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Quinoline-2-carboxylic acid derivative and its use as excitatory amino acids antagonist

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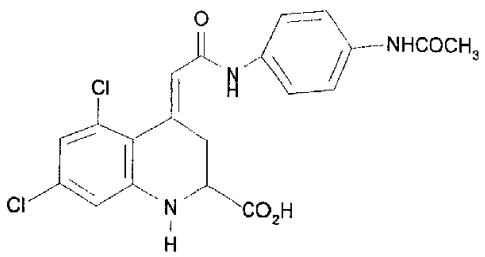
(57) Abstract

The (+) enantiomer of E 4-(4-Acetylamino-phenylcarbamoylmethylene)-5,7-dichloro-1,2,3,4-tetrahydro quinoline 2-carboxylic acid and salt thereof which are antagonist of excitatory amino acids, to processes for their preparation, to pharmaceutical compositions containing them, and to their use in medicine.

QUINOLINE-2-CARBOXYLIC ACID DERIVATIVE AND ITS USE AS EXCITATORY AMINO ACIDS ANTAGONIST

This invention relates to an enantiomer of (±) E 4-(4-Acetylamino-phenylcarbamoylmethylene)-5,7-dichloro-1,2,3,4-tetrahydro quinoline 2-carboxylic acid a potent and specific antagonist of excitatory amino acids, to processes for preparing the same, to pharmaceutical compositions containing it, to its use in medicine.

WO 97/12870 describes inter alia (±) E 4-(4-Acetylamino-phenylcarbamoylmethylene)-5,7-dichloro-1,2,3,4-tetrahydro quinoline - 2-carboxylic acid (A)



(A)

and salts thereof, which have an antagonist action at the strychnine insensitive glycine binding site located on the N-methyl-D- aspartate (NMDA) receptor complex.

We have now found that an enantiomer of compound (A) herein after referred to as the (+) enantiomer exhibits a particular useful profile of activity as a selective antagonist for the strychnine insensitive glycine binding site on the NMDA receptor complex.

The present invention thus provides the (+) enantiomer of (E) 4-(4-acetylamino-phenylcarbamoylmethylene)-5,7-dichloro-1,2,3,4-tetrahydro quinoline 2-carboxylic acid (hereinafter compound (I)) and salts thereof, substantially free of the corresponding (-) enantiomer.

The term substantially free as used herein means that compound (I) contains less than 10% of the (-) enantiomer and preferably less than 5%.

The term (+) enantiomer as used herein refers to the specific enantiomer which is the product of examples 2, 4 and 5.

For use in medicine the salts of compound (I) will be physiologically acceptable thereof. Other salts however may be useful in the preparation of compound I or physiologically acceptable salts thereof. Therefore, unless otherwise stated, references to salts include both physiologically acceptable salts and non-physiologically acceptable salts of compound (I).
Suitable physiologically acceptable salts of compounds of the invention include base addition salts.
Suitable physiologically acceptable base addition salts of compound (I) include alkali metal or alkaline earth metal salts such as sodium, potassium, calcium, and magnesium, and ammonium salts, formed with amino acids (e.g. lysine and arginine) and organic bases (e.g. procaine, phenylbenzylamine, ethanolamine diethanolamine and N-methyl glucosamine).

A preferred salt of compound (I) is the sodium salt.

The compound of the invention and/or physiologically acceptable salts thereof are excitatory amino acid antagonists. More particularly they are potent antagonists at the strychnine insensitive glycine binding site associated with the NMDA receptor complex. As such it is a potent antagonist of the NMDA receptor complex. Compound (I) is therefore useful in the treatment or prevention of neurotoxic damage or neurodegenerative diseases. Thus compound (I) is also useful for the treatment of neurotoxic injury which follows cerebral stroke, thromboembolic stroke, haemorrhagic stroke, cerebral ischemia, cerebral vasospam, hypoglycemia, amnesia, hypoxia, anoxia, perinatal asphyxia cardiac arrest. Compound I is also useful in the treatment of

chronic neurodegenerative diseases such as Huntingdon's disease, Alzheimer's senile dementia, amyotrophic lateral sclerosis, Glutaric Acidaemia type, multi-infarct dementia, status epilecticus, contusive injuries (e.g. spinal cord injury and head injury), viral infection induced neurodegeration (e.g. AIDS, encephalopaties), Down syndrome, epilepsy, schizophrenia, depression, anxiety, pain, neurogenic bladder, irritative bladder disturbances, migraine, headaches, including cluster headaches, tension headache, drug dependency, including withdrawal symptoms from alcohol, cocaine, opiates, nicotine, benzodiazepine and emesis.

The potent and selective action of the compound of the invention at the strychnine- insensitive glycine binding site present on the NMDA receptor complex may be readily determined using conventional test procedures. Thus the ability to bind at the strychnine insensitive glycine binding site was determined using the procedure of Kishimoto H et al. J Neurochem 1981, 37 1015-1024. The selectivity of the action of compound (I) for the strychnine insensitive glycine site was confirmed in studies at other ionotropic known excitatory amino acid receptors. Thus compound (I) was found to show little or no affinity for the kainic acid (kainate) receptor, α-amino-3-hydroxy-5-methyl-4-isoxazole-proprionic acid (AMPA) receptor or at the NMDA binding site.

The compound of the invention has also been found to inhibit NMDA induced convulsions in mice using the procedure Chiamulera C. et al. Psychopharmacology (1990) 102, 551-552.

The neuroprotective activity of the compound of the invention was demonstrated in the middle cerebral artery occlusion preparation in mice, using the procedure described by Chiamulera C. et al., European Journal of Pharmacology, 216 (1992) pp. 335-336.

The invention therefore provides for the use of compound (I) and/or physiologically acceptable salt thereof for use in therapy and in

particular use as medicine for antagonising the effects of excitatory amino acids upon the NMDA receptor complex.

The invention also provides for the use of compound (I) and/or a physiologically acceptable salt thereof for the manufacture of a medicament for antagonising the effects of excitatory amino acids upon the NMDA receptor complex.

According to a further aspect, the invention also provides for a method for antagonising the effects of excitatory amino acids upon the NMDA receptor complex, comprising administering to a patient in need thereof an antagonistic amount of compound (I) and/or a physiologically acceptable salt.

It will be appreciated by those skilled in the art that reference herein to treatment extends to prophylaxis as well as the treatment of established diseases or symptoms.

It will further be appreciated that the amount of the compound of the invention required for use in treatment will vary with the nature of the condition being treated, the route of administration and the age and the condition of the patient and will be ultimately at the discretion of the attendant physician. In general however doses employed for adult human treatment will typically be in the range of 2 to 800mg per day, dependent upon the route of administration.
Thus for parenteral administration a daily dose will typically be in the range 20-800mg, preferably 60-800mg per day. For oral administration a daily dose will typically be within the range 200-800mg, e.g. 400-600mg per day.

The desired dose may conveniently be presented in a single dose or as divided doses administered at appropriate intervals, for example as two, three, four or more sub-doses per day.

While it is possible that, for use in therapy, compound (I) may be administered as the raw chemical, it is preferable to present the active ingredient as a pharmaceutical formulation.

The invention thus further provides a pharmaceutical formulation comprising compound (I) or a physiologically acceptable salt thereof together with one or more pharmaceutically acceptable carriers thereof and, optionally, other therapeutic and/or prophylactic ingredients. The carrier(s) must be 'acceptable' in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

The compositions of the invention include those in a form especially formulated for oral, buccal, parenteral, inhalation or insufflation, implant, or rectal administration. Parenteral administration is preferred.

Tablets and capsules for oral administration may contain conventional excipients such as binding agents, for example, syrup, acacia, gelatine, sorbitol, tragacanth, mucilage of starch or polyvinylpyrrolidone; fillers, for example, lactose, sugar, microcrystalline cellulose, maize-starch, calcium phosphate or sorbitol; lubricants, for example, magnesium stearate, stearic acid, talc, polyethylene glycol or silica; disintegrants, for example, potato starch or sodium starch glycollate, or wetting agents such as sodium lauryl sulphate. The tablets may be coated according to methods well known in the art. Oral liquid preparations may be in the form of, for example, aqueous or oily suspensions, solutions emulsions, syrups or elixirs, or may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may contain conventional additives such as suspending agents, for example, sorbitol syrup, methyl cellulose, glucose/sugar syrup, gelatin, hydroxyethylcellulose, carboxymethyl cellulose, aluminium stearate gel or hydrogenated edible fats; emulsifying agents, for example, lecithin, sorbitan mono-oleate or acacia; non-aqueous vehicles (which may include edible oils), for example, almond oil, fractionated coconut oil, oily esters, propylene glycol or ethyl alcohol; solubilizers such as surfactants

for example polysorbates or other agents such as cyclodextrins; and preservatives, for example, methyl or propyl p- hydroxybenzoates or ascorbic acid. The compositions may also be formulated as suppositories, e.g. containing conventional suppository bases such as cocoa butter or other glycerides.

For buccal administration the composition may take the form of tablets or lozenges formulated in conventional manner.

The composition according to the invention may be formulated for parenteral administration by injection or continuous infusion. Formulations for injection may be presented in unit dose form in ampoules, or in multi-dose containers with an added preservative. The compositions may take such forms as suspensions, solutions, or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilising and/or dispersing agents. Alternatively the active ingredient may be in powder form for constitution with a suitable vehicle, e.g. sterile, pyrogen-free water, before use.

For administration by inhalation compound (I) according to the invention is conveniently delivered in the form of an aerosol spray presentation from pressurised packs, with the use of a suitable propellant, such as dichlorodifluoromethane, tirchlorofluoromethane, dichloro-tetrafluoroethane, carbon dioxide or other suitable propellants, such as dichlorodifluoromethane, trichlorofluoromethane, dichloro-tetrafluoroethane, carbon dioxide or other suitable gases, or from a nebuliser. In the case of a pressurised aerosol the dosage unit may be determined by providing a valve to deliver a metered amount.

Alternatively, for administration by inhalation or insufflation, the compounds according to the invention may take the form of a dry powder composition, for example a powder mix of the compound and a suitable carrier such as lactose or starch. The powder composition may be presented in unit dosage form in, for example, capsules or cartridges

of e.g. gelatin, or blister packs from which the powder may be administered with the aid of an inhaler or insufflator.

The composition according to the invention may also be formulated as a depot preparation. Such long acting formulations may be administered by implantation (for example subcutaneously or intramuscularly) or by intramuscular injection. Thus for example, the compounds of the invention may be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

The compositions according to the invention may contain between 0.1 - 99% of the active ingredient, conveniently from 30- 95% for tablets and capsules and 3-50% for liquid preparations.

In a further aspect the invention provides processes for the preparation of compound 1.

Thus in a first process (hereinafter Process A) compound (I) ((+)-E-4-(4-Acetylamino-phenylcarbamoylmethylene)-5,7- dichloro-1,2,3,4-tetra-hydroquinoline-2-carboxylic acid) may be prepared by esterification of compound A with a suitable chiral alcohol, separating the resultant diastereomeric esters by conventional means e.g. chromatography or crystallisation followed by hydrolysis of the required single diastereomeric ester.

Suitable chiral alcohols for use in the process A include (+)S-indanol, (+)S-methyl mandelate, chiral (C1-4)alkyl lactate: i.e., (+)R or (-)S methly lactate, (+)R t-butyl lactate, (+)R or (-)S ethyl lactate, (-)S isopropyl lactate, (-)S butyl lactate, (+)R isobutyl lactate or chiral aralkyl lactate (i.e. benzyl lactate), (-)S perillyl alcohol, (-)methyl(R)-3-hydroxy-2-methylpropionate, (-)(R)-2-butanol, (-)(S)-2-methyl-1-butanol.

The diastereomeric esters of compound A including the single diastereomeric ester substantially free of the other diastereomeric are novel compounds and represent a further aspect of the invention

The diastereomeric esters of compound A may be prepared by conventional means such as reaction of the chiral alcohol with an activated derivative of the compound A in an aprotic solvent such as ether e.g. tetrahydrofuran. The activated derivative of Compound A may be prepared from compound A using conventional means for preparing activated derivatives of a carboxylic acid groups such as those conveniently used in peptide synthesis.

A particularly convenient method of preparing the diastereomeric esters of compound A is to prepare the activated derivative of compound A in the presence of the chiral alcohol.

Thus for example compound A may be treated with the Mitsunobu combination of reagents, i.e. a dialkyl azo-dicarboxylate such as diethylazodicarboxylate and a triarylphosphine e.g. triphenylphosphine or trialkylphoshine (i.e. tributylphosphine) in the presence of the chiral alcohol.

The reaction conveniently takes place in the presence of a suitable solvent such as an ether (e.g. diethylether or tetrahydrofuran), a halohydrocarbon (e.g. dichloromethane) or a nitrile (e.g. acetonitrile) or a mixture thereof at a temperature ranging from 0-30°.

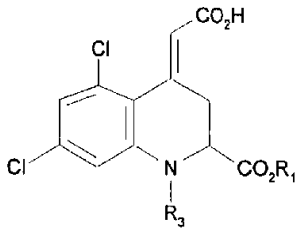
The required single diastereomeric ester of compound A substantially free of the other diastereomers may be obtained from the mixture thereof by conventional means, for example by the use of conventional chromatographic procedures such as preparative HPLC or by fractional crystallization.

Compound (I) may be prepared from the corresponding single diastereomeric ester of compound A by hydrolysis e.g. alkaline

hydrolysis. Thus for example the hydrolysis may be carried using an alkali metal hydroxide e.g. sodium hydroxide or lithium hydroxide in a solvent such as an ether e.g. tetrahydrofuran and water.
Compound (I) may be isolated as the free acid or as a salt thereof.

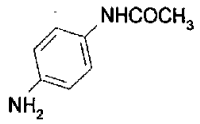
Compound I may also be obtained from racemic compound A by use of chiral HPLC procedures.

Compound (A) may be prepared by reaction of an activated derivative of the carboxylic acid (II) in which R_1 is a carboxylic acid protecting group and R_3 is hydrogen or a nitrogen protecting group



(II)

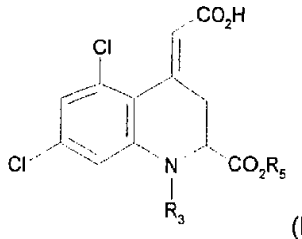
with the amine(III)



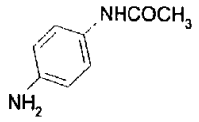
(III)

followed where necessary by subsequent removal of the carboxylic acid protecting group R_1 and any nitrogen protecting group R_3 using the methods and examples described in WO97/12870.

The invention also provides a further process for the preparation of the compound (I) (hereinafter process B) which comprises reacting an activated derivative of the carboxylic acid (IV)) in which R_3 is hydrogen or a nitrogen protecting group and R_5 is a suitable chiral group with the amine (III).



(IV)



(III)

and subjecting the resulting compound to the following reactions:
i) where necessary removal of nitrogen protecting group R_3
ii) separation of the resultant diastereomeric esters
iii) hydrolysis of the required single diastereomeric ester and isolation of the (+) enantiomer as a free acid or a salt thereof, and if desired the subsequent conversion of the free acid of the (+) enantiomer into a salt thereof.

Suitable chiral groups (R_5) for use in the process B are those derived from chiral alcohols such as (+)S-indanol, (+)S-methyl mandelate, chiral (C1-4)alkyl lactate: i.e., (+)R or (-)S methly lactate, (+)R t-butyl lactate, (+)R or (-)S ethyl lactate, (-)S isopropyl lactate, (-)S butyl lactate, (+)R isobutyl lactate or chiral aralkyl lactate (i.e. benzyl lactate), (-)S perillyl alcohol, (-)methyl(R)-3-hydroxy-2-methylpropionate, (-)(R)-2-butanol, (-)(S)-2-methyl-1-butanol.

R_5 is preferably a group derived from a chiral (C1-4)alkyl lactate alcohol.

More preferably R_5 is derived from (+)(R) t-butyl lactate alcohol.

Suitable activated derivatives of the carboxylic acid group include the corresponding acyl halide, mixed anhydride, activated ester such as a thioester or the derivative formed between the carboxylic acid group and a coupling agent, such as that used in peptide chemistry, for example carbonyl diimidazole or a diimide, such as dicyclohexylcarbodiimide.

The reaction is preferably carried out in an aprotic solvent, such as a hydrocarbon, a halohydrocarbon such as dichloromethane or an ether such as tetrahydrofuran.

When R_3 is a nitrogen protecting group, examples of suitable groups include alkoxycarbonyl, e.g. t-butoxycarbonyl, arylsulphonyl e.g. phenysulphonyl or 2-trimethylsilylethoxymethyl.

Alkyl when used as substituent or a part of a substituent group means that the group may be straight or branched. Thus C1-4 alkyl includes methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl or ter-butyl.

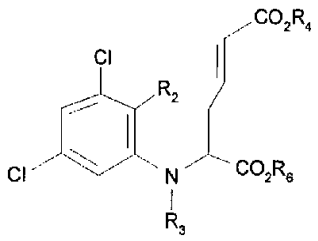
The activated derivatives of the carboxylic acid (IV) may be prepared by conventional means. A particularly suitable activated derivative for use in this reaction is thioester such as that derived from pyridine-2-thiol. These esters may conveniently be prepared by treating the carboxylic acid (II) with 2,2'-dithiopyridine and triphenylphosphine in a suitable aprotic solvent such as an ether e.g. tetrahydrofuran, a halohydrocarbon e.g. dichloromethane, an amide e.g. N,N-dimethylformamide or acetonitrile.

The appropriate diastereomeric derivative can be isolated by conventional means e.g. chromatography or by crystallisation.

The hydrolysis step conveniently takes place using an alkali metal hydroxide e.g. sodium hydroxide or lithium hydroxide in a suitable solvent such as an ether i.e. tetrahydrofuran, water and a mixture

thereof or alkali trialkylmethylsilanolate (e.g. trimethylsilanolate) followed, where desired or necessary, by the addition of a suitable acid e.g. hydrochloric acid to give the corresponding free carboxylic acid.

Compounds of formula (IV) or (II) may be prepared by the cyclisation of a compound of formula (V)



(V)

in which R_2 represents a bromine or iodine atom, R_3 represents hydrogen or a nitrogen protecting group, R_4 represents a hydrogen atom or a suitable carboxylic acid protecting group such as t-butyl group and R_6 represents R_1 or R_5 as defined in formula (II) or formula (IV) respectively, followed by removal of the of carboxylic protecting group R_4 using conventional methods.

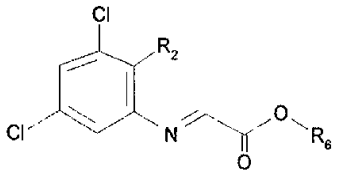
In one embodiment of this process the reaction may be carried out using a catalytic amount of a Palladium (O) complex such as tetrakis(triphenylphosphine)palladium and a suitable organic base such as trialkylamine e.g. triethylamine or inorganic base, e.g. potassium carbonate.

The reaction is conveniently carried out in an aprotic solvent such as acetonitrile or dimethylformamide at a temperature with the range of 20°C to 150°C followed, where necessary or desired, by subsequent removal of the carboxylic acid protecting group R_4 and any protecting group R_3.

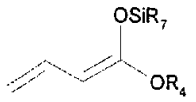
In a further embodiment of the process the reaction is carried out using a catalytic amount of a Pd(II) salt such as palladium acetate, in the presence of a suitable organic base such as trialkyl amine e.g. triethylamine and of a triarylphosphine such as triphenylphosphine.

The reaction is carried out in an aprotic solvent such as acetonitrile or dimethylformamide and preferably with heating, followed, where necessary or desired, by subsequent removal of the carboxylic acid protecting group R_4 and any nitrogen protecting group R_3.

Compounds of formula (V) may be prepared by reacting a imino ester of formula(VI), wherein R_2 and R_6 have the meanings defined above, with a compound of formula (VII) in which R_7 represents a C1-4 alkyl group and R_4 is a hydrocarbysilyl group such as trialkylsilyl, e.g. trimethylsilyl or terbutyldimethylsilyl or a suitable carboxylic acid protecting group such as tert-butyl group, followed, if desired, by the conversion of the group NH into a nitrogen protecting group NR_3.



(VI)



(VII)

The reaction is carried out in an aprotic solvent such as halohydrocarbon e.g. dichloromethane, chlorobenzene or acetonitrile at low temperature e.g. -78°C in the presence of a Lewis acid such as zinc bromide and zinc chloride.

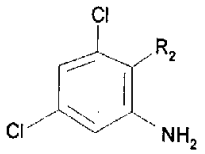
The conversion of the group NH into the nitrogen protected group NR_3 may be obtained using conventional means for introducing such nitrogen protecting groups, e.g. reaction with the group R_3X wherein X is a leaving group e.g. halogen or methanesulphonate.

The process for preparing compounds of formulae (II) or (IV), using the intermediate (V) when prepared from intermediates (VI) and (VII), is novel and forms a further feature of the invention.

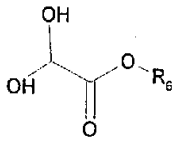
A particularly preferred embodiment of this novel process for preparing compounds of formula (V) is to use an intermediate ester (VI), wherein R_6 group is derived from (+)R t-butyl lactate alcohol. This yields a mixture of diastereomeric *esters* in which the required diastereomeric ester is obtained in diastereomeric excess.

Further, the cyclisation of the compound (V) so obtained using the process conditions described above affords the required compound (IV) as a mixture of diastereomeric esters in which the required diastereomeric ester is also obtained in diastereomeric excess.

Compounds of formula (VI) may be prepared by reaction of amine (IX) with a compound of formula (VIII) wherein R_2, R_6 have the meanings defined for compounds of formula (V).



(IX)



(VIII)

The reaction is preferably carried out in a solvent such as an aromatic hydrocarbon (e.g. benzene toluene or xylene) at a temperature ranging from ambient to the reflux temperature of the reaction mixture.

Compounds of formula (III), (VII), (VIII), and (IX) are either known compounds or may be prepared by analogous methods to those used for known compounds.

Thus compounds of formula (VII) may be prepared according to procedures described in Tetrahedron Letters, Vol. 22, No. 29, pp. 2833 -

2836, 1981. Compounds of formula (VIII) may be prepared according to procedures described in Helvetica Chimica Acta, 1981 Vol. 64, p. 2808 .

In any of the above reactions the carboxylic acids protecting group may be removed by conventional procedures known for removing such groups. Thus compounds where R_1 is a benzyl group, this may be removed by hydrolysis using an alkali metal hydroxide e.g. lithium hydroxide or sodium hydroxide in a suitable solvent such as ethanol or isopropanol, water or mixtures thereof, followed, where desired or necessary, by that addition of a suitable acid e.g. hydrochloric acid to give the corresponding free carboxylic acid.
When R_4 is a t butyl group this may be removed by hydrolysis using organic acids e.g. formic acid.

In any of the above reactions the nitrogen protecting group may be removed by conventional procedures known for removing such groups, for example by acid or base hydrolysis. Thus when R_3 is alkoxycarbonyl e.g. t-butoxycarbonyl or phenylsulphonyl it may be removed by alkaline hydrolysis using for example lithium hydroxide in a suitable solvent such as tetrahydrofuran or an alkanol e.g. isopropanol. Alternatively the alkoxycarbonyl group may be removed by acid hydrolysis.

Physiologically acceptable salts of compound I may be prepared by treating the corresponding acid with an appropriate base in a suitable solvent. For example alkali and alkaline metal salts may be prepared from an alkali or alkaline metal hydroxide, or the corresponding carbonate, bicarbonate or trialkylsilanolate e.g. trimethylsilanolate thereof.

Alternatively alkali or alkaline earth salts may be prepared by direct hydrolysis of carboxylic acid protected derivatives of compound I with the appropriate alkali or alkaline metal hydroxide.

In order that the invention may be more fully understood the following examples are given by way of illustration only.

In the Intermediates and Examples unless otherwise stated:

Melting points (m.p.) were determined on a Gallenkamp m.p. apparatus and are uncorrected. All temperatures refers to ^0C. Infrared spectra were measured on a FT-IR instrument. Proton Magnetic Resonance (^1H-NMR) spectra were recorded at 400 MHz, chemical shifts are reported in ppm downfield (d) from Me_4Si, used as internal standard , and are assigned as singlets (s), doublets (d), doublets of doublets (dd), triplets (t), quartets (q) or multiplets (m). Column chromathography was carrier out over silica gel (Merck AG Darmstaadt, Germany). The following abbreviations are used in text: EA = ethyl acetate, CH = cyclohexane, DCM = dichloromethane, THF = tetrahydrofuran, TFA = trifluoroacetic acid, TEA = triethylamine, DMSO = dimethylsulphoxide, Tlc refers to thin layer chromatography on silica plates. Solution were dried over anhydrous sodium sulphate; r.t. (RT) refers to room temperature.

Intermediate 1

-tert-butyl-(R)-acryloyloxy-2-methylacetate

To a solution of (R)-*tert*-butyl lactate alcohol (4.5 g), triethylamine (9.5 ml) and dimethylaminopyridine (0.73 g) in dry dichloromethane (200ml) was added, at 0°C, a solution of acryloyl chloride (5.5 ml) in dichloromethane (100 ml), and the resulting mixture was stirred for 1hr at 0°C and for an additional hour at room temperature. Then a 1M solution of HCl was added followed by ethyl acetate (600 ml). The organic phase was washed with water and brine. Final purification by column chromatography (cyclohexane/ethyl acetate 85/15) afforded the title compound (4.6g) as a colourless oil.

^{1} H NMR (DMSO) (ppm) 6.36 (dd, 1H), 6.22 (dd, 1H), 5.99 (dd, 1H), 4.89 (q, 1H), 1.40 (d, 3H), 1.39 (s, 9H)
IR (CDCl3) (cm^{-1}) 1727

Intermediate 2

1-tert-butyl-(R)-2(oxoacetoxy)-2-methyl acetate

A solution of intermediate 1 (4.6 g) in THF/H_2O (3/1) (28 ml) was reacted overnight at room temperature with osmium tetroxide (4% wt in water, 5 ml) and sodium periodate (12.3 g). The mixture was taken up with ether (500 ml), the water phase was separated, and the organic phase was dried and concentrated. Final purification by column chromatography (cyclohexane/ethyl acetate 60/40) afforded the title compound (4.1g) as a colourless oil.

^{1}H NMR (DMSO): 6.75 (d, 1H); 6.70 (d, 1H); 5.05 (t, 1H); NMR - 4.85m (1H);1.40m(12H).

Intermediate 3

(3,5-dichloro-iodophenylimino)acetic acid, 1-(R)-(1-tert-butoxycarbonyl)ethyl ester

A solution of intermediate 2 (0.784 g) in toluene (20 ml) was refluxed in Dean-Stark apparatus for 1hr. Then, 3,5-chloro-2-iodoaniline (0.75 g) and $MgSO_4$ (5 g) was added, and the mixture refluxed for 1hr. Then mixture was cooled, filtered through celite to eliminate the $MgSO_4$, concentrated to give the title compound (1.2 g) as a pale yellow oil.

^{1}H NMR (DMSO) (ppm) 7.95 (s, 1H); 7.67 (d, 1H); 7.30 (d, 1H); 5.09 (q, 1H); 1.49 (d, 3H); 1.43 (s, 9H)

IR (Film) (cm^{-1}) 1743

Intermediate 4

(E)-5-(3,5-dichloro-iodophenylimino)hex-2-enedioic acid, 6-[1-(R)-(1-tert-butoxycarbonyl)]-ethyl ester

To a suspension of $ZnCl_2$ (0.36g) in dry dichloromethane (10ml) cooled to -78°C, a solution of intermediate 3 (1.2g) in dry dichloromethane (20ml) was added. Then 1,1 trimethylsilyloxy 1,3 butadiene (1.14g) was added and the resulting mixture was stirred for 2hrs at -30°C. Then a saturated solution of NH_4Cl (20ml) was added followed by ethyl acetate (30ml). The organic phase was washed with brine (20ml) and dried. Purification by column chromatography (cyclohexane/ethyl acetate 50/50) afforded the title compound (1.13g) as a colourless oil (diastereomeric excess 50%).

^{1} H NMR (DMSO) d (ppm) 12.3 (bs, 1H) 7.01 (d, 1H); 6.79 (m, 1H); 6.66 (d, 1H); 5.90 (d, 1H); 5.29 (d, 1H); 4.97 (q, 1H); 4.72 (m, 1H); 2.83 (m, 2H); 1.39 (m, 12H).

Intermediate 5

(E)-4-carboxymethylene-5,7-dichloro-1,2,3,4-tetrahydroquinoline-2-carboxylic acid, [1-(R)-(1-tert-butoxycarbonyl)]ethyl ester

To a solution of intermediate 4 (1.2g) in dry DMF (10ml) was added TEA (0.7ml) and $Pd(PPh_3)_4$ (0.248g). The reaction mixture was heated to 100°C for 1 hour, then ethyl acetate (20ml) was added followed by a 1M solution of HCl (10ml). The organic phase was washed with brine (20ml), dried and concentrated. Final purification by column chromatography (cyclohexane/ethyl acetate 40/60) afforded the title compound (0.5g) as a colourless oil (diastereomeric excess 50%).

^{1} H NMR (DMSO) 12.3 (s, 1H); 7.27 (bs, 1H); 6.73 (d, 1H); 6.45 (d, 1H); 6.40 (s, 1H); 4.79 (q, 1H); 4.29 (m, 1H); 3.61 (m, 1H); 3.13 (m, 1H); 1.35 (m, 10H).

Intermediate 6

(E)-4-[(2-pyridyl)thiocarbonylmethylene]-5,7-dichloro-1,2,3,4-tetrahydroquinoline-2-carboxylic acid, [1-(R)-(1-tert-butoxycarbonyl)]ethyl ester

To a solution of intermediate 5 (0.5g) in dry THF (30ml) was added PySSPy (0.66g) and PPh_3 (0.81g). The reaction mixture was stirred for 1h at room temperature, then the solvent was evaporated and the crude purified by column chromatography (cyclohexane/ethyl acetate 80/20) to give the title product (0.170g) as a yellow foam (diastereomeric excess 60%).

^{1} H NMR (DMSO) p.p.m 8.61 (m, 1H); 7.91 (m, 1H); 7.71 (m, 1H); 7.46 (bs, 1H); 7.45 (m, 1H); 6.88 (s, 1H); 6.78 (d, 1H); 6.76 (d, 1H); 4.81 (q, 1H); 4.36 (m, 1H); 3.73 (dd, 1H); 3.05 (m, 1H); 1.4 (m, 3H); 1.34 (s, 9H).

Example 1

(+/-)(E)-4-acetylamino-phenylcarbamoylmethylene)-5,7-dichloro-1,2,3,4-tetrahydro quinoline-2-carboxylic acid

(diastereomers 1a and 1b)

A solution of diethylazodicarboxylate (0.136ml;) in dry tetrahydrofuran (10ml) was added, dropwise over 5 minutes, to a suspension of (±)(E)-4-(4-acetylamino-phenylcarbamoylmethylene)-5,7-dichloro-1,2,3,4-tetrahydroquinoline-2-carboxylic acid (270mg), triphenylphosphine (228mg) and tert-butyl (R)-(+)-lactate (127mg) in dry tetrahydrofuran (20ml) under a nitrogen atmosphere.

The yellow solution was stirred at 23°C for 30 minutes, then concentrated *in vacuo* and purified by flash chromatography eluting with cyclohexane/ethyl acetate 4:6 to give 240mg of the title compound (a mixture of the two diastereoisomers 1a and 1b). The diastereomers were separated by preparative HPLC (column:Supelcosil LC-CN; phase hexane-tetrahydrofuran 65:35 and after 14 minutes 60:40; flux 10ml/min; λ=260nm) and then further purified on silica, eluting with cyclohexane/ethyl acetate, first 8:2 then 1:1, to give diastereoisomer 1a :91mg as whitish solid, and diastereoisomer 1b 76mg as yellow solid

Example 1: m.p.175-177°C. IR (nujol): 3200 (NH), 1738 (C=O) cm^{-1}. ^{1}H-NMR (DMSO): 10.09-10.05 (2s, 1H); 9.86-9.84 (2s, 1H); 7.64-7.44 (m, 4H); 7.34-7.19 (2d, 1H); 6.73-6.66 (d, 2H); 6.71-6.64 (2s, 1H); 4.76-4.56 (2q, 1H); 4.39-4.26 and 3.73 (m and dd, respectively, 2H); 3.30-2.68 (dd, 1H); 2.00 (s, 3H); 1.34-1.31 (2s, 9H); 1.29-1.15 (2d, 3H). MS: *m/z*=562 $[M+H]^+$

Diastereomer 1a m.p. 206-8°C. T.l.c.ethyl acetate-cyclohexane 7:3, Rf= 047. IR (nujol): 3314 (NH), 1730, 1666, 1656 (C=O) cm^{-1}. ^{1}H-NMR (DMSO): 10.09 (s, 1H); 9.86 (s, 1H); 7.57 (d, 2H); 7.49 (d, 2H); 7.33 (d, 1H); 6.71 (d, 2H); 6.67 (d, 1H); 6.65 (m, 1H); 4.57 (q, 1H); 4.37 (m, 1H); 4.29 (m, 1H); 2.69 (m, 1H); 2.00 (s, 3H); 1.35 (s, 9H); 1.16 (d, 3H). MS: *m/z*=561$[M]^+$, 562 $[M+H]^+$. HPLC: retention time 12.56min (column:Supelcosil LC-CN; phase hexane-tetrahydrofuran 70:30; flux 0.8ml/min; λ =260nm)

Diastereomer 1b: m.p. 105-7°C. T.l.c.ethyl acetate-cyclohexane 7:3, Rf= 040. IR (nujol): 3310 (NH), 1738 and 1659 (C=O) cm^{-1}. ^{1}H-NMR (DMSO): 10.05 (s, 1H); 9.85 (s, 1H); 7.55 (d, 2H); 7.47 (d, 2H); 7.19 (d,

1H); 6.74 (d, 1H); 6.73 (d, 1H); 6.72 (s, 1H); 4.77 (q, 1H); 4.29 (m, 1H); 3.74 (dd, 1H); 3.29 (m, 1H); 2.00 (s, 3H); 1.32 (s, 9H); 1.29 (d, 3H). MS: *m/z*=561$[M]^+$, 562 $[M+H]^+$. HPLC: retention time 15.60min (column:Supelcosil LC-CN; phase hexane-tetrahydrofuran 70:30; flux 0.8ml/min; λ=260nm)

Example 2

(+)-E-4-(4-Acetylamino-phenylcarbamoylmethylene)-5,7- dichloro-1,2,3,4-tetrahydroquinoline-2-carboxylic acid

Lithium hydroxide monohydrate (12.84mg) was added to a solution of diastereoisomer 1a (from Example 1 86mg) in tetrahydrofuran (5ml) and water (2.5ml). The solution was stirred at 23°C for 30 minutes, then concentrated *in vacuo*. The residue was diluted with further water (10ml) and extracted with ethyl acetate (2X15ml). The aqueous layer was acidified with 5% hydrochloric acid until pH=1 and extracted with ethyl acetate (3X15ml). The combined organic extracts were dried and concentrated in vacuo to give the title compound as a whitish solid (56mg). m.p. 224-6°. IR (nujol): 3356-3302 (NH), 3350-2600 (OH); 1724 and 1663-1650 (C=O) cm^{-1}. ^{1}H-NMR (DMSO): 12.71 (s, 1H); 10.10 (s, 1H); 9.87 (s, 1H); 7.56 (d, 2H); 7.50 (d, 2H); 7.10 (d, 1H); 6.70 (d, 2H); 6.68 (m, 1H); 4.11 (m, 1H); 3.87 (m, 1H); 3.08 (dd, 1H); 2.01 (s, 3H). MS: *m/z*=434 $[M+H]^+$.

HPLC: retention time 15.2 min (column:cyclobond I 2000 SN ; phase β-cyclodextrin S Naphthyl ethyl carbamate, phase mobile: methanol buffer ammonium acetate (pH=3); flux 1ml/min. ; λ=260 nm)

$[\alpha]_D=[+16]_{20}$. λ= 598nm, solvent :dimethyl sulphoxide.

Conc=0.26% w/v

Example 3

(E)-4-(4-acetylamino-phenylcarbamoylmethylene)-5,7-dichloro-1,2,3,4-tetrahydroquinoline-2-carboxylicacid,[1-(R)-(1-tert-butoxycarbonyl)]ethyl ester

To a solution of intermediate 6 (0.155g) in dry toluene (20ml) 4-aminoacetanalide (0.05g) was added. The resulting mixture was heated at 100°C for 3hrs, then the solvent was evaporated and the crude

purified by column chromatography (cyclohexane/ethyl acetate 50/50) to give the title compound (0.05 g) as a yellow solid.

^{1}H-NMR (DMSO) d 10.05 (bs, 1H); 9.85 (bs, 1H); 7.55 (d, 2H); 7.47 (d, 2H); 7.19 (dd, 1H); 6.74 (d, 1H); 6.73 (d, 1H); 6.72 (s, 1H); 4.77 (q, 1H); 4.29 (m, 1H); 3.74 (dd, 1H); 3.29 (m, 1H); 2.00 (s, 3H); 1.32 (s, 9H); 1.29 (d, 3H).

IR (nujol) (cm^{-1}) 3310, 1738, 1659.

Example 4

(+)(E)-4-(4-acetylamino-phenylcarbamoylmethylene)-5,7-dichloro-1,2,3,4-tetrahydroquinoline-2-carboxylic acid.

To a solution of example 3 (0.021g) in THF/H_2O (3/1) LiOH (0.005g) was added. After 15mins. the THF was evaporated and water (2ml) was added. The solution was washed with ethylacetate (2x5ml), then a 1M solution of HCl was added and the resulting solution was extracted with ethyl acetate (2x5ml) and evaporated to give the title compound as a pale yellow solid (0.01g).

^{1}H-NMR (DMSO) d 12.71 (s, 1H); 10.10 (s, 1H); (9.87 (s, 1H); 7.56 (d, 2H); 7.50 (d, 2H); 7.10 (d, 1H); 6.70 (d, 2H); 6.68 (m, 1H); 4.11 (m, 1H); 3.87 (m, 1H); 3.08 (dd, 1H); 2.01 (s, 3H).

IR (nujol) (cm-1) 3356-3302, 1724, 1663-1650.

HPLC: retention time 9.7 min (column: cyclobond SN; phase β-cyclodextrin S Naphthyl carbamate, phase mobile: methanol buffer ammonium acetate (pH=5); flux 1ml/min. ; λ=260 nm)

Example 5

(+) 4-(4-Acetylamino-phenylcarbamoylmethylene)-5,7-dichloro-1,2,3,4-tetrahydroquinoline-2-carboxylic acid, sodium salt

Sodium trimethylsilanolate (7.74 mg) was added to a suspension of (+) 4-(4-acetylamino-phenylcarbamoylmethylene)-5,7-dichloro-1,2,3,4-tetrahydroquinoline-2-carboxylic acid (30 mg) in dry tetrahydrofuran (3 ml) under a nitrogen atmosphere. The yellow suspension was stirred at 23°C for 1 hr, then it was concentrated *in vacuo* and the residue was triturated with diethyl ether (5 ml). After filtration the title compound was obtained (27 mg) as a yellow solid.

M.p. 202-5 °C (dec).
IR (nujol): 3400-3000 (NH), 1650 (C=O) cm^{-1}. ^{1}H-NMR (DMSO): 11.71 (bs, 1H); 9.26 (bs, 1H); 7.65 (d, 2H); 7.49 (d, 2H); 6.74 (d, 1H); 6.71 (bs, 1H); 6.52 (s, 1H); 6.50 (d, 1H); 3.51 (m, 1H); 3.29 (m, 1H); 2.64 (m, 1H); 2.01 (s, 3H). MS: *m/z*=456 $[M+H]^+$, 478 $[M+H]^+$. HPLC: retention time 14.28 min.(column: cyclobond I 2000SN; phase b-cyclodextrin S Naphtyl ethyl carbamate, phase mobile: methanol-buffer ammonium acetate, flux 1ml/min; λ=260nm).

Pharmacy Example

Intravenous Infusion	**% w/v**
Compound (I)	0.3 - 0.5
Polysorbate 80	1
tris(hydroxymethyl)aminomethane	0.54
Dextrose solution 5% w/v	qs to volume

Compound (I) and Polysorbate were added to a solution of tris(hydroxymethyl)aminomethane in a 5% aqueous dextrose solution suitable for injection. The solution was filtered through a sterile 0.2 micron sterilising filter and filled in containers before being sterilised by autoclaving.

The affinity of the compound of the invention for strychnine insensitive glycine binding site located on the NMDA receptor complex was determined using the procedure of Kishimoto H. et al J. Neurochem 1981, 37, 1015-1024. The pKi value obtained is 8.8.

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

The reference to any prior art in this specification is not, and should not be taken as, an acknowledgment or any form of suggestion that that prior art forms part of the common general knowledge in Australia.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

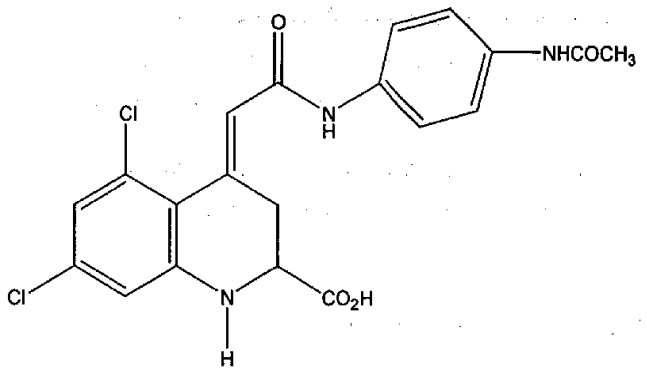
1. (+) (E)4-(4-Acetylamino-phenylcarbamoylmethylene) –5,7-dichloro-1,2,3,4-tetrahydro quinoline 2-carboxylic acid, substantially free of the (-) enantiomer, and salts thereof.

2. (+) (E)4-(4-Acetylamino-phenylcarbamoylmethylene) –5,7-dichloro-1,2,3,4-tetrahydro quinoline 2-carboxylic acid, substantially free of the (-) enantiomer, and physiologically acceptable salts thereof.

3. The sodium salt of (+) (E)4-(4-Acetylamino-phenylcarbamoylmethylene) -5,7-dichloro-1,2,3,4-tetrahydro quinoline 2-carboxylic acid, substantially free of the (-) enantiomer.

4. A process for preparation of a compound as claimed in any one of claims 1 to 3 which comprises:

(a) esterification of compound A with a suitable chiral alcohol,



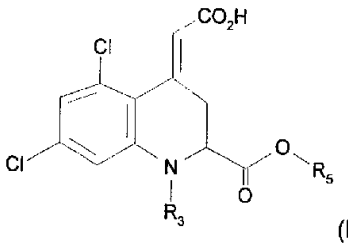
(A)

followed by

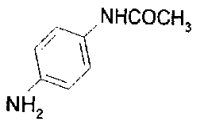
(i) separation of the resultant diastereomeric esters,

(ii) hydrolysis of the required single diastereomeric ester, and isolation of the (+) enantiomer as the free acid or a salt thereof; and if desired the subsequent conversion of the free acid of the (+) enantiomer into a salt thereof;

b) reacting a compound of formula(IV), wherein R_3 is hydrogen or a nitrogen protecting group and R_5 is a suitable chiral group, with the amine(III),



(IV)



(III)

in the presence of a Lewis acid and subjecting the resulting compounds to the following reactions:

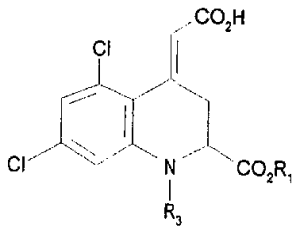
i) where necessary removal of nitrogen protecting group R_3,

ii) separation of the resultant diastereomeric esters,

iii) hydrolysis of the required single diastereomeric ester and isolation of the (+) enantiomer as the free acid or a salt thereof; and if desired the subsequent conversion of the free acid into a salt thereof.

5. A process as claimed in claim 4 wherein the chiral alcohol is (+)R t-butyl lactate alcohol, or R5 is a group derived from (+)R t-butyl lactate alcohol.

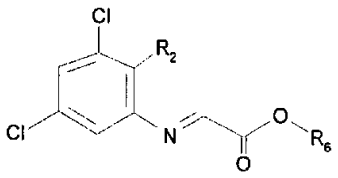
6. A process for the preparation of intermediates of formula (IV), as defined in claim 4 or 5, or intermediates of formula (II)



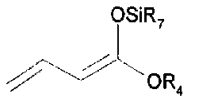
(II)

which comprises the following steps:

i) reacting an imino ester of formula(VI), wherein R_2 represents a bromine or iodine atom and R_6 is a suitable chiral group R_5 as defined above or a carboxylic acid protecting group R_1 as described in formula (II), with a compound of formula (VII) in which R_7 represents a C_{1-4} alkyl group and R_4 is a hydrocarbysilyl group or a suitable carboxylic acid protecting group, followed, if desired, by the conversion of the resultant compound of formula (V) wherein R_3 is hydrogen into a compound of formula (V) wherein R_3 is a nitrogen protecting group and

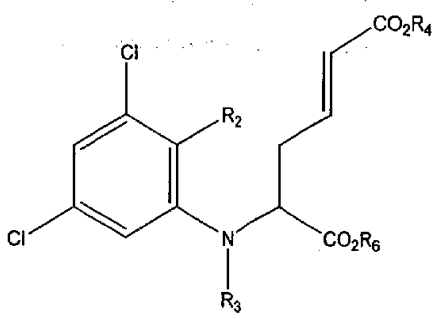


(VI)



(VII)

ii) cyclisation of the resulting compound (V)



(V)

in which R_2 is a bromine or iodine atom, R_3 represents hydrogen or a nitrogen protecting group R_4 is a hydrogen atom or a suitable carboxylic acid protecting group and R_6 represents a suitable chiral group R_5 or a carboxylic acid protecting group R_1 and thereafter, if necessary, removing the carboxylic protecting group R_4 and any nitrogen protecting group R_3.

7. A process as claimed in claims 4 or 5 wherein the intermediate (IV) is prepared according to the process of claim 6.

8. A pharmaceutical composition comprising a compound as claimed in claim 2 or claim 3 in admixture with one or more physiologically acceptable carriers or excipients.

9. The use of a compound as claimed in claim 2 or claim 3 in the manufacture of a medicament for antagonising the effect of excitatory amino acids upon the NMDA receptor complex.

10. A method of treatment of a mammal including man for conditions where antagonising the effects of excitatory amino acids on the NMDA receptor complex is of therapeutic benefit comprising administration of an effective amount of a compound as claimed in claim 2 or claim 3.

11. (+) (E)4-(4-Acetylamino-phenylcarbamoylmethylene) –5,7-dichloro-1,2,3,4-tetrahydro quinoline 2-carboxylic acid, substantially free of the (-) enantiomer, according to any one of claims 1, 2 or 3, substantially as hereinbefore described with reference to the Examples.

DATED this 4th day of January, 2001
Glaxo Wellcome SpA

By DAVIES COLLISON CAVE

Patent Attorneys for the Applicants