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Novel prostaglandin I2 derivative

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(71) Applicant(s)

Kaken Pharmaceutical Co., Ltd.; Asahi Glass Company, Limited

(72) Inventor(s)

Murata, Takahiko; Amakawa, Masahiro; Teradaira, Shin; Matsumura, Yasushi; Konishi, Katsuhiko

(74) Agent / Attorney

Griffith Hack, GPO Box 4164, Sydney, NSW, 2001

(56) Related Art

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Title: NOVEL PROSTAGLANDIN I<sub>2</sub> DERIVATIVE

Abstract: Disclosed is a novel prostaglandin I<sub>2</sub> derivative which is different from known PGI<sub>2</sub> compounds, or a pharmaceutically acceptable salt of the prostaglandin I<sub>2</sub> derivative. More specifically disclosed is a 7,7-difluoro-PGI<sub>2</sub> derivative (formula (1)), particularly wherein R<sup>1</sup> and R<sup>2</sup> independently represent a hydrogen atom or a linear alkyl group having 1 to 3 carbon atoms, and R<sup>3</sup> represents a hydrogen atom, an alkyl group having 1 to 4 carbon atoms, an alkoxyalkyl group, an aryl group, a halogen atom, or a halogenated alkyl group.

Key Points: The PGI<sub>2</sub> derivative is effective for treating defecation trouble, as shown by the defecation trouble score (soft stool score + occult blood score) as follows: normal (0), control (CC), test compound (DD), *=P<0.05 AND **=P<0.01 RELATIVE TO CONTROL, RESPECTIVELY.

添付公開書類:
国際調査報告（条約第21条(3)）

SPECIFICATION
NOVEL PROSTAGLANDIN I₂ DERIVATIVE

TECHNICAL FIELD OF THE INVENTION

[0001] The present invention relates to a 7,7-difluoroprostaglandin I₂ derivative wherein the carboxy group at C-1 of prostaglandin is substituted by a tetrazole group, and two fluorine atoms are bonded at C-7 thereof, and a medicament comprising the same as an active ingredient, specifically, the medicament for the prophylaxis or treatment of an inflammatory bowel disease.

This application claims priority to a patent application Nos. 2008-232133 and 2009-168193 filed in Japan, the contents of which are incorporated herein by this reference.

BACKGROUND OF THE INVENTION

[0002] Inflammation of the digestive tract is observed in the mouth cavity, esophagus, stomach, small intestine, large intestine and anus, and includes acute inflammation and chronic inflammation. When the mucosal epithelia are affected by physical or chemical stimuli, or are infected by bacteria or virus, inflammation is induced, and erosions or ulcerous lesions occur depending on the level of the inflammation. Excessive secretion of gastric acid due to a stress causes gastritis, gastric ulcer or duodenal ulcer. In addition, excessive ingestion of alcohol induces congestion of mucosal blood flow or reflux of gastric acid due to reduced stomach motility, thus causing gastritis, gastric ulcer, duodenal ulcer or esophagitis. Orthopedic patients, rheumatoid arthritis patients and the like under a long term administration of a non-steroidal anti-inflammatory drug suffer from drug-induced gastric ulcer or duodenal ulcer. In
addition, cancer patients develop radiation enteritis with radiation therapy or drug-induced enteritis with anti-cancer drug treatment. Furthermore, patients infected with tuberculosis, amebic dysentery and the like develop infectious enterogastritis such as intestinal tuberculosis and amebic colitis. Besides these, ischemic enteritis and the like are developed by ischemia due to blood flow obstruction. If immunity of patients with inflammation of digestive tract is abnormal, even when the cause is removed, repair of the organ is prevented and conditions become chronic. Of these inflammatory diseases of the digestive tract, the diseases with inflammation in the intestine are referred to as inflammatory bowel disease in a broad sense.

On the other hand, there are inflammatory intestinal diseases of unidentified cause. Ulcerative colitis and Crohn’s disease are two well known diseases, which are inflammatory bowel disease in a narrow sense. Furthermore, it also includes similar diseases such as intestinal Behcet’s disease and simple ulcer. They are intractable chronic gastrointestinal diseases along with repeated remission and relapse, where main etiology of the disease is considered to be less protection of the intestinal epithelium, or abnormal intestinal immune response against enteric bacteria entering into the intestinal tissues.

Ulcerative colitis is a chronic colon disease in which erosions and ulcers are formed in the large intestinal mucosa continuously from the rectum, and symptoms thereof include abdominal pain, diarrhea, bloody stool, fever and the like. On the other hand, in Crohn’s disease, a lesion can occur in any digestive tract from the mouth cavity to large intestine and anus. This disease is characterized by discontinuous longitudinal ulcer and cobblestone-like appearance in the gastrointestinal tract, and the symptoms thereof include abdominal pain, diarrhea, fever, undernutrition due to malabsorption of nutrients, anemia, and the like.
For the prophylaxis and/or treatment of inflammation in inflammatory diseases of the digestive tract, in case of with a known cause, the cause is removed or suppressed. For example, antacid, anticholinergic agent, histamine H2 receptor antagonist, proton pump inhibitor and the like are used against inflammation in gastritis, gastric ulcer, duodenal ulcer and the like to suppress secretion and actions of gastric acid. In other instances, prostaglandin E derivatives and the like are used to supplement prostaglandin E for inflammation induced by a non-steroidal anti-inflammatory drug, which inhibits PGE production.

On the other hand, the prophylaxis or treatment of inflammatory bowel disease in a narrow sense includes drug therapy, nutrition (diet) therapy and surgical therapy. For the drug therapy, 5-aminosalicylic acid preparations (pentasa, salazopyrin), steroids (prednisolone), immunosuppressants (azathiopurine, mercaptopurine and tacrolimus), anti-TNF-α antibodies (infliximab) and the like are used. [0003]

PG derivatives having a tetrazole group instead of the carboxy group at C-1 of prostaglandin (hereinafter to be referred to as PG) have been reported in the following patent documents 1, 2, and non-patent document 1.

Furthermore, 7,7-difluoro PGI2 analogs and manufacturing methods thereof have been reported (patent documents 3 and 4). In addition, 7,7-difluoro PGI2 analogs are described to be useful as prophylactic or therapeutic agents for cardiovascular diseases. [prior art documents]

[patent documents]

[0004]
patent document 1: DE 2405255
patent document 2: WO 03/103664
Problems to be Solved by the Invention

The present invention aims to provide a novel prostaglandin I₂ derivative, which is different from the known PGI₂ analogs as described above, or a pharmaceutically acceptable salt thereof.

Means of Solving the Problems

In an attempt to solve the aforementioned problems, the present inventors have synthesized novel PG analogs conferred with particular properties of fluorine atom and conducted studies to clarify the property and physiological activity thereof. As a result, the inventors have found that a novel 7,7-difluoro PGI₂ derivative, wherein the carboxy group at C-1 of the prostanoic acid skeleton is substituted by a tetrazole group and two fluorine atoms are bonded, is excellent in the property and pharmacological action, and that it is an excellent chemical as a medicament, which resulted in the completion of the present invention.

As far as the present inventors know, PGI₂ analogs having a tetrazole group instead of the carboxy group at C-1 of PG have not been published, further, synthetic examples, property, physiological activity and the like of PGI₂ analogs, wherein C-1 of PG is a tetrazole group and two fluorine atoms are present at C-7 of PG, have not been published at all.

Therefore, the present invention provides a 7,7-difluoro PGI₂ derivative represented by the following formula (1), a pharmaceutically acceptable salt thereof, and a medicament containing the same as an active ingredient.
wherein $R^1$ and $R^2$ are each independently a hydrogen atom or a straight chain alkyl group having a carbon number of 1 to 3, and $R^3$ is a hydrogen atom, an alkyl group having a carbon number of 1 to 4, an alkoxyalkyl group, an aryl group, a halogen atom or a haloalkyl group.
The present invention as claimed herein is described in the following items 1 to 24:

1. A prostaglandin I₂ derivative represented by the formula (1) or a pharmaceutically acceptable salt thereof.

wherein R¹ and R² are each independently a hydrogen atom or a straight chain alkyl group having a carbon number of 1 to 3, and R³ is a hydrogen atom, an alkyl group having a carbon number of 1 to 4, an alkoxyalkyl group, an aryl group, a halogen atom or a haloalkyl group.

2. The prostaglandin I₂ derivative according to item 1, wherein R¹ is a methyl group, or a pharmaceutically acceptable salt thereof.

3. The prostaglandin I₂ derivative according to item 1 or 2, wherein R³ is a methyl group, or a pharmaceutically acceptable salt thereof.

4. The prostaglandin I₂ derivative according to any one of items 1-3, wherein R² is a hydrogen atom, or a pharmaceutically acceptable salt thereof.
5. The prostaglandin \( \text{I}_2 \) derivative according to any one of items 1-4, wherein \( R^1 \) is a methyl group, and \( R^2 \) is a hydrogen atom, or a pharmaceutically acceptable salt thereof.

6. The prostaglandin \( \text{I}_2 \) derivative according to any one of items 1-5, wherein \( R^3 \) is a \( m \)-methyl group, or a pharmaceutically acceptable salt thereof.

7. The prostaglandin \( \text{I}_2 \) derivative according to item 1, wherein \( R^1 \) is a methyl group, \( R^2 \) is a hydrogen atom, and \( R^3 \) is a methyl group, or a pharmaceutically acceptable salt thereof.

8. The prostaglandin \( \text{I}_2 \) derivative according to item 1, wherein \( R^1 \) is a hydrogen atom, \( R^2 \) is a methyl group, and \( R^3 \) is a methyl group, or a pharmaceutically acceptable salt thereof.

9. A medicament comprising the prostaglandin \( \text{I}_2 \) derivative according to any one of items 1-8, or a pharmaceutically acceptable salt thereof as an active ingredient.

10. A method for the prophylaxis or treatment of a disease of the digestive tract in a patient, comprising administering to the patient an effective amount of the prostaglandin \( \text{I}_2 \) derivative according to any one of items 1-8, or a pharmaceutically acceptable salt thereof.

11. The method according to item 10, wherein the disease of the digestive tract is an inflammatory disease of the digestive tract.

12. The method according to item 11, wherein the inflammatory disease of the digestive tract is an inflammatory bowel disease.
13. The method according to item 12, wherein the inflammatory bowel disease is ulcerative colitis or Crohn’s disease.

14. The method according to item 12, wherein the inflammatory bowel disease is intestinal Behcet’s disease or simple ulcer.

15. The method according to item 10, wherein the disease of the digestive tract is ulcerative disease of the digestive tract.

16. The method according to item 15, wherein the ulcerative disease of the digestive tract is gastritis or gastric ulcer.

17. The method according to item 16, wherein the gastritis or gastric ulcer is drug-induced gastritis or gastric ulcer.

18. The method according to item 17, wherein the drug-induced gastritis or gastric ulcer is induced by a non-steroidal anti-inflammatory drug.

19. The method according to item 16, wherein the gastritis or gastric ulcer is induced by alcohol.

20. The method according to item 15, wherein the ulcerative disease of the digestive tract is small intestinal ulcer.

21. The method according to item 20, wherein the small intestinal ulcer is a drug-induced small intestinal ulcer.

22. The method according to item 21, wherein the drug-induced small intestinal ulcer is induced by a non-steroidal anti-inflammatory drug.
23. The method according to item 20, wherein the small intestinal ulcer is induced by alcohol.

24. Use of the prostaglandin \( \text{I}_2 \) derivative according to any one of items 1-8, or a pharmaceutically acceptable salt thereof, in the preparation of a medicament for the prophylaxis or treatment of a disease of the digestive tract.

Effect of the Invention

The novel 7,7-difluoro \( \text{PGI}_2 \) derivative afforded by the present invention can provide a medicament which maintains blood concentration for a long time and exhibits a pharmacological action by parenteral administration or oral administration, and which is for the prophylaxis or treatment of inflammation of the digestive tract or the onset of diarrhea or blood feces in inflammatory bowel disease, or for the prophylaxis or treatment of gastritis or ulcer in gastric ulcer, small intestinal ulcer and the like.

BRIEF DESCRIPTION OF THE DRAWINGS

[Fig. 1] shows the effect on abnormal stool in mouse DSS colitis model.

[Fig. 2] shows the effect on colon shortening in mouse DSS colitis model.

[Fig. 3] shows the effect on abnormal stool in rat DSS colitis model.
[Fig. 4] shows the effect on colon shortening in rat DSS colitis model.

[Fig. 5] shows the effect on colonic tissue injury in rat DSS colitis model.

[Fig. 6] shows the effect on abnormal stool in remission/relapse model of mouse DSS colitis.

[Fig. 7] shows the effect on stool consistency score in mouse T cell transfer model of colitis.

[Fig. 8] shows the effect on fecal occult blood score in mouse T cell transfer model of colitis.

[Fig. 9] shows the effect on body weight decrease score in mouse T cell transfer model of colitis.

[Fig. 10] shows the effect on DAI score in mouse T cell transfer model of colitis.

[Fig. 11] shows the effect on gastric ulcer in rat ethanol induced-gastric mucosal injury model.

[Fig. 12] shows the effect on small intestinal ulcer in rat indomethacin-induced small intestinal injury model.

[Embodiment of the Invention]

(Definition of the compound of the present invention)

In the nomenclature of the compounds in the present specification, the numbers used to show the position in PG skeleton correspond to the numbers in the prostanoic acid skeleton. In the present specification, a group in which a hydrogen atom of an alkyl group is substituted is also indicated as a substituted alkyl group. The same applies to other groups.

In addition, a "lower" organic group such as alkyl group and the like means that the carbon number thereof is not more than 6. The carbon number of the "lower" organic group is preferably not more than 4.

The "alkyl group" may be a straight chain or a branched chain. Unless otherwise specified, the alkyl group is
preferably a lower alkyl group having a carbon number of 1 to 6, and a lower alkyl group having a carbon number of 1 to 4 is particularly preferable. Examples of the alkyl group include a methyl group, an ethyl group, a propyl group, an isopropyl group, a butyl group, an isobutyl group, a sec-butyl group, a tert-butyl group, a pentyl group, a hexyl group and the like. [0014]

The "alkoxy group" is preferably a lower alkoxy group having a carbon number of 1 to 6, particularly preferably an alkoxy group having a carbon number of 1 to 4. The alkoxy group may be a straight chain or a branched chain. Examples of the alkoxy group include a methoxy group, an ethoxy group, a propoxy group, a butoxy group and the like. [0015]

The "alkoxyalkyl group" is an alkyl group substituted by an alkoxy group. The alkoxy group of the alkoxyalkyl group is preferably a lower alkoxy group having a carbon number of 1 to 4, and the alkyl group of the alkoxyalkyl group is preferably a lower alkyl group having a carbon number of 1 to 4. The alkoxyalkyl group is preferably a lower alkoxyalkyl group (that is, the carbon number of the whole alkoxyalkyl group is not more than 6), more preferably a lower alkoxyalkyl group having a carbon number of not more than 4. Examples of the alkoxyalkyl group include a methoxymethyl group, an ethoxymethyl group, a propoxymethyl group, an ethoxyethyl group and the like. [0016]

The "aryl group" is a monovalent aromatic hydrocarbon group optionally having substituent(s). As an aryl group without a substituent, a phenyl group is preferable. As the "substituted aryl group" (an aryl group having substituent(s)), an aryl group wherein one or more hydrogen atoms in the aryl group are substituted by a lower alkyl group, a halogen atom, a halogenated (lower alkyl) group, a lower alkoxy group and the like is preferable. Preferable examples
of the substituted aryl group include a substituted phenyl group, and particular examples thereof include a
monohalophenyl group (e.g., chlorophenyl group, fluorophenyl group, bromophenyl group etc.), a (halogenated lower alkyl)
substituted phenyl group (e.g., trifluoromethylphenyl group etc.) and a (lower alkoxy) phenyl group (e.g., methoxyphenyl
group, ethoxyphenyl group etc.).

The "halogen atom" is a fluorine atom, a chlorine atom, a
bromine atom or an iodine atom.

The "haloalkyl group" is an alkyl group wherein one or
more hydrogen atoms in the alkyl group are substituted by a
halogen atom, and preferred is a lower haloalkyl group having
a carbon number of 1 to 6. Examples of the haloalkyl group
include a fluoromethyl group, a difluoromethyl group, a
trifluoromethyl group, a trifluoroethyl group, a
pentafluoroethyl group, a chloromethyl group, a bromomethyl
group and the like.

As 7,7-difluoro PGI2 derivative represented by formula
(1) of the present invention (hereinafter, also referred as
PGI2 derivative (1) of the present invention), the following
compound is preferable from the aspects of pharmacological
activity and physical property.

That is, R1 and R2 are each independently a hydrogen atom
or a straight chain alkyl group having a carbon number of 1 to 3,
and each independently is preferably a hydrogen atom or a
methyl group. Particularly preferably, one of R1 and R2 is a
hydrogen atom, and the other is a methyl group.

R3 is a hydrogen atom, an alkyl group having a carbon
number of 1 to 4, an alkoxyalkyl group, an aryl group, a
halogen atom or a haloalkyl group, and a hydrogen atom, an
alkyl group having a carbon number of 1 to 4, a lower
alkoxyalkyl group such as a methoxymethyl group and the like,
a halogen atom such as a chlorine atom, a fluorine atom and
the like, or a lower haloalkyl group such as a lower fluoroalkyl group and the like is preferable. Particularly, a hydrogen atom, an alkyl group having a carbon number of 1 to 4, a chlorine atom or a haloalkyl group having a carbon number of 1 to 4 is preferable. As the alkyl group having a carbon number of 1 to 4, a methyl group and an ethyl group are preferable, and as the haloalkyl group having a carbon number of 1 to 4, a trifluoromethyl group is preferable.

As \( R^3 \), a hydrogen atom, a methyl group or a trifluoromethyl group is most preferable.

In addition, \( R^3 \) may be substituted at any of the ortho(o), meta(m) and para(p) positions relative to the position of substitution of the main chain of the prostaglandin skeleton by a benzene ring. \( R^3 \) is particularly preferably substituted at the meta(m) position.

[0019]
(Embodiment of preferable compound of the present invention)

In addition, preferable combinations of \( R^1 \), \( R^2 \) and \( R^3 \) in compound of the present invention are as follows.

\( R^1 \) is a hydrogen atom, \( R^2 \) is a hydrogen atom, and \( R^3 \) is a hydrogen atom.

\( R^1 \) is a hydrogen atom, \( R^2 \) is a hydrogen atom, and \( R^3 \) is a methyl group.

\( R^1 \) is a hydrogen atom, \( R^2 \) is a hydrogen atom, and \( R^3 \) is a chlorine atom.

\( R^1 \) is a hydrogen atom, \( R^2 \) is a hydrogen atom, and \( R^3 \) is a trifluoromethyl group.

\( R^1 \) is a methyl group, \( R^2 \) is a hydrogen atom, and \( R^3 \) is a hydrogen atom.

\( R^1 \) is a methyl group, \( R^2 \) is a hydrogen atom, and \( R^3 \) is a methyl group.

\( R^1 \) is a methyl group, \( R^2 \) is a hydrogen atom, and \( R^3 \) is a chlorine atom.

\( R^1 \) is a methyl group, \( R^2 \) is a hydrogen atom, and \( R^3 \) is a trifluoromethyl group.
R₁ is a hydrogen atom, R₂ is a methyl group, and R₃ is a hydrogen atom.
R₁ is a hydrogen atom, R₂ is a methyl group, and R₃ is a methyl group.
R₁ is a hydrogen atom, R₂ is a methyl group, and R₃ is a chlorine atom.
R₁ is a hydrogen atom, R₂ is a methyl group, and R₃ is a trifluoromethyl group.
R₁ is a methyl group, R₂ is a methyl group, and R₃ is a methyl group.
R₁ is a methyl group, R₂ is a methyl group, and R₃ is a chlorine atom.
R₁ is a methyl group, R₂ is a methyl group, and R₃ is a trifluoromethyl group.
Furthermore, preferable combinations from among those mentioned above are as follows.
R₁ is a methyl group, R₂ is a hydrogen atom, and R₃ is a methyl group.
R₁ is a methyl group, R₂ is a hydrogen atom, and R₃ is a methyl group.

[0020] (Production method of PGI₂ derivative of the present invention)

PGI₂ derivative (1) of the present invention can be produced, for example, based on the methods described in JP-A-07-324081 and JP-A-08-217772 relating to the inventions made by the present inventors. For example, using Corey lactone as a starting material, ω chain is introduced at first, and the lactone is converted by fluorination into ω chain-containing difluoro Corey lactone. Then, an α chain unit is introduced by an addition reaction with an organometallic reagent having a tetrazole group at the terminal and a dehydrating reaction, or Wittig reaction using a phosphonium salt having a tetrazole group at the terminal, and the like, and the hydroxyl group is
deprotected as necessary, whereby PGI₂ derivative (1) can be synthesized.

Alternatively, difluoro Corey lactone is obtained by fluorination from Corey lactone as a starting material. Then, an α chain unit is introduced by an addition reaction with an organometallic reagent having a tetrazole group at the terminal and a dehydrating reaction, or Wittig reaction using a phosphonium salt having a tetrazole group at the terminal, and the like, ω chain is introduced, and the hydroxyl group is deprotected as necessary, whereby PGI₂ derivative (1) can be synthesized.

Alternatively, PGI₂ derivative (1) can also be synthesized by converting a carboxy group of the carboxylic acid derivative described in JP-A-07-324081 to a cyano group and converting the derivative to a tetrazole derivative.

Of these production methods, representative methods are specifically explained using the following chemical formulas.
For example, using Corey lactone (7) as a starting material, ω chain is introduced at first, the obtained Corey lactone derivative (6) containing the ω chain is subjected to
a fluorination reaction to give a chain containing difluoro
Corey lactone derivative (3) having two fluorine atoms at the
α-position of the carbonyl group. Then, the difluorolactone
derivative (3) is reacted with phosphorane derivative (4) to
introduce an α chain unit, whereby PGI₂ derivative (2) with
protected hydroxyl groups can be obtained. The hydroxyl-
protecting group is removed to give PGI₂ derivative (1) of the
present invention.

The phosphorane derivative (4) can be obtained from a
phosphonium salt derivative (5).

Except when Rⁱ - R³ are particular substituents, the
above-mentioned lactone derivative (6) is a known compound.
The above-mentioned novel lactone derivative (6) wherein R¹ -
R³ are particular substituents can be produced by a method
similar to that of known lactone derivatives (6). For example,
novel lactone derivatives (6) can be produced by reacting 3-
aryl-2-oxoalkylphosphonic acid diester with Corey lactone
having a formyl group. Here, the alkyl chain of
alkylphosphonic acid has a carbon number of not less than 3.

R⁴, R⁵ and R⁷ are each independently a hydroxyl-protecting
group. R⁴, R⁵, and R⁷ may be same protecting groups. As the
protecting group, the hydroxyl-protecting group described in
“Shin Jikken Kagaku Koza (New Courses in Experimental
Chemistry) 14, synthesis and reaction of organic compound (V)”
(Maruzen Company, Limited), “Protective Groups in Organic
synthesis” (by T.W. Greene, J. Wiley & Sons) and the like can
be used. Specifically, a triorganosilyl group, an alkoxyalkyl
group, a monovalent group having a cyclic ether structure and
the like can be mentioned. As the triorganosilyl group, a
silyl group wherein 3 groups selected from an alkyl group, an
aryl group, an aralkyl group and alkoxy group are bonded to a
silicon atom is preferable, and a group wherein 3 lower alkyl
groups or aryl groups are bonded to a silicon atom is
particularly preferable. As specific examples of the
protecting group, a tetrahydropyranyl group, a tert-
butyldimethylsilyl group, a tert-butyldiphenylsilyl group, a
triethylsilyl group, a triphenylsilyl group or a
triisopropylsilyl group and the like are preferable.

Particularly, a tetrahydropyranyl group, a tert-
butyldimethylsilyl group, a tert-butyldiphenylsilyl group and
the like are preferable.

[0026]

The hydroxyl-protecting group can be removed easily. The
deprotection method of the protected hydroxyl group can be a
conventional method. For example, the methods described in
"Shin Jikken Kagaku Koza (New Courses in Experimental
Chemistry) 14 synthesis and reaction of organic compound (I),
(II) and (V)" (Maruzen Company, Limited), "Protective Groups
in Organic synthesis" (by T.W. Greene, J. Wiley & Sons) and
the like can be employed.

[0027]

For conversion of lactone derivative (6) to
difluorolactone derivative (3) by a fluorination reaction,
various known fluorination methods can be applied. For example,
a method including reacting with various electrophilic
fluorinating agents in an inert solvent can be employed. The
fluorination can also be performed by the methods described in
the present inventors.

In the fluorination reaction of lactone derivative (6),
an electrophilic fluorinating agent is preferably used. As the
electrophilic fluorinating agent, known or well known
electrophilic fluorinating agent can be used. For example, the
electrophilic fluorinating agents described in "Chemistry of
fluorine "(Kodansha Scientifics Ltd.) by Tomoya Kitazume,
Takashi Ishihara, and Takeo Taguchi and the like can be
mentioned. Specifically, N-fluorosulfonyl amides, N-sulfonyl
imide derivative, acetyl hypofluorite, fluorine gas and the
like can be mentioned.

14
The electrophilic fluorinating agent is preferably used in the presence of an inert solvent. As the inert solvent, ether solvents, hydrocarbon solvents, polar solvents, mixed solvents thereof and the like can be mentioned.

The difluorolactone derivative (3) obtained by the fluorination reaction is then reacted with phosphorane derivative (4) to give PGI₂ derivative (2) wherein the hydroxyl group is protected. The phosphorane derivative (4) is produced from the corresponding phosphonium salt derivative (5), in an inert solvent in the presence of a base, and the formed phosphorane derivative (4) is preferably used directly for the Wittig reaction with difluorolactone derivative (3) without isolation. As the production methods of phosphorane derivative (4) and phosphonium salt derivative (5), the methods described in DE2242239, DE2405255 and the like can be employed. As R₆ for phosphorane derivative (4) or phosphonium salt derivative (5), an aryl group such as a phenyl group, a tolyl group and the like is preferable, and a phenyl group is particularly preferable. As the inert solvent, ether solvents, hydrocarbon solvents, polar solvents, aqueous solvents, alcoholic solvents, mixed solvents thereof and the like can be mentioned.

The hydroxyl-protecting group is removed from the PGI₂ derivative (2) with protected hydroxyl groups obtained by the above method to give PGI₂ derivative (1).

Since PGI₂ derivative (1) of the present invention has an asymmetric carbon in the structure, various stereoisomers and optical isomers are present. The present invention encompasses all of such stereoisomers, optical isomers, and mixtures thereof.

Specific examples of PGI₂ derivative (1) of the present invention include the compound represented by the following formula (8).
(Examples of PGI₂ derivative (1) of the present invention)

A compound wherein, in the formula (8), R¹, R², and R³ have structures shown in the following Table 1 can be mentioned.

[Table 1]

<table>
<thead>
<tr>
<th>Compound</th>
<th>R¹</th>
<th>R²</th>
<th>R³</th>
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<tr>
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<tr>
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<td>H</td>
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</table>

(Features of PGI₂ derivative (1) of the present invention)

PGI₂ derivative (1) of the present invention is the
derivative which is not easily metabolized in the body and has improved stability. Since the carboxy group of the PG skeleton is converted to a tetrazole group, it is not easily metabolized by β-oxidation, which is known as a common metabolic pathway of fatty acid such as prostaglandins. Therefore, it has a prolonged plasma half-life and can maintain an effective plasma concentration for a long time, as compared to a compound having a carboxy group of the PG skeleton. Since the metabolic stability is improved in this way, the bioavailability of drugs can be improved.

[0034]
(Medicament containing PGI₂ derivative (1) of the present invention or a pharmaceutically acceptable salt thereof as active ingredient)

The medicament of the present invention contains PGI₂ derivative (1) and/or a pharmaceutically acceptable salt of PGI₂ derivative (1), and further, a pharmaceutically acceptable carrier and, in some cases, other treatment components.

The medicament of the present invention contains PGI₂ derivative (1) and/or a pharmaceutically acceptable salt of PGI₂ derivative (1), or a hydrate thereof, and further, a pharmaceutically acceptable carrier and, in some cases, other treatment components.

[0035]

When the prophylactic or therapeutic agent of the present invention is administered to patients, the daily dose varies depending on the age and body weight of patients, pathology and severity and the like. Generally, 0.0001 - 10 mg, preferably 0.01 - 1 mg, of the agent is desirably administered in one to several portions. For example, for oral administration, 0.001 - 3 mg is preferable, and 0.01 - 0.5 mg is particularly preferable. For intravenous administration, 0.0001 - 1 mg is preferable, and 0.001 - 0.1 mg is particularly preferable. The dose can be changed as appropriate depending on the disease and its condition. As the
dosing regimen, an injection product of the agent may be desirably administered by continuous drip infusion.

For use as a medicament, the agent can be administered to the body by oral administration and parenteral administration (e.g., intravascular (intravenous, intraarterial) administration, subcutaneous administration, rectal administration etc.). Examples of the dosage form include oral dosage form such as tablet, capsule and syrup, parenteral dosage form such as liquid injection (solution, emulsion, suspension and the like), infusion, suppositories, nasal preparations, patches and inhalations. Oral dosage is particularly desirable.

A preparation in the aforementioned dosage form can be produced by mixing PGI$_2$ derivative (1) of the present invention or a pharmaceutically acceptable salt thereof with additives necessary for formulation such as conventional carriers, excipients, binders and stabilizers, and formulating the mixture in a conventional method. For example, when the preparation is a powder, granule, tablet and the like, it can be produced by using any pharmaceutical carriers preferable for producing a solid dosage form, for example, excipients, lubricants, disintegrants, binders and the like.

These excipient may be, for example, inert diluents, such as calcium carbonate, sodium carbonate, lactose, calcium phosphate and sodium phosphate; granulating agent and disintegrant, such as cornstarch and alginic acid; binder, such as starch, gelatin and gum arabic, and lubricant, such as magnesium stearate, stearic acid and talc. The tablet may be uncoated or coated by a known technique to delay disintegration and absorption in the stomach and the intestine, thus ensuring a sustained release for a longer time. For example, a time delay material, such as glyceryl monostearate or glyceryl distearate may be used.
PGI₂ derivative (1) of the present invention may be provided as a hard gelatin capsule containing a mixture with an inert solid diluent, for example, calcium carbonate, calcium phosphate or kaolin. Alternatively, it may be provided as a soft gelatin capsule containing a mixture with a water miscible solvent, such as propylene glycol, polyethylene glycol and ethanol, or oils, such as peanut oil, liquid paraffin and olive oil.

When the preparation is syrup or liquid, stabilizers, suspending agents, corrigents, aromatic substances and the like may be appropriately selected and used for the production, for example. For injection manufacturing, an active ingredient is dissolved in distilled water for injection together with a pH adjuster such as hydrochloric acid, sodium hydroxide, lactose, sodium lactate, acetic acid, disodium hydrogen phosphate and sodium dihydrogen phosphate, and an isotonic agent such as sodium chloride and glucose, and injection is aseptically prepared. An inactive nonaqueous diluent such as propylene glycol, polyethylene glycol, olive oil, ethanol and polysorbate 80 may be used for formulation of the preparation. Moreover, mannitol, dextrin, cyclodextrin, gelatin and the like may be added, and the mixture is freeze-dried in vacuo to give an injection to be dissolved before use. For stabilization and improvement of drug delivery to a lesion, moreover, a liposome preparation or a lipid emulsion may be formulated by a known method and used as an injection.

In addition, a rectal dosage preparation may be produced by using a suppository base, such as cacao butter, fatty acid triglyceride, fatty acid diglyceride, fatty acid monoglyceride and polyethylene glycol. Furthermore, a water-soluble base, such as polyethylene glycol, polypropylene glycol, glycerol and glycerolgelatin, an oily base, such as white petrolatum,
hard fat, paraffin, liquid paraffin, Plastibase, lanolin and purified lanolin, and the like may be used to adjust the preparation to suitable viscosity and ointment for intrarectal administration can also be produced.

5 [0041]

PGI₂ derivative (1) of the present invention or a pharmaceutically acceptable salt thereof can be administered topically to the skin or mucous membrane, i.e., transdermal or transmucosal administration. As general dosage forms for this purpose, gel, hydrogel, lotion, solution, cream, ointment, sprays, dressing agent, foam preparation, film, skin patch, oblate, implant, sponge, fiber, bandage, microemulsion and the like can be mentioned. As commonly-used carriers, alcohol, water, mineral oil, liquid paraffin, white petrolatum, glycerol, polyethylene glycol, propylene glycol and the like can be mentioned.

[0042]

PGI₂ derivative (1) of the present invention can be mixed with cyclodextrin or an appropriate derivative thereof or a soluble polymer such as polyethylene glycol-containing polymer, for the purpose of use in any of the aforementioned dosage forms, and improving solubility, dissolution rate, bioavailability and stability. For example, drug-cyclodextrin complex and the like have been confirmed to be generally useful for most dosage forms and administration routes. Both inclusion and non-inclusion complexes can be used. As another method for direct complexation with drugs, cyclodextrin can also be used as an auxiliary additive, i.e., carrier, excipient or solubilizer. For these purposes, α-, β- and γ-cyclodextrins and the like are generally used.

[0043]

(Pharmaceutically acceptable salt of PGI₂ derivative of the present invention)

A pharmaceutically acceptable salt of PGI₂ derivative (1) of the present invention is a salt of the moiety of the
tetrazole group of the derivative with a basic substance, which is a compound wherein the hydrogen atom of the tetrazole group is substituted by cation.

Examples of the cation include alkali metal cations such as Na⁺ and K⁺, metal cations (other than alkali metal cations) such as 1/2 Ca²⁺, 1/2 Mg²⁺, 1/2 Zn²⁺ and 1/3 Al³⁺, NH₄⁺, ammonium cations of organic amine and amino acid such as triethanolamine, diethanolamine, ethanolamine, tromethamine, lysine and arginine, and the like. Preferable cation is sodium ion or potassium ion.

[0044]

More particularly, the acceptable salt is a salt produced from a pharmaceutically acceptable nontoxic base such as inorganic base and organic base. As a salt derived from the pharmaceutically acceptable nontoxic inorganic base, lithium salt, copper salt, ferric salt, ferrous salt, manganic salt, manganese salt and the like can be mentioned in addition to the aforementioned sodium salt, potassium salt, calcium salt, magnesium salt, zinc salt, aluminum salt, ammonium salt and the like. Of these, sodium salt, potassium salt, calcium salt, magnesium salt and ammonium salt are preferable, and sodium salt and potassium salt are particularly preferable. A salt derived from a pharmaceutically acceptable nontoxic organic base includes salts with primary, secondary and tertiary amine, substituted amine including naturally occurring substituted amine, cyclic amine, and basic ion exchange resin. Other than the examples of the aforementioned organic amine and amino acid, isopropylamine, diethylamine, triethylamine, trimethylamine, tripropylamine, ethylenediamine, N,N'-dibenzylethylenediamine, 2-diethylaminoethanol, 2-dimethylaminoethanol, morpholine, N-ethyl-morpholine, piperazine, piperidine, N-ethylpiperidine, betaine, caffeine, choline, glucamine, glucosamine, histidine, Hydrabamine, methyl glucamine, polyamine resin, procaine, purine, theobromine and the like can be mentioned.
[0045]
(Use of medicament containing PGI₂ derivative of the present invention or a pharmaceutically acceptable salt thereof as an active ingredient)

A medicament containing PGI₂ derivative (1) of the present invention or a pharmaceutically acceptable salt thereof as an active ingredient shows excellent effects as a drug for diseases of the digestive tract.

The disease of the digestive tract in the present invention includes inflammatory disease and ulcerative disease of the digestive tract, which is a disease with inflammation or ulcer in the epithelial, mucosal or submucosal tissues of the digestive tract, or abnormal proliferation or dysfunction of mucosal epithelium, and which is caused by physical stimuli, chemical stimuli such as by gastric juice, stimuli by drug such as non-steroidal anti-inflammatory drugs and steroids, immune diseases and autoimmune diseases of unknown etiology, mental diseases and the like.

The inflammatory disease of the digestive tract includes inflammatory bowel disease, particularly ulcerative colitis, Crohn's disease, which is a non-specific granulomatous inflammatory disease accompanied by fibrillization or ulceration, intestinal Behçet's disease and simple ulcer. The ulcerative disease of the digestive tract of the present invention includes stomatitis, aphthous stomatitis, esophagitis, esophageal ulcer, gastritis, gastric ulcer and small intestinal ulcer.

Moreover, gastritis and gastric ulcer include drug-induced gastritis, gastric ulcer, alcoholic gastritis and gastric ulcer, and the drug-induced gastritis and gastric ulcer include gastritis and gastric ulcer induced by a non-steroidal anti-inflammatory drug.

Small intestinal ulcer includes drug-induced small intestinal ulcer and alcoholic small intestinal ulcer, and the drug-induced small intestinal ulcer includes small intestinal
ulcer induced by a non-steroidal anti-inflammatory drug.

Particularly, the medicament of the present invention is useful as a prophylactic or therapeutic agent for ulcerative colitis, Crohn’s disease, gastritis, gastric ulcer or small intestinal ulcer.

The present invention is explained in detail in the following by referring to specific examples, which are not to be construed as limiting.

**Example 1**

**Synthesis of methyl (2R)-2-(m-tolyl)propionate**

To (2R)-2-(m-tolyl)propionic acid (12.45 g) were added methanol (14.83 g) and concentrated sulfuric acid (6.46 g), and the mixture was stirred under refluxing for 6 hr. Then, the mixture was neutralized with 10% aqueous sodium carbonate solution, and extracted with hexane. After drying over magnesium sulfate, the residue was concentrated under reduced pressure to give the title compound (12.79 g). The structural property was as described below.

**[0048]**

\[ ^1H-NMR(CDCl_3): \delta 1.49(d, J=7.0\ Hz, 3H), 2.33(s, 3H), 3.64(s, 3H), 3.69(dd, J=14.4, 7.3\ Hz, 1H), 7.06-7.22(m, 4H). \]

**Example 2**

**Synthesis of dimethyl (3R)-2-oxo-3-(m-tolyl)butylphosphonate**

To dimethyl methylphosphonate (1.97 g) was added tetrahydrofuran (THF) (25 mL), and the mixture was cooled to -78°C. n-Butyllithium (1.5 M hexane solution) (10 mL) was added, and the mixture was stirred for 1 hr. Then, a solution of methyl ester {methyl (2R)-2-(m-tolyl)propionate} synthesized in Example 1 (1.34 g) in THF (3.8 mL) was added at -78°C, and the mixture was stirred for 2 hr. The reaction was quenched with 25 mL of saturated aqueous sodium hydrogen carbonate, and the mixture was extracted with ethyl acetate. The extract was
dried over magnesium sulfate, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (hexane/ethyl acetate 5:1 - 1:5) to give the title compound (1.63 g). The structural property was as described below.

[Example 3]

Sodium hydride (55%) (8.75 g) was dispersed in 1,2-dimethoxyethane (DME) (300 mL) and the mixture was ice-cooled. A solution of phosphonate (dimethyl (3R)-2-oxo-3-(m-tolyl)butylphosphonate) (54.7 g) synthesized in Example 2 in DME (50 mL) was added, and the mixture was stirred for 1 hr. To the above-mentioned solution was added a solution of (1S,5R,6R,7R)-6-formyl-7-benzoyloxy-2-oxabicyclo[3.3.0]octan-3-one (50.0 g) in DME (400 mL), and the mixture was stirred for 1 hr. The reaction was quenched with 350 mL of 10% brine, and the mixture was extracted with ethyl acetate. The extract was dried over magnesium sulfate, and the residue was concentrated under reduced pressure. The concentrated crude product was recrystallized from t-butyl methyl ether to give the title compound (64.7 g). The structural property was as described below.

[0052]

$^{1}$H-NMR (CDCl$_3$): $\delta$ 1.39 (d, J=6.7 Hz, 3H), 2.34 (s, 3H), 2.84 (dd, J=22.3, 14.1, 0.6 Hz, 1H), 3.18 (dd, J=22.3, 14.1 Hz, 1H), 3.76 (dd, J=19.3, 11.1 Hz, 6H), 4.00 (dd, J=13.8, 7.0 Hz, 1H), 7.01-7.24 (m, 4H).
[Example 4]

Synthesis of (1S,5R,6R,7R)-6-[(1E,3R,4R)-3-hydroxy-4-(m-tolyl)-1-pentenyl]-7-benzoyloxy-2-oxabicyclo[3.3.0]octan-3-one

A solution of enone{(1S,5R,6R,7R)-6-[(1E,4R)-3-oxo-4-(m-tolyl)-1-pentenyl]-7-benzoyloxy-2-oxabicyclo[3.3.0]octan-3-one} (147.0 g) synthesized in Example 3 in THF (1480 mL) was cooled to \(-40^\circ\text{C}\), (-)-B-chlorodiisopinocampheylborane (1.7 M hexane solution) (721 mL) was added, and the mixture was stirred under ice-cooling for 20 hr. Acetone (183 mL) was added and the mixture was stirred for 3 hr. Aqueous sodium hydrogen carbonate was added, and the mixture was extracted with t-butyl methyl ether. The extract was dried over magnesium sulfate, and concentrated under reduced pressure to give a crude title compound (649.9 g).

[Example 5]

Synthesis of (1S,5R,6R,7R)-6-[(1E,3R,4R)-3-hydroxy-4-(m-tolyl)-1-pentenyl]-7-hydroxy-2-oxabicyclo[3.3.0]octan-3-one

The crude alcohol, {(1S,5R,6R,7R)-6-[(1E,3R,4R)-3-hydroxy-4-(m-tolyl)-1-pentenyl]-7-benzoyloxy-2-oxabicyclo[3.3.0]octan-3-one} (649.9 g) synthesized in Example 4 was dissolved in methanol (740 mL), potassium carbonate (116.3 g) was added, and the mixture was stirred at room temperature for 17 hr. Acetic acid was added to adjust to pH 7, methanol was evaporated, water was added, and the mixture was extracted with ethyl acetate. The extract was purified by silica gel column chromatography (hexane/ethyl acetate=4/1 - 0/1) to give the title compound (22.3 g). The structural property was as described below.

[0055]

\(^1\text{H-NMR}(\text{CDCl}_3): \delta 1.33 (d, J=7.0 \text{ Hz}, 3\text{H}), 1.70 (s, 1\text{H(OH)}), 1.86 (ddd, J=11.3, 7.8, 3.2 \text{ Hz}, 1\text{H}), 2.07 (d, J=4.4 \text{ Hz}, 1\text{H(OH)}), 2.13-2.23 (m, 2\text{H}), 2.34 (s, 3\text{H}), 2.35-2.44 (m, 3\text{H}), 2.47 (d, J=3.8 \text{ Hz}, 1\text{H}), 2.56 (dd, J=18.2, 9.7 \text{ Hz}, 1\text{H}), 2.80 (q, J=7.0 \text{ Hz}, 1\text{H}),
3.79–3.85 (m, 1H), 4.12–4.16 (m, 1H), 4.81 (dt, J=7.0, 3.2 Hz, 1H), 5.27 (ddd, J=15.7, 8.5, 0.6 Hz, 1H), 5.50 (dd, J=15.2, 6.8 Hz, 1H), 6.94–7.20 (m, 4H).

[Example 6]

Synthesis of (1S,5R,6R,7R)-6-[(1E,3R,4R)-3-t-butyldimethylsiloxy-4-(m-tolyl)-1-pentenyl]-7-t-butyldimethylsiloxy-2-oxabicyclo[3.3.0]octan-3-one

To a solution of the diol, {(1S,5R,6R,7R)-6-[(1E,3R,4R)-3-hydroxy-4-(m-tolyl)-1-pentenyl]-7-hydroxy-2-oxabicyclo[3.3.0]octan-3-one} (988 mg) synthesized in Example 5 in N,N-dimethylformamide (DMF) (10 mL) were added at room temperature t-butyldimethylsilyl chloride (1.17 g) and imidazole (1.08 g), and the mixture was stirred for 2.5 hr.

The reaction mixture was poured into saturated aqueous sodium hydrogen carbonate, and the mixture was extracted with hexane/ethyl acetate=2/1 mixture. The extract was dried over magnesium sulfate, concentrated under reduced pressure and purified by silica gel column chromatography (hexane/ethyl acetate 20:1 - 10:1) to give the title compound (1.56 g). The structural property was as described below.

[Example 7]

Synthesis of (1S,5R,6R,7R)-6-[(1E,3R,4R)-3-t-butyldimethylsiloxy-4-(m-tolyl)-1-pentenyl]-7-t-butyldimethylsiloxy-2-oxa-4,4-difluoro-bicyclo[3.3.0]octan-3-one

Tetrahydrofuran (THF) (19 mL) was added to manganese
bromide (1.48 g) and N-fluorobenzenesulfonimide (2.48 g), and
the mixture was stirred for 30 min, and cooled to -78°C. A
solution of the lactone, {{(1S,5R,6R,7R)-6-[(1E,3R,4R)-3-t-
butyldimethylsiloxy-4-(m-tolyl)-1-pentenyl]-7-t-
butyldimethylsiloxy-2-oxabicyclo[3.3.0]octan-3-one} (0.5 g)
synthesized in Example 6 in THF (5 mL) was added, a solution
(0.5 M, 13 mL) of potassium bis(trimethylsilyl)amide in
toluene was added and the mixture was warmed to 0°C over 3.5 hr.
The reaction mixture was poured into saturated aqueous sodium
hydrogen carbonate, and the mixture was extracted with
hexane/ethyl acetate=1/1 mixture. The extract was dried over
magnesium sulfate, concentrated under reduced pressure and
purified by silica gel column chromatography (hexane/ethyl acetate 20:1) to give the title compound (0.32 g). The
structural property was as described below.

1H-NMR(CDCl3):δ-0.08-0.03(m, 12H), 0.82(s, 9H), 0.89(s, 9H),
1.28(d, J=7.0 Hz, 3H), 1.70-1.77(m, 1H), 1.96-2.04(m, 1H),
2.31(s, 3H), 2.60-2.91(m, 3H), 3.82-3.87(m, 1H), 3.99-4.23(m,
1H), 5.00(t, J=6.4 Hz, 1H), 5.06(dd, J=15.7, 7.8 Hz, 1H),
5.33(ddd, J=15.9, 6.7, 1.2 Hz, 1H), 6.88-7.16(m, 4H).

[Example 8]

31F-NMR(CDCl3): -113.1(d, J=279.3 Hz), -91.0(dd, J=279.3, 25.9
Hz).

Synthesis of 4-[(Z)-(1S,5R,6R,7R)-6-[(1E,3R,4R)-3-t-
butyldimethylsiloxy-4-(m-tolyl)-1-pentenyl]-7-t-
butyldimethylsiloxy-2-oxa-4,4-difluoro-bicyclo[3.3.0]octan-3-
ylidene]-1-(tetrazol-5-yl)butane

To a suspension of 4-(tetrazol-5-
yl)butyltriphenylphosphonium bromide (14.0 g) in toluene (390
mL) was added a solution (0.5M, 120 mL) of potassium
bis(trimethylsilyl)amide in toluene, and the mixture was
stirred at 60°C for 1 hr. A solution of the difluorolactone,
{{(1S,5R,6R,7R)-6-[(1E,3R,4R)-3-t-butyldimethylsiloxy-4-(m-
tolyl)-1-pentenyl]-7-t-butyldimethylsiloxy-2-oxa-4,4-difluoro-
bicyclo[3.3.0]octan-3-one} synthesized in Example 7 (4.32 g)
in toluene (130 mL) was added at -10°C, and the mixture was
stirred for 18 hr while warming the mixture to room
temperature. Aqueous sodium hydrogen carbonate was added to
quench the reaction, and the mixture was extracted with
hexane/ethyl acetate=1/1 mixture. The extract was dried over
magnesium sulfate, concentrated under reduced pressure and
purified by silica gel column chromatography (hexane/ethyl
acetate=5/1 - 0/1) to give the title compound (4.1 g). The
structural property was as described below.

{[0061]

^1^H-NMR(CDCl$_3$): δ-0.14-0.01(m, 12H), 0.82(s, 9H), 0.89(s, 9H),
1.23-1.27(m, 3H), 1.82-2.09(m, 5H), 2.21-2.28(m, 1H), 2.31(s,
3H), 2.45-2.53(m, 1H), 2.64-2.73(m, 2H), 2.93-2.97(m, 1H),
3.90(dd, J=11.7, 5.3 Hz, 1H), 4.08-4.09(m, 1H), 4.84-4.87(m,
2H), 5.27(dd, J=15.5, 7.8 Hz, 1H), 5.44(dd, J=15.6, 6.2 Hz,
1H), 6.92-7.16(m, 4H).

^{19^F-NMR(CDCl$_3$):} -112.3(d, J=253.4 Hz), -81.4(dd, J=253.4, 18.7
Hz).

[Example 9]

[0062]

Synthesis of 4-{(Z)-(1S,5R,6R,7R)-6-{[(1E,3R,4R)-3-hydroxy-4-
(m-tolyl)-1-pentenyl]-7-hydroxy-2-oxa-4,4-difluoro-
bicyclo[3.3.0]octan-3-ylidene}-1-(tetrazol-5-yl)butane

THF (81 mL), water (81 mL) and acetic acid (244 mL) were
added to the compound (4.1 g) synthesized in Example 8, and
the mixture was stirred at 35°C for 46 hr. Water (500 mL) was
added and the mixture was extracted with chloroform. The
extract was dried over magnesium sulfate, concentrated under
reduced pressure and purified by silica gel column
chromatography (hexane/ethyl acetate=1/5 - 0/1) and
recrystallized from diethyl ether to give the title compound
(1.1 g). The structural property was as described below.

[0063]

28
Synthesis of dimethyl 2-oxo-3-(m-tolyl)butylphosphonate

Using racemate of 2-(m-tolyl)propionic acid and in the same manner as in the method of Examples 1 - 2, the title compound was synthesized. The structural property was as described below.

Synthesis of (1S,5R,6R,7R)-6-[(1E,3R,4RS)-3-t-butyl(dimethyl)siloxy-4-(m-tolyl)-1-pentenyl]-7-t-butyl(dimethyl)siloxy-2-oxabicyclo[3.3.0]octan-3-one

Using racemate of dimethyl 2-oxo-3-(m-tolyl)butylphosphonate and in the same manner as in the method of Examples 3 - 6, the title compound was synthesized. The structural property was as described below.
Synthesis of (1S,5R,6R,7R)-6-[(1E,3R,4RS)-3-t-butyldimethylsiloxy-4-(m-tolyl)-1-pentenyl]-7-t-butyldimethylsiloxy-2-oxa-4,4-difluoro-bicyclo[3.3.0]octan-3-one

Using (1S,5R,6R,7R)-6-[(1E,3R,4RS)-3-t-butyldimethylsiloxy-4-(m-tolyl)-1-pentenyl]-7-t-butyldimethylsiloxy-2-oxabicyclo[3.3.0]octan-3-one synthesized in Example 11 and in the same manner as in the method of Example 7, the title compound was synthesized. The structural property was as described below.

1H-NMR (CDCl₃): 6 -0.20-0.05 (m, 12H), 0.80-0.90 (m, 18H), 1.19-1.29 (m, 3H), 1.70-2.10 (m, 2H), 2.31 (s, 3H), 2.60-3.05 (m, 3H), 3.84-4.12 (m, 2H), 4.95-5.50 (m, 3H), 6.85-7.20 (m, 4H). 19F-NMR (CDCl₃): -113.6 - -112.8 (m), -91.7 - -90.6 (m).

Synthesis of 4-[(Z)-(1S,5R,6R,7R)-6-[(1E,3R,4RS)-3-t-butyldimethylsiloxy-4-(m-tolyl)-1-pentenyl]-7-t-butyldimethylsiloxy-2-oxa-4,4-difluoro-bicyclo[3.3.0]octan-3-ylidene]-1-(tetrazol-5-yl)butane

Using (1S,5R,6R,7R)-6-[(1E,3R,4RS)-3-t-butyldimethylsiloxy-4-(m-tolyl)-1-pentenyl]-7-t-butyldimethylsiloxy-2-oxa-4,4-difluoro-bicyclo[3.3.0]octan-3-one synthesized in Example 12 and in the same manner as in the method of Example 8, the title compound was synthesized. The structural property was as described below.

1H-NMR (CDCl₃): 6 -0.15-0.05 (m, 12H), 0.80-0.89 (m, 18H), 1.20-1.28 (m, 3H), 1.80-3.05 (m, 14H), 3.90-4.15 (m, 2H), 4.85-4.95 (m, 2H), 5.23-5.58 (m, 2H), 6.90-7.20 (m, 4H). 19F-NMR (CDCl₃): -113.0 - -111.3 (m), -82.0 - -80.7 (m).
Synthesis of 4-[(Z)-(1S,5R,6R,7R)-6-[(1E,3R,4RS)-3-hydroxy-4-(m-tolyl)-1-pentenyl]-7-hydroxy-2-oxa-4,4-difluoro-bicyclo[3.3.0]octan-3-ylidene]-1-(tetrazol-5-yl)butane

Using 4-[(Z)-(1S,5R,6R,7R)-6-[(1E,3R,4RS)-3-butyldimethylsiloxy-4-(m-tolyl)-1-pentenyl]-7-t-butyldimethylsiloxy-2-oxa-4,4-difluoro-bicyclo[3.3.0]octan-3-ylidene]-1-(tetrazol-5-yl)butane synthesized in Example 13 and in the same manner as in the method of Example 9, the title compound was synthesized. The structural property was as described below.

[0073]

\[ \text{IH-NMR (CDCl}_3\text{):} \delta 1.15-1.35 (m, 3H), 1.80-3.00 (m, 11H), 2.29 (s, 3H), 4.05-4.20 (m, 2H), 4.75-4.85 (m, 2H), 5.35-5.70 (m, 2H), 6.95-7.25 (m, 4H). \]

\[ \text{19F-NMR (CDCl}_3\text{):} -114.5 - -112.7 (m), -83.5 - -81.8 (m). \]

[Example 15]

[0074]

Synthesis of 5-[(Z)-(1S,5R,6R,7R)-6-[(1E,3R,4RS)-3-hydroxy-4-(m-tolyl)-1-pentenyl]-7-hydroxy-2-oxa-4,4-difluoro-bicyclo[3.3.0]octan-3-ylidene]pentanoic acid (carboxylate form)

Using (1S,5R,6R,7R)-6-[(1E,3R,4RS)-3-t-butyldimethylsiloxy-4-(m-tolyl)-1-pentenyl]-7-t-butyldimethylsiloxy-2-oxa-4,4-difluoro-bicyclo[3.3.0]octan-3-one synthesized in Example 12 and (4-carboxybutyl)triphenylphosphonium bromide, and in the same manner as in the method of Examples 8 - 9, the title compound was synthesized. The structural property was as described below.

[0075]

\[ \text{IH-NMR (CD}_3\text{OD):} \delta 1.17-1.30 (m, 3H), 1.63-2.79 (m, 11H), 2.29 (s, 3H), 3.75-4.12 (m, 2H), 4.66-4.85 (m, 2H), 5.40-5.58 (m, 2H), 6.95-7.15 (m, 4H). \]

\[ \text{19F-NMR (CD}_3\text{OD):} -118.3 - -117.7 (d, J=250.4Hz), -86.1 - -85.3 (m). \]

[Example 16]
In vitro metabolic stability of the compound of the present invention

A mixture of the compound F and compound J of the present invention described in Table 1 (F:J = 52:41, synthesized in Example 14), and a mixture of compounds wherein the tetrazole groups at C-1 of compound F and compound J are respectively substituted by carboxylic acid (referred to as carboxylate form, F:J = 54:34, synthesized in Example 15) were tested.

First, a mitochondria fraction was prepared from the rat liver according to the following Reference A. Then, in reference to the method of YAMAGUCHI et al. described in the following References B and C, an NADPH-independent D-oxidation reaction was studied. The reaction was carried out at 37°C for 30 min, and stopped with a methanol solution containing a suitable internal standard substance. Each compound was quantified by the internal standard method using a high performance liquid chromatography mass spectrometry apparatus (LC-MS/MS). The compound residual ratio after metabolic reaction of compounds F, J and each carboxylate form thereof in rat mitochondria fraction is shown in the following Table 2 in average ± standard deviation of 3 experiments.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Residual ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compound F</td>
<td>91.6±6.8</td>
</tr>
<tr>
<td>Compound J</td>
<td>90.1±6.9</td>
</tr>
<tr>
<td>Carboxylate form of Compound F</td>
<td>27.8±2.2</td>
</tr>
<tr>
<td>Carboxylate form of Compound J</td>
<td>44.1±2.1</td>
</tr>
</tbody>
</table>

As is clear from the above-mentioned Table 2,
representative compound F and compound J of the present invention are not subject to $\beta$ oxidation in a mitochondria fraction.

[0079]

5 References


[Example 17]

[0080]

Plasma pharmacokinetics after intravenous administration to rats

To verify the in vivo metabolic stability of the compound of the present invention, plasma pharmacokinetics was evaluated after intravenous administration to rats. Male rats (6 weeks old, body weight 160 - 180 g) were acclimated for 1 week, and the animals diagnosed healthy were used. A mixture of compound F and compound J of the present invention described in Table 1 (F:J = 52:41), and a mixture of carboxylate forms of compound F and compound J (F:J = 54:34), and compound F (synthesized in Example 9) were dissolved in a small amount of ethanol and physiological saline was added to prepare test compound solutions. The test compound solutions were instantaneously administered intravenously at 1 mL/kg from the femoral vein of non-fasting rats under light ether anesthesia. Venous blood was drawn from the tail vein 5, 15, 30, 45, 60, 90 and 120 min after administration. The blood was mixed with heparin and centrifuged (3000 rpm, 4°C, 15 min) to obtain plasma. The plasma compound concentration was determined by the internal standard method using LC-MS/MS. The determination range by this method was from 0.1 to 100 ng/mL. The compound concentrations obtained from each rat were
analyzed in a model-independent way using a pharmacokinetics analysis software WinNonlin (ver.3.3), and average ± standard deviation of 3 animals for each group was obtained. The apparent half-life ($t_{1/2}$) in the elimination phase is shown in the following Table 3.

<table>
<thead>
<tr>
<th>test compound</th>
<th>dose</th>
<th>$t_{1/2}$ (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>compound F administered as a mixture with isomers</td>
<td>50 µg/kg (mixture with isomers)</td>
<td>115±31</td>
</tr>
<tr>
<td>compound J administered as a mixture with isomers</td>
<td>50 µg/kg (mixture with isomers)</td>
<td>77±19</td>
</tr>
<tr>
<td>compound F carboxylate form of compound F administered as a mixture with isomers</td>
<td>300 µg/kg</td>
<td>158±15</td>
</tr>
<tr>
<td>carboxylate form of compound J administered as a mixture with isomers</td>
<td>50 µg/kg (mixture with isomers)</td>
<td>9.6±1.7</td>
</tr>
<tr>
<td>carboxylate form of compound J administered as a mixture with isomers</td>
<td>50 µg/kg (mixture with isomers)</td>
<td>8.9±0.3</td>
</tr>
</tbody>
</table>

As is clear from the above-mentioned Table 3, $t_{1/2}$ values of the compound F and compound J of the present invention were about 1 - 2 hr, which were markedly prolonged in comparison with less than 10 min of the carboxylate forms. It suggests that the compound F and compound J of the present invention have excellent metabolic stability.

Prophylactic effect on dextran sodium sulfate-induced colitis model in mice

The prophylactic effect of compound F on ulcerative colitis was examined in dextran sodium sulfate-induced colitis model. The animal model displays inflammation localized to the large intestine, resulting in diarrhea and blood feces, which resembles pathologic condition of the clinical ulcerative
colitis closely (cf.: References D and E).

Female BALB/c mice (6 weeks old, Japan SLC) were purchased, acclimated for 1 week and used for the study. Except the normal group, the mice were allowed to freely drink a dextran sodium sulfate (to be abbreviated as DSS, MP Biochemicals, M.W. 36,000 - 50,000, Lot No. 3439J) solution prepared to 2.2 w/v% for 9 days to induce colitis. Compound F was orally administered at doses of 0.1, 0.3 and 1 mg/kg, once a day, daily, from the start day of DSS drinking (day 0) to one day before autopsy (day 9). To the control group was orally administered a solvent (1 vol% ethanol solution) at 10 mL/kg in the same manner.

Our preliminary study had revealed that mouse feces show a correlation between the water content and shape thereof. Thus, to determine the level of diarrhea, the stool was graded into 6 levels; normal (score 0), spherical stool being not less than 50% (score 1), banana-shaped stool being less than 50% (score 2), banana-shaped stool being not less than 20% (score 3), muddy stool (score 4), watery stool (score 5) (stool consistency score). The fecal occult blood was graded using fecal occult blood slide 5 Shionogi II (Shionogi & Co., Ltd.) into 5 levels; negative (no change of the slide color from yellow, score 0), weakly positive (slightly blue green, score 1), positive (blue green, score 2), moderately positive (clear blue green, score 3) and strongly positive (instantaneous color change to dark blue with color developer, score 4). The sum of the stool consistency score and occult blood score was defined as the stool score. Eight to 10 animals were used for each group, and the results were expressed as average ± standard deviation.

As a result, the body weight gradually increased over the study period without any difference among groups. The control group showed obvious loose stools and occult blood in stools.
from day 4 of DSS drinking. On the day of autopsy (day 9), the
length of the large intestine thereof was clearly shorter than
that of the normal group. Compound F dose-dependently
suppressed the increase in the stool score, which was a
suppressive tendency at 0.1 mg/kg and significant at 0.3 and 1
mg/kg (Fig. 1). Likewise, compound F showed a dose-dependent
suppressive effect on shortening of the large intestine (Fig.
2). Thus, compound F clearly prevented the onset of ulcerative
colitis.

References

Prophylactic effect on dextran sodium sulfate-induced colitis
in rats

Prophylactic effect of compound F on colitis was also
studied in rats. Male SD rats, 7 weeks old, body weight around
210 g - 240 g (Charles River) were purchased, acclimated for 1
week and used for the study. Except the normal group, the rats
were allowed to freely drink a DSS (MP Biochemicals, M.W.
36,000-50,000, Lot No. 4556J) solution prepared to 5.5 w/v% for 8
days to induce colitis. Compound F at doses of 0.3, 1
and 3 mg/kg was orally administered once a day, daily, from
one day before the start day of DSS drinking to one day before
autopsy (day 7). To the control group was orally administered
a solvent (1 vol% ethanol solution) at 5 mL/kg.

On day 8 from the start of DSS drinking, 1.25 w/v% Evans
blue solution was administered at 0.2 mL/100 g from the tail
vein. After 30 min, the rats were subjected to laparotomy
under ether anesthesia and exsanguinated to death. Thereafter,
the large intestine was dissected from just below the cecum to
the anus, and the length was measured with a caliper. After
the contents of the large intestine were removed, the colonic tissue of 7 cm long from the anus was washed 3 times with physiological saline and dried overnight with a vacuum pump. The next day, the dry weight was measured, formamide (2 mL) was added, the dye was extracted at 50°C overnight, and the level thereof was measured at 620 nm. A standard curve was prepared using an Evans blue standard solution, and the amount (mg) of Evans blue in 1 g of the colonic tissue was calculated to estimate degree of colonic tissue injury.

To show the level of diarrhea, the shape of stool was graded into 6 levels, with normal (score 0), rod-like stool being less than 50% (score 1), rod-like stool being not less than 50% (score 2), rod-like stool and partly muddy stool (score 3), muddy stool (score 4) and watery stool (score 5) (stool consistency score). Fecal occult blood score was evaluated by the same method described in Example 18. The sum of stool consistency score and occult blood score was defined as the stool score. Seven to 10 animals were used for each group, and the results are shown in average ± standard deviation.

As a result, the body weight of the control group gradually increased consistently, but the increase was significantly smaller than that of the normal group. The stool score of the control significantly elevated from day 1 of DSS drinking. On the day of autopsy (day 8), the large intestine thereof showed an apparent tissue injury and a significant shortening. In contrast, administration of compound F at 1 mg/kg and 3 mg/kg showed a significant suppressive tendency or significant suppressive effect on these events (Figs. 3, 4, 5). That is, compound F prevents ulcer development in the large intestine and normalizes the organ function, thereby leading suppression of symptoms of diarrhea and blood feces.

[Example 20]
Therapeutic effect on remission/relapse model of dextran sodium sulfate-induced colitis in mice

Next, therapeutic effect of compound F on colitis was studied in a chronic model. Female BALB/c mice, 6 weeks old, body weight about 20 g (Japan SLC) were purchased, acclimated for 1 week and used for the study. The mice were divided into a colitis induction group and a normal group. The colitis induction group was allowed to freely drink a 2.6 w/v% DSS (MP Biochemicals, M.W. 36,000-50,000, Lot No. 4556J) solution to induce colitis. On day 8 when the stool score (defined in Example 18) of the colitis induction group reached about 4.5, the mice were subdivided into a control group, a compound F 1 mg/kg administration group and a salazosulfapyridine (SIGMA, Lot No. 085K1930, hereinafter to be abbreviated as SASP) 100 mg/kg administration group. Then the mice were allowed to drink distilled water instead of DSS solution for 9 days (remission period). After the grouping, the stool score was evaluated every 3-4 days. When the score of the control group reached about 1, the mice were again allowed to drink the DSS solution to cause a relapse (relapse period). The periods of remission and relapse were taken as 1 cycle and the cycle was repeated 5 times. As for the 5th cycle, however, only the remission period was performed.

Compound F at a dose of 1 mg/kg and SASP at a dose of 100 mg/kg were orally administered once a day, daily, for 50 days from the initial remission period (day 8 from the start of 2.6 w/v% DSS drinking) to the fifth remission period (day 57 from the start of 2.6 w/v% DSS drinking). To the control group was orally administered a solvent (1 vol% ethanol solution) at 10 mL/kg. If a mouse had score 0 of both stool consistency score and occult blood score on the last day of each remission period, the mouse was regarded as "in remission". The remission ratio (%) was calculated as a ratio of mice in
remission in each group. Eight to 10 mice were used for each group and the results are shown in average value.

[0093]

As a result, the stool score of the control group increased in the relapse period, and decreased in the remission period. The score was significantly higher than that of the normal group almost throughout the study period (Fig. 6). The remission ratio thereof was 35.5% on average of 5 remission periods (Table 4). Compound F decreased the stool score early in the remission period, and suppressed an increase in the score in the relapse period. The remission ratio thereof was not less than 60% in any remission period, and the average was 66.0%, which was evidently higher than that of the control group. On the other hand, SASP did not show a clear effect on the stool score in either the remission period or the relapse period. The remission ratio thereof was slightly higher in the 1st, 3rd and 4th cycles than that of the control group, conversely lower in the 2nd and 5th cycles, and the average value was equivalent to that of the control group.

As shown above, compound F provides not only a prophylactic effect but also a therapeutic effect, as well as a remission maintaining effect. Moreover, the effects thereof are considered to be far superior to SASP in clinical use.

[0094]

Table 4
Remission Ratio of Remission/Relapse Model of DSS-induced Colitis in Mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of animals</th>
<th>Remission Ratio (%)</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Cycle 1</td>
<td>Cycle 2</td>
<td>Cycle 3</td>
<td>Cycle 4</td>
<td>Cycle 5</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>9</td>
<td>33.3</td>
<td>66.7</td>
<td>11.1</td>
<td>33.3</td>
<td>33.3</td>
<td>35.5</td>
</tr>
<tr>
<td>Compound F</td>
<td>10</td>
<td>60.0</td>
<td>80.0</td>
<td>70.0</td>
<td>60.0</td>
<td>60.0</td>
<td>66.0</td>
</tr>
<tr>
<td>1 mg/kg</td>
<td></td>
<td>50.0</td>
<td>50.0</td>
<td>37.5</td>
<td>50.0</td>
<td>12.5</td>
<td>40.0</td>
</tr>
<tr>
<td>SASP</td>
<td>100 mg/kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

39
Prophylactic effect on CD4*CD25- T cell transfer colitis model in mice

The effect on Crohn's disease, another type of inflammatory bowel disease, was studied. T cell transfer model is well known as a Crohn's disease model, which develops chronic gastritis or enteritis (see: References F, G, H). In addition, it can also be regarded as an animal model of intestinal Behcet's disease or simple ulcer, suffering from similar intestinal ulcer accompanied by activation of T cells (see: References I, J).

Female BALB/cA Jcl mice, 6 weeks old, body weight 19 - 23 g (CLEA Japan, Inc.) and female C.B-17/Icr-scid mice (6 weeks old, CLEA Japan, Inc.) were purchased, acclimated for 1 week and used for the study.

After laparotomy under ether anesthesia, BALB/cA Jcl mice were exsanguinated to death through the abdominal aorta and caudal vena cava, and the spleen was isolated. Splenocytes were prepared from the spleen and then CD4*CD25- T cells were prepared with a CD4* T cell Isolation Kit (No. 130-090-860, Milky Biotech Co., Ltd.) and CD25-Biotin antibodies (No. 130-092-569, Milky Biotech Co., Ltd.). The cells were separated using the autoMACS Separator (Milky Biotech Co., Ltd.). The separated CD4*CD25- T cells were suspended in physiological phosphate buffer solution, and 2.5x10^5 cells per animal were intraperitoneally administered to C.B-17/Icr-scid mice to induce colitis.

One mg/kg of compound F or prednisolone was initially administered at 5 hr before transfer of CD4*CD25- T cells, and orally administered thereafter once a day, daily, for 20 days. To the control group was orally administered a solvent (1 vol% ethanol solution) at 10 mL/kg. A clinical endpoint was the sum
of stool consistency score (0 - 5), fecal occult blood score (0 - 4) and body weight decrease score (0 - 5), termed as the Disease Activity Index score (hereinafter to be abbreviated as DAI score: highest score 14). The stool consistency score was graded for the hardness of stool as normal (0), slightly loose (1), somewhat loose (2), loose (3), considerably loose (4) and diarrhea (5). The fecal occult blood score was evaluated in the same manner as in Example 18. The body weight decrease score was graded for the changes in the body weight as increase (0), decrease of less than 3% (1), decrease of not less than 3% and less than 6% (2), decrease of not less than 6% and less than 9% (3), decrease of not less than 9% and less than 12% (4), and decrease of not less than 12% (5). Eight to 10 mice were used for each group and the results were expressed as average.

As a result, stool consistency score and fecal occult blood score of the control group showed a clear increase from 12 days after T cell transfer and the body weight decrease score showed a clear increase on day 19, all reaching almost maximum 21 days later. Compound F suppressed the increases in both the stool consistency score and the fecal occult blood score by almost half as shown in Fig. 7 and 8, respectively, and prevented the increase in the body weight decrease score almost completely as shown in Fig. 9. On the other hand, though prednisolone suppressed an increase in the fecal occult blood score by almost the same level as the compound F administration as shown in Fig. 8, it failed to show a clear effect on the stool consistency score on day 21 as shown in Fig. 7. In addition, the body weight decrease score remained at higher values than those in the control group over the study period as shown in Fig. 9, and prednisolone clearly worsened the score. As shown in Fig. 10, the DAI score indicated that compound F is comprehensively superior to prednisolone.
Therefore, compound F can suppress the condition of Crohn’s disease, intestinal Behcet’s disease and simple ulcer as well as ulcerative colitis more effectively than existing drugs.

References


Example 22

Effect on ethanol-induced gastric mucosal injury model in rats

The suppressive effect of compound F on gastric mucosal injury was investigated in ethanol-induced gastric mucosal injury model in rats. This model is frequently used as an animal model of human acute gastritis associated with congestive mucosal injury (Reference K).

Male SD rats (7 weeks old, Charles River) were purchased through Oriental BioService Inc., acclimated for 1 week and used for the study. The rats were grouped based on the body weight, placed in a clean cage set with a wire mesh floor one day before the study, fasted for 19 hr (without water for last 3 hr), and orally administered with ethanol (special grade, Nacalai Tesque, Lot No. V8A5862, 1.5 mL) in all groups to induce gastric mucosal injury. Compound F was orally administered at doses of 0.01, 0.1 and 1 mg/kg 30 min before induction of gastric mucosal injury at a volume of 5 mL/kg. To the control group was orally administered a solvent (1 vol% ethanol solution) at 5 mL/kg in the same manner. Eight animals were used for each group.

The rats were bled to death from the abdominal aorta and caudal vena cava under ether anesthesia after 1 hr from the ethanol administration, and the stomach was isolated. The
isolated stomach was immediately filled with 2 vol% neutral
formalin solution (6 mL) and fixed for 15 min. The stomach was
incised along the midline of the greater curvature from the
cardiac part to the pyloric part, and extended on a vinyl
chloride board. The length and width of each ulcer were
measured under a stereomicroscope, the area was calculated,
and the sum thereof was taken as the total ulcer area.

As a result, total ulcer area of the control group
averaged 103 mm². Compound F significantly reduced the total
ulcer area in a dose-dependent manner from 0.01 mg/kg, and
almost completely reduced the area at a dose of 1 mg/kg (Fig.
11). Thus, compound F suppressed the gastric mucosal injury.

Reference
[Example 23]

Effect on indomethacin-induced small intestinal injury model
in rats

The suppressive effect of compound F on small intestinal
injury was investigated using indomethacin-induced small
intestinal injury model in rats. Administration of non-
steroidal anti-inflammatory drugs (NSAIDs) is known to induce
hemorrhagic injury in the small intestine of human. This model
is characterized by mucosal injury of the small intestine
induced by administration of a NSAID, indomethacin, to rat and
shows pathology similar to that of NSAIDs-induced small
intestinal injury or Crohn's disease in human (References L
and M).

Male SD rats, 7 weeks old (Charles River) were purchased,
acclimated for 1 week and used for the study. The rats were
grouped based on the body weight and subcutaneously
administered with indomethacin (SIGMA, Lot No. 19F0018) at 15
mg/5 mL/kg to all groups to induce small intestinal injury.
Compound F at doses of 0.01, 0.1 and 1 mg/kg was orally administered at a volume of 5 mL/kg 30 min before and 6 hr after the subcutaneous administration of indomethacin. To the control group was orally administered a solvent (1 vol% ethanol solution) at 5 mL/kg in the same manner. Eight animals were used for each group.

The rats were intravenously administered with 2 mL of 10 mg/mL Evans blue solution under ether anesthesia 23.5 hr after the indomethacin administration. After 30 min, the rats were bled to death from the abdominal aorta and caudal vena cava under ether anesthesia and the small intestine was isolated. The isolated small intestine was filled with an adequate amount (about 35 mL) of 2 vol% neutral formalin solution, and fixed for about 15 min. Thereafter, the small intestine was entirely incised along the mesenteric attachment site, and extended on a vinyl chloride board. The length and width of each ulcer were measured under a stereomicroscope, the area was calculated, and the sum thereof was taken as the total ulcer area.

As a result, the total ulcer area in the small intestine was about 730 mm² in the control group. In contrast, the compound F administration group significantly reduced the ulcer area in a dose-dependent manner from a dose of 0.1 mg/kg administration, and completely reduced the area at a dose of 1 mg/kg (Fig. 12). Thus, compound F strongly suppressed the small intestinal injury.

References

From the above, compound F showed a superior suppressive action on the direct injury to the gastrointestinal tract mucosa due to alcohol and the like and mucosal regenerative
failure due to NSAIDs and the like. Therefore, compound F is expected to show a protective effect and a tissue repair effect on mucosal injury of the gastrointestinal tract.

As shown in the above-mentioned examples and found with compound F, the compound of the present invention is effective for gastrointestinal tract injury and delay in cure due to immune-related inflammation of digestive tract, drug-induced mucosal injury of the gastrointestinal tract and drug-induced mucosal regenerative failure. Specifically, it is useful for inflammatory bowel disease such as ulcerative colitis and Crohn's disease, alcoholic gastritis or gastric ulcer, small intestinal ulcer and the like, and are not limited to the recited diseases.

INDUSTRIAL APPLICABILITY

[0107] PGI\(_2\) derivative of the present invention is useful as an active ingredient of medicaments. A medicament containing PGI\(_2\) derivative of the present invention as an active ingredient is useful as a medicament for the prophylaxis or treatment of inflammatory diseases and ulcer diseases of the digestive tract. Particularly, it is useful as a medicament for the prophylaxis or treatment of ulcerative colitis, Crohn's disease, gastritis or gastric ulcer, small intestinal ulcer.

This application is based on a Japanese patent application No. 2008-232133 filed on September 10, 2008 and a Japanese patent application No. 2009-168193 filed on July 16, 2009, and the contents disclosed therein including specification, claims, drawings and abstract are hereby entirely incorporated by reference.
0108

It is to be understood that, if any prior art publication is referred to herein, such reference does not constitute an admission that the publication forms a part of the common general knowledge in the art, in Australia or any other country.

0109

In the claims which follow and in the preceding description of the invention, except where the context requires otherwise due to express language or necessary implication, the word "comprise" or variations such as "comprises" or "comprising" is used in an inclusive sense, i.e. to specify the presence of the stated features but not to preclude the presence or addition of further features in various embodiments of the invention.
Claims

1. A prostaglandin I₂ derivative represented by the formula (1) or a pharmaceutically acceptable salt thereof.

\[
\text{R}^1 \quad \text{R}^2 \quad \text{R}^3
\]

wherein \( \text{R}^1 \) and \( \text{R}^2 \) are each independently a hydrogen atom or a straight chain alkyl group having a carbon number of 1 to 3, and \( \text{R}^3 \) is a hydrogen atom, an alkyl group having a carbon number of 1 to 4, an alkoxyalkyl group, an aryl group, a halogen atom or a haloalkyl group.

2. The prostaglandin I₂ derivative according to claim 1, wherein \( \text{R}^1 \) is a methyl group, or a pharmaceutically acceptable salt thereof.

3. The prostaglandin I₂ derivative according to claim 1 or 2, wherein \( \text{R}^3 \) is a methyl group, or a pharmaceutically acceptable salt thereof.

4. The prostaglandin I₂ derivative according to any one of claims 1-3, wherein \( \text{R}^2 \) is a hydrogen atom, or a pharmaceutically acceptable salt thereof.

5. The prostaglandin I₂ derivative according to any one of claims 1-4, wherein \( \text{R}^3 \) is a methyl group, and \( \text{R}^2 \) is a hydrogen atom, or a pharmaceutically acceptable salt thereof.
6. The prostaglandin \( \text{I}_2 \) derivative according to any one of claims 1-5, wherein \( R^3 \) is a \( \text{m}- \) methyl group, or a pharmaceutically acceptable salt thereof.

7. The prostaglandin \( \text{I}_2 \) derivative according to claim 1, wherein \( R^1 \) is a methyl group, \( R^2 \) is a hydrogen atom, and \( R^3 \) is a methyl group, or a pharmaceutically acceptable salt thereof.

8. The prostaglandin \( \text{I}_2 \) derivative according to claim 1, wherein \( R^1 \) is a hydrogen atom, \( R^2 \) is a methyl group, and \( R^3 \) is a methyl group, or a pharmaceutically acceptable salt thereof.

9. A medicament comprising the prostaglandin \( \text{I}_2 \) derivative according to any one of claims 1-8, or a pharmaceutically acceptable salt thereof as an active ingredient.

10. A method for the prophylaxis or treatment of a disease of the digestive tract in a patient, comprising administering to the patient an effective amount of the prostaglandin \( \text{I}_2 \) derivative according to any one of claims 1-8, or a pharmaceutically acceptable salt thereof.

11. The method according to claim 10, wherein the disease of the digestive tract is an inflammatory disease of the digestive tract.

12. The method according to claim 11, wherein the inflammatory disease of the digestive tract is an inflammatory bowel disease.

13. The method according to claim 12, wherein the inflammatory bowel disease is ulcerative colitis or Crohn's disease.
14. The method according to claim 12, wherein the inflammatory bowel disease is intestinal Behcet's disease or simple ulcer.

15. The method according to claim 10, wherein the disease of the digestive tract is ulcerative disease of the digestive tract.

16. The method according to claim 15, wherein the ulcerative disease of the digestive tract is gastritis or gastric ulcer.

17. The method according to claim 16, wherein the gastritis or gastric ulcer is drug-induced gastritis or gastric ulcer.

18. The method according to claim 17, wherein the drug-induced gastritis or gastric ulcer is induced by a non-steroidal anti-inflammatory drug.

19. The method according to claim 16, wherein the gastritis or gastric ulcer is induced by alcohol.

20. The method according to claim 15, wherein the ulcerative disease of the digestive tract is small intestinal ulcer.

21. The method according to claim 20, wherein the small intestinal ulcer is a drug-induced small intestinal ulcer.

22. The method according to claim 21, wherein the drug-induced small intestinal ulcer is induced by a non-steroidal anti-inflammatory drug.

23. The method according to claim 20, wherein the small intestinal ulcer is induced by alcohol.
24. Use of the prostaglandin $\text{I}_2$ derivative according to any one of claims 1-8, or a pharmaceutically acceptable salt thereof, in the preparation of a medicament for the prophylaxis or treatment of a disease of the digestive tract.

25. The prostaglandin $\text{I}_2$ derivative according to any one of claims 1-8, the medicament according to claim 9, the method according to any one of claims 10-23, or the use according to claim 24, substantially as herein described with reference to any one of the Examples.
FIG. 1

![Graph showing stool score and occult blood score for different groups.

<table>
<thead>
<tr>
<th>Compound F (mg/kg/day)</th>
<th>Normal</th>
<th>Control</th>
<th>0.1</th>
<th>0.3</th>
<th>1</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>6</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### p<0.01
** * p<0.05, p<0.01 versus control, respectively
FIG. 2

![Bar graph showing the length of large intestine (mm) with different treatments: normal, control, 0.1 mg/kg/day, 0.3 mg/kg/day, and 1 mg/kg/day of compound F. The graph includes error bars and significance levels.]

- **## p<0.01**
- **## p<0.01 versus control**
FIG. 3

[Bar chart showing stool score (stool consistency score + occult blood score) for different doses of compound F (mg/kg/day).]

- Normal control: 0.3, 1, 3
- p = 0.078

- ** p < 0.01 versus control

---

# p < 0.01
** p < 0.01 versus control
FIG. 4

![Graph showing the effect of different doses of compound F on the length of large intestine (cm). The graph includes bars for normal, control, and various compound F doses (0.3, 1, and 3 mg/kg/day).](image)

- **##**: p<0.01
- *****: p<0.05 versus control
- ******: p<0.01 versus control
**FIG. 5**

![Bar graph showing colonic tissue injury](image)

<table>
<thead>
<tr>
<th></th>
<th>Evans blue amount (mg/dry weight(g))</th>
</tr>
</thead>
<tbody>
<tr>
<td>normal</td>
<td>2</td>
</tr>
<tr>
<td>control</td>
<td>3</td>
</tr>
<tr>
<td>0.3</td>
<td>7</td>
</tr>
<tr>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

### Notes:
- **##** p<0.01
- **** p<0.01 versus control
FIG. 6

The figure shows a graphical representation of stool consistency and occult blood scores over days from the start of drinking DSS. The x-axis represents days from the start of drinking DSS, ranging from 8 to 58 days. The y-axis represents the stool score, which includes consistency score and occult blood score.

Key:
- C: Normal
- ●: Control
- ▲: Compound F 1mg/kg
- ■: SASP 100mg/kg

Legend:
- #, ## p<0.05, p<0.01 for control versus normal, respectively
- *, ** p<0.05, p<0.01 versus control, respectively
FIG. 7

- ● control
- ▲ compound F  1 mg/kg
- ■ prednisolone  1 mg/kg

stool consistency score

9  12  14  16  19  21

days after T cell transfer

* p<0.05 versus control
FIG. 8

- control
- compound F  1 mg/kg
- prednisolone  1 mg/kg

Fecal occult blood score vs. days after T cell transfer.
FIG. 9

- control
- compound F 1 mg/kg
- prednisolone 1 mg/kg

Body weight decrease score vs. days after T cell transfer.
**FIG. 10**

- **control**
- **compound F 1 mg/kg**
- **prednisolone 1 mg/kg**

![Graph showing DA1 score over days after T cell transfer](image)

* p<0.05 versus control
**FIG. 11**

[Bar chart showing area of gastric ulcers (mm²) for different doses of compound F (ng/kg).]

- Control
- 0.01
- 0.1
- 1

**p<0.01 versus control**
**FIG. 12**

![Graph showing the area of small intestinal ulcers (mm²) with control and different doses of compound F (mg/kg)].

- Control
- 0.01 mg/kg
- 0.1 mg/kg
- 1 mg/kg

**p < 0.01 versus control**