δ 1.42 (s, 9H), 4.84 (s, 2H), 7.00-7.72 (m, 3H).

- The above ester (1.08 g, 3.5 mmol) was dissolved in 8 ml of dioxane and a solution of HCl in dioxane (8 ml, 5M) was added. The mixture was refluxed for 2 h during which time a precipitate was formed. This was filtered off and purified by dissolving in 10% NaOH solution, decolourising with activated carbon and reprecipitation with hydrochloric acid (pH=1) to afford 0.37 g (35%) of the title compound. M.p. 280-82°C. ¹H-NMR (DMSO-d₆): δ 4.95 (s, 2H), 7.37 (dd, 1H), 7.60 (m, 2H), 12.00 (br.s, 1H), 13.55 (br.s, 1H).
- 15
- 20

- 25 Analysis: Calculated for C₁₀H₇Cl N₂O₅ · 2H₂O: C, 39.17; H, 3.61; Cl, 11.56; N, 9.13%. Found: C, 39.29; H, 3.72; Cl, 11.30; N, 9.01%.

EXAMPLE 21

30

1-Ethoxycarbonylmethyl-6,8-dichloroquinoxaline-2,3(1H, 4H)-dione

- 35 A mixture of 2,4-dichloro-6-nitroaniline (7.7 g, 37.2 mmol) and SnCl₂ (21.6 g, 111.6 mmol) were mechanically stirred under a nitrogen atmosphere. Hydrochloric acid

(38.5 ml) was added and the mixture was warmed at 75-80°C for 2 h. The mixture was cooled (ice bath) and neutralized (pH=9) with 50% potassium hydroxide solution followed by extraction with ether (3 x 150 ml). The combined organic
5 extracts was dried over sodium hydroxide pellets and filtered. The solvent was evaporated leaving 6.2 g of intermediary 1,2-diamino-3,5-dichlorobenzene, which was dissolved in 200 ml of methanol and stirred under cooling in an ice bath. Hydrochloric acid (2.90 ml) was added
10 followed by addition of glyoxylic acid monohydrate (3.9 g, 42.4 mmol). The ice bath was removed and stirring was continued for 16 h. The precipitate was filtered off, washed with cold methanol and dried to afford 6.0 g isomeric mixture (6:1, ¹H-NMR) of 6,8-dichloroquinoxalin-
15 2(1H)-one and 5,7-dichloroquinoxalin-2(1H)-one, respectively. The isomeric mixture was recrystallized from 2-methoxyethanol (100 ml) to afford 3.4 g of 6,8-dichloroquinoxalin-2-(1H)-one. M.p. 228-229°C. ¹H-NMR (DMSO-d₆): δ 7.88 (m, 2H), 8.30 (s, 1H), 12.20 (s, 1H).

20

Under a nitrogen atmosphere sodium (0.13 g, 5.62 mmol) was dissolved in 25 ml of abs. ethanol and 6,8-dichloroquinoxalin-2(1H)-one (1.10 g, 5.11 mmol) was added. The mixture was refluxed for 20 min., and ethyl iodoacetate
25 (0.729 ml, 6.13 mmol) was added. The reaction mixture was refluxed for 72 h, cooled to room temperature, then poured onto crushed ice and acidified by dilute hydrochloric acid (pH=2). The precipitated compound was extracted with dichloromethane (4 x 30 ml) and purified by column chromatography on silica gel (eluent 10% THF in heptane) to give
30 1-ethoxycarbonylmethyl-6,8-dichloroquinoxalin-2(1H)-one. ¹H-NMR (DMSO-d₆): δ 1.23 (t, 3H), 4.22 (q, 2H), 5.29 (s, 2H), 7.97 (dd, 2H), 8.45 (s, 1H).

35 The above ester, (70 mg, 0.23 mmol) was reacted with 30% H₂O₂ (0.345 ml) in glacial acetic acid (0.92 ml) at 55°C for 4.5 h. The mixture was cooled in an icebath and H₂O

(0.40 ml) was added. The precipitate was filtered off, washed with water and dried to give 35 mg (48%) of the title compound. $^1\text{H-NMR}$ (DMSO-d_6): δ 1.23 (t, 3H), 4.20 (q, 2H), 5.02 (s, 2H), 7.21 (d, 1H), 7.42 (d, 1H), 12.3 (s, 1H).

EXAMPLE 22

10 1-Carboxymethyl-6,8-dichloroquinoxaline-2,3(1H, 4H)-dione

The ester of example 21 (34 mg, 0.107 mmol) was reacted with 0.1 M NaOH (3.2 ml) in a mixture of THF (2.0 ml) and water (0.5 ml) for 26 h at 45°C. The solvent was removed
15 in vacuo and the remanens was dissolved in water (1.0 ml), cooled in an ice bath and the pH was adjusted to 2.5 with 0.5 M hydrochloric acid. The precipitate was filtered off, washed with water and dried to give 20 mg (65%) of the
20 title compound. $^1\text{H-NMR}$ (DMSO-d_6): δ 5.0 (s, 2H), 7.2 (d, 1H), 7.41 (d, 1H), 12.33 (s, 1H), 13.18 (br.s, 1H).

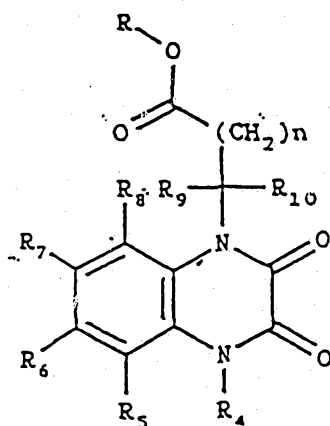
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CLAIMS

1. 1-carboxyalkylquinoxaline-2,3(1H,4H)-dione compounds or tautomeric forms thereof of the formula



(I)

wherein

R represents hydrogen, C_{1-6} -alkyl, including branched chains, or aralkyl and n is an integer from 0 to 5; R^4 represents hydrogen or hydroxy; R^5 , R^6 , R^7 and R^8 independently represent hydrogen, nitro, halogen, alkoxy, aryloxy, aralkoxy, C_{1-6} -alkyl including branched chains, or aryl provided that at least one of R^5 , R^6 , R^7 and R^8 does not represent hydrogen when R represents hydrogen, $-CH_3$ or $-CH(CH_3)_2$ and n is 0 or 1; R^9 represents hydrogen, lower alkyl, or aryl; R^{10} represents hydrogen, or alkyl, and pharmaceutically acceptable salts thereof.

2. Compounds according to claim 1 wherein R^4 is hydrogen and $n = 0$.

3. A method for the preparation of a compound of claim 1, ~~characterized in~~ including:

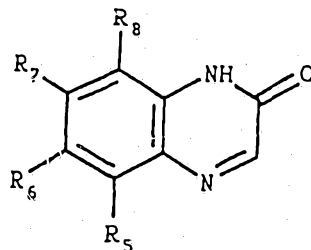
a) alkylating a compound of the formula (II):



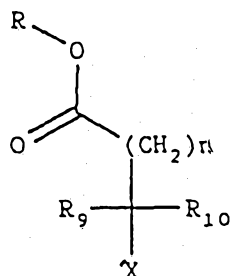
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(II)

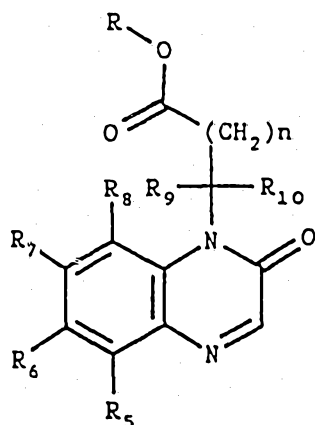


wherein R^5 , R^6 , R^7 and R^8 have the meanings set forth above, with a compound of formula (III)



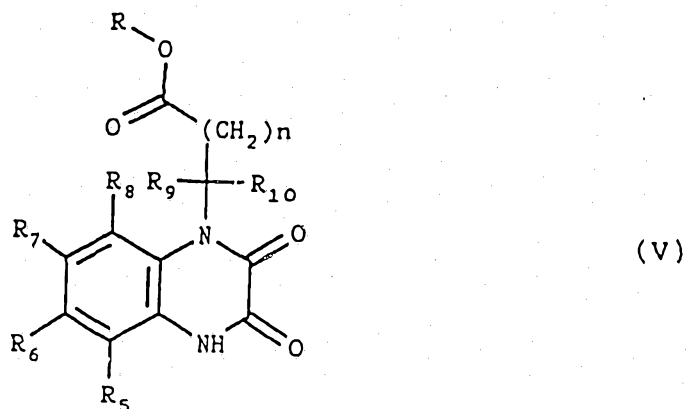
(III)

wherein R , n , R^9 and R^{10} are as previously defined, provided that R is not hydrogen, and X represents a leaving group, preferably bromine or iodine, to form a compound of formula (IV)



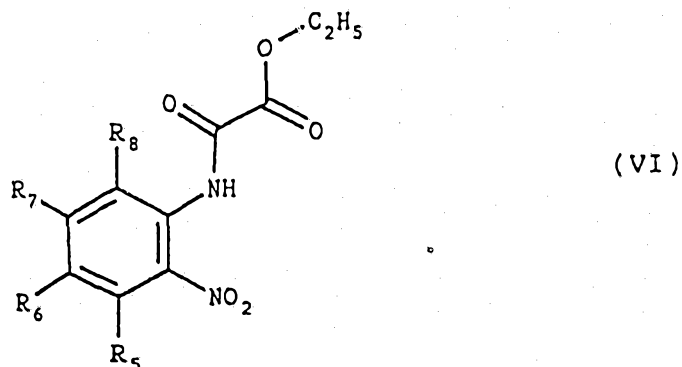
(IV)

wherein R, n, R⁵, R⁶, R⁷, R⁸, R⁹ and R¹⁰ have the meanings set forth above, provided that R is not hydrogen, and oxidation of a compound of formula (IV) with aqueous hydrogen peroxide in acetic acid to form a compound of formula (V):



wherein R, n, R⁵, R⁶, R⁷, R⁸, R⁹ and R¹⁰ have the meanings set forth above, provided that R is not hydrogen, and hydrolysis of a compound of formula (V), or reacting a compound of formula (IV) with aqueous H₂O₂/NaOH mixture and precipitation with a mineral acid, preferably hydrochloric acid, to form a compound of formula I wherein R and R⁴ are hydrogen and n, R⁵, R⁶, R⁷, R⁸, R⁹ and R¹⁰ are as previously defined or

b) alkylating a compound of formula (VI):

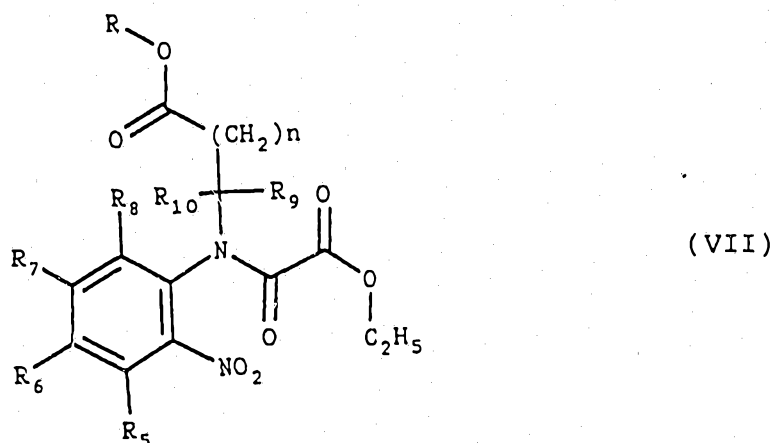


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wherein ~~R, R⁵, R⁶, R⁷, R⁸, R⁹ and R¹⁰~~ and ~~R⁵, R⁶, R⁷ and R⁸~~ have the meanings set forth above, provided that R⁵, R⁶, R⁷ and R⁸ is not NO₂, with a compound of formula (III), in a suitable solvent, e.g. alcohol or DMF and sodium ethoxide or sodium hydride as base, to form a compound of formula (VII)

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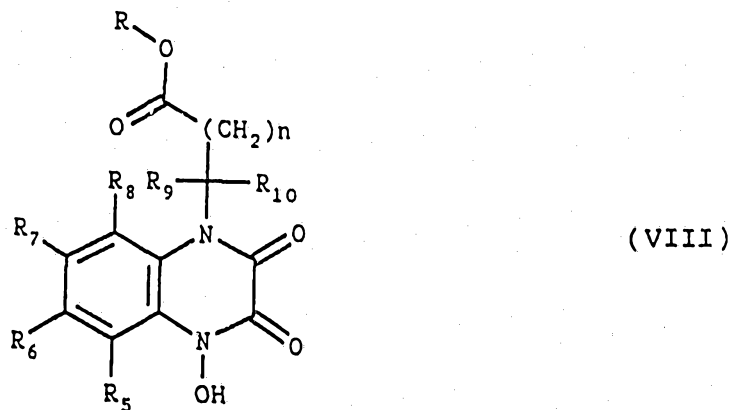


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wherein R is preferably tert-butyl and n and R⁵, R⁶, R⁷, R⁸, R⁹, R¹⁰ have the meanings defined for formula (I), provided that R⁵, R⁶, R⁷ and R⁸ is not NO₂, and reducing a compound of formula (VII) to form a compound of the formula (VIII)

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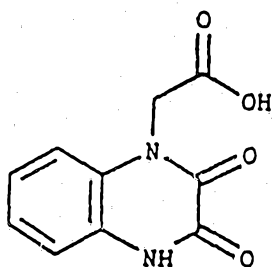
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wherein R is preferably tert-butyl and n and R⁵, R⁶, R⁷,



R^8 , R^9 and R^{10} have the meanings defined for formula (I), provided that R^5 , R^6 , R^7 and R^8 is not NO_2 , and reacting a compound of formula (VIII) to form a compound of formula (I) wherein R represents hydrogen or

c) halogenating or nitrating a compound of formula (IX):



(IX)

to form a compound of formula (I) wherein $n = 0$, and wherein R^4 , R^9 and R^{10} are hydrogen and wherein R^5 , R^6 , R^7 and R^8 are independently hydrogen, and halogen or independently hydrogen and NO_2 .

4. A pharmaceutical composition ^{including} ~~comprising~~ as active component a quinoxaline compound according to claim 1 or a pharmaceutically acceptable salt thereof and a pharmaceutically acceptable carrier or diluent.

5. A pharmaceutical composition according to claim 4 in the form of an oral dosage unit containing about 1-200 mg of the active compound.

6. A method of treating a central nervous system ailment in a person in need of such treatment, characterized in administering to said person an amount of a compound of claim 1 effective in alleviation of such an ailment.

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7. A method of treating a central nervous system ailment in a subject in need of such treatment, ^{including} ~~comprising~~ the step of administering to said subject an amount of a compound of claim 1 which is effective for the alleviation of such ailment in the form of a pharmaceutical composition thereof, in which it is present together with a pharmaceutically acceptable carrier or diluent.

8. A pharmaceutical composition suitable for use in the treatment of a central nervous system ailment, ^{including} ~~comprising~~ an amount of a compound according to claim 1 which is effective for the alleviation of such disorder together with a pharmaceutically acceptable carrier or diluent.

9. The use of a compound according to claim 1 or a pharmaceutically acceptable salt thereof for ^{preparing} ~~the preparation of~~ a medicament useful in the treatment of a central nervous system ailment.

REPLACEMENT SHEET

10. A compound according to claim 1 substantially as
hereinbefore described with reference to any one of the
5 Examples.

DATED : 15 JUNE 1994

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INTERNATIONAL SEARCH REPORT

International Application No PCT/DK 91/00078

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all) ⁶		
According to International Patent Classification (IPC) or to both National Classification and IPC IPC5: C 07 D 241/44, 241/52		
II. FIELDS SEARCHED		
Minimum Documentation Searched ⁷		
Classification System	Classification Symbols	
IPC5	C 07 D	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in Fields Searched ⁸		
SE,DK,FI,NO classes as above		
III. DOCUMENTS CONSIDERED TO BE RELEVANT⁹		
Category *	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
X	Chemical Abstracts, volume 96, no. 3, 18 January 1982, (Columbus, Ohio, US), Borthakur, N et al.: "Studies in 1,2,3,4-tetrahydro-3-oxoquinoxaline-1-acetic acid: a facile oxidation of active methylene group", see page 450, abstract 20066m, & Indian J. Chem. 1981, 20(9), 822- 824 --	1-2
X	EP, A2, 315959 (A/S FERROSAN) 17 May 1989, the claims, example 21-23, 25 -- -----	1-5
* Special categories of cited documents:¹⁰ "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance, the claimed invention cannot be considered novel or cannot be considered to involve an inventive step "Y" document of particular relevance, the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art. "&" document member of the same patent family		
IV. CERTIFICATION		
Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report	
18th June 1991	1991 -06- 25	
International Searching Authority	Signature of Authorized Officer	
SWEDISH PATENT OFFICE	Solveig Gustavsson	

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. ☒ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE¹

This International search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☒ Claim numbers 6...7..., because they relate to subject matter not required to be searched by this Authority, namely:

See PCT Rule 39.1 (iv): Methods for treatment of the human or animal body by surgery or therapy, as well as diagnostic methods.

2. ☐ Claim numbers....., because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim numbers....., because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING²

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.
2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:
3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the the claims. It is covered by claim numbers:
4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

- ☐ The additional search fees were accompanied by applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

**ANNEX TO THE INTERNATIONAL SEARCH REPORT
ON INTERNATIONAL PATENT APPLICATION NO.PCT/DK 91/00078**

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the Swedish Patent Office EDP file on **91-05-29**
The Swedish Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A2- 315959	89-05-17	NONE	