603765

SPRUSON & FERGUSON

COMMONWEALTH OF AUSTRALIA

PATENTS ACT 1952

CONVENTION APPLICATION FOR A STANDARD PATENT

We, SHELL INTERNATIONALE RESEARCH MAATSCHAPPIJ B.V., of Carel van Bylandtlaan 30, 2596 HR, The Hague, the Netherlands hereby apply for the grant of a standard patent for an invention entitled: Carboxy Azetidine Derivatives For Use In The Reduction of Blood Cholesterol Levels. ~~"THERAPEUTIC COMPOUNDS"~~

which is described in the accompanying complete specification.

DETAILS OF BASIC APPLICATION

Number of Basic Application:-
8514075

Name of Convention Country in which Basic Application was filed:-
Great Britain

Date of Basic application:-
4 June 1985

Our address for service is:-

C/- Spruson & Ferguson
Patent Attorneys
Level 33 St Martins Tower
31 Market Street
Sydney New South Wales Australia

DATED this SECOND day of JUNE 1986

SHELL INTERNATIONALE RESEARCH MAATSCHAPPIJ B.V.

By: M.J. Anderson

Registered Patent Attorney.

TO: THE COMMISSIONER OF PATENTS
AUSTRALIA

SPECIFICATION ACCEPTED AND AMENDMENTS
ALLOWED 3-9-90

SBR:JMA:278M

COMMONWEALTH OF AUSTRALIA

Patents Act 1952-55

DECLARATION IN SUPPORT OF A CONVENTION APPLICATION FOR A PATENT

In support of the Convention Application made by SHELL INTERNATIONALE RESEARCH MAATSCHAPPIJ B.V. for a patent for an invention entitled:

"Therapeutic compounds"

I, Onno Aalbers, of Carel van Bylandtlaan 30, The Hague, the Netherlands, do solemnly and sincerely declare as follows:

1. I am authorized by SHELL INTERNATIONALE RESEARCH MAATSCHAPPIJ B.V., the applicant for the patent, to make this declaration on its behalf.

2. The basic application~~(s)~~ as defined by Section 141 of the Act was/~~were~~ made in the United Kingdom on the 4th day of June, 1985 by SHELL INTERNATIONALE RESEARCH MAATSCHAPPIJ B.V.

3. James Brown Morton GELLATLY, of 43 St. Lawrence Forstal, Canterbury, Kent, England, and John Gilbert MARTIN, of 108 Park Drive, Sittingbourne, Kent, England, both British citizens.

~~is~~/are the actual inventor(s) of the invention and the facts upon which the Applicant Company is entitled to make application are as follows: as Assignees of the inventor(s).

4. The basic application~~(s)~~ referred to in paragraph 2 of this Declaration was/~~were~~ the first application~~(s)~~ made in a Convention Country in respect of the invention the subject of the application.

Declared at the Hague

Dated this 5th day of May 1986

Signature of Declarant

LODGED AT SUB-OFFICE
2 JUN 1986
Sydney

To.: The Commissioner of Patents,
Commonwealth of Australia

JB2H04

(12) PATENT ABRIDGMENT (11) Document No. AU-B-58268/86
(19) AUSTRALIAN PATENT OFFICE (10) Acceptance No. 603765

(54) Title
AZETIDINE DERIVATIVES

International Patent Classification(s)
(51)[4] **A61K 031/395**

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8514075 **04.06.85** **GB UNITED KINGDOM**

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(44) Publication Date of Accepted Application : **29.11.90**

(71) Applicant(s)
SHELL INTERNATIONALE RESEARCH MAATSCHAPPIJ B.V.

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(74) Attorney or Agent
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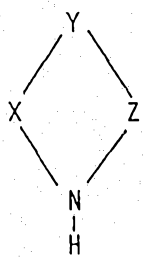
(57) Claim

1. Composition for use in a method for treatment of the human or animal body to reduce blood cholesterol levels, characterised in that the composition comprises a carboxy azetidine derivative of the general formula I or a pharmaceutically acceptable salt, ester, amide, alkylamide, hydrazide or alkylhydrazide thereof:-



I

in which:

X represents one of the groups CH_2, CHR or CR_2;

Y represents one of the groups CHR, CR_2 or $CH.CO_2H$ and

Z represents one of the groups CH_2, CHR, CR_2 or $CH.CO_2H$;

the or each R independently represents an alkyl, alkenyl or cycloalkyl group or an aryl or aralkyl group optionally substituted on the aryl nucleus by one or more of the same or different substituents selected from halogen atoms, alkyl groups and alkoxy groups;

and one but only one of Y and Z must represent a $CH.CO_2H$ group,

.../2

in association with a carrier in a pharmaceutically acceptable state of purity.

12. A method for the treatment, prophylaxis or reduction of blood cholesterol levels in a patient requiring said treatment, prophylaxis or reduction, which method comprises administering to said patient an effective amount of a composition according to any one of claims 1 to 8 or 10.

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FORM 10

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COMMONWEALTH OF AUSTRALIA

PATENTS ACT 1952

COMPLETE SPECIFICATION

(ORIGINAL)

FOR OFFICE USE:

58268/86.

Class Int. Class

Complete Specification Lodged:

Accepted:

Published:

This document contains the amendments made under Section 49 and is correct for printing.

Priority:

Related Art:

Name of Applicant: SHELL INTERNATIONALE RESEARCH MAATSCHAPPIJ B.V.

Address of Applicant: Carel van Bylandtlaan 30, 2596 HR, The Hague, the Netherlands

Actual Inventor(s): JAMES BROWN MORTON GELLATLY and JOHN GILBERT MARTIN

Address for Service: Spruson & Ferguson, Patent Attorneys, Level 33 St Martins Tower, 31 Market Street, Sydney, New South Wales, 2000, Australia

Complete Specification for the invention entitled: Carboxy Azetidine Derivatives For Use In The Reduction of Blood Cholesterol Levels

~~"THERAPEUTIC COMPOUNDS"~~

LODGED AT SUB-OFFICE
- 2 JUN 1986
Sydney

AUSTRALIAN PATENT OFFICE

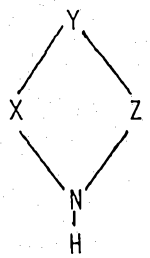
The following statement is a full description of this invention, including the best method of performing it known to us

SBR:JMA:278M

The present invention relates to the therapeutic use of certain carboxylic azetidine derivatives, in particular their use in reducing the level of cholesterol in blood.

European Patent number 0029265 describes the production of plants in which male sterility has been brought about by treatment with certain carboxy azetidine derivatives. It has now been unexpectedly found that this type of compound also exerts a therapeutic effect in mammals, in particular by reducing the level of cholesterol in blood. Cardiovascular diseases, for example ischemic heart diseases, atherosclerosis and hypertension are amongst the more commonly occurring causes of death. These are often caused by insufficient blood flow resulting from atherosclerosis, which is generally associated with elevated levels of blood serum cholesterol. Compounds which reduce the cholesterol level in blood therefore offer a valuable therapeutic tool in reducing the risk of cardiovascular disease.

According to a first embodiment of this invention there is provided a composition for use in a method for treatment of the human or animal body to reduce blood cholesterol levels, characterised in that the composition comprises a carboxy azetidine derivative of the general formula I or a pharmaceutically acceptable salt, ester, amide, alkylamide, hydrazide or alkylhydrazide thereof:-



I

in which:

X represents one of the groups CH_2, CHR or CR_2;

Y represents one of the groups CHR, CR_2 or $CH.CO_2H$ and

Z represents one of the groups CH_2, CHR. CR_2 or $CH.CO_2H$;

the or each R independently represents an alkyl, alkenyl or cycloalkyl group or an aryl or aralkyl group optionally substituted on the aryl nucleus by one or more of the same or different substituents selected from

halogen atoms, alkyl groups and alkoxy groups;
and one but only one of Y and Z must represent a $CH.CO_2H$ group,
in association with a carrier in a pharmaceutically acceptable state of purity.

Preferably the or each R independently represents an alkyl or alkenyl group having up to 6, especially up to 4, carbon atoms, a cycloalkyl group having from 3 to 6 carbon atoms, or a phenyl or benzyl group optionally substituted by one or more, preferably one or two, of the same or different substituents selected from chlorine, fluorine or bromine atoms, alkyl groups having from 1 to 4 carbon atoms and alkoxy groups having 1 to 4 carbon atoms.

More preferably the or each R independently represents a methyl or ethyl group or a phenyl group optionally substituted by one or two substituents selected from fluorine and chlorine atoms and methyl and methoxy groups.

Preferably X represents CH_2, Y represents CHR or $CH.CO_2H$ and Z represents CH_2 or $CH.CO_2H$, one of Y and Z being $CH.CO_2H$.

According to a second embodiment of this invention there is provided a process for the manufacture of a medicament for application in reducing blood cholesterol levels in a human or animal body comprising mixing a carboxy azetidine derivative as defined above with a pharmaceutically acceptable carrier.

The azetidine derivative may for example be the free acid of the general formula I; a hydrolhalide or an alkali metal salt thereof; the amide or hydrazide thereof in which the amide or hydrazide group may be substituted by one or two alkyl, preferably C(1-4) alkyl, especially methyl, groups; an alkyl, alkenyl or aralkyl ester, preferably an alkyl or alkenyl ester

having up to 10, especially up to 7, carbon atoms in the alkyl or alkenyl group; or a hydrohalide of such an amide, hydrazide or ester.

Preferably the azetidine derivative is the free acid of formula I, a hydrohalide or an alkali metal salt thereof, the hydrazide thereof, a C(1-10) alkyl ester thereof or a hydrohalide of said hydrazide or ester. Especially preferred is the free acid of formula I or a C(1-4)alkyl ester, for example the methyl ester, thereof, or a hydrohalide of said acid or ester.

Especially preferred azetidine derivatives are 3-carboxyazetidine, its methyl ester hydrochloride, and 2-carboxy-3-methyl-azetidine.

The azetidine derivative may exist in the form of isomers depending on the meaning of the group X, Y and Z. For example, 2-carboxy-3-methylazetidine exists as geometric isomers depending on the relative positions of the carboxy and the methyl group, and in addition, for each of these geometric isomers, optical isomers exist. As is usual in processes involving biological systems, some isomers may be more therapeutically active than others.

Administration of the azetidine compounds for therapeutic use can be via any of the accepted modes of administration for therapeutic agents. These methods include oral, parenteral, transdermal, subcutaneous and other systemic modes. In order to facilitate administration, the azetidine compounds are suitably formulated in accordance with standard pharmaceutical practice as therapeutic compositions. Accordingly, the invention includes also a therapeutic composition which comprises a carboxy azetidine derivative of the general formula I as defined above in association with a carrier in a pharmaceutically acceptable level of purity. When the intended route of administration is parenteral, the therapeutic composition should, of course, be in a sterile form.

Depending on the intended mode, the compositions may be in the form of solid, semi-solid or liquid dosage forms, such as, for example, tablets, pessaries, suppositories, pills, capsules, powders, liquids, suspensions, or the like, preferably in unit dosage forms suitable for single administration of precise dosages. The compositions, whether or not in unit dosage form, can conveniently be in the form of a pack which comprises such a composition, together with instructions for use in a method of treatment by therapy. The compositions will include a conventional pharmaceutical excipient and an active compound of formula I or the pharmaceutically acceptable salts thereof and, in addition, may include other medicinal agents, pharmaceutical agents, carriers adjuvants, diluents, etc.

For solid compositions, conventional non-toxic solid include, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin, talcum, cellulose, glucose, sucrose, magnesium carbonate, and the like may be used. The active compound as defined above may be formulated as suppositories using, for example, polyalkylene glycols, for example, propylene glycol, as the carrier. Liquid pharmaceutically administerable compositions can, for example, be prepared by dissolving, dispersing , etc. an active compound as defined above and optional pharmaceutical adjuvants in an excipient, such as, for example, water, saline, aqueous dextrose, glycerol, ethanol, and the like, thereby forming a solution or suspension. If desired, the pharmaceutical composition to be administered may also contain minor amounts of nontoxic auxiliary substances such as wetting agents, pH buffering agents and the like, for example, sodium acetate, sodium lauryl sulphate, sorbitan monolaurate, triethanolamine sodium acetate, triethanolamine oleate, etc. Actual methods of preparing such dosage forms are known, or will be apparent, to those skilled in this art; for example, see Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, Pa., 15th Edition, 1975. The composition or formulation to be

administered will, in any event, contain as quantity of the active compound(s), a thereapeutically effective amount, i.e. in an amount effective to achieve cholesterol reduction in the subject being treated.

For oral administration, a pharmaceutically acceptable non-toxic composition is formed by the incorporation of any of the normally employed excipients, such as, for example pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin, talcum, cellulose, glucose, sucrose, magnesium, carbonate, and the like. Such compositions take the form of solutions, suspensions, tablets, pills, capsules, powders, sustained release formulations and the like. Such compositions may contain \%-95% active ingredient, preferably 1-70%.

Parenteral administration is generally characterized by injection, either subcutaneously, intramuscularly or intravenously. Injectables can be prepared in conventional forms, either as liquid solutions or suspensions, solid forms suitable for solution or suspension in liquid prior to injection, or as emulsions. Suitable excipients are, for example, water, saline, dextrose, glycerol, ethanol or the like. In addition, if desired, the pharmaceutical compositions to be administered may also contain minor amounts of non-toxic auxiliary substances such as wetting or emulsifying agents, pH buffering agents and the like, such as for example, sodium acetate, sorbitan monolaurate, triethanolamine oleate, etc.

A more recently devised approach for parenteral administration employs the implantation of a slow-release or sustained-release system, such that a constant level of dosage is maintained. See, e.g., U.S. Patent No. 3,710,795.

The dosage employed is preferably in the range of 0.1 to 200mg active ingredient per day per kg body weight. As indicated above, the therapeutic effect of particular interest is the reduction of cholesterol levels in blood. In order to achieve this effect, the dosage of azetidine compound

administered should normally be at least 100ppm of subject diet, (corresponding approximately to 10-20 mg active compound per day per kg body weight) with the upper limit being determined by other factors such as economics and avoidance of undesired side-effects.

It is also believed that other therapeutic effects may occur, particularly on tissues. For example, rat studies indicate that mortality over a two-year period is reduced, apparently because treated rats suffer less from chronic renal disease than control rats.

The invention is illustrated in the following Examples.

Example 1

Rats (Fischer 344 strain) were fed a standard diet (LAD 2, supplied by K.& K. Greff Chemicals Ltd., Croydon, England) to which had been added varying amounts of test compound. The trial used 75 male and 75 female rats, with 15 of each sex in each dose group. Blood samples taken by retro-orbital bleeding after the animals had been fed for 13, 26 and 52 weeks, and the concentration of cholesterol in the blood plasma determined by a standard enzymatic colorimetric test, kinetically measured (ref. Siedel, J. et al (1981). J. Clin. Chem. Clin. Biochem. 19,838). The test compound was azetidine-3-carboxylic acid, and the results are set out in Table 1 below.

Subsequently, retroorbital blood samples were obtained at week 78 and cardiac blood samples at termination of this 2 year study; data obtained confirm the trends of Table 1. Survival of male rats to study termination was substantially greater in the 1000 ppm group (64% survival) than in the controls or intermediate groups (24-48%). Preliminary observations at necropsy suggest that deaths due to chronic renal disease were fewer in the 1000 ppm group than in the other groups.

TABLE 1

Concn. Test Compound	0 (Control)	Concentration of cholesterol (M Mole/litre)			
		3.0 ppm diet	30 ppm diet	100 ppm diet	1000 ppm diet
Males at 13 weeks	2.3	2.2	2.2	2.1 **	1.9 **
Females at 13 weeks	3.0	3.0	2.8	2.5 **	2.0 **
Males at 26 weeks	2.11	2.09	1.80 *	1.66 **	1.21 **
Females at 26 weeks	3.29	3.15	2.56 **	2.17 **	1.41 **
Males at 52 weeks	2.52	2.55	2.14	2.17 **	1.41
Females at 52 weeks	2.57	3.54	2.95	2.50	1.53

Single and double stars indicate 95 and 99% levels of significant differences, respectively, from controls. (The data for 52 weeks have not been analysed statistically, so no stars are assigned.) In both sexes and at all times there was a reduction in the concentration of plasma cholesterol at 100 and 1000 ppm in the diet of azetidine-3-carboxylic acid (corresponding, respectively to approximately 10 and 100 mg/kg/day).

Example 2

Mice (C57/C3H hybrid) were fed for 13 weeks on a standard laboratory diet (LAD 2, supplied by K.& K. Greff Chemicals Ltd., Croydon, England) to which had been added varying amounts of test compound. The trial used 75 male and 75 female mice, with 15 of each sex in each dose group. Blood samples were taken by retro-orbital bleeding, and the concentration of cholesterol in the blood plasma determined by the standard enzymatic colorimetric test (as used for Ex.). The test compound was azetidine-3-carboyxlic acid, and the results are set out in Table 2 below.

TABLE 2

Concn. Test Compound	Concentration of cholesterol (M Mole/litre)				
	0 (Control)	3.0 ppm diet	30 ppm diet	100 ppm diet	1000 ppm diet
Males	3.15	3.30	3.03	3.71	2.78
Females	2.76	2.78	2.63	2.37	2.22

In both the males and the females, the concentration of cholesterol was less in the group fed 1000 ppm (corresponding approximately to 200 mg/kg/day) of test compound than it was in the control group.

Example 3

Groups of 7 male and 7 female Fischer 344 rats were fed for 5 weeks on standard laboratory diet (LAD 2) containing 2500 ppm azetidine-3-carboxylic acid or 2500 ppm of the methyl ester hydrochloride of azetidine-3-carboxylic acid. A group of 21 males and 21 females were fed LAD 2 only (controls). Cardiac blood samples were obtained at terminal necropsy and the concentrations of plasma cholesterol determined as in Example 1.

Results indicate that the concentrations of plasma cholesterol were less (reductions in males 13-15%; reductions in females 35-40%) in the groups fed azetidine-3-carboxylic acid and the methyl ester hydrochloride of azetidine-3-carboxylic acid than in the controls.

Example 4

Groups of 3 male and 3 female hyperlipidaemic homozygotic rabbits (Froxfield) were fed standard rabbit diet containing 0 (controls) or 2500 ppm azetidine-3-carboxylic acid for eight weeks. Blood samples were obtained prior to initiation of the study (day 0), at day 28 and at termination (day 56) for estimation of local plasma cholesterol (method of Roeschau et al. (1974) Klin Chem., V Klin Biochem., 12, 226). Results are presented in Table 3, and show a marked reduction of plasma cholesterol in the treated animals over the period of the study.

TABLE 3

Treatment/Sex	Plasma cholesterol (m mol l^{-1})		
	Day 0	Day 28	Day 56
CONTROL			
Males	20.3	18.3	17.6
Females	16.7	19.1	22.3
AZETIDINE-3-CARBOXYLIC ACID			
Males	22.4	16.1	16.7
Females	24.5	16.7	13.2*

* One female killed on day 46 due to illness; mean presented is for two survivors only.

Example 5

Pharmaceutical compositions

The following examples illustrate pharmaceutical compositions according to the invention. In the examples, the active ingredient is either 3-carboxyazetidine or its methyl ester hydrochloride. Other azetidine derivatives may be formulated in a similar manner.

Tablet for oral administration

	mg/tablet
Active ingredient	250
Sodium starch glycollate	5
Microcrystalline cellulose	45
Sodium lauryl sulphate	3

The active ingredient and the microcrystalline cellulose are sieved through a 40 mesh screen. The sodium starch glycollate and sodium lauryl sulphate are sieved through a 60 mesh screen. The powders are blended together in a suitable blender until homogeneous. The mixture is then compressed on

appropriate punches on an automatic tablet machine. The tablets may be covered in a thin polymer coat applied by the usual film coating technique. A pigment may be included in the film coat.

Capsule for oral administration

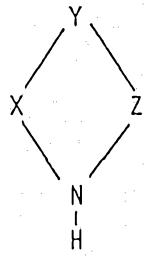
	mg/capsule	mg/capsule
Active ingredient	200mg	2.5
*Starch	150mg	97.0
magnesium stearate	5.0mg	1.0

*a form of directly compressible starch

The active ingredient is sieved and blended with the excipients. The mix is then filled into hard gelatine capsules using suitable machinary. Other doses may be prepared by altering the fill weight.

The claims defining the invention are as follows:

1. Composition for use in a method for treatment of the human or animal body to reduce blood cholesterol levels, characterised in that the composition comprises a carboxy azetidine derivative of the general formula I or a pharmaceutically acceptable salt, ester, amide, alkylamide, hydrazide or alkylhydrazide thereof:-



I

in which:

X represents one of the groups CH_2, CHR or CR_2;

Y represents one of the groups CHR, CR_2 or $CH.CO_2H$ and

Z represents one of the groups CH_2, CHR, CR_2 or $CH.CO_2H$;

the or each R independently represents an alkyl, alkenyl or cycloalkyl group or an aryl or aralkyl group optionally substituted on the aryl nucleus by one or more of the same or different substituents selected from halogen atoms, alkyl groups and alkoxy groups;

and one but only one of Y and Z must represent a $CH.CO_2H$ group,

in association with a carrier in a pharmaceutically acceptable state of purity.

2. Composition as claimed in claim 1 wherein, in the general formula I, X represents CH_2; and Y represents CHR or CHCOOH; and Z represents CH_2 or CHCOOH.

3. Composition as claimed in claim 1 or 2 wherein, in the general formula I, the or each R independently represents an alkyl or alkenyl group having up to 6 carbon atoms, a cycloalkyl group having from 3 to 6 carbon atoms, or a phenyl or benzyl group optionally substituted by one or more of the same or different substituents selected from chlorine, fluorine or bromine atoms, alkyl groups having from 1 to 4 carbon atoms and alkoxy groups having 1 to 4 carbon atoms.

4. Composition as claimed in claim 3 wherein, in the general formula I, the or each R independently represents a methyl or ethyl group

or a phenyl group optionally substituted by one or two substituents selected from fluorine and chlorine atoms and methyl and methoxy groups.

5. Composition as claimed in any one of claims 1 to 4, in the form of the free acid of formula I, a C(1-4)alkyl ester thereof, or a hydrohalide of said acid or ester.

6. Composition as claimed in claim 1 wherein 3-Carboxy azetidine or its methyl ester hydrochloride is the active compound.

7. Composition as claimed in any one of the preceding claims which is in a sterile form.

8. Composition as claimed in any one of the preceding claims in a unit dosage form.

9. A process for the manufacture of a medicament for application in reducing blood cholesterol levels in a human or animal body comprising mixing a carboxy azetidine derivative as defined in claim 1 with a pharmaceutically acceptable carrier.

10. Composition as claimed in claim 1 substantially as hereinbefore described with reference to any one of the examples.

11. A process for the manufacture of a medicament for application in reducing blood cholesterol levels in a human or animal body substantially as herein described with reference to Example 5.

12. A method for the treatment, prophylaxis or reduction of blood cholesterol levels in a patient requiring said treatment, prophylaxis or reduction, which method comprises administering to said patient an effective amount of a composition according to any one of claims 1 to 8 or 10.

DATED this TWENTY-SEVENTH day of SEPTEMBER 1989
Shell Internationale Research Maatschappij BV

Patent Attorneys for the Applicant
SPRUSON & FERGUSON