COMMONWEALTH of AUSTRALIA
Patents Act 1952

APPLICATION FOR A STANDARD PATENT

I/We

Fujisawa Pharmaceutical Co., Ltd.

62 1067

of

3, Doshomachi 4-chome, Higashi-ku, Osaka, 541, Japan

hereby apply for the grant of a Standard Patent for an invention entitled:

A cardioprotective agent and a therapeutic agent for ischemic disease comprising N-containing heterocyclic compounds, and a process for the preparation of the same compounds

which is described in the accompanying complete specification.

Details of basic application(s):-

Number	Convention Country	Date
251771/1987	Japan	5 October 1987
184 195	United States of America	21 April 1988

The address for service is care of DAVIES & COLLISON, Patent Attorneys, of 1 Little Collins Street, Melbourne, in the State of Victoria, Commonwealth of Australia.

DATED this THIRD day of OCTOBER 1988

To: THE COMMISSIONER OF PATENTS

.....
a member of the firm of DAVIES & COLLISON for and on behalf of the applicant(s)

M003289 03/10/88

Davies & Collison, Melbourne

COMMONWEALTH OF AUSTRALIA

PATENTS ACT 1952

DECLARATION IN SUPPORT OF CONVENTION OR NON-CONVENTION APPLICATION FOR A PATENT

Insert title of invention.

In support of the Application made for a patent for an invention entitled: "A CARDIOPROTECTIVE AGENT AND A THERAPEUTIC AGENT FOR ISCHEMIC DISEASE COMPRISING N-CONTAINING HETEROCYCLIC COMPOUNDS,AND A PROCESS FOR THE PREPARATION OF THE SAME COMPOUNDS

Insert full name(s) and address(es) of declarant(s) being the applicant(s) or person(s) authorized to sign on behalf of an applicant company.

I, Shizuo Maeno
of Fujisawa Pharmaceutical Co.,Ltd.
of 3, Doshomachi 4-chome, Higashi-ku,Osaka-shi,
Osaka 541, Japan

do solemnly and sincerely declare as follows :-

Cross out whichever of paragraphs 1(a) or 1(b) does not apply

1(a) relates to application made by individual(s)
1(b) relates to application made by company; insert name of applicant company.

1. (a) ~~I am the applicant for the patent~~

or (b) I am authorized by

FUJISAWA PHARMACEUTICAL CO., LTD.

the applicant..... for the patent to make this declaration on its behalf.

Cross out whichever of paragraphs 2(a) or 2(b) does not apply

2(a) relates to application made by inventor(s)
2(b) relates to application made by company(s) or person(s) who are not inventor(s); insert full name(s) and address(es) of inventors.

2. (a) ~~I am the actual inventor of the invention~~

or (b)

1. Takao TAKAYA
2. Hisashi TAKASUGI
3. Kimio ESUMI
4. Atsushi KUNO
5. Hiroyoshi SAKAI
6. Kazuhiro MAEDA
7. Yoshie SAKAMOTO

See over page for addresses

are the actual inventors..... of the invention and the facts upon which the applicant..... is entitled to make the application are as follows :-

State manner in which applicant(s) derive title from inventor(s)

The actual inventors assigned the invention to the said applicant.

Cross out paragraphs 3 and 4 for non-convention applications. For convention applications, insert basic country(s) followed by date(s) and basic applicant(s).

3. The basic applications..... as defined by Section 141 of the Act were made
in Japan on the 5th October, 1987
by Fujisawa Pharmaceutical Co., Ltd.
in United States of America on the 21st April,1988
by Takao Takaya; Hisashi Takasugi; Kimio Esumi; Atsushi Kuno;
Hiroyoshi Sakai; Kazuhiro Maeda and Yoshie Sakamoto
by.....

4. The basic applications..... referred to in paragraph 3 of this Declaration were the first applications..... made in a Convention country in respect of the invention the subject of the application.

Insert place and date of signature.

Declared at Osaka this 8th day of September,1988

Signature of declarant(s) (no attestation required)

Shizuo Maeno

Shizuo Maeno, Director
Patent & Trademark

Note: Initial all alterations.

DAVIES & COLLISON, MELBOURNE and CANBERRA.

Inventors Addresses

1. 5-87, Suimeidai 1-chome, Kawanishi-shi, Hyogo 666-01

2 14-33, Hamaguchinishi 1-chome, Suminoe-ku, Osaka-shi, Osaka 559

3. 5-11-305, Sumiyoshiyamate 1-chome, Higashinada-ku, Kobe-shi, Hyogo 658

4. 24-6, Shinkofudai 5-chome, Toyono-cho, Toyono-gun, Osaka 563-01

5. 28-134, Kowada-Hirao, Uji-shi, Kyoto 611

6. 481-4, Higashinaka, Yamatotakada-shi, Nara 635

7. 453-403, Hatagasaki, Yonago-shi, Tottori 683

All of Japan, respectively

(12) PATENT ABRIDGMENT (11) Document No. AU-B-23338/88
(19) AUSTRALIAN PATENT OFFICE (10) Acceptance No. 621067

(54) Title
A CARDIOPROTECTIVE AGENT AND A THERAPEUTIC AGENT FOR ISCHEMIC DISEASE COMPRISING N-CONTAINING HETEROCYCLIC COMPOUNDS, AND A PROCESS FOR THE PREPARATION OF THE SAME COMPOUNDS

International Patent Classification(s)
(51)[5] A61K 031/535 A61K 031/54 C07D 213/82 A61K 031/495
(51)[4] A61K 031/44 A61K 031/50

(21) Application No. : 23338/88 (22) Application Date : 03.10.88

(30) Priority Data

(31) Number	(32) Date	(33) Country
62-251771	05.10.87	JP JAPAN
184195	21.04.88	US UNITED STATES OF AMERICA

(43) Publication Date : 01.06.89

(44) Publication Date of Accepted Application : 05.03.92

(71) Applicant(s)
FUJISAWA PHARMACEUTICAL CO. LTD

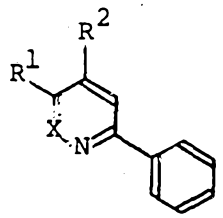
(72) Inventor(s)
TAKAO TAKAYA; HISASHI TAKASUGI; KIMIO ESUMI; ATSUSHI KUNO; HIROYOSHI SAKAI; KAZUHIRO MAEDA; YOSHIE SAKAMOTO

(74) Attorney or Agent
DAVIES COLLISON CAVE , 1 Little Collins Street, MELBOURNE VIC 3000

(56) Prior Art Documents
EP 228845

(57) Claim

1. A method for the treatment of ischemic disease, depressed cardiac metabolism, reperfusion injury or heart diseases which comprises administering to human beings or animals in need thereof a therapeutically effective amount of a compound of the formula:



[I]

wherein R^1 is lower alkyl substituted with a heterocyclic group; carbamoyl substituted with heterocyclic-(lower)alkyl or with lower alkylamino-(lower)alkyl; N-containing heterocycliccarbonyl optionally substituted with lower alkyl; or ureido substituted with lower alkylamino-

.../2

(lower)alkyl; and

R^2 is phenyl substituted with nitro, and

X is =N- or $=C(R^3)-$, in which R^3 is lower alkyl;

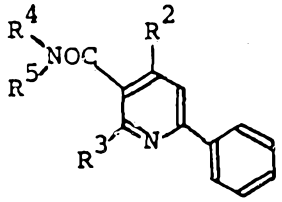
or

R^2 is lower alkyl, and

X is $=C(R^3)-$, in which R^3 is phenyl substituted with nitro;

or its salt, as an active ingredient, optionally in association with a pharmaceutically acceptable, substantially nontoxic carrier or excipient.

4. A process for preparing a compound of the formula:



[I]

wherein R^2 is phenyl substituted with nitro,

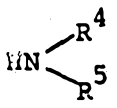
R^3 is lower alkyl, and

R^4 is heterocyclic(lower)alkyl or lower alkylamino(lower)alkyl, and

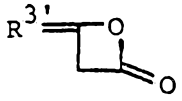
R^5 is hydrogen; or

R^4 and R^5 are taken together with the attached nitrogen atom to form an N-containing heterocyclic group optionally substituted with lower alkyl,

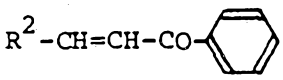
or its salt, which comprises reacting a compound of the formula:



wherein R^4 and R^5 are each as defined above,
or its salt with a compound of the formula:



wherein $R^{3'}$ is lower alkylidene,
then reacting the resultant mixture with a compound of the formula:



wherein R^2 is as defined above,
and ammonia or an agent which liberates ammonia, and finally, if necessary, reacting the resultant mixture with an oxidizing agent.

621067

COMMONWEALTH OF AUSTRALIA

PATENTS ACT 1952

COMPLETE SPECIFICATION

NAME & ADDRESS
OF APPLICANT:

Fujisawa Pharmaceutical Co., Ltd.
3, Doshomachi 4-chome, Higashi-ku
Osaka 541
Japan

NAME(S) OF INVENTOR(S):

Takao TAKAYA
Hisashi TAKASUGI
Kimio ESUMI
Atsushi KUNO
Hiroyoshi SAKAI
Kazuhiro MAEDA
Yoshie SAKAMOTO

ADDRESS FOR SERVICE:

DAVIES & COLLISON
Patent Attorneys
1 Little Collins Street, Melbourne, 3000.

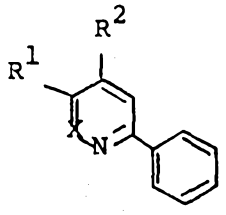
COMPLETE SPECIFICATION FOR THE INVENTION ENTITLED:

A cardioprotective agent and a therapeutic agent for ischemic disease comprising N-containing heterocyclic compounds, and a process for the preparation of the same compounds

The following statement is a full description of this invention, including the best method of performing it known to me/us:-

This invention relates to a cardioprotective agent and a therapeutic agent for ischemic disease comprising N-containing heterocyclic compounds of their salts, and a process for the preparation of the same compound.

Accordingly, one object of this invention is to provide a method for the treatment of ischemic disease, depressed cardiac metabolism, reperfusion injury or heart diseases which comprises administering to human beings or animals in need thereof a therapeutically effective amount of an N-containing heterocyclic compound of the following formula [I] or its salt:



[I]

wherein R^1 is lower alkyl substituted with a heterocyclic group; carbamoyl substituted with heterocyclic(lower)alkyl or with lower alkylamino(lower)alkyl; N-containing heterocycliccarbonyl optionally substituted with lower alkyl; or ureido substituted with lower alkylamino(lower)alkyl; and

R^2 is phenyl substituted with nitro, and

X is =N- or $=\overset{R^3}{\underset{|}{C}}-$ in which R^3 is lower alkyl;

or

R^2 is lower alkyl, and

X is $=\overset{R^3}{\underset{|}{C}}-$, in which R^3 is phenyl substituted with nitro.

Another object of this invention is to provide a process for the preparation of some of the compound [I] or their salts.

Said compounds [I] and their salts possess a reducing effect of reperfusion injury and cardioprotective effect such as an improving or enhancing effect of the depressed cardiac metabolism and said agents are useful in the treatment of ischemia diseases, reperfusion injury and/or heart diseases [e.g. myocardial infarction, heart failure, angina pectoris, etc.] in human beings and animals.

It is known as described in European Patent Application publication No. 228845 that the compound [I] of this invention is useful in the treatment of cerebrovascular diseases such as cerebral apoplexy[e.g. cerebral hemorrhage, carebral infarction, transient cerebral ischemic attack] or the like.

Particulars of the various definitions mentioned in this specification and preferred examples thereof are explained in the following.

The term "lower" used in this specification is intended to mean a group having 1 to 6 carbon atoms and preferably 1 to 4 carbon atoms, unless otherwise provided.

Suitable "lower alkyl" may be straight or branched one such as methyl, ethyl, propyl, isopropyl, butyl, tert-butyl, pentyl, hexyl or the like, in which the most perferable one is methyl.

Suitable "heterocyclic group" may be saturated 5 or 6 membered N-, or N- and S-, or N- and O-containing heterocyclic group such as piperidyl, piperazinyl, morpholinyl, thiomorpholinyl, pyrrolidinyl, imidazolidinyl, pyrazolidinyl, oxazolidinyl or the like, which may be substituted with aforesaid lower alkyl etc..

Preferable examples of "lower alkyl substituted with a heterocyclic group" may be morpholinomethyl, morpholinoethyl, morpholinopropyl, thiomorpholinomethyl, thiomorpholinoethyl, piperazinylmethyl, methyl substituted piperazinylmethyl, or the like.

Suitable "heterocyclic(lower)alkyl" may be the above-mentioned "lower alkyl substituted with a heterocyclic group", or the like.

Preferable examples of "carbamoyl substituted with heterocyclic(lower)alkyl" may be morpholinomethylcarbamoyl, morpholinoethylcarbamoyl, morpholinopropylcarbamoyl, thiomorpholinomethylcarbamoyl, thiomorpholinoethylcarbamoyl, methyl substituted piperazinylethylcarbamoyl, or the like.

Suitable "lower alkylamino(lower)alkyl" may be mono(lower alkyl)amino(lower)alkyl [e.g. methylaminomethyl, ethylaminomethyl, isopropylaminomethyl, 2-methylaminoethyl, 3-methylaminopropyl, 3-methylaminobutyl, etc.], di(lower alkyl)amino(lower)alkyl [e.g. dimethylaminomethyl, dimethylaminoethyl, 2-(N-methyl-N-ethylamino)ethyl, etc.] or the like.

Preferable examples of "carbamoyl substituted with

lower alkylamino(lower)alkyl" may be N-(methylaminomethyl)-carbamoyl, N-(dimethylaminomethyl)carbamoyl, N-(2-dimethylaminoethyl)carbamoyl, N-(2-diethylaminoethyl)-carbamoyl, N-[3-(N-methyl-N-ethylamino)propyl]carbamoyl, or the like.

Suitable "N-containing heterocycliccarbonyl" may be saturated 5 or 6 membered N-, or N- and S-, or N- and O-containing heterocyclic-carbonyl such as 1-pyrrolidinylcarbonyl, 1-imidazolidinylcarbonyl, piperidinocarbonyl, 1-piperazinylcarbonyl, morpholino-carbonyl, thiomorpholinocarbonyl or the like.

The above "N-containing heterocycliccarbonyl" may optionally be substituted with a lower alkyl group as exemplified before.

Preferable examples of "N-containing heterocyclic-carbonyl substituted with lower alkyl" may be 3-methylpiperidinocarbonyl, 4-methylpiperidinocarbonyl, 4-methylpiperazin-1-ylcarbonyl, 2-ethylmorpholinocarbonyl, 2-isopropylthiomorpholin-4-ylcarbonyl, or the like.

Preferable examples of "ureido substituted with lower alkylamino(lower)alkyl" may be 3-(methylaminomethyl)-ureido, 3-(dimethylaminomethyl)ureido, 3-(dimethylamino-ethyl)ureido, 3-(diethylaminoethyl)ureido, 3-(N-methyl-N-ethylaminopropyl)ureido or the like.

Suitable salts of the compound [I] are conventional non-toxic pharmaceutically acceptable salts and may include an organic or inorganic acid addition salt [e.g. formate, acetate, fumarate, trifluoroacetate, maleate, tartrate, methanesulfonate, benzenesulfonate, toluenesulfonate, hydrochloride, hydrobromide, sulfate, phosphate, etc.], or the like.

It is to be noted that each of the compound [I] may include one or more stereoisomers due to asymmetric carbon atom(s), and all of such isomers and a mixture thereof are included within the scope of this invention.

In order to show the usefulness of the compound [I] or their salts used as an ingredient of the cardioprotective agent and the therapeutic agent for ischemic disease, the results of pharmacological tests are shown in the following.

Test Compounds

(a) 3-(2-Morpholinoethylcarbamoyl)-2-methyl-4-(3-nitrophenyl)-6-phenylpyridine

(b) 3-(4-Methylpiperazin-1-ylmethyl)-4-(3-nitrophenyl)-6-phenylpyridazine

(c) 4-Methyl-3-(2-morpholinoethylcarbamoyl)-2-(3-nitrophenyl)-6-phenylpyridine

(d) 2-Methyl-3-(4-methylpiperazin-1-ylcarbonyl)-4-(3-nitrophenyl)-6-phenylpyridine

(e) 3-(2-Dimethylaminoethylcarbamoyl)-2-methyl-4-(3-nitrophenyl)-6-phenylpyridine

(f) 2-Methyl-4-(3-nitrophenyl)-6-phenyl-3-[2-(4-thiomorpholinyl)ethylcarbamoyl]pyridine fumarate

(g) 3-[3-(2-Dimethylaminoethyl)ureido]-2-methyl-4-(3-nitrophenyl)-6-phenylpyridine

The compounds (a) to (e) and (g) were dissolved in 2 equivalent of hydrochloric acid and used as Test Compounds.

Test 1

Method :

The heart was isolated from male guinea-pig weighing 550-650 g and was perfused in the Langendorff mode at 37°C at a constant perfusion pressure of 80 cm H_2O. The perfusion medium was Krebs-Henseleit bicarbonate solution containing 11 mM glucose. The solution was equilibrated with 95% O_2 and 5% CO_2 gas mixture at pH 7.4. A latex balloon was placed in the left ventricular cavity and then left ventricular systolic

(LVSP) and diastolic (LVDP) pressure were measured. Heart rate (HR) was triggered by the pulse pressure. Coronary flow was monitored with electromagnetic flow probe placed on the aorta.

The heart was perfused for 45 min. with the perfusion medium and then for 15 min. with the perfusion medium containing the test compound. The heart was then subjected to the global ischemia by stopping the perfusion. After 35 min. of ischemia, the heart was reperfused by the perfusion medium containing no test compound. At the end of 40 min. reperfusion, the cardiac function was monitored. The heart was immidiately frozen for the estimation of ATP content.

Results :

Test Compound	Concentration (g/ml)	% change from control		
		Cardiac depression (pre-ischemia) LVSP x HR	recovery of ATP content (% increase)	coronary flow
(a)	1×10^{-6}	4.1	96.3	-1.6
	1×10^{-5}	-1.7	89.5	32.0
(b)	1×10^{-5}	-95.8	100.4	-30.6
(c)	1×10^{-5}	-38.9	71.3	31.9
(d)	1×10^{-5}	-29.7	123.6	5.0
(e)	1×10^{-5}	-90.6	76.3	-10.2
(f)	1×10^{-5}	-45.2	64.9	22.5
(g)	1×10^{-5}	-85.7	78.6	-42.7

Test 2

Method :

Male SD rats weighing 270-340 g were anesthetized with sodium pentobarbital. The chest was opened, and then a thread was placed around the left coronary artery near the origin. Both ends of the thread were passed through a small plastic tube. After standing for 5 minutes, test compound was administered intravenously. After 10 minutes, the left coronary artery was occluded for 5 minutes by pulling the thread and pressing the plastic tube to the artery and then reperfusion was achieved by releasing the occlusion for 10 minutes. Electrocardiograms were recorded and analyzed for checking inhibitory rate of incidence of ventricular tachycardia (VT) and ventricular fibrillation (VF) in comparison with control group, and inhibitory rate of mortality as compared with control group was also calculated. 11-18 rats were used for each test group.

Results:

Test Compound	Dose (mg/kg)	Inhibition (%) of incidence as compared with control group		
		VT	VF	Mortality
(a)	1.0	0	28.0	13.5
	3.2	0	60.4	52.5
	10	5.6	82.4	78.9

Test 3

Method :

Ten male SD rats weighing 140-170 g were used for

each group. Test compound was administered intraperitoneally, and then myocardial infarction was induced by administering DL-isoproterenol hydrochloride (120 mg/kg) subcutaneously. After 24 hours, mortality was examined. Survival rats were anesthetized and their hearts were taken out and frozen immediately for the measurement of myocardial ATP content.

Results :

Test Compound	Dose (mg/kg)	Inhibition (%) of mortality *	Recovery (%) of ATP content
(a)	1.0	75	67

* % Change as compared with mortality rate in control group.

Test 4

Method :

Five male ddY mice weighing 27-32 g were used for each group. After test compound was administered intravenously, the right leg was occluded by binding 10 times a rubber band (diameter : 42 mm) round it. After 20 minutes later, the rubber band was cut and thickness of foot pad was measured by a dial gage immediately and 20 minutes after cutting the band. The difference of these two values was regarded as edema.

Results :

Test Compound	Dose (mg/kg)	Inhibition (%) of foot edema
(a)	0.1	12
	1.0	30
	10	42
(d)	1.0	41.5
(f)	1.0	22

As being apparent from the above test results, the compounds [I] and their salts reduce the reperfusion injury and maintain the ATP content against the overload on the heart. Therefore, they are useful as therapeutic agents for ischemic disease and as cardioprotective agents, especially drugs to improve or enhance the depressed cardiac metabolism, and are useful in the treatment of ischemia diseases, reperfusion injury and/or heart diseases [e.g. myocardial infarction, heart failure, angina pectoris, etc.].

The cardioprotective agent and the therapeutic agent for ischemic disease used in the present invention can be administered orally or parenterally to a mammal including human being in a conventional pharmaceutical form such as capsules, micro-capsules, tablets, granules, powders, troches, pills, ointments, suppositories, injection solutions, syrups, and the like.

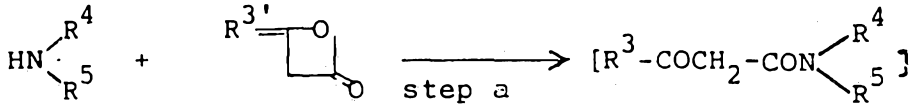
The cardioprotective agent and the therapeutic agent for ischemic disease of the present invention can be produced by the established procedures using various organic or inorganic carriers, which are conventional for pharmaceutical purpose, such as excipient [e.g. sucrose, starch, mannit, sorbit, lactose, glucose, cellulose, talc, calcium phosphate, calcium carbonate, etc.], binding agent [e.g. cellulose, methyl cellulose, hydroxymethyl cellulose, polypropylpyrrolidone, gelatin, gum arabic, polyethylene glycol, sucrose, starch, etc.], disintegrator [e.g. starch, carboxymethyl cellulose, hydroxypropyl starch, sodium bicarbonate, calcium phosphate, calcium citrate, etc.], lubricant [e.g. magnesium stearate, aerosil, talc, sodium laurylsulfate, etc.], flavoring agent [e.g. citric acid, mentol, glycine, orange powders, etc.], preservative [e.g. sodium benzoate, sodium bisulfite, methylparaben, propylparaben, etc.], stabilizer [e.g. citric acid, sodium citrate, acetic acid, etc.], suspending agent [e.g. methyl

cellulose, polyvinylpyrrolidone, aluminum stearate, etc.], dispersing agent [e.g. hydroxypropylmethyl cellulose, etc.], diluting agent [e.g. water, etc.], base wax [e.g. cacao butter, white petrolatum, polyethylene glycol, etc.].

While a dosage or therapeutically effective amount of the cardioprotective agent and the therapeutic agent for ischemic disease of this invention varies according to the age and conditions of each individual patient to be treated, a daily dose of about 0.1-100 mg/kg, preferably 1 to 50 mg/kg of the active ingredient may be generally given for treating diseases.

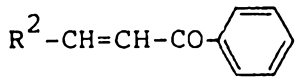
The pharmaceutical compositions of this invention comprises, as an active ingredient, the compound [I] or its salt in an amount of about 0.2 mg to about 500 mg, per dosage unit for oral and parenteral use.

The compound [I], wherein R^1 is carbamoyl substituted with heterocyclic(lower)alkyl or with lower alkylamino-(lower)alkyl, or N-containing heterocycliccarbonyl optionally substituted with lower alkyl, R^2 is phenyl substituted with nitro, and R^3 is lower alkyl, can be prepared by reacting a compound [II] or its salt with a compound [III], then reacting the resultant mixture with a compound [IV] and ammonia or an agent which liberates ammonia, and finally, if necessary, reacting the resultant mixture with an oxidizing agent without isolating intermediate products, as illustrated in the following reaction scheme.



[II] [III]

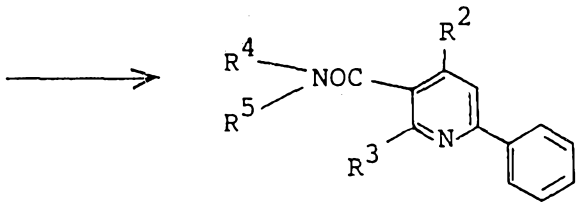
or its salt



[IV]

+

ammonia or an agent which liberates ammonia — step b → oxidation, if necessary — step c →



[Ia]

or its salt

wherein R^2 is phenyl substituted with nitro,
R^3 is lower alkyl,
$R^{3'}$ is lower alkylidene, and
R^4 is heterocyclic(lower)alkyl or lower alkylamino(lower)alkyl, and
R^5 is hydrogen; or
R^4 and R^5 are taken together with the attached nitrogen atom to form an N-containing heterocyclic group optionally substituted with lower alkyl.

Suitable "lower alkylidene" may be methylene, ethylidene, propylidene, butylidene, or the like, in which the most preferable one is methylene.

Each step for preparing the compound [Ia] is explained in more detail in the following.

Step a

Suitable salts of the compound [II] may be the same as exemplified for the compound [I].

This reaction is usually carried out in a conventional solvent such as diethyl ether, tetrahydrofuran, dioxane, benzene, acetone, methyl ethyl ketone, methyl isobutyl ketone or any other solvent which does not adversely influence the reaction.

The reaction temperature is not critical and the reaction is usually carried out under cooling or at ambient temperature.

Step b

Suitable agents which liberate ammonia may be ammonium lower alkanoate[e.g. ammonium formate, ammonium acetate, ammonium propionate, ammonium butyrate, etc.], ammonium carbonate, ammonium hydrogencarbonate, ammonium carbamate or the like.

This reaction is usually carried out in a conventional solvent such as water, alcohol [e.g. methanol, ethanol, propanol, etc.], dioxane, tetrahydrofuran, methylene chloride, chloroform, diethyl ether, benzene,acetone, methyl ethyl ketone, methyl isobutyl ketone or any other organic solvent which does not adversely influence the reaction, or a mixture thereof.

The reaction temperature is not critical, and the reaction is usually carried out under cooling, at ambient temperature or under warming or heating.

Step c

The oxidation reaction can be carried out by a conventional method which is applied for the transformation of an N-containing heterocyclic base to an aromatized N-containing heterocyclic compound, for example, by using an oxidizing agent such as manganese dioxide, lead tetraacetate, mercuric acetate, halogen [e.g. iodine, bromine, etc.], oxygen, hydrogen peroxide, nickel peroxide, sulfur powder, 2,3-dichloro-5,6-dicyano-1,4-benzoquinone, potassium permanganate, or the like.

The present reaction is usually carried out in a conventional solvent such as chloroform, methylene chloride, benzene, toluene, pyridine, ethyl acetate, acetone, methyl ethyl ketone, methyl isobutyl ketone or any other organic solvent which does not adversely influence the reaction.

The reaction temperature is not critical and the reaction is preferably carried out at ambient temperature or under warming to heating.

The obtained compound [Ia] in this step can be separated and isolated from the reaction mixture and purified by methods commonly used for this purpose, for instance, extraction with suitable solvent, column chromatography, reprecipitation, recrystallization and so on.

The compound [Ia] can be prepared more easily and in much better yield by this preparation method without isolation of intermediate products than by the preparation with isolation of intermediate products in each step and than by the preparations described in the above-mentioned European patent application publication No. 228845.

These reaction conditions stated in each step may vary according to the kinds of reactants, solvents and/or other agents.

The following Examples and Reference are given for the purpose of illustrating this invention in more detail.

Example 1

A solution of N-(2-aminoethyl)morpholine (170.5 g) in methyl isobutyl ketone (200 ml) was added portionwise to a solution of diketene (102 ml) in methyl isobutyl ketone (1.3 ℓ) at -30 to -10°C under stirring and the mixture was stirred at -10 to 0°C for one hour. To the resultant solution containing N-(2-morpholinoethyl)-acetoacetamide, was added 3-(3-nitrophenyl)-1-phenyl-2-propen-1-one (220.4 g) and ammonium acetate (100 g) and the mixture was stirred at 80°C for 4.5 hours. The reaction mixture was washed with water and dried over magnesium sulfate. To the filtrate containing 1,4-dihydro-3-(2-morpholinoethylcarbamoyl)-2-methyl-4-(3-nitrophenyl)-6-phenylpyridine was added manganese dioxide (700 g) and the resultant mixture was stirred at 80°C for 2 hours. Manganese dioxide was filtered off and the filtrate was evaporated in vacuo. A mixture of ethyl acetate and water was added to the residue and adjusted to pH 1.0 with 10% hydrochloric acid. The separated aqueous layer was adjusted to pH 9.0 with 20% aqueous potassium carbonate and extracted with a mixture of ethyl acetate and tetrahydrofuran. The extract was washed with brine and dried over magnesium sulfate. The solvent was evaporated in vacuo and the residue was recrystallized from ethanol to give 3-(2-morpholinoethyl-carbamoyl)-2-methyl-4-(3-nitrophenyl)-6-phenylpyridine (154 g, yield 39.6%), which was identified as the compound of Example 9-(2) described in European Patent Application publication No. 228845 and the compound of Reference (3) as mentioned below by their physical data.

Example 2

The following compounds were obtained according to a similar manner to that of Example 1.

(1) 3-(2-Dimethylaminoethylcarbamoyl)-2-methyl-4-(3-

nitrophenyl)-6-phenylpyridine
This compound was identified as the compound of Example 8 described in European Patent Application publication No. 228845.

(2) 2-Methyl-4-(3-nitrophenyl)-6-phenyl-3-[2-(4-thiomorpholinyl)ethylcarbamoyl]pyridine and its fumarate
This compound was identified as the compound of Example 9-(3) described in European Patent Application publication No. 228845.

(3) 2-Methyl-3-(4-methylpiperazin-1-ylcarbonyl)-4-(3-nitrophenyl)-6-phenylpyridine
This compound was identified as the compound of Example 9-(1) described in European Patent Application publication No. 228845.

Example 3

To a solution of 3-(2-morpholinoethylcarbamoyl)-2-methyl-4-(3-nitrophenyl)-6-phenylpyridine (3 g) in ethanol (60 ml) was added concentrated hydrochloric acid (1.68 ml) and water (3.32 ml). The separated crystal was filtered, washed with ethanol (20 ml) and dried in vacuo to give 3-(2-morpholinoethylcarbamoyl)-2-methyl-4-(3-nitrophenyl)-6-phenylpyridine dihydrochloride (2.91 g).

mp : 269-272°C (dec.)

IR (Nujol) : 3175, 1660 cm^{-1}

NMR (D_2O, δ) : 3.00 (3H, s), 3.1-4.3 (12H, m), 7.6-8.2 (7H, m), 8.35-8.65 (2H, m)

Elemental analysis : $C_{25}H_{26}N_4O_4.2HCl$

Calcd. : C 57.81, H 5.43, N 10.79, Cl 13.65
Found : C 57.80, H 5.47, N 10.77, Cl 13.90

Example 4

3-(2-Morpholinoethylcarbamoyl)-2-methyl-4-(3-

nitrophenyl)-6-phenylpyridine monohydrochloride (17.09 g) was obtained according to a similar manner to that of Example 3 from 3-(2-morpholinoethylcarbamoyl)-2-methyl-4-(3-nitrophenyl)-6-phenylpyridine (20 g) and a solution of acetyl chloride (3.5 ml) in ethanol (60 ml).

IR (Nujol) : 3300, 3200, 1660, 1650, 1530, 1355 cm^{-1}

NMR ($CDCl_3$, δ) : 2.66 (3H, s), 2.66-3.5 (6H, m), 3.53-4.20 (6H, m), 7.31-8.76 (9H, m)

Reference

(1) To a solution of diketene (23.0 ml) in diethyl ether (115 ml) was added dropwise a solution of N-(2-aminoethyl)morpholine (34.7 g) in diethyl ether (300 ml) at -40°C. After stirring for 2 hours at the same condition, the resulting precipitates were collected by filtration and dried in vacuo to give N-(2-morpholinoethyl)-acetoacetamide (41.1 g, yield 72.0 %).

IR (Nujol) : 3290, 1715, 1640 cm^{-1}

NMR ($CDCl_3$, δ) : 2.3 (3H, s), 2.4-2.7 (6H, m), 3.2-3.9 (8H, m), 7.3 (1H, s)

(2) Ammonia was bubbled into a solution of N-(2-morpholinoethyl)acetoacetamide (40 g) in a mixture of diethyl ether (400 ml) and tetrahydrofuran (200 ml) at 20°C under water cooling for 3 hours. The resulting precipitates were collected by filtration and dried in vacuo to give N-(2-morpholinoethyl)-3-aminocrotonamide (18.0 g, yield 45.2 %).

mp : 86-93°C

IR (Nujol) : 3300, 3120, 1620 cm^{-1}

NMR ($CDCl_3$, δ) : 2.7-3.0 (9H, m), 3.6-4.3 (6H, m), 4.8 (1H, s)

(3) A mixture of 3-(3-nitrophenyl)-1-phenyl-2-propen-1-one (20 g) and N-(2-morpholinoethyl)-3-aminocrotonamide

(21.9 g) in toluene (200 ml) was refluxed for 55 hours. After allowing to cool at ambient temperature, the reaction mixture was concentrated in vacuo. The residue was subjected to a column chromatography on silica gel eluting with a mixture of ethyl acetate and tetrahydrofuran (100:1 V/V). The fractions containing the object compound were combined and concentrated in vacuo. The residue was recrystallized from ethyl alcohol to give 3-(2-morpholinoethylcarbamoyl)-2-methyl-4-(3-nitrophenyl)-6-phenylpyridine (11.0 g, yield 31.2 %).

Total yield (1) to (3) : 10.2 %

mp : 141-142°C

IR (Nujol) : 3200, 1630, 1565 cm^{-1}

Example 5

3-(2-Morpholinoethylcarbamoyl)-2-methyl-4-(3-nitrophenyl)-6-phenylpyridine (hereinafter referred to as Compound A)	100 g
Hydroxypropylmethyl cellulose	500 g
Lactose	6.87 kg
Low-substituted hydroxypropyl cellulose	1.5 kg
Magnesium stearate	30 g

To a suspension of Compound A and hydroxypropylmethyl cellulose in anhydrous ethanol (5 liters) were added lactose and low-substituted hydroxypropyl cellulose, and the resultant mixture was stirred and then the organic solvent was removed under reduced pressure to give solid dispersion composition. After this composition was converted into granules by a conventional method, the granules were further converted with magnesium stearate into tablets by a conventional method, each of which contains 2 mg of Compound A.

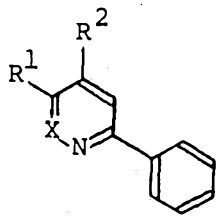
Example 6

Compound A	25.0 g
Methyl cellulose	0.5 g
Polyvinylpyrrolidone	0.05 g
Methyl p-hydroxybenzoate	0.1 g
Polysorbate 80	0.1 g
Lidocain hydrochloride	0.5 g
Distilled water	a proper quantity (total volume 100 ml)

The above-mentioned ingredients were mixed to give 100 ml of an injection suspension.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A method for the treatment of ischemic disease, depressed cardiac metabolism, reperfusion injury or heart diseases which comprises administering to human beings or animals in need thereof a therapeutically effective amount of a compound of the formula:



[I]

wherein R^1 is lower alkyl substituted with a heterocyclic group; carbamoyl substituted with heterocyclic-(lower)alkyl or with lower alkylamino-(lower)alkyl; N-containing heterocycliccarbonyl optionally substituted with lower alkyl; or ureido substituted with lower alkylamino-(lower)alkyl; and

R^2 is phenyl substituted with nitro, and

X is =N- or $=C(R^3)-$, in which R^3 is lower alkyl;

or

R^2 is lower alkyl, and

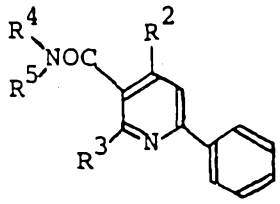
X is $=C(R^3)-$, in which R^3 is phenyl substituted with nitro;

or its salt, as an active ingredient, optionally in association with a pharmaceutically acceptable, substantially nontoxic carrier or excipient.

2. A method according to claim 1 comprising administering a compound of claim 1, wherein R^1 is morpholino(lower)alkylcarbamoyl, R^2 is phenyl substituted with nitro, and X is $=C(R^3)-$, in which R^3 is lower alkyl.

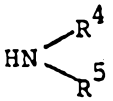
3. A method according to claim 1 or 2 comprising administering a compound of claim 1, which is 3-(2-morpholinoethylcarbamoyl)-2-methyl-4-(3-nitrophenyl)-6-phenylpyridine and its dihydrochloride.

4. A process for preparing a compound of the formula:

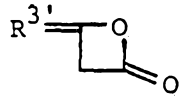


[I]

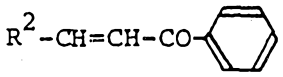
wherein R^2 is phenyl substituted with nitro,
R^3 is lower alkyl, and
R^4 is heterocyclic(lower)alkyl or lower alkylamino(lower)alkyl, and
R^5 is hydrogen; or
R^4 and R^5 are taken together with the attached nitrogen atom to form an N-containing heterocyclic group optionally substituted with lower alkyl,
or its salt, which comprises reacting a compound of the formula:



wherein R^4 and R^5 are each as defined above, or its salt with a compound of the formula:



wherein $R^{3'}$ is lower alkylidene, then reacting the resultant mixture with a compound of the formula:



wherein R^2 is as defined above, and ammonia or an agent which liberates ammonia, and finally, if necessary, reacting the resultant mixture with an oxidizing agent.

5. Processes for the preparation of compounds of the formula [I], substantially as hereinbefore described with reference to the Examples.

DATED this 12th day of December, 1991
Fujisawa Pharmaceutical Co., Ltd.
By Its Patent Attorneys
DAVIES COLLISON CAVE