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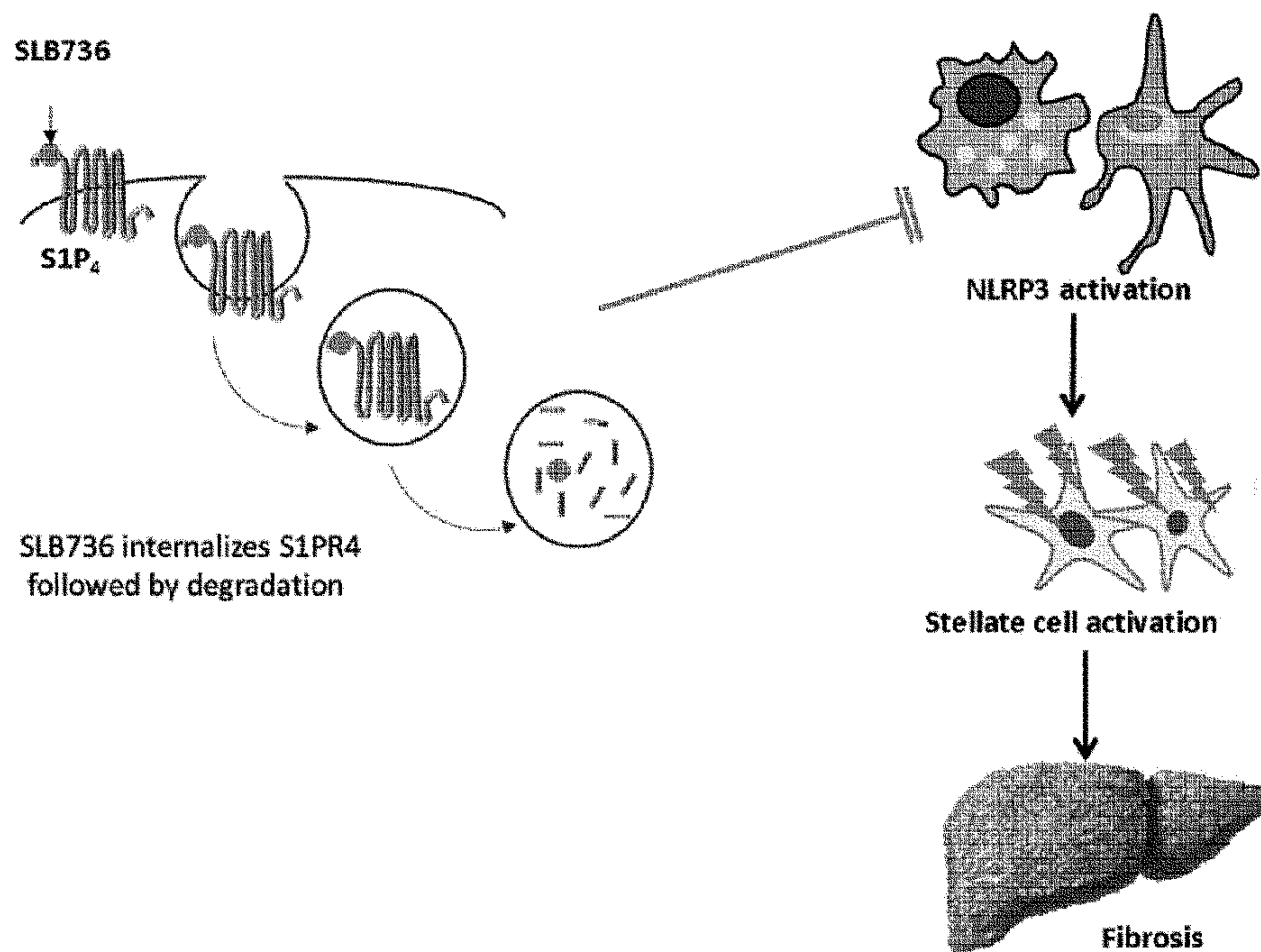
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(54) Titre : COMPOSITION CIBLANT S1PR4 POUR PREVENIR OU TRAITER UNE STEATOHEPATITE NON
ALCOOLIQUE

(54) Title: S1PR4-TARGETING COMPOSITION FOR PREVENTING OR TREATING NON-ALCOHOLIC
STEATOHEPATITIS



(57) **Abrégé/Abstract:**

The present invention relates to a S1PR4-targeting composition for preventing or treating non-alcoholic steatohepatitis and, more particularly, to a pharmaceutical composition and a health functional food composition, both comprising a sphingolipid compound which serves as a functional inhibitor against S1PR4, showing prophylaxis and therapy of non-alcoholic steatohepatitis. The sphingolipid compound of the present invention is expected to be applied as a leading material effective for the prevention or treatment of non-alcoholic steatohepatitis (NASH) as it has the effect of reducing the infiltration of inflammatory cells into hepatic tissues and suppressing fibrosis and decreases a level of liver injury (ALT), inflammation in hepatic tissues, and the expression of a fibrosis-related gene.

【Abstract】

The present invention relates to a S1PR4-targeting composition for preventing or treating non-alcoholic steatohepatitis and, more particularly, to a pharmaceutical composition and a health functional food composition, both comprising a sphingolipid compound which serves as a functional inhibitor against S1PR4, showing prophylaxis and therapy of non-alcoholic steatohepatitis. The sphingolipid compound of the present invention is expected to be applied as a leading material effective for the prevention or treatment of non-alcoholic steatohepatitis (NASH) as it has the effect of reducing the infiltration of inflammatory cells into hepatic tissues and suppressing fibrosis and decreases a level of liver injury (ALT), inflammation in hepatic tissues, the expression of a fibrosis-related gene.

【DESCRIPTION】**【Invention Title】**

S1PR4-TARGETING COMPOSITION FOR PREVENTING OR TREATING
NON-ALCOHOLIC STEATOHEPATITIS

5

【Technical Field】

The present invention relates to an S1PR4-targeting
composition for preventing or treating non-alcoholic
steatohepatitis, and more particularly to a pharmaceutical
composition and a nutraceutical (health functional food)
composition for preventing or treating nonalcoholic
steatohepatitis containing a sphingolipid compound which
acts as a functional antagonist of S1PR4.

15 **【Background Art】**

Myriocin, isolated from *Cordyceps* fungi, has been
known for various pharmacological activities thereof for a
long time and has been found to be effective in preventing
the onset of non-alcoholic fatty acid liver disease
(NAFLD) (Yang et al., Am. J. Physiol. Endocrinol. Metab.
2009; 19435851, Kurek et al., Liver Int. 2014; 24106929,
Kasumov et al., PLoS One. 2015; 25993337). However,
experimentation on animals has shown that this substance
caused death from severe digestive disorders at 10 times a
normal dose due to the low safety margin thereof. For this

25

reason, the substance has not developed into a drug. In addition, sphingolipid compounds containing myriocin have been considered to have low development potential as drugs due to the low solubility thereof in water.

5 However, FTY720 (Fingolimod) developed from myriocin derivatives has been approved to have an indication to relapsing-remitting multiple sclerosis (RRMS), which is a degenerative neurological disease that often occurs in western young people. Efforts to develop drugs based on
10 sphingolipids are ongoing. FTY720 is an oral drug, which acts as a modulator of sphingosine 1-phosphate (S1P) receptors expressed in lymphocytes and various neuron cells on the immune system to reduce recycling and migration of pathogenic lymphocytes to the central nervous
15 system.

 Meanwhile, FTY720 acts non-selectively on subtypes of S1P receptors (S1P1, S1P2, S1P3, S1P4 and S1P5), resulting in problems due to lymphocyte reduction (lymphopenia) in blood or severe side effects centered in the cardiac
20 circulatory system such as bradycardia and arrhythmia. Thus, there is a need for the development of a therapeutic agent having selectivity for the S1P receptor.

 Therefore, as a result of extended efforts to develop an independent compound selectively acting on the S1P
25 subtype receptor among sphingolipid compounds, the present

inventors have found that the compound according to the present invention specifically binds to the S1P4 receptor present in macrophages and thus acts as an functional inhibitor (antagonist), thereby exerting effects of preventing or treating non-alcoholic steatohepatitis (NASH). Based on this finding, the present invention has been completed.

【Disclosure】

【Technical Problem】

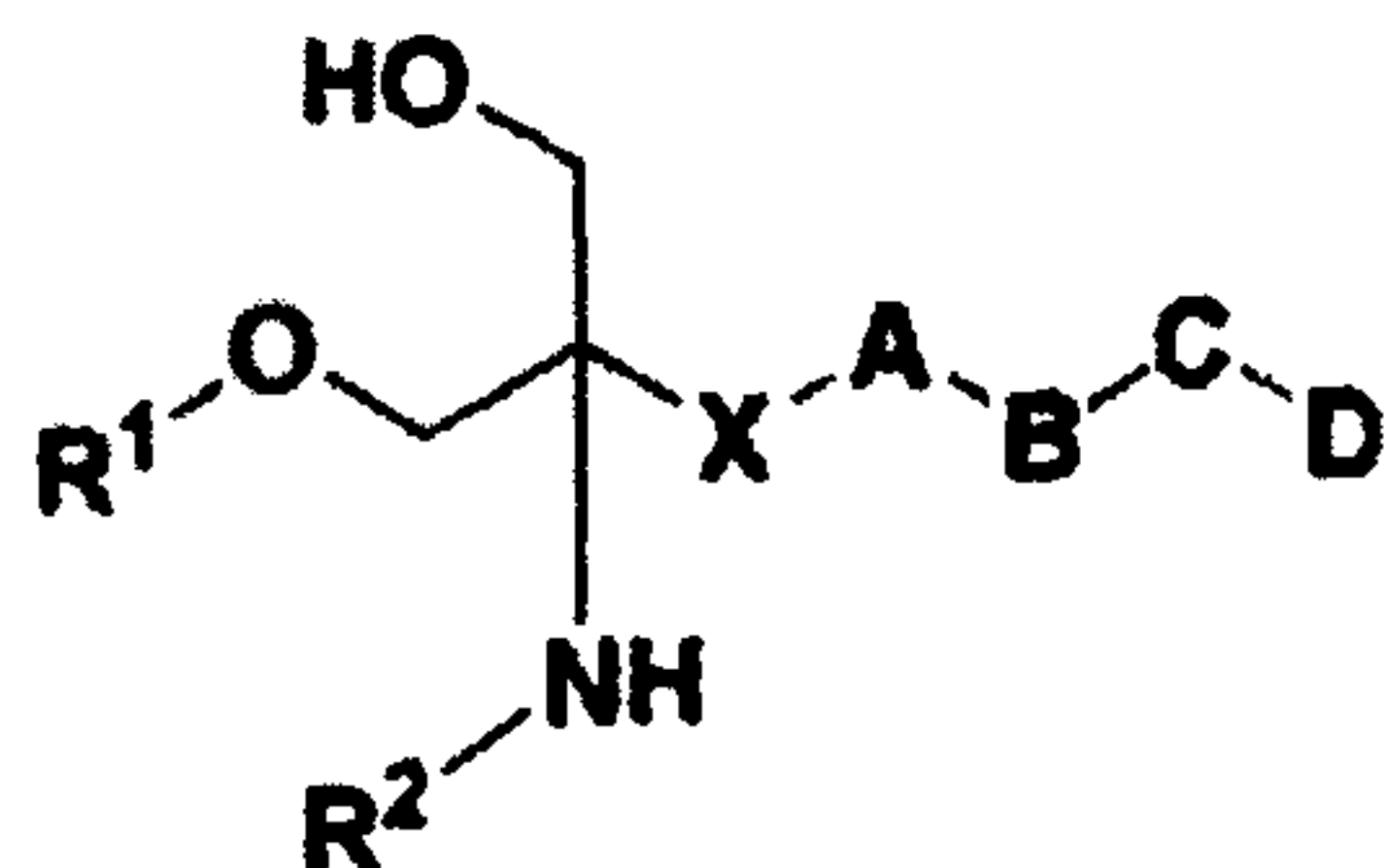
Therefore, it is one object of the present invention to provide a pharmaceutical composition for preventing or treating nonalcoholic steatohepatitis.

It is another object of the present invention to provide a functional health food for preventing or treating nonalcoholic steatohepatitis.

【Technical Solution】

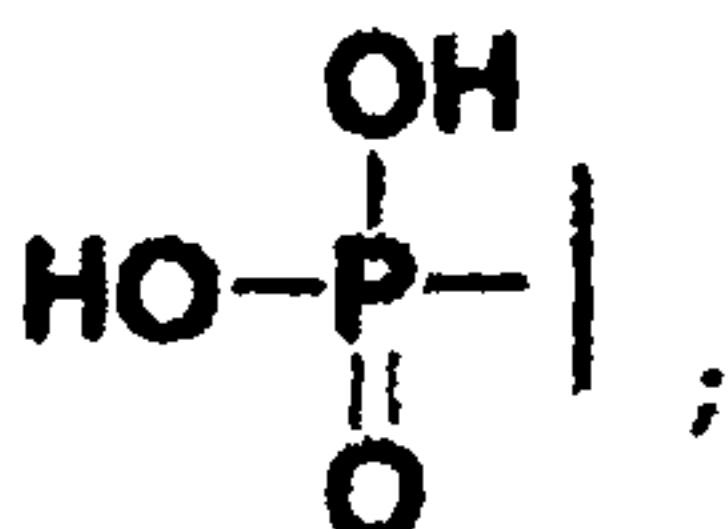
Therefore, the present invention has been made in view of the above problems, and provides a pharmaceutical composition for preventing or treating non-alcoholic steatohepatitis (NASH) containing a compound represented by the following formula 1, an optical isomer thereof or a pharmaceutically acceptable salt thereof as an active ingredient:

[Formula 1]



5

wherein

R¹ is hydrogen orR² is hydrogen, or unsubstituted or substituted C₁₋₅

straight or branched alkyl carbonyl, wherein the

10 substituted alkyl carbonyl is substituted with one or more

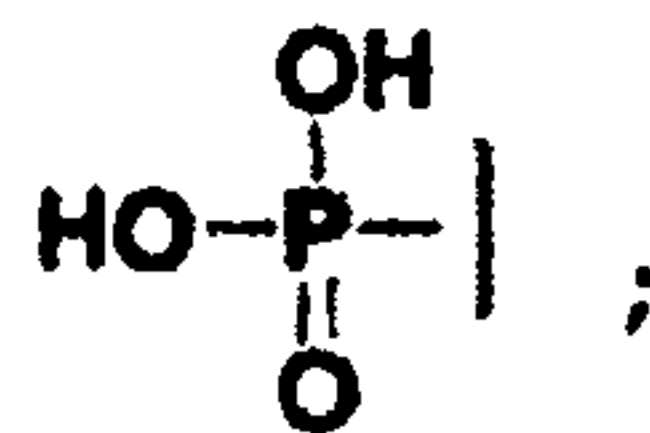
substituents selected from the group consisting of

hydroxy, halogen, cyano, nitro and amino;

A is a five-membered ring heteroarylene containing

one or more heteroatoms selected from the group consisting

15 of N, O and S;

B is C₁₋₁₁ straight or branched alkylene;C is a single bond or C₆₋₁₀ arylene;D is -H, or C₁₋₁₅ straight or branched alkyl; andX is a single bond, C₁₋₅ alkylene, C₂₋₅ alkenylene or20 C₂₋₅ alkynylene.Preferably, R¹ is hydrogen orR² is hydrogen, or C₁₋₃ straight or branched alkyl carbonyl;

A is a five-membered ring heteroarylene containing

25 one or more heteroatoms selected from the group consisting

of N, O and S;

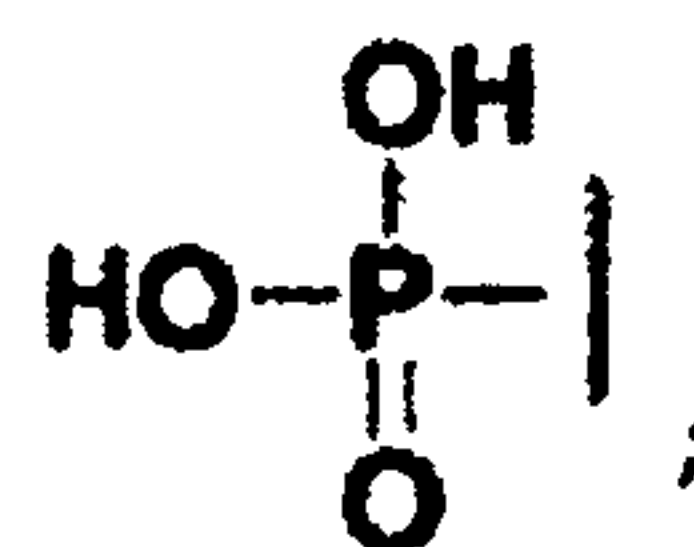
B is C₁₋₉ straight or branched alkylene;

C is a single bond or C₆₋₁₀ arylene;

D is -H, or C₃₋₁₂ straight or branched alkyl; and

5 X is a single bond, C₁₋₃ alkylene, C₂₋₃ alkenylene or C₂₋₃ alkynylene.

More preferably, R¹ is hydrogen or



R² is hydrogen or acetyl;

10 A is a five-membered ring heteroarylene containing one or more heteroatoms selected from the group consisting of N, O and S;

B is C₂₋₈ alkylene;

C is a single bond or phenylene;

15 D is -H, or C₆₋₁₀ straight or branched alkyl; and

X is a single bond, -CH₂CH₂-, -CH=CH- or -C≡C-.

Preferred examples of the active ingredient represented by Formula 1 according to the present invention may include the following compounds:

20 (1) 2-amino-2-(2-(3-decylisoxazol-5-yl)ethyl)propane-1,3-diol;

(2) 2-amino-2-(2-(1-decyl-1H-1,2,3-triazol-4-yl)ethyl)propane-1,3-diol;

(3) 2-amino-2-((3-octylisoxazol-5-yl)ethynyl)propane-1,3-diol;

25

(4) 2-amino-2-(2-(3-octylisoxazol-5-yl)ethyl)propane-1,3-diol;

(5) 2-amino-2-(hydroxymethyl)-4-(3-octylisoxazol-5-yl)butyl dihydrogen phosphate;

5 (6) 2-amino-2-((3-decylisoxazol-5-yl)ethynyl)propane-1,3-diol;

(7) 2-amino-4-(3-decylisoxazol-5-yl)-2-(hydroxymethyl)butyl dihydrogen phosphate;

10 (8) 2-amino-2-(2-(3-(4-hexylphenethyl)isoxazol-5-yl)ethyl)propane-1,3-diol;

(9) 2-amino-2-((3-dodecylisoxazol-5-yl)ethynyl)propane-1,3-diol;

(10) 2-amino-2-(2-(3-dodecylisoxazol-5-yl)ethyl)propane-1,3-diol;

15 (11) 2-amino-4-(3-dodecylisoxazol-5-yl)-2-(hydroxymethyl)butyl dihydrogen phosphate;

(12) 2-amino-2-(2-(1-octyl-1H-1,2,3-triazol-4-yl)ethynyl)propane-1,3-diol;

20 (13) 2-amino-2-(2-(1-octyl-1H-1,2,3-triazol-4-yl)ethyl)propane-1,3-diol;

(14) 2-amino-2-((1-decyl-1H-1,2,3-triazol-4-yl)ethynyl)propane-1,3-diol;

(15) 2-amino-2-(2-(1-(4-hexylphenethyl)-1H-1,2,3-triazol-4-yl)ethyl)propane-1,3-diol;

25 (16) 2-amino-2-(1-butyl-1H-1,2,3-triazol-4-yl)propane-1,3-

diol;

(17) 2-amino-2-(3-dodecylisoxazol-5-yl)propane-1,3-diol;

(18) (E)-2-amino-2-(2-(3-decylisoxazol-5-yl)vinyl)propane-1,3-diol;

5 (19) (E)-2-amino-2-(1-butyl-1H-1,2,3-triazol-4-yl)propane-1,3-diol;

(20) 2-amino-2-(2-(3-(8-phenyloctyl)-isoxazol-5-yl)ethyl)propane-1,3-diol;

10 (21) 2-amino-2-(2-(1-(8-phenyloctyl)-1H-1,2,3-triazolebutyl-4-yl)propane-1,3-diol;

(22) N-(2-(1-dodecyl-1H-1,2,3-triazol-4-yl)-1,3-dihydroxypropane-2-yl)acetamide;

(23) N-(2-(3-dodecylisoxazol-5-yl)-1,3-dihydroxypropane-2-yl)acetamide;

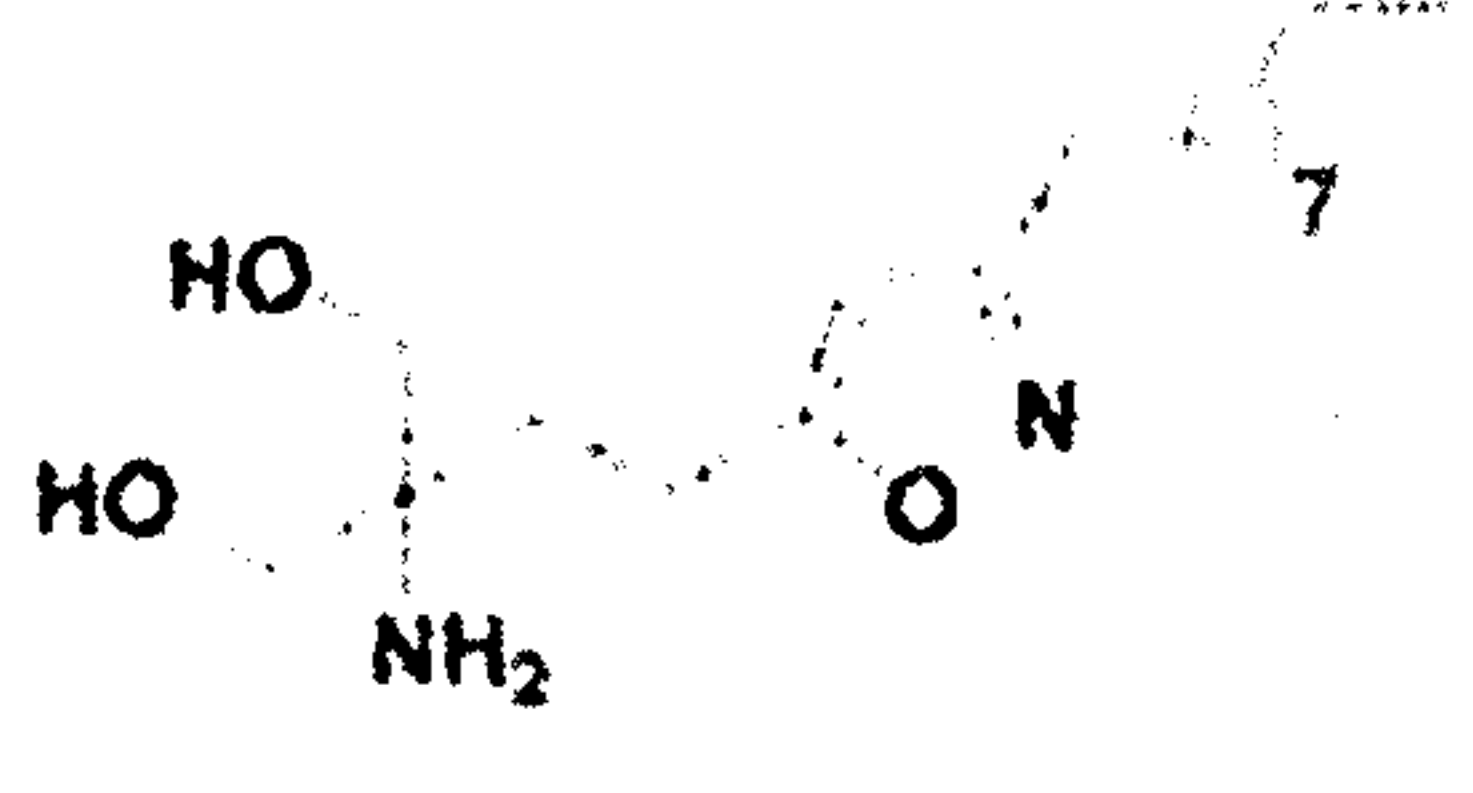
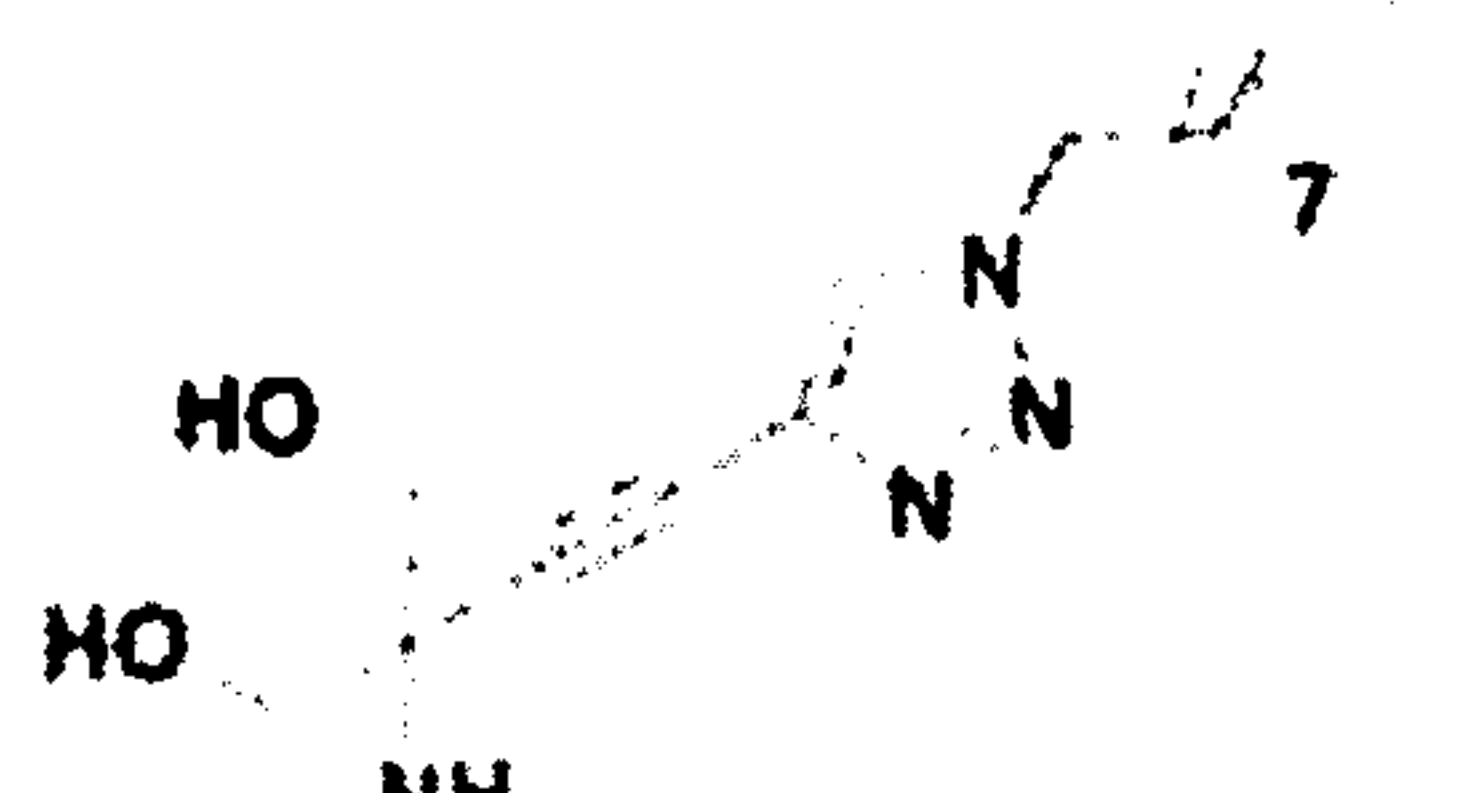
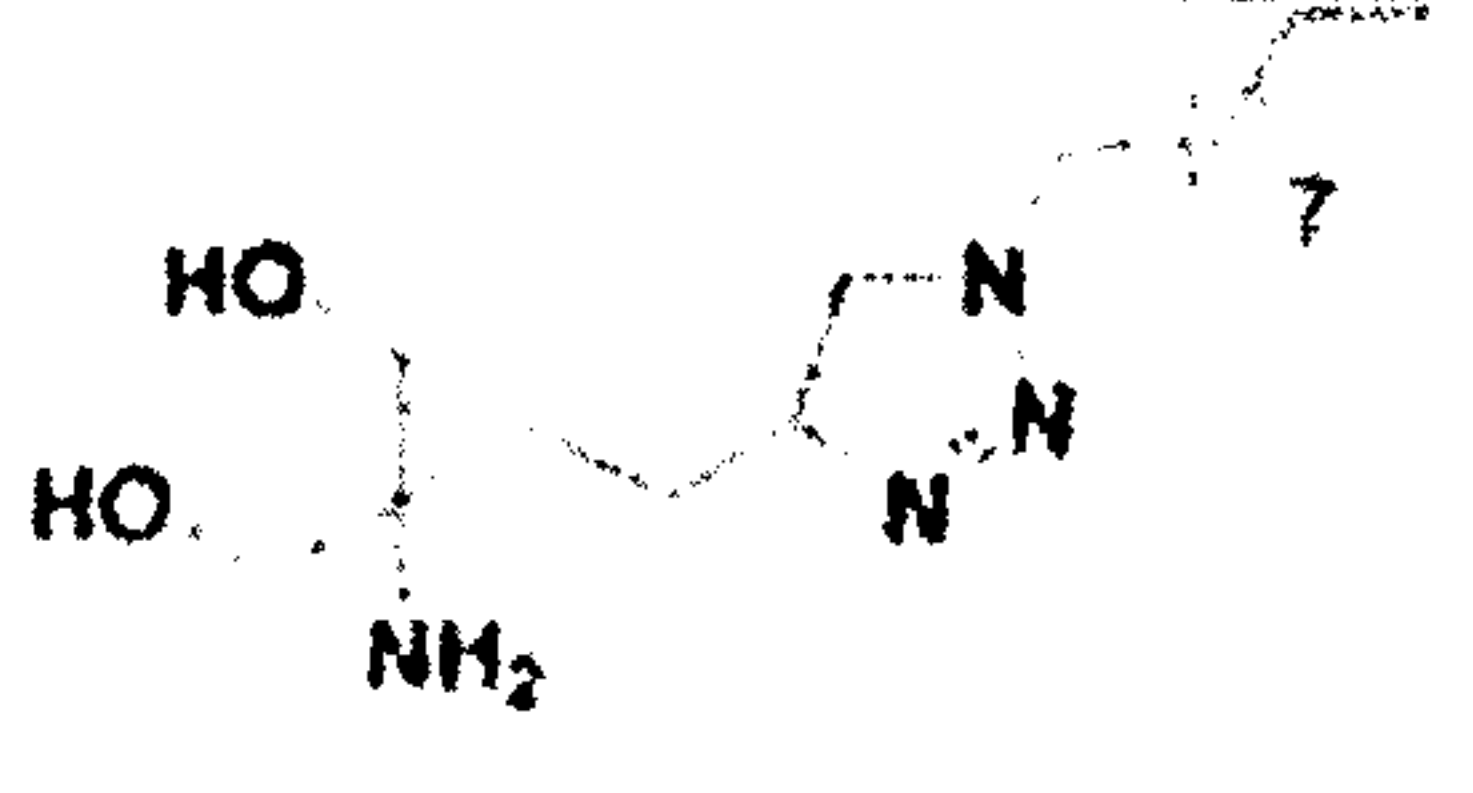
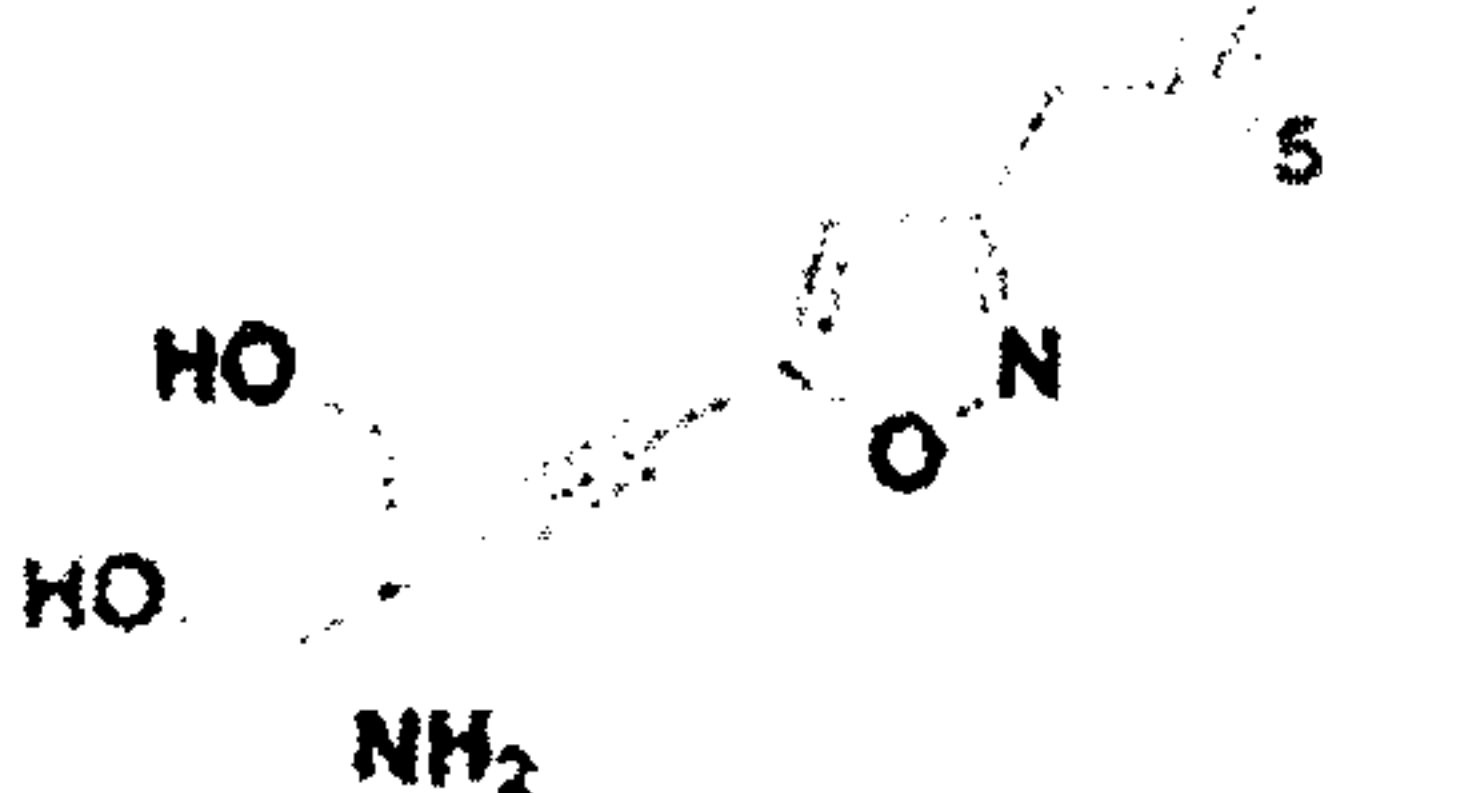
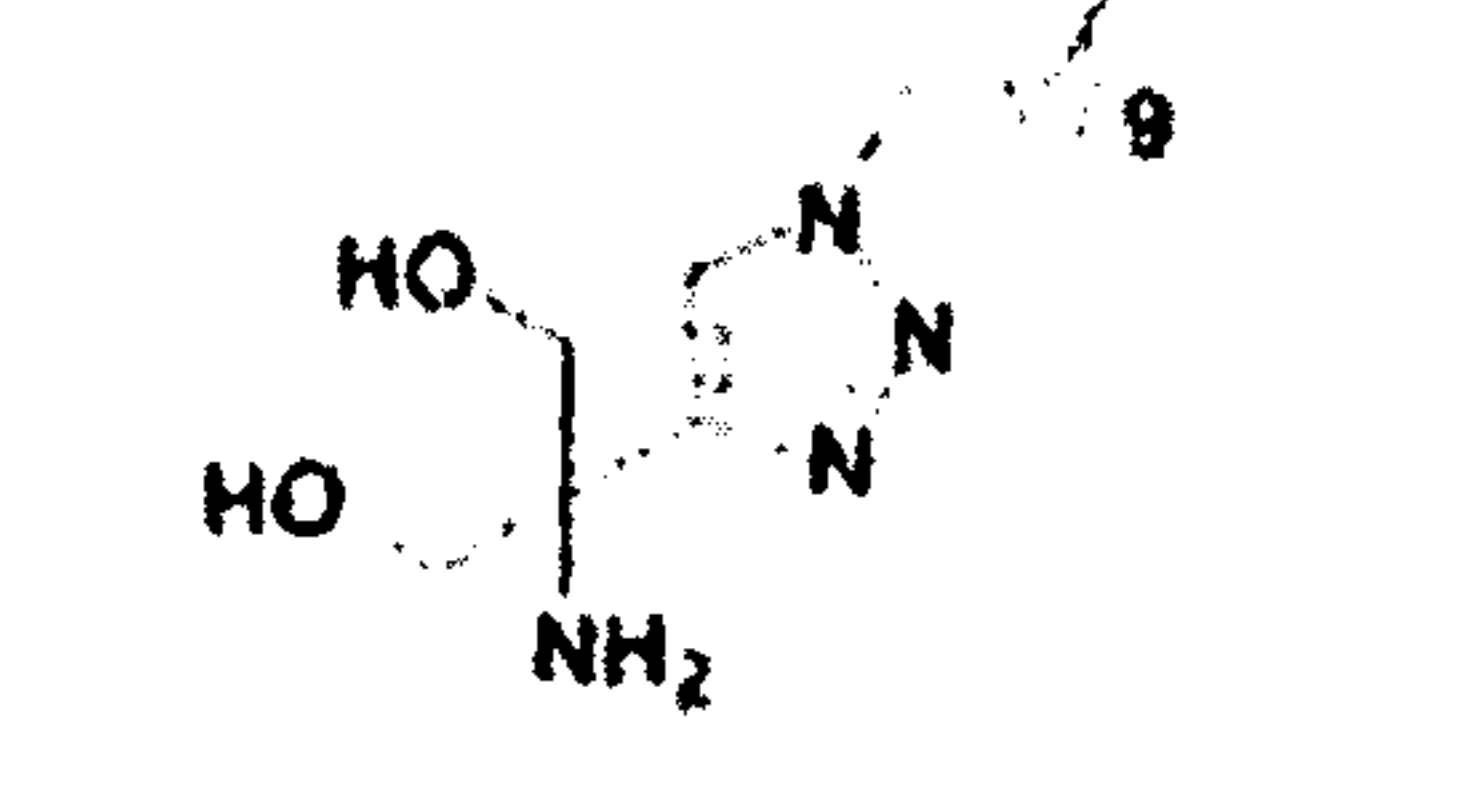
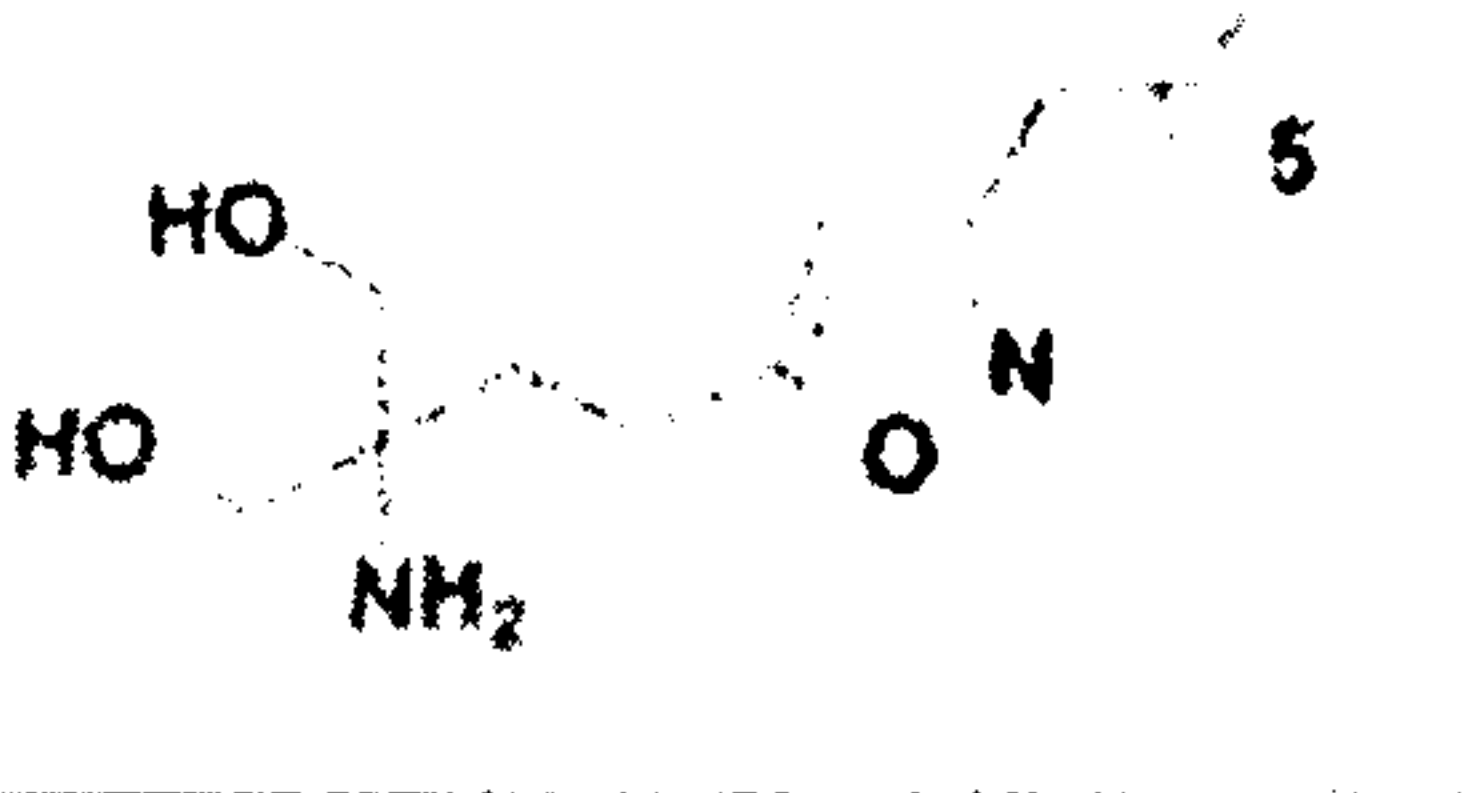
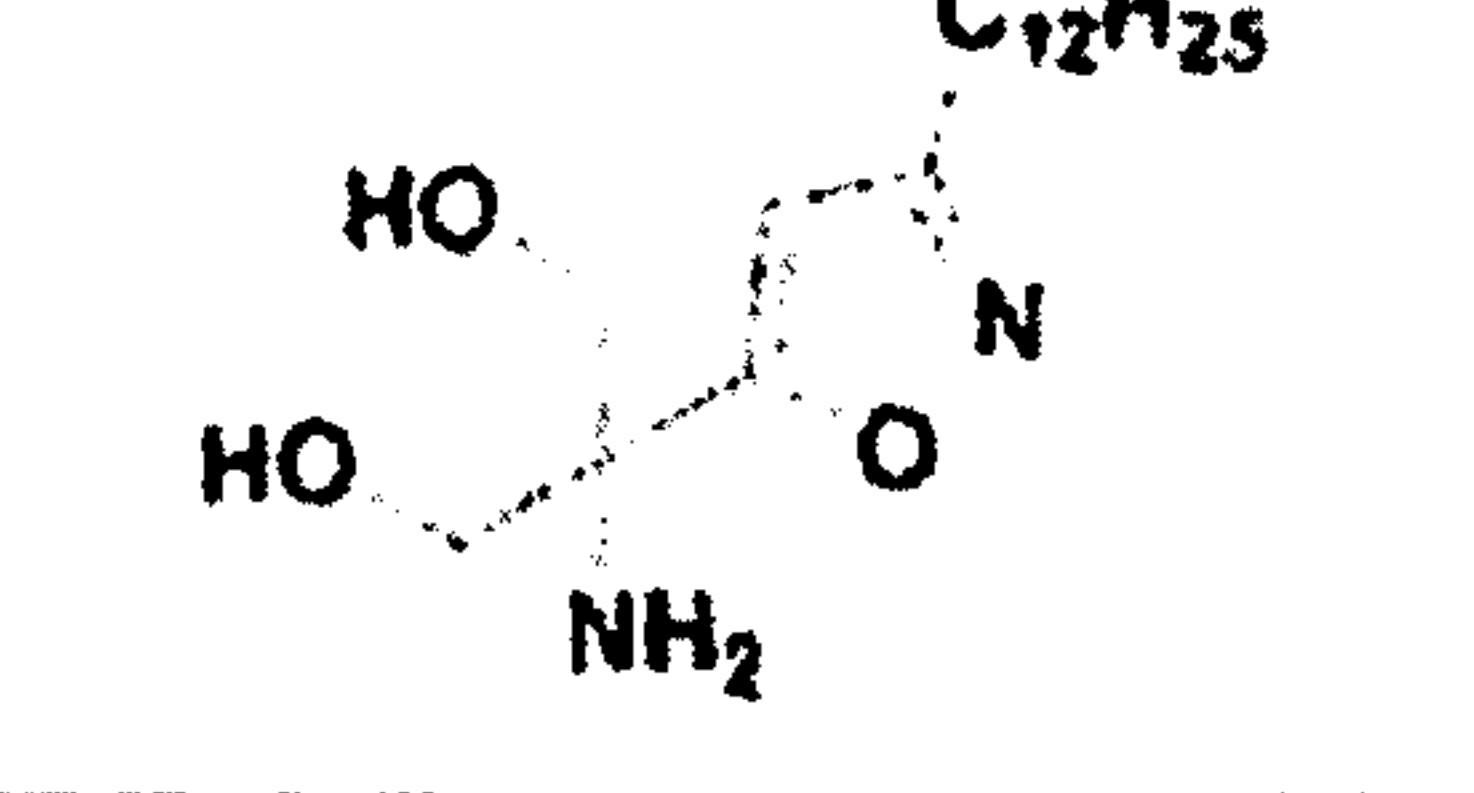
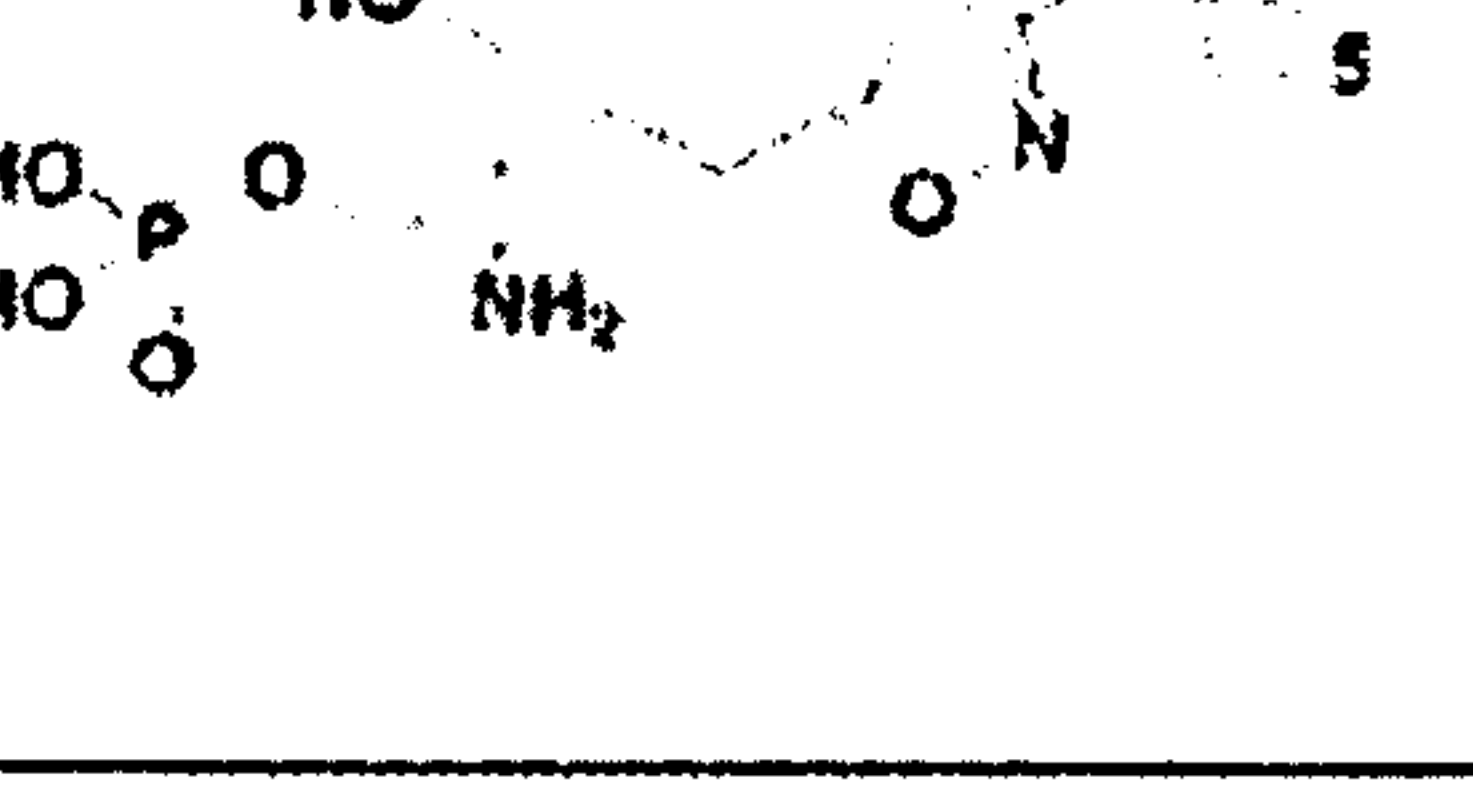
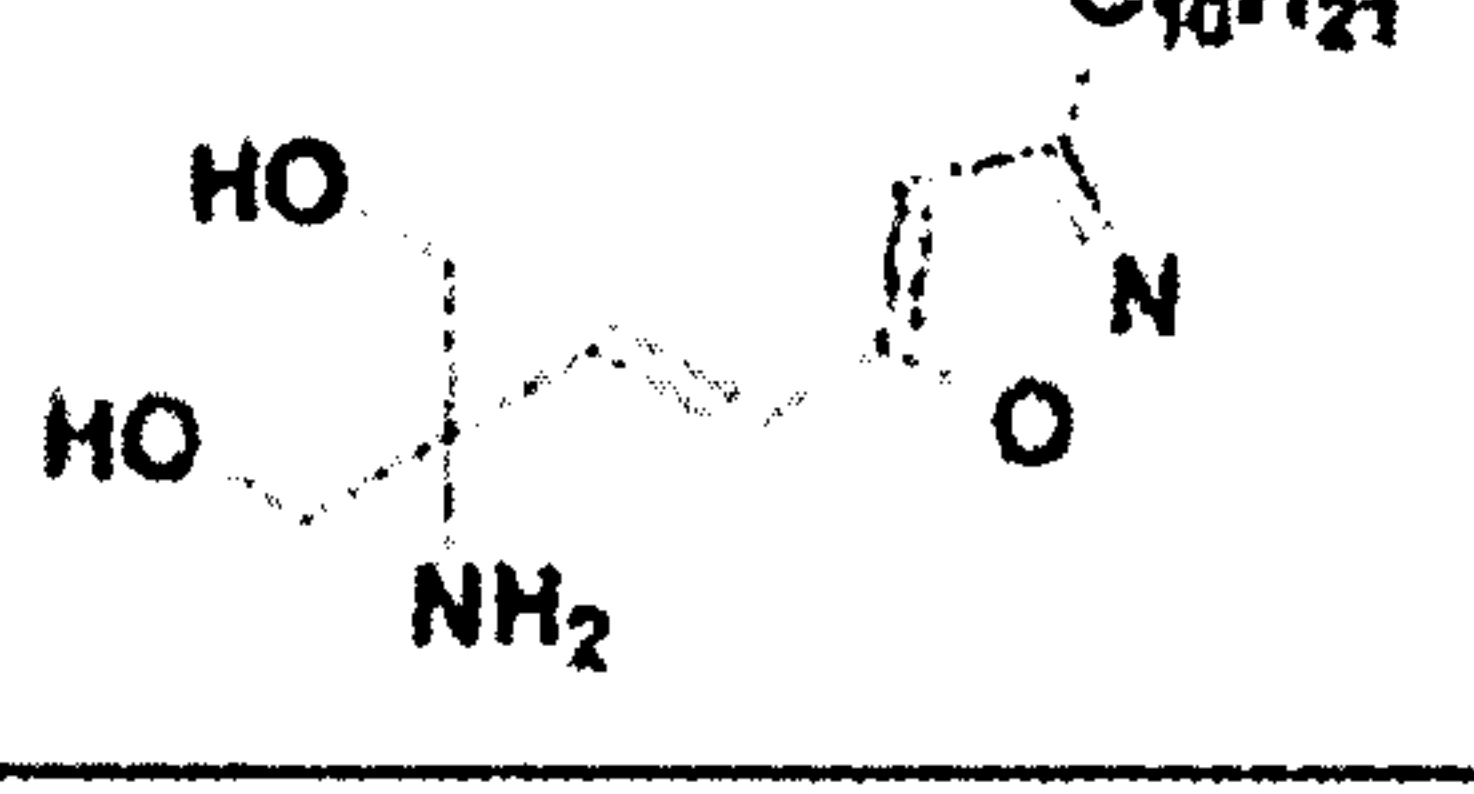
15 (24) N-(4-(1-decyl-1H-1,2,3-triazol-4-yl)-1-hydroxy-2-(hydroxymethyl)butan-2-yl)acetamide;

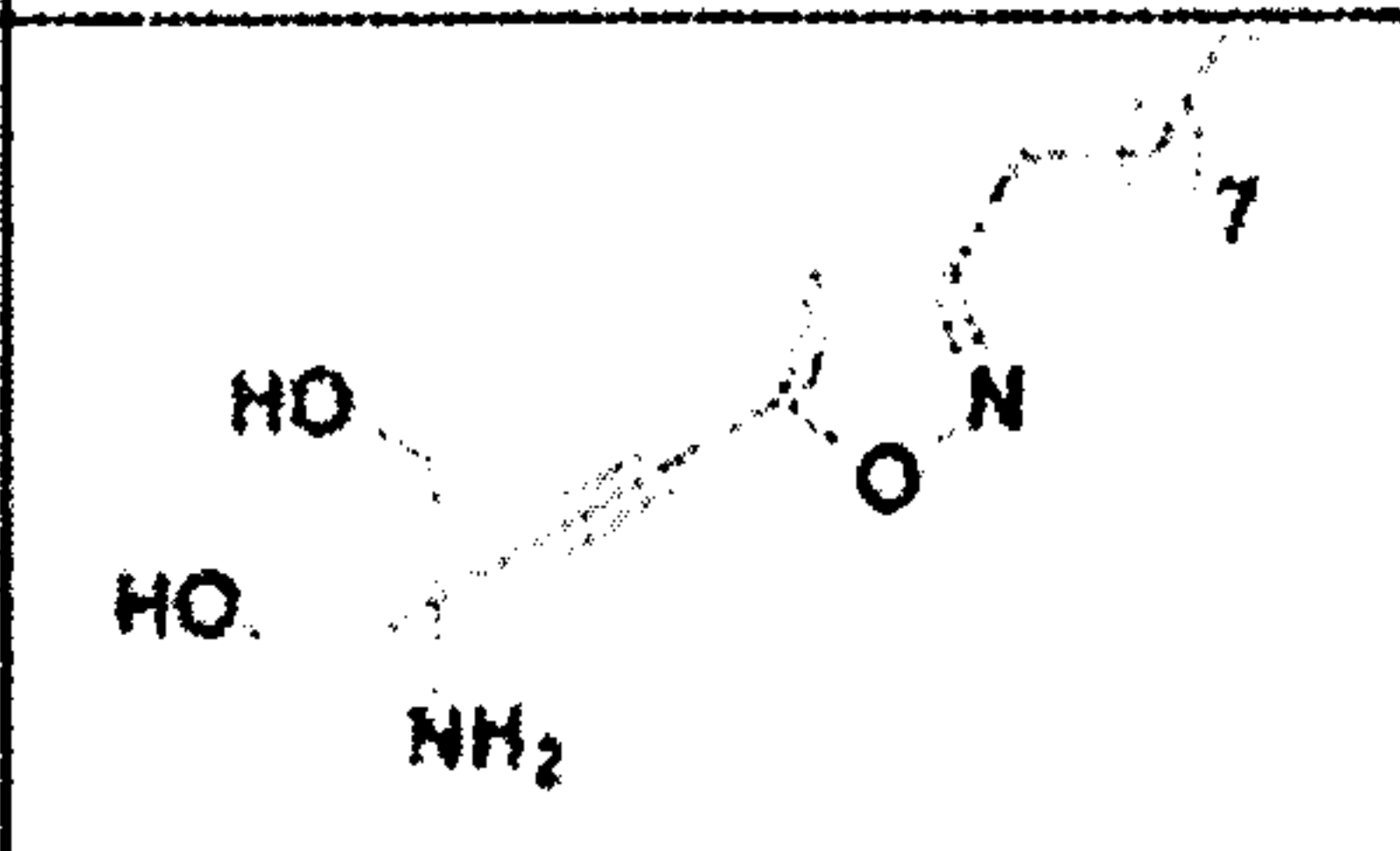
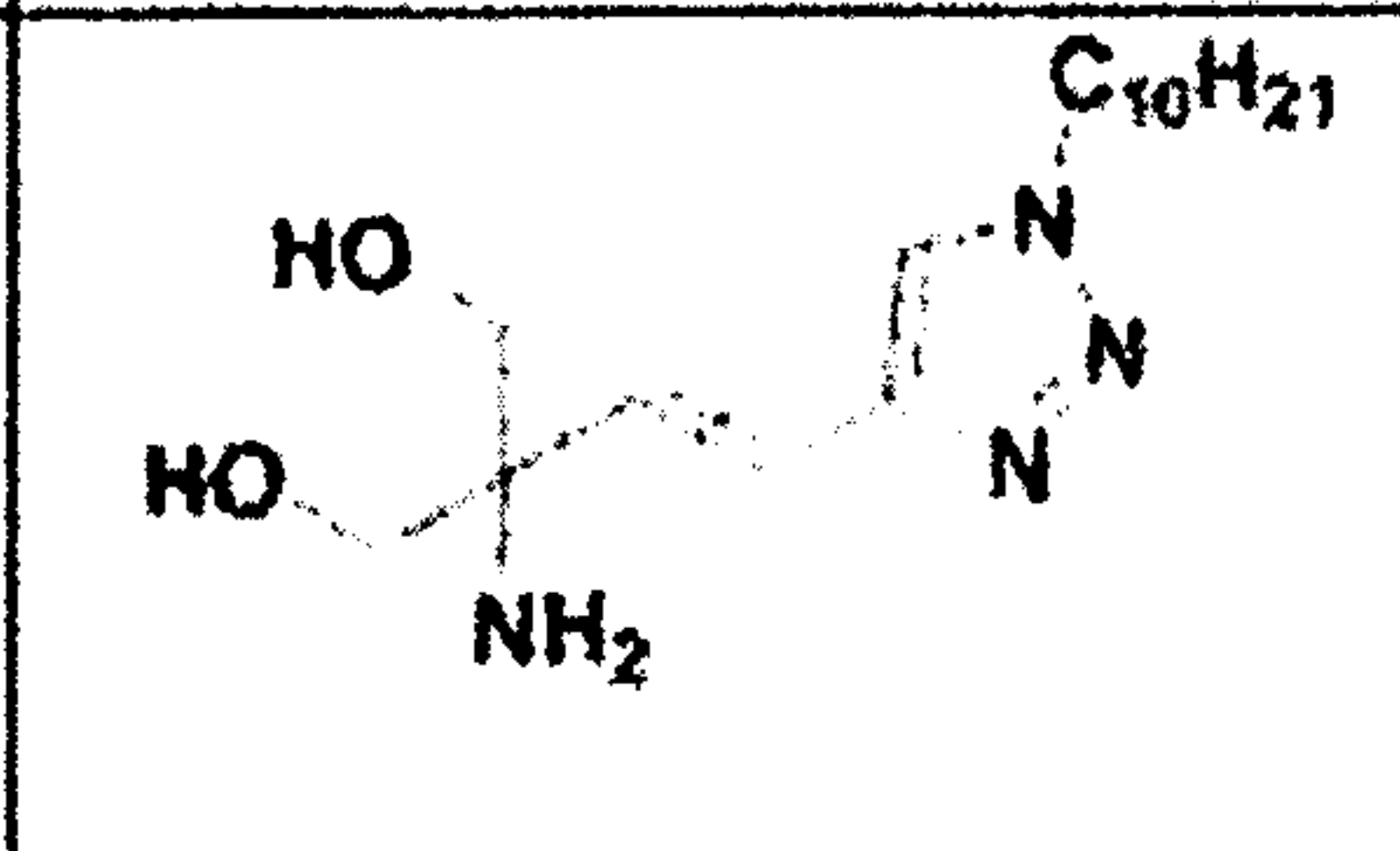
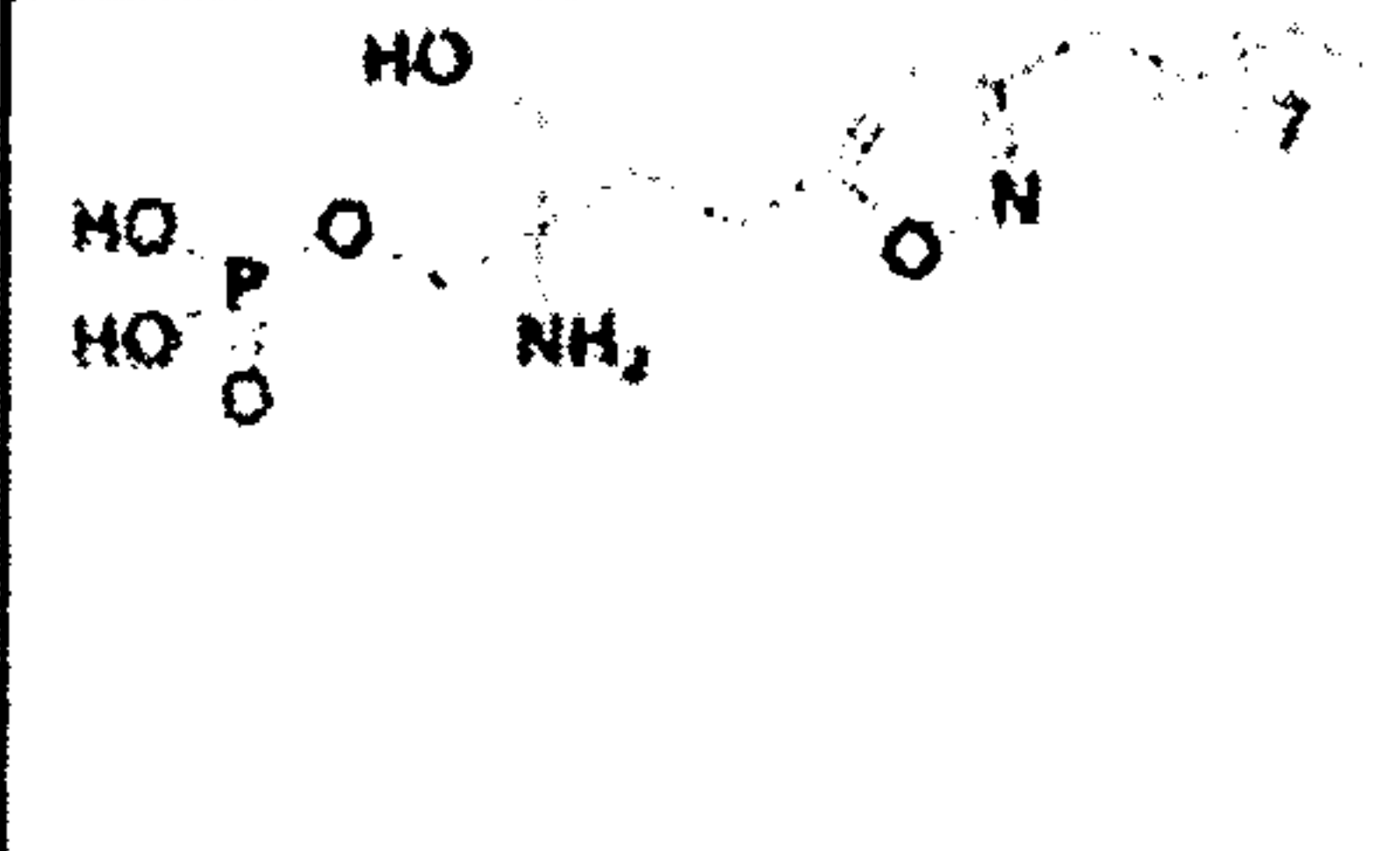
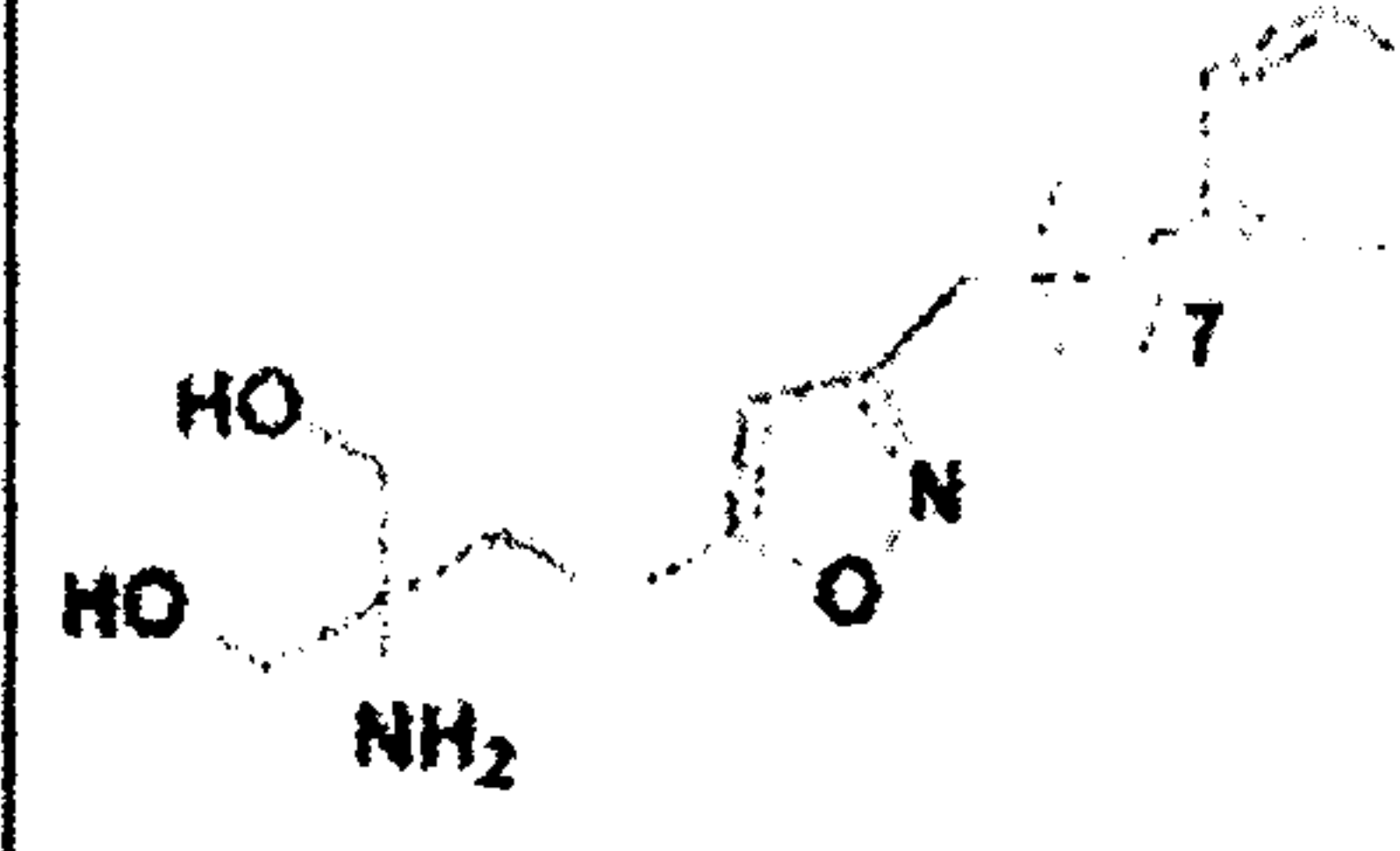
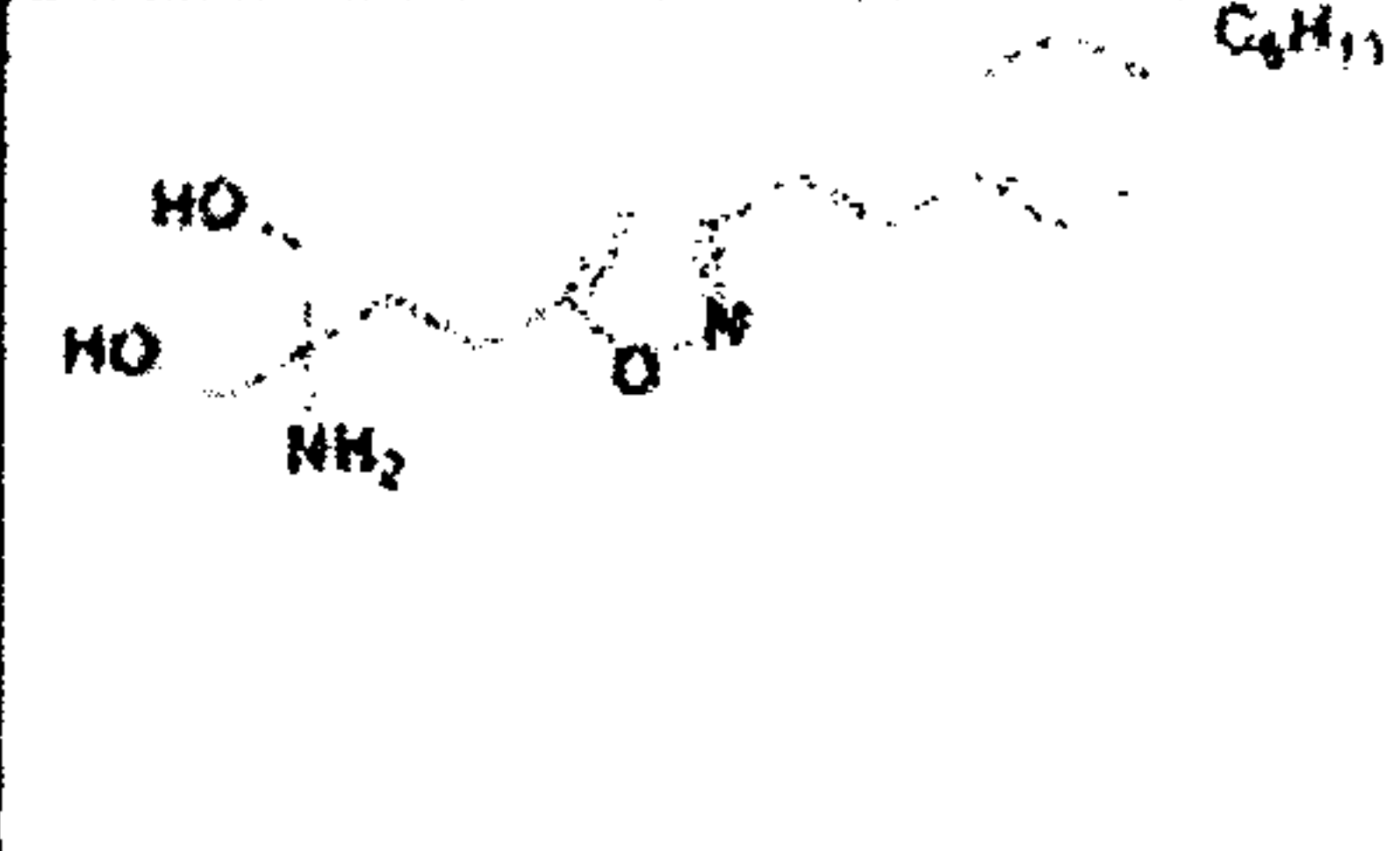
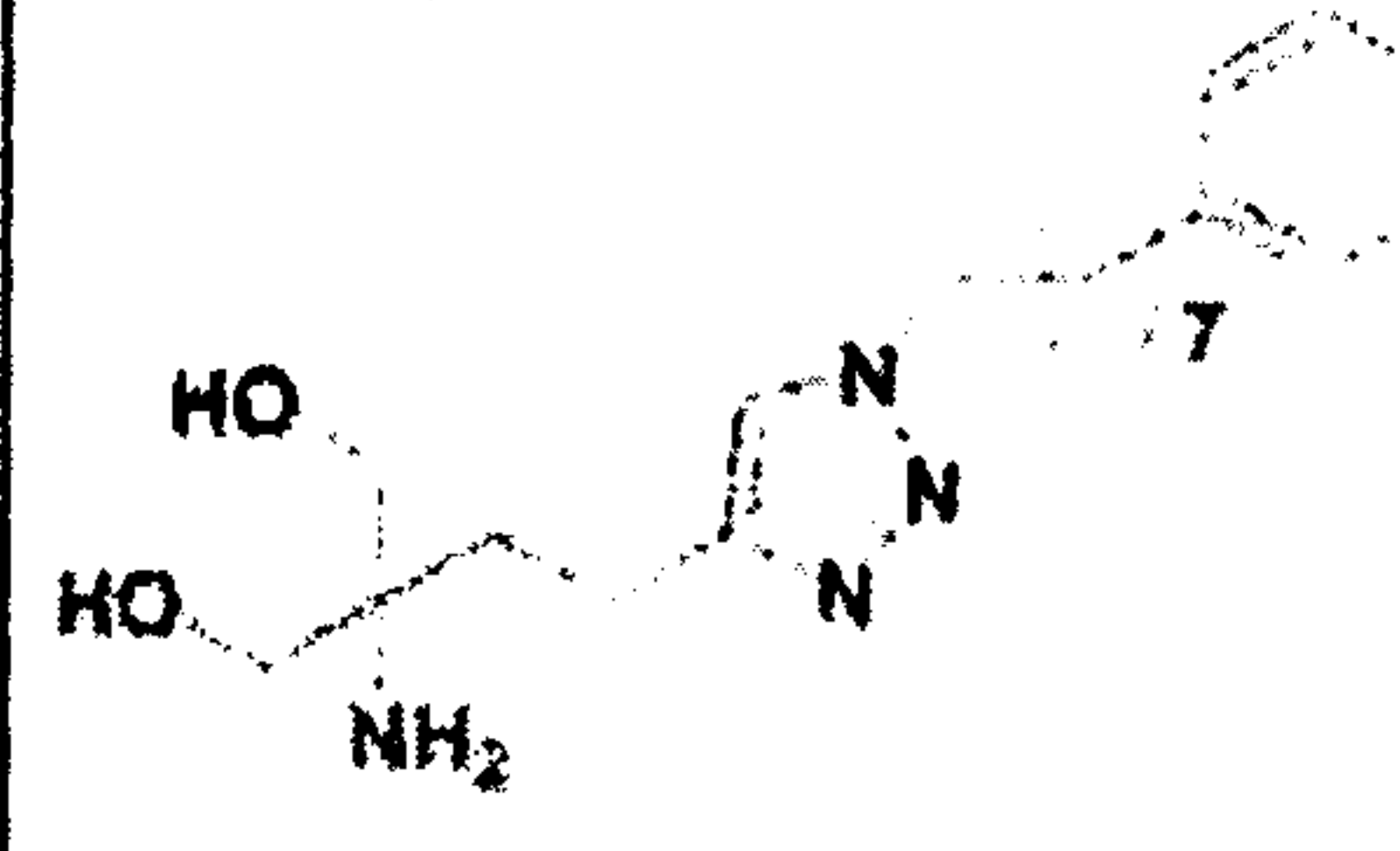
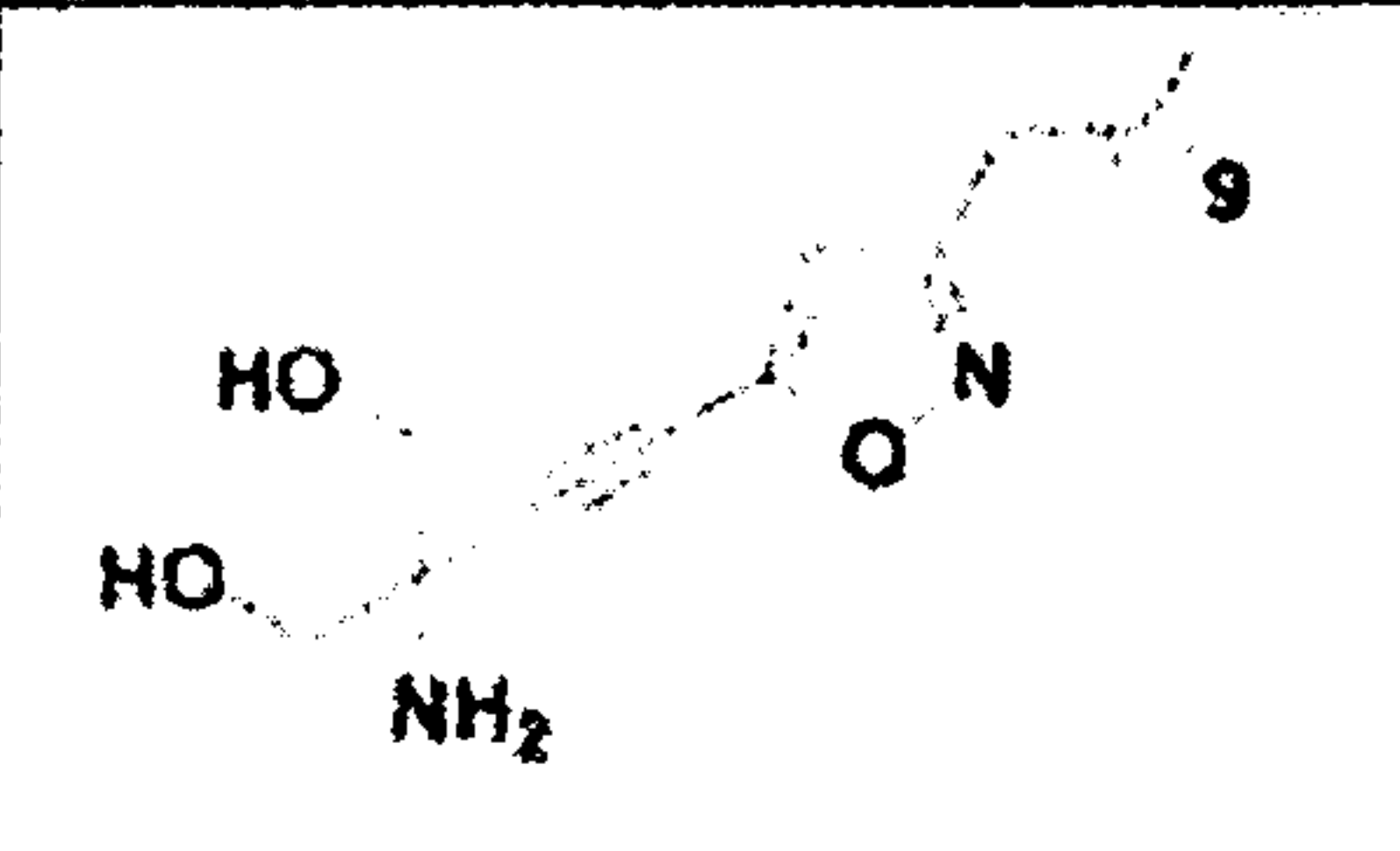
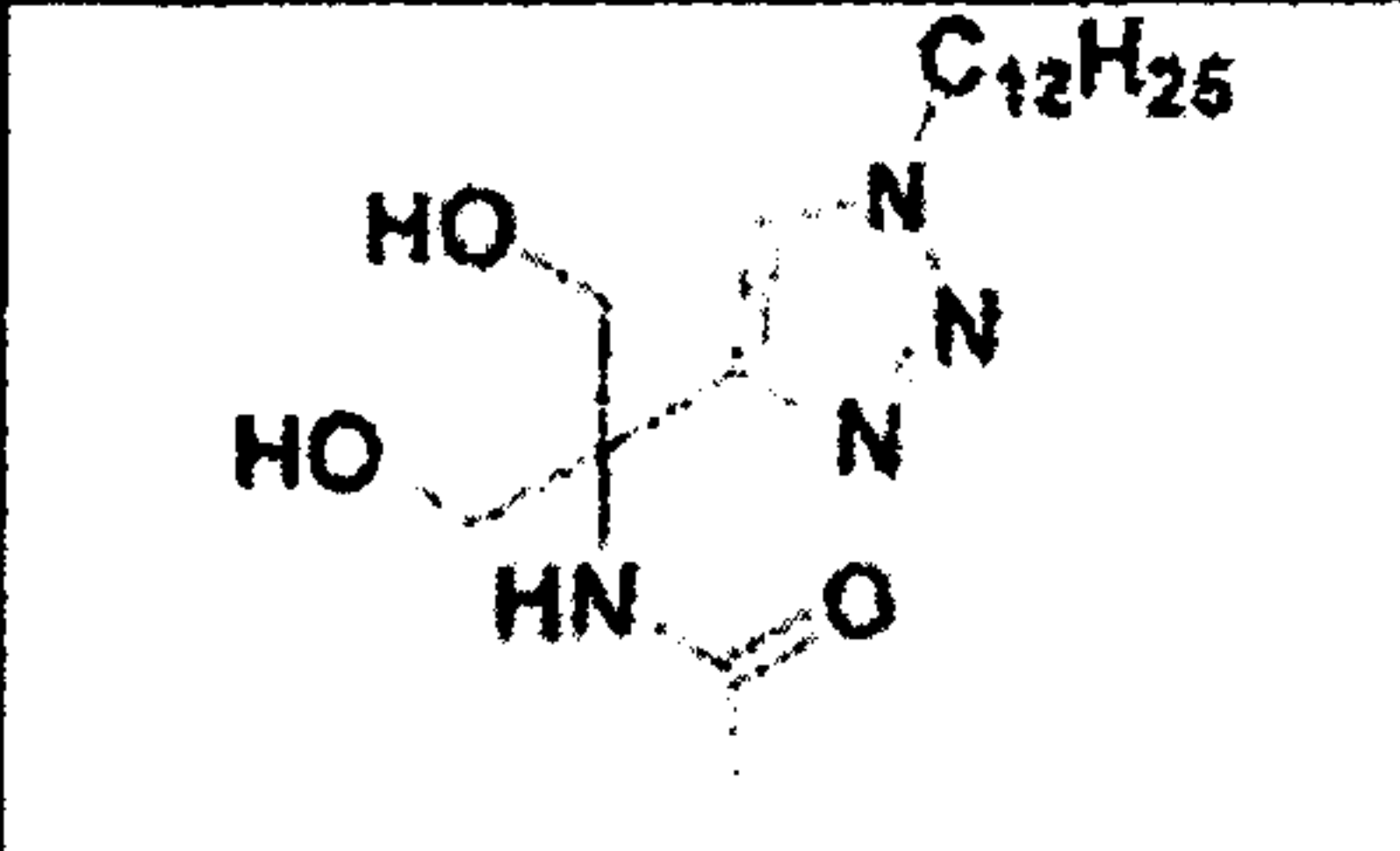
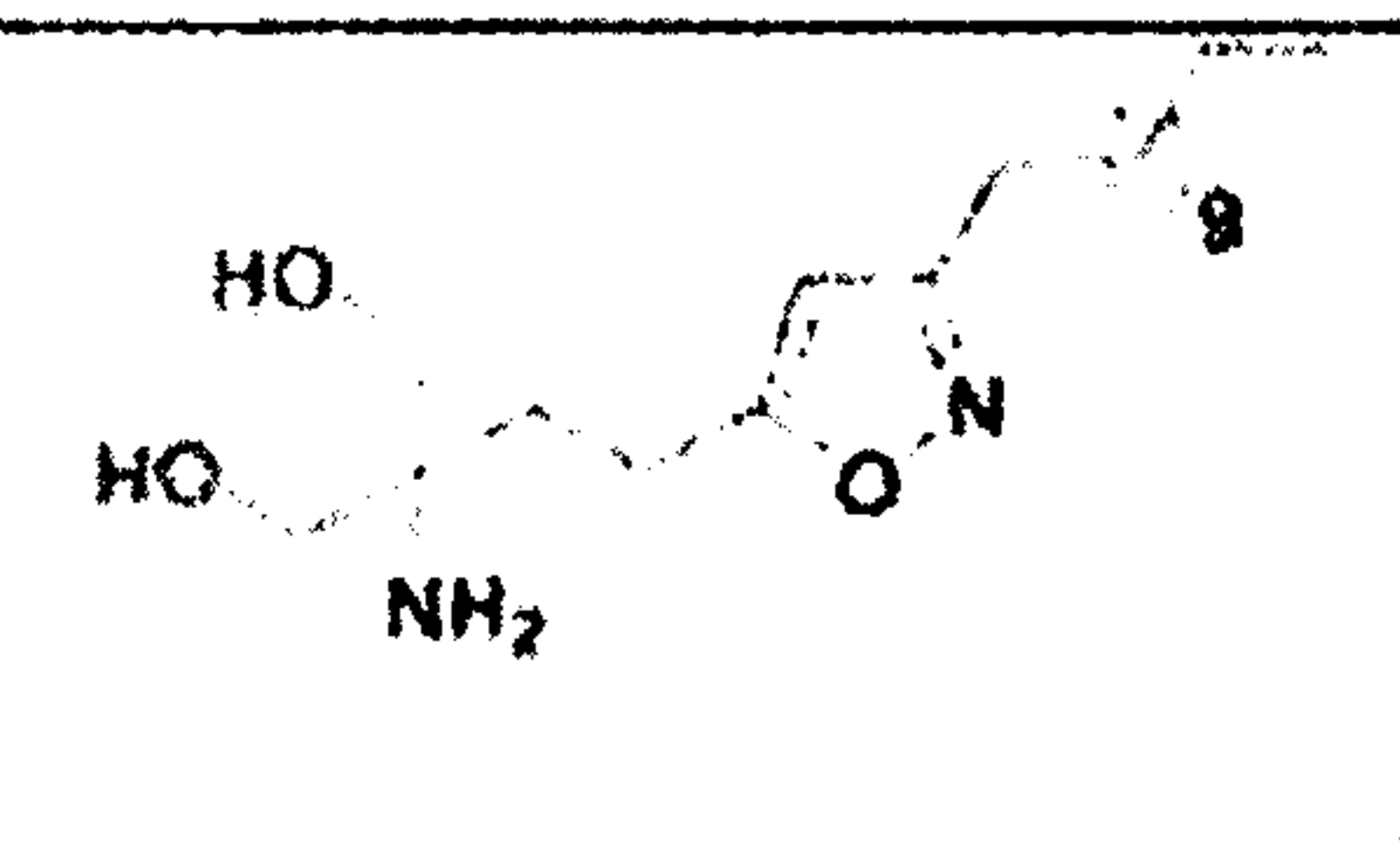
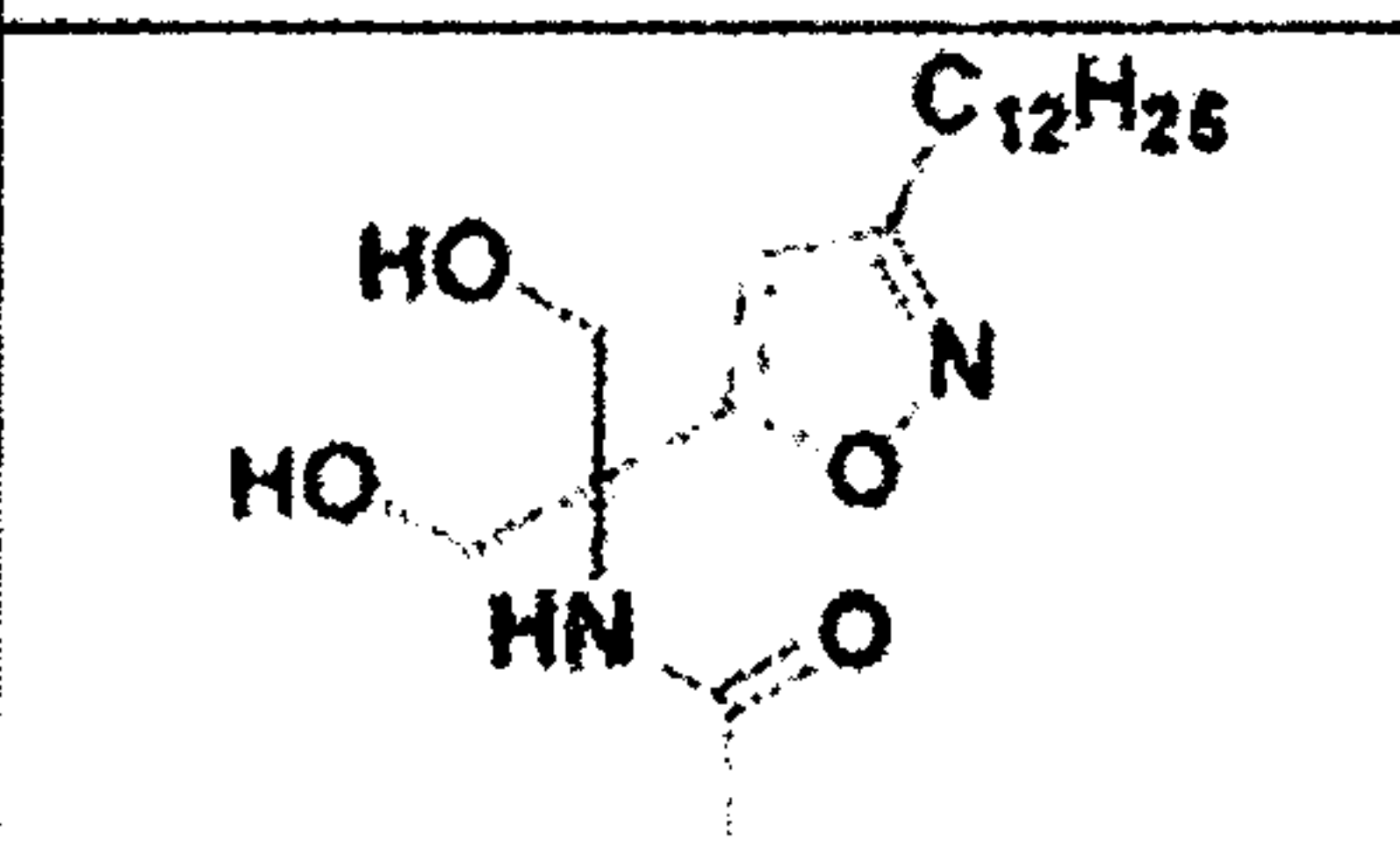
(25) N-(4-(3-decylisoxazol-5-yl)-1-hydroxy-2-(hydroxymethyl)butan-2-yl)acetamide; and

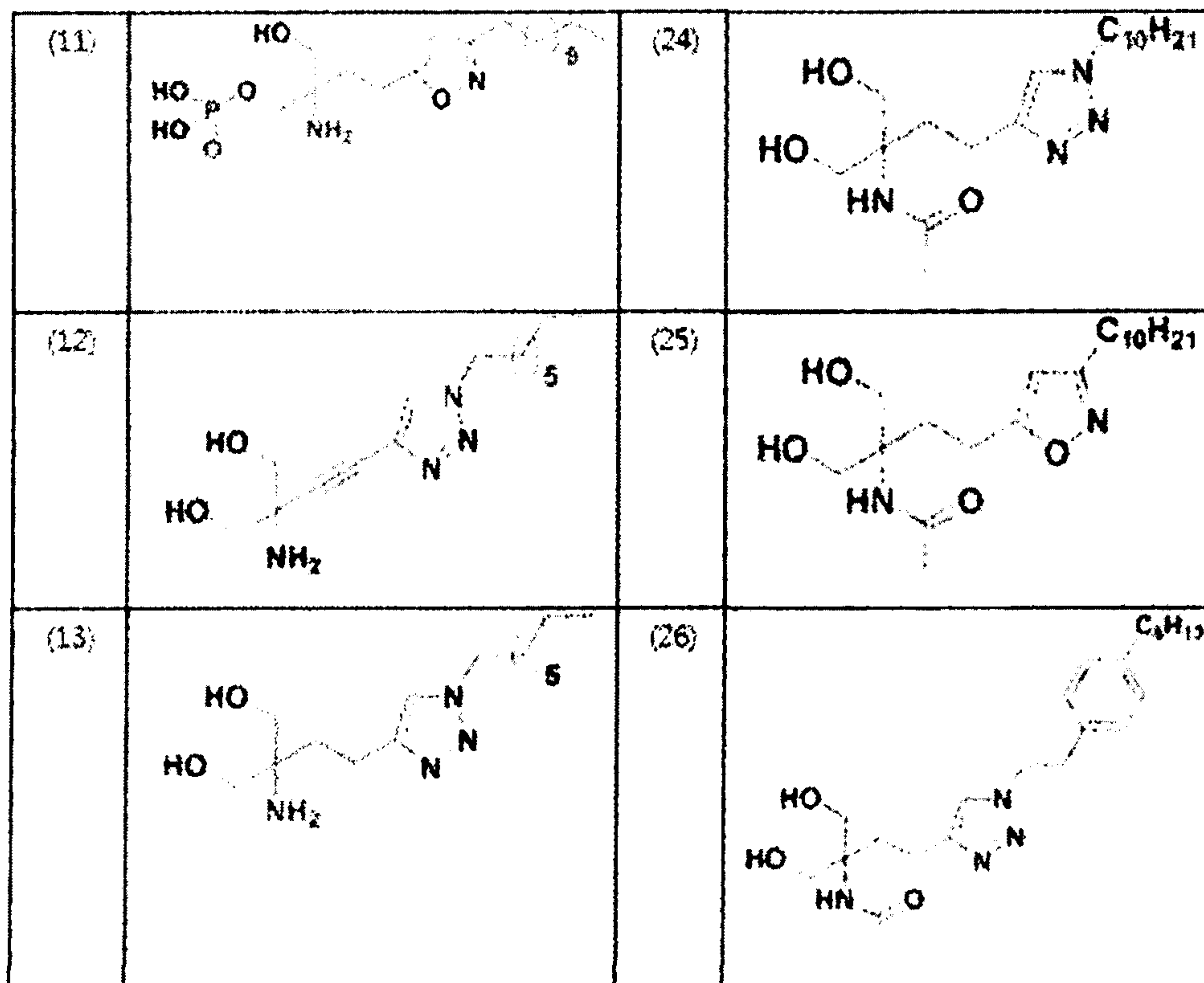
20 (26) N-(4-(1-(4-hexylphenethyl)-1H-1,2,3-triazol-4-yl)-1-hydroxy-2-(hydroxymethyl)butan-2-yl)acetamide.

The chemical structures of the compounds (1) to (26) are shown in Table 1 below.

[Table 1]

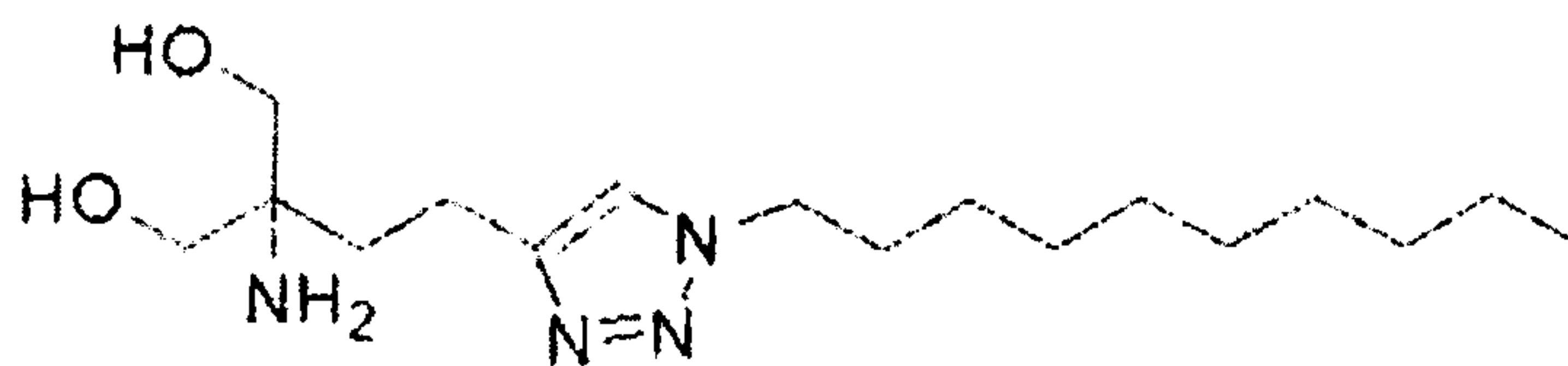
No	Chemical structure	No	Chemical structure
(1)		(14)	
(2)		(15)	
(3)		(16)	
(4)		(17)	
(5)		(18)	

(6)		(19)	
(7)		(20)	
(8)		(21)	
(9)		(22)	
(10)		(23)	



More preferably, the active ingredient according to the present invention is 2-amino-2-(2-(1-decyl-1H-1,2,3-triazol-4-yl)ethyl)propane-1,3-diol, which is a compound represented by the following Formula 2. In the following detailed description of the invention, the compound represented by the following Formula 2 is referred to as "SLB736".

[Formula 2]



The compound represented by Formula 1 of the present
 5 invention may be used in the form of a pharmaceutically
 acceptable salt, and as the salt, an acid addition salt
 formed by a pharmaceutically acceptable free acid is
 useful. The acid addition salt is obtained from inorganic
 acids such as hydrochloric acid, nitric acid, phosphoric
 10 acid, sulfuric acid, hydrobromic acid, hydroiodic acid,
 nitrous acid and phosphorous acid, non-toxic organic acids
 such as aliphatic mono- and di-carboxylates, phenyl-
 substituted alkanoates, hydroxy alkanoates and alkane
 dioates, aromatic acids, aliphatic and aromatic sulfonic
 15 acids, and organic acids such as acetic acid, benzoic
 acid, citric acid, lactic acid, maleic acid, gluconic
 acid, methanesulfonic acid, 4-toluenesulfonic acid,
 tartaric acid and fumaric acid. Examples of the
 pharmaceutically nontoxic salt include sulfate,
 20 pyrosulfate, bisulfate, sulfite, bisulfite, nitrate,
 phosphate, monohydrogen phosphate, dihydrogen phosphate,
 metaphosphate, pyrophosphate chloride, bromide, iodide,
 fluoride, acetate, propionate, decanoate, caprylate,
 acrylate, formate, isobutyrate, caprate, heptanoate,
 25 propiolate, oxalate, malonate, succinate, suberate,

sebacate, fumarate, maleate, butyne-1,4-dioate, hexane-
1,6-dioate, benzoate, chlorobenzoate, methyl benzoate,
dinitrobenzoate, hydroxybenzoate, methoxybenzoate,
phthalate, terephthalate, benzenesulfonate,
5 toluenesulfonate, chlorobenzene sulfonate, xylene
sulfonate, phenylacetate, phenylpropionate,
phenylbutyrate, citrate, lactate, β -hydroxybutyrate,
glycolate, malate, tartrate, methane sulfonate, propane
sulfonate, naphthalene-1 sulfonate, naphthalene-2-
10 sulfonate, mandelate and the like.

The acid addition salt according to the present
invention can be prepared through a conventional method,
for example, by dissolving a derivative of Formula 1 in an
organic solvent such as methanol, ethanol, acetone,
15 methylene chloride or acetonitrile, adding an organic or
inorganic acid thereto and filtering and drying the
resulting precipitate, or distilling a solvent and excess
acid under reduced pressure, drying and crystallizing the
resulting product in an organic solvent.

20 Also, a pharmaceutically acceptable metal salt can
be prepared using a base. An alkali metal or alkaline
earth metal salt is, for example, obtained by dissolving
the compound in an excess of an alkali metal hydroxide or
alkaline earth metal hydroxide solution, filtering the
25 insoluble compound salt, and evaporating and drying the

filtrate. At this time, it is pharmaceutically suitable to prepare a sodium, potassium or calcium salt as the metal salt. Also, the corresponding salt is obtained by reacting an alkali or alkaline earth metal salt with a suitable silver salt (e.g., silver nitrate).

Furthermore, the present invention includes not only the compound represented by Formula 1 and pharmaceutically acceptable salts thereof, but also solvates, optical isomers, hydrates and the like that can be prepared therefrom.

As used herein, the term "prevention" refers to any action that inhibits or delays the onset of nonalcoholic steatohepatitis by administering the pharmaceutical composition of the present invention to a subject.

As used herein, the term "treatment" refers to any action that ameliorates or positively affects symptoms of nonalcoholic steatohepatitis by administering the pharmaceutical composition of the present invention to a subject.

The active ingredient of the present invention specifically binds to sphingosine 1-phosphate receptor 4 (S1PR4) and then removes the same in cells, thereby inhibiting the activity of S1PR4.

In addition, the active ingredient of the present invention inhibits the activity of the inflammation-

regulatory complex (inflammasome).

In addition, the active ingredient of the present invention inhibits IL- β production in macrophages.

Specifically, as shown in FIG. 1, the compound of the present invention acts as a functional antagonist for this receptor, based on the mechanism of specifically binding to S1PR4 of macrophages and then inducing S1PR4 into cells and removing the same, thereby inhibiting the activity of NLRP3 inflammasome and preventing or treating nonalcoholic steatohepatitis (NASH).

The pharmaceutical composition of the present invention may further include a pharmaceutically acceptable carrier. In the present invention, the term "pharmaceutically acceptable" means that a compound is commonly used in the pharmaceutical field, while neither irritating an organism that administers the compound nor inhibiting the biological activity or properties of the administered compound.

In the present invention, the type of carrier is not particularly limited and any carrier can be used as long as it is commonly used in the art. Non-limiting examples of the carrier include saline, sterile water, Ringer's solution, buffered saline, albumin injection solutions, lactose, dextrose, sucrose, sorbitol, mannitol, xylitol, erythritol, maltitol, maltodextrin, glycerol, ethanol and

the like. These may be used alone or in combinations of two or more thereof.

Also, the pharmaceutical composition of the present invention may be added with other pharmaceutically acceptable additives such as excipients, diluents, antioxidants, buffers or bacteriostatic agents, and may be optionally added with fillers, extenders, wetting agents, disintegrants, dispersants, surfactants, binders or lubricants.

The pharmaceutical composition of the present invention may be formulated into a variety of formulations suitable for oral or parenteral administration. Non-limiting examples of the formulation for oral administration include troches, lozenges, tablets, aqueous suspensions, oily suspensions, prepared powders, granules, emulsions, hard capsules, soft capsules, syrups or elixirs and the like.

In order to formulate the pharmaceutical composition for oral administration, a binder such as lactose, saccharose, sorbitol, mannitol, starch, amylopectin, cellulose or gelatin, an excipient such as dicalcium phosphate, a disintegrant such as corn starch or sweet potato starch, or a lubricant such as magnesium stearate, calcium stearate, sodium stearyl fumarate or polyethylene glycol wax may be used, and a sweetener, fragrance, syrup

or the like may be also used.

Furthermore, in the case of a capsule, a liquid carrier such as fatty oil may be additionally used in addition to the above-mentioned substances.

5 Non-limiting examples of the parenteral formulation include injection solutions, suppositories, powders for respiratory inhalation, spray aerosols, ointments, powders for application, oils, creams and the like.

10 In order to formulate the pharmaceutical composition for parenteral administration, a sterile aqueous solution, a non-aqueous solvent, a suspension, an emulsion, a freeze-dried preparation, an external preparation and the like may be used. The non-aqueous solvent and the suspension may be propylene glycol, polyethylene glycol,
15 vegetable oils such as olive oil, injectable esters such as ethyloleate or the like.

Further, more specifically, when the pharmaceutical composition of the present invention is formulated into an injection solution, the composition of the present
20 invention is mixed in water with a stabilizer or buffer to prepare a solution or suspension, which is then formulated for ampoule or vial unit administration. In addition, when the pharmaceutical composition of the present invention is formulated into an aerosol, a propellant or the like may
25 be combined with an additive so as to disperse the water-

dispersed concentrate or the wet powder.

In addition, when the pharmaceutical composition of the present invention is formulated into an ointment, cream or the like, an animal oil, vegetable oil, wax, paraffin, starch, tragacanth, cellulose derivatives, polyethylene glycol, silicone, bentonite, silica, talc, zinc oxide or the like may be used as a carrier.

A pharmaceutically effective amount (effective dosage) of the pharmaceutical composition of the present invention may vary depending on the formulation method, administration method, administration time and/or administration route of the pharmaceutical composition or the like, and may vary depending on various factors including the type and extent of the response to be achieved by administering the pharmaceutical composition, the type, age, body weight, general state of health, state or extent of the disease, gender, diet, or excretion of the subject, to which the composition is administered, and the ingredients of drug compositions simultaneously or sequentially administered to the subject, as well as various similar factors well known in the pharmaceutical field, and those skilled in the art can easily determine and prescribe a dosage effective for the desired treatment.

The dosage of the pharmaceutical composition of the

present invention to obtain more preferable effects is preferably 0.1 mg/kg to 1,000 mg/kg, more preferably 10 mg/kg to 500 mg/kg per day. The pharmaceutical composition of the present invention may be administered once a day,
5 or may be administered several times in respective portions. Therefore, the dosage does not limit the scope of the present invention in any aspect.

The administration route and administration method of the pharmaceutical composition of the present invention
10 may be independent of each other and are not particularly limited, and any administration route or method may be used, as long as the pharmaceutical composition can be delivered to the desired site. The pharmaceutical composition may be administered by oral or parenteral
15 administration.

The method for parenteral administration includes, for example, intravenous administration, intraperitoneal administration, intramuscular administration, transdermal administration, subcutaneous administration or the like.
20 The composition may be applied, sprayed or inhaled to the disease site, but the present invention is not limited thereto.

The present invention also provides a health functional food composition for preventing or ameliorating
25 non-alcoholic steatohepatitis (NASH) containing the

compound represented by Formula 1, an optical isomer thereof or a pharmaceutically acceptable salt thereof as an active ingredient.

The food composition of the present invention may be prepared into any one formulation selected from the group consisting of a tablet, granule, powder, capsule, liquid solution and pill. The food composition according to the present invention may be formulated into a powder, liquid, tablet, soft capsule, granule, tea bag, instant tea or drink containing the compound represented by Formula 1 as an active ingredient. The content of the active ingredient may be appropriately determined depending on the purpose of use (prevention or amelioration). In general, the amount of the active ingredient contained in the food composition may be added in an amount of 0.1 to 90% by weight of the total food weight. However, in the case of prolonged administration for health and hygiene or health maintenance, the amount may be below the range of content defined above. In addition, the food composition according to the present invention may also contain other pharmaceutical compositions or natural products that do not impair the main effect of the present invention in addition to the active ingredient described above and preferably have an effect synergistic with the main effect.

The food composition formulated in the form described above may be added to food as it is or may be used in combination with other food or food ingredients, and may be appropriately used according to conventional methods.

5 Examples of the food include drinks, meat, sausages, bread, biscuits, rice cakes, chocolate, candies, snacks, confectionery, pizza, ramen, other noodles, gums, dairy products including ice creams, various soups, beverages, alcoholic beverages and vitamin complexes, dairy products
10 and dairy-processed products, and include all functional foods in the conventional sense.

When the food composition of the present invention is a drink, it contains the active ingredient of the present invention as an essential ingredient in a predetermined
15 ratio. There are no particular limitations as to other ingredients used to prepare the drink, and various flavoring agents or natural carbohydrates may be contained as additional components, like common beverages. Examples of such natural carbohydrates include conventional sugars
20 including monosaccharides such as glucose and fructose, disaccharides such as maltose and sucrose and polysaccharides such as dextrin and cyclodextrin, and sugar alcohols such as xylitol, sorbitol and erythritol. In addition to the substances described above, a flavoring
25 agent such as a natural flavoring agent or a synthetic

flavoring agent may be used. The proportion of the natural carbohydrate is generally about 1 to 20 g, preferably about 5 to 12 g with respect to 100 ml of the composition of the present invention.

5 Also, the food composition of the present invention may contain a variety of nutrients, vitamins, minerals (electrolytes), flavoring agents such as synthetic flavoring agents and natural flavoring agents, colorings and enhancers (such as cheese or chocolate), pectic acid
10 and salts thereof, alginic acid and its salts, organic acids, protective colloidal thickeners, pH adjusters, stabilizers, preservatives, glycerin, alcohols, carbonation agents used for carbonated drinks, and the like. In addition, the food composition of the present
15 invention may contain flesh for producing natural fruit juices, fruit juice beverages and vegetable beverages. These components can be used independently or in combination. The proportion of such additives is not critical, but is generally determined within the range of
20 about 0.1 to about 20 parts by weight with respect to 100 parts by weight of the active ingredient of the present invention.

 The present invention also provides a pharmaceutical composition for preventing or treating nonalcoholic
25 steatohepatitis containing a functional antagonist of

S1PR4 as an active ingredient.

The present invention also provides a health functional food composition for preventing or ameliorating non-alcoholic steatohepatitis containing a functional antagonist of S1PR4 as an active ingredient.

In one embodiment of the present invention, the inhibition of S1PR4 expression through S1PR4 shRNA as well as the sphingolipid compound according to the present invention provides the effect of reducing IL-1 β production in macrophages and the effects of preventing the onset of NASH and treating NASH. Therefore, the functional antagonist for S1PR4 according to the present invention may be not only a compound that acts as a functional antagonist against S1PR4 including the sphingolipid compound according to the present invention, but also the antibody against S1PR4 that inhibits the activity of S1PR4 protein, and antisense nucleotides, aptamers, siRNAs, shRNAs, miRNAs and RNAi for mRNA of S1PR4 that inhibits S1PR4 expression. In the present invention, the antibody against S1PR4 may be a monoclonal antibody or a polyclonal antibody.

The present invention also provides a method for preventing or treating nonalcoholic steatohepatitis (NASH) including administering to a subject a composition containing the compound represented by Formula 1, an

optical isomer thereof, or a pharmaceutically acceptable salt thereof as an active ingredient.

The present invention also provides the use of composition containing the compound represented by Formula 1, an optical isomer thereof, or a pharmaceutically acceptable salt thereof as an active ingredient for preventing or treating nonalcoholic steatohepatitis (NASH).

10 **【Advantageous effects】**

The sphingolipid compound of the present invention has effects of reducing deposition of lipids in liver tissue, reducing infiltration of inflammatory cells and inhibiting fibrosis, and is also expected to be applicable as a leading substance effective for the prevention or treatment of non-alcoholic steatohepatitis (NASH) by reducing liver function (ALT) levels, liver tissue inflammation and expression of fibrosis-related genes.

20 **【Brief Description of the Drawings】**

The above and other objects, features and other advantages of the present invention will be more clearly understood from the following detailed description taken in conjunction with the accompanying drawings, in which:

25 FIG. 1 is a schematic diagram showing a mechanism for

treating nonalcoholic steatohepatitis of a compound according to the present invention;

FIG. 2 shows the result identifying the inhibitory effect of the sphingolipid compound SLB736 according to the present invention on the activity of NLRP3 inflammation-regulating complex;

FIG. 3 shows the result identifying the inhibitory effect of the SLB736 compound according to the invention on S1PR4 expression in a mouse liver tissue model;

FIG. 4 shows the result identifying the inhibitory effect of the SLB736 compound according to the present invention on S1PR4 expression in a mouse macrophage model;

FIG. 5 shows the result identifying that the SLB736 compound of the present invention is not recycled to the cell membrane after being introduced into the cells;

FIG. 6 shows the result identifying the inhibitory effect of reducing IL- β production of S1PR4 shRNA in mouse macrophages;

FIG. 7 shows the result of microscopic examination for identifying the effect of SLB736 on the prevention of steatosis and lobular inflammation in liver tissue of a NASH-induced mouse model;

FIG. 8 shows the result of microscopic examination for identifying the effect of SLB736 on the prevention of fibrosis in liver tissue of a NASH-induced mouse model;

FIG. 9 shows the result of evaluation on the NASH prevention effect of SLB736 in liver tissue of the NASH-induced mouse model based on the CRN scoring system;

FIG. 10 shows the result identifying the expression inhibition effects of inflammatory and fibrosis markers in liver tissue of the mouse model;

FIG. 11 shows the result identifying the therapeutic effect of SLB736 in liver tissue of the mouse model (A - treatment of steatosis and lobular inflammation; B - treatment of fibrosis); and

FIG. 12 shows the result identifying that lymphopenia does not appear in blood after administration of SLB736, compared to after administration of FTY720.

【Best mode】

Hereinafter, the present invention will be described in more detail with reference to the following examples. However, it will be obvious to those skilled in the art that these examples are provided only for illustration of the present invention and should not be construed as limiting the scope of the present invention.

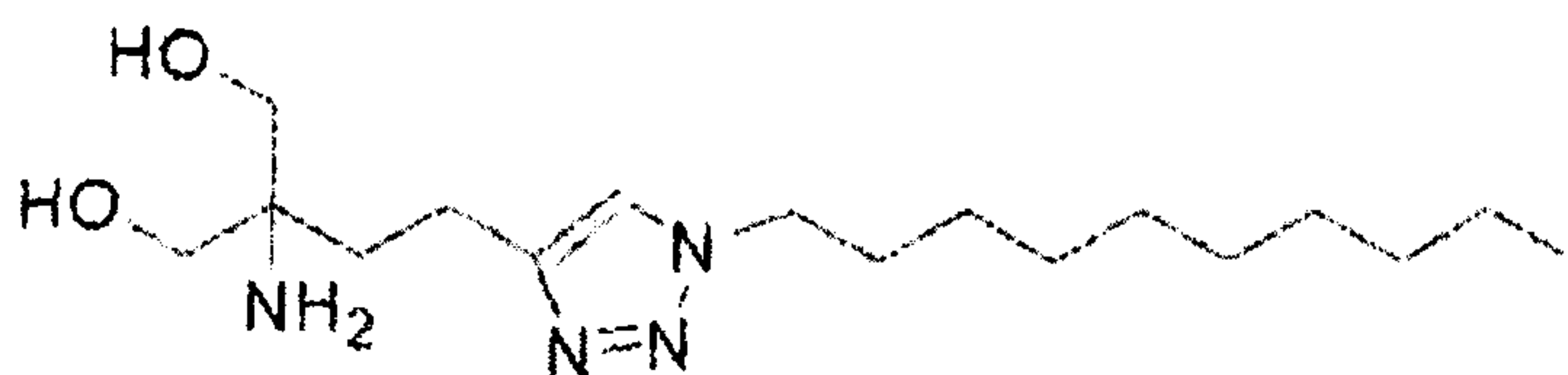
Example 1. Selection of sphingolipid candidates

Fifteen types of sphingolipid compounds structurally similar to myriocin and FTY720 (fingolimod), among about 600

types of sphingolipid compounds held by a sphingolipid material bank, were obtained in order to develop lead candidates for the development of nonalcoholic steatohepatitis (NASH) drugs.

5 The result of measurement of agonistic activity of S1P receptors (sphingosine 1-phosphate receptors) using the GPCR activity measurement method of DiscoverX Corp. showed that 2-amino-2-(2-(1-decyl)-1H-1,2,3-triazol-4-yl)ethylpropane-1,3-diol (hereinafter referred to as "SLB736") has an
10 agonistic effect specific for S1PR4.

[Formula 2]



Example 2. Inhibitory activity of NLRP3 inflammation-regulating complex

It is well known that the treatment of macrophages with lipopolysaccharide (LPS) results in activation of inflammation-regulating complex (inflammasome), thereby increasing IL-1 β production (Mariathasan et al., Nat. Rev. Immunol. 2007; 17186029). In order to determine the inhibitory effect of the activity of inflammation-regulating complex (NLRP3 inflammasome), which is a
25

representative inflammation-regulating protein, the IL-1 β production of candidate substances upon the treatment of macrophages with LPS was measured and evaluated.

5 First, 12 hours after treatment of Raw cells (mouse macrophage lines) with LPS (100 ng/ml, Sigma-Aldrich), the concentration of IL-1 β in the medium was found to be markedly increased.

10 The macrophages were treated with myriocin in different concentrations as a control group and were then treated with the same concentration of LPS for 24 hours. As a result, it was found that 10 μ M of myriocin effectively reduced IL-1 β production. Based on this, experiments were conducted to determine the effect of sphingolipid candidates on the inhibition of IL-1 β .

15 The result of treatment with the known sphingolipid derivative drug, FTY720, showed that FTY720 exhibits an inhibitory effect of IL-1 β production similar to 10 μ M myriocin at a concentration of 0.1 μ M, which is a much lower concentration than myriocin. On the other hand, when
20 treating with SLB736, the compound represented by Formula 2 according to the present invention, at 0.1 μ M or 1.0 μ M and then with LPS, it was found that the inhibitory effect of IL-1 β production was excellent at each concentration to a significant extent to FTY720 (FIG. 2).

25

Example 3: Identification of effects of SLB736 on inhibition of expression of S1PR4 in tissue and cells

In order to determine whether or not SLB736, the compound of the present invention, acts as a functional antagonist of S1PR4, changes in S1PR4 protein expression were observed after administration of candidate substances to nonalcoholic steatohepatitis (NASH) model mice and macrophages thereof.

It is well known that the administration of a methionine choline-deficient diet (MCDD) to mice results in non-alcoholic steatohepatitis. The mice (C57BL/6N, Orient Bio, Inc., Korea) used in the experiments were divided into three groups, and MCDD alone, 1 mg/kg of MCDD and FTY720, and 1 mg/kg of MCDD and SLB736 were administered to each group for 6 weeks. Then, changes in the expression of S1PR4 protein in liver tissue of mice were measured through Western blotting. As a result, it was found that the expression of S1PR4 was significantly increased in the mice administered with MCDD alone compared to the control (con) mice not administered with MCDD, and the amount of S1PR4 protein was decreased in the mice administered with FTY720 or SLB736 in combination with MCDD (FIG. 3).

In addition, Raw cells, which are mouse macrophage lines, were treated with a FTY720 or SLB736 substance, and

were then treated with LPS, and S1PR4 expression was then measured. As a result, the expression of S1PR4 was significantly increased in cells treated only with LPS, compared to the control group (con) without any treatment, whereas treatment with FTY720 or SLB736 in combination with LPS was found to significantly reduce the expression level of S1PR4 (FIG. 4).

This result demonstrates that the SLB736 compound of the present invention functions to reduce the expression level of S1PR4 protein.

Example 4. Identification of activity as functional antagonist of S1PR4

Cell lines overexpressing S1PR4-EGFP were used to identify whether or not SLB736 and FTY720 actually internalize S1PR4 into cells and thereby act as functional antagonists.

Specifically, the cell lines overexpressing S1PR4-EGFP were incubated in a cover glass allowing for microscopic observation and were then treated with a vehicle (dimethyl sulfoxide hydrochloric acid, 100 nM), S1P (100 nM; agonist, positive control group), FTY720 (100 nM) and SLB736 (1 μ M). 30 minutes and 2 hours after treatment with each material, the cell lines were fixed with a fixing solution and observed with a fluorescence

microscope. At this time, the cells were cultured after treatment with a protein synthesis inhibitor (cycloheximide) in order to inhibit the synthesis of new receptors, were first observed with a fluorescence microscope 30 minutes after each material treatment, and were then washed. Then, the cells were further cultured for 1 hour 30 minutes and were secondarily observed, and cell images were obtained.

The results are shown in FIG. 5. It could be seen that S1PR4 was internalized in the medium treated with S1P, FTY720 and SLB736 after cell culture for 30 minutes, and in S1P (agonist)-treated cells after cell culture for 2 hours, S1PR4 was recycled to the cell membrane, whereas FTY720 and SLB736 were not recycled thereto. This indicates that SLB736 according to the present invention acts as a functional antagonist of S1PR4, like FTY720.

Example 5. Identification of inhibition of IL-1 β production by S1PR4 shRNA

In addition, after infecting Raw cells, the macrophage line, with S1PR4 shRNA lentivirus, viable cells capable of inhibiting S1PR4 expression were selected. When treating the selected cells with LPS, changes in IL-1 β production were measured and were compared with control groups (Vec, Vec + LPS) not infected with shRNA lentivirus.

As a result, it could be seen from FIG. 6 that the IL-1 β concentration was significantly reduced in the macrophage line infected with S1PR4 shRNA lentivirus (shS1PR4 + LPS) compared to the control group (Vec + LPS).

5

Example 6. Identification of effects of SLB736 on prevention of NASH onset and treatment of NASH

In order to identify the effect of the SLB736 compound on the prevention of onset of non-alcoholic steatohepatitis (NASH), an animal model administered with MCDD for 6 weeks was used.

Generally known characteristic microscopic findings of NASH include steatosis, ballooning degeneration of hepatocytes, lobular inflammation, perisinusoidal fibrosis and the like. In 2005, the NASH clinical research network (CRN) designed a detailed grading system for lesions corresponding to nonalcoholic steatohepatitis and thus proposed the nonalcoholic fatty liver disease activity score (NAS), which is widely used in various research due to the excellent reproducibility thereof. Accordingly, the present inventors have scored the grade of steatosis, location of steatosis, lobular inflammation and the extent of fibrosis, from the results of liver tissue observation of animal models, based on the CRN classification system. Based on this, the effect of SLB736, which is the target

compound in the present invention, on the prevention or progression of non-alcoholic steatohepatitis caused by MCDD was evaluated.

Specifically, eight-week-old mice (C57BL6/N, Orient Bio Inc., Korea) were divided into two groups, the control group was fed only with MCDD for 6 weeks to induce the onset of NASH, and the experimental group was fed with SLB736 in a dose of 1 mg/kg/day for six weeks in combination with MCDD, liver tissue was collected from each mouse and was observed with a microscope, and the degree of steatosis, lobular inflammation and fibrosis was observed. The result showed that the degree of steatosis and lobular inflammation (see black triangle in FIG. 7) were significantly reduced in mice administered in combination with SLB736, and the degree of fibrosis was also significantly reduced in the SLB736 administration group.

In order to determine this quantitatively, evaluation was conducted according to the NAS system of the CRN. The result showed that, as shown in FIG. 9, the degree of steatosis, lobular inflammation and the degree of fibrosis were all significantly alleviated compared to the control group.

In addition, the expression levels of TGF- β , TNF- α and MCP-1 were measured in order to identify the effects

of the SLB736 compound on the expression of inflammatory markers and fibrosis markers in liver tissue. Specifically, RNA was extracted from liver tissue of the animal model and mRNA was measured using Q-PCR. The result showed that the expression of all of TGF- β , TNF- α and MCP-1 was reduced to a significant level in mice administered with SLB736 (FIG. 10).

This shows that the compound SLB736 of the present invention is effective in preventing nonalcoholic steatohepatitis (NASH).

A NASH-induced animal model obtained by administering MCDD to 8-week-old mice (C57BL6/N, Orient Bio Inc., Korea) for 4 weeks was used in order to identify the effect of the SLB736 compound on the treatment of non-alcoholic steatohepatitis (NASH). Specifically, the NASH-induced model mice were divided into two groups, the control group was further fed with MCDD for 4 weeks, the experimental group was fed with SLB736 at a dose of 5 mg/kg/day for 4 weeks in combination with MCDD, and liver tissue was collected from each mouse and observed with a microscope to determine the degree of steatosis, lobular inflammation and fibrosis.

The result showed that the control group that had been continuously fed with MCDD was found to have steatosis, lobular inflammation and fibrosis in liver

tissues due to the administration of MCDD, indicating the onset of NASH. On the other hand, the mice administered with SLB736, in combination with a general diet, exhibited a considerable reduction in the degree of steatosis and lobular inflammation (FIG. 11), and the SLB736 administration group also exhibited a considerable reduction in the degree of fibrosis (FIG. 11).

Example 7. Comparison of Lymphopenia in blood between administration of SLB736 and FTY720

FTY720, which acts as a functional antagonist of S1PR, has been reported to cause the serious side effect, lymphopenia in the blood. Therefore, when the SLB736 of the present invention was administered, changes of leukocytes and lymphocytes in the blood were measured to determine the possibility of side effects. On the day of autopsy, blood was collected from the posterior vein using a syringe and whole blood was refrigerated in an EDTA-2K CBC bottle. The leukocytes and lymphocytes in the whole blood were measured using an automated analyzer.

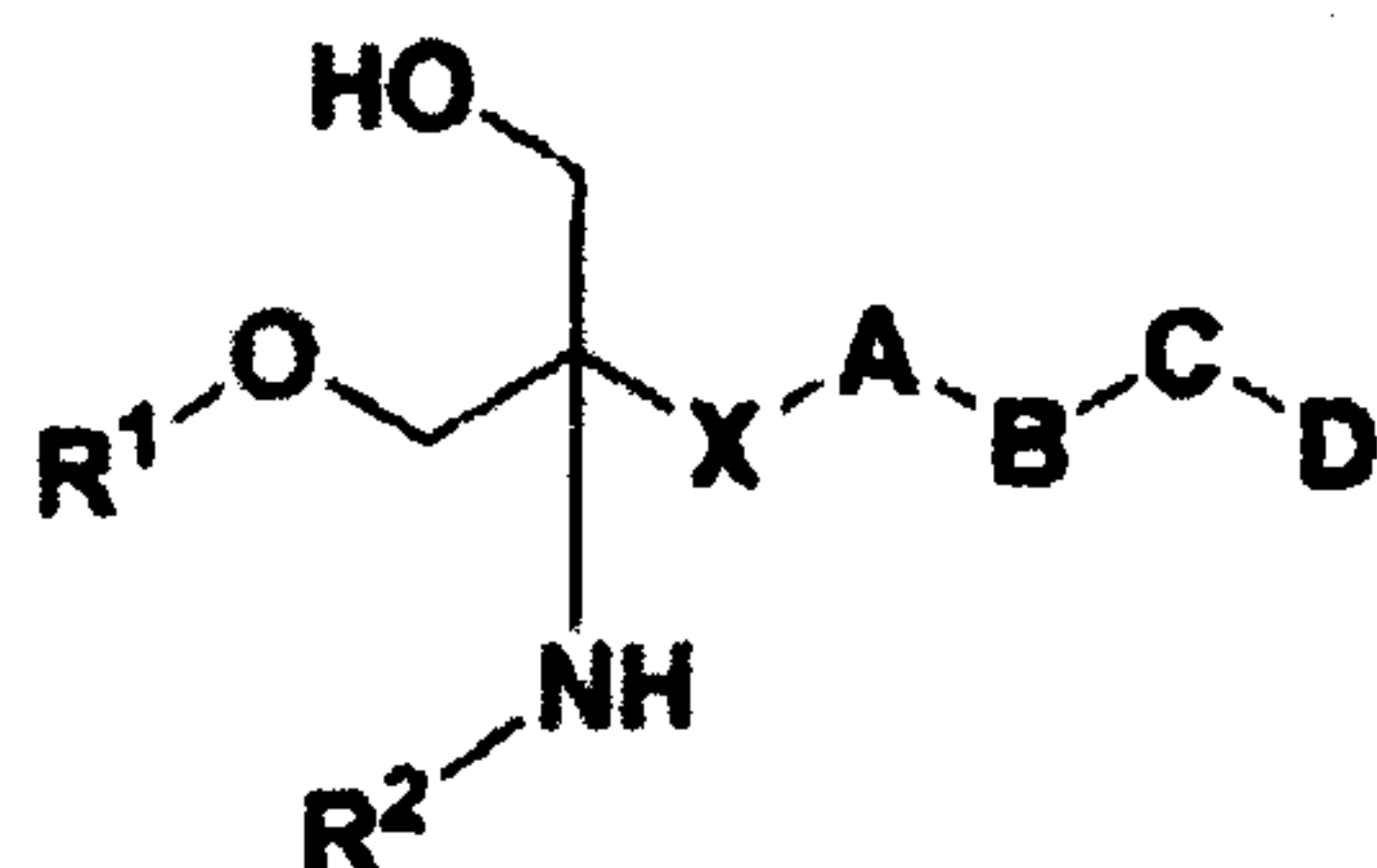
As shown in FIG. 12, the result showed that the mice administered with SLB736 had no reduction of lymphocytes and leukocytes in blood, which means that administration of FTY720 causes no side effects.

10 **【Claims】**

【Claim 1】

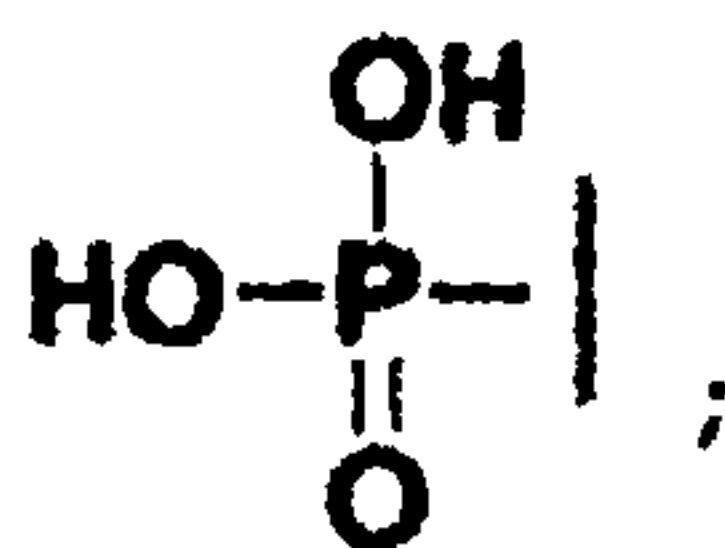
A pharmaceutical composition for preventing or treating non-alcoholic steatohepatitis (NASH) comprising a compound represented by the following formula 1, an optical isomer thereof or a pharmaceutically acceptable salt thereof as an active ingredient:

[Formula 1]



wherein

R¹ is hydrogen or



15 R² is hydrogen, or unsubstituted or substituted C₁₋₅ straight or branched alkyl carbonyl, wherein the substituted alkyl carbonyl is substituted with one or more substituents selected from the group consisting of hydroxy, halogen, cyano, nitro and amino;

20 A is a five-membered ring heteroarylene containing one or more heteroatoms selected from the group consisting of N, O and S;

B is C₁₋₁₁ straight or branched alkylene;

C is a single bond or C₆₋₁₀ arylene;

25 D is -H, or C₁₋₁₅ straight or branched alkyl; and

X is a single bond, C₁₋₅ alkylene, C₂₋₅ alkenylene or C₂₋₅ alkynylene.

【Claim 2】

5 The pharmaceutical composition according to claim 1, wherein



R² is hydrogen, or C₁₋₃ straight or branched alkyl carbonyl;

10 A is a five-membered ring heteroarylene containing one or more heteroatoms selected from the group consisting of N, O and S;

B is C₁₋₉ straight or branched alkylene;

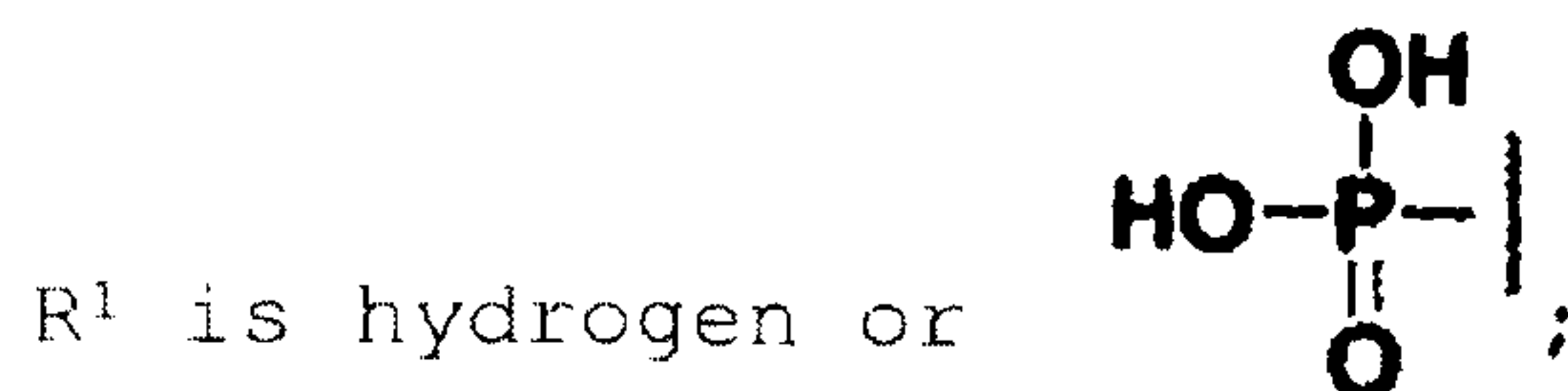
C is a single bond or C₆₋₁₀ arylene;

15 D is -H, or C₃₋₁₂ straight or branched alkyl; and

X is a single bond, C₁₋₃ alkylene, C₂₋₃ alkenylene or C₂₋₃ alkynylene.

【Claim 3】

20 The pharmaceutical composition according to claim 1, wherein



R² is hydrogen or acetyl;

25 A is a five-membered ring heteroarylene containing

one or more heteroatoms selected from the group consisting of N, O and S;

B is C₂₋₈ alkylene;

C is a single bond or phenylene;

5 D is -H, or C₆₋₁₀ straight or branched alkyl; and

X is a single bond, -CH₂CH₂-, -CH=CH- or -C≡C-.

【Claim 4】

The pharmaceutical composition according to claim 1,
10 wherein the compound represented by Formula 1 comprises any one selected from the group consisting of the following compounds:

(1) 2-amino-2-(2-(3-decylisoxazol-5-yl)ethyl)propane-1,3-diol;

15 (2) 2-amino-2-(2-(1-decyl-1H-1,2,3-triazol-4-yl)ethyl)propane-1,3-diol;

(3) 2-amino-2-((3-octylisoxazol-5-yl)ethynyl)propane-1,3-diol;

(4) 2-amino-2-(2-(3-octylisoxazol-5-yl)ethyl)propane-1,3-diol;
20

(5) 2-amino-2-(hydroxymethyl)-4-(3-octylisoxazol-5-yl)butyl dihydrogen phosphate;

(6) 2-amino-2-((3-decylisoxazol-5-yl)ethynyl)propane-1,3-diol;

25 (7) 2-amino-4-(3-decylisoxazol-5-yl)-2-

(hydroxymethyl)butyl dihydrogen phosphate;

(8) 2-amino-2-(2-(3-(4-hexylphenethyl)isoxazol-5-yl)ethyl)propane-1,3-diol;

(9) 2-amino-2-((3-dodecylisoxazol-5-yl)ethynyl)propane-1,3-diol;

(10) 2-amino-2-(2-(3-dodecylisoxazol-5-yl)ethyl)propane-1,3-diol;

(11) 2-amino-4-(3-dodecylisoxazol-5-yl)-2-(hydroxymethyl)butyl dihydrogen phosphate;

(12) 2-amino-2-(2-(1-octyl-1H-1,2,3-triazol-4-yl)ethynyl)propane-1,3-diol;

(13) 2-amino-2-(2-(1-octyl-1H-1,2,3-triazol-4-yl)ethyl)propane-1,3-diol;

(14) 2-amino-2-((1-decyl-1H-1,2,3-triazol-4-yl)ethynyl)propane-1,3-diol;

(15) 2-amino-2-(2-(1-(4-hexylphenethyl)-1H-1,2,3-triazol-4-yl)ethyl)propane-1,3-diol;

(16) 2-amino-2-(1-butyl-1H-1,2,3-triazol-4-yl)propane-1,3-diol;

(17) 2-amino-2-(3-dodecylisoxazol-5-yl)propane-1,3-diol;

(18) (E)-2-amino-2-(2-(3-decylisoxazol-5-yl)vinyl)propane-1,3-diol;

(19) (E)-2-amino-2-(1-butyl-1H-1,2,3-triazol-4-yl)propane-1,3-diol;

(20) 2-amino-2-(2-(3-(8-phenyloctyl)-isoxazol-5-

yl)ethyl)propane-1,3-diol;

(21) 2-amino-2-(2-(1-(8-phenyloctyl)-1H-1,2,3-triazolebutyl-4-yl)propane-1,3-diol;

(22) N-(2-(1-dodecyl-1H-1,2,3-triazol-4-yl)-1,3-dihydroxypropan-2-yl)acetamide;

(23) N-(2-(3-dodecylisoxazol-5-yl)-1,3-dihydroxypropan-2-yl)acetamide;

(24) N-(4-(1-decyl-1H-1,2,3-triazol-4-yl)-1-hydroxy-2-(hydroxymethyl)butan-2-yl)acetamide;

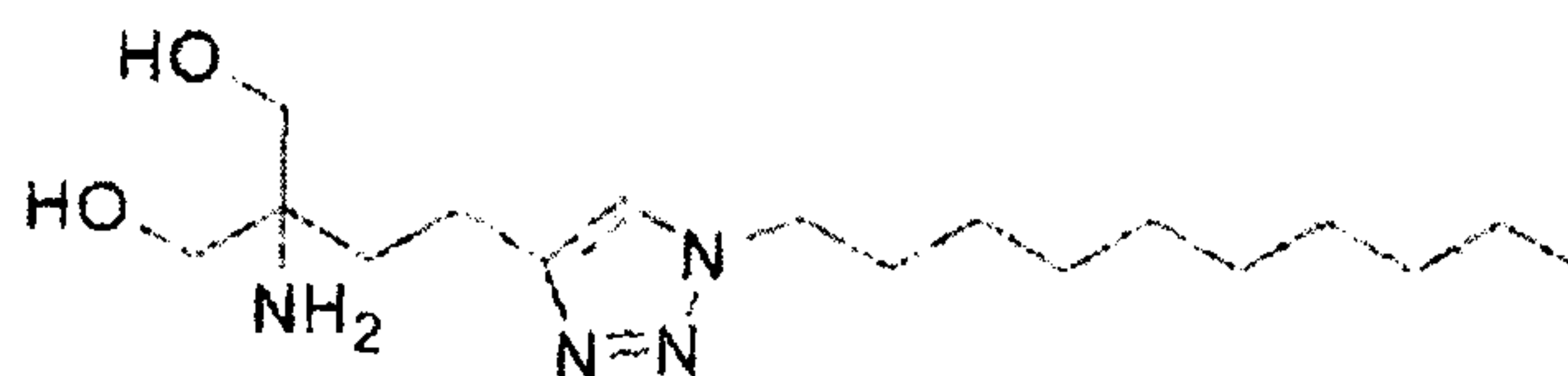
(25) N-(4-(3-decylisoxazol-5-yl)-1-hydroxy-2-(hydroxymethyl)butan-2-yl)acetamide; and

(26) N-(4-(1-(4-hexylphenethyl)-1H-1,2,3-triazol-4-yl)-1-hydroxy-2-(hydroxymethyl)butan-2-yl)acetamide.

【Claim 5】

The pharmaceutical composition according to claim 1, wherein the compound represented by Formula 1 is a compound represented by the following Formula 2:

[Formula 2]



【Claim 6】

The pharmaceutical composition according to claim 1,
 wherein the active ingredient inhibits activity of
 sphingosine 1-phosphate receptor 4 (S1PR4) by specifically
 binding to the S1PR4 and then removing the S1PR4.

5

【Claim 7】

The pharmaceutical composition according to claim 1,
 wherein the active ingredient inhibits activity of an
 inflammation-regulating complex (inflammasome) in
 macrophages.

10

【Claim 8】

The pharmaceutical composition according to claim 7,
 wherein the active ingredient inhibits IL- β production in
 macrophages.

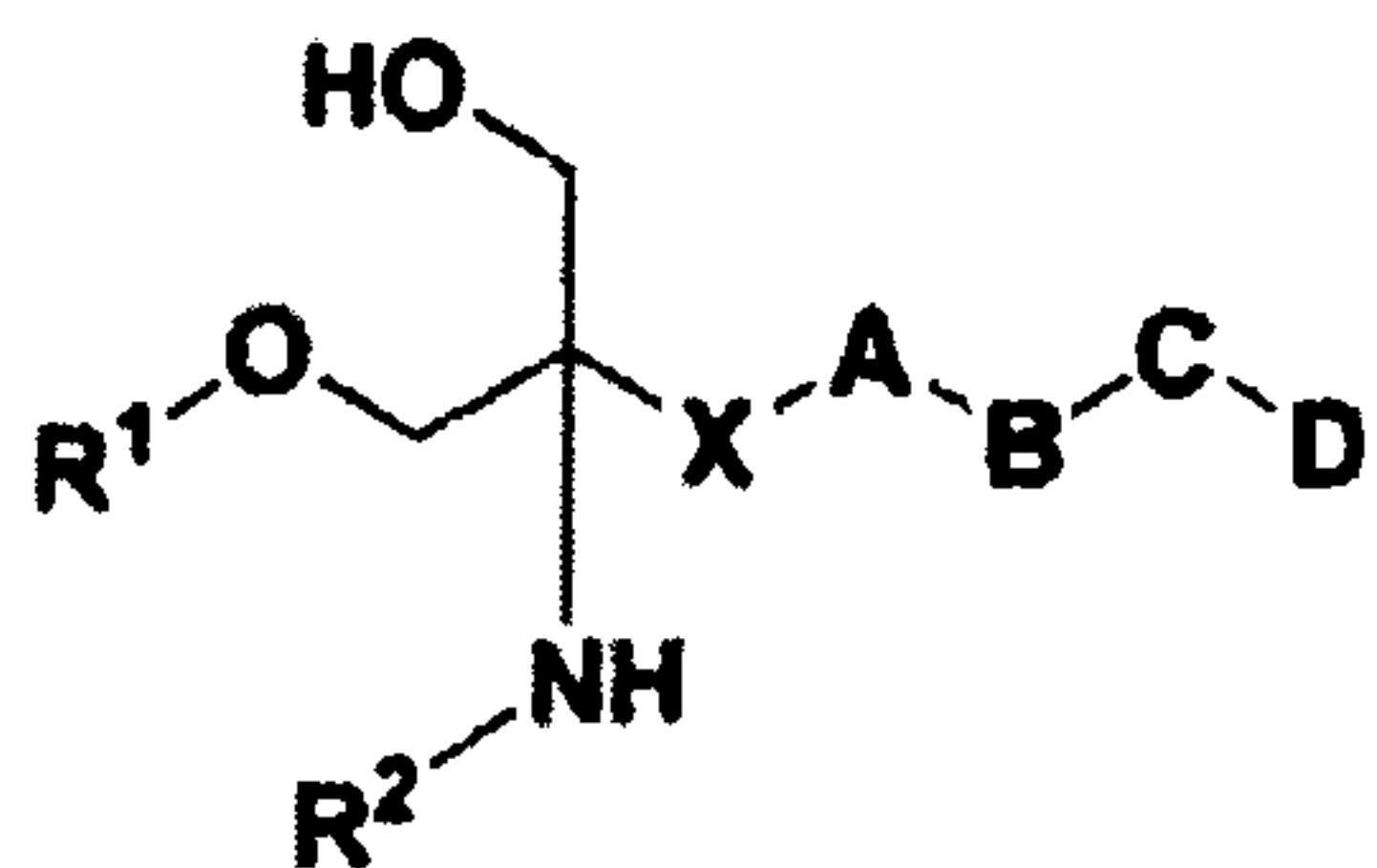
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【Claim 9】

A health functional food composition for preventing
 or treating non-alcoholic steatohepatitis (NASH)
 comprising a compound represented by the following formula
 1, an optical isomer thereof or a pharmaceutically
 acceptable salt thereof as an active ingredient:

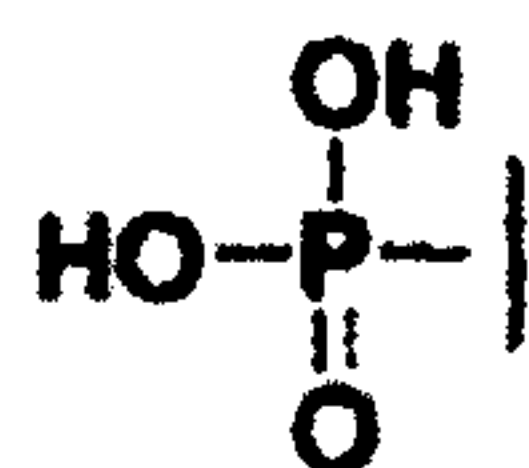
20

[Formula 1]



25

wherein



R¹ is hydrogen or ;

5 R² is hydrogen, or unsubstituted or substituted C₁₋₅ straight or branched alkyl carbonyl, wherein the substituted alkyl carbonyl is substituted with one or more substituents selected from the group consisting of hydroxy, halogen, cyano, nitro and amino;

10 A is a five-membered ring heteroarylene containing one or more heteroatoms selected from the group consisting of N, O and S;

B is C₁₋₁₁ straight or branched alkylene;

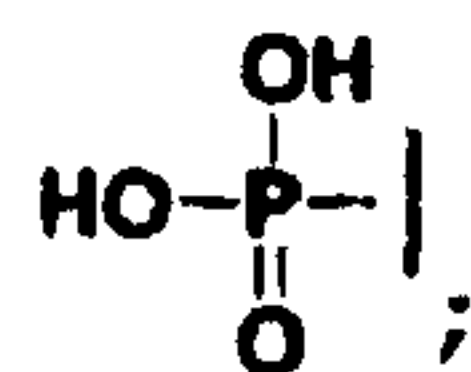
C is a single bond or C₆₋₁₀ arylene;

15 D is -H, or C₁₋₁₅ straight or branched alkyl; and

X is a single bond, C₁₋₅ alkylene, C₂₋₅ alkenylene or C₂₋₅ alkynylene.

【Claim 10】

20 The health functional food composition according to claim 9, wherein



R¹ is hydrogen or

R² is hydrogen, or C₁₋₃ straight or branched alkyl carbonyl;

25 A is a five-membered ring heteroarylene containing

one or more heteroatoms selected from the group consisting of N, O and S;

B is C₁₋₉ straight or branched alkylene;

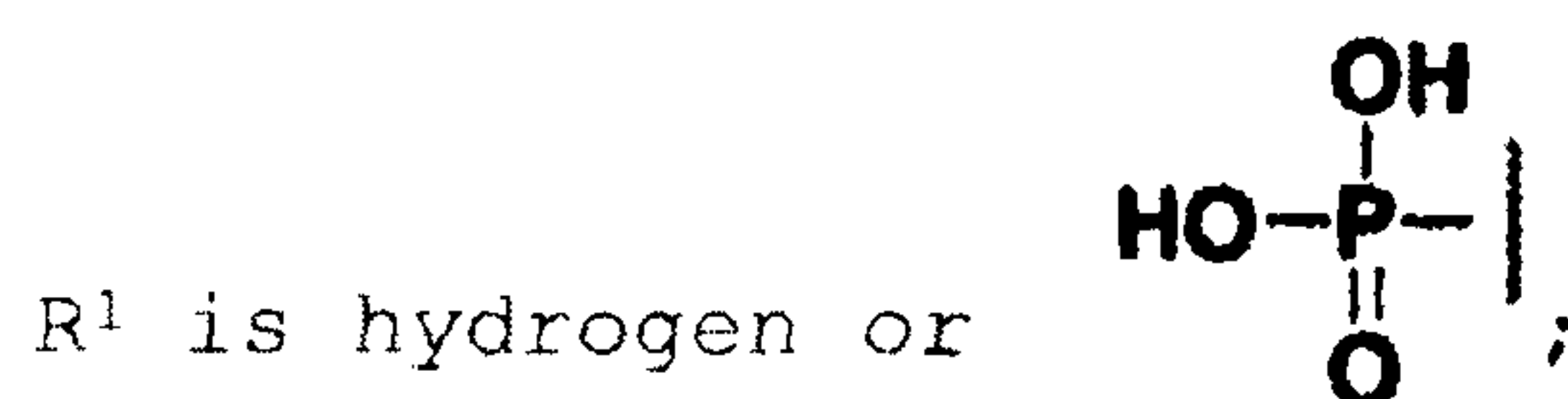
C is a single bond or C₆₋₁₀ arylene;

5 D is -H, or C₃₋₁₂ straight or branched alkyl; and

X is a single bond, C₁₋₃ alkylene, C₂₋₃ alkenylene or C₂₋₃ alkynylene.

【Claim 11】

10 The health functional food composition according to claim 9, wherein



R² is hydrogen or acetyl;

15 A is a five-membered ring heteroarylene containing one or more heteroatoms selected from the group consisting of N, O and S;

B is C₂₋₈ alkylene;

C is a single bond or phenylene;

20 D is -H, or C₆₋₁₀ straight or branched alkyl; and

X is a single bond, -CH₂CH₂-, -CH=CH- or -C≡C-.

【Claim 12】

25 The health functional food composition according to claim 9, wherein the compound represented by Formula 1

comprises any one selected from the group consisting of the following compounds:

- (1) 2-amino-2-(2-(3-decylisoxazol-5-yl)ethyl)propane-1,3-diol;
- 5 (2) 2-amino-2-(2-(1-decyl-1H-1,2,3-triazol-4-yl)ethyl)propane-1,3-diol;
- (3) 2-amino-2-((3-octylisoxazol-5-yl)ethynyl)propane-1,3-diol;
- (4) 2-amino-2-(2-(3-octylisoxazol-5-yl)ethyl)propane-1,3-
10 diol;
- (5) 2-amino-2-(hydroxymethyl)-4-(3-octylisoxazol-5-yl)butyl dihydrogen phosphate;
- (6) 2-amino-2-((3-decylisoxazol-5-yl)ethynyl)propane-1,3-diol;
- 15 (7) 2-amino-4-(3-decylisoxazol-5-yl)-2-(hydroxymethyl)butyl dihydrogen phosphate;
- (8) 2-amino-2-(2-(3-(4-hexylphenethyl)isoxazol-5-yl)ethyl)propane-1,3-diol;
- (9) 2-amino-2-((3-dodecylisoxazol-5-yl)ethynyl)propane-
20 1,3-diol;
- (10) 2-amino-2-(2-(3-dodecylisoxazol-5-yl)ethyl)propane-1,3-diol;
- (11) 2-amino-4-(3-dodecylisoxazol-5-yl)-2-(hydroxymethyl)butyl dihydrogen phosphate;
- 25 (12) 2-amino-2-(2-(1-octyl-1H-1,2,3-triazol-4-

yl)ethynyl)propane-1,3-diol;

(13) 2-amino-2-(2-(1-octyl-1H-1,2,3-triazol-4-yl)ethyl)propane-1,3-diol;

(14) 2-amino-2-((1-decyl-1H-1,2,3-triazol-4-yl)ethynyl)propane-1,3-diol;

(15) 2-amino-2-(2-(1-(4-hexylphenethyl)-1H-1,2,3-triazol-4-yl)ethyl)propane-1,3-diol;

(16) 2-amino-2-(1-butyl-1H-1,2,3-triazol-4-yl)propane-1,3-diol;

(17) 2-amino-2-(3-dodecylisoxazol-5-yl)propane-1,3-diol;

(18) (E)-2-amino-2-(2-(3-decylisoxazol-5-yl)vinyl)propane-1,3-diol;

(19) (E)-2-amino-2-(1-butyl-1H-1,2,3-triazol-4-yl)propane-1,3-diol;

(20) 2-amino-2-(2-(3-(8-phenyloctyl)-isoxazol-5-yl)ethyl)propane-1,3-diol;

(21) 2-amino-2-(2-(1-(8-phenyloctyl)-1H-1,2,3-triazolebutyl-4-yl)propane-1,3-diol;

(22) N-(2-(1-dodecyl-1H-1,2,3-triazol-4-yl)-1,3-dihydroxypropan-2-yl)acetamide;

(23) N-(2-(3-dodecylisoxazol-5-yl)-1,3-dihydroxypropan-2-yl)acetamide;

(24) N-(4-(1-decyl-1H-1,2,3-triazol-4-yl)-1-hydroxy-2-(hydroxymethyl)butan-2-yl)acetamide;

(25) N-(4-(3-decylisoxazol-5-yl)-1-hydroxy-2-

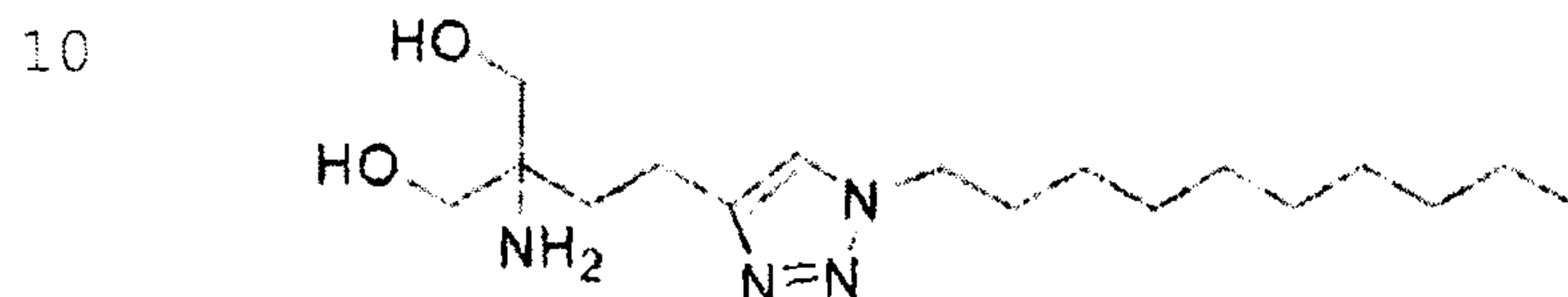
(hydroxymethyl)butan-2-yl)acetamide; and

(26) N-(4-(1-(4-hexylphenethyl)-1H-1,2,3-triazol-4-yl)-1-hydroxy-2-(hydroxymethyl)butan-2-yl)acetamide.

5 **【Claim 13】**

The health functional food composition according to claim 9, wherein the compound represented by Formula 1 is a compound represented by the following Formula 2:

[Formula 2]



15 **【Claim 14】**

The health functional food composition according to claim 9, wherein the active ingredient inhibits activity of sphingosine 1-phosphate receptor 4 (S1PR4) by specifically binding to the S1PR4 and then removing the S1PR4.

20 **【Claim 15】**

The health functional food composition according to claim 9, wherein the active ingredient inhibits activity of an inflammation-regulating complex (inflammasome) in macrophages.

25

【Claim 16】

The health functional food composition according to claim 15, wherein the active ingredient inhibits IL- β production in macrophages.

【Claim 17】

A pharmaceutical composition for preventing or treating nonalcoholic steatohepatitis comprising a functional antagonist of S1PR4 as an active ingredient.

【Claim 18】

The pharmaceutical composition according to claim 17, wherein the functional antagonist of S1PR4 inhibits activity of S1PR4 by specifically binding to the S1PR4 and then removing the S1PR4.

【Claim 19】

The pharmaceutical composition according to claim 17, wherein the functional antagonist of S1PR4 inhibits activity of an inflammation-regulating complex (inflammasome) in macrophages.

【Claim 20】

The pharmaceutical composition according to claim 19,

wherein the functional antagonist of S1PR4 inhibits IL- β production in macrophages.

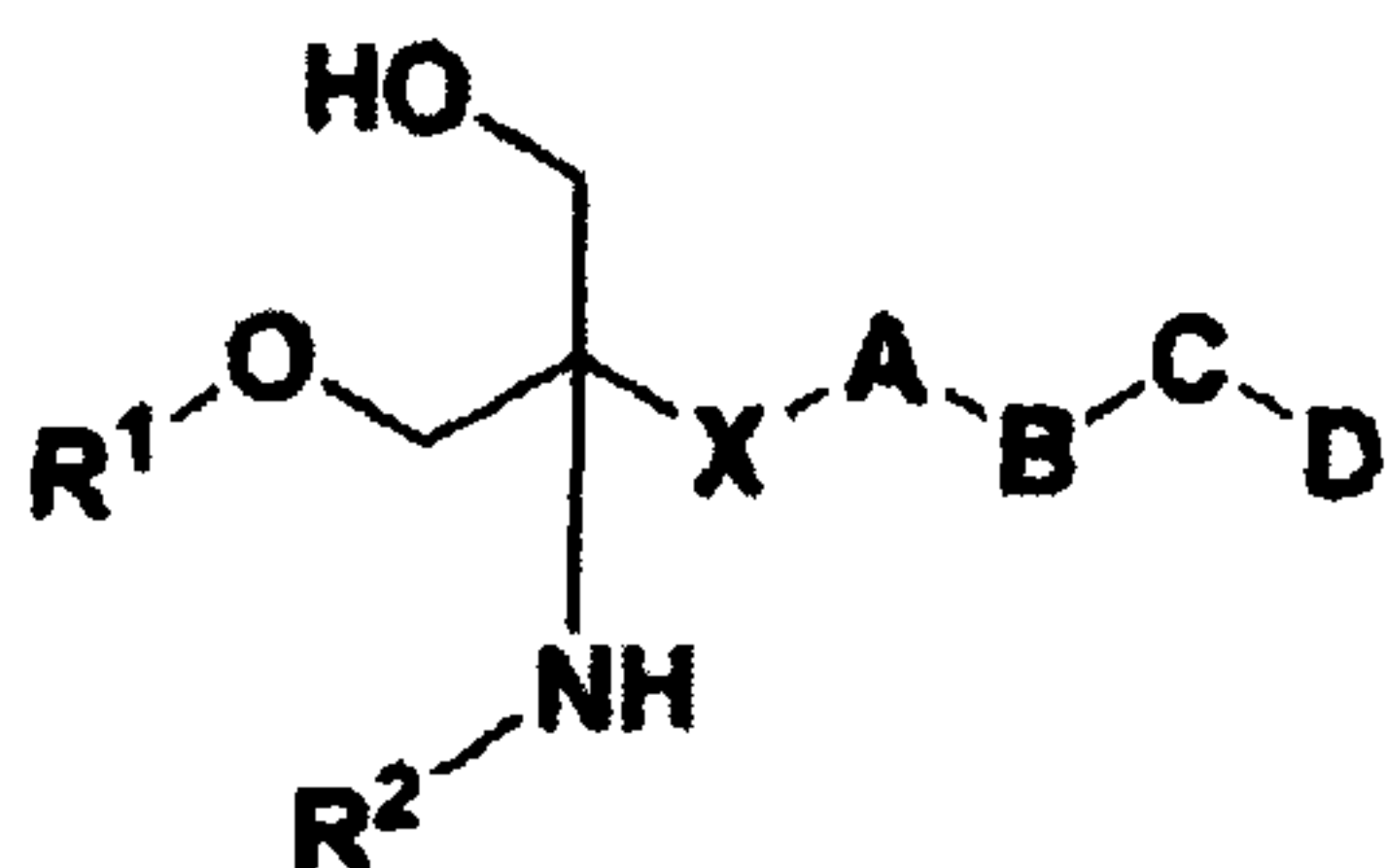
【Claim 21】

5 A health functional food composition for preventing or treating nonalcoholic steatohepatitis comprising a functional antagonist of S1PR4 as an active ingredient.

【Claim 22】

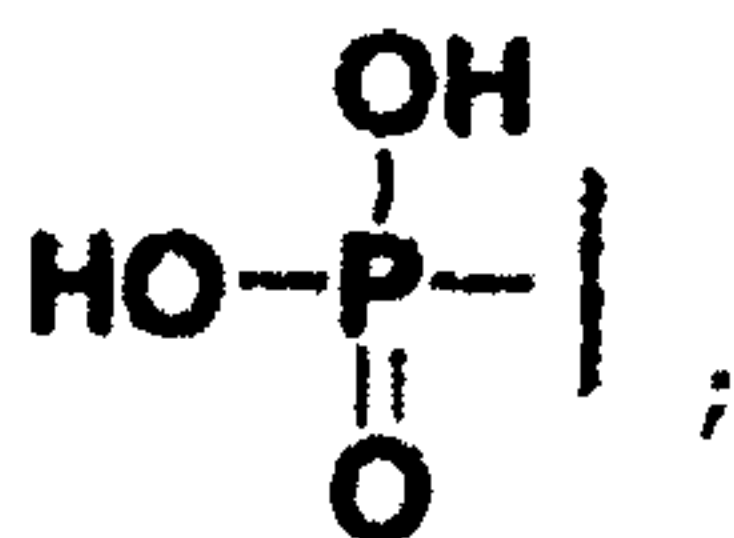
10 A method for preventing or treating nonalcoholic steatohepatitis (NASH) comprising administering to a subject a pharmaceutical composition comprising a compound represented by the following Formula 1, an optical isomer thereof, or a pharmaceutically acceptable salt thereof as
15 an active ingredient:

[Formula 1]



wherein

R¹ is hydrogen or



R² is hydrogen, or unsubstituted or substituted C₁₋₅ straight or branched alkyl carbonyl, wherein the
25 substituted alkyl carbonyl is substituted with one or more

substituents selected from the group consisting of hydroxy, halogen, cyano, nitro and amino;

A is a five-membered ring heteroarylene containing one or more heteroatoms selected from the group consisting of N, O and S;

B is C₁₋₁₁ straight or branched alkylene;

C is a single bond or C₆₋₁₀ arylene;

D is -H, or C₁₋₁₅ straight or branched alkyl; and

X is a single bond, C₁₋₅ alkylene, C₂₋₅ alkenylene or C₂₋₅ alkynylene.

【Claim 23】

The method according to claim 22, wherein

R¹ is hydrogen or $\begin{array}{c} \text{OH} \\ | \\ \text{HO}-\text{P}-| \\ || \\ \text{O} \end{array};$

R² is hydrogen, or C₁₋₃ straight or branched alkyl carbonyl;

A is a five-membered ring heteroarylene containing one or more heteroatoms selected from the group consisting of N, O and S;

B is C₁₋₉ straight or branched alkylene;

C is a single bond or C₆₋₁₀ arylene;

D is -H, or C₃₋₁₂ straight or branched alkyl; and

X is a single bond, C₁₋₃ alkylene, C₂₋₃ alkenylene or C₂₋₃ alkynylene.

【Claim 24】

The method according to claim 22, wherein

5 R¹ is hydrogen or $\begin{array}{c} \text{OH} \\ | \\ \text{HO}-\text{P}- \\ || \\ \text{O} \end{array};$

R² is hydrogen or acetyl;

A is a five-membered ring heteroarylene containing one or more heteroatoms selected from the group consisting of N, O and S;

10 B is C₂₋₈ alkylene;

C is a single bond or phenylene;

D is -H, or C₆₋₁₀ straight or branched alkyl; and

X is a single bond, -CH₂CH₂-, -CH=CH- or -C≡C-.

15 **【Claim 25】**

The method according to claim 22, wherein the compound represented by Formula 1 comprises any one selected from the group consisting of the following compounds:

20 (1) 2-amino-2-(2-(3-decylisoxazol-5-yl)ethyl)propane-1,3-diol;

(2) 2-amino-2-(2-(1-decyl-1H-1,2,3-triazol-4-yl)ethyl)propane-1,3-diol;

(3) 2-amino-2-((3-octylisoxazol-5-yl)ethynyl)propane-1,3-diol;

25

(4) 2-amino-2-(2-(3-octylisoxazol-5-yl)ethyl)propane-1,3-diol;

(5) 2-amino-2-(hydroxymethyl)-4-(3-octylisoxazol-5-yl)butyl dihydrogen phosphate;

5 (6) 2-amino-2-((3-decylisoxazol-5-yl)ethynyl)propane-1,3-diol;

(7) 2-amino-4-(3-decylisoxazol-5-yl)-2-(hydroxymethyl)butyl dihydrogen phosphate;

10 (8) 2-amino-2-(2-(3-(4-hexylphenethyl)isoxazol-5-yl)ethyl)propane-1,3-diol;

(9) 2-amino-2-((3-dodecylisoxazol-5-yl)ethynyl)propane-1,3-diol;

(10) 2-amino-2-(2-(3-dodecylisoxazol-5-yl)ethyl)propane-1,3-diol;

15 (11) 2-amino-4-(3-dodecylisoxazol-5-yl)-2-(hydroxymethyl)butyl dihydrogen phosphate;

(12) 2-amino-2-(2-(1-octyl-1H-1,2,3-triazol-4-yl)ethynyl)propane-1,3-diol;

20 (13) 2-amino-2-(2-(1-octyl-1H-1,2,3-triazol-4-yl)ethyl)propane-1,3-diol;

(14) 2-amino-2-((1-decyl-1H-1,2,3-triazol-4-yl)ethynyl)propane-1,3-diol;

(15) 2-amino-2-(2-(1-(4-hexylphenethyl)-1H-1,2,3-triazol-4-yl)ethyl)propane-1,3-diol;

25 (16) 2-amino-2-(1-butyl-1H-1,2,3-triazol-4-yl)propane-1,3-

diol;

(17) 2-amino-2-(3-dodecylisoxazol-5-yl)propane-1,3-diol;

(18) (E)-2-amino-2-(2-(3-decylisoxazol-5-yl)vinyl)propane-1,3-diol;

5 (19) (E)-2-amino-2-(1-butyl-1H-1,2,3-triazol-4-yl)propane-1,3-diol;

(20) 2-amino-2-(2-(3-(8-phenyloctyl)-isoxazol-5-yl)ethyl)propane-1,3-diol;

(21) 2-amino-2-(2-(1-(8-phenyloctyl)-1H-1,2,3-triazolebutyl-4-yl)propane-1,3-diol;

(22) N-(2-(1-dodecyl-1H-1,2,3-triazol-4-yl)-1,3-dihydroxypropan-2-yl)acetamide;

(23) N-(2-(3-dodecylisoxazol-5-yl)-1,3-dihydroxypropan-2-yl)acetamide;

15 (24) N-(4-(1-decyl-1H-1,2,3-triazol-4-yl)-1-hydroxy-2-(hydroxymethyl)butan-2-yl)acetamide;

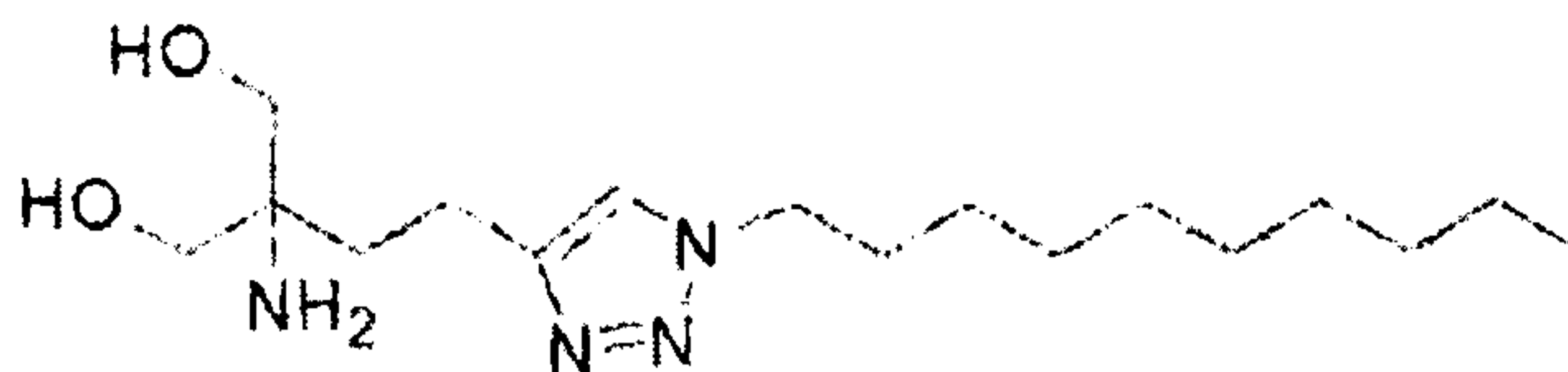
(25) N-(4-(3-decylisoxazol-5-yl)-1-hydroxy-2-(hydroxymethyl)butan-2-yl)acetamide; and

(26) N-(4-(1-(4-hexylphenethyl)-1H-1,2,3-triazol-4-yl)-1-hydroxy-2-(hydroxymethyl)butan-2-yl)acetamide.

【Claim 26】

The method according to claim 22, wherein the compound represented by Formula 1 is a compound represented by the following Formula 2:

[Formula 2]

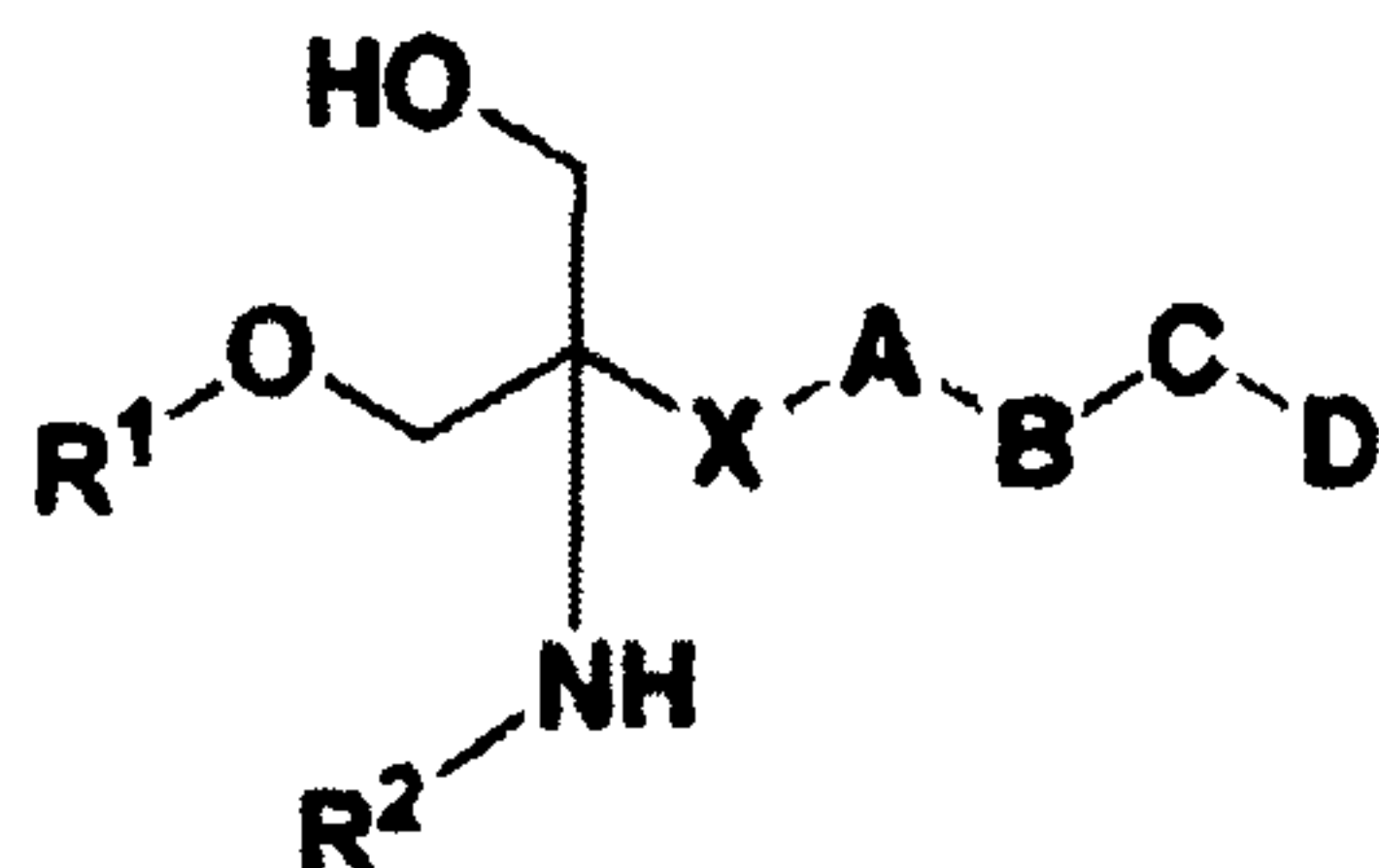


5

【Claim 27】

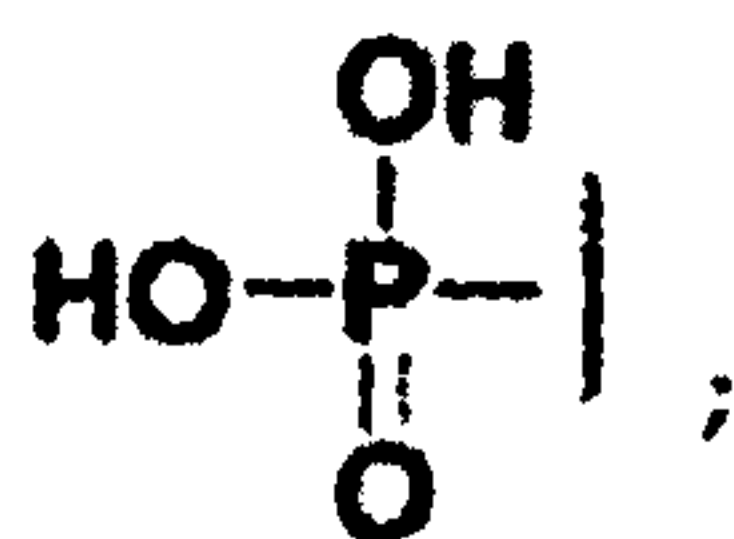
Use of a composition comprising a compound represented by the following Formula 1, an optical isomer thereof or a pharmaceutically acceptable salt thereof as an active ingredient for preventing or treating nonalcoholic steatohepatitis (NASH):

[Formula 1]



15

wherein

R¹ is hydrogen orR² is hydrogen, or unsubstituted or substituted C₁₋₅

straight or branched alkyl carbonyl, wherein the substituted alkyl carbonyl is substituted with one or more substituents selected from the group consisting of hydroxy, halogen, cyano, nitro and amino;

A is a five-membered ring heteroarylene containing one or more heteroatoms selected from the group consisting

25

of N, O and S;

B is C₁₋₁₁ straight or branched alkylene;

C is a single bond or C₆₋₁₀ arylene;

D is -H, or C₁₋₁₅ straight or branched alkyl; and

5 X is a single bond, C₁₋₅ alkylene, C₂₋₅ alkenylene or
C₂₋₅ alkynylene.

【Claim 28】

The use according to claim 27, wherein

10

R¹ is hydrogen or $\begin{array}{c} \text{OH} \\ | \\ \text{HO}-\text{P}-| \\ || \\ \text{O} \end{array};$

R² is hydrogen, or C₁₋₃ straight or branched alkyl
carbonyl;

15 A is a five-membered ring heteroarylene containing
one or more heteroatoms selected from the group consisting
of N, O and S;

B is C₁₋₉ straight or branched alkylene;

C is a single bond or C₆₋₁₀ arylene;

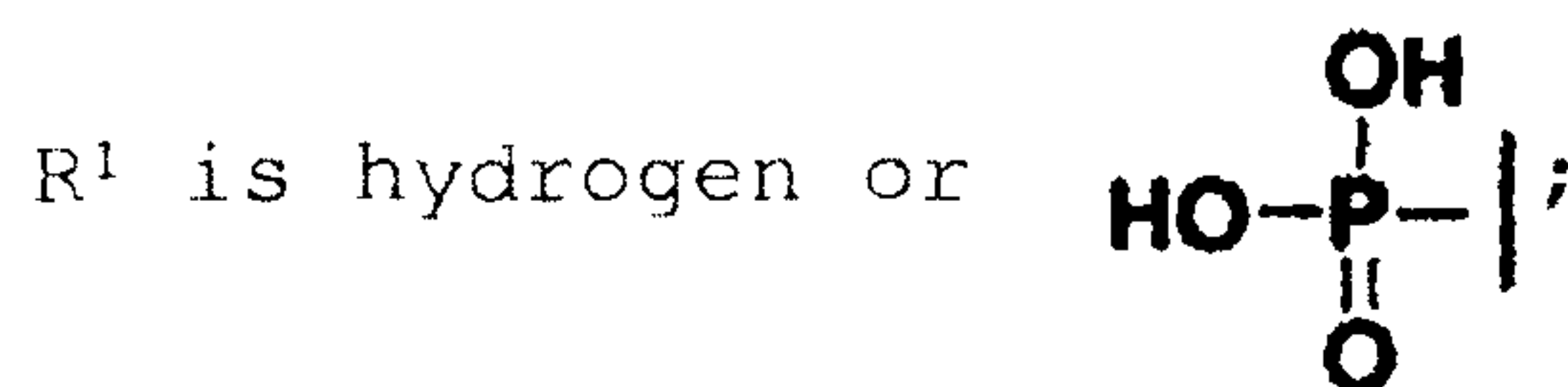
D is -H, or C₃₋₁₂ straight or branched alkyl; and

20 X is a single bond, C₁₋₃ alkylene, C₂₋₃ alkenylene or
C₂₋₃ alkynylene.

【Claim 29】

The use according to claim 27, wherein

25



R² is hydrogen or acetyl;

A is a five-membered ring heteroarylene containing
 5 one or more heteroatoms selected from the group consisting
 of N, O and S;

B is C₂₋₈ alkylene;

C is a single bond or phenylene;

D is -H, or C₆₋₁₀ straight or branched alkyl; and

10 X is a single bond, -CH₂CH₂-, -CH=CH- or -C≡C-.

【Claim 30】

The use according to claim 27, wherein the compound
 represented by Formula 1 comprises any one selected from
 15 the group consisting of the following compounds:

(1) 2-amino-2-(2-(3-decylisoxazol-5-yl)ethyl)propane-1,3-
 diol;

(2) 2-amino-2-(2-(1-decyl-1H-1,2,3-triazol-4-
 yl)ethyl)propane-1,3-diol;

20 (3) 2-amino-2-((3-octylisoxazol-5-yl)ethynyl)propane-1,3-
 diol;

(4) 2-amino-2-(2-(3-octylisoxazol-5-yl)ethyl)propane-1,3-
 diol;

(5) 2-amino-2-(hydroxymethyl)-4-(3-octylisoxazol-5-
 25 yl)butyl dihydrogen phosphate;

(6) 2-amino-2-((3-decylisoxazol-5-yl)ethynyl)propane-1,3-diol;

(7) 2-amino-4-(3-decylisoxazol-5-yl)-2-(hydroxymethyl)butyl dihydrogen phosphate;

5 (8) 2-amino-2-(2-(3-(4-hexylphenethyl)isoxazol-5-yl)ethyl)propane-1,3-diol;

(9) 2-amino-2-((3-dodecylisoxazol-5-yl)ethynyl)propane-1,3-diol;

10 (10) 2-amino-2-(2-(3-dodecylisoxazol-5-yl)ethyl)propane-1,3-diol;

(11) 2-amino-4-(3-dodecylisoxazol-5-yl)-2-(hydroxymethyl)butyl dihydrogen phosphate;

(12) 2-amino-2-(2-(1-octyl-1H-1,2,3-triazol-4-yl)ethynyl)propane-1,3-diol;

15 (13) 2-amino-2-(2-(1-octyl-1H-1,2,3-triazol-4-yl)ethyl)propane-1,3-diol;

(14) 2-amino-2-((1-decyl-1H-1,2,3-triazol-4-yl)ethynyl)propane-1,3-diol;

20 (15) 2-amino-2-(2-(1-(4-hexylphenethyl)-1H-1,2,3-triazol-4-yl)ethyl)propane-1,3-diol;

(16) 2-amino-2-(1-butyl-1H-1,2,3-triazol-4-yl)propane-1,3-diol;

(17) 2-amino-2-(3-dodecylisoxazol-5-yl)propane-1,3-diol;

25 (18) (E)-2-amino-2-(2-(3-decylisoxazol-5-yl)vinyl)propane-1,3-diol;

(19) (E)-2-amino-2-(1-butyl-1H-1,2,3-triazol-4-yl)propane-1,3-diol;

(20) 2-amino-2-(2-(3-(8-phenyloctyl)-isoxazol-5-yl)ethyl)propane-1,3-diol;

5 (21) 2-amino-2-(2-(1-(8-phenyloctyl)-1H-1,2,3-triazolebutyl-4-yl)propane-1,3-diol;

(22) N-(2-(1-dodecyl-1H-1,2,3-triazol-4-yl)-1,3-dihydroxypropan-2-yl)acetamide;

10 (23) N-(2-(3-dodecylisoxazol-5-yl)-1,3-dihydroxypropan-2-yl)acetamide;

(24) N-(4-(1-decyl-1H-1,2,3-triazol-4-yl)-1-hydroxy-2-(hydroxymethyl)butan-2-yl)acetamide;

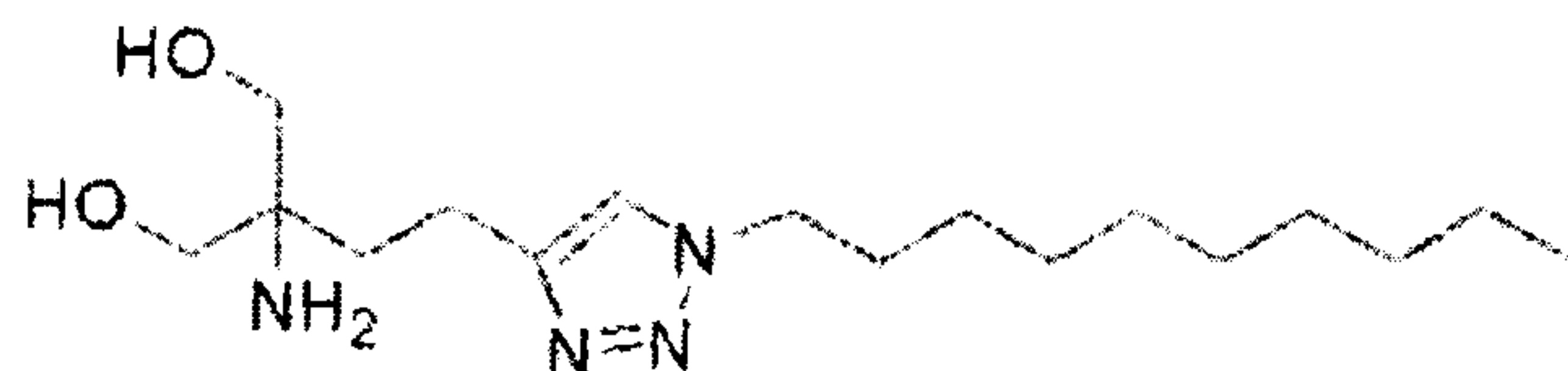
(25) N-(4-(3-decylisoxazol-5-yl)-1-hydroxy-2-(hydroxymethyl)butan-2-yl)acetamide; and

15 (26) N-(4-(1-(4-hexylphenethyl)-1H-1,2,3-triazol-4-yl)-1-hydroxy-2-(hydroxymethyl)butan-2-yl)acetamide.

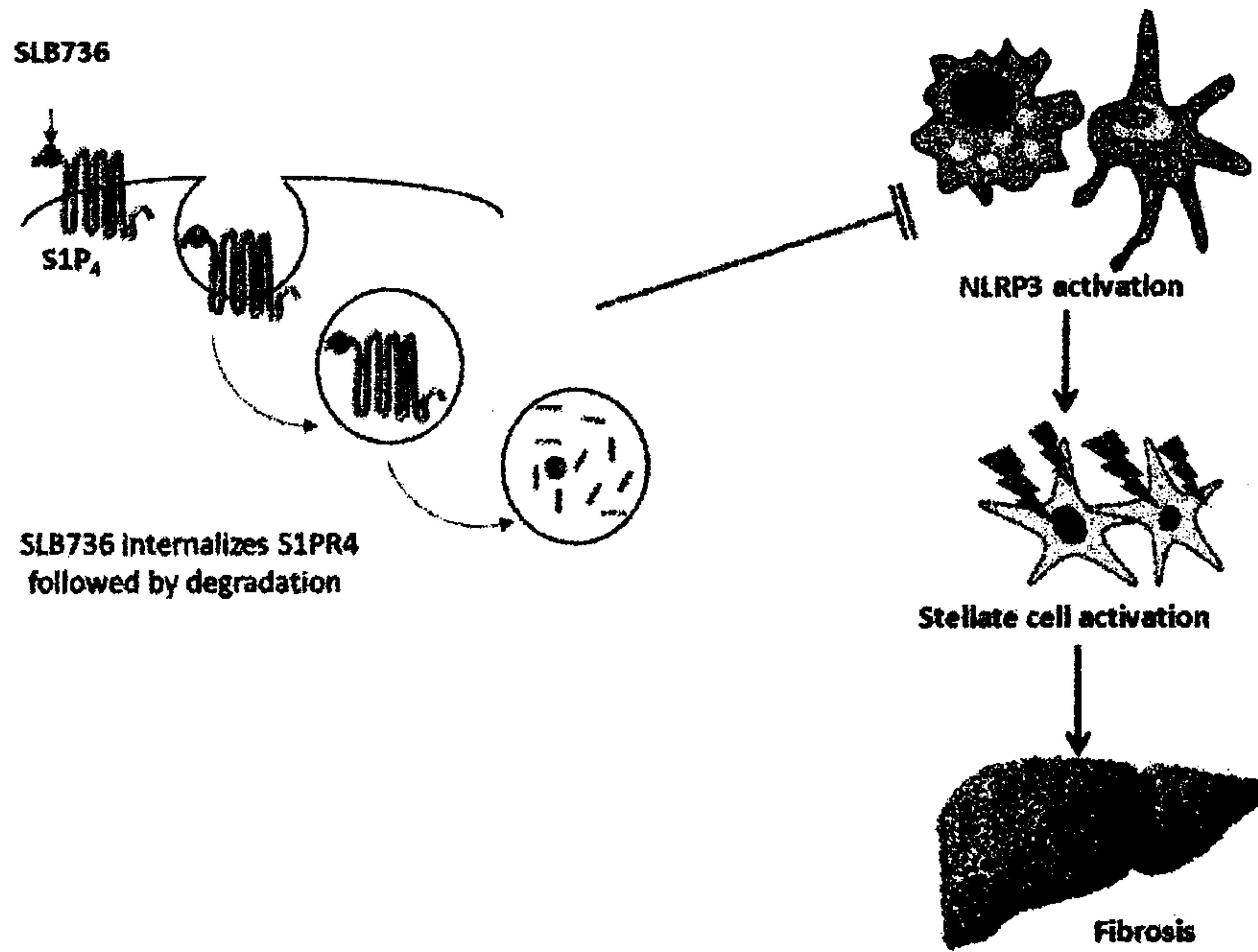
【Claim 31】

20 The use according to claim 27, wherein the compound represented by Formula 1 is a compound represented by the following Formula 2:

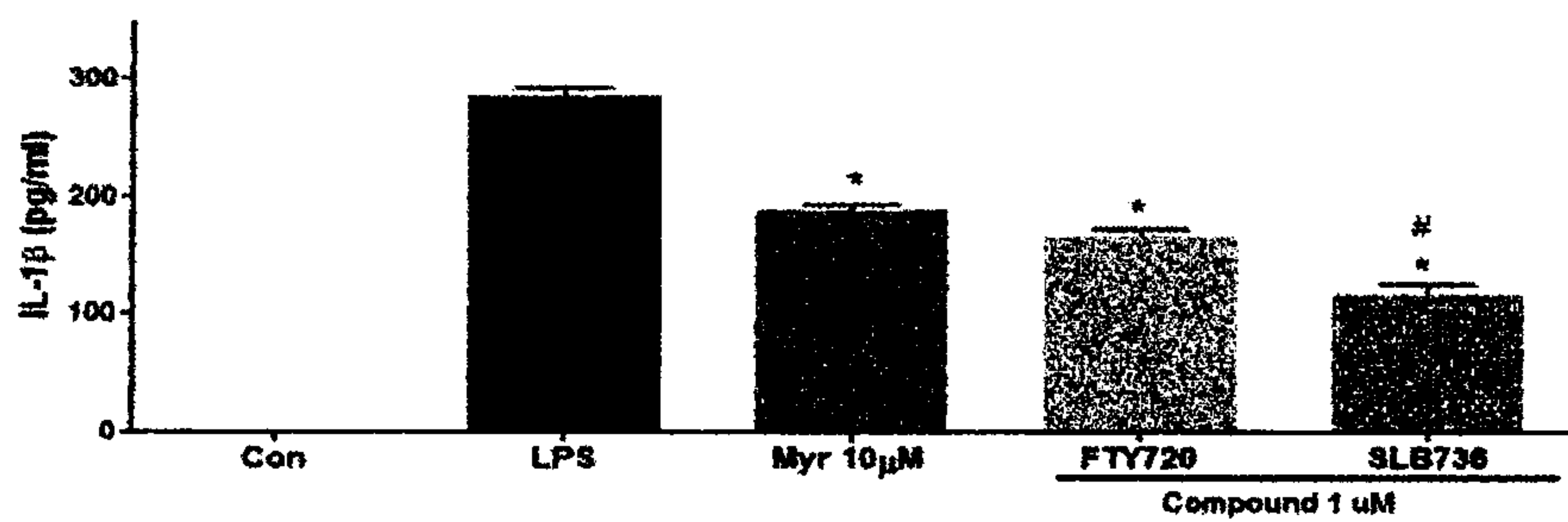
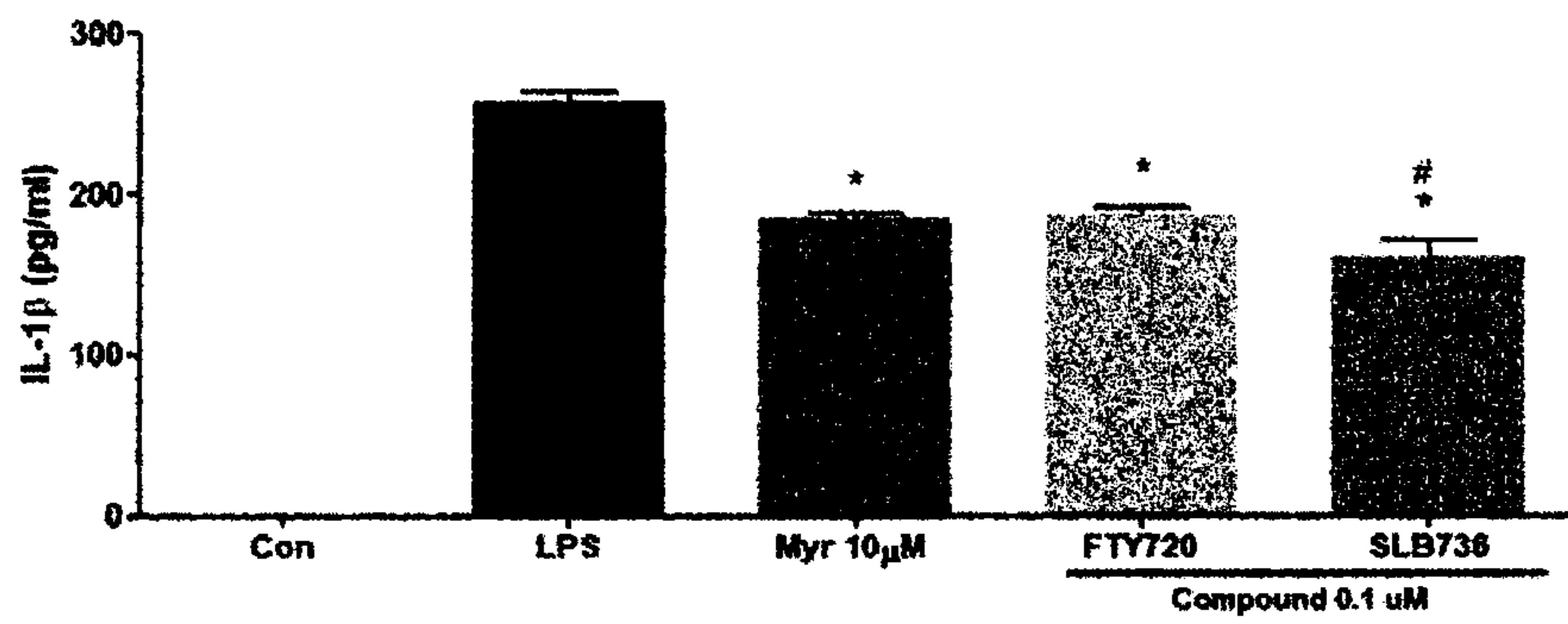
[Formula 2]



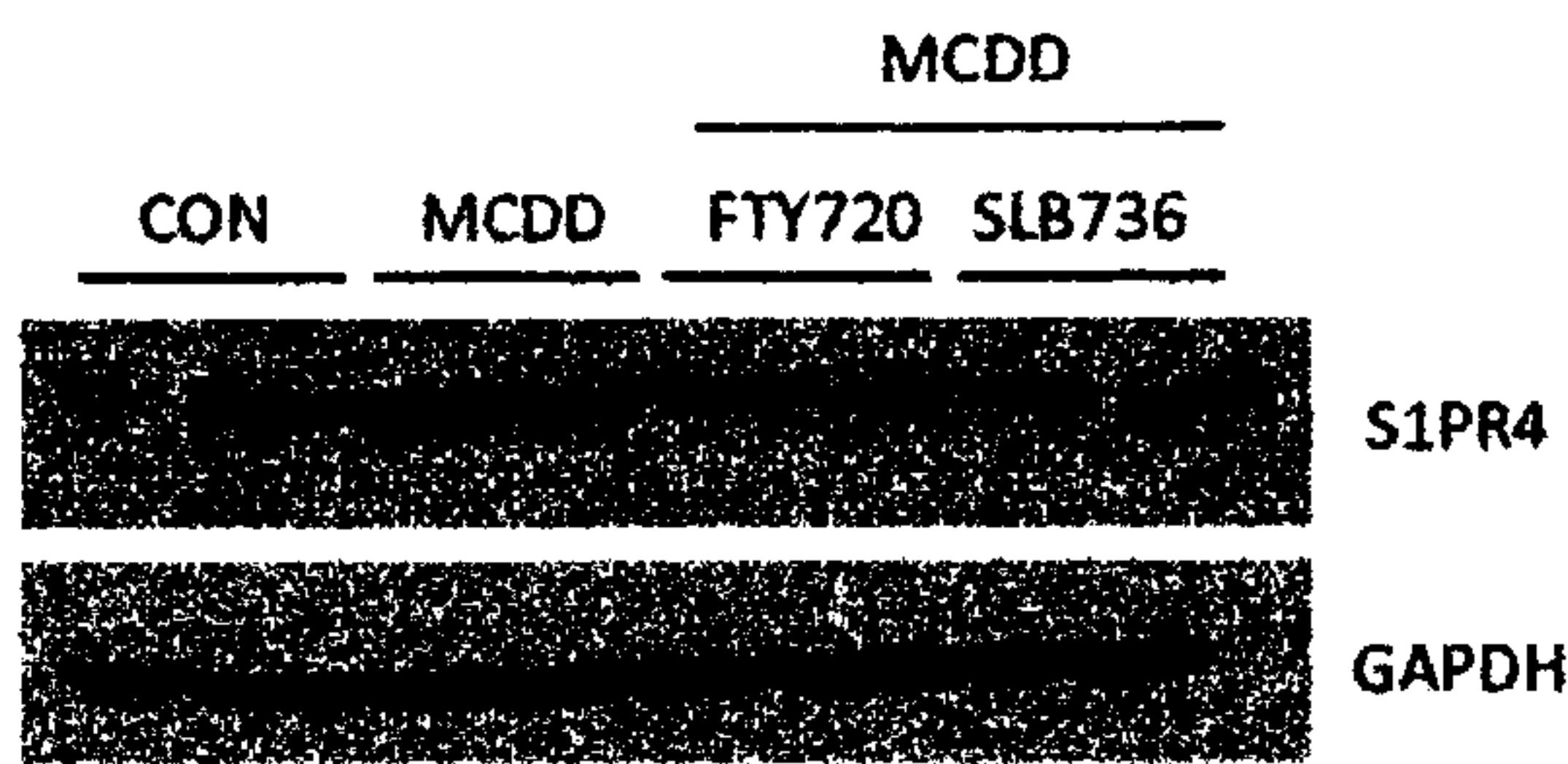
【Fig.1】



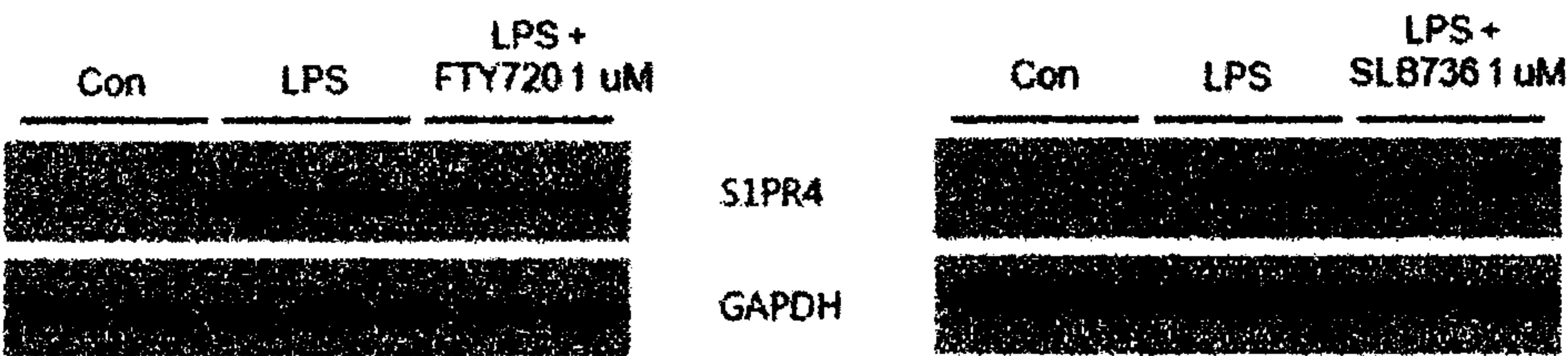
【Fig.2】



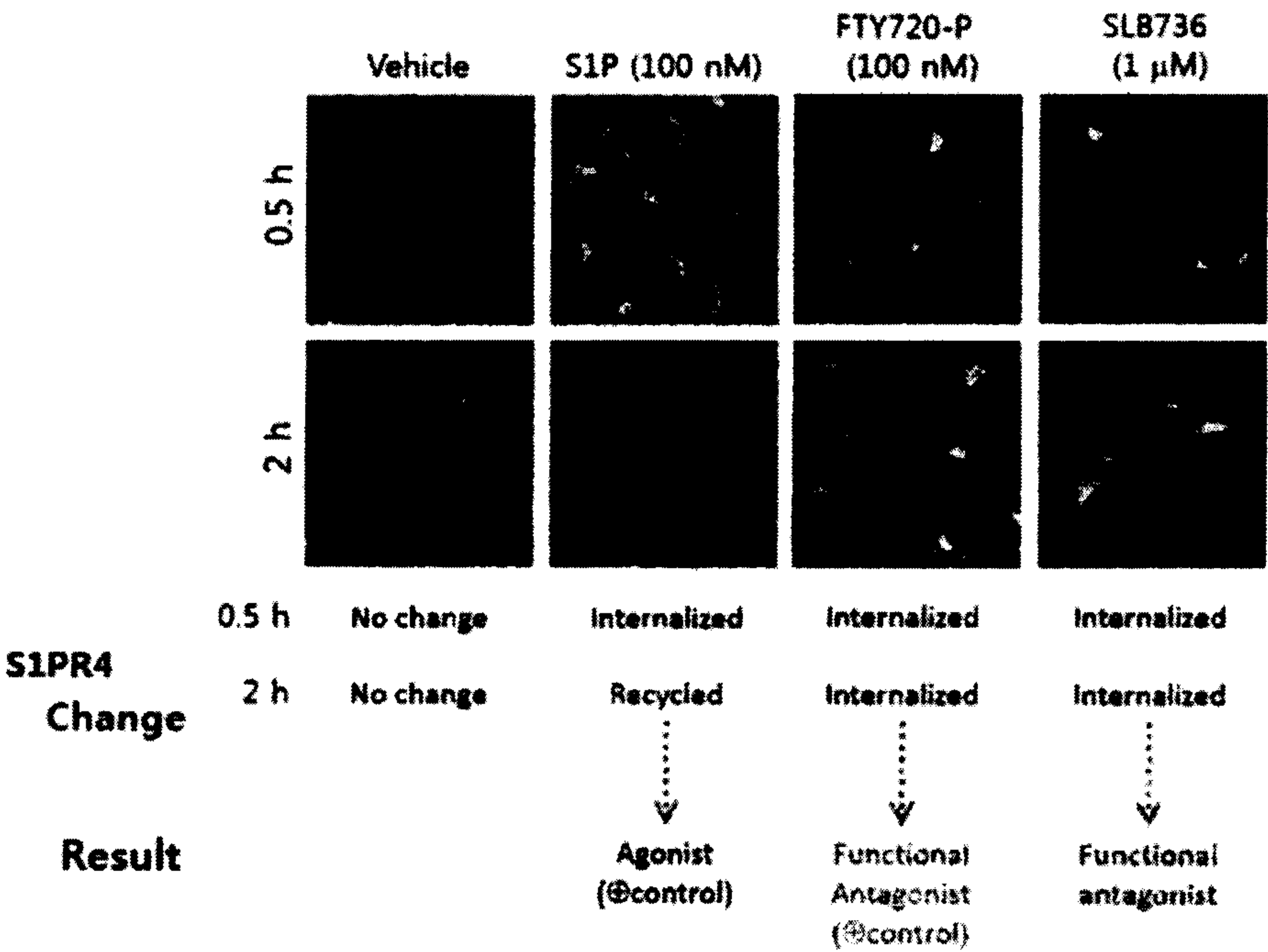
【Fig.3】



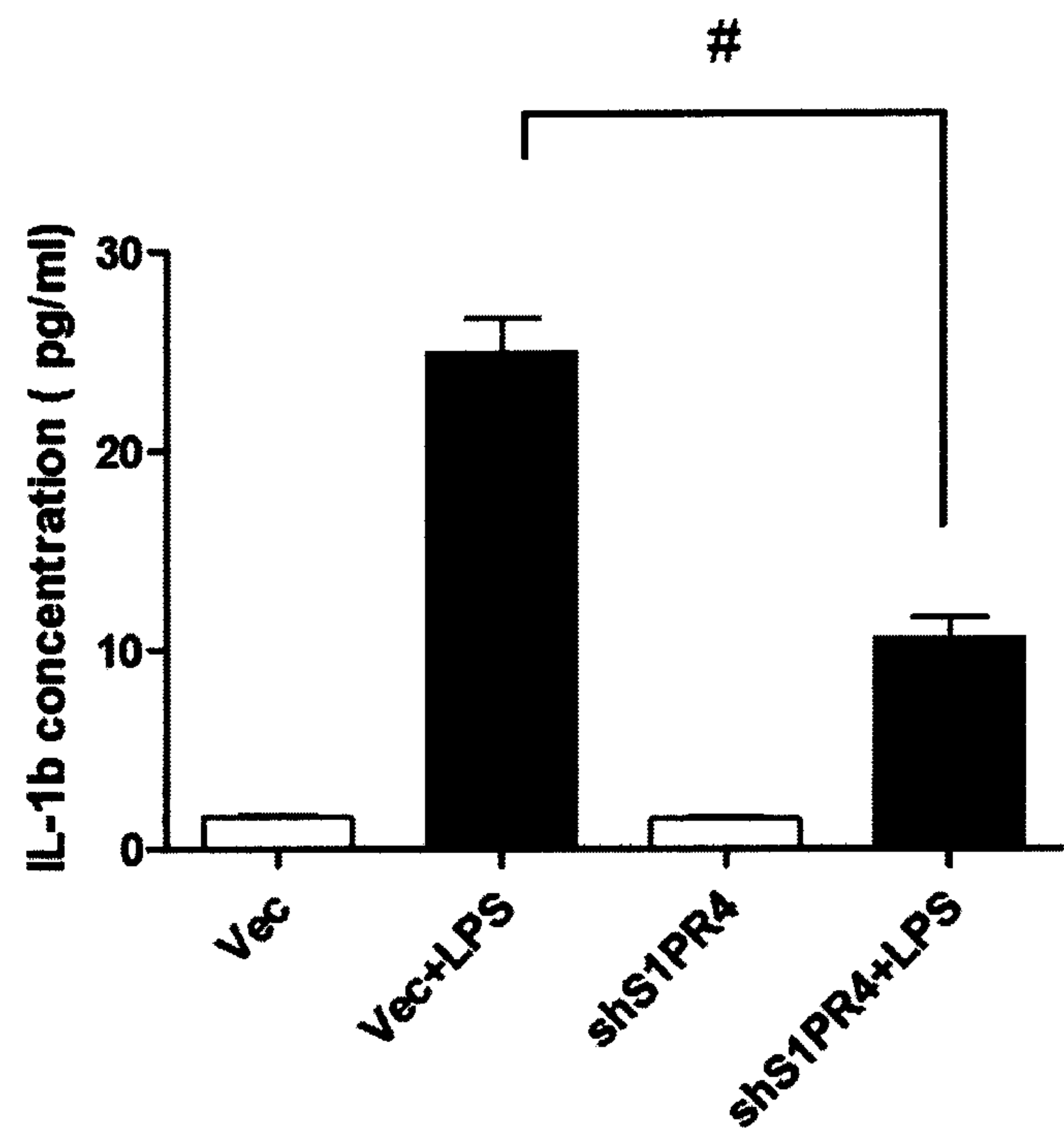
【Fig.4】



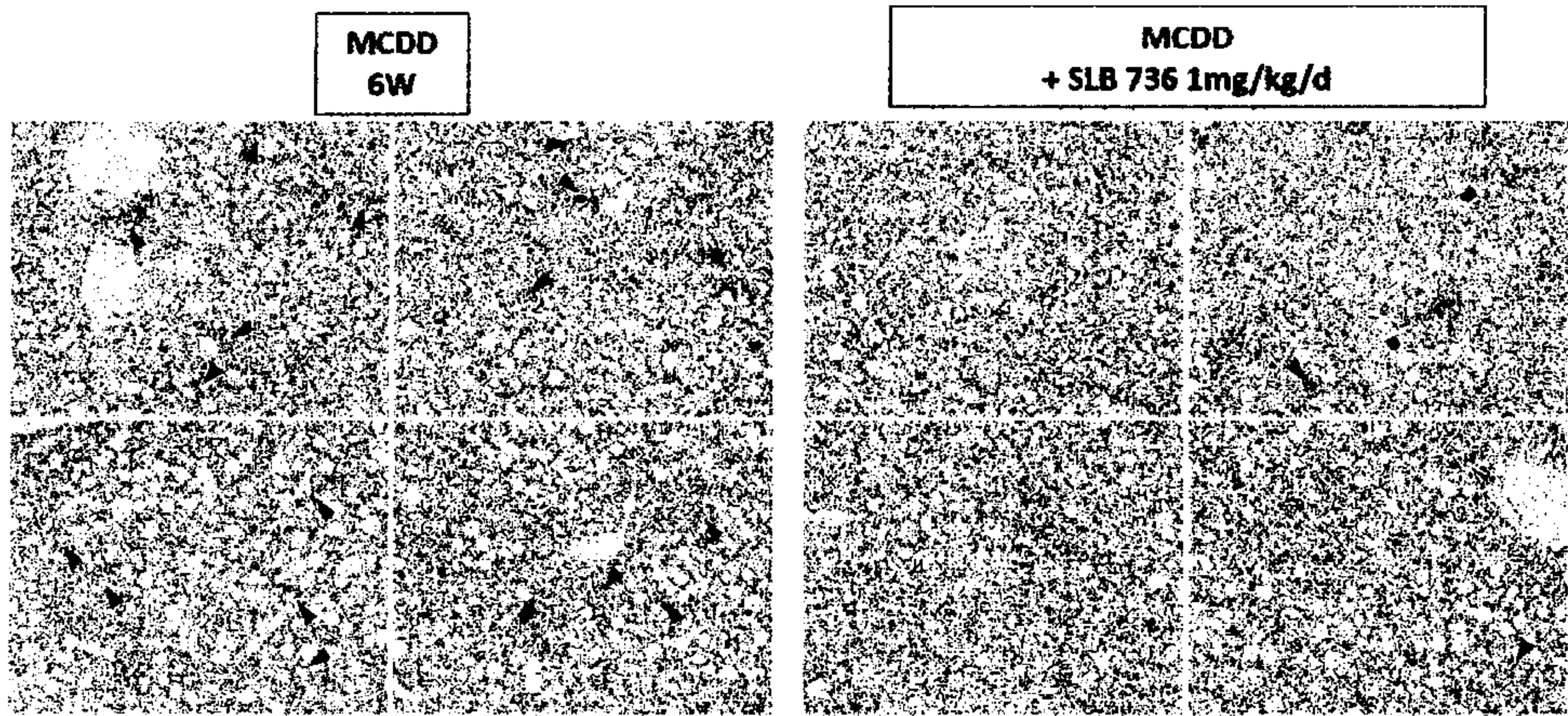
【Fig.5】



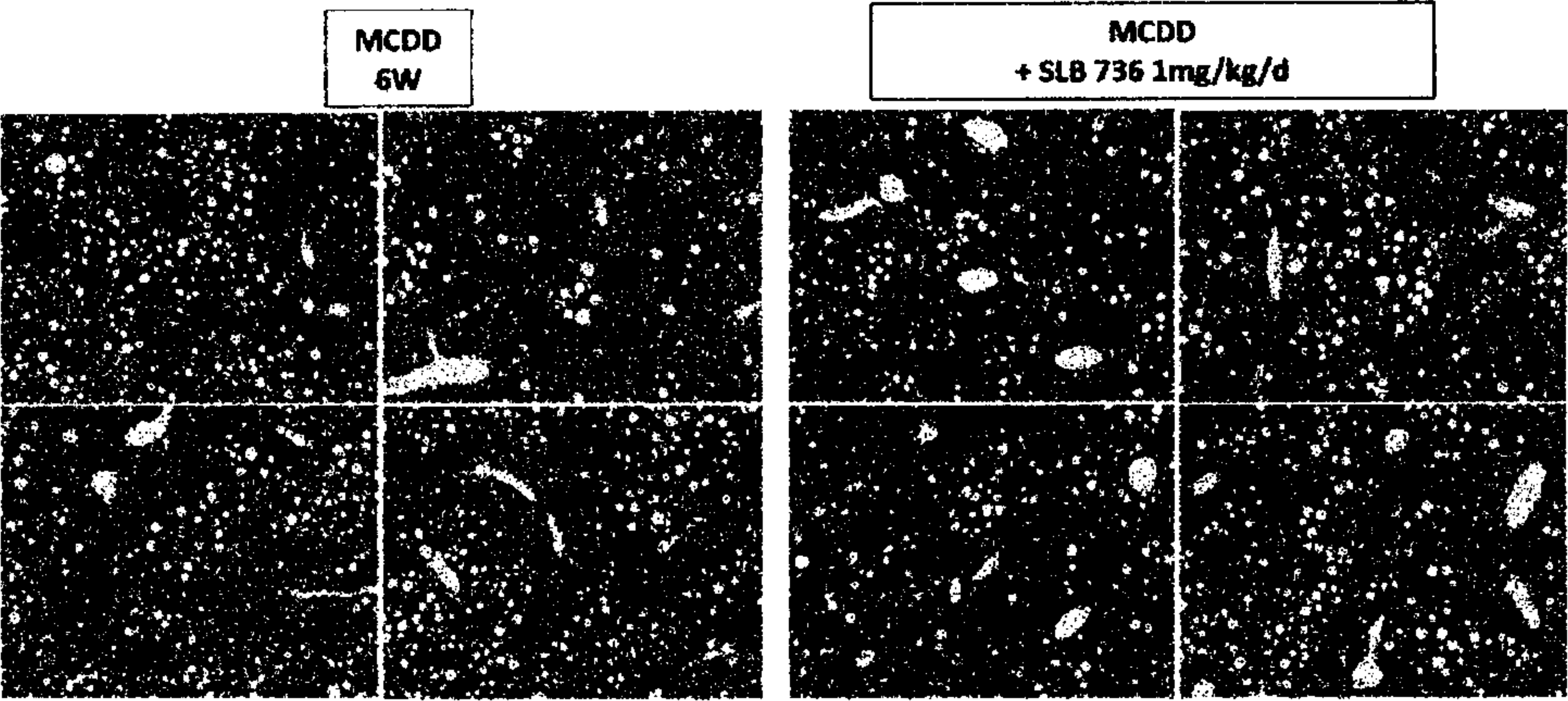
【Fig.6】



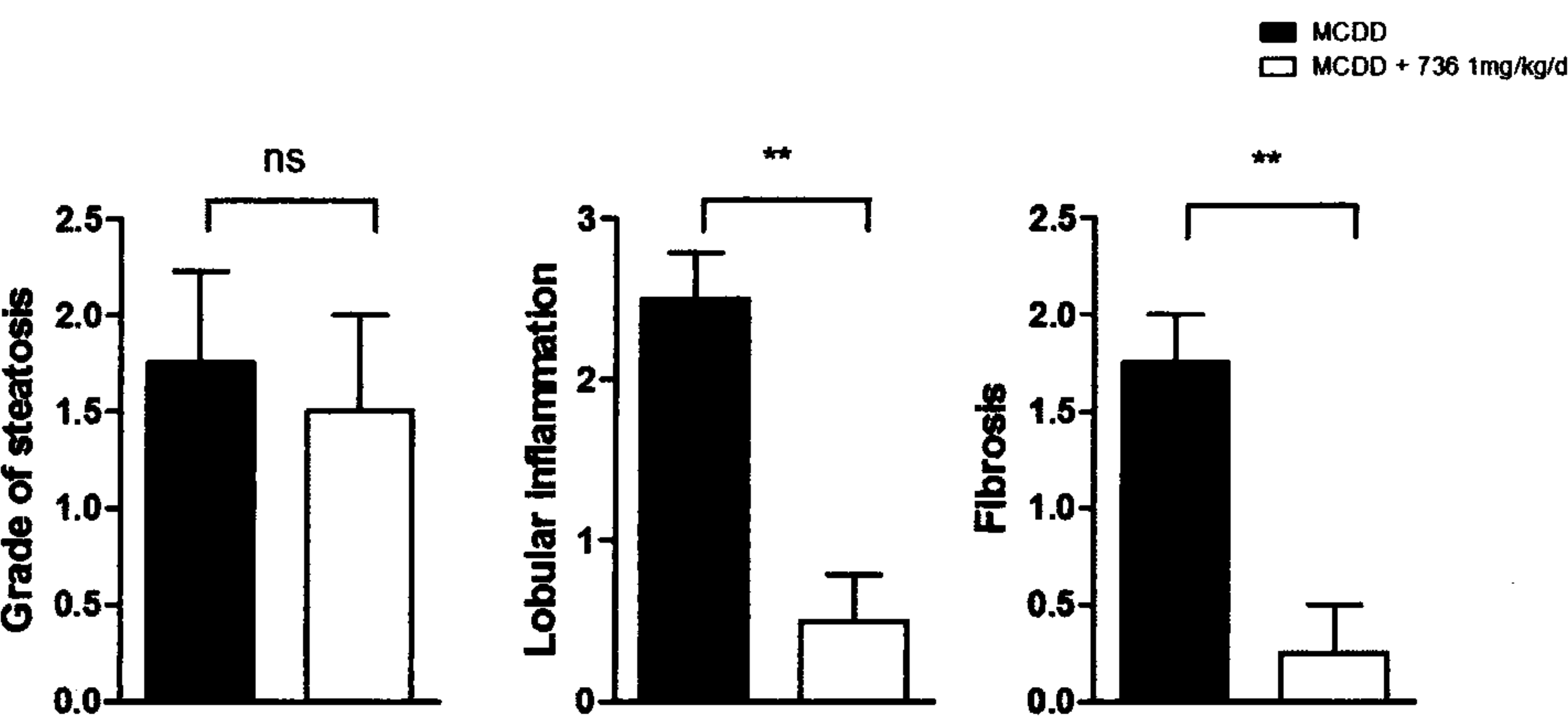
【Fig.7】



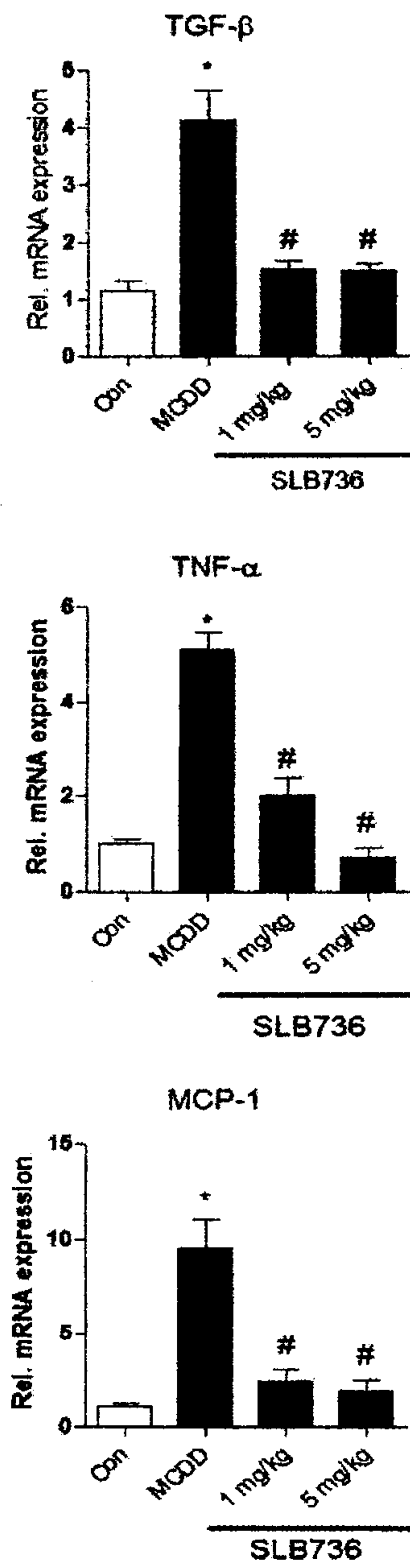
【Fig.8】



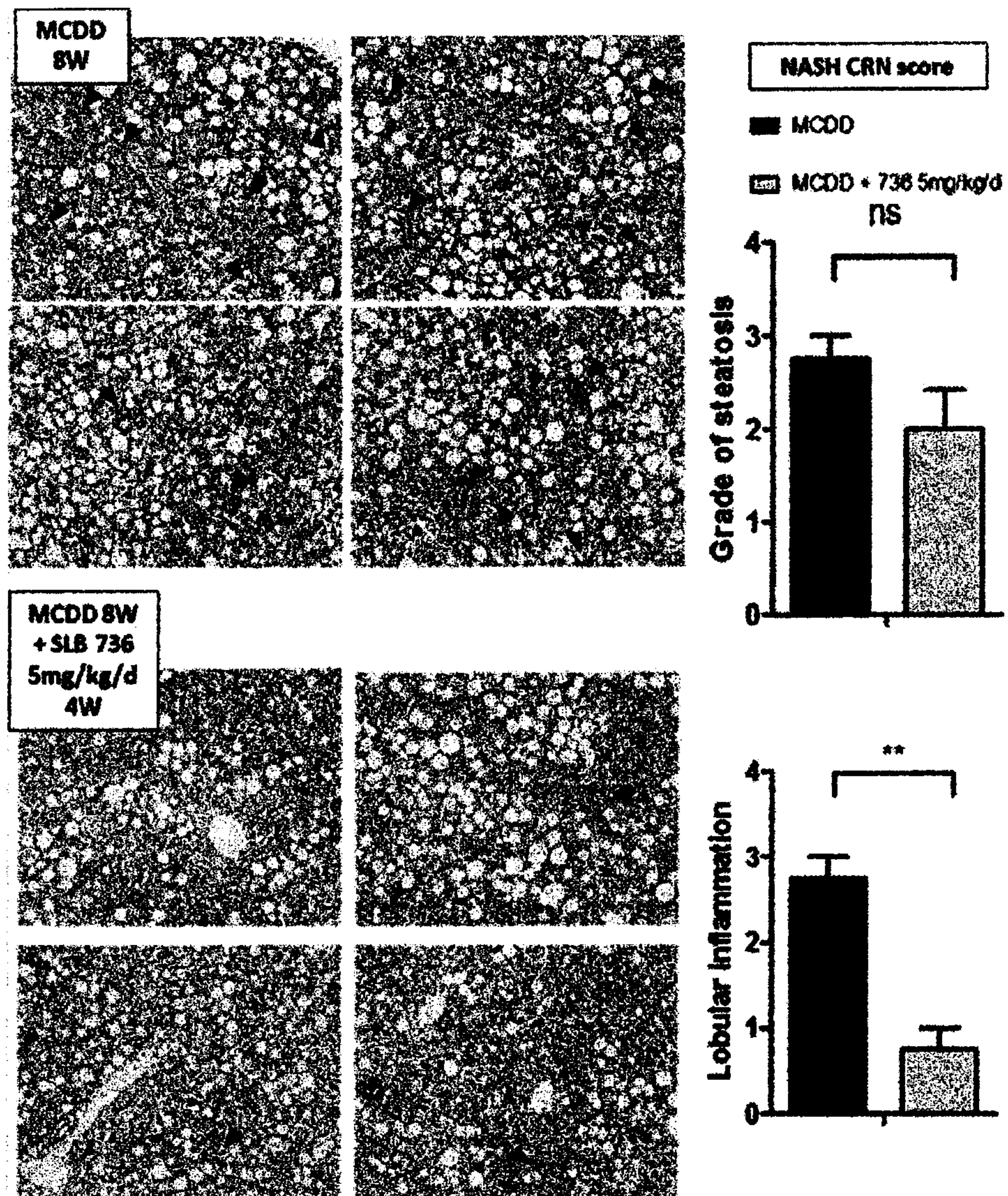
【Fig.9】



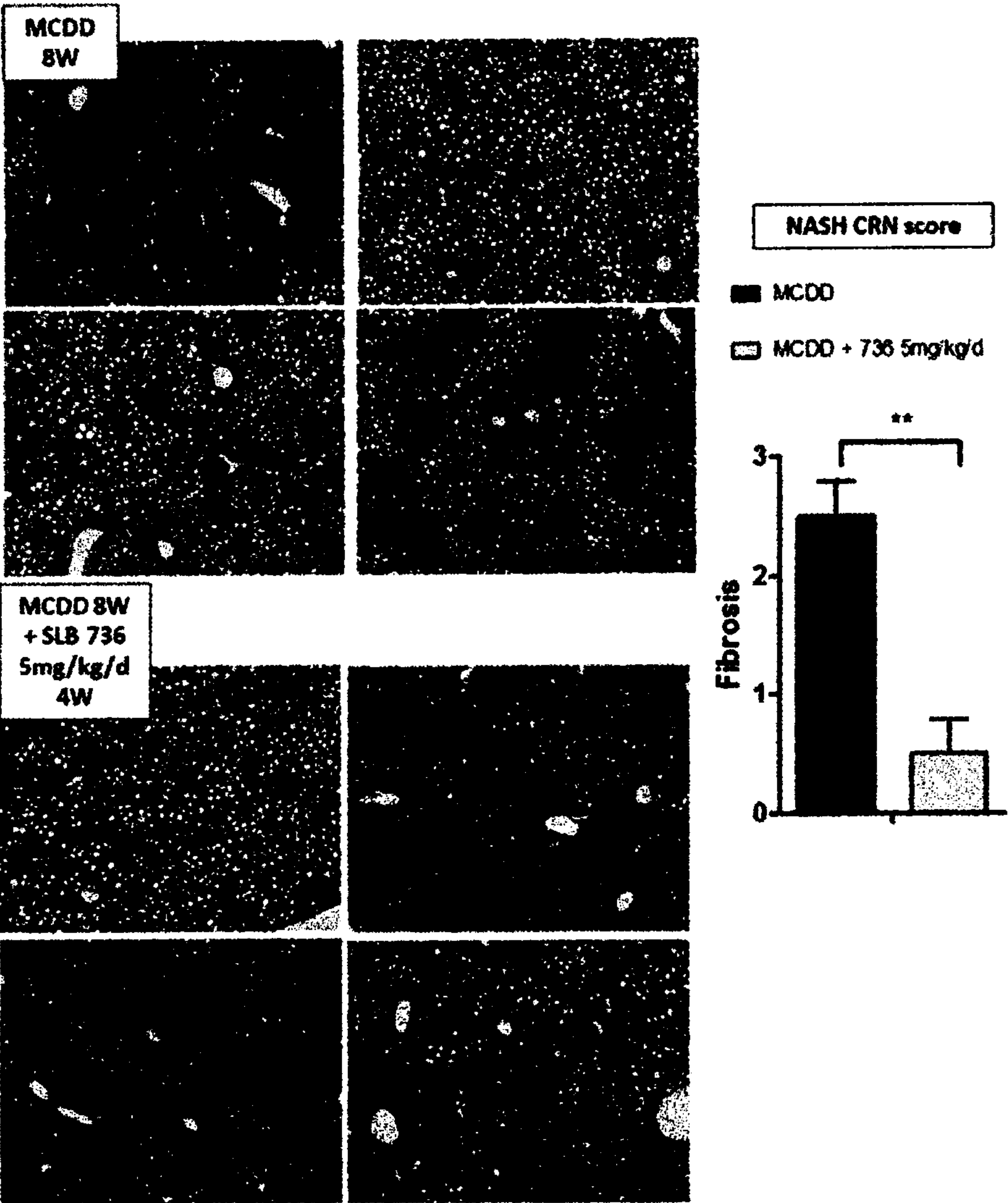
【Fig.10】



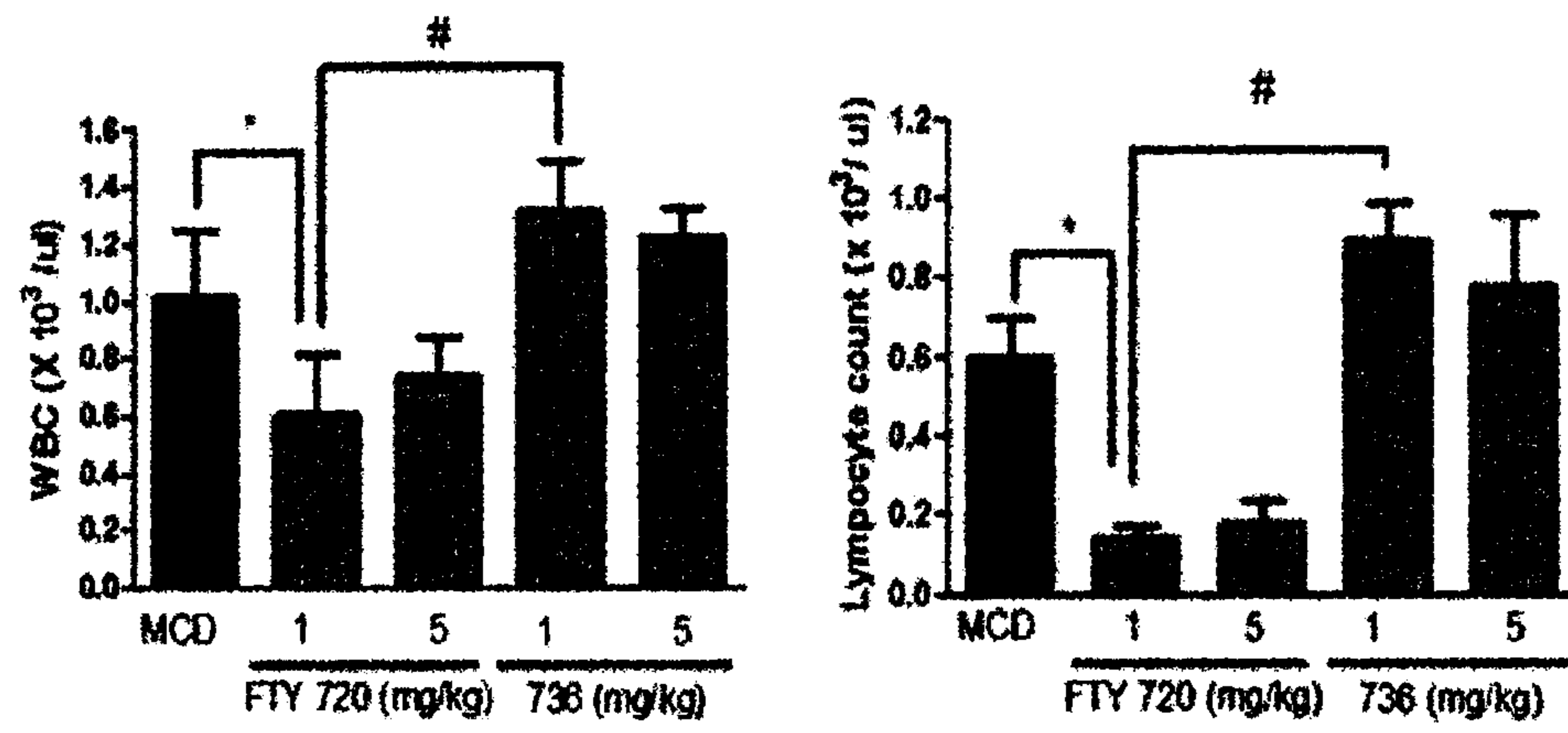
【Fig.11a】



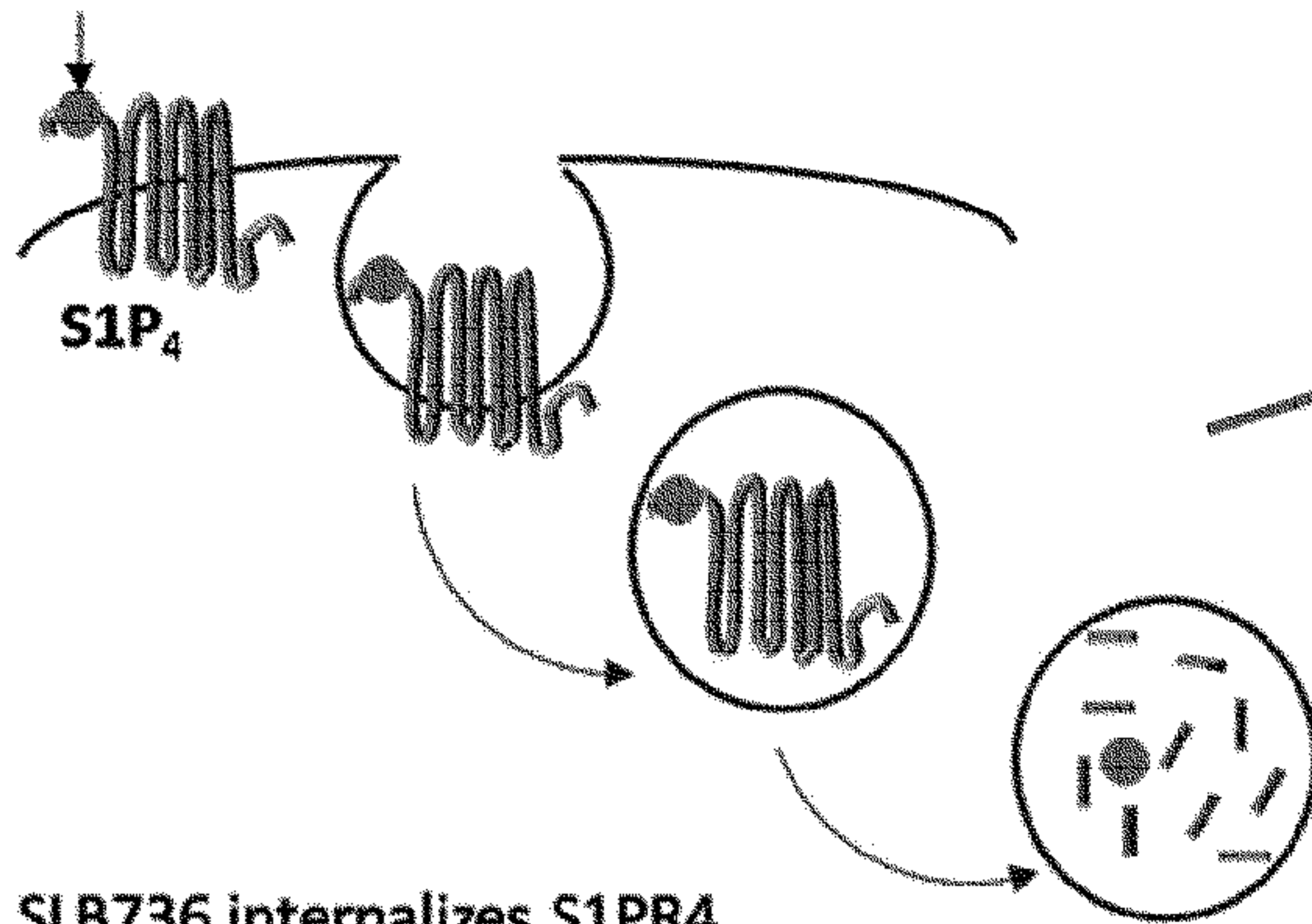
【Fig.11b】



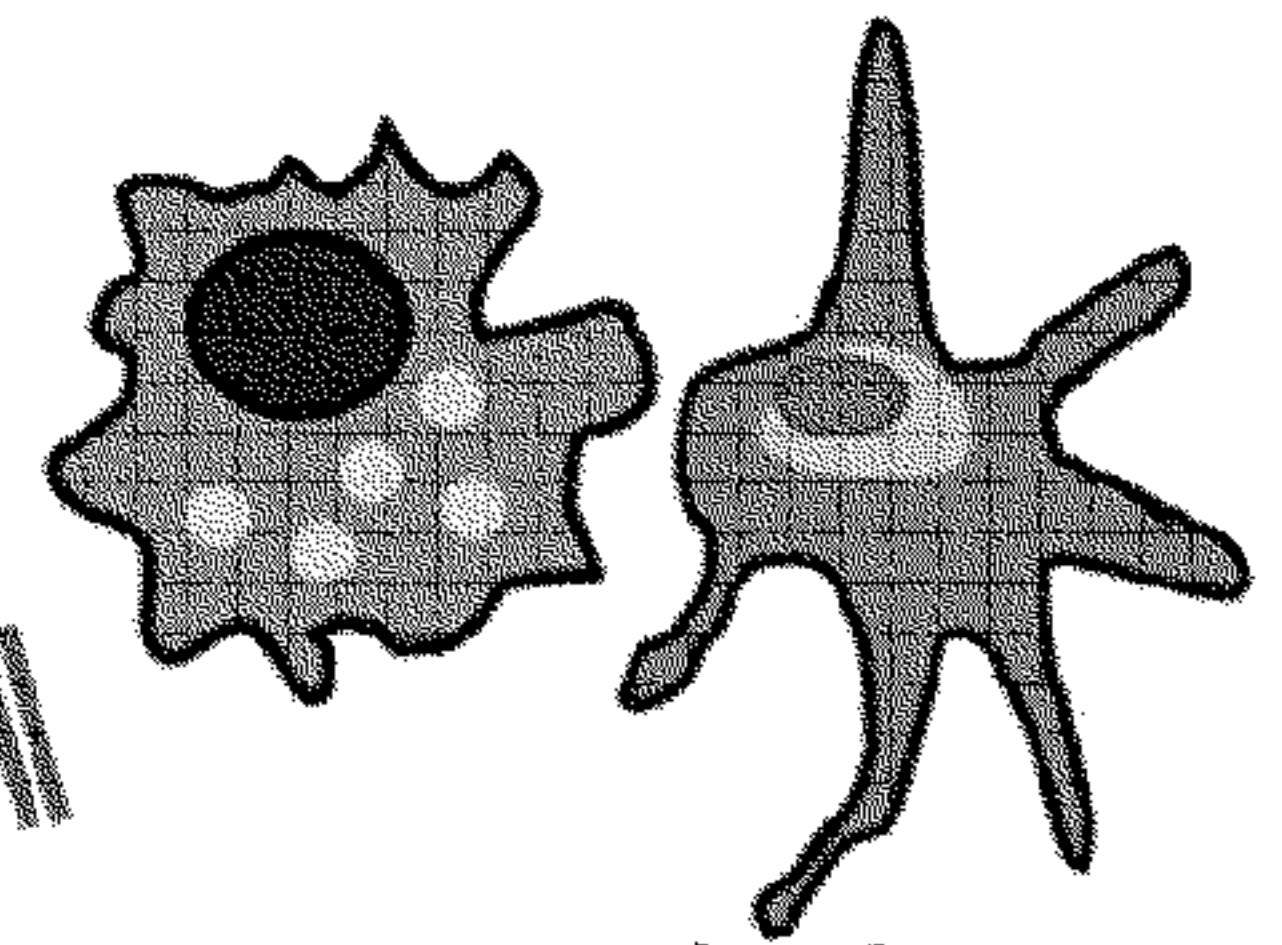
【Fig.12】



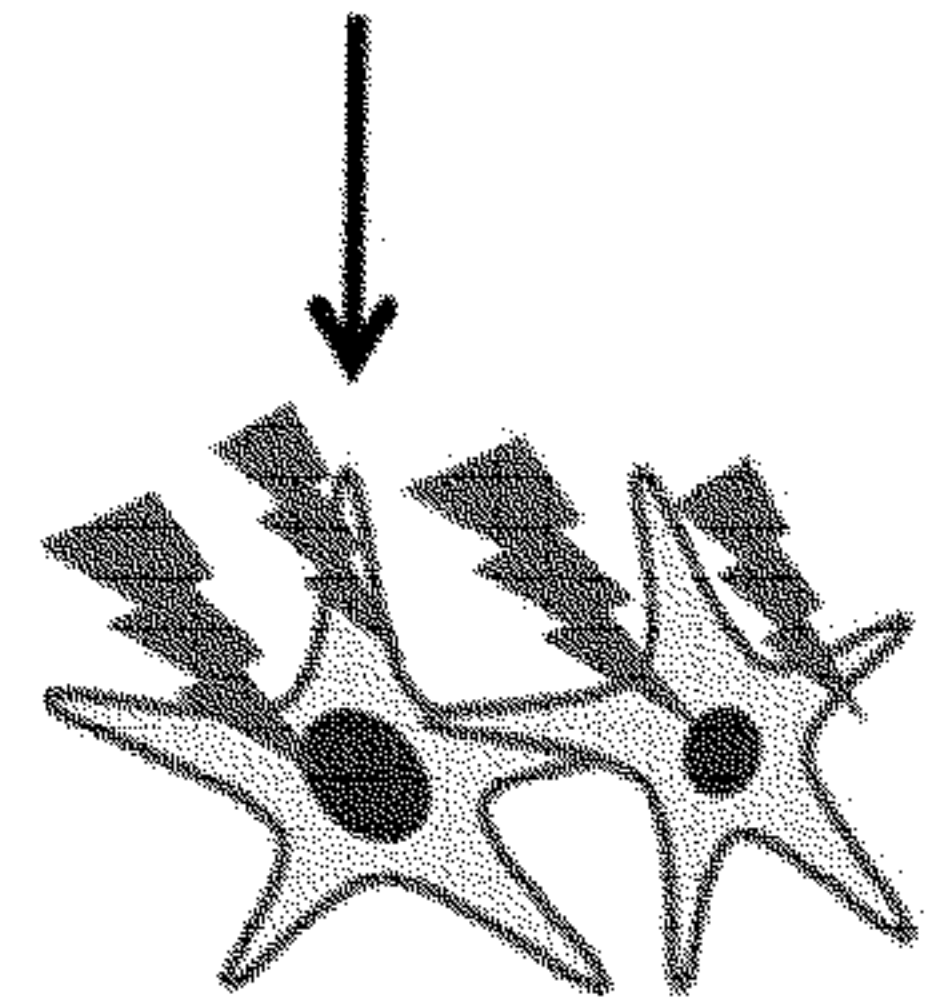
SLB736



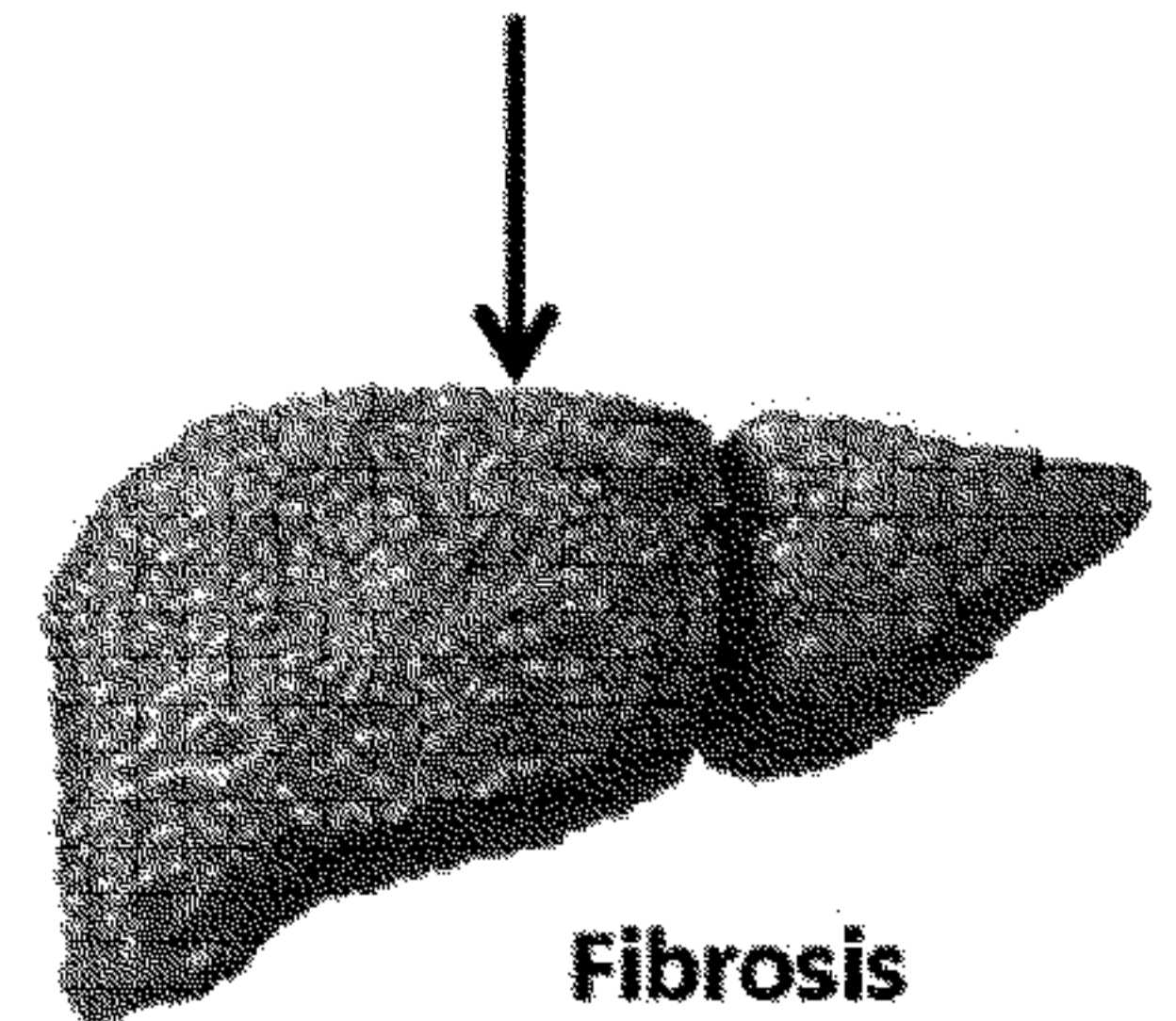
**SLB736 internalizes S1PR4
followed by degradation**



NLRP3 activation



Stellate cell activation



Fibrosis