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(71) Applicant *(for all designated States except US)*: **OTSUKA PHARMACEUTICAL CO., LTD.** [JP/JP]; 9, Kanda-Tsukasamachi 2-chome, Chiyoda-ku, Tokyo, 1018535 (JP).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **NAKAJIMA, Atsushi** [JP/JP]; c/o Yokohama City University, 9-1, Fukuura 3-chome, Kanazawa-ku, Yokohama-shi, Kanagawa, 2360004 (JP). **FUJITA, Koji** [JP/JP]; c/o Yokohama City University, 9-1, Fukuura 3-chome, Kanazawa-ku, Yokohama-shi, Kanagawa, 2360004 (JP). **YONEDA, Masato** [JP/JP]; c/o Yokohama City University, 9-1, Fukuura 3-chome, Kanazawa-ku, Yokohama-shi, Kanagawa, 2360004 (JP). **MUKEDA, Osamu** [JP/JP]; c/o OTSUKA PHARMACEUTICAL CO., LTD., 7-1, Dosho-machi 1-chome, Chuo-ku, Osaka-shi, Osaka, 5410045 (JP). **ICHIMURA, Takako** [JP/JP]; c/o OTSUKA PHARMACEUTICAL CO., LTD., 7-1, Dosho-machi 1-chome, Chuo-ku, Osaka-shi, Osaka, 5410045 (JP).

(74) Agents: **TANAKA, Mitsuo** et al.; AOYAMA & PARTNERS, IMP Building, 3-7, Shiromi 1-chome, Chuo-ku, Osaka-shi, Osaka, 5400001 (JP).

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(54) Title: CARBOSTYRIL DERIVATIVES INCLUDING CILOSTAZOL FOR TREATING FATTY LIVER

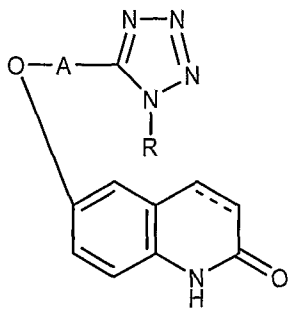
(57) Abstract: The invention relates to a medicament for preventing and/or treating fatty liver which comprises as an active ingredient cilostazol or a pharmaceutically acceptable salt thereof.

DESCRIPTION

CARBOSTYRIL DERIVATIVES INCLUDING CILOSTAZOL FOR TREATING FATTY LIVER

Technical Field

The invention relates to a medicament for preventing and/or treating fatty liver. More particularly, it relates to a medicament for preventing and/or treating fatty liver which comprises as an active ingredient a carbostyril derivative of formula (1):



(1)

wherein A is a lower alkylene group, R is a cycloalkyl group, the bonding between 3- and 4-positions of the carbostyril skeleton is a single bond or a double bond, or a salt thereof.

Background Art

The carbostyril derivatives of formula (1) or salts thereof and the process for the preparation thereof are disclosed in JP-63-20235-B. And it is known that the carbostyril derivatives (1) have platelet aggregation

inhibitiory action, phosphodiesterase (PDE) inhibition action, antiulcer, hypotensive action and antiphlogistic action, and are useful as an antithrombotic agent, a drug for improving cerebral circulation, an antiinflammatory agent, an antiulcer drug, an antihypertensive drug, an antiasthmatic drug, a phosphodiesterase inhibitor, etc.

In addition, JP-9-157170-A reports that the carbostyril derivative may be useful as an agent for promoting hepatocyte growth factor (HGF) production. Therefore, the hepatocyte growth action thereof is expected to be useful in the case of treating a disease such as fulminant hepatic failure or promoting the liver regeneration after hepatectomy.

These days, an interest in metabolic syndrome has been increasing. With regard to the evaluation of the syndrome, visceral fat is adopted as one of the criteria thereof. Amongst the visceral fat, especially an interest in fatty liver is increasing. Fatty liver is in a condition that fat is built up in the liver, which causes little subjective symptom. However, the condition could get worse when left untreated, and not a few patients suffering from fatty liver may progress in hepatitis, cirrhosis and liver cancer.

Fatty liver can be roughly classified into alcoholic fatty liver and non-alcoholic fatty liver. As patients of

metabolic syndrome increase, there is increasing interest in non-alcoholic steatohepatitis (NASH) in Japan that is a disease of fatty liver accompanied with symptoms of necrosis of hepatocyte, inflammation, and/or fibril formation, which affects even non-drinkers. Although the cause of NASH has not been sufficiently clarified yet, it is thought that fatty liver may be one of the causes at least. Therefore, a strict nutrition therapy is important for the treatment thereof, and drug therapy is also considered for intractable symptom (M. Yoneda, et al., *Folia Pharmacologica Japonica*, Vol.128 (2006), No.4 235-238).

Thus, fatty liver has seriously critical factors to progress toward severe cases, however, there are few effective medicaments for preventing and treating fatty liver. It has been still desired to develop an effective medicament for fatty liver, especially NASH whose cause has not been sufficiently clarified yet and non-alcoholic fatty liver which might progress toward NASH.

Disclosure of Invention

As mentioned above, no satisfied medicament for preventing and treating fatty liver has been found yet, and thus it has been desired to develop a medicament for preventing and treating fatty liver in Japan and other

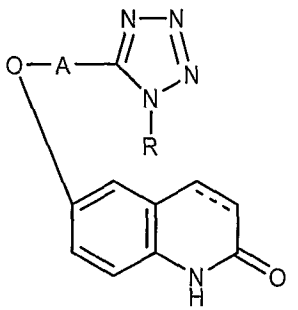
countries. In addition, there has been no/few medicament for treating NASH because NASH is a newly uprising disease. Furthermore, the condition of NASH is thought to be composed of a variety of factors which are complicatedly related, and thus it is not enough to treat the disease with a single medicament, and usually plural medicaments must be used for the treatment. From the viewpoint of the need for treating this new disease, NASH, it has been desired to develop a new medicament for the treatment or apply an existent medicament to the treatment.

The present inventors have intensively studied a new medicament for preventing and/or treating fatty liver and have found that a carbostyril derivative of the above formula (1), especially 6-[4-(1-cyclohexyl-1H-tetrazol-5-yl)butoxy]-3,4-dihydrocarbostyril (cilostazol) or a salt thereof is useful for preventing and/or treating fatty liver. As mentioned above, it has been already known that the carbostyril derivative has a hepatocyte growth action as an agent for promoting HGF, however, it is very surprising and unexpected from the mechanistic viewpoint that the derivative may be used for treating fatty liver though the target organ of both the diseases is the same liver.

The present inventors have found for the first time that 6-[4-(1-cyclohexyl-1H-tetrazol-5-yl)butoxy]-3,4-

dihydrocarbostyril (cilostazol) or a salt thereof could improve the treatment of fatty liver on model animals suffering from fatty liver, and then have accomplished the present invention.

The present invention provides a medicament for preventing and/or treating fatty liver comprising as an active ingredient a carbostyril derivative of the general formula:



(1)

wherein A is a lower alkylene group, R is a cycloalkyl group, the bonding between 3- and 4-positions of the carbostyril skeleton is a single bond or a double bond, or a salt thereof.

The present invention also provides a medicament for preventing and/or treating fatty liver comprising as an active ingredient 6-[4-(1-cyclohexyl-1H-tetrazol-5-yl)butoxy]-3,4-dihydrocarbostyril (cilostazol) or a salt thereof.

The present invention also provides a composition for preventing and/or treating fatty liver comprising the above-mentioned carbostyril derivative (1).

The present invention also provides use of the above-mentioned carbostyril derivative (1) in preparation of a medicament for preventing and/or treating fatty liver.

The present invention also provides a method for preventing and/or treating fatty liver which comprises administering an effective amount of the above-mentioned carbostyril derivative (1) to a patient in need thereof.

According to the present invention, 6-[4-(1-cyclohexyl-1H-tetrazol-5-yl)butoxy]-3,4-dihydrocarbostyril (cilostazol) or a salt thereof may be useful for preventing/treating NASH through preventing and/or treating fatty liver and inhibiting the initial stage of NASH. Furthermore, the invention provides a medicament for preventing and/or treating NASH comprising as an active ingredient the above-mentioned carbostyril derivative (1).

Brief Description of Drawings

Fig. 1 shows the result of the Hematoxylin-eosin-stained liver in the control group rats to which only a general diet was given for 6 weeks.

Fig. 2 shows the result of the Hematoxylin-eosin-stained liver in the CDAA group rats to which the CDAA diet was given for 6 weeks.

Fig. 3 shows the result of the Hematoxylin-eosin-stained liver in the cilostazol-administration group rats

to which the CDAA diet + 6 mg/kg of cilostazol was given every day for 6 weeks.

Fig. 4 shows the result of the Hematoxylin-eosin-stained liver in the aspirin-administration group rats to which the CDAA diet + 5 mg/kg of aspirin was given every day for 6 weeks.

Fig. 5 shows the result of the Hematoxylin-eosin-stained liver in the ticlopidine-administration group rats to which the CDAA diet + 10 mg/kg of ticlopidine was given every day for 6 weeks.

Fig. 6 shows the result of the Masson-stained liver in the CDAA group rats to which the CDAA diet was given for 6 weeks.

Fig. 7 shows the result of the Masson-stained liver in the cilostazol-administration group rats to which the CDAA diet + 6 mg/kg of cilostazol was given every day for 6 weeks.

Fig. 8 shows the result of the Masson-stained liver in the aspirin-administration group rats to which the CDAA diet + 5 mg/kg of aspirin was given every day for 6 weeks.

Fig. 9 shows the result of the Masson-stained liver in the ticlopidine-administration group rats to which the CDAA diet + 10 mg/kg of ticlopidine was given every day for 6 weeks.

Fig. 10 shows serum level of triglyceride in each rat

of the CDAA group, the ticlopidine-administration group, the aspirin-administration group, the cilostazol-administration group, and the control group, which was 6 weeks after the administration of medicaments started.

Best Mode for Carrying Out the Invention

In the above carbostyril derivative (1), the cycloalkyl group includes C_3-C_8 cycloalkyl groups such as cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, and cyclooctyl. Preferred cycloalkyl group is cyclohexyl. The lower alkylene group includes C_1-C_6 alkylene groups such as methylene, ethylene, propylene, butylene, and pentylene, among which preferred one is butylene.

Preferable carbostyril derivative (1) is 6-[4-(1-cyclohexyl-1H-tetrazol-5-yl)butoxy]-3,4-dihydrocarbostyril, which has been put on the market in the trade name of cilostazol as an antiplatelet agent.

The carbostyril derivative (1) can be easily converted to a salt thereof by getting it treated with a pharmaceutically acceptable acid. The acid includes, for example, inorganic acids such as hydrochloric acid, sulfuric acid, phosphoric acid, and hydrobromic acid; and organic acids such as oxalic acid, maleic acid, fumaric acid, malic acid, tartaric acid, citric acid, and benzoic

acid.

These carbostyril derivatives (1) and salts thereof and processes for preparation thereof are disclosed in JP-63-20235-B.

The carbostyril derivatives of formula (1) may be used in bulk or preferably in the form of a pharmaceutical preparation with a conventional pharmaceutical carrier or diluent. The dosage form in the present invention includes, but not limited thereto, for example, the dosage forms exemplified in JP-10-175864-A, and typically an oral solid dosage form such as tablets, capsules, and particles; various liquid preparations suitable for oral administration; and also a parenteral preparations such as injections and suppositories. The dose of the carbostyril derivative (1) is not limited to a specific range. The carbostyril derivatives (1) or a salt thereof may be used in an amount of 100 to 400 mg/day per an adult (50 kg of body weight), which is administered once a day or two to several times per day. The carbostyril derivative (1) may be included in 0.1 - 70 % (w/w) per the composition of the preparation, preferably 50 - 100 mg per a dosage unit of the preparation.

The preparation for injection is usually prepared in the form of a liquid preparation, an emulsion, or a suspension, which are sterilized and further are preferably

made isotonic to the blood. The preparations in the form of liquid, emulsion or suspension are usually prepared by using conventional pharmaceutical diluents such as water, ethyl alcohol, propylene glycol, ethoxylated isostearyl alcohol, polyoxylated isostearyl alcohol, and polyoxyethylene sorbitan fatty acid esters. These preparations may be prepared by mixing the carbostyril derivative (1) with an isotonic agent such as sodium chloride, glucose, glycerin in an amount sufficient for making isotonic and may further be prepared by mixing with conventional solubilizers, buffers, anesthetizing agents, and optionally colorants, preservatives, fragrant materials, flavors, sweetening agents, and other medicaments.

The preparations of the invention such as tablets, capsules, liquid for oral administration may be prepared by a conventional method. The tablets may be prepared by mixing the carbostyril derivative (1) with conventional pharmaceutical carriers such as gelatin, starches, lactose, magnesium stearate, talc, gum arabic, and the like. The capsules may be prepared by mixing the carbostyril derivative (1) with inert pharmaceutical fillers or diluents and filling hard gelatin capsules or soft capsules with the mixture. The oral liquid preparations such as syrups or elixirs are prepared by mixing the carbostyril derivative (1) with sweetening agents (e.g. sucrose),

preservatives (e.g. methylparaben, propylparaben), colorants, flavors, and the like. The preparations for parenteral administration may also be prepared by a conventional method, for example, by dissolving the carbostyril derivative (1) of the present invention in a sterilized aqueous carrier, preferably water or a saline solution. Preferred liquid preparation suitable for parenteral administration is prepared by dissolving about 50 - 100 mg of the carbostyril derivative (1) in water and an organic solvent and further in a polyethylene glycol having a molecular weight of 300 to 5000, in which preferably a lubricant such as sodium carboxymethyl-cellulose, methylcellulose, polyvinylpyrrolidone, and polyvinyl alcohol may be incorporated. Preferably, the above liquid preparations may further comprise a disinfectant (e.g. benzyl alcohol, phenol, thimerosal), a fungicide, and further optionally an isotonic agent (e.g. sucrose, sodium chloride), a topical anesthetic, a stabilizer, a buffer, and the like. In view of keeping stability, the preparation for parenteral administration may be put in a capsule, followed by removing the aqueous medium by a conventional lyophilizing technique. The preparation can be recovered into a liquid preparation by dissolving it in an aqueous medium when used.

Example

Fisher 344 rats were fed only a general diet or a choline-deficiented, L-amino acid-defined (CDAA) diet to prepare a control group and a CDAA group as an animal model for fatty liver, respectively. Three medicament-treated groups were prepared by administering the following three medicaments to the CDAA groups every day. (Aspirin and ticlopidine are medicaments having the same platelet aggregation inhibitiory action as cilostazol.)

6 mg/kg of cilostazol

5 mg/kg of aspirin

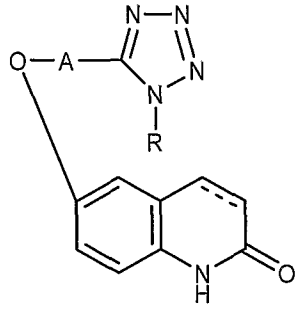
10 mg/kg of ticlopidine

Six weeks after the administration of medicaments started, livers of the rats in each medicament-treated group were taken out and stained with Hematoxylin-eosin staining and Masson staining, in which lipid droplets were observed. The results of the Hematoxylin-eosin staining are shown in Fig.1 - Fig. 5, and the results of the Masson staining are shown in Fig.6 - Fig. 9. According to these results, the lipid droplet of liver in the cilostazol-administration group (i.e. administered 6 mg/kg of cilostazol + CDAA) was markedly less than that of the CDAA group without cilostazol administration or that of aspirin/ticlopidine administration groups. In addition, the amount of triglyceride in the liver as well as the serum level of

triglyceride in those rats were measured. The serum levels of triglyceride in each rat of every groups are shown in Fig. 10. As is seen from the results, it was found that the serum level of triglyceride in the cilostazol-administration (6 mg/kg) group was significantly lower than that in the control group and in other medicaments-treated groups. Therefore, it was found that cilostazol is a useful medicament for the prevention and/or treatment of fatty liver.

CLAIMS

1. A medicament for preventing and/or treating fatty liver comprising as an active ingredient a carbostyril derivative of the general formula:



(1)

wherein A is a lower alkylene group, R is a cycloalkyl group, the bonding between 3- and 4-positions of the carbostyril skeleton is a single bond or a double bond, or a salt thereof.

2. The medicament of claim 1 wherein the active ingredient is 6-[4-(1-cyclohexyl-1H-tetrazol-5-yl)butoxy]3,4-dihydrocarbostyril or a salt thereof.

3. Use of the carbostyril derivative or a salt thereof as set forth in claim 1 or 2 in preparation of a medicament for preventing and/or treating fatty liver.

4. A method for preventing and/or treating fatty liver which comprises administering an effective amount of the

carbostyril derivative or a salt thereof as set forth in claim 1 or 2 to a patient in need thereof.

Fig. 1

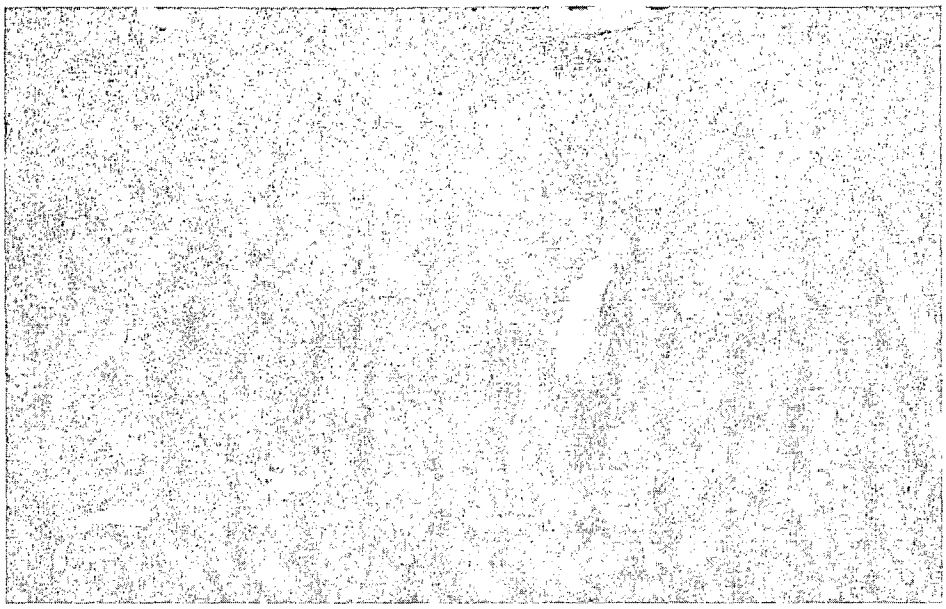


Fig. 2

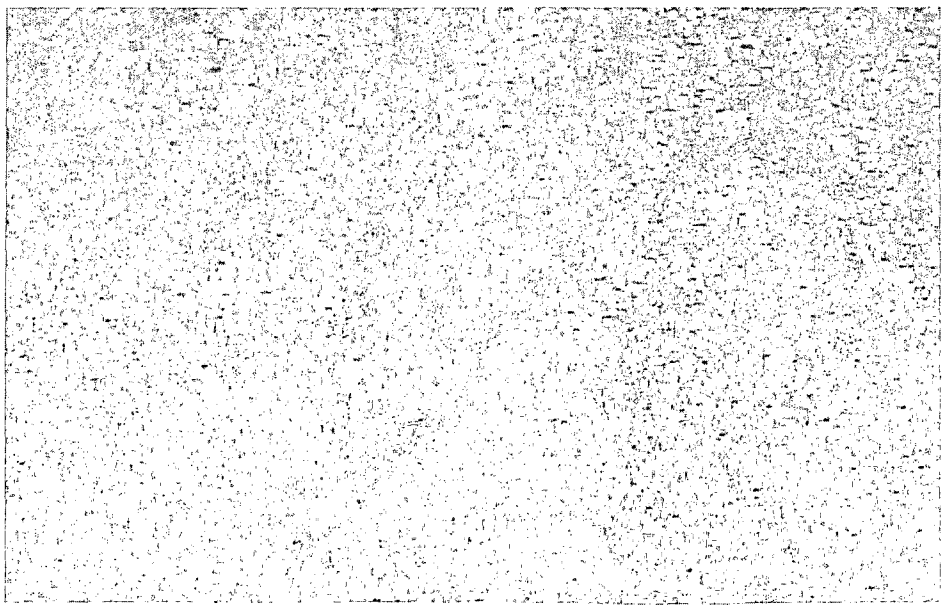


Fig. 3

Fig. 4

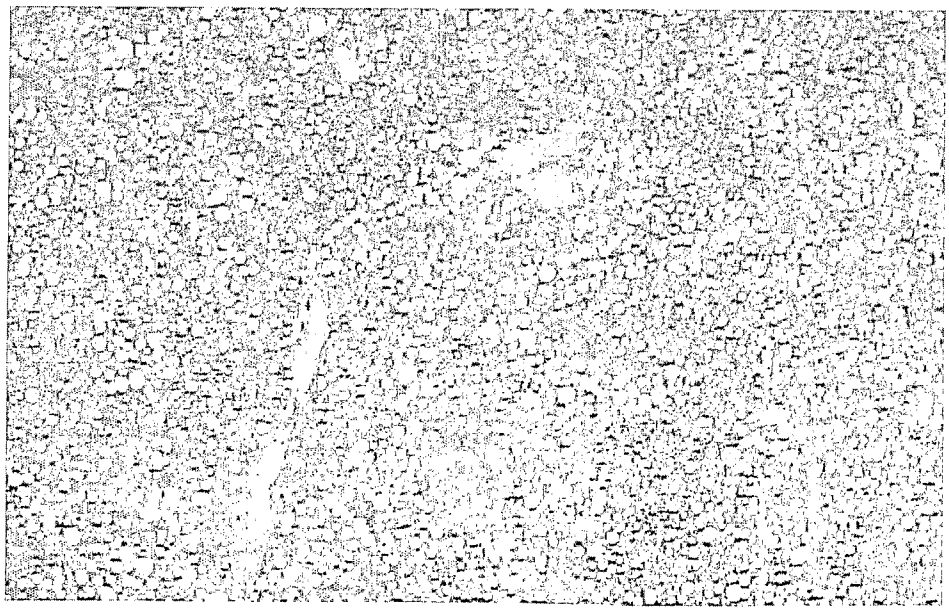


Fig. 5

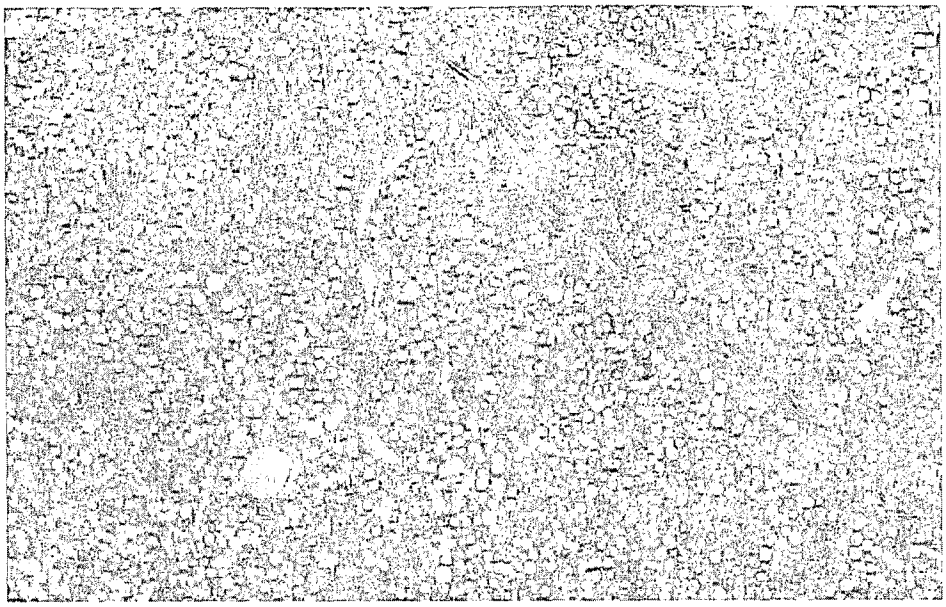


Fig. 6

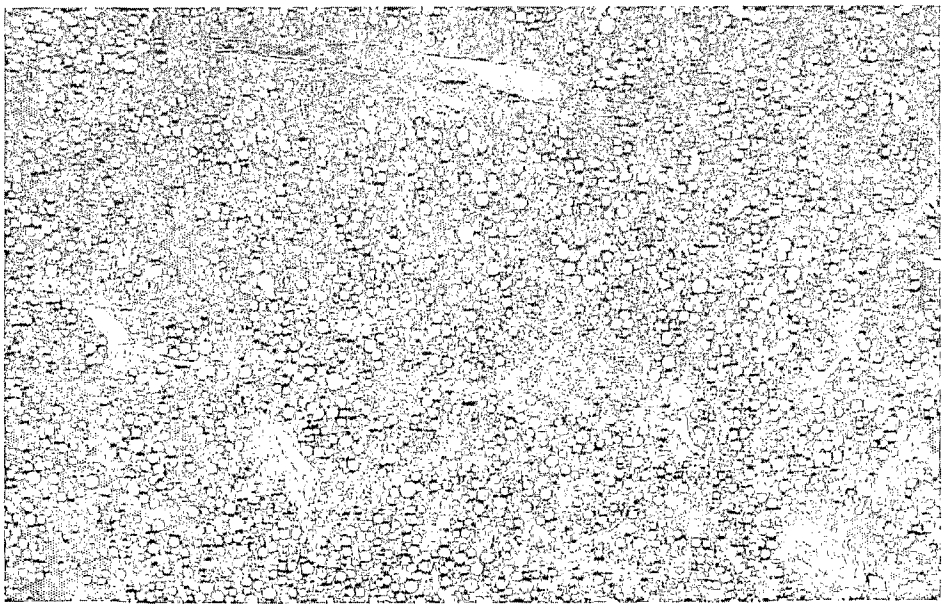


Fig. 7

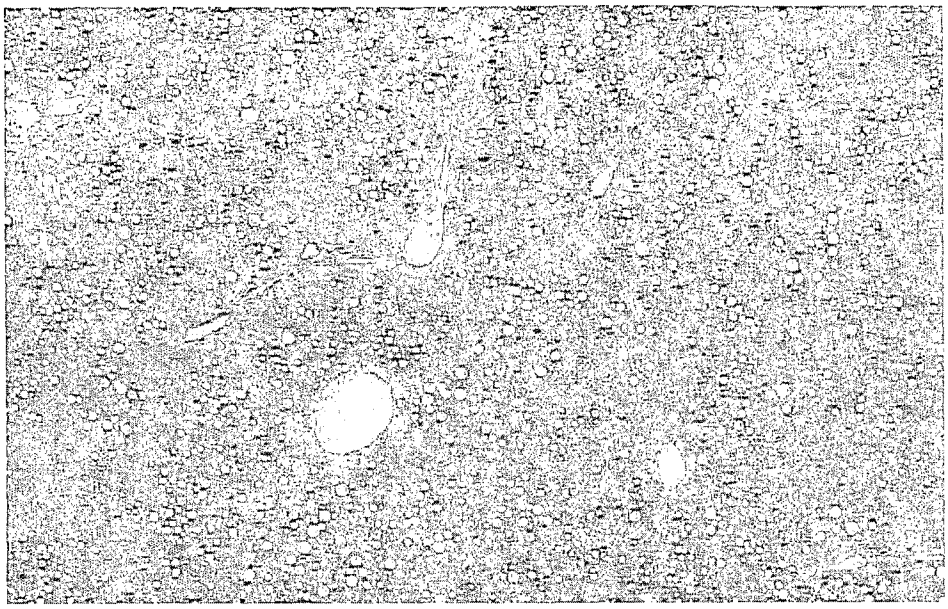


Fig. 8

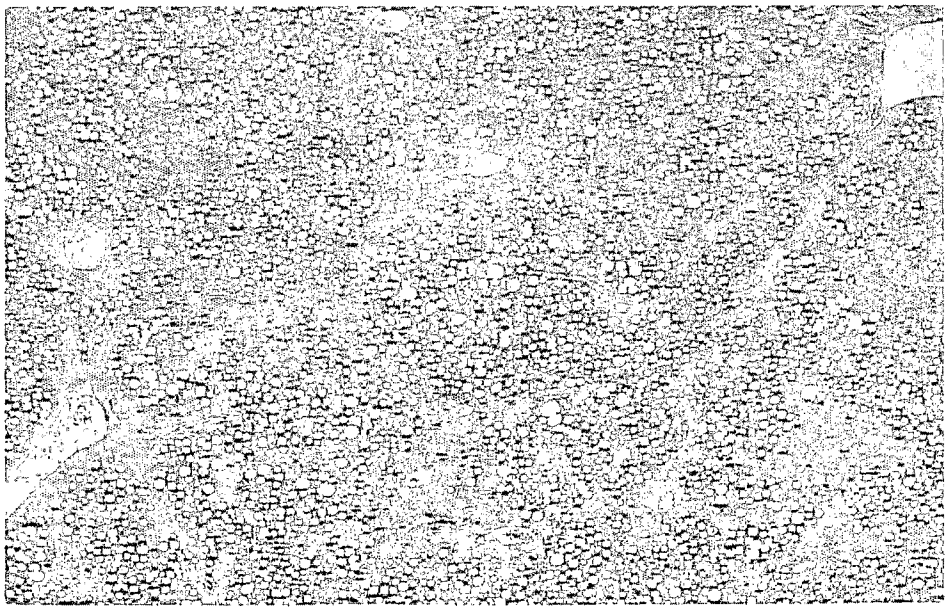


Fig. 9

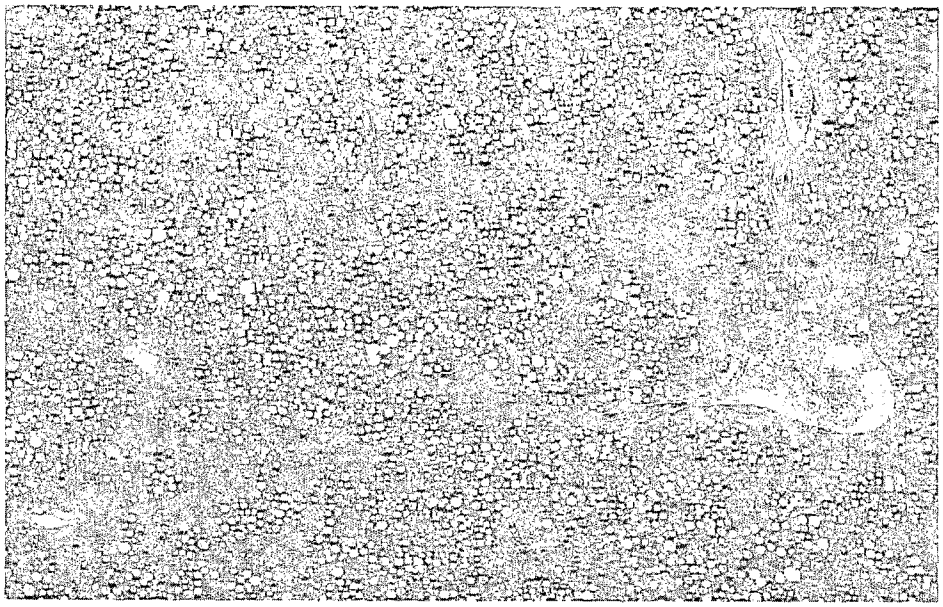
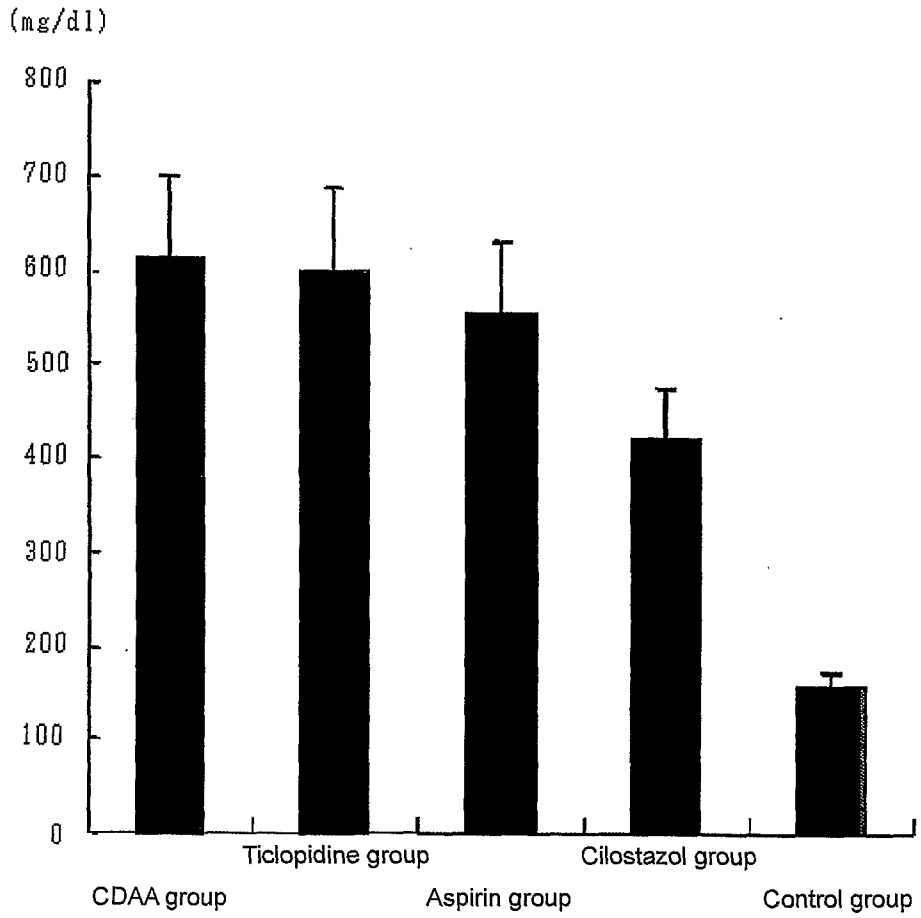


Fig. 10


(mg/dl)
800
700
600
500
400
300
200
100
0
Ticlopidine group
Cilostazol group
CDAA group
Aspirin group
Control group

INTERNATIONAL SEARCH REPORT

International application No
PCT/JP2008/062771

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K31/4709 A61P1/16

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, WPI Data, EMBASE, CHEM ABS Data, SCISEARCH, PASCAL

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	FUJITA KOJI ET AL: "Novel therapeutic approach for nafld using antiplatelet agents in an animal model" HEPATOLOGY, vol. 46, no. 4, Suppl. S, October 2007 (2007-10), pages 762A-763A, XP002493755 & 58TH ANNUAL MEETING OF THE AMERICAN-ASSOCIATION-FOR-THE-STUDY-OF-LIVE R-DISEASES; BOSTON, MA, USA; NOVEMBER 02 -06, 2007 ISSN: 0270-9139 the whole document	1-4

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 August 2008	15/09/2008

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Albrecht, Silke

INTERNATIONAL SEARCH REPORT

International application No
PCT/JP2008/062771

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	FUJITA K ET AL: "Effectiveness of antiplatelet drugs against experimental non-alcoholic fatty liver disease" GUT, [Online] 2 July 2008 (2008-07-02), XP009104963 [retrieved on 2008-08-28] the whole document	1-4
Y	JP 09 157170 A (OTSUKA PHARMA CO LTD) 17 June 1997 (1997-06-17) cited in the application page 2, paragraph 3 claims	1-4
Y	KOSONE TAKASHI ET AL: "HGF ameliorates a high-fat diet-induced fatty liver" AMERICAN JOURNAL OF PHYSIOLOGY - GASTROINTESTINAL AND LIVER PHYSIOLOGY, vol. 293, no. 1, 29 March 2007 (2007-03-29), pages G204-G210, XP002493822 ISSN: 0193-1857 the whole document	1-4
Y	MATSUMOTO K ET AL: "HGF: ITS ORGANOTROPHIC ROLE AND THERAPEUTIC POTENTIAL" CIBA FOUNDATION SYMPOSIUM, AMSTERDAM, NL, vol. 212, 1 January 1997 (1997-01-01), pages 198-214, XP008005072 ISSN: 0300-5208 page 206, paragraph 1	1-4
Y	TAHARA MINORU ET AL: "Hepatocyte growth factor leads to recovery from alcohol-induced fatty liver in rats" JOURNAL OF CLINICAL INVESTIGATION, vol. 103, no. 3, February 1999 (1999-02), pages 313-320, XP002493823 ISSN: 0021-9738 the whole document	1-4

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/JP2008/062771

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
JP 9157170 A	17-06-1997	JP 3944257 B2	11-07-2007